On-Transient of Insect Electroretinogram: Its Cellular Origin

Abstract. *Electrical responses to light stimuli were recorded intracellularly from the retinula cells and the laminar cells of the wild type and the x-7 mutant of* Drosophila melanogaster *in order to determine the cellular origin of the on-transient of the insect electroretinogram. The response of the retinula cell of the mutant x-7 closely resembles that of the wild type even though the on-transient is absent from the electroretinogram of x-7. Neither contains a spike-like component that can be identified with the on-transient of the electroretinogram. However, a spike-like response resembling the on-transient in latency and time course has been obtained from one of the several types of cells in the lamina of the wild type. Moreover, the same response could not be obtained from the laminar cells of the mutant x-7. Thus, the on-transient does not appear to originate from the retinula cell but probably from one or more of several types of cells in the lamina.*

Much work has gone into deciphering the mass response of the eye recorded as the electroretinogram (ERG). In many insects the ERG consists of a corneal positive, spike-like "on-transient," followed by a sustained negative wave that lasts throughout the entire illumination period. After cessation of the light stimulus, a corneal negative "off-transient" follows. Evidence accumulated to date strongly suggests that the sustained negative wave consists mainly of the receptor potential whereas the off-transient has its origin in the higher order elements (*1–3*). The site of origin of the on-transient is less certain. Some investigators have implicated the retinula cell or its axon (*4, 5*), whereas others have presented evidence that it occurs in the postsynaptic elements (*1, 6, 7*). Recently, Eichenbaum and Goldsmith (*7*) showed that the on-transient is absent in a compound eye of the fly consisting only of the retinula cells and their axons; such an eye was obtained by transplanting the larval eye disk to the abdomen of the host larva and allowing it to metamorphose to adult form. Their results suggest that neither the retinula cell nor its axon is responsible for the on-transient.

We elected to reexamine this problem using ERG mutants of *Drosophila* and to attempt to identify the cell responsible for the on response. We have compared the intracellular responses from the retinula and the laminar cells of *Drosophila melanogaster* wild type with those of the mutant x-7. The mutant x-7 is an allelic form of *tan* (*8*) and was isolated in our laboratory (*3*). It is non-phototactic, and its ERG lacks both the on- and off-transients (*3*). Histologically, no obvious differences have been observed between the retina and lamina of x-7 and those of the wild type either with the light or electron microscope (*3*).

The experimental animals were males from wild-type and x-7 strains or from the same strains placed on a white-eye (*w* or *bw;st*) (*8*) background. Intracellular recordings were made by means of micropipettes filled with 2*M* KCl or 6 percent aqueous solution of Procion yellow (*9*). The KCl electrodes had resistances of over 100 megohms. The electrode was introduced into the retina from a small hole made on the cornea with a glass micropipette having a large tip diameter and used as the reference electrode. The reference electrode was then inserted into the proboscis. In order to identify the cell from which the response was obtained, the cell was injected with Procion yellow (*9*). Since the screening pigments in the eye would obscure the marked cell, only white-eyed strains were used for this work. We found no evidence that the genes used to provide the white-eye background have any effect on either the retinular or the laminar activity.

The intracellularly recorded receptor potential of *Drosophila melanogaster* is similar to those obtained from larger flies (*4, 10*) both in waveform and time course, although the amplitude tends to be smaller. It is a depolarizing potential consisting of a dynamic phase, appearing at the leading edge of the potential, and a steady phase lasting the whole illumination period (Fig. 1, A and B). The dynamic phase is small or absent at low stimulus intensities but becomes increasingly prominent as the stimulus intensity is raised.

If the on-transient is generated by the retinula cell, one would expect it to appear in the intracellular recording from the retinula cell of the wild-type fly whereas no on-transient should be recorded from the retinula cell of x-7. As may be seen in Fig. 1, this is not the case. Except for the small "off effect" displayed by the wild-type response, there is no obvious difference in the waveform of the intracellularly recorded receptor potential of wild type (Fig. 1B) and x-7 (Fig. 1A) over a 4-log unit range of stimulus in-

