ELECTRICAL STUDIES ON THE COMPOUND EYE OF LIGIA OCCIDENTALIS DANA (CRUSTACEA: ISOPODA)*

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INTRODUCTION

Thorough study of the compound eye electroretinogram has not previously been extended to the Crustacea. Therefore exploratory studies on the eye of *Ligia* were initiated and extended. This eye has revealed certain features of unusual interest. Among these are very rapid dark adaptation, high flicker fusion frequency, and the establishment by a brief flash of light of a very slowly decaying facilitation in the electroretinogram. In addition responses of the type recorded from the isolated *Ligia* retina have not been recorded from the isolated retina of any other compound eye.

Structure

The *Ligia* eye shares the general structural features of the typical insect or crustacean compound eye. In each ommatidium light passes through the cornea and crystalline cone to enter the zone of the retinula cells. This sensory zone will be briefly described. Fixed (Susa), bleached (Mayer's chlorine), and stained (Masson) sections, prepared in this laboratory and not yet described elsewhere, reveal that each ommatidium contains eight retinula cells. These form a cylinder extending from the crystalline cone to the basement membrane, a distance of about 120 μ for ommatidia at the center of the eye. Seven of the cells are columnar, have basally situated nuclei, and contribute rhabdomeres to the central axis of the retinula cylinder. Of these seven cells five have an average diameter of about 12 μ, two of about 6 μ; the two are adjacent cells. All the rhabdomeres are in intimate contact with the apex of the crystalline cone. The *Ligia* eye appears to be of the appositional type.

The eighth retinula cell, equal in length to the other seven, is not columnar but spindle-shaped; its nucleus lies at the middle of the long axis of the cell, occupying its greatest diameter (7 to 8 μ); proximal and distal to the nucleus the cell tapers to about 2.5 μ. There is no rhabdomere and no part of this peripherally situated cell reaches the central axis of the retinula cylinder.

From the base of each retinula cell a nerve fiber arises, penetrates the basement membrane, and proceeds toward the optic ganglion. Cross-sections just below the basement membrane show eight fibers per ommatidium; five have diameters of about

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9 μ, two of about 4.5 μ, and one of about 1.5 to 2 μ. These fibers belong respectively to the five larger and two smaller retinula cells with rhabdomeres and to the eccentric cell. *En route* to the optic ganglion the fibers become grouped into ensheathed bundles; at the same time the larger fiber diameters diminish until, quite close to the optic ganglion, all fibers have a rather uniform diameter of about 1.5 to 2 μ. The bundle sheaths merge with the sheath surrounding the optic ganglion. The sections thus far studied have not permitted resolution of fiber and synaptic relations in the optic ganglion.

A study by Hanström (1924) includes the histology of the optic ganglion of *Ligydia occidentalis* (= *Ligia occidentalis*). Nerve fibers from the ommatidia enter the lamina ganglionaris where they all synapse with second order units. Two other synaptic centers, the medulla externa and medulla interna, lie in that order central to the lamina. Between the medulla interna and the brain lies the optic nerve. Fig. 1 indicates these areas as they appear in the opened *Ligia* head.

The "isolated retina" will mean the structures which remain distal to a cut through the retinula nerve fibers as they enter the lamina. "Electroretinogram" or "ERG" will refer to potentials evoked by illumination and recorded from points anywhere in the eye or on the optic ganglion, and will be qualified whenever necessary to indicate whether a particular response originates in an intact eye or in one from which the optic ganglion has been removed.

Methods

Ligia occidentalis, the common isopod of rocky California shores, can be taken in large numbers at any time. Large specimens of about 3 cm. body length were maintained on damp sand in the laboratory at 15°C. until used. Generally they were kept in the dark for about 24 hours before experiments began and none of those used had been in the laboratory for more than a week.

When the responses of the intact eye in the intact animal were to be studied the thorax and the posterior half of the head were imbedded in a plaster of Paris block. The sessile eyes, perfectly immobilized, protruded at one end of the block, the abdomen with its pleopod gills at the other. The pleopods were kept damp during experiments. Animals so mounted survive for days.

When recording from the inner surface of the retina and the optic ganglion the following technique was used. The terga were removed from the anterior half of the thorax. The exposed gut was severed in the head and with its attached digestive glands was peeled back away from the head. The isolated head was pinned to a small piece of cork on the end of a short dowel rod. The dorsal skeleton of the head, the muscles of the mouth parts, and the remaining gut were removed. The preparation (Fig. 1) now consisted of eyes, head, CNS, and ventral head skeleton.

Both types of preparation were fixed in the vise of a mounting platform. The cornea of the experimental eye was oriented in the terminal focus of the optical system. This included a 100 watt concentrated arc lamp as a source of white light (Buckingham and Dreibert, 1946), a condensing lens system, 15 cm. of water, a variable speed rotary shutter with interchangeable sector discs cutting the beam at a focus, a manual shutter, and a carriage for Wratten neutral tint filters. The circular cross-section of the beam as it emerged from the terminal lens subtended 22° at the cornea. The

terminal focus had a diameter of 1.9 mm. The maximal illuminance of a surface placed in the focus was 15,000 foot-candles. This is unit intensity throughout the paper. The units used are applicable to the *Ligia* eye only if the luminosity function of wave length for *Ligia* is the same as for the human. Since a luminosity curve has not been determined for *Ligia* foot-candles in this paper may measure only relative illuminance.

The anteroposterior dimension of the *Ligia* eyes used was about 2.5 mm., the dorsoventral dimension about 2 mm. The focus covered most of the eye. Within the corneal area of the focus were facets of about 900 ommatidia. Because of eye curvature and the direction of the incident light a gradient of illumination is expected, with constant corneal illumination, at the receptor level through the stimulated ommatidial field.

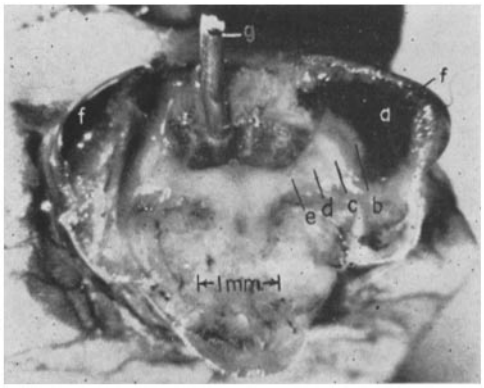

FIG. 1. View into the opened head of *Ligia* from a position above, behind, and to the left: *a*, inner retinal surface; *b*, *lamina ganglionaris, c, medulla externa, d, medulla interna, e,* optic nerve; *f*, cornea; *g*, pin.

Sharp pointed needle electrodes of stainless steel were positioned by micro manipulators. The electrodes were uninsulated; care was exercised to assure that only the tips were acting as leads. A D.C. amplifier with an input impedance of 2 megohms, which served to minimize electrode polarization, was used in experiments in which ERG wave form was important. For other experiments a condenser-coupled amplifier was used. Responses appeared on the screen of a dual beam oscilloscope.

RESULTS

A. Experiments on the Intact Animal

1. Wave Form and Polarity of the Ligia Electroretinogram.—Records of the most frequently encountered type of response to high intensity light stimuli are reproduced in Fig. 2. In all responses of this type the lead from the illuminated eye records initial negativity. The first response of both the 5 msec. *a* and the 650 msec. *b* series, that of the dark-adapted eye, differs from succeeding ones in: (1) lower rate of rise and fall of all response components; (2) lesser peak amplitude of the on-effect, and in *b* of the off-effect; (3) lesser amplitude of the

positive deflection immediately following the on-effect in *a* and *b*. This positive deflection is not a pure off-effect in *a* since it appears much the same in *b* where it is clearly not an off-effect. Stimuli of 5 msec. allow insufficient time for development of a discrete off-effect.

