Linear Relations between Stimulus Amplitudes and Amplitudes of Retinal Action Potentials from the Eye of the Wolf Spider

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ABSTRACT Incremental photic stimuli have been used to elicit small amplitude retinal action potentials from light-adapted ocelli of the wolf spider, Lycosa baltimoriana (Keyserling) in order to see whether or not the amplitudes of these potentials are linearly related to the stimulus amplitudes. Sine wave variations of light intensity around a mean elicit sine wave variations in potential which contain inappreciable harmonics of the stimulus frequency and whose amplitudes are linearly related to the stimulus amplitudes. Likewise, the responses to the first two periodic Fourier components of incremental rectangular wave stimuli of variable duty cycle are directly proportional to the amplitudes of these components and have phases dependent only on the frequencies and phases of these components. Thirdly, a linear transfer function can be found which describes the amplitudes and phases of responses recorded at different frequencies of sine wave stimulation and this transfer function is sufficient to predict the responses to incremental step stimuli. Finally, it is shown that flash response amplitudes are linearly related to incremental flash intensities at all levels of adaptation. The relations of these linear responses to non-linear responses and to physiological mechanisms of the eye are discussed.

INTRODUCTION

Considerable effort has been expended to show just what sort of non-linearities the eye exhibits (Rushton, 1961; Stevens, 1961), but there has been remarkably little study of the eye under conditions where its responses might be linear. It should not be difficult to obtain linear responses of the eye, for by restricting stimuli so that only a small portion of the eye's non-linear characteristics are traversed, its responses should be satisfactorily linear. Moreover, there are compelling reasons for going to the trouble to obtain linear responses, for it should then be quite simple and straightforward to analyze and describe the dynamic and transient properties of these responses.
as compared to those of the eye responding over its non-linear range. In particular, it should be possible to apply to the responses of the eye powerful analytical techniques used to characterize the responses of linear, physical systems to such stimuli as, for example, sine waves.

Sine wave stimuli have been used to characterize the temporal properties of the human eye. To a human observer, a sine wave photic stimulus (that is, a stimulus dimmed and brightened with time in a sinusoidal manner around a mean intensity) can give the same sensation of flicker elicited by the more usual sorts of square wave experimental stimuli. Indeed, as was discovered by Ives (1922) and deLange (1961), if these stimuli are of high enough frequency and are adjusted in amplitude so as to just give the sensation of flicker, then it is not possible to distinguish between the flicker caused by the sine wave and the flicker caused by the square wave. On the assumption that the threshold flicker response is linear because it is so small, and hence that the temporal response of the human eye may be described by its sine wave response, deLange (1954, 1958, 1961) and others (Ives, 1922; Cobb, 1934; Levinson, 1959; Forsyth, 1960; Kelly, 1961b; Matin, 1962) have sought to account for the way the human eye resolves flicker by analogy with low pass, passive, linear physical filters. It is, perhaps, precarious to conclude from manipulations upon the stimulus (as have been performed in these psychophysical experiments) that the responses of the human eye are indeed linear at flicker threshold, but it should be possible to further test this analogy by measurements of amplitudes and waveforms of electrical responses of the eye to the same sorts of flicker stimuli as were used in the psychophysical experiments.

A few sine wave analyses of the electrical flicker responses of visual systems have already been reported. van der Tweel (1961), and Hermann and Stark (1963), have found that, to a first approximation, visual responses to incremental step stimuli may be predicted from the amplitudes and phases of the responses to sine wave stimuli, as would be expected of linear systems. However, these authors reported only moderate success in obtaining linear responses. Better success was obtained using small incremental flashes by DeVoe (1962) and by Kirschfeld (1961), who also recorded both linear and non-linear responses to sine wave stimuli. In addition, Hagins et al. (1960) have found that transretinal voltages from the squid eye may be linear with light intensities covering 5 orders of magnitude. Obviously therefore, linear responses not only should be but can be obtained from eyes, and sine wave analyses of these responses are very much in order.

