

Frequency Characteristics in the Visual System of *Drosophila*

Genetic Dissection of Electroretinogram Components

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ABSTRACT Various *Drosophila* mutants were used to dissect the electroretinogram (ERG) frequency response into components of different origins. The ommochrome granules in the receptor cell body are known to migrate in response to light, limiting the amount of light entering the rhabdomere. Comparison between the ERG frequency responses of the wild type and the mutant lacking the ommochrome granules indicates that the pigment migration reduces the amplitude gain at frequencies below 0.5 Hz. The ERG of *Drosophila* compound eyes consists of contributions from receptor cells and the second-order cells in the lamina. Mutants with defective laminae showed a high-frequency cutoff with a corner frequency of about 20 Hz, while in wild type the response peaked in that frequency region. These results suggest that the lamina contributes mainly to the high-frequency components of the ERG transfer function. The shot noise model (Dodge et al., 1968) has been tested in *Drosophila* by comparing the frequency response of the genetically isolated receptor component and the power spectrum of the noise superimposed on the intracellular receptor potential. The results are consistent with the hypothesis that the receptor potential consists of a summation of small discrete potentials (bumps). In a mutant in which the bumps exhibit latency dispersion in response to a dim flash, the receptor showed a poor high-frequency response, the corner frequency being lowered to about 1–2 Hz. The slope of the cutoff was approximately 20 dB/dec indicating that the latency dispersion in this mutant is the major limiting factor in temporal resolution. Light-evoked high frequency oscillations have been observed in the ERG of another mutant. The oscillation was found sharply turned to light flickering at about 55 Hz.

INTRODUCTION

For small changes in light intensity, both vertebrate and invertebrate visual systems can be treated as linear systems (deLange, 1958; DeVoe, 1963; Kuiper and Leutscher-Hazelhoff, 1965; Cleland and Enroth-Cugell, 1966; Pinter, 1966). The main advantage of linear system analysis is its simplicity in manipulation and interpretation of results. Small signal frequency analysis is now widely

adopted for describing and predicting the response dynamics of various elements in visual systems (see, for example, Knight et al., 1970; Pinter, 1972; Ratliff et al., 1974; Toyoda, 1974). The method involves a small-amplitude sinusoidal modulation of the light stimulus which gives rise to a sinusoidal response of the same frequency but generally of different amplitude and phase. In a linear system, the amplitude gain and the phase shift at all modulating frequencies (transfer function) fully specify the input-output relation of the system. The electroretinogram (ERG) is most suitable for this kind of analysis when long, stable intracellular recordings are difficult to obtain. In this paper we present a stepwise genetic dissection of the frequency response of the *Drosophila* ERG, namely, the use of single gene mutants to remove step by step the different components from the wild-type ERG. In this manner we were able to identify and study in the ERG frequency response the different contributions originating from the kinetics of the pigment granule migration, the lamina response, and the receptor potential.

The compound eye of *Drosophila* consists of about 700 subunits called ommatidia. Each ommatidium contains eight photoreceptors: six peripheral retinal cells (R_{1-6}) and two central retinal cells ($R_{7,8}$). The data obtained to date indicate that R_{1-6} , R_7 , and R_8 are associated with three different action spectra (Eckert, 1972; McCann and Arnett, 1972; Minke et al., 1975; Harris et al., 1976). Proximal to the receptor layer lies the lamina, the first synaptic region of the fly optic lobes. The lamina receives input from R_{1-6} , while $R_{7,8}$ bypass the lamina and project onto the higher visual center (Trujillo-Cenoz, 1965; Boschek, 1971; Minke et al., 1975).

The retinula cell body of *Drosophila* contains ommochrome granules (Nolte, 1961). As in other dipterans (Kirschfeld, 1969; Stavenga, 1975), these granules migrate in response to light, limiting the light flux through the rhabdomeres (Franceschini, 1972, 1975). To determine the contribution of the pigment migration process to the frequency characteristics of the ERG, we compared the responses of wild-type and white-eyed flies. Two white-eyed stocks, having little or no ommochrome pigment, were used: a sex-linked mutant white (*w*) and a double mutant brown; scarlet (*bw;st*) (Lindsey and Grell, 1968).

The ERG of the *Drosophila* compound eye has a complex waveform (Fig. 1, top trace) and consists of the contributions from receptor cells and the second-order cells in the lamina. The corneal-positive on-transient and the negative off-transient arise in the lamina and most of the sustained negative component reflects depolarization of receptor cells (for reviews, see Goldsmith and Bernard, 1974; Pak, 1975). Mutations which selectively eliminate the lamina components in the ERG are therefore highly desirable. One such, a third chromosome mutant, *ora*^{JK84} (outer rhabdomeres absent)¹ isolated by Koenig and Merriam (1975), has only vestigial rhabdomeres of R_{1-6} cells. Since the central cells $R_{7,8}$ are the only functional receptors in this mutant (Harris et al., 1976), the lamina receives no input. Therefore, the ERG of this mutant consists solely of the responses of the R_7 and R_8 . There is also available an X-linked mutant sevenless, *sev*^{LY3}, in which the R_7 rhabdomere is missing (Harris et al., 1976). Therefore,

¹ The superscript in the mutant name is the allele designation. For sake of convenience, all the allele designations will be omitted after the first appearance.

the ERG of the double mutant *sev;;ora* consists only of the R_8 response. Indeed, the ERGs of the above two mutants are similar to the intracellularly recorded receptor response in time course (see Fig. 1, and Alawi and Pak, 1971).

Another X-chromosome mutant *nonA*^{P49} (no on-transient A) was isolated at

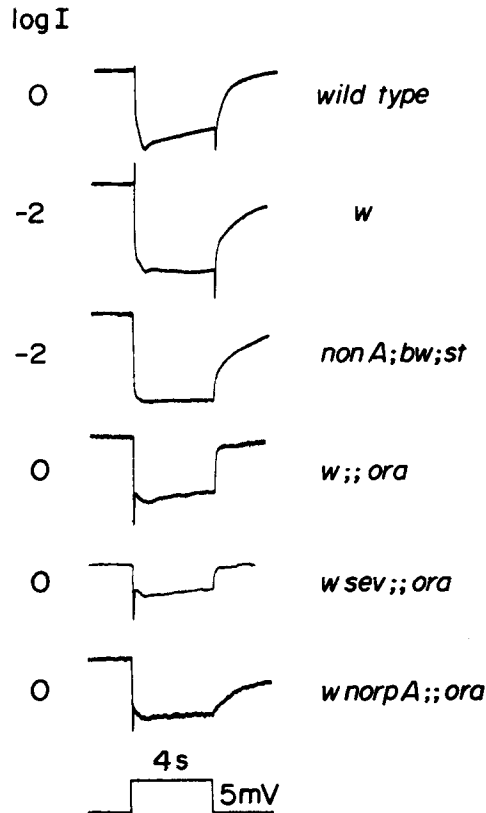


FIGURE 1. ERG waveforms recorded from dark-adapted flies of various strains. All mutants are placed on white-eyed background, combined with either *w* or *bw;st* to remove the screening pigments. On the left, log intensities to the 4-s stimuli are indicated. The white-eyed fly (*w*) is more sensitive to light because of the lack of screening pigments. The absence of the on- and off-transients in the *nonA;bw;st* ERG suggests a defective lamina. The *w;;ora* ($R_{7,8}$ only) and *w sev;;ora* (R_8 only) ERGs look like the intracellularly recorded receptor response. The slow repolarization after light-off in the ERG of *w norpA;;ora* reflects the effect of dispersion in latency distribution of bump generation.