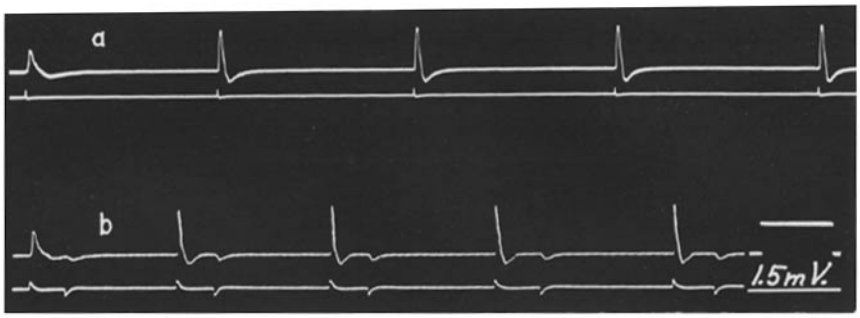

FIG. 2. Typical ERG of fast type *Ligia* eye. Subcorneal leads; indifferent lead from non-illuminated eye. Negativity of active lead gives upward deflection. Stimulus of maximum intensity, log I = 0; stimulus duration in *a* 5 msec., in *b* 250 msec. One stimulus every second, signal on lower trace. First stimulus of each series to dark-adapted eye. D.C. amplification.

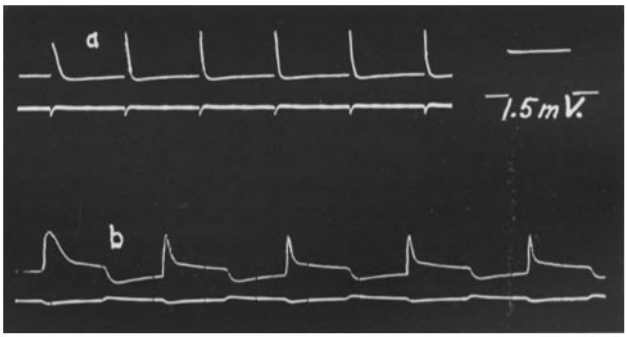

FIG. 3. ERG of slow type *Ligia* eye. Same conditions as in Fig. 2 except that stimulus duration in *b* is 500 msec.

The equilibrium wave form is attained rapidly; it may be seen in the responses to the fourth stimuli. The two series were continued for about thirty stimuli and no further changes appeared. In other cases (see Fig. 4) the amplitude of the on-effect to 250 msec. and longer stimuli first increased, then decreased to some lower equilibrium level than that attained by the fourth response. The initial amplitude increase of the on-effect is part of a complex of changes which has been termed "facilitation"; its properties will be described later. With high

intensity stimuli of the order of 2 msec. duration in a 1/second series, the on-effect attains by the fourth response a facilitated amplitude which is indefinitely constant. With stimuli of 250 msec. there is usually an initial facilitation frequently followed by a decline. With stimuli of 500 msec. a decline from the facilitated amplitude has always appeared.

If the positive off-effect were eliminated from *b* of Fig. 2 the responses of the 5 msec. series would resemble those of the 250 msec. series quite closely. The base line is maintained after the early positivity in *b* in the presence of the stimulating light, while in *a* the stimulating light has been off for some time before the base line is regained. Eyes giving the type responses shown in Fig. 2 will be referred to as "fast" eyes.

The responses of the "slow" eye of Fig. 3 were obtained under the same experimental conditions as those of Fig. 2 except that in Fig. 3 *b* 500 msec. stimuli were used. The conspicuous differences from the fast eye lie in: (1) the great reduction of the positive phase following the negative on-effect in *a*, and the absence of the early positivity in *b*; (2) the failure to assume the base line potential while the stimulating light was on in *b*; (3) the absence of facilitation in *b*.

Such slow eye responses were rarely encountered in freshly collected intact animals, more often in those kept in the laboratory for a number of days. They appeared frequently in the fresh preparations with opened heads which form the subject of Results, *B*.

The terms "fast" and "slow" have been, and will continue to be, based upon characteristic responses to high intensity stimulation. Fig. 4 illustrates changes in the ERG of a fast eye as a function of stimulus intensity. The wave form at low intensity ($\log I \leqq -3.99$) is similar to that of the slow eye at high intensity. The early positivity becomes more pronounced as intensity increases; facilitation likewise increases with intensity.

The slow eye has been associated with preparations in less than perfect condition. Certain qualitative data from slow eyes are quite consistent with data from fast eyes. Among these are polarity of on- and off-effects, and presence of facilitation (generally present in the slow eye only with very brief stimuli). Such consistencies are considered sufficient reason for including slow eye data in Results, *B*. The rare slow eyes encountered while working with intact animals have not been included in any of the following data of Results, *A*.

2. Facilitation.—When the dark-adapted eye is presented with certain series of stimuli of equal intensity and duration facilitation appears in the ERG. A second stimulus applied at an appropriate interval after a first evokes a response whose component deflections have greater amplitudes and rates of rise and fall than their counterparts in the response to the first stimulus. One aspect of this complex, the change in amplitude of the on-effect, has been chosen for detailed study.

The records of Fig. 5 represent a typical experiment in which two 5 msec. stimuli separated by a constant dark interval of 1 second were presented to the dark-adapted eye at different light intensities; at least 2½ minutes separated pairs of stimuli. From the records of Fig. 5 the curves of Fig. 6 were drawn. The solid curves illustrate the height of on-effect elicited by the first and second flashes at different intensities; the dashed curve shows that facilitation becomes proportionately greater with increase of intensity. (Experiments with constant

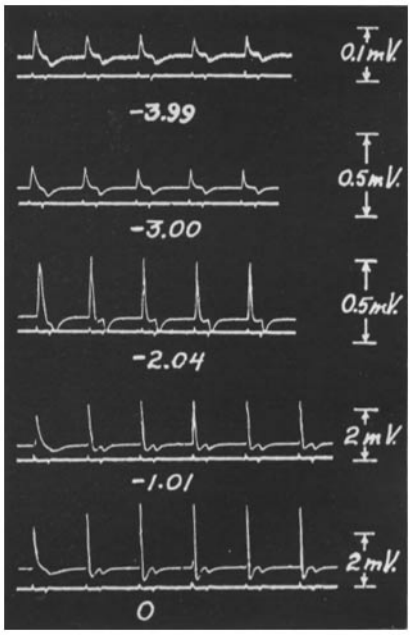

FIG. 4. Fast type ERG at different light intensities. Stimulus duration of 250 msec.; one stimulus every second, signal on lower trace. Log I given below respective records. Leads as in Fig. 2. Eye dark-adapted before each series. D.C. amplification.

test flash were not performed.) Facilitation of amplitude is not apparent with the lowest intensities but close inspection of Fig. 5 shows that responses to flash 2 at low intensities have somewhat greater initial rates of rise from the base line. (In the superimposed response pairs the response to flash 2 is always the first to rise and fall.) At lower intensities members of the response pairs appear to have different latencies, though a very gradual rise of the first response could cause the latency to appear greater than is actually the case. With the higher intensity response pairs it is quite impossible to measure any latency differences between the first and second members. The latency of the response to flash 2 at log I = −5.06 is approximately 20 msec.; that of the

responses at log I = 0 is approximately 6 msec. No precise latency determinations have been carried out in this study.

