THE LANDING RESPONSES OF INSECTS

II. THE ELECTRICAL RESPONSE OF THE COMPOUND EYE OF THE FLY, *LUCILIA SERICATA*, UPON STIMULATION BY MOVING OBJECTS AND SLOW CHANGES OF LIGHT INTENSITY

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An investigation of the landing response of the fly, *Lucilia sericata* (Goodman, 1960) has shown that the legs are lowered from their retracted flight position in response to a visual stimulus. Experiments showed that the moment at which the legs were lowered did not depend upon an estimate by the fly of its distance from the landing surface but that its approach to a surface appeared to be measured in terms of the product of: the number of ommatidia stimulated, the logarithm of the relative decrease of intensity by which they were stimulated and the rate at which they were stimulated. It seemed that when the sensory product of these three factors rose to a certain level the legs were lowered. As far as the response it gave was concerned the fly did not discriminate between a large landing surface moving slowly towards it and a smaller one moving faster, between a large pale surface and a smaller darker one or even between a moving object and a decrease in light intensity provided that the product: number of ommatidia stimulated × decrease of intensity × rate of decrease was the same in each case. In fact the fly behaved as though its perception of movement was based upon the multiplication together of the response of successively stimulated ommatidia.

Hassenstein (1951) and Hassenstein & Reichardt (1956) have shown that perception of movement by the beetle, *Chlorophanus*, is based upon the evaluation of the contrasts presented to successive ommatidia by a system of multiplication at some level in the central nervous system. They suppose that the physiological interaction takes place between the after-effect resulting from stimulation of one visual unit and the response of the subsequently stimulated unit. The rate of stimulation is important in that the sooner the second unit is stimulated the less will the after-effect of the first have faded.

Recordings have been made from the retinular layer of the compound eye of *Lucilia* during stimulation by a moving object and by slow changes of light intensity to determine whether there is any physiological evidence that the fly is perceiving the approach of the landing surface in this manner. Since much of the work on the electrical responses of the insect eye has involved recording from a dark-adapted eye stimulated by comparatively brief flashes of white or monochromatic light (Crescitelli & Jahn, 1939; Bernhard, 1942; Jahn & Wulff, 1942; Autrum, 1950; Ruck, 1958; and many others), it was hoped that such recordings would also yield some information on the response of a light-adapted eye to small, slow changes of intensity.

MATERIALS AND METHODS

The wings and legs were removed from each specimen of *L. sericata* and the ventral surface was attached by wax to a horizontal supporting rod (Fig. 1). The head was secured to the thorax by a narrow bridge of wax. Silver wire electrodes were used of diameter 0·15 mm. sealed into glass holders with 0·5 cm. of wire exposed at the tip. The tip of the recording electrode was sharpened by electrolysis. The glass electrode holders were in turn held by two micromanipulators. A very fine slit was made in the intact eye and the recording electrode was inserted approximately 0·2 mm., at which depth it should lie within the retinular cell layer of the eye. The neutral electrode was inserted into a slit in the thorax. The electrodes were connected to a Nagard d.c. amplifier and the recordings were displayed on a cathode-ray oscilloscope. The preparation was screened in a small metal box having Perspex walls.

Stimulation by an approaching object

During flight *L. sericata*, as do many other insects, folds the first two pairs of legs up under the body and, as it approaches a landing surface, lowers and extends them forward to grasp the surface. This landing response was studied by projecting a suitable surface towards the fly through a measurable distance at a measurable uniform speed. The same apparatus, which has been fully described in a previous paper (Goodman, 1960), was used in the following experiments. The diameter of the disk used as a landing surface, its distance from the fly, the speed at which it travelled and the distance through which it travelled could all be varied independently. In addition, by the appropriate use of shaded disks the contrast between the approaching disk and its background could be varied. The second beam of the oscilloscope was connected via a 4 V. battery to a circuit which was broken when the disk began to move and closed when it ceased to move. A record of the stimulus was obtained in this way. The fly on its supporting rod with electrodes inserted was clamped with the centre of the head in line with the centre of the approaching disk (Figs. 1 A, B and 2 A).

