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THE TIME COURSE OF THE ELECTRORETINOGRAM OF COMPOUND EYES IN INSECTS AND ITS DEPENDENCE ON SPECIAL RECORDING CONDITIONS

BY M. KUGEL*
Lehrstuhl für Biokybernetik, Universität Tübingen, F.R.G.

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SUMMARY

Electroretinograms recorded from slowly moving insects like the mealworm beetle *Tenebrio molitor* and the stick insect *Carausius morosus* are shown to be distorted by the use of electrodes of stainless steel and silver/silver chloride wires, unless they are used in conjunction with amplifiers having extremely high input resistance. Undistorted electroretinograms can also be recorded using micropipettes filled with a suitable electrolyte. The undistorted ERG of *Tenebrio molitor* is monophasic, as described by Autrum (1950) for *Dixippus*, and as expected from his rule.

INTRODUCTION

Electroretinograms (ERGs) elicited by rectangular light stimuli from the compound eyes of insects may be classified as 'monophasic' or 'diphasic'. Autrum (1950) postulated that ERGs of slowly moving insects belong to the monophasic or '*Dixippus*'-type, whereas the diphasic ERG is typical for the eyes of fast-flying insects ('*Calliphora*'-type). Support for this hypothesis has been provided by some investigations (e.g. Kirschfeld, 1959, 1961; Hassenstein, 1957; Dudek & Koopowitz, 1973) but not by others (Ruck, 1958; Mazokhin-Porshnyakov, 1969; Yinon & Auerbach, 1969; Yinon, 1970*a*, b, 1971). However, it may be misleading to compare results obtained with different recording techniques. The present study investigates this by employing different electrodes and an amplifier having variable input resistance to record ERGs from the compound eye of the mealworm beetle *Tenebrio molitor*. Yinon & Auerbach (1969) and Yinon (1970*a*, *b*, 1971) recorded diphasic ERGs from *Tenebrio*, in apparent controversion of the hypothesis of Autrum (1950). It will be shown that their results must be considered a consequence of their recording technique.

METHODS

Experimental animals were adults of both sexes of the mealworm beetle *Tenebrio molitor* L., the stick insect *Carausius morosus* L., and the blowfly *Calliphora erythrocephala* Meig. All animals were bred at a temperature of about 20 °C, with relative air-humidity of 50–60% and with artificial day–night illumination (30 lux).

The following types of electrodes were used: (1) entomological stainless-steel

* Present address: Sektion Neurophysiologie, Universität Ulm, F.R.G.

needles with tip diameter of about 15 μm; (2) silver/silver chloride wires (Ag/AgCl) with tip diameter of about 20 μm, plated electrolytically in 0·5 M-KCL at 1 mA per cm^2 for 45 min in the dark, and used immediately after production; (3) glass micropipettes with outer tip diameter of about 15 μm, filled with insect Ringer solution. The pipettes were connected to the amplifier via Ag/AgCl wires. The composition of the Ringer solution was (in g/l H_2O): NaCl, 7·6; KCl, 0·75; $CaCl_2$, 0·22; $MgCl_2$, 0·19; $NaHCO_3$, 0·37; NaH_2PO_4, 0·48.

The insects were fastened with a special glue (Stabilit express, Henkel) on a black cork surface which was placed on a microdrive. Legs and antennae were amputated and the head was fixed. The recording electrode was inserted just beneath the cornea of the compound eye (100–200 μm depth); the reference electrode into the subcuticular region of pro- or mesothorax. The position of both recording and reference electrodes did not affect the shape of the ERG. Both electrodes were of the same type in a given experiment.

The potentials were preamplified with a d.c. amplifier (F. Haer 74–20–1) which allowed a stepwise variation of the input resistance up to 10^5 MΩ. A d.c.-coupled oscilloscope was used for monitoring the ERGs which were photographed from the screen. In all oscillograms downward deflection indicates negative polarity.

