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SEPARATION OF RECEPTOR AND LAMINA POTENTIALS IN THE ELECTRORETINOGRAM OF NORMAL AND MUTANT *DROSOPHILA*

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INTRODUCTION

A variety of blind or partially blind *Drosophila* mutants have recently been found (Benzer, 1967; Pak, Grossfield & White, 1969; Hotta and Benzer, 1969; Cosens & Manning, 1969; Pak, Grossfield & Arnold, 1970). Some of them seem to have defects in the photoreceptor cells (Cosens & Manning, 1969; Pak *et al.* 1970) others have been interpreted as lacking some important neural events following light reception (Pak *et al.* 1969; Hotta & Benzer, 1969). These findings were the results of electroretinogram (ERG) measurements.

Up to the present time the ERG of *Drosophila* has been described as consisting of five components. These are (1) a fast and (2) a slow cornea-positive on-effect (Hengstenberg & Götz, 1967), two cornea-negative sustained potentials (Pak *et al.* 1969) one (3) with short the other (4) with long rising and decay times and (5) a cornea-negative off-effect. In addition a second, delayed cornea-negative off-effect (6) should probably be added (see: Results 2*b*).

Although dividing up the ERG into components is a necessary prerequisite for its analysis, it finally would be desirable to know (*a*) the cell types involved in the ERG; (*b*) their individual contributions to the ERG; (*c*) the extracellular conditions for (*b*) and (*d*) the functional significance of the ERG in vision.

The only cell type so far identified is the retinula cell which produces the fast negative sustained potential (3) (receptor potential) (Pak *et al.* 1969; Bernhard, 1942; Autrum & Gallwitz, 1951; Wolbarsht, Wagner & Bodenstein, 1965). The phasic components (1) and (5) may in analogy to other dipterans be attributed to the activity of the *lamina ganglionaris* (lamina potential) (Autrum & Gallwitz, 1951). Since the lamina consists of ten or more different cell types (Trujillo-Cenóz, 1965; Boschek, 1970; Braitenberg & Strausfeld, 1971) a simple explanation for the lamina potential cannot be expected.

In *Drosophila* the ERG mutants may provide a new approach to this analysis. One can compare their ERGs to that of wild type and correlate the abnormalities in the ERGs with the behavioural and morphological defects of the mutants. However, for this comparative study to be useful more has to be known about the ERG itself.

Thus this communication will deal primarily with the wild-type ERG. A simple model for the ERG will be proposed which includes the extracellular conditions for its appearance. In addition, it will be shown that the lamina potential can be isolated

experimentally, and some of its functional properties will be described. Finally the ERGs of three mutants which seem to have lamina defects will be compared to that of wild type.

MATERIAL AND METHODS

The wild-type strain *Drosophila melanogaster* 'Berlin' and the eye-colour mutant *white* were used for the ERG analysis. The mutant strains *tan*[1] and *ebony*[11] were kindly provided by Dr S. Benzer, California Institute of Technology, Pasadena, U.S.A. The behavioural mutant opm 2 was selected for the absence of an optomotor response and has a single mutation around position 56 (± 5) on the X-chromosome (M. Heisenberg & K. G. Götz, unpublished). The double mutants *white*-opm 2, *white-tan* and *white-ebony* were obtained from crosses between the above mutants.

For ERG measurements flies were lightly anaesthetized with ether and glued via the thorax to the tip of a steel needle using a 1 : 2 mixture of resin and beeswax. Legs, wings, proboscis and the head were immobilized by small droplets of the same mixture, leaving the respiratory movements of the abdomen unimpaired. The cornea was punctured at the desired position with an electrolytically sharpened tungsten needle vibrating in axial direction at 400 cps. Two or three micropipettes filled with 'Drosophila-Ringer' solution (0·13 M-NaCl, 4·7 mM-KCl and 1·9 mM-$CaCl_2$) were used (see Fig. 3). Electrode A was inserted dorsally into the thoracic-abdominal junction and had a tip diameter of 50 μm; electrode B with a tip diameter of 10–15 μm was placed just below the cornea; both contained 0·2 % agar in addition to the Ringer solution. Electrode C had a tip diameter of 3–5 μm and was inserted in the centre of the eye parallel to the long axis of the ommatidia at this position. Signals from the electrodes B and C were amplified with a high input-impedance DC amplifier and observed on an oscilloscope. In the text the probing electrode is always marked (+) the indifferent electrode (−). Positive-going potential changes with respect to the (+) electrode are shown as upward deflexions in the graphs.

White light from a quartz-iodine light bulb in a Zeiss microscope lamp and a mechanical shutter with an opening and closing time of about 1 ms were used for stimulation. If not stated otherwise flash duration was 0·65 s. Intensity was adjusted by neutral density filters and will be indicated in the text as % of the standard intensity. The standard illumination measured at the position of the fly was E = 890 lx. This would be found in the centre of a half sphere with a luminance B = 280 cd/m^2. The light source subtended an angle of 50 min of arc.

For sinusoidal modulation the light was first depolarized then passed through two polarizing filters one rotating at the desired speed and finally was depolarized again.

Blue light was obtained using a Kodak gelatine filter No. 47B which has a broad transmission band around 430 mμ. The optical glass fibres used for stimulation of small numbers of receptor cells were kindly provided by A. Jacobsen, Jenaer Glaswerk Schott & Gen., Mainz, Germany. The fibres had a diameter of 20 μm and at a length of 500 mm had a transmission above 0·5 for wavelengths between 400 and 900 mμ.