Stimulation by a decrease of intensity

The fly was mounted on its support inside a matt black metal box with the centre of its head in line with the centre of an opal glass screen (see Fig. 2 B). The d.c. illumination of this screen could be increased or decreased at a uniform rate over a wide range of intensities. The angle subtended at the fly's eye by the screen could be varied between 10° and 200°. This apparatus has also been fully described in the preceding paper. A record of the stimulus could be obtained on the second beam of the oscilloscope by a device similar to that described above.

Stimulation by brief flashes of light

In a few cases the flies were stimulated with a brief flash of white light for comparative purposes. They were placed 5 cm. from an opal glass screen of diameter 4 cm. in a black metal box. The light source was a 24 V., 250 W. bulb run from two 12 V. car batteries and the illumination of the screen was of the order of 2 log. ft. lamb. The duration of the flash was controlled by placing a camera shutter between the screen and the light source.
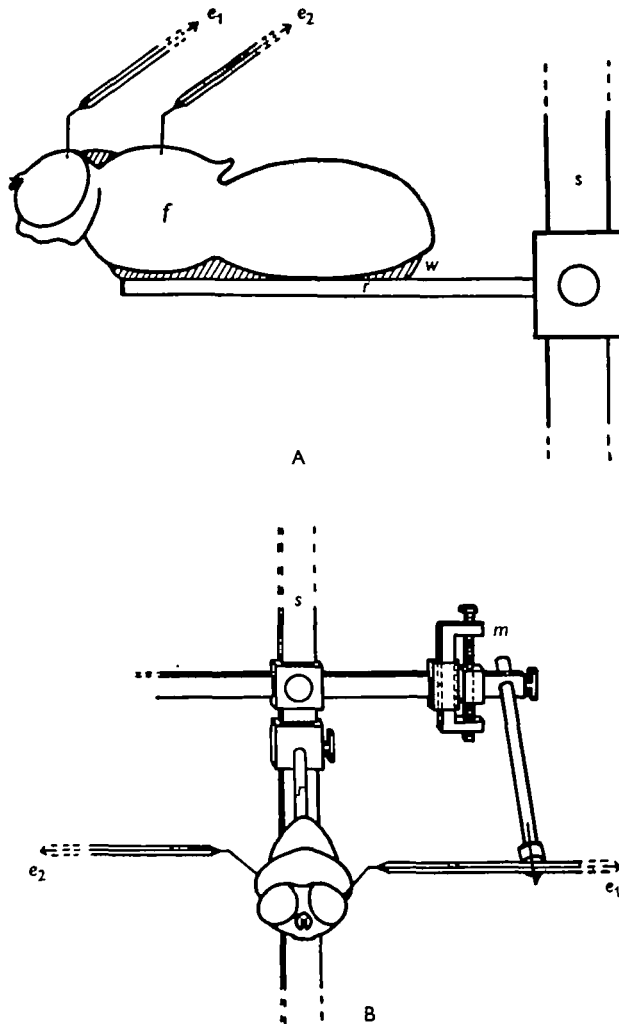
Fig. 1. (A) A lateral view of the preparation: e_1 and e_2, recording and neutral electrodes inserted in the eye and the thorax respectively and leading off to a d.c. amplifier; f, fly with legs and wings removed; s, adjustable support; w, wax attaching fly to rod, r, and head to thorax. Electrode holders omitted. (B) The fly viewed from in front and above showing one electrode holder, m.