Purdue University (Pak, 1975). The absence of the on- and off-transients in the ERG (Fig. 1) suggests a defective lamina in this mutant, for these fast transients are thought to originate in the lamina. The use of the above mutants, *ora*, *sev;;ora*, and *nonA* enabled us to observe the frequency responses of $R_{7,8}$ and R_8 cells separately and to analyze the receptor and lamina contributions in the ERG frequency response.

Small discrete unitary potentials (bumps) have been recorded from photore-

ceptor cells of several arthropods (Yeandle, 1957; Adolph, 1964; Scholes, 1965; Kirschfeld, 1966), including *Drosophila* (Wu and Pak, 1975). It is thought that these bumps summate to form the receptor potential (Rushton, 1961; Fuortes and Yeandle, 1964; Dodge 1968; Wu and Pak, 1975). On the basis of a shot noise model, Dodge et al. (1968) proposed a way quantitatively to test this idea. They pointed out that if the variation in the latency of occurrence of the bumps is negligible in comparison with the time course of individual bumps, the square of the frequency response amplitude should be proportional to the power spectrum calculated from the fluctuations in the receptor potential under steady illumination. In *Limulus* photoreceptors, the occurrence of the bumps usually exhibits large dispersion of latencies (Fuortes and Yeandle, 1964). Quantitative studies indicate that the dynamical response of *Limulus* photoreceptors can be predicted from the measured latency dispersion and the power spectrum of the bump noise (Wong et al., 1976; Wong and Knight, 1977). In normal *Drosophila* photoreceptors, the bumps show negligible variation in latency in response to brief pulses of light (Pak et al., 1976; Wu, 1976). Therefore we set out to compare the power spectrum of the bump noise with the square of the frequency response of the genetically isolated receptor component of the ERG.

A gene on the X chromosome, designated no receptor potential *A* (*norpA*), has been found to affect the phototransduction process (Pak, 1975). An allele of this cistron, *norpA*^{H52}, isolated by M. Heisenberg, is temperature sensitive (Deland and Pak, 1973). At room temperature, bumps in this mutant exhibit a large dispersion in latency distribution in response to a dim flash (Pak et al., 1976). The effect of bump latency dispersion on the receptor potential can be observed in the ERG of the double mutant *norpA*; *ora* (*norpA* mutant placed on a mutant background lacking the *R*₁₋₆ rhabdomeres, see Fig. 1), since in this double mutant only the receptor potentials contribute to the ERG. If bumps indeed summate to form the receptor potential, the *norpA* receptor should have poor response to high-frequency stimulation because of the latency dispersion.

Finally, a second chromosome mutant, receptor oscillation *A* (*rosA*) isolated in Pak's laboratory, shows light-evoked oscillations in the ERG. An attempt was made to demonstrate the resonant nature of the oscillation.

MATERIALS AND METHODS

Preparation and Recording

Due to their lack of screening pigments, white-eyed flies are more sensitive to light and give responses to a wider range of stimulus intensities (Alawi et al., 1972; Pak and Lidington, 1974). In the present study, all the above described mutants were combined with either *w* or *bw*; *st* to remove the screening pigments.

For ERG recordings flies were first immobilized by chilling and were fixed on their sides to glass slides with a rosin-beeswax mixture. The flies recovered from the effect of cooling in a few minutes, and respiration was unimpaired by these preparatory steps. The ERG was recorded by use of glass microelectrodes filled with physiological saline. The recording electrode just penetrated the cornea and the reference electrode pierced the thorax. The tip of a fiber optics waveguide was positioned within 0.5 cm from the eye. After amplification, the voltage signal was displayed on an oscilloscope and sampled on-line by a digital PDP 11-45 computer (Digital Equipment Corp., Maynard, Mass.). The

voltage signal was also recorded on a Brush pen recorder (Gould Inc., Cleveland, Ohio). The techniques for the intracellular recordings have been described previously (Wu and Pak, 1975).

Light Stimulus

For frequency response measurements, the light source was a Sylvania Glow Modulator Tube R1131C (Sylvania Electric, New York). The spectrum of the light contains wavelengths from 360 to 600 nm with a sharp spectral line at about 580 nm. A 3-foot fiber optics light guide (American Optical LGM) guided the light stimulus from the glow tube to the preparation. The unattenuated light intensity (the intensity $\log I = 0$ in the figures) at the preparation level was 7.5×10^{14} photons/cm²-s when measured at 520 nm over a 40-nm bandwidth. The light stimulus was attenuated by means of neutral density filters. The sinusoidal stimulation was obtained by frequency modulation of the 0.5-ms light pulses originating from the glow tube, i.e. the frequency of the light pulses varied in a sinusoidal manner. The carrier frequency was 500 Hz for the experiments of Figs. 2-4 and 600 Hz for Figs. 5-9.

For intracellular recordings, the light source was a 150-W xenon arc lamp attached to a Bausch & Lomb High Intensity Monochromator (Bausch & Lomb Inc., Rochester, N. Y.). The unattenuated intensity of the 520 nm stimulus (half peak bandwidth = 16 nm) was about 3×10^{14} photons/cm²-s at the level of the preparation.

Frequency Response Measurement

For each fly, the intensity-evoking half-saturated response (σ) was determined in order to provide a reference for the effective intensity of the stimulus. To measure the frequency response at a specific mean light intensity, the following steps were taken for each data point. (a) The voltage output was sampled for 5 s in the dark before the light stimulus was presented to determine the base-line. (b) The stimulus was applied for 10 s to allow the response to reach a steady-state amplitude. The sinusoidal modulation about the mean intensity was then applied, and the voltage response was sampled. (c) Afterwards, the preparation was allowed to dark adapt for 90-120 s before proceeding to the next round.

Peak-to-peak modulation of the light intensity was 40% of the mean (modulation index $m = 0.2$) in the experiments illustrated in Fig. 2-4 and 60% ($m = 0.3$) for Figs. 5-9. These rather large modulation amplitudes greatly improved the signal-to-noise ratio and seemed to have little effect on the linearity of the response. The amplitude gain was defined by $(\delta r/r)/(\delta s/s)$, where $\delta r/r$ is the ratio of the modulated response amplitude to the mean voltage response, and $\delta s/s$ the ratio of the modulation to the mean stimulus intensity.

The computer was employed for sampling and processing of experimental data. Response was sampled at 60 points/s for 20 s for modulation frequencies below 20 Hz, and 600 points/s for 2 s for frequencies above 20 Hz. The data were placed into 32 bins, i.e. each complete cycle of the sine wave was divided into 32 parts, and the average of the sampled data points in each part was calculated and stored. The data in the 32 bins accumulated in each additional cycle of the sinusoid until the end of the modulation. The grand average for each of the 32 parts was then computed from the accumulated sums. From the 32 averaged values, the Fourier coefficients of the fundamental and the harmonics were calculated. The amplitude gain and phase shift were computed from the coefficients of the fundamental. The harmonic content provided a basis for judging the linearity of the response. We have arbitrarily defined the corneally negative ERG to be in phase with the stimulus such that the phase plots start out at low frequencies with near-zero degree phase shift.