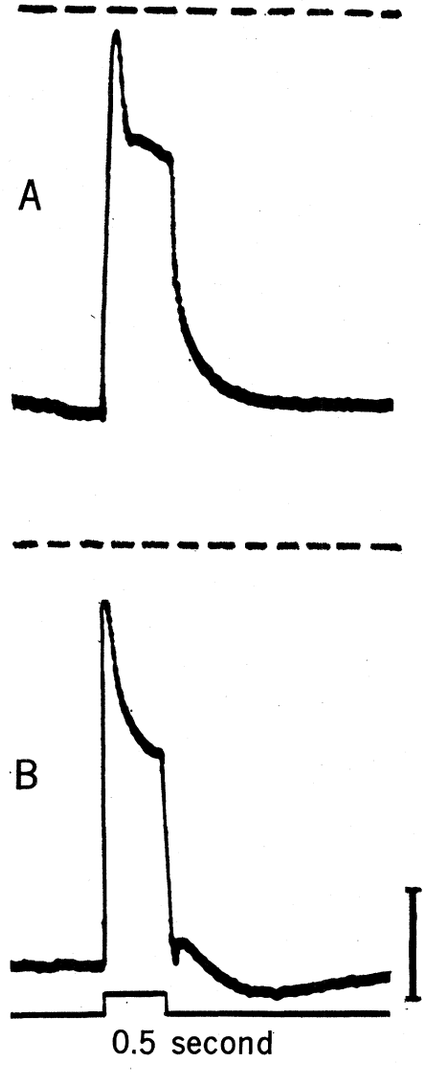

Fig. 1. Intracellular response to illumination obtained from the retinula cell of (A) x-7 mutant and (B) wild-type *Drosophila melanogaster*. A blue-green light stimulus (wavelength, 500 nm) 0.5 second long was used. The resting level of the membrane potential is indicated by the dashed line. Scale, 5 mv.

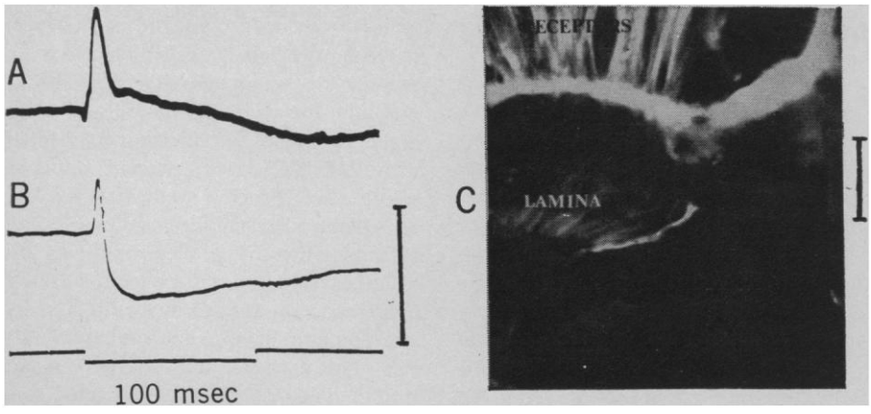

Fig. 2. (A) An intracellular recording from a cell in the lamina. (B) An extracellularly recorded ERG response. In both cases the stimulus was a 500-nm wavelength applied for 100 msec. Scale, 10 mv. (C) A histological section of the compound eye of a wild-type fly placed on a white-eye background, showing the fluorescent dye injected into a laminar cell after a record similar to that shown in (A) had been obtained. The white-eye background was used to eliminate the screening pigments, which otherwise would mask the dye spots. Scale, 40 μm.

tensity. Neither contains the spike-like component at the onset of the response that can be associated with the ERG on-transient. Even at much faster sweep speeds no evidence of any inflections due to spike-like components has been observed on the rising phases of the retinular responses.

The resting potentials of the retinula cells appear to be rather low, amounting to only about −20 mv. However, the low resting potentials do not appear to be due to possible injuries which might abolish the on-transient. In the first place, the retinular responses have been held for over an hour without substantially affecting the waveforms. Moreover, the spike-like component can be readily recorded from the laminar cells by means of the same type of electrodes, even though their resting potentials are also low. The resting potentials of *Drosophila* visual cells probably are normally low, although reliable measurements of resting potentials are difficult to make with the high-resistance electrodes required in this work.

Some investigators have suggested that the dynamic phase observed in intracellular recording may be responsible for the on-transient of the ERG (*5*). If this were true, one would not expect to find the dynamic phase in the receptor potential of x-7, since the x-7 ERG does not contain the on-transient. In reality the dynamic phase is just as prominent in the receptor potential of x-7 as in the wild type (Fig. 1, A and B). Moreover, as Eichenbaum and Goldsmith (*7*) have pointed out, the properties (polarity and time course) of the dynamic phase are very different from those of the on-transient. Our findings agree with their observations. Also, the latency of the dynamic phase is shorter than that of the on-transient.