One 5 msec. high intensity stimulus presented to a dark-adapted eye conditions that eye so that another 5 msec. stimulus of the same intensity delivered within 2 minutes of the first elicits a facilitated response. The facilitated state decays with increasing interval between conditioning and test flash. Fig. 7

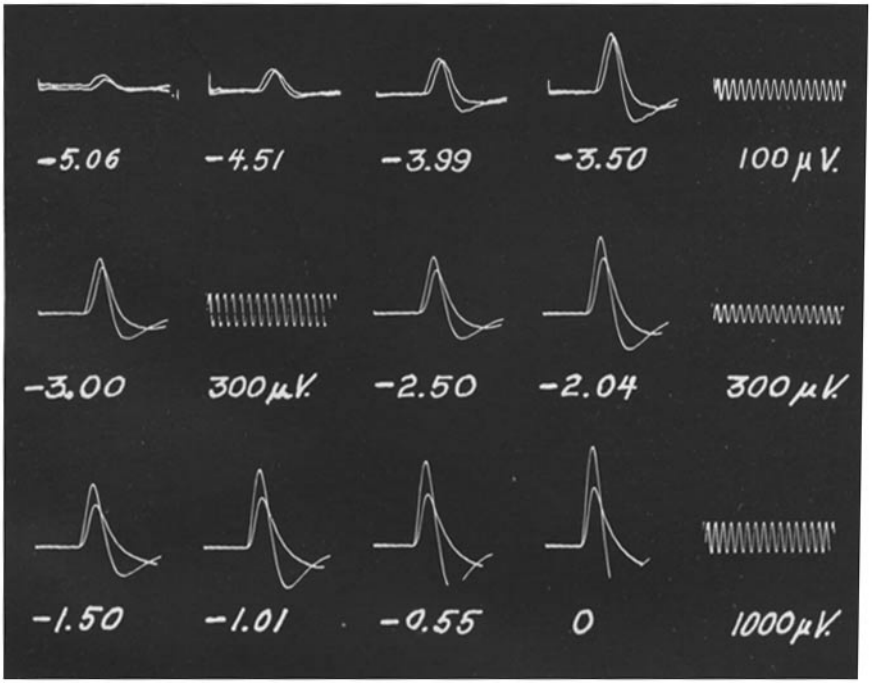

FIG. 5. Facilitation at different light intensities. Superimposed response pairs to stimuli of 5 msec. duration; 1 second interval between members of each pair. Eye dark-adapted prior to each pair. Leads as in Fig. 2. Each 60 cycle calibration applies to records left of itself. Time constant = 0.5 second.

represents one of several experiments in which the ratio of on-effect amplitudes to test and conditioning stimuli was determined as a function of the interval between the two stimuli. Between each pair of stimuli at least 2½ minutes elapsed before presenting the first flash of the next pair.

The facilitated state was always found to be fully developed if a 1 second interval separated conditioning and test flashes. The experiment of Fig. 8 illustrates the development of the facilitated state during the 1st second. The interval given below the records is elapsed time from the beginning of the

conditioning stimulus to the beginning of the test stimulus. The test flash fails to evoke an on-effect of its own up to some interval between 25 and 60 msec. From this interval up to about 250 msec. the second on-effect grows till it equals the amplitude of the first, and beyond 250 msec. continues to increase. Between ½ second and 1 second the full facilitated amplitude is at-

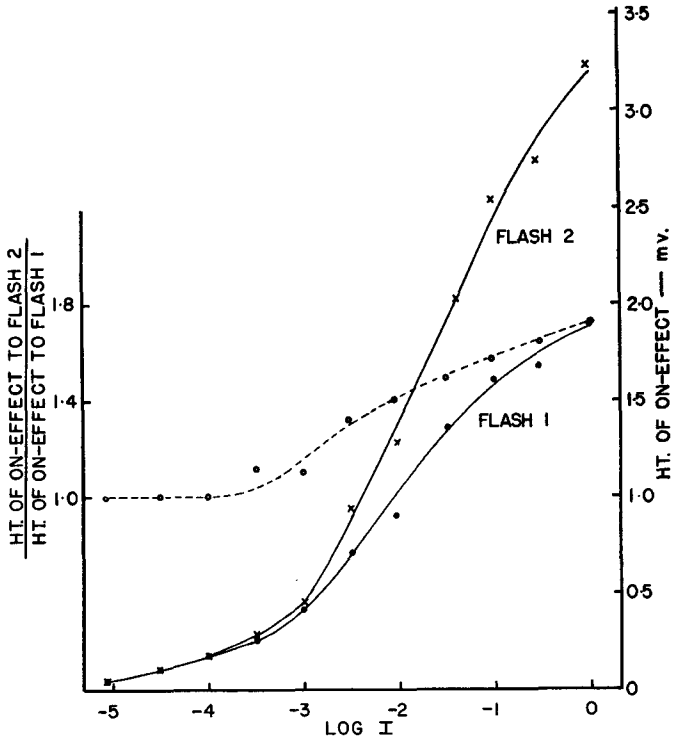

FIG. 6. Facilitation at different light intensities. A plot of Fig. 5 data. Ordinate values for solid curves at right, for dashed curve at left.

tained and is maintained in this case through the 3rd second; a slight decline is visible by the 4th.

Another property of the *Ligia* ERG may be noted in Fig. 8. A comparison of the response to a single 5 msec. flash (first record) with that to two such flashes separated by a 5 msec. dark period (second record) indicates that the second flash has not summed with the first to increase the height of the on-effect. Therefore the height of the on-effect was determined within the 10 msec. before the second flash arrived. Two experiments on different animals were performed to determine that stimulus duration just sufficient to produce a maximal on-effect at a given intensity. It was found that between log I = 0

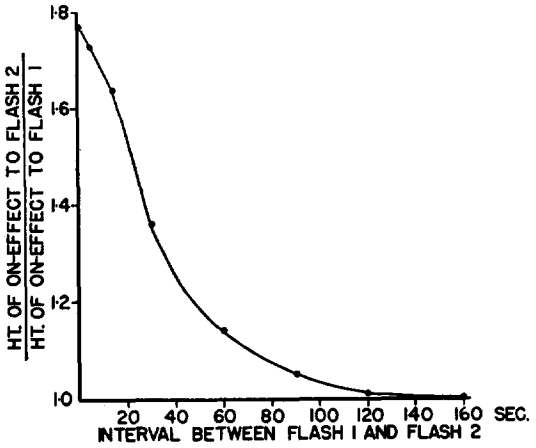

FIG. 7. Decay of the facilitated state. Log I = 0, duration = 5 msec. for both flashes. Eye dark-adapted before flash 1.

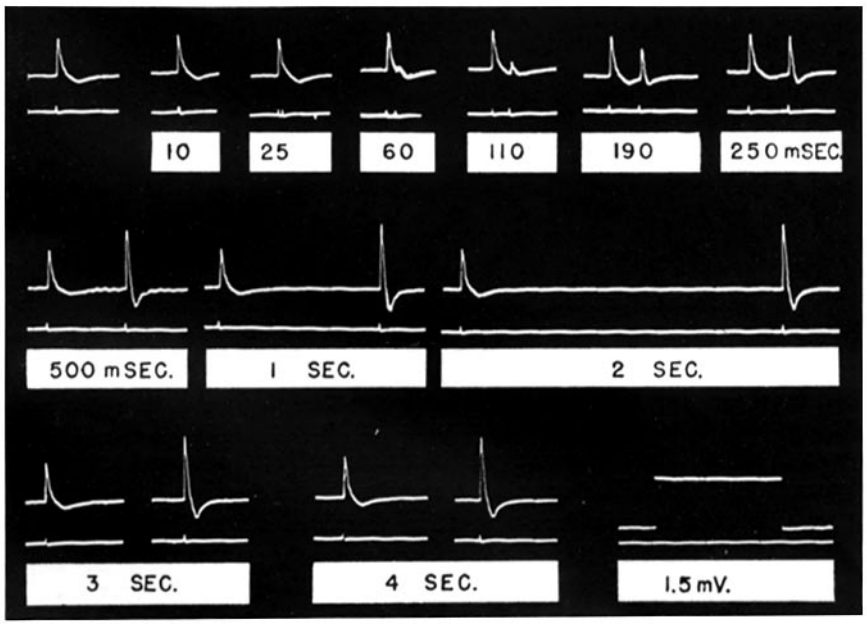

FIG. 8. Growth of the facilitated state. Log I = 0, duration = 5 msec. for all stimuli. First record is response to a single flash. Intervals given below other records. Eye dark-adapted prior to each pair of stimuli. Leads as in Fig. 2. D.C. amplification.

and log I = −3 stimuli of 10 to 12 msec. were sufficient and necessary to produce maximal on-effects.