RESULTS

(1) *Extracellular conditions for the ERG*

(*a*) *Lateral homogeneity*

The receptor potential of the ERG is supposed to be the sum of the potentials of the retinula cells in all parts of the eye. In *Drosophila*, however, there is no experimental basis to this assumption. It may well be that only the cells adjacent to the electrode contribute to the ERG which in turn implies large lateral potential gradients in the extracellular space between illuminated and non-illuminated areas of the eye. As one

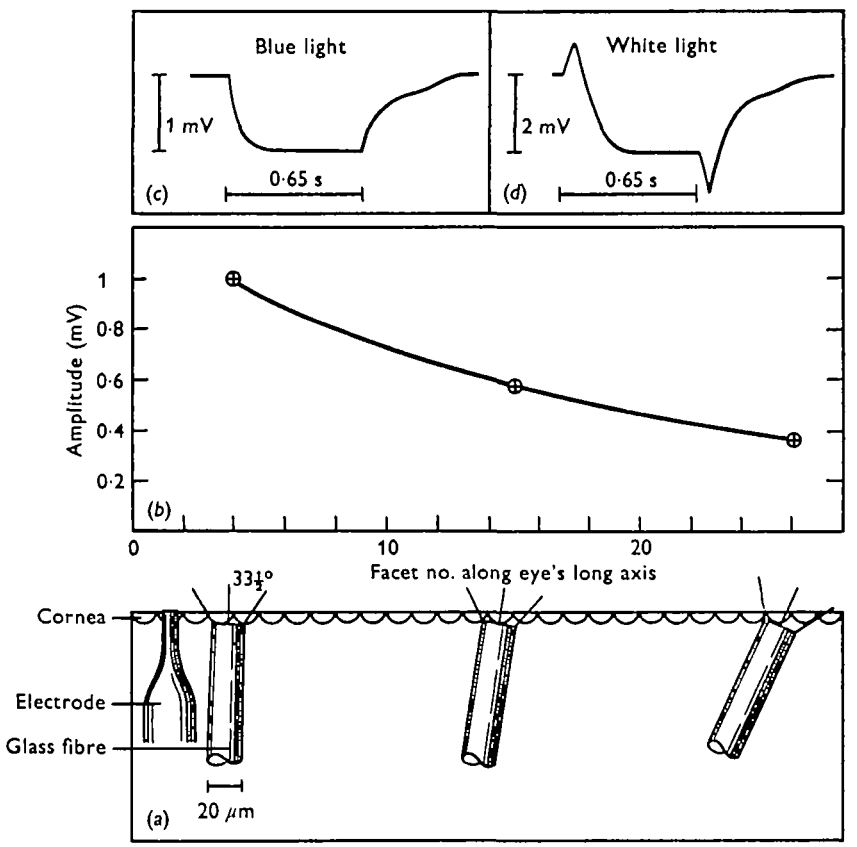

Fig. 1. ERG from small groups of receptor cells; (*a*) schematic drawing of the stimulus condition: the electrode is on the left; to the right along the eye's long axis the positions of the light-transmitting fibre are shown where measurements were taken; (*b*) amplitude of ERG (blue light) at the glass-fibre positions shown; (*c*) shape of ERG with blue light (about 3–4 facets stimulated); (*d*) shape of ERG with white light (20–30 facets stimulated).

of the alternatives the ERG might be a co-operative phenomenon, i.e. meaning that stimulation of single or of a few units elicits no ERG at all. Experiments using a point source of light that would ideally stimulate only 7 or 8 rhabdomers (Scholes, 1969; Trujillo-Cenóz & Melamed, 1966; Braitenberg, 1967; Kirschfeld, 1967) provide no simple answer to this problem since the contribution of the large number of indirectly illuminated receptor cells to the ERG is not known.

A semiquantitative experiment was therefore designed to record the ERG of small numbers of retinula cells at various distances from the (+) electrode. For this the red-eyed wild strain 'Berlin' was used. The (+) electrode was placed just below the cornea at one end of the eye's long axis and an optical glass fibre, 20 μm in diameter, was placed with its cut end on the cornea and was used for illumination (Fig. 1*a*). The intensity of white light at the far end of the fibre was about 100 times the standard intensity since all the light was focussed on to this spot. It could be observed under the microscope that about three to four facets directly under the glass fibre were maximally illuminated whereas 20 to 30 facets around the fibre were illuminated indirectly by scattered light passing through the screening pigment of the eye. This could be avoided using blue light (Kodak Filter No. 47b) for which absorption in the screening pigment is comparatively high. Under these conditions the ERG was a monophasic cornea-negative sustained potential (Fig. 1*c*). Only in a few cases very small phasic components could be observed. Since blue light caused a normal diphasic ERG if large areas of the eye were illuminated, the lack of the phasic components was attributed to the small number of receptor cells stimulated.

The amplitude of the response was 1 mV near the electrode and 0·35 mV at the far end of the eye (Fig. 2*b*). This might have been due to the fact that the angle at which the fibre touched the eye also decreased with increasing distance. Using white light the shape of the ERG was normal (Fig. 1*d*). But unlike the receptor potential the on- and off-effects did not decrease with increasing distance between glass fibre and electrode which again might be explained by the change in the angle between eye surface and the glass fibre.

This shows that indeed all parts of the eye contribute to the ERG although they may do so to various degrees. Thus no large lateral resistances divide the *Drosophila* eye extracellularly; the whole laterally repetitive structure of the retina can be roughly regarded as one compartment in so far as extracellular electrical activity is concerned.

(*b*) *Different layers in the eye*

In thin sections the eye appears as a series of layers parallel to the cornea: retinula cell layer, basement membrane, lamina, etc (Fig. 3). The question arises whether this structure has any significance for the ERG. Possibly some of these morphological discontinuities constitute resistance barriers for the extracellular current flow.

To test this the steady potential of the eye at various depths below the surface was measured in the dark and under illumination. In order to be able to stimulate the eye homogeneously at high intensity while inserting an electrode from the front the mutant *white* was used for this and for the following experiments. It has been demonstrated that the lack of screening pigment does not alter the main properties of the ERG (Hengstenberg & Götz, 1967).

