Recording of Retinal Action Potentials from Single Cells in the Insect Compound Eye

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ABSTRACT Electrical responses were recorded intracellularly from the compound eyes of a fly (*Lucilia*) and of several dragonflies (*Copera*, *Agriocnemis*, and *Lestes*). An ommatidium of the dragonflies is made up of four retinula cells and a rhabdom composed of three rhabdomeres while the *Lucilia* has an ommatidium of seven independent retinula cells and rhabdomeres. The intracellular responses presumably recorded from the retinula cell had the same wave form in the two groups of insects: The responses were composed of two components or phases, a transient spike-like potential and a slow one maintained during illumination. The membrane potential, in the range of −25 to −70 mv., was influenced by the level of adaptation, and it was transiently depolarized to zero by high levels of illumination.

INTRODUCTION

It seems well established that the receptor layer of the arthropod compound eye responds to photic stimulation with a slow potential termed the electroretinogram (ERG). This is supported by several experiments in which ERG's were recorded from the receptor layer detached from the optic lobes (Jahn and Wulff, 1942; Bernhard, 1942; Autrum and Gallwitz, 1951; Hartline, Wagner, and MacNichol, 1952). However, it is not yet known what cells in the receptor layer give rise to the slow potential. In the *Limulus* compound eye Hartline, Wagner, and MacNichol (1952) succeeded in recording spikes and slow potentials (ommatidial action potential) intracellularly from an ommatidium. Their results were later confirmed by several authors including Tomita (1956), MacNichol (1958), and Fuortes (1958 and 1959). Though the eccentric cell is considered as the generation site of spike potentials there are several different views on the origin of the ommatidial action potential.

In this connection intracellular recording of action potentials from the receptor layer of the compound eye seemed to give substantial information on the site of slow potential generation. Kuwabara and Naka (1959) have al-

ready reported briefly on the intracellular recording of action potentials from the compound eye of the fly, *Lucilia*. The present paper confirms their results, and describes several additional features of the intracellularly recorded retinal action potentials in *Lucilia* and several dragonflies.

MATERIAL AND METHOD

Structure of the Insect Ommatidium[1]

The compound eye of the fly, *Lucilia* (*Cyclorrhapha*), and the compound eyes of the dragonflies, *Copera*, *Agriocnemis*, and *Lestes* (Zygoptera), were studied. Methods of recording electrical responses and stimulating apparatus were the same as described by Naka and Kuwabara (1959 *a*). A corneal area about 1 mm. in diameter was stimulated. Intensity of illumination was controlled by three neutral filters each having an absorption of 90 per cent. The intensity of illumination without any filters is referred to as 1 or unit intensity. The microelectrodes used were 3 M KCl-filled glass capillaries with resistance of more than 30 megohms measured in 3 M KCl solution (more than 100 megohms in Ringer solution). The electrical resistance of the KCl-filled microelectrode was different in Ringer and in 3 M KCl solution. For convenience of measurement the resistance was checked in 3 M KCl solution. To record intracellular responses from small retinal cells of diameter less than 10 micra the microelectrode must have (*a*) a very high resistance, and (*b*) a long taper near its tip. The same type of microelectrode could also pick up spike and slow potentials intracellularly from the toad retina (Naka, Inoma, Kosugi, and Tong, 1960). Recording electrodes were led into 12AU7 cathode-follower input stages (Tomita and Torihama, 1956) succeeded by D.C. amplifiers combined with a dual beam oscilloscope. All experiments were conducted at room temperatures of 25 to 30°C.

The compound eye of *Lucilia* and Zygoptera shares the general features of a typical insect apposition eye, the receptor layer lying between the cornea and basement membrane as shown in Fig. 1. The receptor layer is made up of individual units, the ommatidia, having the shape of a cylinder, extending the whole length of the receptor layer. An insect ommatidium includes elongated receptor cells (retinula cells), rhabdom or rhabdomeres, pigment cells, and dioptric apparatus (corneal lens and crystalline cone). The proximal process of the retinula cell passes through the basement membrane as a post-retinal fiber extending to the optic ganglion. The main constituents of the ommatidium are the retinula cells and the rhabdom or rhabdomeres which are regarded by several authors as the loci of photochemical reactions (*cf.* Goldsmith and Philpott, 1957). Arrangements of these two structures in an ommatidium differ in *Lucilia* and in the Zygoptera; an ommatidium of *Lucilia* includes seven independent retinula cells and rhabdomeres while in the Zygoptera a rhabdom formed of three rhabdomeres is surrounded by four

