Linear Superposition of Retinal Action Potentials to Predict Electrical Flicker Responses from the Eye of the Wolf Spider, *Lycosa baltimoriana* (Keyserling)

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ABSTRACT Retinal action potentials were elicited from light-adapted posterior median ocelli of the wolf spider *Lycosa baltimoriana* (Keyserling) by rectangular shaped photic stimuli representing 8 per cent increments or decrements of the background illumination. Responses to trains of recurrent incremental or decremental flashes were successfully predicted by graphical linear superposition of a single flash response, which was repeatedly drawn and added to itself at intervals equal to the period of the intermittent stimulus. Incremental stimuli inverted to form decremental stimuli elicited responses which were also inverted. Responses to single incremental flashes were successfully predicted by linear superposition of the response to one incremental step stimulus, which was inverted and added to itself at an interval equal to the duration of the flash.

INTRODUCTION

Retinal action potentials have been recorded from a large number of arthropod species, but with one exception, those of arachnids in general and of spiders in particular appear not to have been described. The exception is the electrical response from an eye of an unidentified spider recorded by Hartline, Wagner, and MacNichol (1952); the response of this spider eye has a very simple waveform. Available descriptions of the histology of spider eyes (see below) portray no synapses or ganglia within these eyes; the simple electrical response recorded at the cornea of the spider eye most likely represents the massed response of primary visual receptor cells. Thus recordings from the spider eye are well suited to the study of temporal properties of visual receptor cells as manifested in the retinal action potential. This is particularly true of temporal properties revealed by intermittent photic stimulation. Using the spider eye, it is not necessary to resort to surgical techniques (Autrum and Gallwitz, 1951; Ruck, 1961 *c*) or to other means (Autrum and Hoffmann, 1960; Ruck, 1961 *b*) if the flicker responses of arthropod visual receptor cells are to be studied in isolation. A second advantage of the spider retinal action potential for the study of flicker lies in its simplicity. As will be demonstrated, there are in the electrical flicker responses of wolf spider eyes no interactions of the type which, in the retinal action potentials from vertebrate eyes for example, enhance *a*-waves at certain frequencies of intermittent stimulation (Granit, 1955). For these reasons, this investigation of temporal visual properties manifested in the retinal action potential during intermittent stimulation makes use of the eyes of spiders.

The present work is an attempt to extend to electrical flicker responses an hypothesis of temporal visual properties originating in and hitherto supported primarily by psychophysical investigations of flicker. Briefly, this hypothesis states that at or near flicker fusion, the eye responds to waveforms of intermittent photic stimuli much as a linear, low pass, electrical filter acts upon waveforms of periodic input voltages (deLange, 1958). That is, under appropriate stimulus conditions the visual system is a linear system. Kirschfeld (1961) has shown that the electrical responses of weevil eyes may be linear under incremental stimulus conditions. A way in which the linearity of electrical responses elicited by intermittent stimulation may be tested can be seen from the following: If an intermittent stimulus presented to the eye is of sufficiently low frequency, the electrical response to any one of the flashes comprising the intermittent stimulus will just die away before the next flash is presented. The electrical flicker response will then be the sum of single flash responses placed end to end. If, however, the frequency of intermittent stimulation is any higher than this, the response to any one flash will not just die away before the next flash is presented. If the stimulus conditions are correct, then as a consequence of the linear filter hypothesis stated above the flicker response at high frequencies of intermittent stimulation should also be the sum of single flash responses, no longer placed end to end, but now overlapping to a considerable extent. In other words, the superposition property of linear systems should hold. In the results to be presented, this possibility will be tested using the electrical responses from incrementally stimulated, light-adapted wolf spider eyes.

The Spider Visual System Spiders possess only simple ocelli; these are generally eight in number and may be arranged in two or three rows on the front of the cephalothorax. As a consequence of differing embryological development, spider ocelli exhibit dimorphism. Widmann (1908) distinguished two types: inverted (or prebacillar) eyes, in which the axon bifurcations and cell bodies of the retinal cells are proximal to the rhabdom-bearing rods, and erect (or postbacillar) eyes, in which the rhabdom-bearing rods are situated between the distal cell body and the proximal axon origination. Only the anterior median pair of eyes are prebacillar; the remainder are postbacillar. In neither kind of eye has any author described ganglia or synapses, such as are found for example in insect dorsal ocelli (Ruck, 1961 *a*). Widmann (1908), Scheuring (1914), and Homann (1950) have all portrayed the optic nerve fibers of spiders as simple extensions of the retinal cells. In wolf spiders, the embryological development of the postbacillar eyes is consistent with this picture (Homann, 1961). In view of the small dimensions of the retinal cells (4 to 5 μ (Widmann, 1908)), it would be desirable to have confirmation of these light microscope studies from electron microscope studies, but aside from the work of Miller (1957) on the structure of spider rhabdoms, this is not available.

On the basis of these authors' work, it is to be expected that the only electrically active cells within the spider ocellus will be primary visual sense cells, and the electrical records to be presented bear this out. During maintained illumination of the eye of the wolf spider, the cornea becomes purely negative with respect to an indifferent electrode placed elsewhere on the animal. In arthropods such purely cornea-negative potentials elicited during illumination represent the primary depolarizations of the distal ends of receptor cells (Bernhard, 1942; Autrum and Gallwitz, 1951; Hartline, Wagner, and MacNichol, 1952; Naka, 1961). Other corneal potentials also ascribed to receptor cells (Ruck, 1961 *a*) have not been recorded from wolf spider eyes.

The choice of wolf spiders for the present work was dictated by two considerations. First, the eyes of wolf spiders are large and well developed; the wolf spiders, unlike most web-spinning spiders, stalk their prey visually (Homann, 1931). The posterior median (postbacillar) eyes used in the present investigation may be as large as 1 mm in diameter and possess as many as 4500 retinal cells (Homann, 1931). Since all these retinal cells are grouped under one lens, conditions are good for the recording of the massed activity of large numbers of cells.

A second consideration is the relation of the ocelli to the optic lobes of the supraesophageal ganglion. The advantages of recording from the simple spider ocellus are lost in the jumping spiders, whose optic lobes are closely applied to the back of the ocelli (Hanström, 1919). Thus although jumping spider eyes are even more highly developed and specialized than those of wolf spiders (Homann, 1928), their retinal action potentials are complicated by the presence of on- and off-effects (DeVoe, 1961) similar to those attributable to ganglionic discharges in both "fast" (Ruck, 1961 *c*) and some "slow"

(Jahn and Wulff, 1942) insect compound eyes. In wolf spiders, however, the optic lobes are located at some distance from the eyes (Hanström, 1919); although no figures for the length of the optic nerve in wolf spiders are available, personal observation suggests that this length may be over 1 mm. No potentials attributable to the optic lobes have ever been recorded from the corneas of wolf spider eyes.

MATERIALS AND METHODS

Large adult wolf spiders of the species *Lycosa baltimoriana* (Keyserling) were used in these experiments; some animals were as large as 1 inch in body length and 2.5 inches in leg spread. Preliminary experiments designed to reveal any gross differences between retinal action potentials recorded from the various eyes revealed none; the electrical responses from both prebacillar and postbacillar eyes possessed the simple waveforms to be illustrated. The electrical responses from the posterior median eyes, which were the largest and most conveniently located for stimulating and recording, were therefore selected for study.

