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STUDIES ON THE ORIGINS OF ERG COMPONENTS OF THE CRAYFISH COMPOUND EYE

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Anthony Kam-Cheong Tsoi and Wilkin Wai-Kuen Cheung (1985) Studies on the origins of ERG components of the crayfish compound eye. *Bull. Inst. Zool., Academia Sinica* 24(1): 117-124. The origins of the crayfish's ERG was investigated by recording with microelectrodes at various locations of the compound eye. Previous experiments suggested that the diphasic ERG recorded extracellularly in the flies, moths, butterflies and some other rapidly flying diurnal insects were a summation of potentials generated from regions above and below the basement membrane. Since the two components are opposite in sign, they tend to cancel out one another depending on their relation in time and magnitude. This complexity causes fundamental problems when compound ERG is used in electrophysiological experiments. It is therefore important to study the dynamics of this phenomenon. Detailed analysis of the contribution of individual components is difficult because in these insects the basement membrane is rather thin, so that the components are always compounded. However, in the crayfish, the basement membrane is thicker and it is therefore possible to separate the potentials generated from regions above and below it. Moreover, it would be interesting to find if the basement membrane of the crayfish had been punctured and hence lost its insulating properties, the diphasic ERG recorded would be similar to that recorded in flies, moths and butterflies.

The electroretinogram, or ERG, is a mass electrical response of the eye to a light stimulus. In the almost 100 years since the insect electroretinogram was first recorded (Dewar and McKendrick, 1873), it has been the focus of many studies. The interest that it has generated is due to several factors, including the ease with which it can be detected, and its considerable variability and complexity. Its function as a clinical tool for retinal physiology and assessment has long been recognized by clinicians and researchers.

However, there is a considerable body of criticism against the use of ERG in the study of vision, mainly due to its difficulty in interpretation. It was evident that not all species of organisms produced ERG of similar shapes and that different locations of recording electrode gave further complications in the interpretation (Koopowitz, 1974).

Perhaps the most valid criticism on the interpretation of the ERG in arthropods is that the retina comprises an outpocket of the brain and therefore the ERG components may be contributed by the neurons of the optic lobe as well as from the retinal cells. As a result, a 'pure' ERG indicating retinal electrophysiology cannot usually be found.

In the past decade, the isolation and analyses of ERG components have been investigated (Autrum and Hoffmann, 1960; Heisenberg, 1971; McReynolds and Gorman, 1970; Naka and Kuwabara, 1959). However,

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they studied only the temporal aspects of the ERG components. Naka and Kuwabara (1959) found that the ERG of the crayfish *Procambarus* consisted of two components which responded to the onset and persistence of illumination respectively. However, the origins of the ERG components remained unidentified until the late 1960's (Heisenberg, 1971; Pak *et al.*, 1969; Swihart, 1969, 1971). Heisenberg (1971) suggested that the ERG components might originate from the retina and lamina.

The work of these investigators, however, did not solve the problem about the variations of ERG features in different organisms. The question whether different waveforms of ERG recorded in the compound eyes of different kinds of organisms are basically analogous and comparable to one another remains to be solved. The aim of the present study is an attempt to answer this problem. It intends to show that waveforms of ERG recorded in different species of insects and crustacea, though apparently different, are comparable to one another. This study also attempts to give an explanation of the variations of ERG found in different recording locations by searching the dynamics of interaction between ERG components. Two experiments were done in this study: The first one was a demonstration of the two monophasic ERG found in the crayfish *Cambarus sp.* at different recording locations. The second experiment demonstrated that the ERG may change from monophasic to diphasic under certain conditions.

MATERIALS AND METHODS

a. Preparation

The preparation was similar to that described by Naka and Kuwabara (1959). The compound eye was removed from the crayfish *Cambarus sp.* with the rest of the eyestalk. A small hole was made at the centre of the corneal surface so as to insert the microelectrode vertically into the receptor layer.

b. Saline

The crayfish saline used is Van Harreveld's solution (Harreveld, 1936). The solution can be prepared following ingredients: 12.00 g NaCl, 0.40 g KCl, 1.99 g $CaCl_2.2H_2O$, 0.53 g $MgCl_2.6H_20$, 0.20 g $NaHCO_3$ per litre distilled water.