[1] Detailed description of the fine structure of the Zygoptera compound eye will appear elsewhere.

retinula cells. An electron micrograph of a cross-section of an ommatidium of Zygoptera (*Agriocnemis*) is shown in Fig. 2. In the ommatidium two retinula cells each have their own rhabdomere; the third rhabdomere is shared by the other two retinula cells. In the retinula cell mitochondria may be seen around the rhabdom, and in the peripheral part of the cell there are numerous double

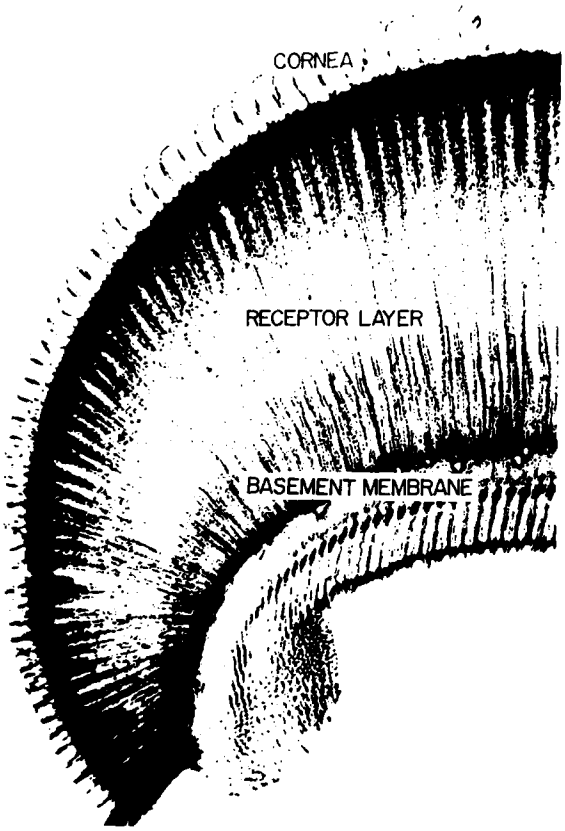

FIGURE 1. Microphotograph of the insect apposition eye (*Agriocnemis*). The receptor layer composed of structural units, the ommatidia, is spaced between the corneal surface and the basement membrane.

membrane profiles which are supposedly the endoplasmic reticulum. The rhabdomere is built up of closely packed submicroscopic hexagonal compartments or honeycombs with diameters down to a few hundred A as reported by several authors (Goldsmith and Philpott, 1957; Miller, 1957; Wolken, Capenos, and Turano, 1957; Fernández-Morán, 1958). As described by Miller (1957) one end of the tubular structure or microvillus is continuous with the cytoplasm of the retinula cell while the other end is closed making contact with the adjoining rhabdomere. From electron microscopic obser-

vation the diameters of the retinula cells are 5 to 8 micra in the Zygoptera and 8 to 10 micra in *Lucilia* while diameters of the rhabdomere are about 1.5 and 3 micra respectively.