The steady potential was found to be 30 to 80 mV positive throughout the eye (Fig. 2). This value was not constant during the experiment because of slow drift within the preparation and because of sudden changes arising due to the mechanical displacement of tissue during the advancement of the electrode. However, two results of this experiment were very obvious. (1) The large negative steady potentials located at synaptic regions, reported to be present in some other insects (Burtt & Catton, 1964), could not

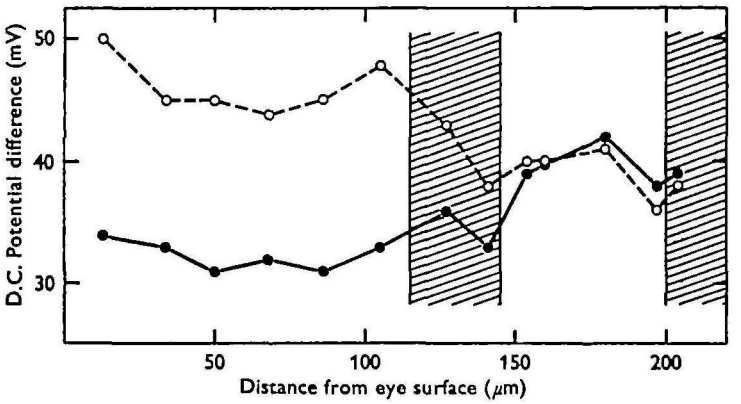

Fig. 2. Resting potential in the extracellular space of the eye and the lamina as a function of electrode depth. The path of the electrode is indicated in Fig. 3 as a scale in the centre of the eye; ●—●, under constant light (standard intensity); ○- -○, in the dark. Resting potential normally did not change after 1 min of adaptation. In the shaded zones the shape of the ERG changed drastically (see text).

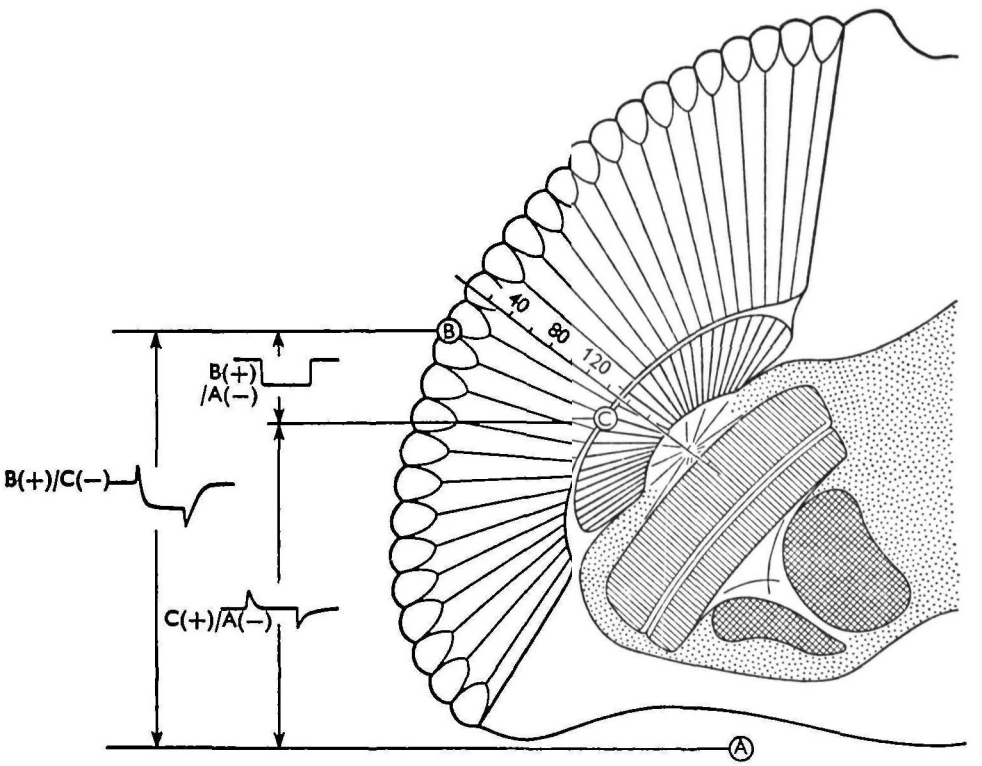

Fig. 3. Drawing after a photograph by R. Hengstenberg of a horizontal thin section through the left eye and optic lobes of *Drosophila*. The preparation was fixed in glutaraldehyde – OsO_4 and embedded in Araldite. The circles with the letters A–C in the drawing show positions of electrodes. Electrode A is in the thorax. On the left are shown schematic representations of the ERG between sites B and A and the monophasic receptor potential between B and C. The difference between these two should be measured with the electrodes at C and A. The oblique scale shows the distance from the cornea in microns.

be found in *Drosophila* at the depth of the lamina. (2) But in agreement with Burtt Catton's results the steady potential in the light-adapted state was always smaller than in the dark-adapted state by a certain value which was dependent upon the intensity. This difference was remarkably constant throughout the retinula cell layer but disappeared in the lamina region. Therefore, at about the depth of the basement membrane over a distance of 30 μm, a potential difference of more than 15 mV must exist, implying an extracellular resistance large compared to the resistances in the retinula cell layer. The only alternative to this conclusion would be a secondary EMF at this level of the eye supporting the activity of the receptor cells. This possibility seems unlikely and will not be discussed further in this paper. The corresponding result was obtained if the eye was penetrated from the other side through the back of the head. This extracellular barrier will be called the 'receptor barrier'. It will be shown in the next part of this account that in such penetration experiments the shape of the ERG changes drastically in the region of the receptor barrier.