Estimation of Power Spectrum

The intracellular voltage response was first stored on magnetic tape, which accepted a frequency band from 0 to 1,000 Hz. The autocovariance function $C(\tau)$ of the steady-state component of the receptor potential was computed according to the formula

$$C(\tau) = \overline{\{v(t) - \bar{v}\} \{v(t + \tau) - \bar{v}\}}, \quad (1)$$

where $v(t)$ is the voltage at time t and τ is the time lag. The calculation was based on 750 points sampled at 8-ms intervals. The power spectrum was obtained by calculating the finite Fourier cosine transform of the first 250 points of the autocovariance function. Smoothing of the power spectrum was achieved by averaging the nonoverlapping groups of five neighboring points in the spectrum. The whole system was calibrated by sine waves of known frequencies.

RESULTS

Up to four flies from each mutant strain were studied. Results presented in the following figures are based on single flies because the stimulus intensity for each fly was not strictly controlled with reference to σ , the intensity-eliciting half-saturated response, of each individual fly. However, the frequency response data were found to be highly reproducible from fly to fly of the same mutant strain. The distinct, systematic differences between the frequency responses of various mutants reported here are well outside the ranges of the variation among flies of the same mutant strain and appear to be due mainly to the effect of different mutations.

Measurements on the same individual fly were often repeated two or three times. Both amplitude gain and phase data showed relatively large variation at very low (<0.3 Hz) and high frequencies (>50 Hz). The variations, however, were less than 10% in the cases checked. We attribute a substantial part of the variation at the low-frequency end to slow, light-correlated voltage drift during the recordings, probably due to the movement of the thorax picked up by the reference electrode. The larger variation in the high frequency end is probably caused by the lower signal-to-noise ratio at high frequencies.

The ERG responses appeared approximately linear at frequencies between 1 and 50 Hz, as determined by the waveform of the response and the small harmonic content. Second and third harmonics were usually less than 10% of the fundamental. For frequencies below 1 Hz, distortion of the sinusoidal waveform was evident even by visual inspection of the strip chart recordings.

Effect of Light Intensity on the Drosophila ERG

As has been described previously, the eye color pigments of all mutants used in this work were eliminated genetically because white-eyed flies are more sensitive to light and give responses to a wider range of light intensity. Thus, we have used the white-eyed strain w as a control stock and extensively studied the relationship between light intensity and the ERG frequency response in w flies. Fig. 2 shows the amplitude and phase plots of the transfer function of the white-eyed flies (w) at -4 and -2 log intensities. The half-saturation intensity (σ) determined from the stimulus-response curve, was -2.1 log units. The most distinctive feature of the amplitude gain plot at low intensity ($\log I = -4$, Fig. 2)

is a steep high-frequency cutoff (3-dB frequency = 20 Hz).² At a higher intensity ($\log I = -2$) the cutoff shifts to higher frequencies. In addition, the gain is suppressed in the low frequency end (<10 Hz), resulting in a peak at about 20 Hz in the plot (Fig. 2).

In a minimum phase system, some phase lead would be expected when amplitude gain peaked. However, no phase lead was observed at the intensity

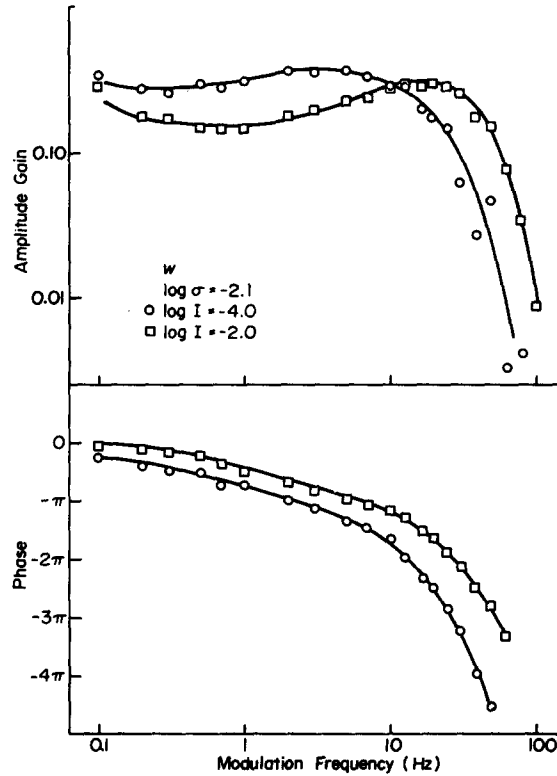


FIGURE 2. Frequency response of the white-eyed (*w*) fly at two different light intensities. For this and all following figures, σ represents the light intensity which evokes the half-maximum response, and I denotes the mean light intensity of the modulated stimulus in log units. The intensity $\log I = 0$ corresponds to the unattenuated intensity described in Materials and Methods. Continuous curves are fitted by eye.

$\log I = -2$ (Fig. 2). This indicates that the ERG, which is composed of responses of the retina and lamina summing in parallel, is not likely to be a minimum phase system.

Effect of Pigment Migration

The presence or absence of pigment migration seems to be reflected by the

² The 3-dB frequency is defined here as the frequency at which the frequency response amplitude is $1/\sqrt{2}$ of the amplitude of the low-frequency plateau.

waveforms of the ERG in response to a step increase of light. The ERG of white-eyed flies (*w*) reaches a steady state soon after the onset of light, while the ERG of wild-type flies continues to decline during a stimulus of 4 s duration (Fig. 1), presumably because of continued diminution in effective light intensity caused by migration of the pigment granules. Since the presence of pigment migration provides a gain control mechanism for the photoreceptor, one would expect some systematic difference in the frequency responses between the mutant *w*, which lacks pigment granules, and the wild-type fly.

Fig. 3 illustrates the transfer function of wild-type flies at $\log I = 0$ ($\log \sigma = -1.1$). At comparable light intensities (with reference to σ) the wild-type ERG shows overall a slightly larger amplitude gain than the *w* ERG (Fig. 3), which may be due to a pleiotropic effect of *w* mutation. Nevertheless the general profile of the amplitude gain plot of wild-type flies resembles that of white-eyed (*w*) flies

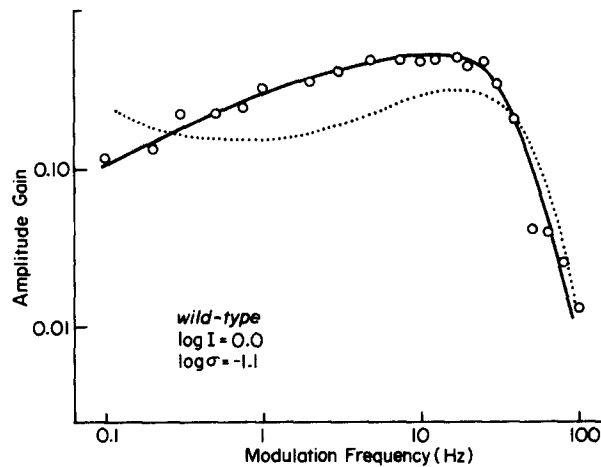


FIGURE 3. Frequency response of the wild-type fly. The dotted curve is the frequency response of *w* ($\log I = -2$, $\log \sigma = -2.1$) copied from Fig. 2.

except for the gently sloping cutoff at low frequency not found in *w* (Fig. 3). As in the case of white-eyed flies, the gain peaks at about 20 Hz. In comparison with white-eyed flies the wild type shows reduced gain (with reference to that at the peak) at frequencies below 0.5 Hz. Reduced amplitude gain at these frequencies is consistent with the reported migration time constant of 1–2 s (Franceschini, 1972).