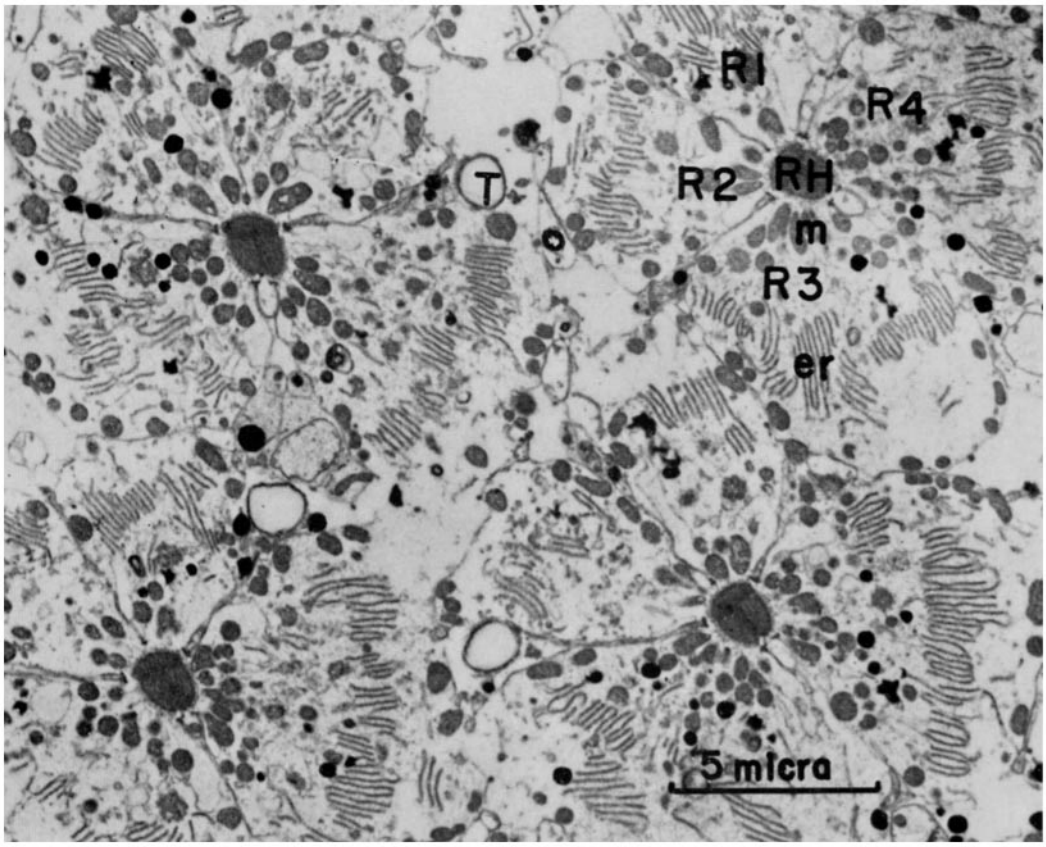

FIGURE 2. Electron micrograph of a cross-section of the receptor layer of the compound eye of the dragonfly, *Agriocnemis*. OsO_4–fixed, methylmethacrylate-embedded, and photographed by Akashi tronscope. *R1* to *4*, retinula cells, *RH*, rhabdom, *T*, trachea, *m*, mitochondria, *er*, endoplasmic reticulum. Photographed by Mr. Y. Tominaga.

ELECTRICAL RESPONSE OF THE COMPOUND EYE

Intracellular Recording from the Zygoptera Compound Eye

The response most frequently recorded from the receptor layer of the Zygoptera compound eye was a monophasic negative potential of a few millivolts. Sometimes a minute movement of the electrode resulted in a sudden decrease in the resting potential of −25 to −70 mv. This is shown in Fig. 3 in which

action potentials and resting potential were recorded on a sweep of very low speed. At A a monophasic negative response was recorded and then the electrode was slightly advanced and the resting potential suddenly shifted by about 70 mv. toward the negative side. The electrode was kept unchanged at this position and the preparation was subjected to illumination at B and C. Responses at B and C were completely different from the response at A; the polarity was reversed and amplitudes were greatly increased to more than 50 mv. Further movement of the electrode resulted in a sudden return of the resting potential to the original level and a response was recorded at D at this position of the electrode.

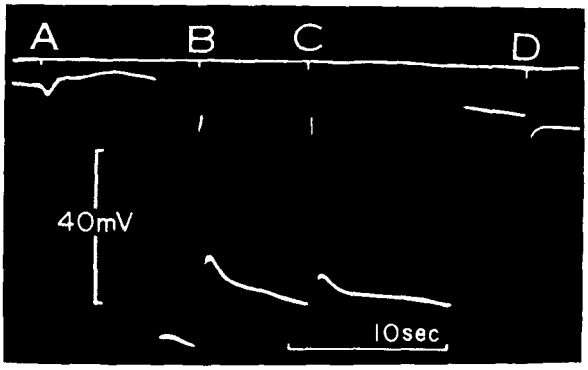

FIGURE 3. Intra- (B and C) and extracellular (A and D) recording from the Zygoptera compound eye. Illuminations, 40 msec. in duration, are given at A, B, C, and D, signals being shown on the upper beam. Only the peaks of the early spike-like component are visible in B and C. Upward deflection indicates positivity in all records.