(2) *The lamina potential*

(*a*) *Separation of receptor and lamina potential*

It has been described recently that the receptor potential of the ERG can be measured separately by placing one electrode (+) on the surface of the cornea and the other (−) 'near the basement membrane' (Pak *et al.* 1969). In fact if the penetrating electrode (−) is advanced in small steps one detects no receptor potential for the first 100 μm. Only after the electrode has passed the receptor barrier can the receptor potential be recorded.

The above finding has an important consequence. Since between the cornea and the thorax a normal diphasic ERG can be recorded, and between the cornea and a point just proximal to the receptor barrier a monophasic receptor potential can be detected, one should be able to record the difference between these two with the probing electrode at the receptor barrier and the indifferent electrode in the thorax (Fig. 3). To demonstrate this a 3-electrode-experiment was designed where one electrode (B) was placed on the cornea, the second (C) proximal to the receptor barrier and the third (A) in the thorax. The three responses are shown in Fig. 4. The bottom row shows the normal ERG, the upper row the receptor potential. The middle row is obviously the difference between the former two although the three curves represent responses to successive light flashes.

This experiment was continued in two ways. (*a*) If the electrode at the receptor barrier (C) was advanced another 40–60 μm the response between B (+) and C (−) changed to a diphasic ERG and the response between A (−) and C (+) either disappeared, accompanied by a drop of the steady potential to zero, or it changed abruptly to an inverse ERG of small amplitude probably due to penetration of the medulla. (*b*) If prior to the advancement of the C-electrode the B-electrode was also placed proximal to the receptor barrier the same response in magnitude and shape as shown in the middle column of Fig. 4, but with the opposite polarity, was observed between electrodes B (−) and C (+). This demonstrates that the response is produced close to the receptor barrier by a tissue which is distinct from the rest of the brain. Since this tissue is most probably the lamina the response will be called the 'lamina potential'.

The same result for the origin of the diphasic components of the ERG has been obtained previously for *Calliphora* by ablation experiments (Autrum & Gallwitz, 1951).

The lamina potential contains at least five components and seems to consist of the activity of several cell types (Figs. 4, 9). Two on- and two off-effects on top of a very slow negative sustained potential were clearly distinguished. The shape depended, however, to a certain degree upon the depth of the C (+) electrode. At about 140 μm beneath the retinal surface just proximal to the receptor barrier the lamina potential appeared as illustrated in Fig. 9*a*. If the electrode was advanced 30 μm the lamina potential changed (Fig. 9*b*). The slow negative sustained potential disappeared and a fast sustained positive potential could be observed to replace it. If the electrode was advanced another 20 μm into the eye the off-effects also disappeared.

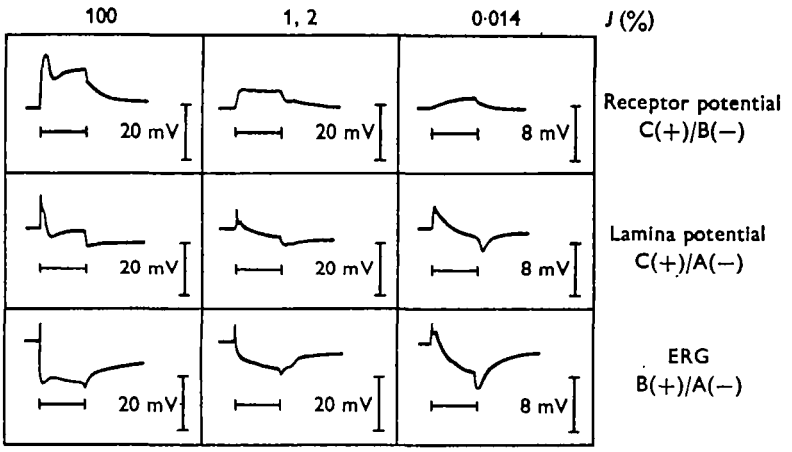

Fig. 4. Separation of ERG into receptor potential and lamina potential at various intensities; electrode positions are indicated in Fig. 3. The horizontal bar in each square shows time and duration of the light flash (0·65 s).

(*b*) *Properties*

Since the functional role of the lamina at high intensities is not known, the experiments were concentrated on the low-intensity range or on low flash contrast (small $\Delta I/I$) at high ambient light intensity (Fig. 5). For both conditions behavioural (Kirschfeld & Reichardt, 1970) and electrophysiological (Scholes, 1969) experiments as well as anatomical observations (Braitenberg, 1967; Kirschfeld, 1967) have demonstrated in *Musca* the functional involvement of the lamina. It turned out that the shape of the lamina potential at low intensities or at high intensity but low flash contrast was much simpler, consisting merely of a phasic positive on-effect and a phasic negative off-effect (Figs. 4, 5) resembling the negative first derivative in time of the receptor potential. In addition, it was observed that with decreasing intensity or contrast the receptor potential decreased faster than the lamina potential (Figs. 4, 5). Near the threshold of the ERG only the lamina potential was observed.

It has been shown earlier in *Calliphora* that the diphasic ERG can be elicited by light flashes of much higher frequency than the surgically or pharmacologically isolated receptor potential or the monophasic ERG of other insects (Hoffmann, 1959; Autrum & Hoffmann, 1957).

To see whether the lamina potential is responsible for this, the 3-electrode-experiment was repeated using as a stimulus sinusoidally modulated light of different frequencies. The average intensity was 0·085 % standard intensity, modulation was over 99 %. The electrodes were in the same positions as indicated in Fig. 3. The results (Fig. 6*a*) show that the amplitude of the receptor potential decreases between 1 and 10 cps whereas the lamina potential amplitude has its maximum in this range. At high frequencies the ERG amplitude is almost entirely determined by that of the lamina potential which for instance at 8 cps is four times that of the receptor potential.