Receptor and Lamina Contributions

NonA mutation is thought to disrupt the functioning of the lamina (see Introduction). The amplitude plot of the white-eyed *nonA* mutant (*nonA;bw;st*) shows a high-frequency cutoff at a stimulus intensity of 1.3 log units above σ (Fig. 4). At the highest available intensity (2.3 log units above σ) suppression of the low frequency response (between 0.5 and 10 Hz) is seen in addition to the high-frequency cutoff causing a slight hump in the plot at about 15 Hz (Fig. 4).

The amplitude gains of *w*; *ora* and *w sev*; *ora* ERGs are shown in Fig. 5. Their phase data are very similar to those of *nonA*; *bw*; *st* shown in Fig. 4 and not presented here. Since the axons of R_7 and R_8 do not synapse in the lamina, the ERG responses of these mutants consist only of the receptor responses. As in *nonA* flies (Fig. 4), the amplitude gain plots of *w*; *ora* and *w sev*; *ora* show simple cutoff at high frequencies. In *w*; *ora* flies light adaptation seems to suppress only the low-frequency response, while leaving the high-frequency response relatively unaffected (compare $\log I = -1.6$ and 0 in Fig. 5). The same is true for *w sev*; *ora* flies (not shown).

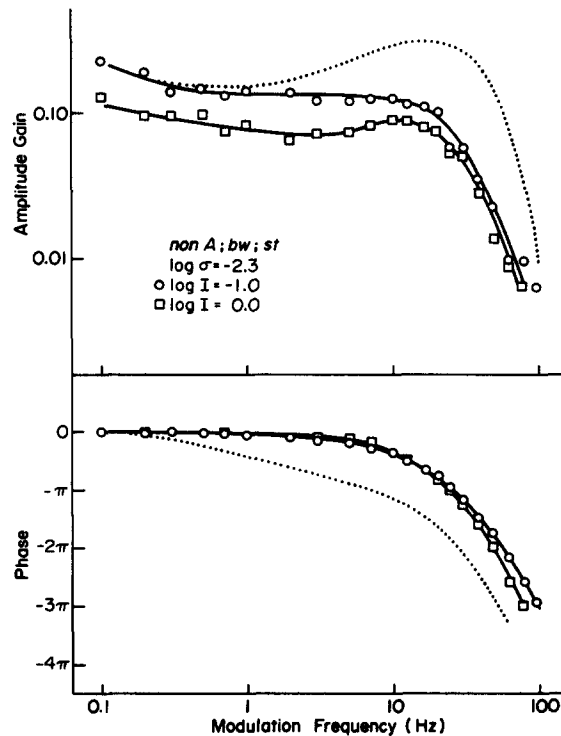


FIGURE 4. Frequency response of the *nonA*; *bw*; *st* fly at the intensity of $1.3 \log$ units above σ and at the highest available intensity. Dotted curves are the frequency response of *w* ($\log I = -2$) copied from Fig. 2.

In the case of *w* flies, in which the lamina is functional, the high-frequency response is considerably enhanced at comparable intensities (Figs. 4 and 5). The improvement of high frequency response in *w* flies thus seems to be due to the contribution from the lamina.

Receptor Noise Spectrum and Frequency Response

In normal *Drosophila* photoreceptors, the bumps show negligible variation in latency distribution; therefore the frequency response of the receptor potential would be determined mainly by the time course of individual bumps. The time

course of individual bumps can be determined by the power spectrum of their resulting random summation, the receptor potential. The power spectrum so measured is expected to be proportional to the square of the frequency response amplitude.

As illustrated in Fig. 6, we have compared the square of the frequency response of the genetically isolated receptor component of the ERG and the power spectrum of the bump noise. The triangles show the relative power spectral density of the steady-state receptor potential recorded intracellularly from the control strain *w* ($\log I = -2$, $\log \sigma = -2.7$). The other two symbols show the squares of the normalized ERG responses of the mutants *nonA;bw;st* ($\log I = -1$, $\log \sigma = -2.3$) and *w sev;;ora* ($\log I = 0$, $\log \sigma = -1.8$), computed from Figs. 4 and 5, respectively. The close fit of these three quantities seems to agree

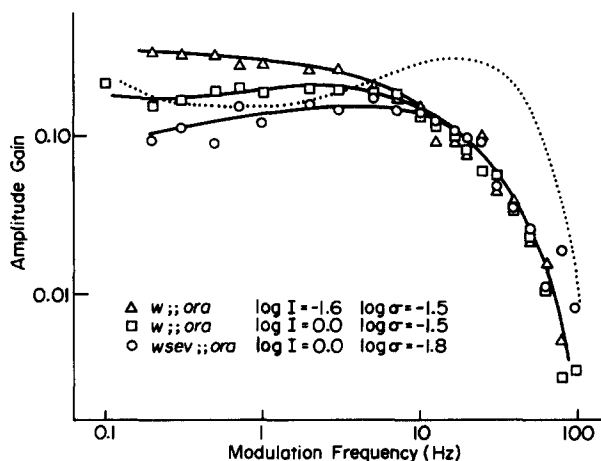


FIGURE 5. Frequency response of the *w;;ora* and *w sev;;ora* flies. The dotted curve is the frequency response of *w* ($\log I = -2$) copied from Fig. 2.

with the notion that the frequency response of the receptor potential is mainly determined by the bump shape in normal fly photoreceptors.

Effect of Latency Dispersion of Bumps

In *norpA* flies quantum bumps exhibit a large dispersion in latency distribution in response to light, but the individual bumps have a normal time course (Pak et al., 1976). Therefore, the *norpA* receptor is expected to have poor response to high-frequency stimulation because of the latency dispersion. This notion was examined by studying the frequency response of the ERG by use of the mutant *w norpA;;ora* (see Introduction). As compared with *w;;ora* ($R_{7,8}$ only), the *w norpA;;ora* fly did show a poor high frequency response, the corner (3-dB) frequency being lowered from about 15 Hz for *w;;ora* to between 1 and 2 Hz for *w norpA;;ora* (Fig. 7). The light intensity was found to exert little effect on the observed slope of the cutoff (not shown). The amplitude gain of *w norpA;;ora* displays a slope of about 20 dB/dec at the high frequency cutoff and a 3-dB frequency of about 1-2 Hz, and therefore obviously departs from the fit shown

in Fig. 6. (The cutoff slope of the curve in Fig. 6 is about 40 dB/dec and the 3-dB frequency about 20 Hz.)