RESULTS

The electrical response to stimulation by brief flashes of white light

Dark-adapted flies were stimulated by flashes of white light of 0·2, 0·5 and 1·0 sec. duration. The illumination potentials recorded resemble those described by Autrum (1950) for *Calliphora* (see Fig. 3 A). Upon exposure to light a positive potential of brief duration, the on-wave, was formed. When the illumination ceased a steep negative potential formed which decayed rapidly, occasionally overshooting into a smaller positive potential before returning to the base-line; this was the off-wave. The plateau between the on- and the off-waves was slightly negative relative to the resting potential of the dark-adapted eye.
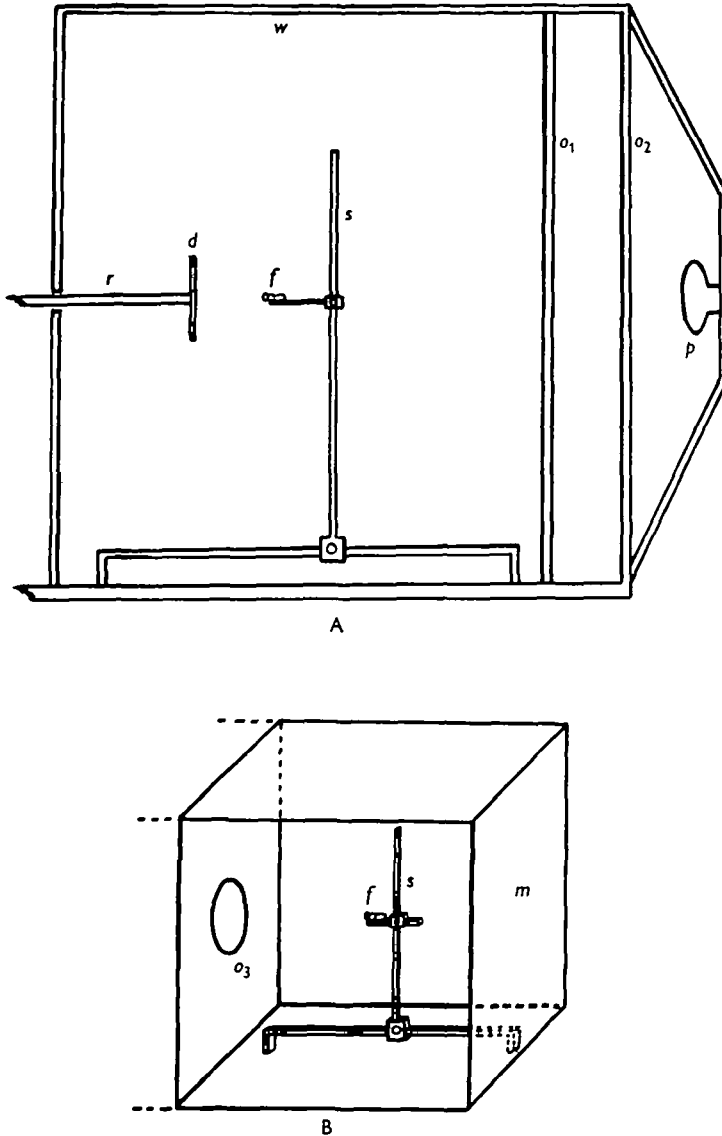
Fig. 2. (A) Section through the white-walled box, w, in which the fly, f, is mounted, s, with the centre of its head in line with the centre of the disk, d. The disk was screwed to a rod r and could be projected towards the fly through a known distance at a given uniform speed. The box was illuminated by the d.c. source p placed behind two opal glass screens, o_1 and o_2. For further details of the apparatus see Goodman (1960). The electrodes and their holders, together with the screening box, are omitted. (B) The black metal box, m (with one side removed), in which the fly, f, was mounted facing an opal glass screen, o_3. Details of the method by which the illumination of the opal glass screen was changed are given in Goodman (1960).

Stimulation by an approaching object

(a) A dark object against a light background

The light-adapted fly was stimulated by a black disk, 9 cm. in diameter, travelling 30 cm. towards it at a speed of 35 cm./sec. The disk approached against a matt white background illuminated at 1·5 log. ft. lamb. If the resting potential of the eye is