3. Dark Adaptation.—The course of dark adaptation was followed in *Ligia*

by a method which utilized the height of on-effect in response to brief stimuli of constant duration and intensity as an index of changing sensitivity of the eye. The method will be described in reference to the experiment of Fig. 9. First the animal was allowed to dark adapt after mounting in the experimental apparatus for a period of 45 minutes. This time has been found more than adequate for equilibration with the near total darkness of the experimental room. Then curve *a* describing the height of the on-effect as a function of log I was determined. The stimulus duration here and for all points in the figure was 5 msec. Next the eye was steadily illuminated for 5 minutes at log I = −2.04. At the end of the 5th minute the adapting light was removed, and at subsequent intervals in the dark the height of the on-effect was determined with test stimuli at log I = 0. These heights are plotted in curve A as a function of time in

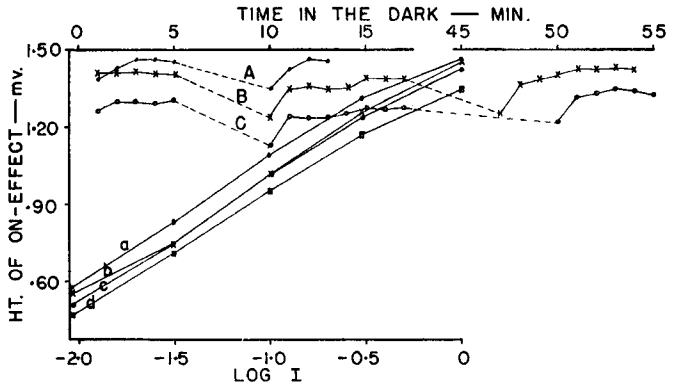

FIG. 9. Dark-adaptation curves. See text for explanation.

the dark. Then curve *b* was determined in the same manner as curve *a*. The eye was illuminated for 5 minutes at log I = −1.01; test flashes followed at log I = 0, and curve B was determined in the same manner as A. Then curve *c* was obtained. The eye was adapted 5 minutes at log I = 0, and test flashes determined curve C. The experiment ended with the curve *d* determinations.

An effort was made in dark-adaptation experiments to maintain amplitude increments attributable to facilitation approximately constant. To this end a constant 1 minute interval was used to separate all test flashes. Exceptions to this occurred (Fig. 9) between minutes 5 and 10, and after minute 17; after the longer dark periods the curves rise in each case, indicating addition of facilitated increments to the following several responses.

Each dark adaptation curve of Fig. 9 should be considered relative to the log I *vs.* height of on-effect curve which preceded it. The dark-adaptation curve following exposure to log I = −2.04 shows that 3 minutes sufficed for the eye to regain fully the sensitivity it had prior to light adaptation. The other two

curves show that a longer time was required when the intensity of the adapting light was higher. However, the sensitivity change as judged by change of amplitude of on-effect is confined to a small range; the response at the end of the 1st minute is equal to, or exceeds, the response elicited by a stimulus of only 0.47 log unit lower intensity presented when the eye had full sensitivity.

4. Flicker Fusion Frequency (f.f.f.).—The criterion of the f.f.f. was the disappearance from the ERG of responses to flickering light. All f.f.f. determinations were made with a light:dark ratio of 1 at a temperature of about 22°C.

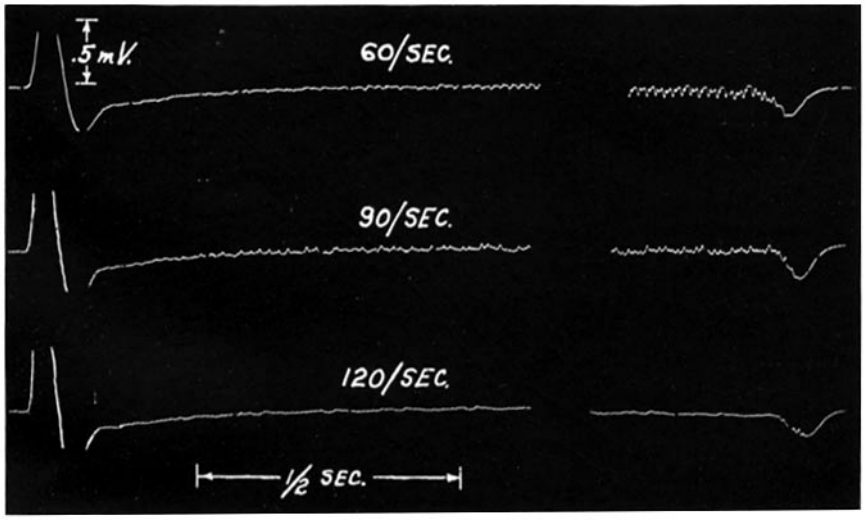

FIG. 10. Responses to flickering light. Log I = 0 for all three records. Gaps represent 6 seconds. Leads as in Fig. 2. This animal gave upper curve of Fig. 11. Time constant = 0.5 second.

Fig. 10 shows responses to flicker exposures of 60, 90, and 120 flashes/second. The gaps represent 6 seconds. The growth of the response from the outset of exposure (60/second and 90/second) is evident and represents in part at least the development of facilitation in the flicker situation. (The preceding dark periods were not carefully controlled here.) The 120/second response represents the f.f.f. at log I = 0; the eye which supplied these records gave the upper curve of Fig. 11.

The f.f.f. determinations of Fig. 11 were obtained from five animals on the same day. After positioning in the experimental apparatus 20 minutes were allowed for equilibration before making the first determination at log I = −4. A determination was considered satisfactory if it could be repeated with small error two or three times. Essentially the same results could be obtained by gradually increasing the flicker rate to the fusion point as by decreasing the

flicker rate to the fusion point; the former proved the easier technique and was regularly used. Fig. 11 shows that the highest intensity (15,000 foot-candles) was insufficient to produce maximal f.f.f. values. Values from other experiments were quite similar providing the animals were fresh.

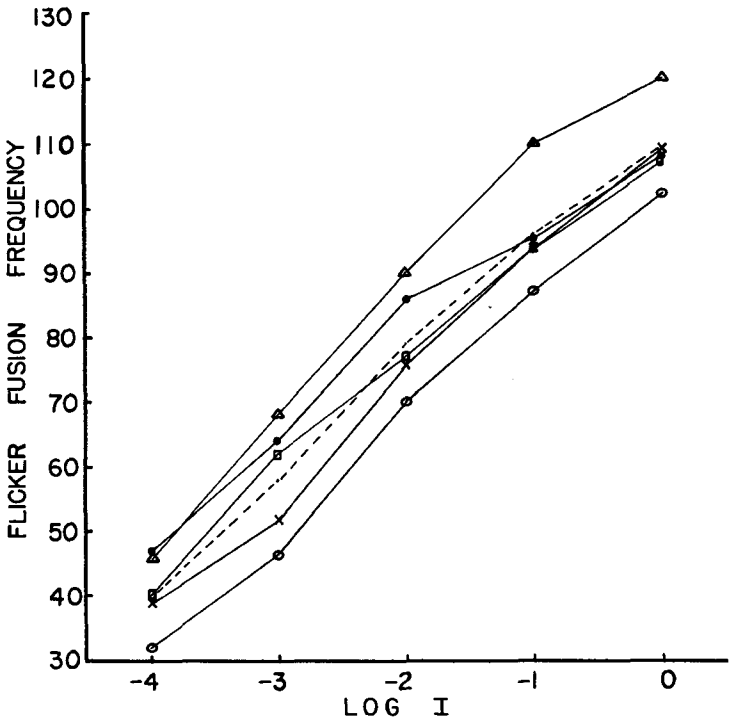

FIG. 11. Flicker fusion frequency. The rate of flicker at which responses to each flash disappeared from the ERG is plotted as a function of log I. Each solid curve a separate animal; dashed curve is the average for the five animals.