c. Electrode

Glass capillary tubing was pulled to a fine point by a micropipette puller. The electrode were filled with 3 M KCl. The electrode used in this study had a resistance value of about 10 megaohms.

d. Electrical measurement

The electrical potential was picked up by a biological amplifier and the signal displayed on an oscilloscope.

e. Stimulating light

White light from a tungsten halogen lamp was used as light source. The intensity could be attenuated by neutral density filters allowing a peak of 5mV with 1mV per division on the screen (Kong *et al.*, 1980). The light was collimated with glass lens and focused on the eye. The exposure time was varied by an electronic shutter delivering square pulses of light 100msec. duration. For ease in comparison, the duration of light stimulus could be fixed or varied from 100 msec. to 500 msec.

f. Histological preparation

Eyes were fixed with formal saline, dehydrated through an alcohol series and stained with Haematoxylin and Eosin and sectioned at 7 μm.

g. Experimental conditions

After 30 minutes of dark adaptation, the electrode was advanced and the ERG was recorded every 10 μm. Responses from three regions were recorded by camera. That is, the regions distal to, at and proximal to the basement membrane.

It is noteworthy that the positions of the three regions can be located with reference to the basement membrane. As the electrode was advancing from the region above the basement membrane to the region below it, a reverse

in polarity of the ERG occurred. Such polarity reversal therefore indicates that the electrode has penetrated through the basement membrane (Naka and Kuwabara, 1959; Swihart, 1969). Shaw (1975) also found that a rise in resistance could also be recorded if the electrode reached the basement membrane. In this study, both indicators were used to infer the position of the basement membrane. Other positions relative to the location of the basement membrane were determined using the scales read on the micromanipulator.

RESULTS AND DISCUSSION

Histology

The internal structures of the eye are similar to that described by Waterman *et al.* (1969), Kong and Goldsmith (1977). The length of the ommatidium and the distance between various regions were measured (Fig. 1). These measurements are very important for the estimation of the locations of the recording electrode.