regarded as the base-line, the corneal region of the eye became negative with respect to the neutral electrode as the disk began to move forward. The negative potential continued to develop as long as the disk was moving. When movement ceased it decayed rapidly. The base-line with the disk in the new position, 0·5 cm. from the eye, was very slightly positive in relation to the old base-line (see Fig. 3 C). This negative
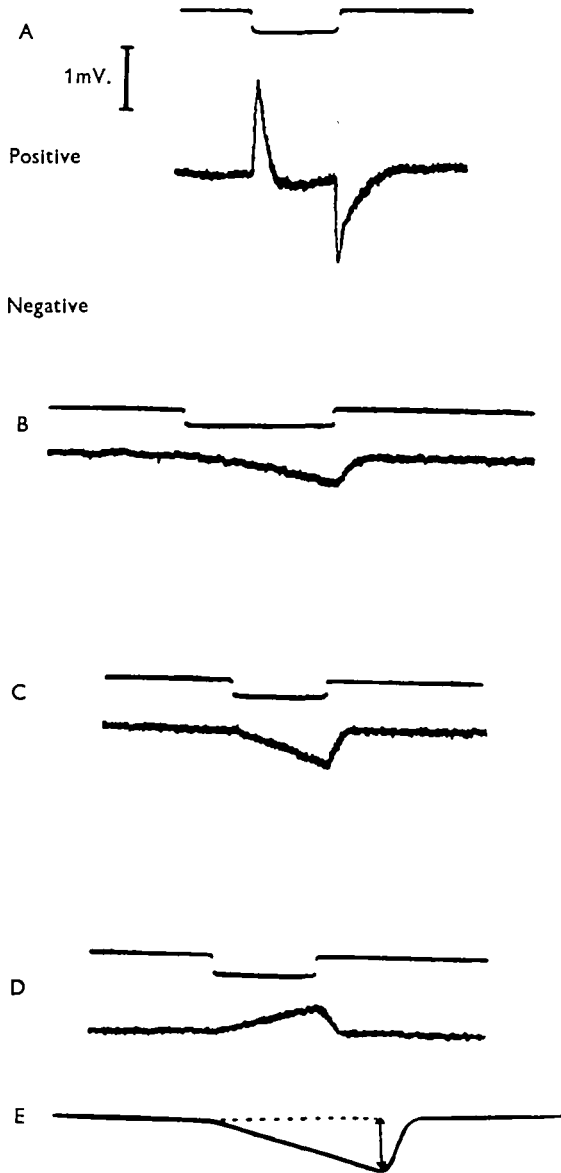
A

1mV.

Positive

Negative

B

C

D

E

Fig. 3. (A) The electroretinogram of the dark-adapted eye of *Lucilia* on stimulation with a flash of white light of 0·5 sec. duration. (B) The negative potential developed as a black disk 9 cm. in diameter approaches at a speed of 20 cm./sec. against a white background. Movement of the disk is shown on the upper trace. (C) The same with a speed of approach of 35 cm./sec. (D) The positive potential developed as a white disk of diameter 9 cm. approaches at a speed of 32 cm./sec. against a black background. (E) The point at which measurement of the size of the potential recorded was made. This was the moment just before the disk stopped moving.

potential closely resembles the off-wave described above, the only apparent difference being its slower development accompanying the much slower decrease of light intensity.

(b) A light object against a dark background

A white disk was projected towards the fly against a matt black background under conditions similar to those described above. A positive potential developed as soon as the disk began to move (see Fig. 3D). When the disk stopped moving there was a return to the base-line. The new base-line was in fact very slightly negative with respect to the old one.

The relationship of the size of the negative potential developed to the number of ommatidia stimulated by a dark approaching object, its rate of approach and the change of intensity which it produced

(a) The number of ommatidia stimulated

The means of estimating the number of ommatidia stimulated by a change of light intensity when the disk approached has been discussed in an earlier paper (Goodman, 1960). It was concluded that under certain conditions, observed throughout these experiments, the number of ommatidia stimulated by an approaching disk is directly proportional to the increase in the angle subtended at the eye by the disk. It was convenient therefore to use the increase in angle (hereafter known as θ) as a measure of the number of ommatidia stimulated by the movement of a disk.

The fly was stimulated by a black disk, 9 cm. in diameter, travelling towards it at a speed of 40 cm./sec. against a white background. The distance travelled by the disk was varied so that the increase in the angle subtended at the eye could be varied between 20° and 60°. The contrast presented to successive ommatidia and the rate of stimulation were kept constant. The fly was stimulated 10 times by the disk at each value of θ with a 5 min. interval between each test. The negative potential developed at the moment at which the disk stopped moving was measured in each case (see Fig. 3E) and its relationship to θ examined (see Fig. 4). The negative potential recorded was directly proportional to the increase in the angle subtended at the eye by the disk and hence to the number of ommatidia stimulated. Eleven other flies were stimulated in a similar manner but with differences in the range of values of θ. A different rate of stimulation was used in some cases. In each case the potential developed was directly proportional to θ.