B. Experiments on the Exposed Optic Pathway and the Isolated Retina

1. Experiments with Opened Head Preparations.—These preparations were moistened only by the fluids remaining after dissection; a moist chamber was not used. Experiments were limited to about 30 minutes.

In about twenty experiments an electrode tip was moved along the inner retinal surface from the periphery toward and onto the optic ganglion. This active electrode was coupled with an inactive one situated under the cornea of the dark eye. One such experiment is illustrated by Fig. 12. The numbered active electrode positions indicated on the diagram of the optic pathway represent the positions from which the correspondingly numbered responses were

recorded. Before each of the brief series of responses the eye was in the dark for 2 minutes.

When the electrode is positioned peripherally on the retina responses are the same as obtained with the active electrode placed subcorneally. As the electrode is moved toward the optic ganglion ERG amplitude gradually decreases. The electrode can be placed at points still on the retina (about as far from the

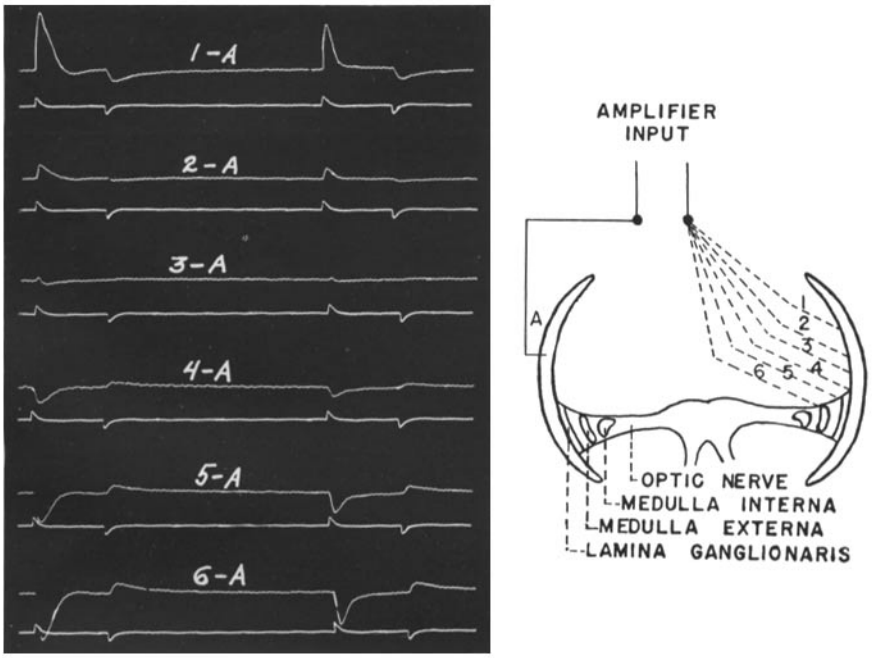

FIG. 12. Responses along the optic pathway. Lead combinations indicated on diagram and above responses. Lead A subcorneal and indifferent in the non-illuminated eye; negativity of a numbered lead gives upward deflection. Stimulus durations = 250 msec., signal on lower trace. Log. I = 0. 2 minutes' dark adaptation preceded each series. D.C. amplification.

lamina ganglionaris as the lamina is from the medulla externa) from which only very small signs of electrical activity can be detected. Central to such points the ERG inverts and increases to a maximum amplitude at the level of the medulla externa. Central to the medulla externa the ERG gradually declines until at the brain it can rarely be detected.

In most experiments on the opened head the voltage was considerably lower than in the average intact preparation, the wave form approached that of the intact slow eye and facilitation was generally absent. Such differences from the normal were present in even the most carefully dissected preparations.

Experiments were conducted to determine what changes, if any, accompany deganglionation of those eyes whose responses prior to deganglionation most closely approached the normal. A fine pointed sliver of razor blade edge was used to sever the retinula nerve fibers as they entered the lamina. The animal of Fig. 13 was first mounted with optic pathway exposed but intact. The upper record was taken. The experimental eye was then deganglionated; the lower record is the response of the deganglionated eye. The two responses are nearly identical.

Experiments of the Fig. 12 type were performed with deganglionated eyes. Fig. 14 illustrates one such experiment with the added refinement of a third electrode. Electrode 1 was fixed and subcorneal in the illuminated eye; electrode

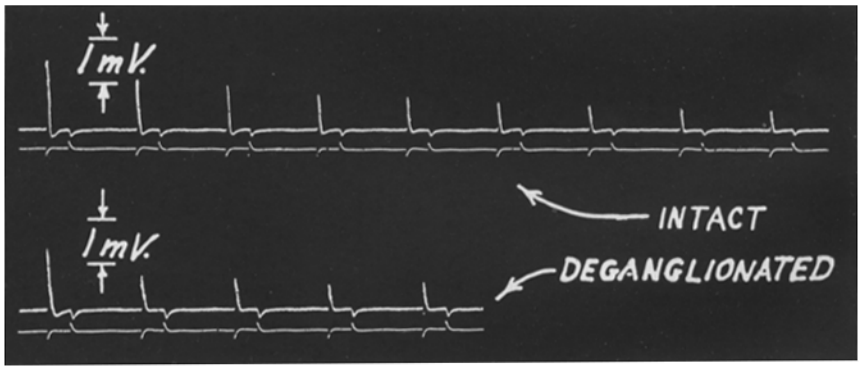

FIG. 13. Responses of an opened head preparation with and without the optic ganglion. Stimulus duration = 250 msec., log I = 0. After recording the upper series the animal was dismounted, deganglionated, and mounted as before to record the lower series. Leads as in Fig. 2. D.C. amplification.

A was fixed and subcorneal in the dark eye; the third electrode was movable and had the positions 2, 3, and 4. A switch selected desired pairs of lead combinations. Connections were arranged so that negativity at any electrode in the illuminated eye produced an upward deflection when coupled with electrode A. Additional switches made it possible to record the animal responses, the stimulus signal and the 60 cycle time base on the same triggered sweep. First the eye was equilibrated with a series of 50 msec. flashes of log I = 0 at 1 flash/second. This stimulation was continuous throughout the experiment. A response appeared on the oscilloscope screen once per second when the animal was switched in. The camera shutter was opened for one response at a time. Two responses from different leads were superimposed; then the film was advanced a half frame to record the stimulus signal, and advanced again to record the time base. This experiment demonstrates the essential aspects of the polarity reversal phenomenon in the isolated retina.

2. Responses of the Isolated Retina in the Intact Animal.—An attempt was made to determine the cause of the variable decline of the opened head preparations. Neither mechanical damage to retina and optic ganglion nor chemical damage traceable to liberated digestive fluids was a likely factor. Interruption of the circulation, however, accompanied all dissections and its effects had not been isolated; this factor was studied.