From these observations it seems adequate to conclude that the electrode was inserted into a cell in the receptor layer of the Zygoptera compound eye, change in the resting potential representing the membrane potential of the cell. Accordingly responses at A and D were recorded extracellularly while responses at B and C were obtained intracellularly from the receptor layer of the compound eye.

In Fig. 3 the response at B started from the membrane potential of −70 mv. and the amplitude of the response was 62 mv. while the response at C started from the membrane potential of −57 mv. and its amplitude was 50 mv. In both cases, however, the peaks of responses reached to the same level of potential, nearly to the zero level, though original values of membrane potentials and amplitudes of action potentials were different. In Fig. 4 in which illuminations were repeatedly given, the peaks of responses reached to the same level of potential while the membrane potential decreased by 10 mv. during the first few flashes. These observations indicate that the maximal amplitude

of the response was closely related to the level of the membrane potential. To elucidate these observations the resting potential was plotted against the amplitude of the response obtained with illuminations of the maximum intensity. Fig. 5, in which abscissae represent the membrane potentials and ordinates the amplitudes of responses, shows no appreciable deviation from the hypothetically drawn relation; the amplitude of action potential equals the membrane potential. The membrane potential can thus be supposed to depolarize to zero with high intensity illumination.

In the records of Fig. 3 the membrane potential did not return to the original level following illumination at B and C; *i.e.*, the membrane potential decreased about 10 mv. after illumination. The same decrease in the mem-

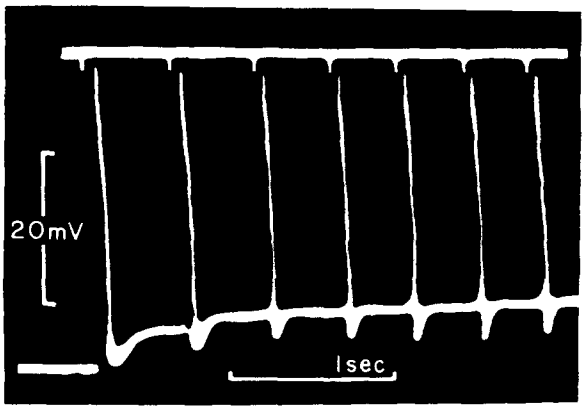

FIGURE 4. Responses recorded by repeated illuminations of about 40 msec. in duration. The upper beam represents the zero level of the resting potential in this and in Figs. 6–8.

brane potential after illumination, or after depolarization, was also observed in Fig. 4 in which illuminations were given repeatedly, about 2 flashes per second. In this record the membrane potential decreased 10 mv. during the first few flashes and it remained at this level during repeated illuminations. On the other hand, the membrane potential increased when the preparation was dark-adapted and this increase often exceeded the original level before illumination. Therefore, the level of the membrane potential was not fixed but dependent on the level of adaptation. Latency of the intracellular response was about 7 msec. when illumination was unit intensity and it increased to about 15 msec. when illumination was decreased to $\frac{1}{1000}$ in intensity.

The two records in Fig. 6 show relations between the wave forms of action potentials and the durations of illumination which were 40 msec. in A and 2 sec. in B. The response to illumination of 40 msec. was a spike-like potential which reached to the zero level of the resting potential (*i.e.*, depolarized the membrane) while the response to illumination of 2 sec. was a spike-like po-

tential followed by a slow one maintained during illumination. In both records there appeared a prolonged after-depolarization due to light adaptation as described above. Comparing these responses in Fig. 6 with those of Figs. 3 and 4 it becomes apparent that plots shown in Fig. 5 represent the relation between the amplitudes of maximal spike-like response and the membrane potential. The small polyphasic potential which appeared at "off" of illumination in the response of Fig. 6 B was due to effects of the extracellular