The present paper is a study of the sine wave responses of the eye of the wolf spider, *Lycosa baltimoriana* (Keyserling), in which the responses measured are retinal action potentials. This paper has two aims, first, to show that using periodic and other stimuli of small amplitude, the responses of the wolf spider eye are as linear as may be desired, and thus secondly, that the
sine wave responses of the wolf spider eye provide a quite sufficient description of the responses to all sorts of other stimuli. The advantages of the wolf spider eye for this type of study were given in an earlier paper (DeVoe, 1962), where it was concluded that electrical potentials at the cornea represent only depolarizations of primary receptor cells. Guilio (1962), who used nicotine to abolish possible synaptic potentials in the eye of the domestic spider *Tegenaria*, has concluded that corneal potentials from the eyes of this spider are also primary receptor potentials. Linear descriptions of electrical responses from wolf spider eyes may therefore also be used to gain insight into the temporal properties of visual receptor cells themselves.

**METHODS**

The methods for recording from the eyes of wolf spiders were very nearly those described in an earlier paper (DeVoe, 1962). Large, intact, adult wolf spiders were restrained in a clamp. Electrical contact was made with the illuminated eye (the posterior median ocellus) and with an electrically indifferent location (the contralateral posterior lateral ocellus) by means of salt bridges in the form of saline-filled micropipettes. Silver-silver chloride electrodes made contact with the saline in the pipettes. For protection from stray light during strong stimulation, the “indifferent” eye was shielded from light on the illuminated eye by a small piece of aluminum foil waxed to the animal. (It seems unlikely that much light from the illuminated eye could reach the indifferent eye through the intervening tissues, since the proximal portions of all retinal cells are wrapped in pigment cells (Widmann, 1908) and the eyeball husk likewise appears heavily pigmented.)

Signals were led from the electrodes to a direct coupled, differential preamplifier, the output of which was displayed on the upper beam of a Tektronix 502 oscilloscope when step and flash stimuli were used. When sinusoidal and other periodic stimuli were used, the preamplifier output was led to a second dc amplifier and then to a tuned amplifier, a General Radio 762-B vibration analyzer. This tuned amplifier was first used to advantage by Granit and Wirth (1953) to resolve periodic electrical responses in the presence of much noise, which is greatly reduced at off-tune frequencies. Another advantage of this type of tuned amplifier is that any component of the retinal action potential of the tune frequency will be amplified with almost zero (±8°) phase shift. This means, for one thing, that if the amplifier is first tuned to the stimulus frequency or one of its harmonics, and if the retinal action potential component at the tune frequency is thereby selectively amplified, then by measuring the phase of the output of the tuned amplifier on one beam of the oscilloscope versus the phase of the stimulus waveform on the other, the phase shift occurring in the eye has been measured. Likewise, the amplitude of this frequency component of the retinal action potential may be read from the meter of the tuned amplifier. The vibration analyzer thus permits measurements of the amplitudes and phases of retinal action potentials relative to stimulus amplitudes and phases at the frequencies of the stimuli and in the presence of much noise. Unfortunately, the tuning range of this instrument extends down only to 2.5 cps.
Both transient stimuli and sinusoidal or other periodical stimuli were used. Transient stimuli were generated by the dual light source optical stimulator previously described (DeVoe, 1962). Changes in intensity of background illumination were made by inserting neutral density filters between the elements of the objective lens, thereby equally attenuating the light from both sources. The intensity to which all others were referred was the unattenuated output of the tungsten lamp in the stimulator; this averaged 32,000 foot-candles at the eye.

Sinusoidal and other periodic photic stimuli were generated by a Sylvania R1131-C glow modulator tube powered by an electronic driver designed at the Bell Telephone Laboratories (McDonald, 1960) and kindly lent by Dr. J. Levinson. The mean current through the glow tube was 12.5 ma; this corresponded to a mean intensity at the eye of ca. 1270 foot-candles.

Sinusoidal variations in light intensity were generated as variations around a mean intensity because, of course, light intensity cannot be a negative quantity. Therefore, the amplitudes of sinusoidal variations around this mean intensity could not exceed the mean intensity, so that these amplitudes were expressed as percentages of the mean intensity. In this paper the term per cent modulation refers to 100 times the ratio of $\frac{1}{2}$ the peak-to-peak amplitude of the sinusoidal stimulus to the mean intensity. As an example, 100 per cent modulation refers to a sinusoidal change in intensity, the minimum intensity of which is zero and the maximum intensity of which is twice the mean intensity. Per cent modulation as defined here is 100 times the ripple ratio $r$ defined by deLange (1958) and 100 times the modulation ratio $m$ defined by Kelly (1961 a).