Resonant Nature of rosA Responses

Light-evoked high frequency oscillations have been observed in the ERG of the mutant *rosA*. Once induced, the oscillation was maintained for about 7–8 s and then gradually subsided while the stimulus was still applied. The oscillation frequency ranged from about 45 Hz at the beginning to about 90 Hz at the end of oscillations (Fig. 8, top trace). As demonstrated in the transfer function (Fig.

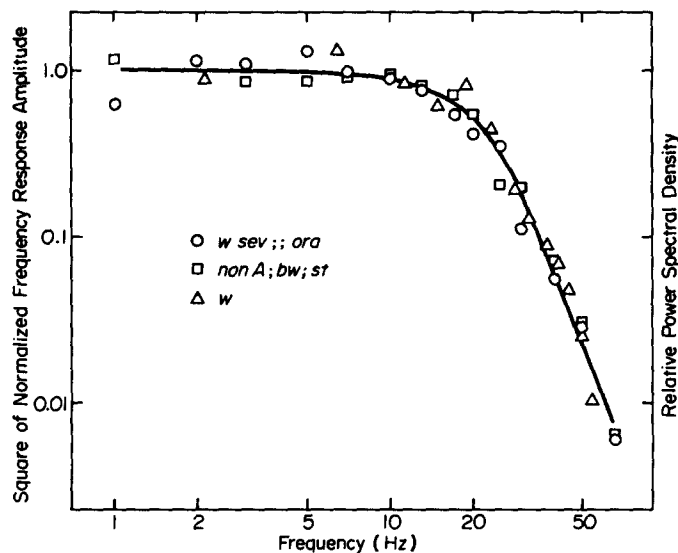


FIGURE 6. Comparison of the normalized power spectrum of the intracellular bump noise and the squares of normalized frequency responses of the ERG receptor component. The triangles show the relative power spectral density of the bump noise recorded intracellularly from the control strain *w* (520 nm, $\log I = -2$, $\log \sigma = -2.7$). The other two symbols show the squares of the normalized ERG frequency responses of the mutants *nonA; bw; st* ($\log I = -1$, $\log \sigma = -2.3$) and *w sev;; ora* ($\log I = 0$, $\log \sigma = -1.8$) computed from Figs. 4 and 5, respectively. The continuous curve is fitted by eye to data points.

9), the *rosA* response (after the oscillation subsided) was found to be sharply tuned to light flickering at about 55 Hz. The lower traces of Fig. 8 show the responses to the sinusoidally flickering light of various frequencies. The resonant nature of the response is evident. The tuning frequency varied slightly in different animals, and the quality of tuning also changed slightly in different experimental runs on the same fly.

DISCUSSION

Lamina Contribution

The ERGs of the mutants *w;; ora* and *w sev;; ora* consist only of receptor responses. The difference between their transfer functions and that of the *w* ERG

(Fig. 5) indicates that the lamina is mainly responsible for the high frequency response of the *Drosophila* ERG.

The observation on *nonA* (defective lamina) flies further supports this notion. The *nonA;bw;st* amplitude gain plot (Fig. 4) resembles those of *w;ora* and *w sev;ora* flies (Fig. 5). The strongest available stimulus does not cause enhancement of the high-frequency response as in the case of *w* (Fig. 4). Moreover, the phase plots of the three flies *nonA;bw;st*, *w;ora* and *w sev;ora* are very similar, whereas *w* shows some additional phase shift of about 0.75π in the vicinity of 20

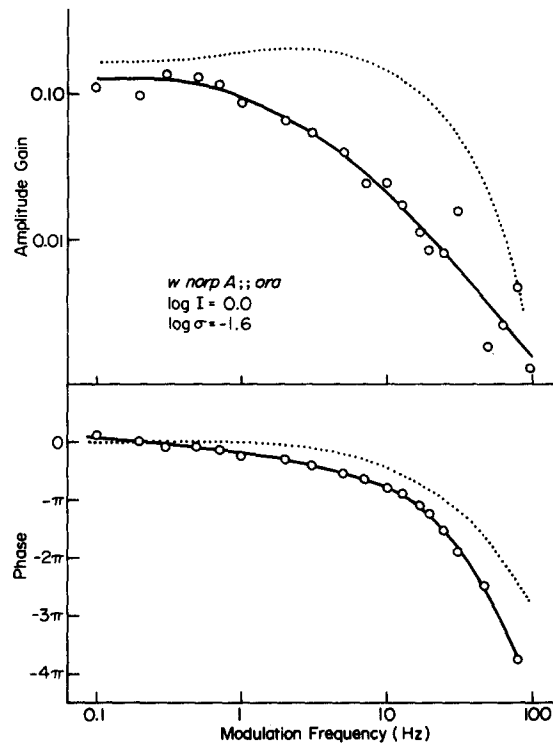


FIGURE 7. Frequency response of the *w norpA; ora* fly. Note that the cutoff (3-dB) frequency is lowered to about 1-2 Hz and the slope of the cutoff is approximately 20 dB/dec. Dotted curves are the frequency response of *w;ora* ($\log I = 0$, $\log \sigma = -1.5$) shown in Fig. 5.

Hz (the peaking frequency in the *w* amplitude plot, see Fig. 4 for comparison), indicating that the dominant components in the *w* high-frequency response are generated at a stage more central to the receptor. Heisenberg (1971) has measured the frequency responses of the receptor and lamina components by differential recordings with tips of two electrodes placed at different depths of the compound eye. His results also indicate that the lamina potential is the dominant component in the high-frequency response of the *Drosophila* ERG. However, a quantitative comparison with our results presented here is not possible because of the large modulation of light intensity in his experiments (the intensity at troughs being less than 1% of that at peaks).

As observed in *w* flies, the *Drosophila* ERG exhibits different frequency characteristics at different light intensities. The enhancement of high-frequency response due to contributions from the lamina is best seen at high light intensity, while the low-intensity response shows simply a steep high frequency cutoff (Fig. 2).

Direct measurements of the frequency responses of R_{1-6} cells and their post-synaptic elements, L_1 and L_2 lamina neurons, have been obtained by intracellular recordings in the larger fly *Calliphora* (Järvilehto and Zettler, 1973). The $L_{1,2}$ neurons respond to moderate and high-intensity stimuli with a rapid hyperpo-

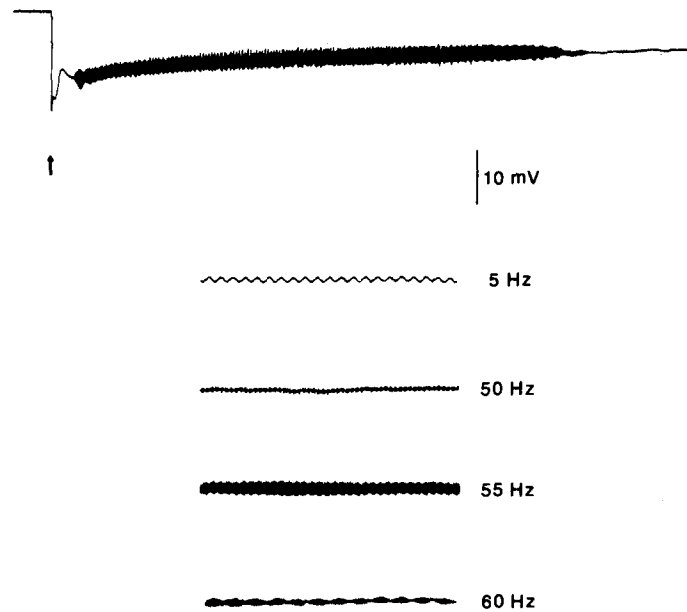


FIGURE 8. *Top trace*: light-evoked oscillations in the ERG of *w;rosA*. The stimulus was a step of light. Arrow indicates onset of light stimulus. The oscillation frequency sweeps from about 45 Hz at the initiation to about 90 Hz as the oscillation amplitude gradually subsides. *Lower traces*: responses to sinusoidally flickering light at various modulation frequencies. The sinusoidal modulation was applied after the oscillation subsided. The resonant nature of the response is evident in these records. The 5 Hz sinusoidal response marks the time scale for this figure.