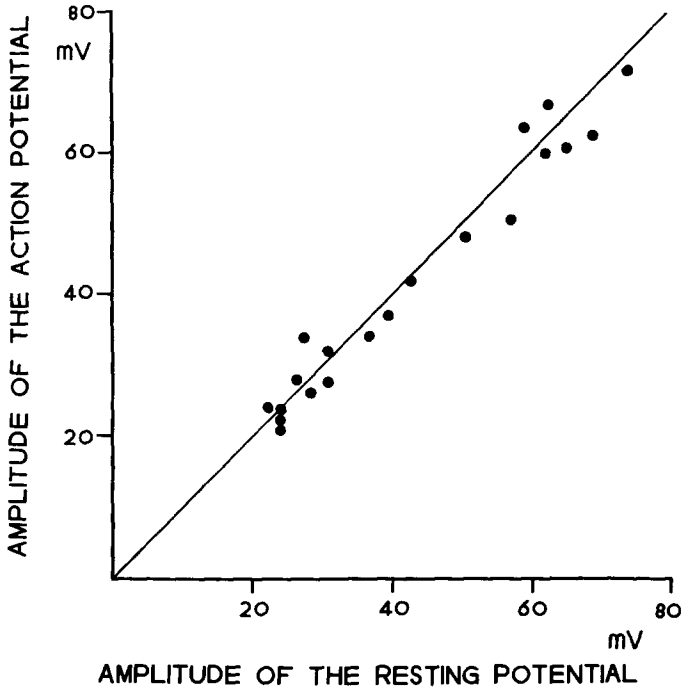

FIGURE 5. Relation between the amplitude of response and the membrane potential. Abscissae represent the membrane potential and ordinates the maximal amplitude of the response.

electric field. In the present experiment in which responses were recorded between the microelectrode in the receptor layer and the indifferent one in the Ringer pool, effects of the extracellular electric field produced by the activity of other cells sometimes appeared even in the intracellular recording, though they were very small in amplitude. When the indifferent electrode was placed on the receptor layer, no distortion of the response was observed.

Effects of change in the intensity of illumination were observed by increasing the intensity of illumination by tenfold while keeping the duration constant (Fig. 7). Under moderately dark-adapted conditions the response to very low intensity illumination ($1/1000$ of unit intensity) was a sustained potential with

a small initial elevation (Fig. 7 A). When the intensity of illumination was increased by tenfold the initial elevation was greatly increased in amplitude and it took on the wave form of a typical spike-like potential (Fig. 7 B). While the sustained potential increased by 50 per cent in amplitude, the initial elevation increased as much as three times. Further increase in the intensity of illumination did not give rise to any appreciable increase in the amplitude of the spike-like potential, indicating that the intensity of illumination was strong

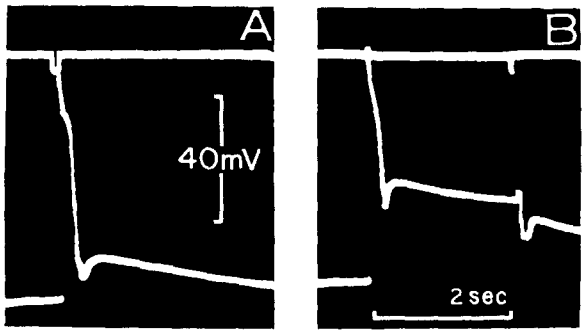

FIGURE 6. Response obtained with illuminations (maximal intensity) of 40 msec. in A and 2 sec. in B.

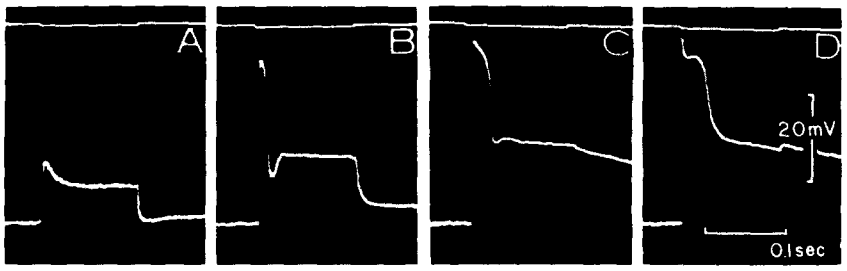

FIGURE 7. Responses obtained with illuminations of different intensities, 1/1000, 1/100, 1/10, and 1 from A to D.

enough for full generation of this response. The increase in the intensity of illumination also caused an increase in the amplitude of the after-depolarization, resulting in a decrease of the off effect. As shown in Fig. 7 C and D, the off effect, the return of the sustained potential to the resting level, became much smaller and finally reversed its polarity. The polarity reversal of the off effect seems to have been brought about as the amplitude of the after-depolarization exceeded that of the sustained potential. There sometimes appeared a small negative phase just after the spike-like potential as in Fig. 6 and also in Fig. 7 B and C. Though the nature of the negative phase is unknown, it was most conspicuous when illumination was moderate in intensity.