Intact wolf spiders were restrained in a kind of spider clamp, from which they could be released at the end of the experiment. The clamp consisted of two pieces of sponge rubber; one contained a hole for the spider's abdomen and cephalothorax, and both pieces could be clamped between two pieces of metal by means of thumbscrews. The spiders were first anesthetized with humidified, 100 per cent CO_2 gas; their legs were then spread fully extended and were lightly clamped between the two pieces of sponge rubber. Spiders thus restrained could still wiggle about due to appendage articulations. Therefore the movement of the cephalothorax, on the front of which the eyes are located, was prevented as follows: The hair on the posterior carapace was removed by gently scraping with a needle, and tackiwax (Central Scientific Company) was carefully melted onto the hair-free area. A small wooden stick was then waxed to the carapace and to the metal clamping the sponge rubber. Waxes other than tackiwax were found to be too brittle and came loose from the carapace as the spider attempted to escape. The spiders were allowed to recover from anesthetization before the experiments proceeded.

Signals were led off from the cornea of the illuminated eye with a salt bridge in the form of a saline-filled micropipette. (For use as saline, an 0.23 M sodium chloride solution was found to be satisfactory; this is the sodium concentration found by the author in preliminary analyses of tarantula hemolymph (which, from a South American species, is isosmotic with 0.244 M NaCl (Rouschal, 1940)), but is somewhat greater than the sodium concentration used in a spider Ringer by Parry and Brown (1959).) The end of the pipette was ground flat and had a diameter of 0.3 to 0.4 mm. The indifferent electrode was an identical saline-filled pipette placed on an unilluminated eye; from this eye stray illumination elicited negligible potentials under the incremental stimulus conditions employed. At other indifferent locations noise, drift, and the resistance of the preparation were greater. Silver-silver chloride electrodes made contact with the saline solutions in the pipettes. All potentials were recorded with a differential input, direct coupled preamplifier led to the upper beam of a tektronix 502 oscilloscope.

There was considerable noise generated both within the preamplifier and by virtue of the fairly high resistance of the preparation (400 to 700 thousand ohms, inclusive of the electrode resistance). It was therefore necessary to insert a single stage, low pass filter between the preamplifier and the oscilloscope. The filter had a time constant of 1.6 msec. and had a frequency response down about 2 per cent at 60 CPS; as flicker fusion occurred at stimulus rates between 40 and 50 CPS for the stimulus conditions used here, no signal was lost by insertion of the filter.

The DC drift of the preamplifier and oscilloscope was negligible compared to the drift originating at the preparation. In order to reduce this drift to tolerable proportions, it was necessary to remove waterproofing waxes from the corneas of the eyes with which the pipettes made contact. The removal of these waxes was accomplished by gently scraping the corneas with the thin glass from a broken glass bubble so that drops of saline placed on the cornea were quickly absorbed. In general, it was satisfactory if the preparation was DC-stable for the duration of an oscilloscope sweep (that is, for at least one-half second); at best, the base line varied by no more than $\pm 75\ \mu v$ from a mean position during 5 minutes. DC stability was found to be much improved 2 to 3 hours following preparation of the animal, presumably because an equilibration of saline evaporation and saline diffusion at the animal-pipette junction had occurred.

All experiments were conducted at temperatures between 19 and 22°C.

The outputs of two light sources were superimposed by means of a beam splitter into two coaxial images of nearly equal size. These were focused at the base of the illuminated eye, which is the approximate location of the iris and of the nodal point (Homann, 1931). The exit pupil of the objective lens is thus seen in Maxwellian view and subtends approximately 30° at the eye. The posterior median eye has a visual perimeter 80° wide by 140° high (Homann, 1931); all of the retina is therefore not directly illuminated.

A source of constant background illumination was furnished by a Sylvania R1131-C glow modulator tube run at 30 ma; this maintained the illuminated eye at a fixed level of light adaptation. Because these animals are diurnal and were intact during the experiments, the background illumination was left on throughout each experiment without fear of fatiguing the preparation, as might occur with excised preparations. Furthermore, maintainence of constant background illumination obviated any changes in retinal sensitivity due to the pigment migration known to occur in these eyes (Scheuring, 1914; *cf.* Bernhard and Ottoson, 1960).

A 6 volt microscope lamp run from a regulated DC power supply furnished the light for incremental flashes; the onset and termination of these flashes were controlled by an electromagnetic shutter. The shutter was pulsed on and off by a power amplifier driven by two tektronix 162 waveform generators. A second light beam passing through the shutter fell on a photocell, the output of which was taken to the lower beam of the oscilloscope to signal onset and termination of the incremental flashes.

The image of the microscope lamp filament at the eye had a diameter of 0.041 inch (about 1 mm); as this was a bit smaller than the image of the glow modulator source at the eye, only fully light-adapted retinal cells were stimulated by the incremental flashes. With the aid of a Macbeth illuminometer, the illumination upon the eye due to the tungsten light source was found to be 30,000 to 33,000 foot-candles (mean of 32,000 foot-candles). By comparing the peak amplitudes of response of the dark-adapted eye to long flashes from the tungsten source and from the glow source, it was found that the effective illumination upon the eye from the glow tube was about one-tenth that due to the tungsten source. In the experiments to be described, the full output of the glow tube furnished background illumination, while a neutral density filter inserted into the tungsten beam reduced its intensity to an average value representing 8 per cent of the background illumination. It is not feasible to express the incremental intensity from the tungsten source more closely than this, because the output of the glow tube slowly decreased about 0.1 log unit during these experiments, and thus slowly altered the background: flash intensity ratio. However, the conclusions to be drawn from these experiments depend neither on the absolute value of the illumination from either light source nor upon the absolute value of their relative intensities.

A Grass oscillograph camera was used to photograph the oscilloscope traces. In spite of attempts to minimize noise and DC drift in the preparation, there was still enough left relative to the low signal levels to make imperative averaging of the responses. The DC drift in the preparation made impractical the averaging of responses by superposition of successive oscilloscope sweeps in one photographic exposure. Instead, ten to forty separate exposures of the oscilloscope traces were taken for each stimulus configuration. These records were then projected onto graph paper, traced, and an average drawn by eye.

RESULTS

Steady-State Flicker Responses There will first be considered steady-state flicker responses, that is, flicker responses in which all transient response to the onset of intermittent stimulation has ceased to be evident. Steady-state flicker responses have occupied the attentions of most workers in the field of flicker, and those from the spider eye are the simplest. Examples of records obtained of steady-state electrical flicker responses of the light-adapted wolf spider eye stimulated by recurrent 8 per cent incremental flashes are shown in the right-hand column of Fig. 1; in the left-hand column are shown averaged responses. The flicker responses are more or less sinusoidal; there is no evidence of any rapid on- or off-effects such as occur in records of flicker responses from fly eyes (Autrum, 1950) or of any synchronized, spike-like activity such as occurs in records of flicker responses from dragonfly ocelli (Ruck, 1961 *b*). As the separations between the flashes constituting the intermittent stimuli decrease, the amplitudes of the flicker responses decrease. At the smallest separation, the variations constituting the flicker response are barely detectable. Disappearance of the flicker response in this manner may be said to constitute flicker fusion.