(b) The rate at which the disk approached

Five flies were in turn stimulated with a black disk, 9 cm. in diameter, approaching against a white background illuminated at 1·5 log. ft. lamb., and giving a brightness contrast of 0·9. The distance travelled by the disk was such that θ was 60°. The speed of approach was varied at intervals of 5 cm./sec., ten tests being given at each speed. The negative potential recorded was directly proportional to the rate of decrease of intensity (hereafter known as V) induced by the disk as it approached up to a relative decrease of 1·8/sec. (see Fig. 5).
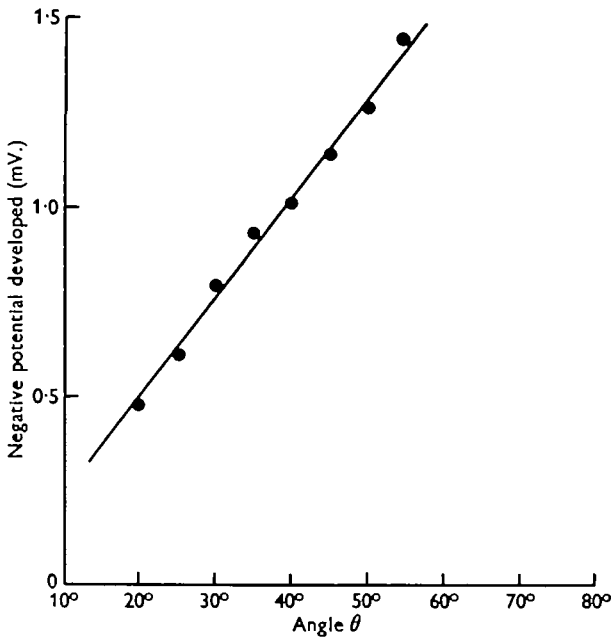
Fig. 4. The relationship between the size of the negative potential recorded and the increase in the angle subtended at the eye by the disk as it approached (angle *θ*).
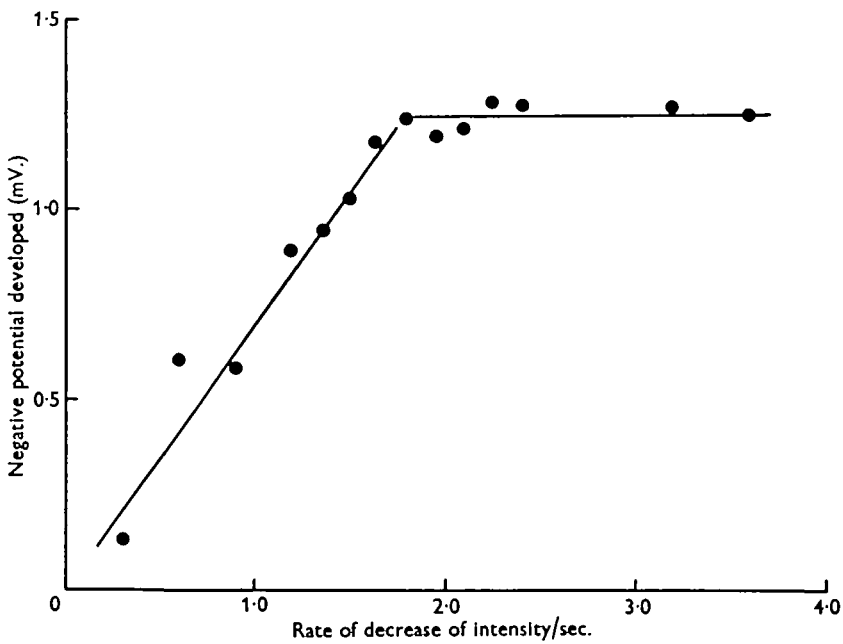
Fig. 5. The relationship between the negative potential recorded and the rate of the decrease of intensity produced by the approaching disk.