In Fig. 8 responses were recorded from the same preparation under fresh and deteriorating conditions; *i.e.*, A was recorded a few minutes after decapitation while B was recorded after the preparation had been allowed to deteriorate for 30 minutes. In A the response was a typical spike-like potential followed by a slow potential maintained during illumination while in B it was a slow sustained potential lacking an initial peak. The difference between the response from the fresh and deteriorating preparations was absence of the spike-like potential in the latter preparation. Light adaptation also brought about similar disappearence of the spike-like potential. When the preparation had deteriorated the latency of the off effect became remarkably long and less

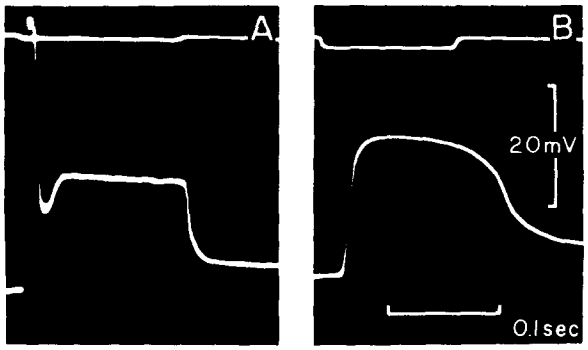

FIGURE 8. Responses obtained from a preparation under different conditions; from a fresh preparation (A) and from poor preparation (B). Durations of illumination, 130 msec. in A and 110 msec. in B.

conspicuous, while light adaptation resulted in decrease in the latency of the off effect. Under these conditions no polarity reversal of the off effect was observed.

Intracellular Recording from the Lucilia Compound Eye

From the receptor layer of the *Lucilia* compound eye responses were recorded intracellularly as in the Zygoptera. The response recorded intracellularly from the receptor layer of the *Lucilia* compound eye had nearly the same wave form as in the Zygoptera, being composed of an initial spike-like potential followed by a slow sustained one. The peak of the action potential reached to the zero level of the resting potential when illumination was strong enough. Typical intracellular responses obtained with illumination of 1 sec. are shown in Fig. 9 in which intensities of illumination were 1 in A and $\frac{1}{1000}$ in B. The response to low intensity illumination was a sustained potential with an initial elevation while increase in the intensity of illumination caused the appearance of a spike-like potential in response to "on" of illumination. When the response

was obtained with illumination of moderate intensity a negative phase followed the spike-like potential as in the Zygoptera (*cf.* Figs. 6 and 7). However, the depolarization following cessation of illumination was not so visible as in the case of the Zygoptera and the polarity reversal of the off effect was observed only exceptionally. It was not decided whether this difference in the intracellular records represented any functional difference between the two groups of compound eyes. In Fig. 10 intra- (upper record) and extracellularly (lower record) recorded responses are shown. Extracellular responses from the *Lucilia* receptor layer were usually diphasic with a positive on and negative

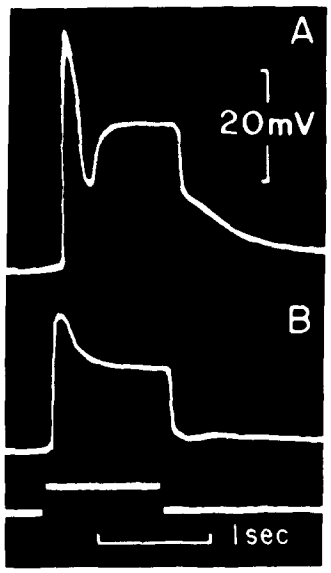

FIGURE 9. Intracellular responses from *Lucilia*. Intensities of illumination, 1 in A and 1/1000 in B. Durations of illumination, about 1 sec.