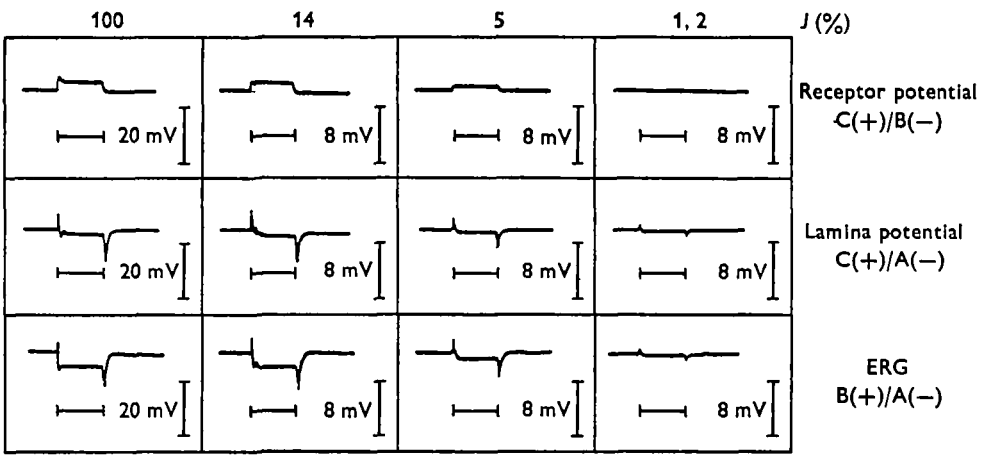

Fig. 5. Receptor and lamina potentials at high ambient light intensity (50 % standard intensity) and different stimulus intensities. Electrode positions are indicated in Fig. 3.

(3) *Mutant ERGs*

Three mutants were used for this study: *ebony*, *tan* and opm 2. The ERGs of *tan* and *ebony* have been studied by several investigators (Pak *et al.* 1969; Hotta & Benzer, 1969). It was found that these mutants showed a normal receptor potential whereas the phasic components were partially missing. All three mutants have poor phototaxis as tested with a technique developed by Benzer (1967) for mutant selection; however, in the optomotor response and in slow phototaxis at low light intensity they seem to be only partially abnormal (K. G. Götz & M. Heisenberg, unpublished). In these experiments all mutants carried the additional mutation *white* in order to be able to compare the results to those in the preceding sections. The ERG analysis now makes it possible to measure the lamina potential in a simple 2-electrode experiment by choosing the appropriate stimulus conditions, since at high stimulus frequencies low intensity or low flash contrast ($\Delta I/\bar{I}$) the wild-type ERG consists predominantly of the lamina potential.

First, the ERG measurements were repeated for the three mutants (Fig. 7). All three showed monophasic cornea-negative sustained potentials over the whole intensity range roughly comparable in amplitude to the receptor potential in the wild type. No off-effects were observed under these conditions. The on-effects in some cases seemed to be present but hidden in the steep edge of the receptor potential. Near threshold,

where the wild-type ERG consists only of the lamina potential, no responses could be elicited from the three mutants.

A similar result was obtained for small-contrast light flashes at high intensity. Under conditions where in the wild-type ERG mainly the sharp on- and off-effects of the lamina potential are seen, only small monophasic depolarizations were observed with *tan* and opm 2, while *ebony* in addition showed a very small on-effect (Fig. 8).

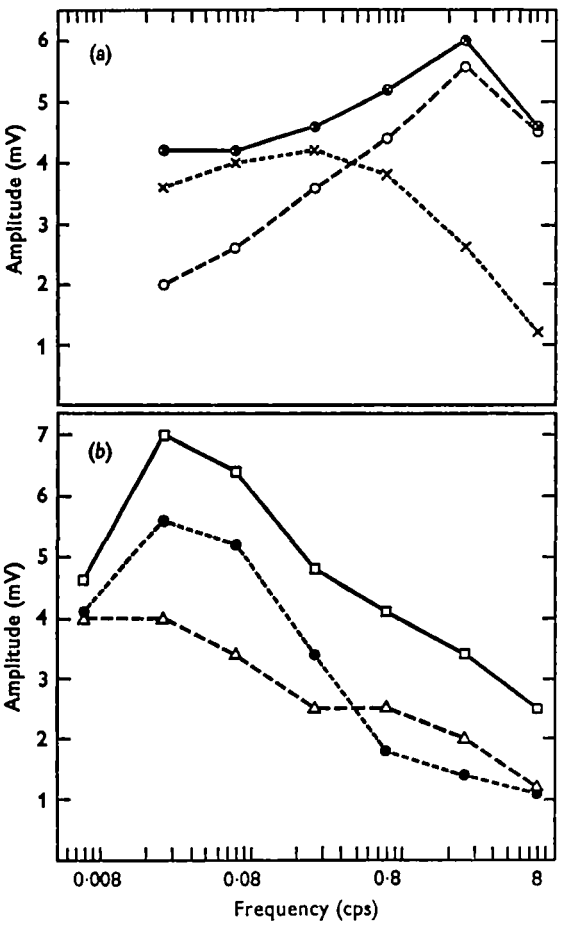

Fig. 6. Frequency dependence of the *Drosophila* ERG. (*a*) Amplitude of wild-type ERG (⊗–⊗), receptor potential (×–×) and lamina potential (○–○) as a function of the stimulus frequency. Average intensity was 0·085% max. standard intensity; modulation > 99%. Electrode positions are indicated in Fig. 3(*b*). Amplitude-frequency function of mutant ERGs; *ebony*, △—△; *tan*, ○—○; opm 2, □—□; stimulus conditions as in (*a*).