(c) The contrast between the stimulating object and the background

Contrast in these experiments was confined to differences in physical brightness only, grey disks of a wide range of shades being projected against a white background. The contrast between the disk and its background was measured in terms of the relative brightness difference between the disk and its background $(I_1 - I_2) I_1$, where I_1 is the brightness of the background and I_2 is the brightness of the disk measured directly in log. ft. lamb. (Weston, 1945). θ was kept constant at 80° and the disks approached the flies at 25 cm./sec. Nine flies were each stimulated 10 times with grey disks of eight different shades. Fig. 6 shows that the negative potential recorded was directly proportional to the relative brightness contrast (hereafter known as C) between the approaching object and its background.
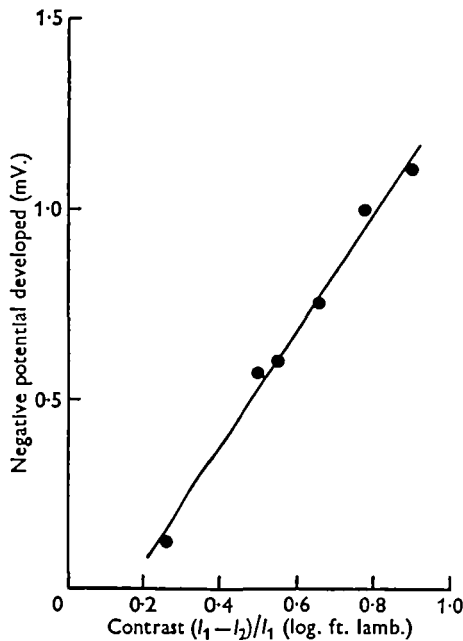

Fig. 6. The relationship between the negative potential recorded and the relative decrease of intensity produced by the approaching disk.

The relationship between the size of the potential developed and the preceding three variables

The experiments described above show that the negative potential developed was directly proportional to each of the three variables over the experimental ranges used, ranges within which all degrees of response could be elicited from the intact insect from an occasional slight lowering of the tarsi to 100 % full response. The behaviour experiments had shown that the percentage of landing responses given by the flies was directly proportional to the product of these three variables. A constant value of θVC produced a constant response irrespective of how the three component factors were varied. The effect on the electroretinogram of varying the values of θ, V and C, whilst keeping their product constant, was therefore determined. Table 1 shows the relationship between a constant product of θVC achieved in various ways, and the

size of the negative potential developed. The size of the negative potential developed remains fairly constant in spite of the variation in stimulus parameters.

Table 1. *The potential change (mV.) developed on stimulation with each of the combinations of θ, V and C used to produce a constant value of θVC*

Fly	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
I	0·30	0·32	0·31	0·28	0·32	0·30	0·25	0·29	0·31	0·28
II	0·41	0·44	0·41	0·39	0·41	0·41	0·39	0·38	0·39	0·39
III	0·38	0·35	0·40	0·38	0·39	0·35	0·37	0·38	0·35	0·40
IV	0·59	0·60	0·59	0·58	0·66	0·58	0·61	0·62	0·59	0·62

The relationship between the electroretinogram and values of θVC well above those which normally produce 100% landing response

Some attempt was made to determine the effect of greatly increasing the value of θVC as a stimulus by increasing the value of V, the speed of approach of the disk being the variable whose range could most conveniently be extended. As can be seen from Fig. 5, at very high speeds of approach the potential formed became independent of the rate of decrease of intensity. It then remained constant up to the highest value of V which could conveniently be obtained. It appears that the negative potential developed varies directly as the rate of decrease of intensity over the range within which the percentage of landing responses is directly proportional to the rate of decrease. At very high values of θVC, values above which 100% landing response was always given, the negative potential developed became independent of the increase in the value of θVC.

Stimulation by a decrease of light intensity

Variation of the number of ommatidia stimulated and the rate and degree of their stimulation could be achieved without using a moving object by mounting the preparation in front of an opal glass screen and decreasing the light intensity of the screen. Under these circumstances, however, the ommatidia were stimulated simultaneously rather than successively. The change in light intensity was measured in terms of the relative brightness decrease $(I_3 - I_4)/I_3$, where I_3 was the original intensity and I_4 the new intensity measured directly in log. ft. lamb.

When the eye was stimulated in this way the corneal region became negative with respect to the neutral electrode and this negative potential continued to develop as long as the light intensity was changing. When the decrease ceased it decayed rapidly, sometimes swinging over into a small positive potential. In fact the form of the potential change was exactly similar to that produced by a dark object approaching against a light background.