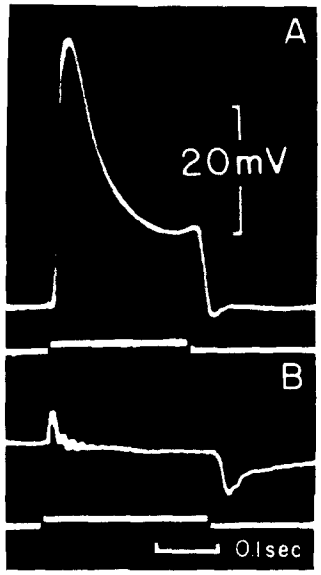

FIGURE 10. Intra- (A) and extracellular (B) responses from *Lucilia*. Durations of illuminations, about 220 msec. in A and 280 msec. in B.

off effect of less than 5 mv. The wave form of the extracellular record varied according to conditions of the preparation as already reported by Naka and Kuwabara (1959 *a*). On the other hand the intracellular record was fairly consistent in its waveform making any direct correlation between the wave forms of intra- and extracellular responses very difficult. However, the slow negative potential in the extracellular recording seems to reflect the activity of the slow sustained potential in the intracellular recording because both potentials had nearly the same wave form with opposite polarity and also because they were the components most resistant to physiological decline (*cf.* Naka and Kuwabara, 1959 *a*).

In a series of records in Fig. 11 relations between the wave forms of both

intra- and extracellular records and the duration of illumination are shown. In this figure the extracellular record had a very complicated wave form with a polyphasic on and diphasic off effect. In the extracellular record the positive potentials at on of illumination were fairly independent of the duration of illumination. The amplitude of the spike-like potential in the intracellular response was independent of the duration of illumination beyond 10 msec., indicating that illumination of 10 msec. was sufficient to induce a full size response at this intensity of illumination. In this record the slow potential appeared in the response when the duration of illumination was more than 30 msec. and an increase in the duration of illumination brought about a corresponding increase in the duration of the slow potential.

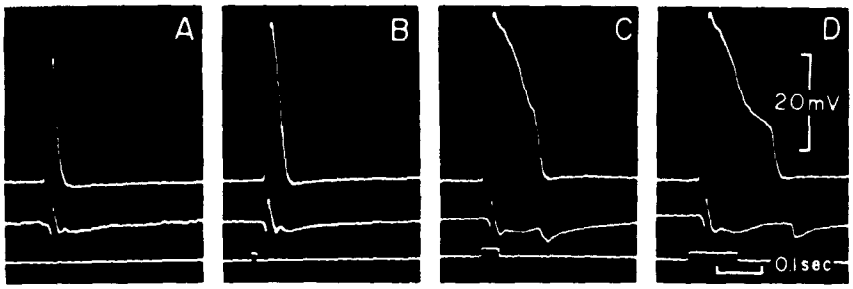

FIGURE 11. Intra- (upper) and extracellular (lower) responses recorded by illuminations of different durations, 10, 15, 30, and 100 msec. from A to D.

DISCUSSION

Recent studies of the fine structure of the insect compound eye (Wolken, Capenos, and Turano, 1957; Goldsmith and Philpott, 1957; Fernández-Morán, 1958) revealed that there are two types of ommatidium in the insect apposition eye; in one type, as in *Lucilia,* individual rhabdomeres are not joined to form a rhabdom while in the other type, as in the Zygoptera, the rhabdomeres are joined to form a rhabdom. According to these observations and also confirmed in the present paper a rhabdomere is composed of numerous microstructures, hexagonal compartments or honeycombs, assumed to be the site of photochemical reactions by Goldsmith and Philpott (1957) and also by Wolken, Mellon, and Contis (1957). In the retinula cell of the Zygoptera quite a number of mitochondria are observed to cluster around the rhabdom giving substantial support to their contention that the rhabdomere is the site of the photochemical reactions.