A Kron-Hite 400-A low frequency oscillator was used to generate sine waves, and two Tektronix 162 waveform generators were used to generate rectangular waves of varying duty cycle. To remove any dc component of these waveforms, the outputs of both stimulus sources were capacitatively coupled through a 10 second time constant coupling to the input of the glow modulator tube driver. The lowest frequency rectangular waves used were at 2.5 cps; their waveforms drooped by at most 2 per cent. This distortion had a negligible effect upon the amplitudes of their fundamentals.

A. RESULTS USING PERIODIC STIMULI

Responses to Sine Wave Stimuli It was first desired to see whether or not linear electrical responses might be elicited from the wolf spider eye by small amplitude sine wave stimuli, that is, whether the amplitudes of these responses might be linearly related to the stimulus amplitudes and whether the responses might be undistorted sine waves. Of course, small amplitude stimuli elicit small amplitude responses, and thus because of the noise in the recording system, responses to sine wave stimuli appeared much as did responses to square wave stimuli previously illustrated (DeVoe, 1962, Fig. 1). In order to measure amplitudes and waveforms of these small amplitude responses, it was therefore necessary to use tuned amplification.

To begin with, the relation between sine wave stimulus amplitudes and
amplitudes of the selectively amplified electrical responses was measured. The results obtained over a large stimulus range at two frequencies of stimulation are shown in Fig. 1. Over these ranges the response amplitudes change very nearly linearly with stimulus amplitudes. Similar linear stimulus-response relations have also been found at 20 cps and 30 cps in another experiment. This correlates well with the linear superposition of responses previously found to be valid in at least the range of 3 to 50 cps (DeVoe, 1962). In the remaining experiments to be described, sinusoidal and other periodic

![Graph showing the relation between sinusoidal stimulus amplitude and flicker response amplitude.](image)

**Figure 1.** Relation between sinusoidal stimulus amplitude and flicker response amplitude. Ordinate: peak-to-peak flicker response amplitude (expressed as μvolts response), read from meter of the vibration analyzer tuned to stimulus frequency. Abscissa: stimulus amplitude expressed as per cent modulation (defined as one-half peak-to-peak stimulus amplitude divided by the mean intensity). Results of one experiment. Mean I = ca. 1270 foot-candles.

stimulus amplitudes were restricted to those which elicited only small (10 to 20 μv peak-to-peak) responses in order that the eye might always respond within its linear range.

Secondly, harmonic analyses were performed upon the recorded retinal action potentials by varying the tune frequency of the vibration analyzer while stimulating the eye with a constant frequency sine wave. Results were obtained when the response amplitudes were 10 μv peak-to-peak at the stimulus frequencies of 2.5, 10, and 25 cps and are shown in Fig. 2. Any harmonics of these stimuli are inappreciable compared to the noise recorded at 0 per
cent modulation ("no stimulus"); this noise was 2 to 4 per cent of the maximum response amplitudes shown. When the stimulus amplitude was increased to elicit 20 μv peak-to-peak response at the stimulus frequency (in this case 10 c/s), the noise remained about the same, that is, became now about 1 to 2 per cent of the maximum amplitude, but any harmonics of the

![Graph showing harmonic content of responses to sinusoidal stimuli.](image)

**Figure 2.** Harmonic content of responses to sinusoidal stimuli. Relative response amplitudes read from meter of the vibration analyzer at each frequency. Stimulation at 2.5, 10, and 25 c/s; stimulus amplitudes elicited 10 μv volts peak-to-peak responses at the stimulus frequency. Response amplitudes at frequencies other than the stimulus frequencies are 2 to 4 per cent of those at the stimulus frequencies. Data represented by filled circles, solid lines drawn by eye. Results of one experiment. Mean I = ca. 1270 foot-candles.

stimulus frequency still remained undetected in the noise. It is concluded that residual non-linearities in the spider eye which can generate harmonics are inappreciable when retinal action potentials of only 10 to 20 μv peak-to-peak are elicited by sine wave stimuli.

In a linear system, the amplitude of a sine wave response is dependent on both the stimulus amplitude and the stimulus frequency, whereas the phase of the sine wave
response with respect to that of the sine wave stimulus is a function of frequency only. The linearity of the spider eye may be judged by this criterion too. In the experiment from which the results of Fig. 1 were taken, the phases of all responses at 10 cps were constant to within the limits of error (±8°) for all stimulus amplitudes and were constant at 25 cps for responses of up to 30 μv in amplitude. In this experiment, responses above 40 μv in amplitude had a different phase, but in subsequent experiments, variations in phase of response at a constant frequency have been attributable to instrumental error. Thus the responses of the wolf spider eye show the phase relations expected of a linear system.