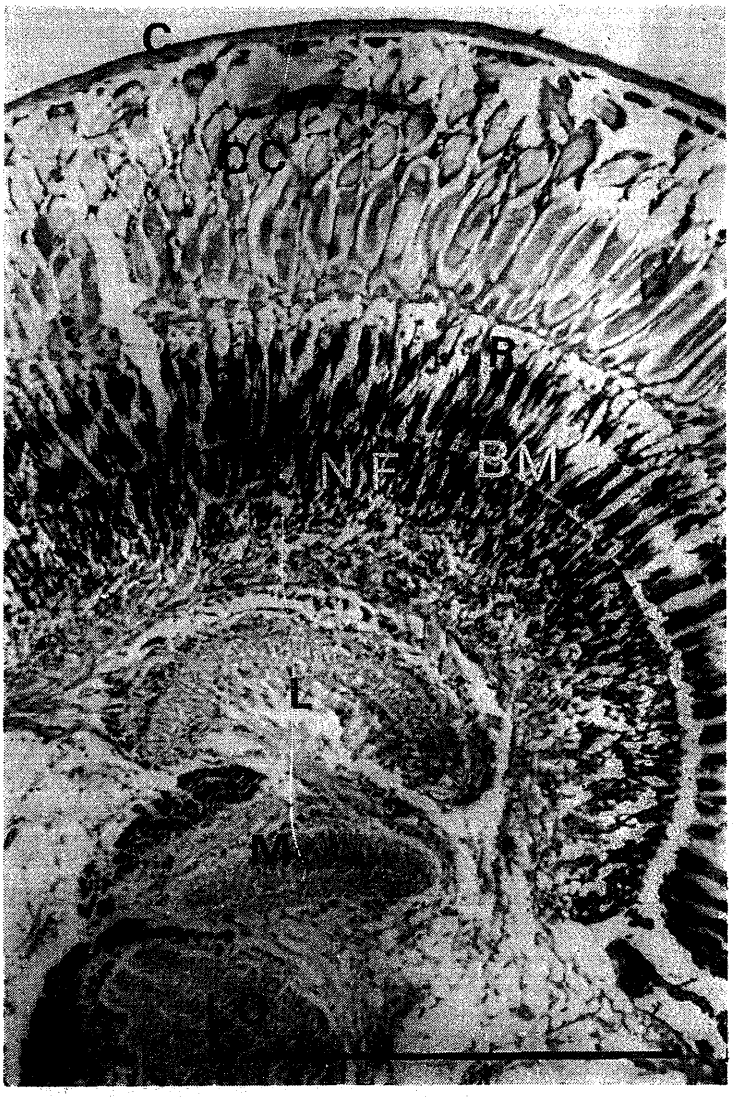

Fig. 1. Longitudinal section of crayfish (*Cambarus* sp.) compound eye, showing cornea (C), crystalline cone (CC), retinula (R), basememt membrane (BM), nerve fibres (NF), lamina (L), medulla (M), and lobula (LO). Vertical bar: 500 μm.

The ERG in different recording locations (First experiment)

a. The receptor ERG

As noticed from Fig. 2, the ERG is purely monophasic with a negative amplitude, similar to that recorded by Stieve *et al.* (1978) on the retina of the crayfish *Astacus leptodactylus*. This is believed to represent a summation of receptor depolarization. Increasing light intensity would only increase the amplitude; however, the monophasic nature remained unchanged.

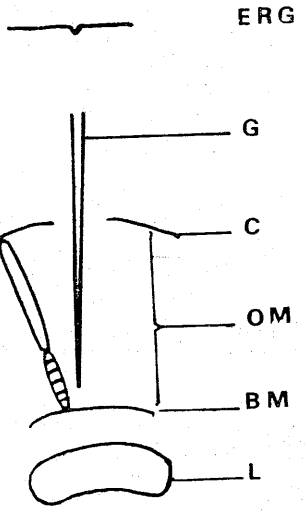

Fig. 2. The ERG recorded at region distal to the basement membrane, with glass electrode (G), cornea (C), ommatidia (OM), basement membrane (BM) and lamina (L) represented diagrammatically. The duration of light stimulus was 100 msec.

b. Recording at the basement membrane

Figure 3 shows that the resting potential

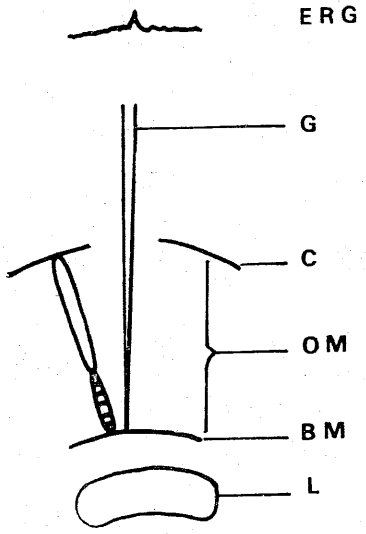

Fig. 3. The ERG recorded at the basement membrane. Abbreviations used are as above.

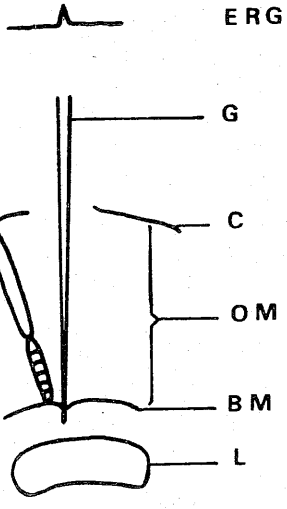

Fig. 4. The ERG recorded at region proximal to the basement membrane. Abbreviations used are as above.

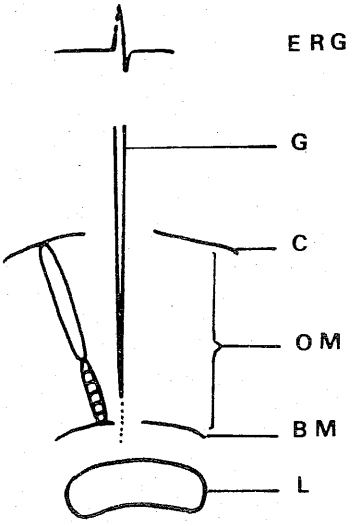

Fig. 5. The diphasic ERG recorded when the basement membrane had been ruptured. Abbreviations used are as above.

recorded in this position is highly irregular and light stimulation apparently causes little or no potential change. Also, the resistance recorded by the same electrode at this point was 3 to 4 times that measured in other locations. These results agree with Heisenberg's (1971) and Shaw's (1977) description of the "resistance barrier".

c. The laminal ERG

The ERG recorded from the region below the basement membrane is shown in Fig. 4. Like the retinal ERG, it is monophasic but is different in having a positive amplitude. Shaw (1968) and Heisenberg (1971) believed that it represented a summation of laminal hyperpolarization. As can be seen from the photographic traces, increasing the light intensity would only increase the amplitude; the monophasic nature remained unchanged.