larizing transient at the onset of response, and a rapid depolarizing overshoot at the offset. The phasic property of the response is reflected in the frequency dependence of the amplitude gain at the synapse. The gain of the $L_{1,2}$ response increases with frequency, ranging from about 3 at 6 Hz to about 8 at 80 Hz.

Phasic responses have been recorded from the *Drosophila* lamina under moderate and high light intensities (Alawi and Pak, 1971; Heisenberg, 1971). The origin of the fast on-transient in the *Drosophila* ERG has been identified with a spike-like response recorded intracellularly from some lamina cells (Alawi and Pak, 1971). Other intracellular recordings of an unidentified depolarizing cell type in the lamina have shown that the response to a step of light increment is an

effective on-off response with a very small steady state (unpublished observation).

At low intensities, however, the $L_{1,2}$ response becomes smoother and lacks the rapid transients (Autrum et al., 1970; Järvillehto and Zettler, 1973). Therefore the appearance of phasic components of the lamina responses at high light intensities may explain the enhancement of the amplitude gain of high-frequency responses observed in *Drosophila* ERG at increased light intensities (Fig. 2).

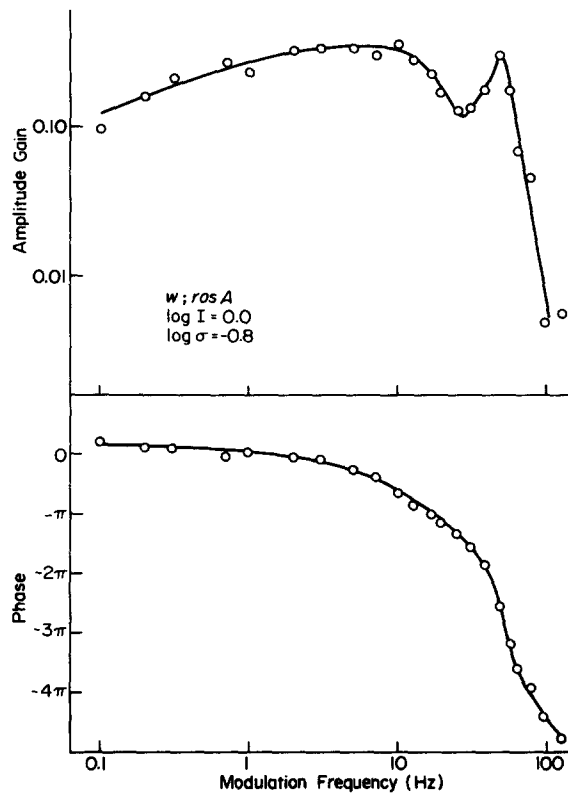


FIGURE 9. Frequency response of the *w;rosA* fly. Note the amplitude and phase characteristics in the neighborhood of tuning frequency (≈ 55 Hz).

In the present study, we have compared the different contributions of the lamina and receptors to the different frequency regions in the frequency response of the *Drosophila* ERG. The problem examined here, however, does not bear direct linking to the dispute over the roles played by the lamina in the slow- and fast-responding eyes of various insect species (Autrum, 1958; Ruck, 1958, 1961).

Receptor Component

As described previously, the *w;ora* ERG reflects the $R_{7,8}$ response and the *w sev;ora* ERG the R_8 response. The R_{1-6} response is thought to be dominant in

the ERG of *nonA;bw;st* (defective lamina), at least at lower light intensities (Fig. 4), because the R_{1-6} system has a greater number of receptors and larger rhabdomeres (Kirschfeld, 1969). However, the slow time course of repolarization in the *nonA* ERG indicates that some slow components of the lamina response are still present in the mutant *nonA*. The receptor response, observed by means of intracellular recordings (Alawi and Pak, 1971; Alawi et al., 1972) and of the isolated ERG components in the mutants *ora* and *sev;;ora* (Fig. 1), shows rapid repolarization after the cessation of light stimulus.

In all experiments conducted under the same stimulus conditions, we found that the *w sev;;ora* response had a smaller (about 0.3 log) amplitude gain at low frequencies than the *w;;ora* response, although both types of response had the same high-frequency cutoff (Fig. 5, log I = 0). This difference seems to indicate that the R_7 contribution to the ERG of *w;;ora* flies is confined to low frequencies. Alternatively, this might be a consequence of the fact that R_7 is a UV receptor and R_8 a blue receptor (Minke et al., 1975; Harris et al., 1976). Since UV components are strongly attenuated by the fiber optics light guide used in these experiments (see Materials and Methods), the effective intensity of the stimulus for R_7 would be lower than that for R_8 . Light adaptation seems to suppress the low-frequency response of the *Drosophila* photoreceptor, while leaving the high-frequency response relatively unaffected. At low light intensities we have observed in both *w;;ora* (Fig. 5, log I = -1.6) and *w sev;;ora* (not shown) flies elevated gains at the low-frequency plateau. At comparable light intensities (with reference to σ), the frequency response of *w sev;;ora* (Fig. 5) appeared very similar to that of *nonA;bw;st* (Fig. 4, log I = -1) except in the low frequency end (<0.7 Hz) where the residual slow lamina components in *nonA* flies may make considerable contribution to the ERG. Furthermore, the phase plots of *nonA;bw;st*, *w;;ora* and *w sev;;ora* are very similar. This resemblance suggests that the R_{1-6} response may have frequency characteristics similar to $R_{7,8}$. We found no strong evidence indicating that R_{1-6} , R_7 , and R_8 have any distinct difference in the frequency response.

Fuortes and Hodgkin (1964) pointed out that the photoreceptor potential can be formally fitted by the equation for a linear filter containing several identical stages of exponential delay. Evidence for multiple stages lies in the phase plots in Figs. 4 and 7. For a single stage, the maximum phase shift would be $\pi/2$. As the high-frequency phase shifts are much more than $\pi/2$, there must be more than one stage. In terms of this model the observed cutoff of approximately 20 dB/dec in the *norpA* mutant indicates that one of the first-order stages has become dominant in the observed frequency range, i.e. its time constant is much greater than those of the other stages. In comparison to the *w;;ora* response, the *w norpA;;ora* response showed a progressively larger phase shift with increasing frequency (Figs. 5 and 7). The phase shift of the *w norpA;;ora* fly was about 0.25π at between 1 and 2 Hz, consistent with the assumption of a dominant single-stage RC filter. The corner (3-dB) frequency of about 1–2 Hz here corresponds to a time constant of about 100–150 ms, i.e. $\tau = 1/2\pi f_c$, where f_c is the corner frequency and τ the time constant. Therefore, the prominent feature of the latency distribution can be described as an exponential distribution with a mean

of about 100–150 ms, which is much longer than the duration of individual bumps (about 40 ms, Wu and Pak, 1975; for further discussion, see Wu, 1976).