Responses to Complex Periodic Stimuli It was next desired to see if the electrical responses of the spider eye might be linear with small amplitude periodic stimuli which were not purely sine waves. One convenient way of calculating the response of a linear system to such complex periodic stimuli is to calculate what its response to each Fourier component of the stimulus would be and then algebraically add (superpose) these solutions. The amplitude of the response to each Fourier component will be directly proportional to both the amplitude of the component and to the relative amplitude of response which a purely sine wave stimulus of its frequency would elicit. Conversely, the linearity of a system may be examined by performing a harmonic analysis upon the waveforms of its responses to complex periodic stimuli and finding out whether or not the response components at the frequencies of the Fourier components of the stimulus are proportional to the amplitudes of these components in the way described above. This sort of harmonic analysis, using tuned amplification, has been tried upon the flicker responses of the spider eye to small amplitude, incremental, rectangular wave stimuli to see if they are linear.

First of all, the response to only the first Fourier component (the fundamental) of a square wave stimulus was measured and compared with the response to a sine wave of the same frequency and amplitude. The experimental reasoning was as follows: According to Fourier, the fundamental of a square wave of 50 per cent duty cycle (50 per cent relative “on-time”) has a peak-to-peak amplitude 1.27 times as large as the peak-to-peak square wave amplitude. Now if a square wave stimulus and a sine wave stimulus of the same frequency are both to elicit the same amplitude of response (chosen in this experiment to be 10 μv peak-to-peak) at this frequency, then the peak-to-peak amplitude of the sine wave stimulus should be 1.27 times as great as the peak-to-peak amplitude of the square wave, providing the eye is responding linearly. The average ratio of measurements made at nine stimulus frequencies is, within experimental error, 1.27, as is shown in Table I. This ratio holds at both high and low stimulus frequencies, and the phases of the responses at each frequency are the same (within experimental error) for both sine wave and square wave stimuli. It is therefore evident that from
the amplitude and frequency of the Fourier fundamental, the response component at this frequency may be uniquely determined. This, of course, is as it should be for a linear system.

Not only the response component at the frequency of the stimulus fundamental but also the response components at harmonic frequencies may be measured by tuned amplification. In the following experiments, responses to both the first and second Fourier components were so measured. In these sorts of experiments, it was necessary to vary something other than the frequency of the stimulus, because the second Fourier component has twice the frequency of the fundamental, by definition, and at high stimulus frequencies the response to the second Fourier component may become immeasurably small while the response to the fundamental is still appreciable (witness the increased stimulus amplitudes in Table I required at high frequencies to elicit the same response amplitude as at low frequency). A measurable amount of response to the second Fourier component of a rectangular wave stimulus should however be obtainable if the frequency of the stimulus is kept constant and low enough and the duty cycle varied instead.

According to Fourier, the equation for a rectangular wave \( S(t) \) of variable duty cycle \( \beta \) is the following:

\[
S(t) = \frac{A}{\beta} \begin{cases} \sin(\omega t) & \text{for } 0 \leq t < \beta \frac{T}{2} \\ \sin(\pi t - \omega \beta \frac{T}{2}) & \text{for } \beta \frac{T}{2} \leq t < T \\ 0 & \text{else} \end{cases}
\]
\[ S(t) = \beta I + \frac{2I}{\pi} \sum_{n=1}^{\infty} \frac{\sin n\beta\pi}{n} \cos \frac{\pi n \omega t}{\lambda} \tag{1} \]

\[ \beta = \text{light duty cycle} \quad (0 \leq \beta \leq 1) \]

\[ I = \text{peak-to-peak intensity} \]

The DC component \( \beta I \) of the rectangular waves was removed by capacitative coupling, preventing any changes in mean stimulus intensity with changes in duty cycle, and only rectangular waves with a constant peak-to-peak amplitude (which was 9.6 per cent of the mean stimulus intensity) were used. In addition, the rectangular wave stimulus was set at 4 cP. At 4 and 8 cP, the relative amplitudes of response (to sine waves) are just about equal (cf. Table II), and hence it may be determined whether the amplitudes of response at these frequencies to the rectangular wave stimulus are now proportional only to the amplitudes of the first and second Fourier components, respectively, as would follow for a linear system.