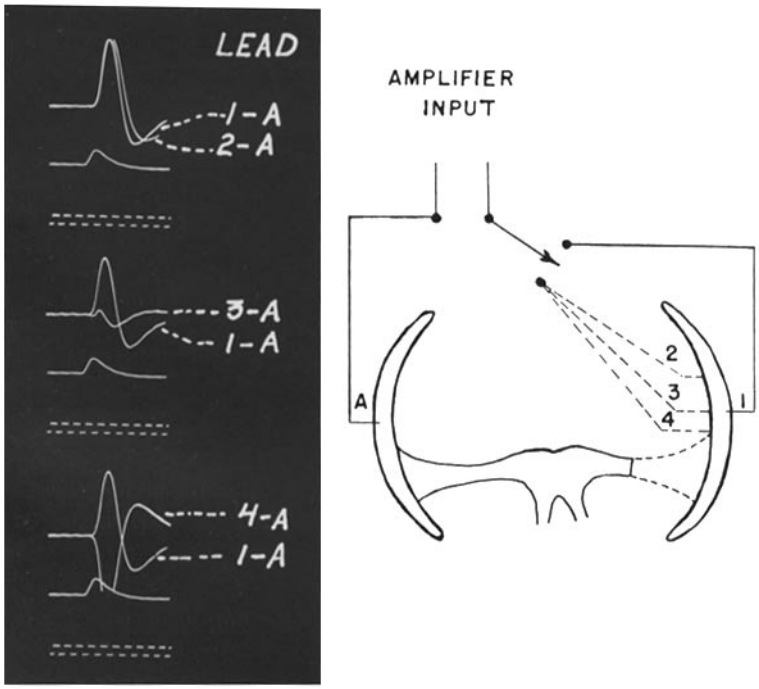

FIG. 14. Responses of the isolated retina. Continual stimulation at log I = 0 at a rate of 1/second; stimulus duration = 10 msec. One response from each of two lead combinations superimposed. Lead A subcorneal in non-illuminated eye; negativity at any numbered lead in the illuminated eye gives upward deflection. Stimulus signal, then 60 cycle time base below retinograms. Time constant = 0.5 second.

A fresh intact animal was mounted in plaster and after dark adaptation was stimulated with high intensity flashes of $\frac{1}{8}$ second duration at a rate of 1 flash/second. Fig. 15 *a* shows the responses to the first two flashes of the series; facilitation is evident. Less than 1 minute later the equilibrium amplitude and wave form were attained; *b* shows an equilibrium response taken at 4 minutes. At 5 minutes the animal was bled by partially removing one tergum at the posterior end of the thorax. Within 30 sec. of the operation the ERG had visibly changed. Responses *c* and *d* were taken at 2 minutes and 9 minutes

respectively after the cut. The amplitude of all ERG components, declined progressively after bleeding.

In several experiments in which animals were bled while fully dark-adapted and the state of the eye subsequently tested with occasional pairs of 5 msec. flashes, the responses were of normal wave form and amplitude and showed normal facilitation for at least 8 minutes. With longer flashes and more continuous stimulation the eye very soon lost the ability to facilitate and the ERG amplitude dropped. The immediate effect of bleeding appears to be a loss or retardation of normal recovery (dark-adaptation) processes.

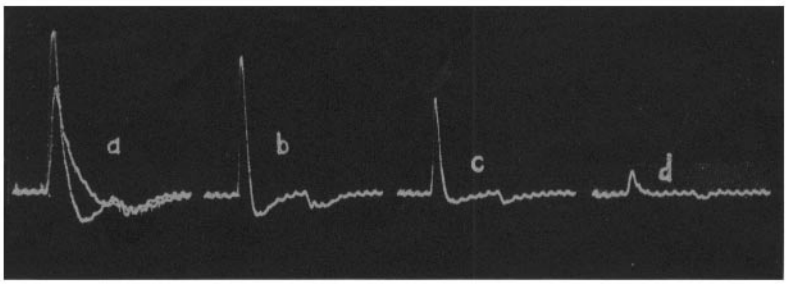

FIG. 15. Effects of bleeding. Intact animal mounted in plaster and dark adapted. Stimulation then continuous with $\frac{1}{8}$ second flashes of log I = 0 at 1/second. First 2 responses in *a*; *b* is an equilibrium response taken at 4 minutes. Animal was then bled; *c* taken at 2 minutes and *d* at 9 minutes after the operation. Leads as in Fig. 2. 60 cycle artefact present.

In order to evaluate a possible role of the optic ganglion in producing some feature of the normal ERG it appears necessary to remove the optic ganglion without interrupting the circulation. This may be done with an intact animal mounted in plaster. Contact of retina and optic ganglion (Fig. 1) is restricted to a rather small anterioventrally situated area. A thin razor blade was passed normally through the cornea into the head dividing the eye into a posterior part nervously isolated from the ganglion, and an anterior part retaining nervous connection with the ganglion. The blade remained imbedded to seal its own cut, preventing bleeding, and also acted as an opaque partition which completely shielded the anterior part of the eye from the stimulating light. Responses from five posterior retinas isolated by this method were the same with respect to wave form, facilitation, and dark adaptation as those from the same area of the eyes before the isolating cuts.

Every qualitative aspect of the ERG recorded from the eyes of normal intact animals has been observed in one or another isolated retina. There is no evidence for any contribution by the optic ganglion to the ERG.

DISCUSSION

A. Wave Form and Polarity of Compound Eye Electroretinograms and the Allocation of Component Potentials

Wave form and polarity of compound eye ERG's vary considerably among species. The fact that in some eyes ERG's may be experimentally altered in a differential manner has suggested that more than one electrical process is involved. A number of studies have been concerned with identification of the parts of the eye from which the separate processes originate. The following conception of the compound eye ERG has emerged. (1) The photoreceptors upon illumination become more negative at their distal ends (extracellular recording). Negativity of a corneal electrode is the first recorded response. (2) The receptor negativity is sustained during illumination unless (3) the optic ganglion responds, after the initial negativity, with a potential opposed to that produced by the receptors. Receptor and ganglion potentials may then sum to produce such features as on- and off-peaks in the ERG and a return to the base line potential during illumination.

This conception is supported by the following evidence. The receptor layer of the *Limulus* compound eye is anatomically isolated from the first obvious synaptic region. When recording extracellularly the distal ends of the ommatidia become more negative to the proximal ends on illumination; the response is monophasic (Hartline, Wagner, and MacNichol, 1952).

Some insect ERG'S resemble the *Limulus* response; others are more complex. Removal of the optic ganglion from insects of the latter group simplifies the ERG to the monophasic negative type.

Before deganglionation the ERG of *Calliphora erythrocephala* consists of a spike-like positive on-effect, a sustained base line or near base line potential during illumination, and a spike-like negative off-effect (Autrum, 1950); with high amplification a slight and very quickly terminated negativity may be seen to precede the on-effect. After deganglionation the ERG is monphasic and negative (Autrum, 1952; Autrum and Gallwitz, 1951). The ERG of the intact *Dixippus morosus* is monophasic and negative; a ganglion response in this type eye does not arise (Autrum, 1950, 1952) because of greater anatomical distance between retina and ganglion than in eyes giving the *Calliphora*-type response.

According to Bernhard (1942) the receptor layer of the *Dytiscus marginalis* eye is responsible for two negative ERG components, while the optic ganglion contributes a third which is opposed in sign to the other two. The ganglion response was removed by deganglionation and cocaine.

Jahn and Wulff (1942) concluded that the optic ganglion of *Trimerotropis maritima* and *T. citrina* was the site of origin of an ERG component opposed to the negative receptor potential. The ganglionic component could be removed by deganglionation and inferred from various leads along the optic pathway.

Hartline, Wagner, and MacNichol (1952) very briefly reported that deganglionation of a house-fly eye altered the ERG from a polyphasic wave form to a monophasic one.