(a) The number of ommatidia stimulated

The number of ommatidia stimulated when the intensity of the screen is decreased can be regarded as directly proportional to the angle subtended at the eye by the screen, hereafter known as angle θ_2, provided that the value of the angle is between 20° and 100° (Goodman, 1960). Nine flies were stimulated by a relative decrease of intensity of 0·69, θ_2 being varied between 20° and 100° at intervals of 10°. Fig. 7 shows that the negative potential recorded was directly proportional to θ_2 and hence to the number of ommatidia stimulated.

(b) The rate of decrease of intensity

Seven flies were in turn stimulated by a relative decrease of intensity of 0·69 at various rates of decrease, θ_2 being kept constant at 20°. The negative potential recorded was in the case of each fly tested directly proportional to the rate of the decrease of intensity (see Fig. 8).
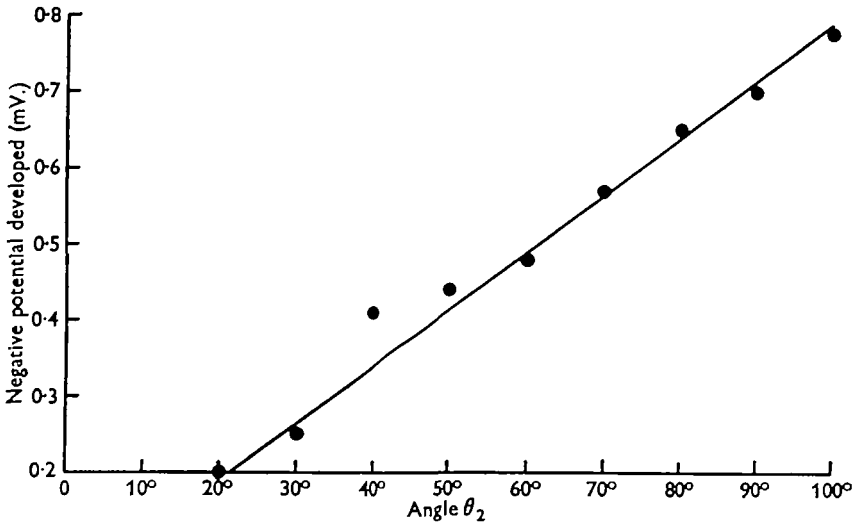

Fig. 7. The relationship between the negative potential recorded and the angle (θ_2) subtended at the eye by the opal glass screen.

(c) The relative decrease of intensity $(I_3 - I_4)/I_3$

I_3 was maintained at 1·5 log. ft. lamb. and I_4 was such that relative decreases of 0·43, 0·54, 0·69, 0·75, 0·83, 0·90, 0·95 and 0·98 were obtained. θ_2 was set at 20° and the rate of the decrease was kept constant. Eight preparations were stimulated in turn by these values of $(I_3 - I_4)/I_3$. Fig. 9 shows that the size of the negative potential recorded was directly proportional to the relative decrease of intensity with which the fly was stimulated.