With the stimulus described above, the amplitude, according to Fourier, of the first component of the rectangular wave varies with duty cycle as \( \sin \beta\pi \), and the amplitude of the second component varies with duty cycle as \( (\sin 2\beta\pi)/2 \). These variations in amplitude as functions of duty cycle are plotted in Fig. 3 as solid lines for the first \((n = 1)\) and second \((n = 2)\) Fourier components. The short vertical bars represent the relative amplitudes of the responses at 4 cP and at 8 cP and were read directly from the meter of the tuned amplifier; the range of measurements is indicated by the length of each bar. The results have been scaled only to make the relative amplitudes of stimulus and response of 4 cP equal at 50 per cent duty cycle. Therefore, the excellent fit between the remainder of the experimental amplitudes and the amplitudes of the first two Fourier components shows that it is quite sufficient to consider only the amplitudes (and frequencies) of the Fourier components of a rectangular wave stimulus when calculating the response of the spider eye to this stimulus (at least as concerns the first two periodic Fourier components).

Likewise, the phases of the responses were found to be dependent only on the phases and frequencies of these two Fourier components and not upon their amplitudes. For example, the second Fourier component changed sign when the duty cycle increased from less than 50 per cent to more than 50 per cent, and the phases of the responses to this component likewise changed by 180° at a duty cycle of 50 per cent. (No such phase change of 180° was expected in the responses to the first Fourier component and none occurred). Furthermore, the phases of the responses to the first and second Fourier components were constant for all duty cycles and were equal to the phases of the responses to sine wave stimuli of 4 cP and 8 cP, respectively. The phase of the response to a 4 cP sine wave stimulus and to the first Fourier component (which was at 4 cP) was \(-29°\). The phase of the response to an 8 cP sine wave...
stimulus was $-89^\circ$ (although the amplitudes of response at 4 and 8 c/s were the same, the phases were not), while the phase of the response to the second Fourier component (which was at 8 c/s) was $-88^\circ$. Thus both under rectangular wave stimulation and under sine wave stimulation, the phases of the responses behaved as would those of a linear system.

This same experiment has been repeated using a 10 c/s rectangular wave stimulus, and the variations of response amplitudes to the first and second Fourier components as functions of duty cycle agreed as well with those predicted as in Fig. 3. However, the amplitude of response to a 20 c/s sine wave stimulus was less than that to a 10 c/s sine wave stimulus, and in keeping with this, all the amplitudes of the responses to the second Fourier component
relative to the amplitudes of responses to the first Fourier component of the 10 cps rectangular wave were less than in Fig. 3. This shows however that if the effect of frequency of a Fourier component of a rectangular wave is taken into account when determining the response to it, then the amplitude of this response remains proportional to the amplitude of the Fourier component.

<table>
<thead>
<tr>
<th>Frequency of stimulation (CPS)</th>
<th>Stimulus amplitude, per cent modulation</th>
<th>Phase shift ±8°</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>3.6 ± 0.4</td>
<td>-22°</td>
</tr>
<tr>
<td>3.8</td>
<td>2.8 ± 0.1</td>
<td>-25°</td>
</tr>
<tr>
<td>5.0</td>
<td>2.3</td>
<td>-34°</td>
</tr>
<tr>
<td>6.0</td>
<td>2.5 ± 0.5</td>
<td>-48°</td>
</tr>
<tr>
<td>7.6</td>
<td>2.8</td>
<td>-54°</td>
</tr>
<tr>
<td>8.1</td>
<td>2.6 ± 0.3</td>
<td>-77°</td>
</tr>
<tr>
<td>10.0</td>
<td>2.9 ± 0.1</td>
<td>-90°</td>
</tr>
<tr>
<td>12.5</td>
<td>3.4</td>
<td>-113°</td>
</tr>
<tr>
<td>15.1</td>
<td>4.5 ± 0.1</td>
<td>-151°</td>
</tr>
<tr>
<td>20.1</td>
<td>6.0</td>
<td>-172°</td>
</tr>
<tr>
<td>25.1</td>
<td>9.0 ± 1.0</td>
<td>-207°</td>
</tr>
<tr>
<td>30.0</td>
<td>13.2 ± 1.4</td>
<td>-236°</td>
</tr>
<tr>
<td>35.5</td>
<td>12.5</td>
<td>-268°</td>
</tr>
<tr>
<td>40.0</td>
<td>22.7 ± 0.3</td>
<td>-287°</td>
</tr>
<tr>
<td>45.0</td>
<td>29.4</td>
<td>-315°</td>
</tr>
<tr>
<td>50.1</td>
<td>36.5 ± 4.5</td>
<td>-330°</td>
</tr>
<tr>
<td>55.1</td>
<td>43.5</td>
<td>-364°</td>
</tr>
<tr>
<td>64.6</td>
<td>89.0 ± 2.0</td>
<td>-399°</td>
</tr>
<tr>
<td>70.0</td>
<td>98.2</td>
<td>-413°</td>
</tr>
</tbody>
</table>