Along with the steady-state flicker responses, there were recorded the responses to single incremental flashes of the same duration and intensity as those comprising the intermittent stimuli. In the experiment whose results are shown in Fig. 1, all flashes were of 25 msec. duration; the response to one

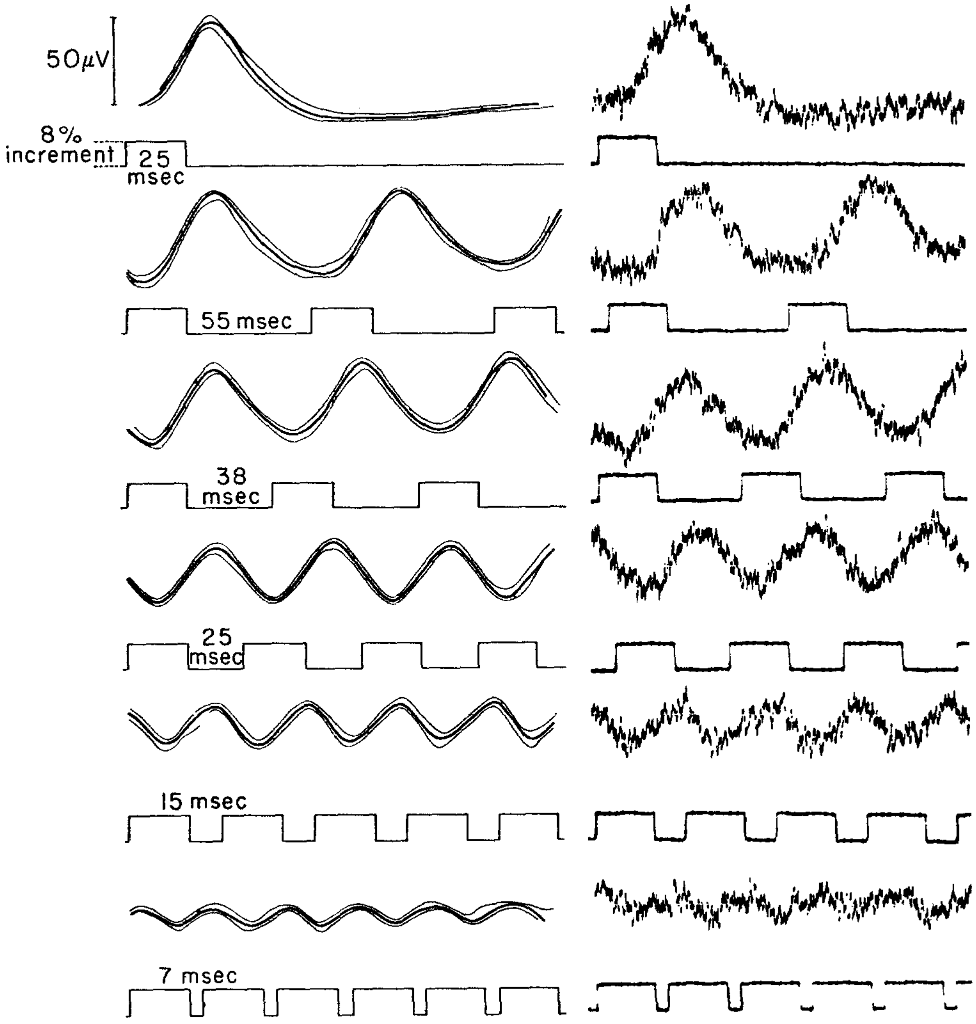

FIGURE 1. Recorded and averaged retinal action potentials elicited by 8 per cent incremental stimuli. In the right-hand column are shown typical records of recorded responses to single and recurrent 25 msec., 8 per cent incremental flashes. In the left-hand column averaged tracings (heavy lines) of such responses and variations around the average (thin lines) are shown. The upper trace in each record is the response; in this and subsequent figures upward deflection indicates corneal negativity with respect to an indifferent electrode. The lower trace in each record is from a photocell; upward deflection indicates opening of the shutter, downward deflection indicates closing of the shutter. A calibration marker of 50 µv is in the upper left-hand corner; flash duration and separations of flashes (in milliseconds) are indicated with the stimulus traces. In all subsequent figures thin lines denoting the variations around the averaged responses have been omitted for reasons of clarity, but the same order of variation occurs throughout.

25 msec. flash is shown at the top of the figure. It was proposed above that algebraic summation of single flash responses such as this might account for the waveforms of flicker responses. This possibility may be tested graphically by repeatedly drawing the response to the single flash shifted each time along the time axis by an interval equal to the period of the intermittent stimulus. The flicker response is predicted by adding the ordinates algebraically with reference to base line. Steady-state flicker responses remain after the response to the first flash of the stimulus dies away. Such a graphical superposition is shown in Fig. 2; there the die-away time of the response to the first flash is indicated as *kP* msec. After *kP* msec. the sum of ordinates (shown as open circles) is periodic at intervals *P*; this sum is the predicted steady-state flicker response.

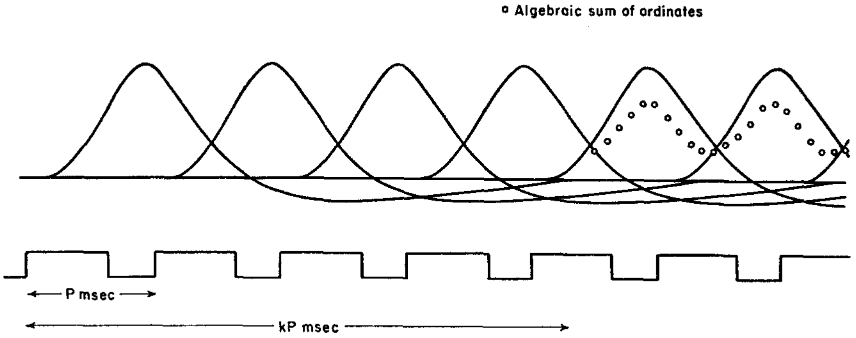

FIGURE 2. The process of superposition. The averaged response to a 25 msec., 8 per cent incremental flash (from Fig. 1) has been drawn repeatedly at intervals *P*. After the time *kP*, when the response to the first flash has died away, the ordinates have been algebraically added with respect to base line. The resultant sum, indicated by the open circles, constitutes the superposition.

The comparison of the predicted steady-state flicker responses with those recorded is shown in Fig. 3. The solid lines are the averages from the records of Fig. 1, while the filled circles are the predicted responses obtained from the graphical superposition of the averaged response to the 25 msec. flash. There is good correspondence between recorded and predicted flicker responses; all aspects of the flicker response waveforms and amplitudes appear to be successfully predicted. It is significant that linear superposition of the response to a single flash is quite as valid at high stimulus frequencies (that is, at small flash separations) as at low stimulus frequencies, for it means that the frequency of stimulation at which the flicker response will "fuse" can be readily found from consideration of the response to a single flash. To know how the eye responds to a single 8 per cent incremental flash is to know how the eye responds to all periodic presentations of this flash.