As a further test the frequency dependence of the mutant ERGs was compared to that of wild type (Fig. 6*b*). The amplitudes of the mutant ERGs had no maximum around 3 cps and were very small in the high-frequency range. For *tan* and opm 2 unusually large amplitudes around 0·03 cps were observed. These originated mainly from the lamina potential as could be shown in 3-electrode experiments. The frequency dependence of the receptor potentials apparently had not changed.

Finally the lamina potentials of the mutants were studied separately in 3-electro[illegible] experiments. As expected some remnants of the lamina potential, especially of the on-effects, were detected in all three mutants, but they differed in shape, size and other properties (Fig. 9*a*). The lamina potential of opm 2 was invariably the smallest.

In all experiments the ERGs of *tan* and opm 2 were found to be very similar. However, under certain stimulus conditions (ambient light 2% standard intensity;

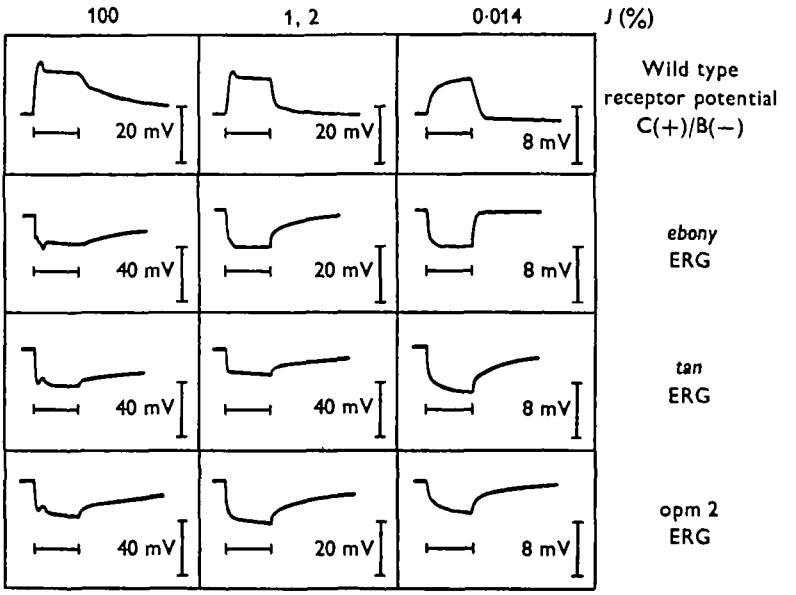

Fig. 7. ERGs of the mutants *ebony*, *tan* and opm 2 to 0·65 s light flashes of various intensities. No off-effects are observed. In *ebony* and *tan* at high intensity traces of on-effects are possibly obscured by the sharp edge of the receptor potential. The mutant ERGs are compared to the receptor potential of wild type.

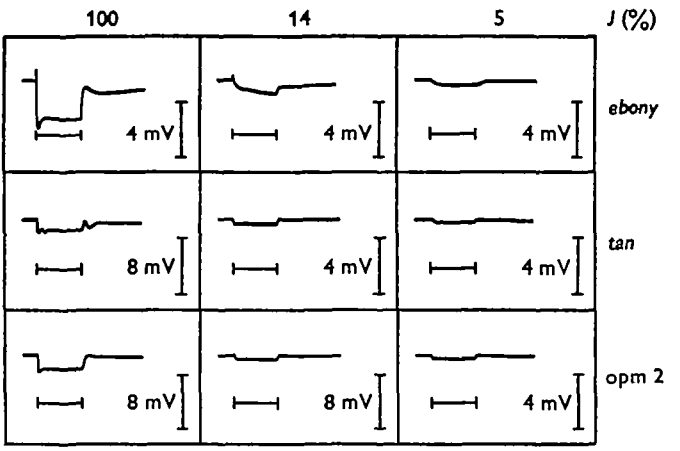

Fig. 8. ERGs of the mutants *ebony*, *tan* and opm 2 at high ambient light intensity (50% standard intensity) and different stimulus intensities. In the ERG of *ebony* a sharp on-effect and in that of *tan* a delayed off-effect are observed (see also Fig. 10).