For stimulation, a d.c.-operated 12 v, 100 W halogen lamp was used, and the light beam directed on to the eye of the animal by means of an optical system. The animals were placed in an electrically shielded, light-proof cage. The intensity of the light beam could be reduced with neutral-density filters (NG·9, Schott & Gen.) over 7 log units. The stimulus duration was controlled by a photographic shutter. With the highest-intensity stimulus (log $I=0$ relative units) an illumination of about 4×10^4 lux was measured at the corneal surface.

RESULTS AND CONCLUSIONS

Fig. 1 shows illumination potentials from the compound eye of *Tenebrio molitor* recorded by means of the three different electrodes (columns A, B, C) with different input resistances (R_E) of the preamplifier (rows *a*, *b*, *c*). Eyes were dark-adapted (5 min) except for curves labelled 'L', where the eyes were light-adapted (see Fig. 1 legend).

The potentials recorded with steel needles and input resistances of 1 or 10 MΩ (Fig. 1 A (a), (*b*)), resemble those recorded by Yinon & Auerbach (1969) and Yinon (1970*a*, *b*, 1971). They show a steep negative-going 'ON' response followed by a decay to the baseline, and a positive-going 'OFF' response after cessation of the stimulus. Yinon employed entomological steel needles of 13 μm tip diameter and 30 KΩ resistance, but did not state the amplifier input resistance.

The potentials recorded with Ag/AgCl electrodes and an R_E of 1 MΩ (Fig. 1 B (*a*)) also showed negative-going ON peaks, but no measurable positive-going OFF peaks. Following the ON peak the potential returned very slowly to a negative plateau value, which remained constant throughout the further illumination (Fig. 1 B (*f*)).

With Ringer-filled microelectrodes, similar potentials were recorded with different values of R_E. These potentials resembled those recorded with the other electrodes at an input resistance of 10^5 MΩ.