The prediction of steady-state flicker responses from the response to a single incremental flash is not limited to flashes 25 msec. in duration. Similarly good correspondence between recorded flicker responses and flicker responses predicted on the basis of superposition of responses to single flashes is also found when the single flashes are 13, 16, 20, and 32 msec. in duration. Thirty-two msec. is the longest flash duration tested for which the peak amplitude of the response to a flash is dependent on the flash duration; for flashes 40 msec. and longer in duration, the peak amplitude (but not the peak-to-peak amplitude) of the response to single flashes is constant and independent of the flash duration. So far as determination of peak flash response amplitude is concerned, 40 msec. may be considered the "critical exposure" (Hartline, 1928). The length of the critical exposure has been shown to have no bearing on responses to intermittent stimulation from ganglion cells of the cat's eye (Enroth, 1952). The same is true for flicker responses from the spider eye; flicker responses elicited by recurrent flashes 40 msec. and longer in duration may also be predicted by linear superposition of the response to a single flash. An example is given in Fig. 4 of recorded flicker responses elicited by 80 msec., 8 per cent incremental flashes; there is excellent agreement between recorded flicker responses (averages shown as solid lines) and predicted flicker responses (filled circles). Similar agreement is found when using flashes 40, 50, 63, and 100 msec. in duration. Flash durations greater than 100 msec. have not been

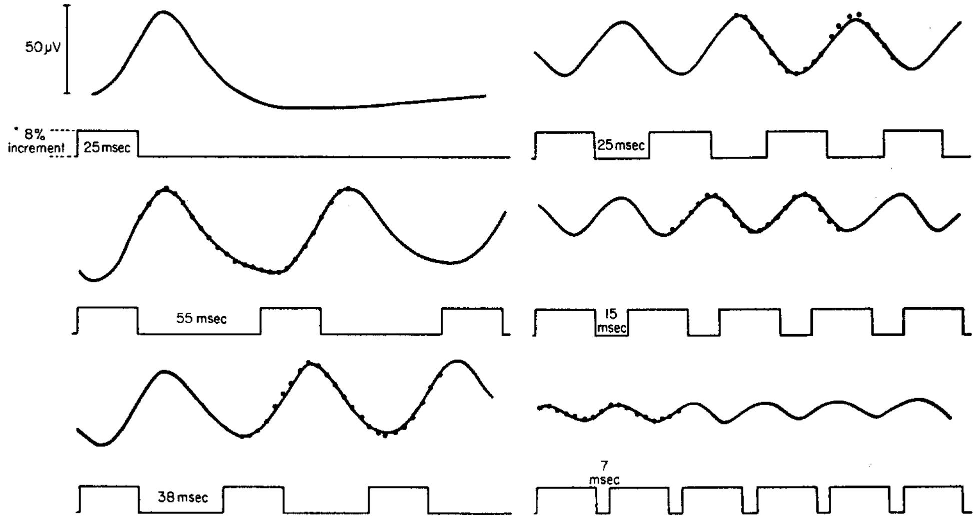

FIGURE 3. Prediction of flicker responses to periodic 25 msec. flashes. The heavy lines are averaged responses previously shown in Fig. 1. The filled circles are the resultants of superposition (performed as in Fig. 2) of the single flash response shown in the upper left-hand corner separated by the time intervals indicated. Other details as in Fig. 1.

tested. All in all, flash durations from 13 to 100 msec., and stimulus frequencies from 3 CPS to 50 CPS have been used. The prediction of steady-state flicker responses by linear superposition of responses to single 8 per cent incremental flashes is valid throughout these ranges.

Flicker Responses to Trains of Flashes To the onset of a train of flashes, the spider eye responds with periodic variations of potential superimposed on a transient which dies away, leaving only a steady-state flicker response. In Fig. 5 are shown flicker responses recorded using trains of 8 per cent incremental flashes; there are also shown flicker responses predicted from superposition of responses to single flashes. To predict the flicker response to a flash train, the response to a single flash is drawn repeatedly as in Fig. 2, and all ordinates are added from the point on the time axis at which the response to the first flash leaves the base line until the point on the time axis when the tail of the response to the last flash dies away. As was the case above, the flicker responses predicted on the basis of this superposition of single flash

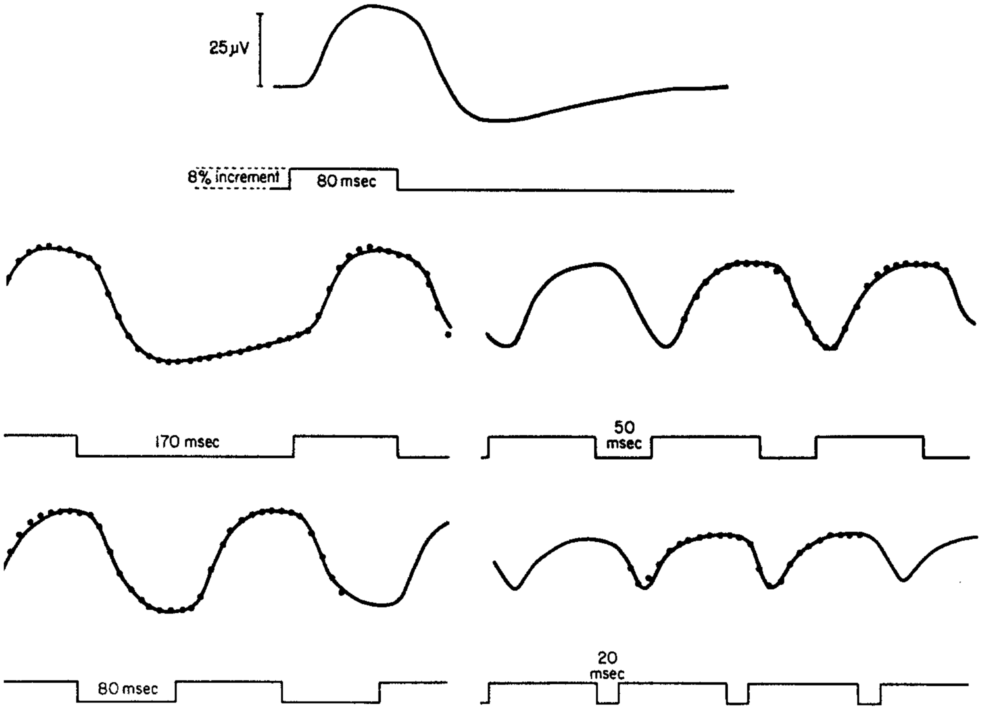

FIGURE 4. Prediction of flicker responses to periodic 80 msec. flashes. At the top is shown the averaged response to a single 80 msec., 8 per cent incremental flash; in the bottom rows averaged flicker responses to recurrent 80 msec. flashes are depicted as solid lines. Flicker responses predicted from superposition of the response to the single 80 msec. flash are shown as filled circles.