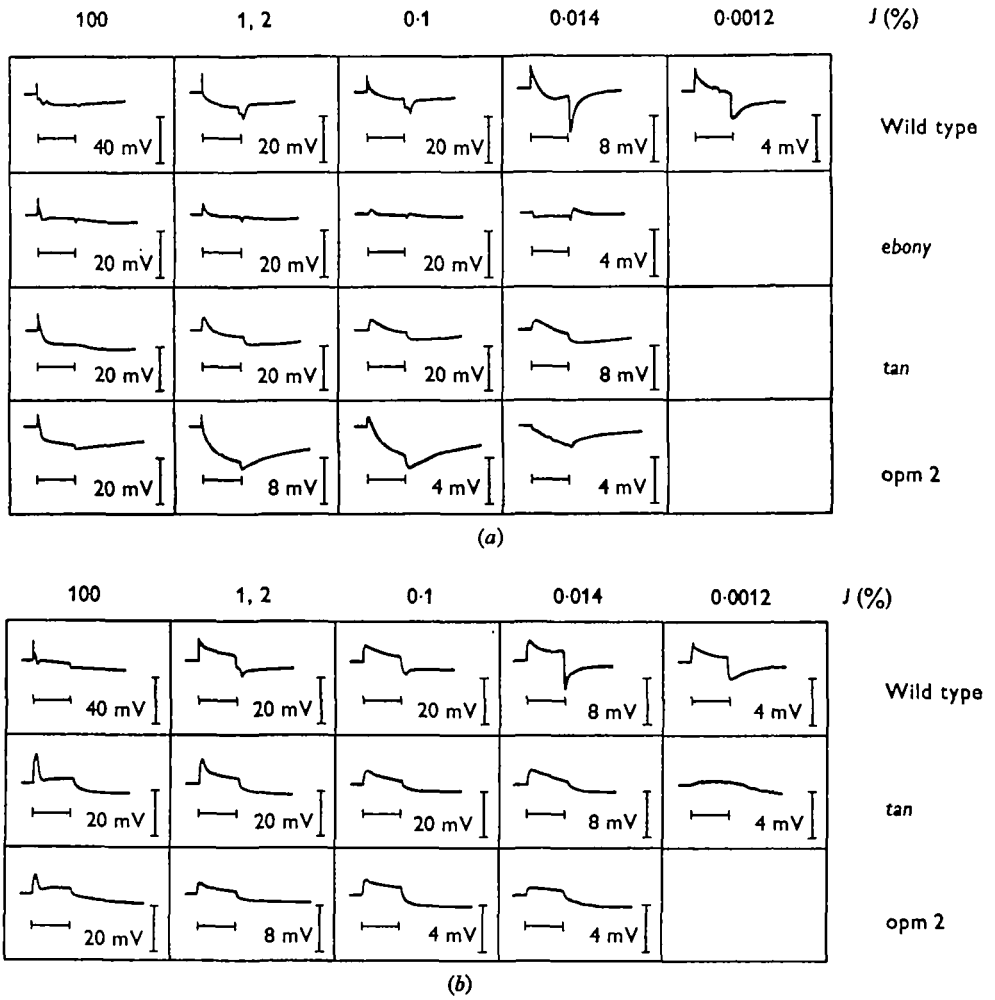

Fig. 9. Lamina potential of wild type and the mutants *ebony*, *tan* and opm 2. (*a*) Electrodes are placed as shown in Fig. 3; electrode C is just proximal to the receptor wall, except for *ebony* where it may be 10–20 μm deeper. (*b*) Electrode C is advanced 30 μm into the lamina. In wild type, *tan* and opm 2 the lamina potential shows a systematic difference from that at the first position. In *ebony* this was not tested.

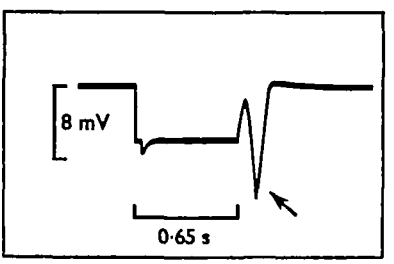

Fig. 10. ERG of *tan*; ambient light: 2% standard intensity: flash: 14% standard intensity The arrow points at the huge delayed off-effect.

flash 14% standard intensity) a huge off-effect appeared in the ERG of *tan* with latency of about 100 ms (Fig. 10). This peak was part of the lamina potential; it may correspond to the delayed 2. off-effect (6) at high intensity in wild type. This peak was never observed in opm 2.

DISCUSSION

(*a*) *Compartments*

The concept of the 'receptor barrier' has, up to this point, been developed from ERG measurements alone. As judged by the experiments and theoretical arguments it must lie between the rhabdomers and the ends of the retinula cell axons. Most probably it lies just distal to the lamina. The extracellular space in the receptor layer bordered by the receptor barrier has to be regarded as one compartment completely separated from the rest of the fly. It is a striking observation in non-shrunken thin sections of the *Drosophila* head that the retinula cell layer is sealed off from the brain by a ring of air sacs with the lamina in the middle (Fig. 3). In electron-micrographs of *Musca domestica* there are several structures near the proximal ends of the retinal cells (e.g. basement membrane, desmosomes, epithelial cell folds; C. B. Boschek, personal communication) which might be the material correlate of the receptor barrier.

In a somewhat different sense the lamina can also be regarded as a distinct compartment for the ERG. However, it seems from the experiments described in sections 1*b* and 2*a* that throughout the lamina high extracellular resistances or at least several tangential layers of high extracellular resistance exist and that the discontinuity separating it from the rest of the brain consists mainly in a sudden decrease of this extracellular resistance. The reason for this assumption is that at different depths in the lamina the contributions of the different cell types to the lamina potential vary to a certain degree and that at about 200 μm from the surface the lamina potential invariably disappears and occasionally the steady potential of the reference electrode is reached.

The reason that in *Drosophila* one records the lamina potential as part of the ERG must be that no low-resistance pathways from the receptor barrier to the reference electrode exist which by-pass the lamina. Again this is supported by the observations on the general structure of the eye and the lamina. It is clearly not the case for the *medulla* and might also be different for the lamina of those insects which have a simple monophasic ERG.

The functional significance of such resistance barriers is unknown. However, if only the retinula cells provide the EMF for the receptor potential it has to be expected that *in vivo* the receptor membrane depolarization proximal to the receptor barrier is smaller than it is distal to it by the amount measured in the receptor potential. Thus it appears that those fractions of the excitations in the retinula cell layer and in the lamina which constitute the ERG participate (if they participate at all) only indirectly in the data-processing chain of the individual visual units which otherwise would be difficult to reconcile with the observed lateral homogeneity. Nevertheless, the ERG can be a convenient and useful indicator for some of the events in this data-processing chain.

(*b*) *Components*

Intracellular recordings from the lamina of *Musca domestica* (Scholes, 1969) and also in the locust (Shaw, 1968) have demonstrated depolarizing and hyperpolarizing units which at low intensity responded to light with monophasic sustained potentials. It is possible therefore that the lamina potential at low intensity is the superposition of two sustained potentials with opposite polarity and different time constants. At high intensity, however, this is certainly more complicated. The fast on-effect in *ebony* and the delayed off-effect in *tan* suggest that at least these components are originally phasic and independent of one another.

Since the retinula cell axons of the cells 1–6 end in the lamina and those of retinula cells 7 (Cajal & Sanchez, 1915) and 8 (Strausfeld, 1970) even pass through it, one has to expect that the lamina potential still contains a fraction of the receptor potential. The extent of this cannot be decided at this stage of the analysis.