The Frequency Response of the Spider Eye The foregoing results have demonstrated that the amplitudes of the flicker responses of the spider eye are not only directly proportional to the amplitudes of sine wave stimuli or to the amplitudes of the Fourier components of complex periodic stimuli, but are also proportional to some function of the frequency of the stimulus. If this function, called a transfer function, were known, then it would be possible to calculate the flicker response of the spider eye to any known periodic stimulus of any frequency using the Fourier analysis outlined above. In this section a transfer function will be derived to describe the response of the spider eye to different frequencies of sine wave stimulation.

In Table II are shown the sine wave stimulus amplitudes needed to elicit
Figure 4. Amplitude and phase characteristics of flicker responses to sine wave stimuli. Experimental data from Table II indicated by filled circles; solid lines represent a transfer function fitted to data (see text). Top ordinate: relative db = $-20 \log_{10}$ of the stimulus amplitude (expressed as per cent modulation + 2.8) required to elicit 10 $\mu$volts peak-to-peak flicker response amplitude. Bottom ordinate: phase shift of response relative to stimulus, in degrees. Abscissa: $2\pi$ times the stimulus frequency, on a log scale. Mean $I = ca. 1270$ foot-candles.
10 µv peak-to-peak response amplitude at various stimulus frequencies. Because there is direct proportionality between stimulus and response amplitudes, as was shown above, measurements made of variations in stimulus amplitudes as functions of frequency may be directly converted into variations in response amplitudes as functions of frequency. A convenient way of expressing the variations in response amplitude (and phase) as a function of frequency is in a Bode plot (Aseltine, 1958). This has been done in Fig. 4. The solid lines represent the following transfer function, which is given in Laplace transform notation:

\[ T(s) = \frac{k(s + 4)e^{-0.005s}}{(s + 21)(s + 130)^3} \]  

\[ k = 10.3 \times 10^6 \mu v/\text{per cent modulation} \]  

In common with transfer functions describing responses of other visual receptors (Kirschfeld, 1961; Hermann and Stark, 1963), this one contains a latent period or transport delay, in this case of 8 msec. The transport delay describes a phase shift linear with frequency and by definition does not affect the amplitude of the response at any frequency.

This transfer function fits the experimental points quite well, except perhaps at the lowest frequency phase measurements. Thus from this transfer function (or from the solid lines in Fig. 4), it becomes a simple matter to calculate the response to any sine wave stimulus and therefore also to any combination of sine wave stimuli comprising the Fourier series of a complex periodic stimulus.

The transfer function was fitted to the experimental points on the basis of the following: First, at the highest stimulus frequencies, the amplitude of response decreases at 18 db/octave, which suggests that there are at least 3 poles in the transfer function. For frequencies greater than 8 cps (ω > 50), the experimental points are well fitted assuming a triple pole at ω = 130. It is difficult to fit the remainder of the experimental points, because there are no data below 2.5 cps (the lower frequency limit of the tuned amplifier). However, it is known that there is a dc response (DeVoe, 1962, Fig. 8), and so if it is assumed that the response drops off below 5 to 6 cps at no more than 6 db/octave, the remaining points are fitted as shown by a single zero at ω = 4 and another pole at ω = 21 in the transfer function.