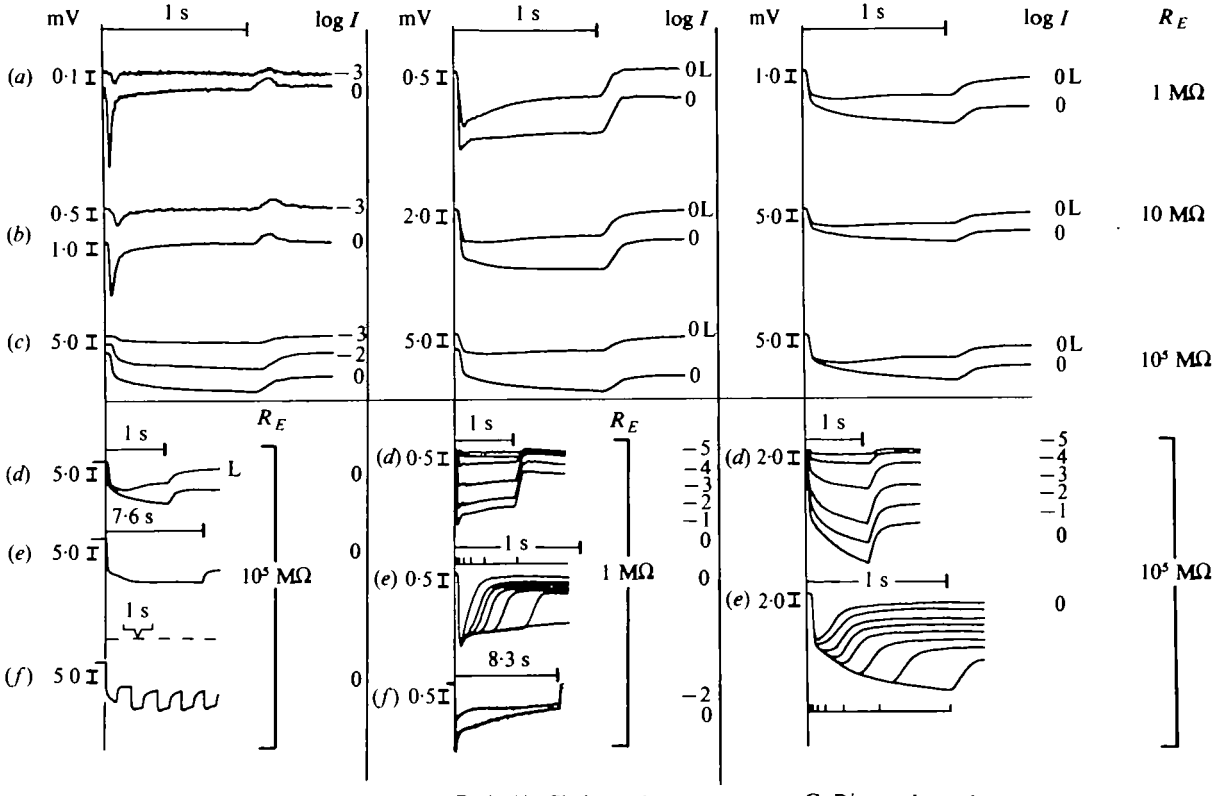

Fig. 1. Illumination potentials recorded from the compound eye of *Tenebrio molitor*. Steel, Ag/AgCl and micropipette electrodes were used for the traces in columns A, B and C respectively. R_E: Amplifier input resistance. Numbers on the left of each trace indicate mV per vertical calibration bar. Log I: stimulus intensity (rel. log units). The stimulus duration was: in B (e), 0·008, 0·033, 0·066, 0·125, 0·25, 0·5 and 1 s; and in C (e), 0·008, 0·166, 0·033, 0·066, 0·125, 0·25, 0·5 and 1 s. Potentials were recorded from the dark-adapted (5 min) eye except those labelled 'L', which were elicited from the light-adapted eye, pre-stimulated with three stimuli of 1 s duration and an inter-stimulus interval of 1 s as shown in A (f). The intensity of the prestimulus was log I = 0 (rel. log units). All other parameters are indicated in the figure. Except C (e), all potentials were recorded from the eye of one individual. The recording electrodes were inserted at the same position upon the eye in all cases, although the position of the electrodes did not affect the shape of the potentials.