Very little is known about the slow negative sustained potential (4). It appears in part with the receptor potential and in part with the lamina potential but in the lamina it is confined only to the distal portion. It is present in the mutants and is completely suppressible by ambient light.

(*c*) *Functions*

So far the only known function of the lamina is the summation of the excitations of the retinula cells 1–6. Theoretically only one type of interneurone in the lamina would be sufficient for this. However, at least nine other cell types are present in *Musca* and *Calliphora* (Braitenberg & Strausfeld, 1971; Strausfeld, 1970). The three properties of the lamina potential described here suggest for which functions to search.

The first follows from the observation that with decreasing intensity the receptor potential decreases faster than the lamina potential, so that near the low threshold of the ERG only the lamina potential is found whereas at high intensity the receptor potential is larger than the lamina potential. This might represent the summation function of the first interneurones in the lamina which receive input from retinula cells 1–6. The lamina units recorded in *Musca* (Scholes, 1969) do not have this property; however, hyperpolarizing cells with a larger response to low-intensity light flashes, and with a lower saturation level compared to the retinula cells, have been described in the lamina of the locust (Shaw, 1968). How these units might interact is unknown.

The second property of the lamina is postulated because of its response to low-contrast light flashes at high intensity. Again, with decreasing contrast ($\Delta I/I$) the receptor potential decreases faster than the lamina potential. Some kind of adaptation has to be involved in the mechanisms responsible for this function.

The most striking feature of the lamina potential is its frequency dependence. The shape of the lamina potential elicited by low-intensity light flashes has the characteristics of a high-pass filter which can be derived from the ratio of the receptor and lamina potential amplitudes at different stimulation frequencies (Fig. 6*a*). This might be an incidental consequence of the summation character of the ERG and the time constants of the components but it might also represent a data-processing step between light reception and spike-train formation. It should be mentioned that the maximum of the opto-

motor response as a function of pattern speed in *Drosophila* is around 1 cps in flight (Götz 1964) and around 3 cps for walking animals (K. G. Götz & H. Wenking, in preparation). In the model for movement detection, as formulated by Reichardt & Varjú (1959), a high-pass filter (D) modifying the visual input has been postulated for *Chlorophanus*. At low intensity this filtering process may be the one appearing in the lamina potential. Therefore, lamina mutants like *ebony* (see Fig. 6*b*) possibly provide an opportunity to locate some of the components of the motion-control system. It should be possible to retrieve these postulated functions of the lamina in higher-order unit recordings, for instance in the movement-sensitive units in the optic lobes of *Musca* or *Calliphora* (McCann, 1970). However, these cells have not been studied sufficiently in this respect.

(*d*) *Mutants*

A few conclusions can be drawn from the mutant ERG experiments. The mutants selected for this study have normal or only slightly reduced receptor potentials but their lamina potentials are disturbed. The lamina potentials are not completely lost and what remains is specific and different in the three mutants. The mutant *ebony* shows a nearly normal fast on-effect but no off-effects, whereas in *tan* the fast *on*-effect is diminished and a huge delayed off-effect can be observed. Finally in the mutant opm 2 the whole lamina potential is reduced. Therefore, the electrophysiological defects cannot, in all of the mutants, be explained by a current leak around the lamina or by a general loss of the high extracellular resistances within the lamina. It seems more likely that either specific resistance changes or abnormalities in the excitability of certain cell types have occurred in at least some of the mutants, and that by these defects the high-pass filter properties as well as the summation and adaptation functions of the lamina are severely disturbed.

If one considers the few behavioural experiments so far carried out it is likely that in *tan* and opm 2 the neural defects caused by the mutation are not confined to the lamina since the optomotor response of these mutants at high intensity, where synaptic interaction in the lamina seems to be unnecessary, is also disturbed. The receptor cells, however, seem to be functionally unimpaired since light sensitivity in slow phototaxis is only diminished by a factor of about 50 for which the loss of lamina function would account (M. Heisenberg & K. G. Götz, unpublished).

In *ebony* the optomotor response at high intensity is basically normal (K. G. Götz & M. Heisenberg, unpublished) and it remains to be seen whether high intensity is needed for the lamina to function sufficiently or whether movement detection in *ebony* is performed without the lamina.

A detailed discussion of the possible neuronal defects in these three mutants has to wait for a quantitative behavioural analysis.

SUMMARY

1. In *Drosophila* the retinula cells and the cells in the *lamina ganglionaris* contribute to the ERG. This is due to extracellular resistance barriers across these cells; one of these is situated near the proximal ends of the rhabdomeres separating the retinula cell layer from the rest of the fly, the other is situated either within several layers or homogeneously distributed throughout the lamina. Because of their different origin,

two components of the ERG, the receptor potential and the lamina potential can be separated experimentally.

2. At high light intensity the receptor potential is larger than the lamina potential. However, under stimulus conditions where the receptor potential is very small (*a*) at low light intensity, (*b*) at high intensity but low flash contrast ($\Delta I/I$), (*c*) at high frequency of stimulation, the lamina potential exceeds the receptor potential. It is suggested that these properties reflect summation and adaptation of the sensory input within the lamina. The shape of the lamina potential has, under these conditions, the characteristics of a high-pass filter and may improve the fly's response to high stimulus frequencies.

3. The ERGs of the mutants *ebony*, *tan* and opm 2 have normal or nearly normal receptor potentials but at the same time demonstrate severe defects in the lamina potentials. In *ebony* a fast on-effect at high intensity, and in *tan* a delayed off-effect at high intensity, are still present. The mutant opm 2 shows very little lamina activity at all. The difference of the defects in the three mutants argues against non-specific current leaks in or around the lamina. Therefore it is most likely that the lamina properties of summation, adaptation, and high-pass filtering, are largely lost in the three mutants. This is supported by behavioural experiments.

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