Comparison between the ERG found in the crayfish Cambarus and other flying insects

The crayfish's ERG observed in the above experiment was all simple monophasic, whereas that observed in rapidly flying insects were complex and diphasic (Autrum, 1950; Swihart, 1971). Even in another species of crayfish *Procambarus clarkii* studied by Naka and Kuwabara (1959), the ERG was also diphasic.

To answer the question whether the strictly monophasic ERG found in the crayfish of this experiment was due to the insulating property of the basement membrane (Shaw, 1977), a second experiment was performed (Fig. 5). This is similar to the first experiment except that recording was made when the electrode was being withdrawn from the region below the basement membrane to the region above it.

The ERG recorded at three regions when the electrode was withdrawn (Second experiment)

When the electrode was withdrawn from a position about 100 μm below the basement membrane for only 10–20 μm, a small negative component appeared. As the electrode was withdrawn further this component increased and a very significant diphasic ERG could be observed (Fig. 5). Increasing the light intensity would increase both the positive and the negative amplitudes.

An obvious explanation for the above observations was that the negative ERG was generated from the retinal cell and the positive component was generated from the region below the basement membrane, probably the lamina. The two components, in an intact compound eye, were separated or insulated from each other by the basement membrane. However, when the basement membrane was pierced, it allowed the two components summated to give a diphasic ERG. However, the difference between the two components was not simply a reverse of polarity. As noted by Wolbarsht *et al.* (1965) and Heisenberg (1971), the negative component was different from the positive component in that the former was a sustained potential and the latter was a transient one (Heisenberg, 1971; Naka and Kuwabara, 1959). In the present study, such features of the two components can be obviously seen when the duration of light exposure was prolonged to 500 msec (Fig. 6). The response in Fig. 6a was observed when the electrode was above the basement membrane. When comparing with Fig. 2, the response here is sustained. Fig. 6b was recorded when the electrode had penetrated through the basement membrane and reached the region below it. Since the electrode was tapered in itself and the hole in the basement membrane was plugged, therefore the response is positively monophasic. Finally, when the electrode was withdrawn and the two components began to mix, a diphasic ERG was obtained (Fig. 6c). In this ERG, the negative component is sustained and the positive component is transient like their counterparts in the monophasic responses.

To test that the response in Fig. 6c is a linear sum of the two in Figs. 6a and 6b, the amplitudes of the latter two traces were measured on a graph paper and added at discrete