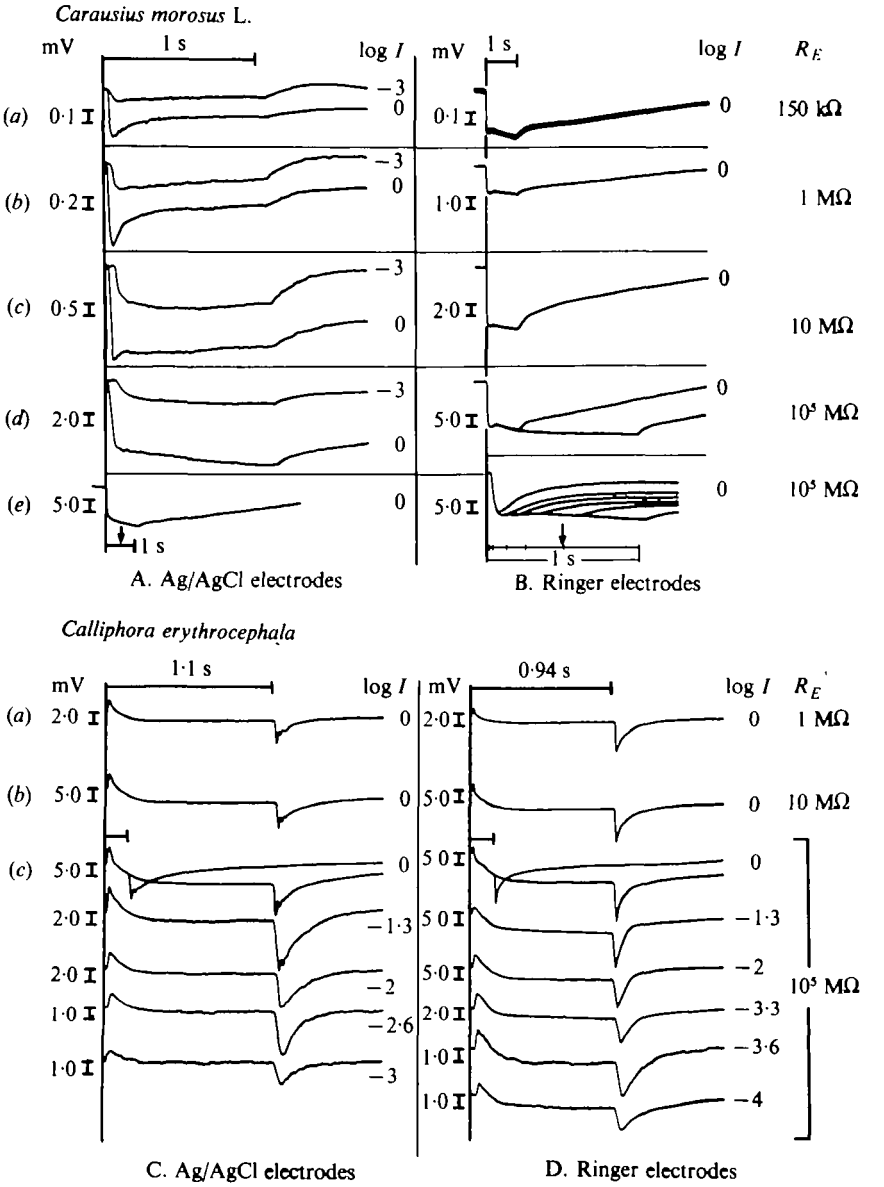

Fig. 2. Illumination potentials from the compound eyes of *Carausius morosus* and *Calliphora erythrocephala*. For labelling see legend to Fig. 1. The stimulus duration was: in B (*d*), 1 and 4·7 s; in B (*e*), 0·008, 0·033, 0·125, 0·25, 0·5 and 1 s; in C (*c*) and D (*c*), 0·125 and about 1 s. Other parameters, see figure. The potentials of *Carausius* were elicited from one eye in one animal, those of *Calliphora* in C and D from two eyes in two different animals.

Light adaptation resulted in the monophasic potentials reaching their maximum earlier than with dark adaptation (Fig. 1).

The shape of the ERG of *Carausius morosus* depended upon the properties of electrodes and the value of R_E in the same fashion as that of *Tenebrio molitor*, whereas

the shape of the ERG of *Calliphora erythrocephala* did not, and was always diphasic (Fig. 2).

These results demonstrate that the ERGs of both slowly moving insects investigated here are monophasic, if recorded either by means of Ringer electrodes or metal electrodes with an amplifier of high input resistance. This is in accordance with the rule of Autrum (1950) and contrary to the finding of Yinon (1970*a*, *b*, 1971). The recording, in this study, of diphasic potentials with metal electrodes can be attributed to the polarization of these electrodes. The properties of metal electrodes are discussed extensively by Schwann (1963).

To examine the transmitting properties of the electrodes used, two of the same type were dipped into an insect Ringer solution (depth about 2 mm; distance from each other, 5 mm). One of them was connected to an oscilloscope, the other to a pulse generator providing rectangular voltage pulses at a frequency of 0·5 pulses/s and an amplitude of 10 mV. Generator and oscilloscope had a common ground. Only the potentials transmitted by Ringer electrodes were not distorted. They were not distorted even at low values of R_E and at frequencies up to 60 pulses/s. Steel needles, and, to a lesser extent, Ag/AgCl electrodes, have the transmitting properties of a high-pass filter. Similar results were obtained using the body of *Tenebrio* instead of the Ringer solution as the medium between the electrodes. It can therefore be assumed that the body of the animal does not contribute to the distortion of the true ERG.

Because a polarizable electrode has the transmitting properties of a high-pass filter, it transmits voltages only if their change in time is sufficiently fast. The lower the imput resistance of the amplifier, the more current flows through the electrode, and the more the electrode is polarized. This accounts for the (gradual) transition of the diphasic potentials at low input resistance. Since the truly diphasic ERG of the *Calliphora* type (positive-going ON peak, negative plateau and negative-going OFF peak) changes fast enough in time, electrodes with the properties of high-pass filters do not influence appreciably the time-course of the ERG. The only effect of increasing amplifier input resistance can be to increase the amplitude of the potential due to the change in the voltage divider formed by electrode and amplifier input.

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