The maximum phase lag expected from a system described by these poles and the zero is 270° at infinite frequency, whereas there is a monotonically increasing lag reaching 413° at the highest frequency used. Although the phase lag might approach some limiting value were the response measurable at higher frequencies, it suffices to describe all phase shifts by adding a phase lag of 0.45°/ω, linear with frequency. This phase lag of 0.45°ω has been converted to a transport delay of 8 ± 1 msec in the transfer function.
B. RESULTS USING TRANSIENT STIMULI

Calculation of the Step Response  One of the advantages possessed by a transfer function of a linear system, such as equation (2) above, when written in the Laplace transform variable s, is that it not only describes the responses to sine wave stimuli, but that it describes the responses to transient (and indeed all other) stimuli as well (Aseltine, 1958). This is a most useful property, for it means that the sufficiency of the above transfer function may be further tested by seeing whether or not it does correctly predict the response to some other stimulus, such as a transient stimulus. A desirable transient response to predict is the response to an incremental step, because the step response itself can be further used to predict responses to all sorts of rectangular shaped incremental stimuli (DeVoe, 1962). The calculated response \( R(t) \) to an 8 per cent incremental step is given by the following equation:

\[
R(t) = 7.21[1 + 7.21e^{-7.21t} - (51.3 \times 10^3t^2 + 916t + 8.21)e^{-100t}]_{\mu V} \tag{3}
\]

This calculated step response is shown in Fig. 5 as open circles; the solid lines are averaged responses to 8 per cent incremental steps recorded during two other, different experiments. The agreement between the calculated and measured responses is remarkably good even though neither step response was recorded from the animal from which the frequency response was recorded and no scaling of amplitudes was necessary to make the calculated and recorded step responses fit each other. The upper of the two recorded step responses was the one used previously (DeVoe, 1962) for the prediction of responses to other rectangular shaped incremental stimuli.

Thus the linear transfer function (Equation 2) fitted to the sine wave responses of the wolf spider eye is quite sufficient to describe transient responses of this eye too. It is of course to be supposed that some other transfer function could equally well be fitted to the same experimental data, but then, the purpose of deriving a linear transfer function was only to show that this linear systems approach could completely (even if not uniquely) describe the electrical responses of the wolf spider eye to small amplitude stimuli.

It follows from the complete success of applying linear systems analysis to the small amplitude responses of the wolf spider eye that now the response to any sufficiently small stimulus waveform may be calculated. For example, the responses to the traditional sorts of rectangular shaped flicker stimuli may be determined in either of two ways, first, by algebraically adding (superposing) a number of step responses separated at the appropriate intervals and having the appropriate signs (DeVoe, 1962), or, second, by calculating the amplitudes and phases of the sine wave responses to each of the periodic Fourier components of the stimulus and then algebraically adding all these.
sine wave responses. Either way, the calculated responses will be the same, because both approaches are based on the same transfer function. Alternatively, any response in the linear range may be calculated wholly analytically by any of a number of formulae valid for linear systems, as for example the convolution integral (Aseltine, 1958).

**Linear Responses at Different Levels of Background Illumination** The only sine wave responses that have been recorded are those that have been described here; that is, those that have been recorded at mean $I = 1270$ foot-candles. Additional experiments demonstrate linear relations between stimulus and response amplitudes at other levels of background illumination too, and hence that the sinusoidal analysis performed above should be equally possible. For example, such linear relations have been demonstrated by linear superposition of flash responses to predict flicker responses at six different background levels (those of Fig. 6) (DeVoe, 1962). At dark adaptation also the eye will respond linearly to small flashes which negligibly affect its sensitivity. In Fig. 6 are shown peak amplitudes of responses to 25 msec. incremental
Figure 6. Relations between incremental flash intensity and flash response amplitude at various background illuminations. Increment flash intensity and background light intensity expressed as foot-candles illumination upon the eye. Open circles represent peak flash response amplitudes to 25 msec flashes. Straight lines drawn by inspection. Results from three experiments on one animal.
flashes presented against the designated background illuminations. (At dark adaptation there was, of course, no background illumination.) At all background intensities, there is a linear relation between the peak amplitudes of the responses and the incremental flash intensities. It may be added that at any one background intensity, all flash responses are simply versions of one another scaled up or down in amplitude. Thus the spider eye did not respond linearly just because the background intensity used earlier happened to have been chosen; it responds linearly whenever incremental stimuli are sufficiently small, regardless of the background illumination (if any).