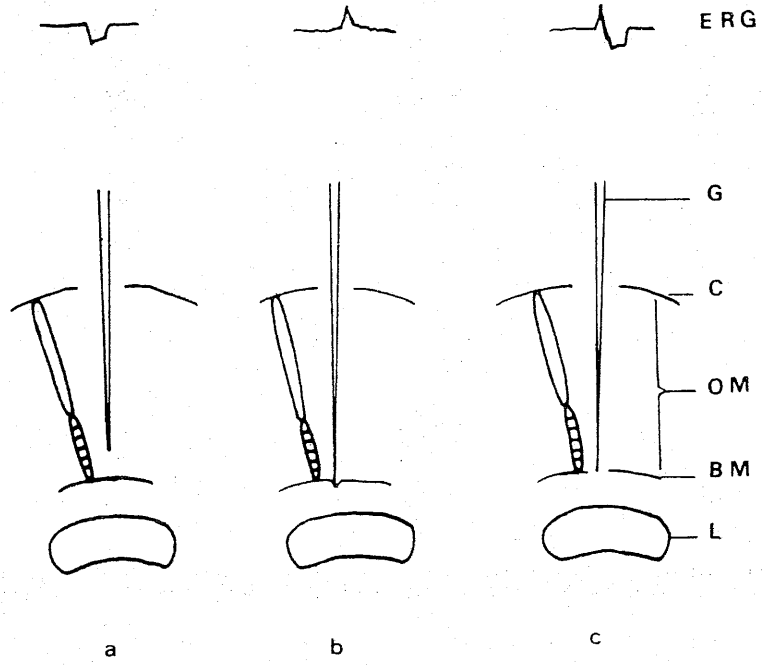

Fig. 6. Recordings made at different locations with duration of light stimulus 500 msec:
(a) at region distal to the basement membrane.
(b) at region proximal to the basement membrane.
(c) at the basement membrane after the electrode had withdrawn from position 'b'.
Abbreviations used are as above.

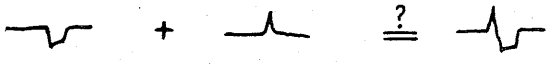

Fig. 7. Summation of observed ERG components deduced from Fig. 6.

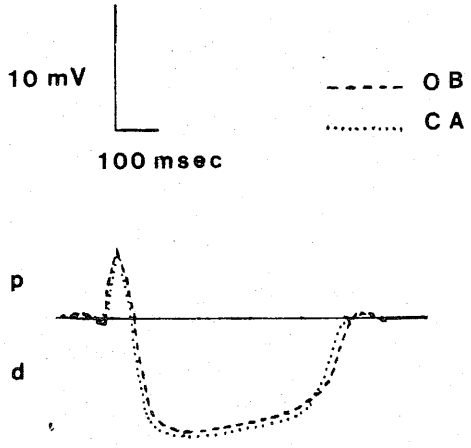

Fig. 8. Comparison between the observed ERG (OB) and the calculated ERG (CA) obtained from the distal region component (d) and the proximal region component (p).

points in time and compared with the observed ERG in Fig. 6c (Fig. 7). It can be seen that the calculated and observed traces are very similar (Fig. 8).

CONCLUSION

In the past decade, the origin of the positive components of the ERG has been a controversial issue. Ruck (1962) believed that they were due to passive current fluxes associated with activity in the receptor axon. Swihart (1972) suggested that the eccentric cells which was found in moth and butterfly to be the source of ERG positive component. Autrum (1950, 1952, 1958) considered that it was the distal neuropile (lamina) to be the origin of positive component. Obviously, the findings of the present study supported Autrum's postulation.

The occurrence of the diphasic ERG found in other insects is probably due to their thinner basement membrane. The crayfish

has a comparatively thicker basement membrane, therefore its intact eye has monophasic ERGs.

It should be pointed out that the positive component isolated in this experiment should not be regarded as a 'pure' component generated from the lamina, since the positive component *per se* may be composed of many components generated from different types of neurons in the lamina (Trujillo-Cenoz, 1965). In referring to the work of Nassel (1977) on the microscopic investigation on the type and arrangement of neurons in the crayfish optic lamina, there were five classes of neurons which summed up to no less than twelve types of neuronal cells were identified. Therefore, the work on the isolation of ERG components still has a long road to go.

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蜥蜴的視網膜電圖的分子電位來源研究

蔡 錦 昌　　張 偉 權

這實驗是用微電極管放在蜥蜴複眼基底膜內的不同位置，來找出視網膜電圖的來源。以往的研究，多以爲那在蒼蠅、蛾、蝶及其牠晝出夜伏的昆蟲看到的雙相視網膜電圖，是由基底膜上部和基底膜下部所產生的不同電位彼此合成而產生。由於這兩個電位彼此不同極，故在合成的時候，因强弱和時間上的差異而互相消減。這種複雜的現象使視網膜電圖應用在電生理學實驗時，產生了基本的繁雜問題。故我們研究其中的機制是一件十分重要的事。

由於一般昆蟲複眼中的基底膜較薄，故要從較複雜的合成電位中分析那個是構成電位的分子組成，這是很困難的。但在蜥蜴的複眼，由於基底膜較厚，故可分隔基底膜上部和下部所產生的分子電位。再者，當蜥蜴的基底膜被刺破因而失去絕緣性時，所產生的雙相電位的形狀，就與在蒼蠅、蛾和蝶所看到的極爲相似。