However, the possibility that the on-transient is generated in the retinula cell axon but not recorded by the electrode in the soma cannot be discarded. In the case of *Musca*, Eichenbaum and Goldsmith (*7*) have tested this hypothesis using the isolated, intact photoreceptor cells obtained by transplantation. They found that the axons of these cells do not respond to photic stimulation with spikes. In this connection, an electrical model of the *Drosophila* retinula cell that we have constructed is also revealing. We find that if a stimulus resembling the on-transient in frequency composition is applied to the proximal end of the axon of the model, almost 20 percent of its amplitude can be detected at the distal end of the soma. It thus appears unlikely that the on-transient, if generated in the axon, could escape detection by an electrode placed in the soma.

If the on-transient of the ERG does not originate from the retinula cell or its axon, where does it come from? The most likely possibility appears to be one of the several types of cells in the lamina. Several different types of activity, both depolarizing and hyperpolarizing, have been obtained intracellularly from the lamina of the wild-type fly. The response we encountered most frequently is shown in Fig. 2A and is compared with the wild-type ERG (Fig. 2B) (*11*). The dye spot obtained after a recording similar to the one displayed in Fig. 2A is shown in Fig. 2C. The response was obtained after a d-c drop of 25 to 30 mv. It consists of a depolarizing, graded, spike-like potential 10 to 15 msec long with an amplitude of 15 to 20 mv at the "on" followed by a depolarizing steady response of much smaller amplitude (Fig. 2A). (By contrast the time course of the dynamic component of the retinula response is about an order of magnitude slower than the spike response of the laminar cell.) Both the latency and time course of the spike-like response of the laminar cell resemble those of the on-transient of the ERG (Fig. 2, A and B) and, indeed, depend on stimulus intensity in much the same way as those of the on-transient of the ERG. If the x-7 mutant, whose ERG lacks the on-transient, is used in these experiments no spike-like response can be obtained from the lamina even though d-c drops signaling penetrations of cells are readily obtained and dye spots similar to the one displayed in Fig. 2C have been recovered. We thus conclude that in all likelihood the on-transient of the ERG represents an extracellular current flow associated with the light-evoked potential in one or more of the several types of cells in the lamina. However, we are open to the possibility that these cells may be glial cells in the lamina.

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11. To our knowledge the records obtained by F. Zettler and M. Järvilehto from *Calliphora* [*Z. Vergl. Physiol.* **68**, 202 (1970)] are the only previously published intracellular records of laminar responses of the higher Diptera that are accompanied by electrode marking experiments. Their records show the waveforms of the laminar and retinular responses to be quite similar. Their subsequent investigations, however, seemed to indicate that these were recorded from the processes of the retinula cells [M. Järvilehto and F. Zettler, *ibid.* **69**, 134 (1970)]. Recently the same group also reported on a hyperpolarizing monopolar neuron in the lamina of *Calliphora* [H. Autrum, F. Zettler, M. Järvilehto, *ibid.* **70**, 414 (1970)].
12. We thank Drs. J. Grossfield, L. Pinto, and T. H. Goldsmith for advice and criticism of the manuscript. Supported in part by NSF grant GB-24666.

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Hycanthone Resistance: Development in Schistosoma mansoni

Abstract. *Following the administration of relatively high doses of the antischistosomal drug hycanthone to mice and hamsters infected with* Schistosoma mansoni, *a number of the worms survived. After a period of 6 to 12 months these parasites resumed production of viable eggs that gave rise to schistosomes that proved resistant to hycanthone and to two other related antischistosomal compounds. This drug resistance has remained stable for three subsequent generations of worms.*

The activity of the thioxanthone derivative hycanthone against *Schistosoma mansoni* in experimental animals and man has been reported (*1–5*). It has been stated that a single intramuscular injection of 50 mg per kilogram of body weight to mice infected with *S. mansoni* results in the elimination of over 91 percent of the worms and that only one-fourth of this dose is required to destroy 98 percent of these parasites in hamsters (*2, 3*).