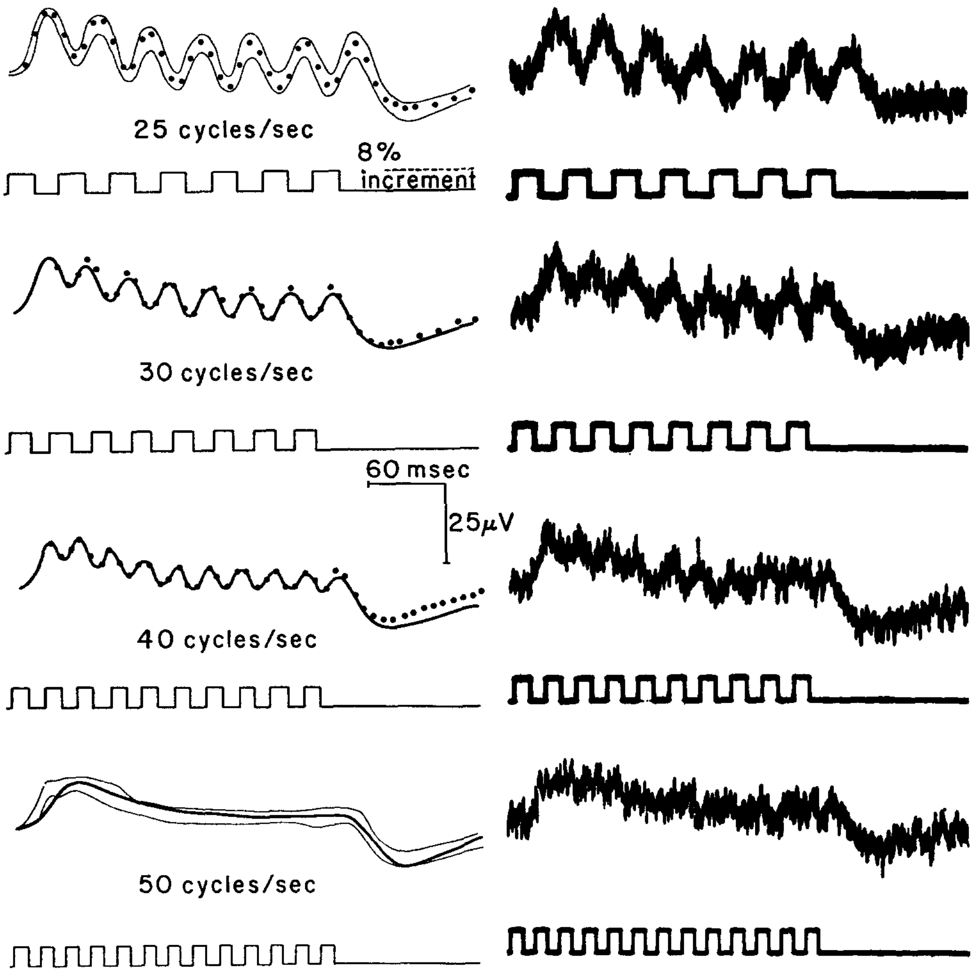

FIGURE 5. Flicker responses to trains of flashes. In the right-hand column are shown typical records of responses to short trains of recurrent 8 per cent incremental flashes; flash durations are equal to flash separations. In the left-hand column are shown tracings from such records. At 25 CPS and 50 CPS it was not possible to construct an average; the limits of variation are shown instead. At 30 CPS and 40 CPS the average is indicated by the heavy line. Filled circles represent the responses to the trains of flashes predicted from superposition of responses to the appropriate duration flashes (shown in Fig. 8). The heavy line superimposed upon the response to the 50 CPS intermittent stimulus is the averaged response to a 250 msec., 4 per cent incremental flash.

responses describe the features of the recorded responses to periodic flash trains, including (*a*) undulations about a transient which dies away, (*b*) steady-state flicker responses, (*c*) a steady-state, mean response level, different from the base line, around which the sinusoidal variations occur, and (*d*) the responses to the termination of the intermittent stimuli. For reasons unknown, the prediction of responses remaining after termination of the intermittent stimuli is least satisfactory.

Because periodic variations in the flicker response at 50 CPS are too small to be resolved from the noise in the recording system, it may be said that the flicker response is fused at this frequency. Presumably the remaining steady-state, mean response magnitude corresponds to perceived brightness of fused flicker in psychophysical experiments; therefore, by the Talbot-Plateau law, this steady-state mean response should be the same whether to an intermittent stimulus too rapid to be resolved or to a constant stimulus of the same mean intensity. Upon the envelope of the response to the intermittent stimulus of 50 CPS, there has been superimposed the response to a 4 per cent incremental, 250 msec. stimulus, shown as the heavy line. The steady-state responses to the intermittent and constant stimuli are the same, but this identity does not extend to the transient associated with the onset and termination of each stimulus. Rather, the response to the constant stimulus has a lower rate of rise, as is to be expected of the response to a lower peak intensity stimulus (Kirschfeld, 1959). Such initial disparity between electrical responses to constant and fused intermittent stimulation was not seen, however, in the retinal action potentials recorded from the cat's eye (Creed and Granit, 1933).

Finally, it is found that the mean response level around which the sinusoidal variations occur is, at steady-state, identical at all frequencies of stimulation illustrated. The applicability of the Talbot-Plateau law is thus not restricted to fused flicker responses from the spider, although it is limited to the steady-state case.

Responses to Decremental Stimuli It can be argued that the stimuli producing steady-state flicker are composed either of flash increments, periodically repeated, or, alternatively, of decrements, periodically repeated, in what would otherwise be a constant illumination. From the latter point of view the steady-state response to intermittent stimulation might be predictable by superposition of responses to single decrements in the illumination.

In order that the responses to flashes and decrements might be measured under nearly identical conditions, the constant illumination whose intensity was decreased for the duration of the decrement was the sum of the constant background illumination used in the previous experiments plus the 8 per cent incremental illumination previously turned on to provide the stimulus. In this experiment, however, turning the 8 per cent incremental illumination off comprised the stimulus. This method equates the mean intermittent illumination (when flash duration and flash separation are equal), but not the background illuminations in the two modes of stimulation. The results, as illustrated in Fig. 6, indicate that there is negligible effect of the 8 per cent difference between constant illuminations upon the responses.

The response to a single 32 msec., 8 per cent decrement is pictured at the top of Fig. 6. In the left-hand column and upper right-hand column, steady-state flicker responses for 32 msec. decrements (flash separations) are illustrated; the filled circles represent the superposition of the decrement response. It is clear that superposition of decrement responses does predict the recorded flicker responses; moreover, these same flicker responses may be predicted by superposition of responses to increments as well. In the upper right-hand column of Fig. 6 the open squares represent the superposition of responses to 32 msec. flashes. Thus the steady-state flicker responses may be predicted either from superposition of responses to flashes generating the intermittent stimuli, or from superposition of responses to the decrements separating the flashes.