The view that the earliest sign of the receptor response is a negative deflection (recorded corneally) is not, however, supported by all the published data on the compound eye. The ERG's of the following insects begin with positive deflections: *Melanoplus differentialis* with low intensity stimulation (Jahn and Crescitelli, 1939 *a*); *Samia cecropia* (Jahn and Crescitelli, 1939 *b*); *Chalenius diffinis* in the evening (Jahn and Crescitelli, 1940); *Dytiscus fasciventris* during the day phase (Jahn and Wulff, 1943); *Cynomya cadaverina* with low intensity stimulation (Wulff and Jahn, 1947); *Galleria mellonella* (Taylor and Nickerson, 1943). It is possible that earlier negativity was present but of such low amplitude as to have been overlooked at the amplifications used. If it is true, however, that the earliest phase of certain insect ERG's is positive it is reasonable to assume that the structure responsible is among the photoreceptors, not in the optic ganglion.

That positive ERG components may originate in the receptor layer is indicated also by the *Ligia* data. The initial deflection from an intact fast eye is negative, immediately followed by an early positive deflection; during sustained illumination the base line potential is regained, and there is a positive off-effect. After the ganglion has been totally removed the response is the same.

The fact that the ERG of the isolated *Ligia* retina displays features which in the insect have been attributed to the optic ganglion implies either that *Ligia* photoreceptors respond in a manner fundamentally different from insect photoreceptors, or that after deganglionation the isolated insect retinas were no longer able to respond in a normal manner. Removal of the optic ganglion is less likely to damage the receptors in *Ligia* than in the insects. In *Ligia* the ganglion covers little of the inner retinal surface; even at the area of contact the sense cell axons are sufficiently long (150 to 200 μ) so that a micro knife carefully used does not come into contact with the retinal basement membrane. In the insects the ganglion covers most of the inner retinal surface and the sense cell axons are very short; cutting the ganglion away or simply lifting it out of the head seems likely to damage the sensory layer to some extent. The possibility that the changes in the insect ERG following deganglionation are injury changes may not be excluded.

The receptor cells of each ommatidium of *Ligia* are of three histological types (Introduction). It may be that each type is responsible for a different aspect of the ERG. It is well known that insect retinula cells are also of more than one type (Hanström, 1927). Bernhard (1942) reported two ERG components of retinal origin in *Dytiscus*, both with the same polarity. Each ommatidium of the *Limulus* eye contains two likely photoreceptor cell types, and Wulff (1950) reported two component ERG potentials, both with the

same polarity. Application of the microelectrode technique to the compound eye will doubtless aid in assigning the components of the ERG to the responsible structures; the work of Hartline, Wagner, and MacNichol (1952) represents a beginning in this direction. The microelectrode technique in the hands of Ottoson and Svaetichin (1952) has produced evidence that the polyphasic ERG of the frog originates in the receptors alone; positive on and off, and negative on-and-off responses, appear to originate in rods and cones respectively.

Polarity reversal of the *Ligia* ERG observed with leads from the central retina and optic ganglion cannot yet be satisfactorily explained. It occurs even in the absence of the ganglion (Fig. 14). Except for polarity reversal the wave form is the same wherever recorded. It seems likely that electrical properties of the path along which the ERG spreads from the receptors are responsible for the reversal. Histological changes occur along the same gradient as the polarity changes. The retinula cell axon layer deepens along any meridian from retinal periphery toward ganglion, and the axons become grouped into ensheathed bundles. It may be that the bundle sheaths have capacitative properties such that a surface electrode making contact with them records the inverse of the polarity changes occurring within the sheaths. The fact that these polarity changes can occur in the absence of the ganglion in *Ligia* casts some doubt on the interpretation of Jahn and Wulff (1942) in which similar ERG polarity changes with electrode position were used to infer the existence of an optic ganglion potential in *Trimerotropis*.

B. Dark Adaptation and Flicker Fusion Frequency

The range of sensitivity change in *Ligia* following intense light adaptation is small. The data of Fig. 9 are replotted in Fig. 16 to permit comparison with data from other studies. Each point on an A, B, or C curve (Fig. 9) represents an on-effect amplitude in response to a test flash of log I = 0 during dark adaptation; the corresponding height of on-effect on the previously determined *a*, *b*, or *c* curve represents a response to some lower, or equal, log I value when the eye was fully dark-adapted. The difference between log I = 0 and the other log I value measures the diminished sensitivity consequent upon light adaptation. This difference is plotted in Fig. 16 as a function of time in the dark and called log reciprocal sensitivity (log 1/sens.).

This plot brings data of the present method which uses a constant test flash to evoke a varying response into a form suitable for comparison with data obtained through use of a varying test flash to evoke a constant response, as for example a threshold. Data of the constant response method and the present one have been shown to mean the same thing in *Limulus* when either ERG amplitude or nerve impulses are studied (Hartline and McDonald, 1947). Their analysis depended on securing curves like the *a*

curve of Fig. 9 for different levels of light adaptation. The curves for different light-adapted levels were parallel, a necessary condition if the two methods are to produce the same data. When the curves are parallel only one of them is needed. The assumption has been made that these curves, if determined for *Ligia*, would be parallel. The sensitivity range of importance is so small in *Ligia* that little error is likely to be introduced by this assumption.

The upper curve of Fig. 16 will be compared with human dark-adaptation data, obtained with a non-electrical constant response method, from Fig. 2 of Hecht, Haig, and Chase (1937). A 30° light-adapting field, seen through a 1 mm.^{2} pupil, was centered 30° nasally on the retina. Retinal illumination, expressed in photons, was converted into foot-chandles' illumination at the pupil to permit comparison with corneal illumination of *Ligia*. Photons/10.7

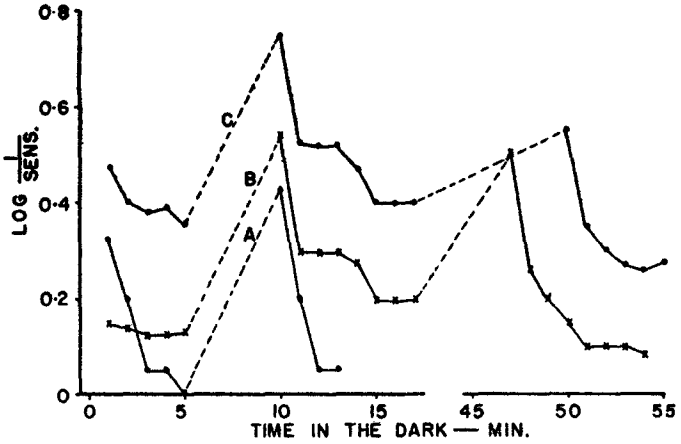

FIG. 16. Dark-adaptation curves replotted from Fig. 9. See text for explanation.

= foot-candles. No attempt was made to base comparison on conditions of equal illumination of the receptors.

Following exposure to 37,290 foot-candles for 2 minutes, log 1/sens. for the rods after 1 minute of dark adaptation was about 4.6, for the cones about 1.45. For *Ligia*, after exposure to 15,000 foot-candles for 5 minutes, log 1/sens. after 1 minute dark adaptation was 0.47. The *Ligia* was less sensitive than in the fully dark-adapted state by a factor of less than 3, the rods by a factor of almost 40,000, and the cones by a factor of about 28.

An outstanding aspect of Autrum's work is the classification of insect ERG's into functionally significant categories. Eyes showing sharply di- or polyphasic ERG's dark adapt more rapidly, have much higher flicker fusion frequencies, and lower absolute thresholds than eyes with predominantly monophasic negative ERG's. Eyes of the first type belong to diurnal, swiftly flying species which include *Calliphora*, *Apis*, adult-*Aeschna*, *Vespa*, *Bombus*, and *Eristalis*. Eyes of the second type occur in nocturnal, more sedentary

forms which include *Dixippus*, *Tachycines*, and larval-*Aeschna* (Autrum 1950, 1952).