It should be noted that the initial peaks before the steady states in the ERGs of *w;;ora*, *w sev;;ora*, and *w norpA;;ora* (Fig. 1) cannot be inferred from the frequency responses with simple cutoffs in these mutants (Figs. 5, 7). The frequency response measurements reported here were based on small sinusoidal changes superimposed on steady light background, therefore they fail to represent the highly nonlinear initial peaks of the receptor potential (Fuortes and Hodgkin, 1964) in response to a large step increase of light intensity as shown in Fig. 1.

Receptor Frequency Response and Noise Spectrum

We have verified in *Drosophila* photoreceptors the shot noise model of Dodge et al. (1968). That lends further support to the hypothesis that the receptor potential is a summation of unitary bumps. By using the mutant *norpA*, we have demonstrated that the dispersion in the latency distribution of bump generation can be a notable source of discrepancy in predicting the frequency response of the receptor potential from the time course of individual bumps. However, our data on both *w;;ora* and *w sev;;ora* flies do not suggest a drastic improvement in the high-frequency response of the receptor potential with increasing light conditions, and thus differ in this aspect from the results in *Limulus* (Dodge et al., 1968). The difference is also indicated by the autocovariance of the shot noise of the intracellular receptor potential. In contrast with a twofold shortening of the time course observed in *Limulus* (Dodge et al., 1968), there is only a slight shortening of the time scale in the autocovariance of *Drosophila* receptor noise as the light intensity increases by 4 log units (Wu, 1976).

In contrast to our observation on *Drosophila*, frequency responses of *Calliphora* reticular cells also display some peaking in the high-frequency range under high-intensity illumination (Leutscher-Hazelhoff, 1975). However, the power spectrum of the steady-state receptor potential in *Calliphora* is not yet reported.

Oscillations in the rosA ERG

The phase plot of the *rosA* response (Fig. 9) shows a behavior similar to that of an ideal simple harmonic resonator. In the ideal resonator, there is a phase shift of π in the neighborhood of resonant frequency, a change between phase lag and lead passing the resonant frequency. The observed phase shift between 40 and 65 Hz is about 1.75π , approximately equal to the shift due to resonance (π) plus that due to the smooth drop in the curve in this region (slope \approx three-eighths π per 0.1 unit of the frequency axis).

There are at least two possible ways in which oscillations can result. They can be due to individual cells or to the interactions among different populations of cells. Ratliff et al. (1970) have induced oscillations of neuronal activities in *Limulus* lateral eyes by taking advantages of the lateral inhibition among different populations of neurons. With the proper time delay between a spot and an annulus stimulation, the impulses in the optic nerve fiber were found to tune to stimulus light flickering at certain frequencies. The frequency of the oscillations

mediated by such synaptic activities, however, was found to be very low (<5 Hz). Moreover, at present there does not appear to be either anatomical or physiological evidence for lateral interactions at the reticular cell level in *Drosophila*. Järvillehto and Zettler (1973), however, have obtained evidence for lateral inhibition at the level of lamina neurons in *Calliphora* by showing that the angular sensitivity curves of the lamina neurons are considerably narrower than that of the R_{1-6} receptor cells.

Similar voltage oscillations have also been recorded intracellularly from reticular cells of the *rosA* mutant (M. Wilcox, private communication). If the oscillations in *rosA* were to be ascribed to individual cells, the cell activity would have to be remarkably homogeneous. Since the ERG is an ensemble of signals from all responding cells in the eye, the manifestation of oscillations in the ERG requires a synchronous activity in a large population of cells. Oscillations are usually found in systems with feedback with a proper phase shift. Once its structural origin is localized, the oscillations resulting from the *rosA* mutation may throw light on the dynamics of interaction among intercellular or intracellular elements.

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REFERENCES

- ADOLPH, A. R. 1964. Spontaneous slow potential fluctuations in the *Limulus* photoreceptor. *J. Gen. Physiol.* **48**:297-322.
- ALAWI, A. A., and W. L. PAK. 1971. On-transient of insect electroretinogram: its cellular origin. *Science (Wash. D. C.)*. **172**:1055-1057.
- ALAWI, A. A., V. JENNINGS, J. GROSSFIELD, and W. L. PAK. 1972. Phototransduction mutants of *Drosophila melanogaster*. In *Advances in Experimental Medicine and Biology*. Vol. 24. G. B. Arden, editor. Plenum Publishing Corp., New York.
- AUTRUM, H. 1958. Electrophysiological analysis of the visual systems in insects. *Exp. Cell Res. Suppl.* **5**:426-439.
- AUTRUM, H., F. ZETTLER, and M. JÄRVILEHTO. 1970. Postsynaptic potentials from a single monopolar neuron of the ganglion opticum I of the blowfly *Calliphora*. *Z. Vgl. Physiol.* **70**:414-424.
- BOSCHEK, C. B. 1971. On the fine structure of the peripheral retina and lamina ganglionaris of the fly, *Musca domestica*. *Z. Zellforsch. Mikrosk. Anat.* **118**:369-409.
- CLELAND, B., and C. ENROTH-CUGELL. 1966. Cat retinal ganglion cell responses to changing light intensity: sinusoidal modulation in the time domain. *Acta Physiol. Scand.* **68**:365-381.