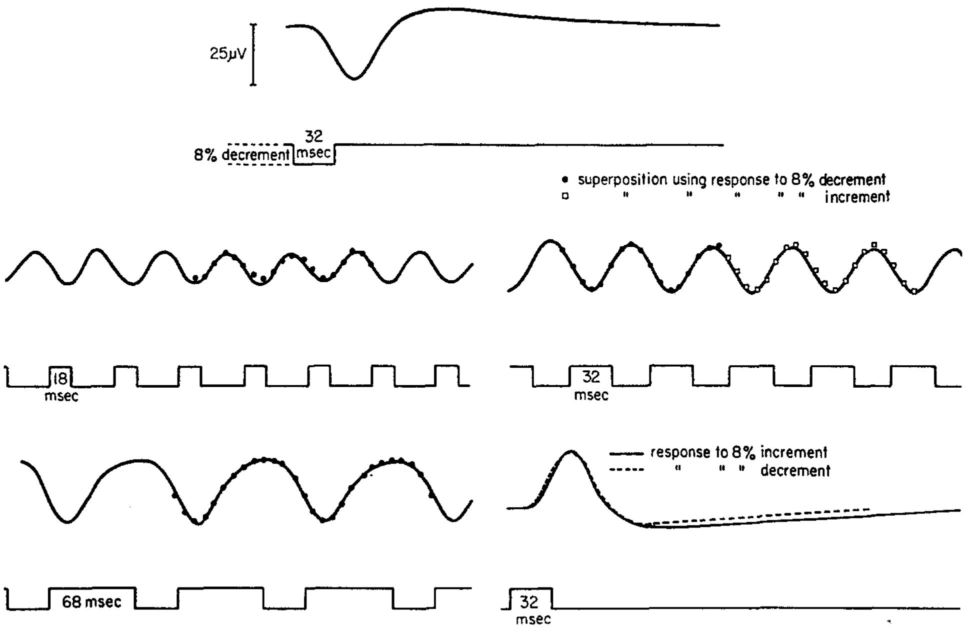

FIGURE 6. Prediction of flicker responses from decremental responses. At the top is shown the averaged response to a 32 msec., 8 per cent decrement. In the middle row and bottom left are shown flicker responses to recurrent, 32 msec. decrements. The filled circles are flicker responses predicted from superposition of responses to single 32 msec., 8 per cent decrements; open squares (in middle right) are flicker responses predicted from superposition of responses to 32 msec., 8 per cent increments. At the bottom right, the averaged response to a 32 msec. increment (solid line) is compared with the averaged response to a 32 msec., 8 per cent decrement (dotted line; drawn inverted).

In addition, comparison (in the lower right-hand corner of Fig. 6) of the responses to 8 per cent, 32 msec. increments and decrements shows that these responses are nearly identical save for sign. That is, when stimuli are, so to speak, "mirror images" (as though the waveform of one were reflected in the base line to yield the other), the responses are also mirror images.

Flicker Responses at Different Light Duty Cycles The results described in the preceding paragraph permit explanation of an allied phenomenon. If the frequency of intermittent stimulation is maintained constant, then the light duty cycle, that is, the proportion of the cycle occupied by the flash, may be varied. The variations in flicker response waveforms as functions of light duty cycle may be found by comparing flicker responses elicited by intermittent stimuli of the same frequency but having different flash durations. A collection of flicker responses at 10 CPS recorded during one experiment is shown in Fig. 7; the flash durations are given under the record of each stimulus waveform. The solid lines are the averages of recorded flicker responses; the filled circles are flicker responses predicted using the responses to single flashes of the durations indicated. The agreement between recorded and predicted flicker responses is good, as was stated above to be the case when using flash durations of 32 to 80 msec. The range of light duty cycles (32 to 80 per cent) used in the experiment illustrated in Fig. 7 is not great enough to demonstrate the quantitative dependence of flicker response upon light duty cycle; this quantitative dependence will be considered in a subsequent paper. In the results of Fig. 7 it can be seen that flicker responses at light duty cycles greater than 50 per cent are nearly the mirror images of flicker responses at light duty cycles

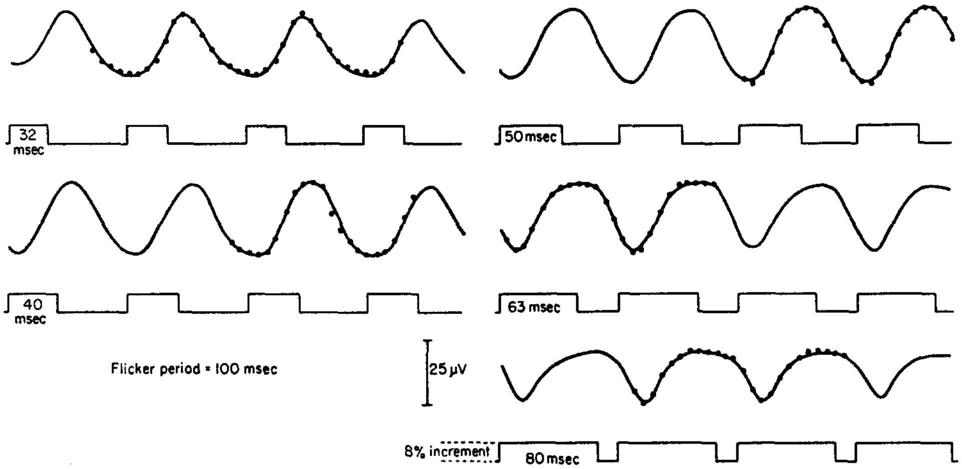

FIGURE 7. Flicker responses measured at various light duty cycles. The solid lines ar averages of flicker responses to 10 CPS intermittent, 8 per cent incremental stimuli com prised of flashes of the durations indicated. The filled circles are flicker responses pre dicted by superposition of the responses to single flashes of the durations indicated.

less than 50 per cent. For example, the flicker response elicited by 63 msec. flashes separated by 37 msec. is nearly the same as the flicker response elicited by 40 msec. flashes separated by 60 msec., only upside down. To a lesser extent, the flicker response to 80 msec. flashes separated by 20 msec. is the mirror image of the flicker response to 32 msec. flashes separated by 68 msec. These results are readily explicable on the basis that responses to incremental flashes are mirror images of the responses to decremental flashes, and that flicker responses may be predicted by superposition of either.

Prediction of Flash Responses from Step Responses In addition to incremental and decremental flashes, incremental and decremental step stimuli are also mirror images of each other. The responses to incremental and decremental step stimuli likewise appear to be mirror images of each other. For practical purposes, the response to an incremental step in intensity is measured as the first part of the response to an incremental flash sufficiently long that the response achieves a steady-state amplitude. The response to the termination of this long flash may be taken to be the response to a decremental step of equal size. The responses to onset and termination of a 250 msec., 8 per cent incremental flash are shown as solid lines at the top and middle, respectively, of the left-hand column of Fig. 8. The response to the incremental step has been drawn upside down as filled circles and has been superimposed on the decremental step response. As the close correspondence shows, the incremental and decremental step responses are approximately mirror images of each other. Not enough experiments of this kind have been performed to know whether or not the response to a step decrement is always exactly the mirror image of the response to a step increment, but the correspondence is close enough to support the conclusion drawn above, namely that mirror image stimuli elicit mirror image responses.