The *Calliphora* eye light adapts to a negligible degree; the ERG has the same amplitude a few seconds after exposure to intense light as in the fully dark-adapted state. Figs 6 and 23 of Autrum (1950), the curves of which may not have been obtained from the same animal, were used to determine the range of sensitivity change in *Dixippus*. Fig. 6 describes the height of on-effect as a function of time in the dark following a 1 minute exposure to a circular extended source of 10 mm. diameter placed 185 mm. from the eye. The brightness of this source was about 41,800 candles/foot.^{2} and should have produced somewhat higher illuminance at 185 mm. than the highest adapting intensity used with *Ligia*. After 1 minute of dark adaptation log 1/sens. is about 1.6 for *Dixippus*, as compared with 0.47 for a *Ligia* eye exposed five times as long. *Dixippus* therefore undergoes a greater sensitivity change than *Calliphora* or *Ligia*, not nearly so great as the human rods, but about the same as the human cones.

Flicker fusion frequency determinations have been made for a number of insect compound eyes using the ERG technique. Values for *Ligia* may be compared directly with these. All the following values were determined with equal light and dark fractions per flicker cycle for eye areas containing many ommatidia. The *Ligia* figure was determined at 22°C.; temperatures are not stated for the insect data. All light intensities except one are expressed in foot-candles. The meaning of the intensities as given is somewhat limited because different light sources were used by the different authors, and the spectral sensitivities of the species concerned are imperfectly, or not at all, known. No intensity values accompanied Autrum's f.f.f. figures for *Aeschna*, but since he compares these directly with f.f.f. = 265/second for *Calliphora* it is assumed that the same stimulus conditions applied for both species. The value, 14.8 watts/cm.^{2} (white light) for *Apis*, was not converted into foot-candles; however, the average f.f.f. for two bees from a local hive, determined with an illuminance of 15,000 foot-candles, is listed. Wherever one of the following values is subject to some uncertainty it is enclosed in parentheses.

Species	F.F.F.	Light intensity	Authors
Apis mellifera	300/sec.	14.8 watts/cm.^{2}	Autrum and Stoecker, 1950
" "	250/sec.	15,000 foot-candles	Present study
Calliphora erythrocephala	265/sec.	9,300 "	Autrum, 1950
Aeschna cyanea (adult)	170/sec.	(9,300 ")	Autrum and Gallwitz, 1951
Ligia occidentalis	110/sec.	15,000 "	Fig. 11
Dytiscus fasciventris			
day phase	72/sec.	10,000 "	Jahn and Wulff, 1941
night phase	58/sec.	10,000 "	" " " "
Aeschna cyanea (larva)	40/sec.	(9,300 ")	Autrum and Gallwitz, 1951
Dixippus morosus	40/sec.	9,300 "	Autrum, 1950
Tachycines asynamorus	(40/sec.)	(9,300 ")	" "

Under comparable experimental conditions the f.f.f. value for *Ligia* is intermediate between those for the slow insect eyes and those for the fast. The figures suggest that with examination of additional species a series of gradually integrading values may appear.

Referring to the series' extremes Autrum (1950, 1952) points out that f.f.f. (as well as ERG wave form and dark-adaptation rates) correlate well with ecological situation. High f.f.f. serves as an index of high "temporal resolving power"; *i.e.*, the ability to resolve rapidly consecutive visual stimuli as discrete sensory impressions rather than as a blur. Significantly, high f.f.f. is characteristic of diurnal, rapid flying insects which orient to their surroundings on the wing. Low f.f.f. values hence low "temporal resolving power," are found among nocturnal, more sedentary insects; the eyes of these forms, however, have higher absolute sensitivity than those of the diurnal fliers. *Ligia*, with an intermediate f.f.f., occupies a more or less intermediate ecological situation in the sense that it is frequently exposed on sunlit rocks and sand, but does not fly. In response to movements of nearby persons or objects, *Ligia* rapidly takes cover. It is likely that high f.f.f. in *Ligia* is a sign of high sensitivity to movement, and that this is of advantage to the animal in avoiding predation by, for example, shore birds.

Autrum (1952) presents evidence for his interesting hypothesis that a potential originating in certain "Lokalzellen" of the lamina ganglionaris of fast insect eyes not only opposes the receptor potential to produce the characteristic fast eye ERG wave form, but also inhibits photochemical bleaching. This inhibition is held to account for the extraordinarily rapid dark-adaptation and high f.f.f. of the fast eye; since an optic ganglion potential is not elicited in slow eyes or deganglionated fast eyes, photochemical bleaching is not opposed, with the result that dark-adaptation rates and f.f.f. values are low. While an electric potential arising within the eye may influence the course of photopigment breakdown, there is reason (see above) to doubt that the lamina ganglionaris is the source of such a potential. In view of the diversity of retinula cell types in compound eyes and the varied receptor performance of which compound eyes are capable, including wave length discrimination (Autrum and Stumpf, 1953; Fingerman and Brown, 1953; von Buddenbrock, 1952) and a "Purkinje shift" (Fingerman and Brown, 1952), there is reason to believe that the physiological basis on which differences between fast and slow eyes rest lies at the photoreceptor cell level.

C. Facilitation

A 5 msec. flash of high intensity delivered to a dark-adapted *Ligia* eye can induce a state of facilitation in the mechanism responsible for the ERG which persists for more than 2 minutes. Between conditioning and test stimuli no electric sign has been observed which indicates the existence of the facili-

tated state. Preliminary experiments indicate that interruption of the circulation interferes with both facilitation and dark adaptation; both phenomena are probably sensitive to reduction of retinal oxygen supply. Clarification of the facilitation mechanism awaits future experiments.

Higher responsiveness following prior illumination has been reported in other eyes but in no case is the mechanism clear. Hartline, Wagner, and MacNichol (1952) mention enhanced excitability of ommatidial nerve fiber discharge following brief subliminal light stimuli. The human eye (Motokawa, 1949) when presented with a brief conditioning light stimulus shows enhanced excitability, not to light, but to an electric current passed through the eye; the enhancement may persist for 6 seconds. The excised frog eye shows the effect in optic nerve activity for as long as 5 minutes. Granit (1947) discusses "differentiation velocity" of the vertebrate ERG; he describes increased rates of rise and fall of ERG components caused by persisting effects of prior illumination. Armington (1952) reports that the amplitude of the X-wave of the human ERG (associated with red color vision) may be greater within the first few minutes after light adaptation than in the fully dark-adapted state. This situation in the vertebrate may bear fundamental similarity to facilitation in *Ligia*.

SUMMARY

The ERG of the compound eye in freshly collected *Ligia occidentalis*, in response to high intensity light flashes of $\frac{1}{8}$ second or longer duration, begins with a negative on-effect quickly followed by an early positive deflection, rapidly returns to the baseline during illumination, and ends with a positive off-effect. As the stimulus intensity is decreased the early positivity progressively decreases and the rapid return to the baseline is replaced by a slowing decline of the negative on-effect. Responses were recorded with one active electrode subcorneally situated in the illuminated eye, the reference electrode in the dark eye.

The dark-adapted eye shows a facilitation of the amplitude and rates of rise and fall of the on-effect to a brief, high intensity light stimulus. This facilitation may persist for more than 2 minutes.

Following light adaptation under conditions in which the human eye loses sensitivity by a factor of almost 40,000 the *Ligia* eye loses sensitivity by a factor of only 3.

The flicker fusion frequency of the ERG may be as high as 120/second with a corneal illumination of 15,000 foot-candles.

Bleeding an otherwise intact animal very rapidly results in a decline of amplitude, change of wave form, and loss of facilitation in the ERG.

When the eye is deganglionated without bleeding the animal the isolated retina responds in the same manner as the intact eye.

Histological examination of the *Ligia* receptor layer showed that each ommatidium contains three different retinula cell types, each of which may be responsible for a different aspect of the ERG.

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