Intracellularly recorded action potentials from the receptor layer of the compound eye of two groups of insects were nearly the same in nature being composed of two components or phases, a spike-like potential and a slow potential. These two potentials differed in several points: (*a*) The spike-like

potential responded only to on of illumination. (*b*) With high intensity illumination the spike-like potential was a transient depolarization of the membrane potential toward the zero level. (*c*) The slow potential maintained during illumination was rather stable while the spike-like potential was labile, being abolished by light adaptation or by deterioration of the preparation. However, it is not known whether these two components or phases are associated with different reactions in a cell or are two phases of a reaction. Further examination of the relations of wave forms of intra- and extracellularly recorded responses

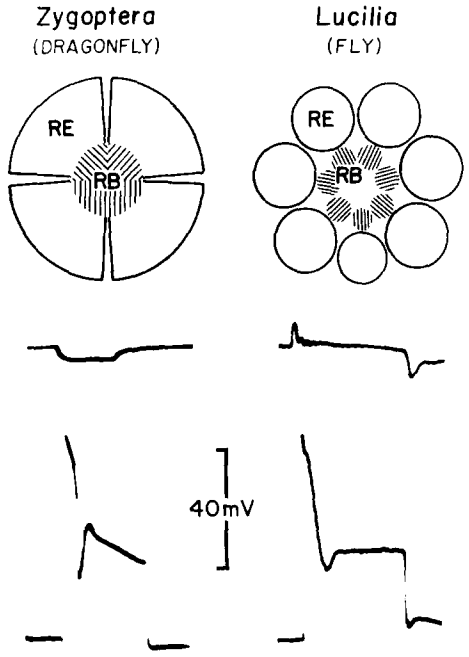

FIGURE 12. Comparison of structure and response from the compound eye of Zygoptera (*Agriocnemis*) and *Lucilia*. Schema of an ommatidium (upper row), extra- (middle row), and intracellular (lower row) responses. *RE*, retinula cell, *RB*, rhabdom or rhabdomere.

will give some suggestions on this question. It is worthy of note that two components recorded extracellularly from the compound eye of the crayfish showed nearly the same characteristics (Naka and Kuwabara, 1959 *b*).

The amplitude of the intracellular response was markedly influenced by the level of the membrane potential which increased or decreased under dark or light adaptation; the spike-like potential in maximal response completely depended upon the membrane potential. This can partly explain changes in the amplitude of extracellular responses under various conditions of adaptation.

As is apparent from electron microscopic observations on the insect omma-

tidium, there are only two structures, retinula cells and rhabdomeres, that can be postulated as a site of slow potential generation. The rhabdomere is very small in diameter and, moreover, it is an elaboration of the plasma membrane of the retinula cell. On the other hand, the retinula cell, the primary sense cell, with a diameter five times as large as that of a rhabdomere seems to offer much as a suitable target for penetration of the microelectrode. Though there is no direct evidence, these considerations favor a view that the electrode was impaled in one of the retinula cells and the response was obtained intracellularly from the cell. However, this assumption does not exclude the rhabdomere as a possible site of origin of a part or phase of intracellular response because the rhabdomere has, as revealed by the electron microscopic observations, a cytoplasmic connection with the retinula cell by means of microvilli (Miller, 1957; Fernández-Morán, 1958). The same type of response was also recorded intracellularly from the compound eye of the crayfish, *Procambarus* (unpublished observations), indicating that the response is not a peculiar characteristic of the insect compound eye but is one of the fundamental processes in photoreception in the arthropod compound eye in general.

The relation between the arrangement of cells in an ommatidium, extra-, and intracellular responses from the compound eye of Zygoptera and *Lucilia* is summarized in Fig. 12. Judging from the wave form of the extracellularly recorded response the compound eye of *Lucilia* belongs to the first eye of Autrum's classification (Autrum, 1950) of the electrical response from the insect compound eye. Though he included the dragonflies among the fast eyes, the extracellularly recorded response from the Zygoptera compound eye is a typical one for a slow eye (Fig. 12, middle row). It is very interesting that the same responses were recorded intracellularly from two types of compound eye which differed remarkably in the wave form of extracellular response and also in the arrangement of cells in the ommatidium. However, the results of the present study are not sufficient to make clear the relation between the wave forms of intra- and extracellular responses and this problem seems to require further investigation.

The author wishes to express his thanks to Professor M. Kuwabara and Dr. H. Morita for their interest during the whole course of the experiment. His thanks are due to Dr. P. R. Ruck for his kindness in reading the manuscript and also to Mr. Y. Tominaga for his kindness in allowing him free use of the electron micrographs.

This work was supported in part by the Science Research Fund of the Ministry of Education of Japan. It is Contribution No. 96 from the Department of Biology, Faculty of Science, Kyushu University.

Received for publication, April 7, 1960.

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