At flash durations shorter than about 230 msec., the response to the step increment does not achieve its final steady-state amplitude before the response to the step decrement (the termination of the flash) is elicited. The flash response may thus be the resultant of superposition of the responses to stimulus steps of opposite sign. To test this possibility, it is sufficient to utilize only the measured response to a step increment. If the responses to mirror image steps are likewise mirror images, nothing is lost by superposing only the response to a step increment. In the lower left-hand corner of Fig. 8, the superposition to predict responses to flashes is shown. The full lines represent the step response shown in the upper left-hand corner. This same response but with opposite sign is taken as the response to a step decrement. The filled circles are the resultant. In the right-hand column the measured responses (solid lines) to 8 per cent incremental flashes are compared with the responses predicted on the basis of superposition of step responses (filled circles). The

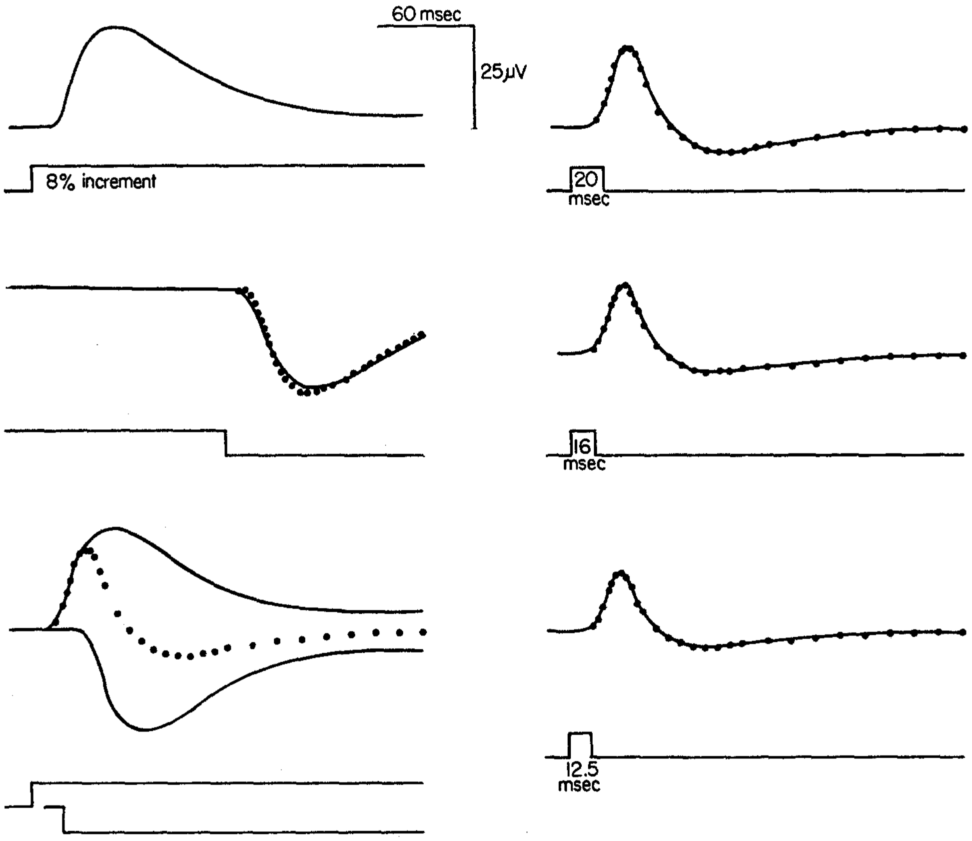

FIGURE 8. Prediction of flash responses from step responses. Upper left, averaged response to the onset of a 250 msec., 8 per cent incremental flash. Middle left, averaged response to the termination of the 250 msec. flash (shown as the solid line) and mirror image of the response to the onset of the 250 msec. flash (shown as filled circles). Bottom left, superposition to predict the flash response. The step response (upper left) has been drawn twice, the second time upside down and displaced on the time axis. The filled circles represent the algebraic sum with respect to base line. Right-hand column, responses to 8 per cent incremental flashes of duration indicated are shown as solid lines; flash responses predicted by superposition of two identical step responses are shown as filled circles.

excellent agreement admits the conclusion that responses to single 8 per cent incremental flashes may be predicted solely from a knowledge of the response to *one 8 per cent step increment in intensity.*

In turn, of course, responses to any periodic flash configuration may be predicted. For example, the measured responses to 20 msec., 16 msec., and 12.5 msec. flashes, illustrated in the right-hand column of Fig. 8, were, in turn, used to predict the responses to flash trains of 25 CPS, 30 CPS, and 40

CPS, respectively, previously illustrated in Fig. 5. Thus under the stimulus conditions that have been used in these experiments, wherein 8 per cent incremental stimuli have been superimposed on a steady background illumination, measured flicker responses, both transient and steady-state, may be deduced from a knowledge of the response to one, 8 per cent step incremental stimulus.

The following rule is therefore a summary of the experimental results presented. To predict the response to an 8 per cent incremental (or decremental) flash of any duration, draw the response to one 8 per cent step increment, then reflect this response in the base line, shift it the duration of the flash (or decrement) along the base line, draw again, and take the algebraic sum. To predict the response to any intermittent stimulus configuration using periodic 8 per cent incremental (or decremental) flashes, superpose responses to the appropriate 8 per cent incremental (or decremental) flash drawn repeatedly along the base line at an interval equal to the period of the intermittent stimulus.

DISCUSSION

The results of this investigation demonstrate that flicker responses from wolf spider eyes may be simply related to responses elicited by rectangular shaped, incremental stimuli through the graphical procedure of linear response superposition. The success of linear response superposition shows that, in the final analysis, the temporal properties of the spider eye manifested during intermittent incremental stimulation need not be considered apart from those temporal properties of the eye manifested in response to the much simpler incremental step stimulus. This conclusion is completely in keeping with the hypothesis of deLange (1958) that at or near flicker fusion the eye may respond much as a linear filter. From the point of view of the spider retinal action potential, the responses illustrated here were of finite size and were not, for the most part, fused. From the point of view of behavioral responses, there is as yet no information whether the intermittent incremental stimuli used would be seen as fused or flickering, and hence whether from behavioral responses to intermittent light, linear filter action of the eye could be demonstrated. However, in the weevils *Hylobius* and *Chlorophanus*, linear visual filters postulated on the basis of behavioral responses to intermittent light have been demonstrated by means of electrical flicker responses as well (Kirschfeld, 1961).