- DELAND, M. C., and W. L. PAK. 1973. Reversibly temperature sensitive phototransduction mutant of *Drosophila melanogaster*. *Nat. New Biol.* **244**:184-186.
- DELANGE, H. 1958. Research into the dynamic nature of the human fovea-cortex systems with intermittent and modulated light. I. Attenuation characteristics with white and colored light. *J. Opt. Soc. Am.* **48**:777-784.
- DEVOE, R. E. 1963. Linear relations between stimulus amplitudes and amplitudes of retinal action potentials from the eye of the wolf spider. *J. Gen. Physiol.* **47**:13-32.
- DODGE, F. A., B. W. KNIGHT, and J. TOYODA. 1968. Voltage noise in *Limulus* visual cells. *Science (Wash. D. C.)*. **160**:88-90.
- ECKERT, H. 1972. Spectral sensitivities of receptor systems of the fly *Musca*. *Naturwissenschaften*. **59**:80-81.
- FRANCESCHINI, N. 1972. Pupil and pseudopupil in the compound eye of *Drosophila*. In *Information Processing in the Visual System of Arthropods*. R. Wehner, editor. Springer-Verlag, Berlin.
- FRANCESCHINI, N. 1975. Sampling of the visual environment by the compound eye of the fly: Fundamentals and applications. In *Photoreceptor Optics*. A. W. Snyder and R. Menzel, editors. Springer-Verlag, Berlin.
- FUORTES, M. G. F., and A. L. HODGKIN. 1964. Changes in time scale and sensitivity in the ommatidia of *Limulus*. *J. Physiol. (Lond.)*. **172**:239-263.
- FUORTES, M. G. F., and S. YEANDLE. 1964. Probability of occurrence of discrete potential waves in the eye of *Limulus*. *J. Gen. Physiol.* **47**:443-463.
- GOLDSMITH, T. H., and G. D. BERNARD. 1974. The visual system of insects. In *The Physiology of Insecta*. Vol. 2. E. Rockstein, editor. Academic Press, Inc., New York.
- HARRIS, W. A., W. S. STARK, and J. A. WALKER. 1976. Genetic dissection of the photoreceptor system in the compound eye of *Drosophila melanogaster*. *J. Physiol. (Lond.)*. **256**:415-439.
- HEISENBERG, M. 1971. Separation of receptor and lamina potentials in the electroretinogram of normal and mutant *Drosophila*. *J. Exp. Biol.* **55**:85-100.
- JÄRVILEHTO, M., and F. ZETTLER. 1973. Electrophysiological-histological studies on some functional properties of visual cells and second order neurons of an insect retina. *Z. Zellforsch. Mikrosk. Anat.* **136**:291-306.
- KIRSCHFELD, K. 1966. Discrete and graded receptor potentials in the compound eye of the fly (*Musca*). In *The Functional Organization of the Compound Eye*. C. G. Bernhard, editor. Pergamon Press, Oxford.
- KIRSCHFELD, K. 1969. Optics in the compound eye. In *Processing of Optical Data by Organisms and by Machines*. W. Reichardt, editor. Academic Press, Inc., New York.
- KNIGHT, B. W. 1973. A stochastic problem in visual neurophysiology. In *Stochastic Differential Equations*. J. B. Keller and H. P. McKean, editors. American Mathematical Society SIAM-AMS Proceedings. **6**.
- KNIGHT, B. W., J. TOYODA, and F. A. DODGE. 1970. A quantitative description of the dynamics of excitation and inhibition in the eye of *Limulus*. *J. Gen. Physiol.* **56**:421-437.
- KOENIG, J., and J. R. MERRIAM. 1975. Autosomal ERG mutants. *Drosophila Information Service*. In press.
- KUIPER, J. W., and J. T. LEUTSCHER-HAZELHOFF. 1965. Linear and nonlinear responses from the compound eye of *Calliphora erythrocephala*. *Cold Spring Harbor Symp. Quant. Biol.* **30**:419-428.
- LEUTSCHER-HAZELHOFF, J. T. 1975. Linear and non-linear performance of transducer and pupil in *Calliphora* retinula cells. *J. Physiol. (Lond.)*. **246**:333-350.

- LINDSLEY, D. L., and E. H. GRELL. 1968. Genetic Variations of *Drosophila melanogaster*. Carnegie Institution of Washington Publication, Oak Ridge, Tenn.
- MCCANN, G. D., and D. W. ARNETT. 1972. Spectral and polarization sensitivity of the dipteran visual system. *J. Gen. Physiol.* **59**:534-558.
- MINKE, B., C.-F. WU, and W. L. PAK. 1975. Isolation of light-induced response of the central retinula cells from the electroretinogram of *Drosophila*. *J. Comp. Physiol.* **98**:345-355.
- NOLTE, D. J. 1961. The pigment granules in two compound eyes of *Drosophila*. *Heredity*. **16**:25-38.
- PAK, W. L. 1975. Mutations affecting the vision of *Drosophila melanogaster*. In Handbook of Genetics. Vol. 3. R. C. King editor. Plenum Publishing Corp., New York.
- PAK, W. L., and K. J. LIDINGTON. 1974. Fast electrical potential from a long-lived, long-wavelength photoproduct of fly visual pigment. *J. Gen. Physiol.* **63**:740-756.
- PAK, W. L., S. E. OSTROY, M. C. DELAND, and C.-F. WU. 1976. Photoreceptor mutant of *Drosophila*: Is protein involved in intermediate steps of phototransduction? *Science (Wash. D. C.)*. **194**:956-959.
- PINTER, R. B. 1966. Sinusoidal and delta function responses of visual cells of the *Limulus* eye. *J. Gen. Physiol.* **49**:565-593.
- PINTER, R. B. 1972. Frequency and time domain properties of retinular cells of the desert locust (*Schistocera gregaria*) and the house cricket (*Acheta domesticus*). *J. Comp. Physiol.* **77**:383-397.
- RATLIFF, F., B. W. KNIGHT, and N. MILKMAN. 1970. Superposition of excitatory and inhibitory influences in the retina of *Limulus*: Effect of delayed inhibition. *Proc. Natl. Acad. Sci. U. S. A.* **67**:1558-1564.
- RATLIFF, F., B. W. KNIGHT, F. A. DODGE, and H. K. HARTLINE. 1974. Fourier analysis of dynamics of excitation and inhibition in the eye of *Limulus*: amplitude, phase and distance. *Vision Res.* **14**:1155-1168.
- RUCK, P. 1958. A comparison of the electrical responses of compound eyes and dorsal ocelli in four insect species. *J. Insect Physiol.* **2**:261-274.
- RUCK, P. 1961. Photoreceptor cell response and flicker fusion frequency in the compound eye of the fly *Lucilia sericata* (Meigen). *Biol. Bull. (Woods Hole)*. **120**:375-383.
- RUSHTON, W. A. H. 1961. The intensity factor in vision. In Light and Life. W. D. McElroy and H. B. Glass, editors. Johns Hopkins Press, Baltimore, Md.
- SCHOLES, J. 1965. Discontinuity of the excitation process in locust visual cells. *Cold Spring Harbor Symp. Quant. Biol.* **30**:517-527.
- STAVENGA, D. G. 1975. Optical qualities of the fly eye—an approach from the side of geometrical, physical and waveguide optics. In Photoreceptor Optics. A. W. Snyder and R. Menzel, editors. Springer-Verlag, Berlin.
- TOYODA, J.-I. 1974. Frequency characteristics of retinal neurons in the carp. *J. Gen. Physiol.* **63**:214-234.
- TRUJILLO-GENOZ, O. 1965. Some aspects of the structural organization of the intermediate retina of dipterans. *J. Ultrastruct. Res.* **13**:1-33.
- WONG, F., B. W. KNIGHT, and F. A. DODGE. 1976. Dispersion of latencies in *Limulus* photoreceptors. The Association for Research in Vision and Ophthalmology Abstracts. April: 17.
- WONG, F., and B. W. KNIGHT. 1977. The adapting-bump model in *Limulus* ventral photoreceptors. The Association for Research in Vision and Ophthalmology Abstracts. April: 119.

- WU, C.-F. 1976. Photoreceptor voltage fluctuations and phototransduction processes in wild-type and mutant *Drosophila melanogaster*. Ph.D. thesis. Purdue University, West Lafayette, Ind.
- WU, C.-F., and W. L. PAK. 1975. Quantal basis of photoreceptor spectral sensitivity of *Drosophila melanogaster*. *J. Gen. Physiol.* **66**:149-168.
- YEANDLE, S. 1957. Studies on the slow potentials and the effects of cations on the electrical responses of the *Limulus* ommatidium. Ph.D. thesis. Johns Hopkins University, Baltimore, Md.