DISCUSSION

It might be supposed that differences in the temporal pattern of the response of the visual units concerned would enable the fly to discriminate at some level in the central nervous system between disks moving at different speeds or between successive stimulation of units as a disk approaches and their simultaneous stimulation when the light intensity of the surroundings is decreased. Extracellular recording in the retinular layer of course gives no information on this point. Whether this type of discrimination is possible for the fly or not, the behaviour experiments show that it is not utilized in the landing reaction but that the insect behaves as if the response of individual visual units is multiplied together until, at a certain level of sensory input, the landing reaction is in some way initiated. The development of a negative potential in the retinular layer as an object approaches whose size is directly related to the product of the number of ommatidia stimulated and the amount and rate of the decrease by which
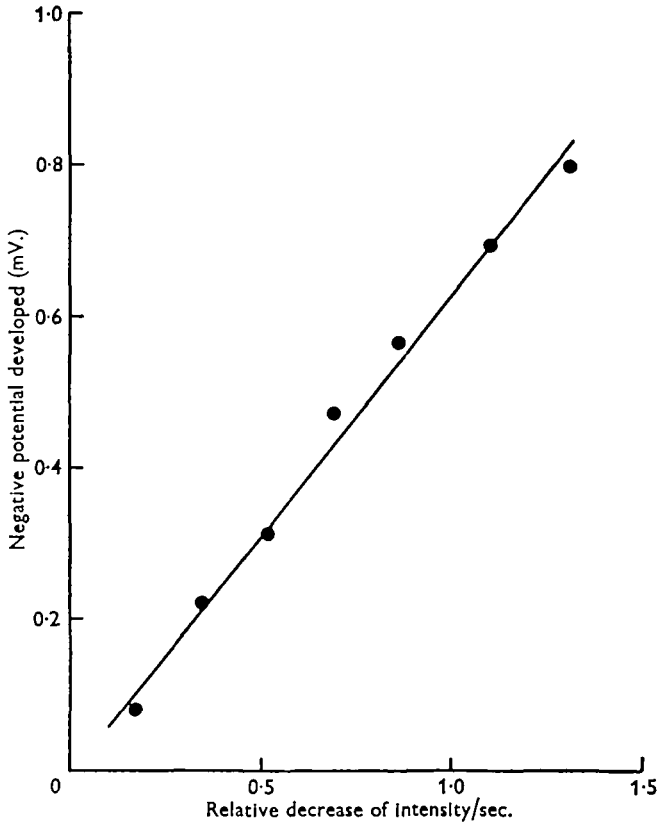
Fig. 8. The relationship between the negative potential recorded and the rate of the decrease of intensity of the opal glass screen.
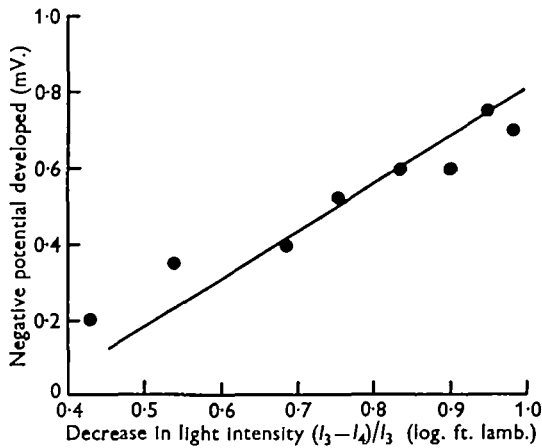

Fig. 9. The relationship between the negative potential recorded and the relative decrease of intensity across the opal glass screen.

they are stimulated, over the range of stimuli which elicit the landing reaction, lends some support to this view. So, too, does the fact that the size of the potential change recorded remains constant provided the eye is stimulated by a constant value of θVC,

no matter how the relative values of the three components are varied. The same relationship exists between θVC and the potential developed when the ommatidia are stimulated simultaneously by a decrease of intensity, suggesting that, under these conditions, the responses of individual units are being continuously multiplied together at some level. Initiation of the landing reaction appears to depend solely on the attainment of a certain value of θVC as the sensory input and to be independent of the manner in which this arises.

It is generally accepted that the diphasic electroretinogram is a complex one, being the sum of two or more component potentials, but the form and nature of the components and the way in which the off-effect arises are not established. The potential change recorded here closely resembles the off-effect recorded by Autrum (1950) and Hassenstein (1957) from this type of eye, but the origin of this particular potential and its relationship, if any, to the motor output cannot be established from these recordings. All that can be said at this stage is that, whatever other effects this type of stimulus produces in the eye, a potential change occurs whose size is related to the stimulus in such a way as to suggest that multiplication of the responses of individual visual units has taken place and which, if it in some way controls the motor output of the insect, could form the basis of this landing reaction.

SUMMARY

1. The electroretinogram of *Lucilia sericata* has been recorded whilst the fly was stimulated by an approaching object and by a slow change of light intensity.

2. The approach of a dark object against a light background was accompanied by the gradual development of a negative potential which decayed rapidly when movement ceased.

3. The size of the potential change was found to be directly proportional to (a) the number of ommatidia stimulated by the approaching object, (b) the rate of the decrease and (c) the amount of the decrease of intensity which it produced.

4. A similar response could be produced by stimulating the fly with a decrease of intensity, the negative potential developed being directly proportional to the number of ommatidia stimulated, to the rate and to the amount of the decrease.

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