The present results also demonstrate the validity of the linear filter hypothesis for responses at low frequencies of intermittent stimulation. The linear filter hypothesis has been mainly supported by psychophysical evidence that complex intermittent stimulus waveforms are resolved as though only the

Fourier fundamentals of these waveforms were present (Ives, 1922; deLange, 1958; Levinson, 1959; Forsyth, 1960; Kelly, 1961); the eye is considered to "filter out" all harmonics such that they contribute nothing to the threshold recognition of flicker. At low frequencies of intermittent stimulation (below about 10 CPS) such "linear short-cut" methods are no longer applicable (deLange, 1961). It is then necessary to consider the contributions to flicker threshold recognition from harmonics as well. Only partial success has so far been enjoyed in attempts to account for low frequency psychophysical flicker responses induced by rectangular shaped intermittent stimuli (deLange, 1961). Conclusions concerning the validity of the linear filter hypothesis drawn from the present results are not, however, restricted to any range of stimulus frequencies. Low frequency flicker responses from the spider eye are as readily accounted for by linear response superposition as are high frequency flicker responses. There is no dependence on the validity of linear short-cut methods.

Linear response superposition has been well illustrated by the simple wolf spider retinal action potentials, thus fully justifying the choice of this animal as the subject for experimentation. Linear response superposition is less well illustrated by the responses of more complex visual systems. In point of time, linear response superposition was first suggested with reference to the more complex vertebrate retinal action potential (Piper, 1911), but Creed and Granit (1933) advanced reasons why such superposition of retinal action potentials should fail, at least for those from the initially dark-adapted cat's eye. Linear superposition of electrical responses from the human eye has recently been reported by Howarth (1961); responses to flashes were best approximated by superposition of responses at "on" and "off" to incremental flashes presented against strong background illumination. Superposition was not achieved at low levels of background illumination. Likewise, van Hof (1960) noted that linear superposition of human cortical potentials evoked by repetitive photic stimulation of the eye occurred only some time following the onset of stimulation; it is probable that his "organization time" was devoted to light-adapting the eye.

Prior light adaptation thus appears necessary for success of linear response superposition. Wolf spider retinal action potentials whose superposition has been documented here were, as stated, elicited from previously light-adapted eyes. A different situation results, however, when there is no background illumination. The author has shown (DeVoe, 1961) that flash responses from dark-adapted eyes may not be used to predict flicker responses to recurrent flashes separated by darkness, for all intermittent stimuli light-adapt the eye to some extent. On the other hand, it is not the prior establishment of any given level of light adaptation but the maintenance of that level which is necessary for the success of linear response superposition. For example, at levels of background illumination 0.4 to 4.0 log units below that used in the experiments of this paper, linear response superposition is quite as valid for predicting flicker responses as for the examples given here. As in the present results, it is only necessary that the incremental flash stimuli be small enough (less than 11 per cent incremental at the background illumination used in the experiments presented in this paper). If the incremental stimuli are too large, each flash light-adapts the eye to some extent and changes the sensitivity of the eye from what it was originally. The light adaptation caused by a single flash does not appear to decrease the response to that flash to the extent that it decreases the responses to flashes following, for example, in a flash train. The result is that responses to single flashes and to recurrent flashes are no longer directly comparable by linear superposition (DeVoe, 1961). Thus linear response superposition is successful under those light-adapted conditions where the state of adaptation does not change appreciably from one presentation to the next of the various stimulus waveforms.

"Suppression" as a Determinant of Flicker Response Amplitude Arden, Granit, and Ponte (1960) found that the amplitude of *b*-wave flicker retinal action potentials from the eye of the cat became larger and more nearly like that of the single flash retinal action potential as the separation of the recurrent flashes increased. They attributed this increase in flicker response amplitude to a neural suppression which decayed exponentially in proportion to the period of darkness separating successive flashes. An alternative explanation can be given in terms of linear response superposition. Fig. 9B shows data taken from the eye of the spider; it can be seen from the straight line relation between $\log \frac{A_s - A_f}{A_s}$ and flash separation that an increase in flicker response amplitude with increase in flash separation likewise obeys an exponential relationship. In this case, however, the exponential relationship could have been predicted from the response to a single flash of each duration used, as each point in Fig. 9B represents a successful prediction of a flicker response by linear superposition of a single flash response. It is difficult to see how information regarding neural suppression could have been present in the responses to single flashes; rather, it seems probable that these exponential relations are but consequences of the waveforms used in linear superposition. This probability is further strengthened by the existence of a similar exponential function relating the peak-to-peak flash response amplitude at a number of flash durations to the maximum possible peak-to-peak flash response amplitude, occuring at flash durations greater than about 230 msec. This is shown in Fig. 9A for flash response amplitudes calculated by superposition of step responses; significantly, the time constants calculated for the straight lines in Fig. 9 are nearly the same for both plots. From these

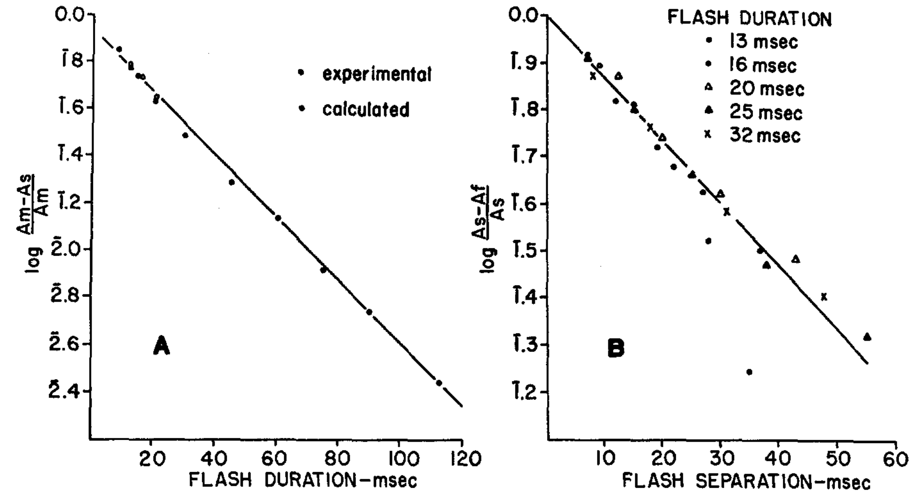

FIGURE 9. Relation between step response amplitude, flash response amplitude, and flicker response amplitude. A, log $[(A_m - A_s)/A_m]$ *versus* flash duration in milliseconds; A_s = peak-to-peak amplitude elicited by 8 per cent incremental flashes, A_m = maximal peak-to-peak response amplitude (elicited by a 250 msec., 8 per cent incremental flash). Open circles obtained using peak-to-peak amplitudes of recorded flash responses illustrated in Fig. 8; filled circles obtained using peak-to-peak amplitudes of flash responses calculated from superposition of the step response illustrated in Fig. 8. Straight line fitted on basis of least squares yields a time constant of 33 msec. B, log $[(A_s - A_f)/A_s]$ *versus* flash separation during intermittent stimulation; A_s = peak-to-peak response amplitude to given durations of 8 per cent incremental flashes, A_f = peak-to-peak flicker response amplitudes to the 8 per cent incremental flashes recurrently presented. Straight line fitted on basis of least squares yields a time constant of 32 msec.

data obtained from spider eyes, it is apparent that some sort of response waveform superposition is sufficient to account for the suppression postulated by Arden *et al.* (1960). Recognizing that the stimulus conditions utilized by these workers were not those most conducive to response superposition, it may nonetheless prove worthwhile to reinvestigate suppression in the light of the results of this paper.

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