DISCUSSION

Non-Linear Responses of the Wolf Spider Eye  It must not be inferred from the results presented here that the wolf spider possesses a visual system which is unique in responding linearly to any and all stimuli which are presented to it. On the contrary, the stimuli used, while selected especially to elicit linear responses, must be considered to be rather restricted. In general, they were either very small incremental stimuli or they were stimuli which elicited very small responses. Others have reported linear responses from various eyes when using similarly restricted stimuli but have also found as well that the more usual sorts of large amplitude stimuli elicit non-linear responses (Hagins et al., 1960; Kirschfeld, 1961). The same is true for the wolf spider eye, which responds non-linearly to stimuli with amplitudes very much larger than those described either here or previously (DeVoe, 1962). A description of these non-linearities has been given elsewhere (DeVoe, 1961). For the wolf spider eye, it is as yet uncertain where the transitions between linear and non-linear responses lie or, indeed, what is the cause of these transitions. In other visual systems, the stimulus limits within which the eye responds linearly may be functions not only of stimulus amplitudes or of background illumination but also of stimulus frequency (deLange, 1957; Kirschfeld, 1961). There is some evidence that the same holds true for the wolf spider eye (DeVoe, 1961).

Physiological Conclusions  The remainder of this discussion will try to show what sort of physiological conclusions may be drawn from the purely formal linear system analysis which has been the subject of this paper.

First of all, complete descriptions of the responses of visual systems by formal analogy with linear systems are certainly preferable to piecemeal descriptions of various features of responses, such as rates of rise, amplitudes of response, flicker fusion frequencies, etc. The responses of visual systems in their linear ranges can be calculated for all arbitrary stimuli by means of well developed techniques (Aseltine, 1958). In turn, these calculated responses can be used as critical tests for the sufficiency of current hypotheses of the inner workings of receptor cells or of visual systems.
Secondly, the linear systems analogy provides a means for distinguishing between features of responses due, on the one hand, to separate physiological mechanisms and, on the other hand, to the same physiological mechanism. One feature is the latent period of the electrical response to transient stimuli. In the wolf spider and other visual systems which have been analyzed by sine wave analysis (Kirschfeld, 1961; Hermann and Stark, 1963), the latent period is formally identical with a transport delay element (see the Results section). This transport delay or latent period is quite separate from and in addition to the beginning of the S-shaped rise, however slow, in the response of minimum phase systems to transient stimuli (see Machin and Pringle, 1960, for a description of the dynamic properties of various delay elements). It has been proposed that the latent period of the eye might be the time required for the products of an autocatalytic, photochemical reaction to reach threshold (Wulff, Fry, and Linde, 1955) or might be the time for an initially slowly rising electrical response to reach an appreciable size (Jones, Green, and Pinter, 1962), but neither proposed mechanism would behave under sine wave stimulation as would a transport delay element.

Another response feature is “sensory hyperpolarization” (Granit, 1955), which is an undershoot, at “off,” of the baseline to which the receptor potential eventually returns (Katz, 1950; Hartline, Wagner, and MacNichol, 1952; Kirschfeld, 1959). No separate physiological process needs to be invoked to explain sensory hyperpolarization in a linear system, however. The response at “off” will be the same as the response at “on,” but will be of opposite sign (DeVoe, 1962). Thus if there is overshoot (resulting from sensory adaptation) at “on,” there will be undershoot (or sensory hyperpolarization) at “off.” If in a linear system overshoot is missing, so is undershoot (Kirschfeld, 1961). The processes responsible for overshoot are, in a linear system, responsible for undershoot as well.

Finally, there is some evidence that linear visual properties may not only be useful for formal calculations of receptor responses, but that they may also be related to behavioral responses to visual stimuli. Kirschfeld (1961) has found good correspondence between the linear responses of beetle retinal cells as manifested in the retinal action potential and the filter properties of a “transmission stage with brief time constant” (Reichardt, 1962) predicted on the basis of optomotor responses. Similarly, a sort of brightness enhancement (Bartley, 1938) has been found for the positive phototropic response of certain flies towards intermittent light sources; stimuli of 15 to 22 cps are more attractive than is even a constant light source of the same mean intensity (Dolley, 1923; Mast and Dolley, 1924). This correlates well with the greater amplitudes of the retinal action potentials of the horsefly Tabanus elicited by sinusoidal stimuli of 10 to 20 cps (Kirschfeld, 1961). Thus there is the possibility that descriptions of the responses of the input stages (the ret-
inal cells) of visual systems by formal analogy with linear systems may eventually permit calculation of the responses at each successive stage in the visual system.

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