In an attempt to explore the mode of the antischistosomal action of hycanthone, it was noted that its onset of action is very slow. After the intramuscular administration of 60 mg of hycanthone per kilogram to mice infected with a Puerto Rican strain of *S. mansoni,* a complete shift of the worms from the mesenteric veins to the liver was observed only after 9 to 10 days. After 3 weeks most of the parasites recovered in the liver remained motionless when placed in 75 percent horse serum, but many of them exhibited motor activity when they were incubated in the same medium containing 5-hydroxytryptamine ($5 \times 10^{-5} M$) (*6*). These surviving worms were damaged functionally and morphologically. For example, alterations in the reproductive system of the females were similar to those observed after administration of other antischistosomal compounds (*7, 8*), the glycogen stores of the males were greatly reduced, both sexes appeared stunted, and their wet weight was decreased. Four to 12 months after the administration of the drug, live worms were found in 257 out of a total of 268 mice. Most of the schistosomes were located in the mesenteric veins. While after 4 months the reproductive system of most females gave the appearance of being damaged, a progressive recovery occurred thereafter, and after 12 months few, if any, abnormalities were noted. This coincided with the reappearance of live eggs in the liver of the host. The miracidia hatching from these eggs were infective to the intermediate host of *S. mansoni*, the snail *Biomphalaria glabrata.* After a period of 5 to 6 weeks the snails that had been infected with these miracidia were shedding cercariae that, in turn, were infective to mice and gave rise to adult schistosomes resistant to hycanthone. Intramuscular administration of hycanthone (80 mg/kg) to the host produced no hepatic shift, no damage to the female reproductive system, no weight loss, and no glycogen depletion in the progeny of the worms that had recovered from the effects of the drug. Administrations of the same dose, repeated four times at intervals of one or several days, were equally ineffective. Miracidia hatched from eggs produced by these hycanthone-resistant worms (F_1) gave rise to a second generation (F_2) of hycanthone-resistant schistosomes. This resistance has remained stable for two subsequent generations (F_3 and F_4).

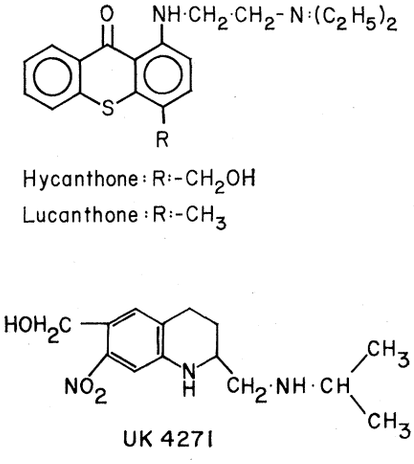

Fig. 1. The structures of hycanthone, lucanthone, and UK4271 (an aminoalkyl tetrahydroquinoline).

The appearance of this type of drug resistance was observed without exception in 163 mice infected with *S. mansoni* originating from eggs deposited in the livers of nine out of nine schistosome-infected mice to which a single dose of hycanthone (30 or 60 mg/kg) had been administered 6 to 12 months previously. This was not limited to the mouse host. A similar pattern was observed in hamsters infected with *S. mansoni.* The treatment of these animals with hycanthone (16 mg/kg, single intramuscular dose) resulted at first in a complete hepatic shift, but 8 months thereafter paired worms (approximately 10 to 20 percent of the number found before treatment) had reestablished themselves in the mesenteric veins. The miracidia hatched from the eggs of these worms gave rise, in hamsters, to schistosomes that were resistant to hycanthone. Intramuscular administration to these hamsters of doses of hycanthone as high as 80 mg/kg had no effect on the worms. This resistance has remained stable in the hamster host for two subsequent generations (F_2 and F_3).

Hycanthone-resistant schistosomes exhibited cross-resistance to two chemically related antischistosomal compounds, lucanthone (*9*) and an aminoalkyl tetrahydroquinoline (UK4271) (*10*) (Fig. 1). An alkylaminoalkyl group in the side chain is a structural feature common to these three compounds. By contrast, hycanthone-resistant schistosomes proved as susceptible to the effects of a nitrovinylfuran (*11, 12*) as hycanthone-susceptible worms.

It remains to be determined whether, in geographic areas where schistosomiasis is endemic, eggs excreted by human subjects previously treated with hycanthone give rise to strains of schistosomes resistant to this drug. Furthermore, the dose of hycanthone used in humans is 5 to 20 times lower than those employed in this study for hamsters and mice, respectively. Yet