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The Effects of Increased Temperature on Electroretinograms of Temperature-Sensitive Paralysis Mutants of *Drosophila melanogaster**

(Diptera/vision/gynandromorphs/*shibire*^{ts}/receptor potential)

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ABSTRACT **Mutations within the *shibire* locus of *Drosophila melanogaster* are non-complementing alleles which result in reversible paralysis at 29° but retention of normal locomotor behavior at 22°.**

Electroretinograms of six of the mutants have been recorded at various temperatures. Two changes occurred in the electroretinograms of flies carrying most of the alleles at high temperature: they lost the "on"- and "off"-transients of the normal electroretinogram, and the fast decay of the receptor potential was attenuated. For flies with four of the alleles, a base-line oscillation was also observed.

Analysis of electroretinograms of mosaic *shibire* flies indicates that the loss of the transients can be attributed to both a pre-synaptic and a post-synaptic effect.

The study of the visual system of Diptera has recently received a great deal of attention, stemming from the recovery of a number of nonphototactic mutants of *Drosophila melanogaster*, many of which show alterations in the electroretinogram (ERG) (1–3). The ERG responses of these mutants are varied: some show loss of certain components of the ERG, while others show severe reduction or loss of the entire response. In all cases studied so far, however, the primary alteration produced by the mutation has remained undetermined.

In this laboratory, we have been working with a number of sex-linked, temperature-sensitive (ts) paralytic mutants (4). The phenotype of these mutants is rapid paralysis at 29° and normal mobility at 22°. One allelic series of these mutants, *paralytic*^{ts}, has already been shown to have no effect on the ERG recorded at the non-permissive temperature (5). In this report, we describe an effect of elevated temperatures on the ERG responses of another allelic series of paralytic mutants, *shibire*^{ts} (*shi*^{ts}).

MATERIALS AND METHODS

Recording of the ERG. Flies were lightly anesthetized with carbon dioxide and mounted in soft wax on the end of a section of glass rod. The flies were held in the wax by their legs, proboscis, and tip of the abdomen. The reference electrode was inserted either into the thorax below the scutellum or into the proboscis. Two kinds of recording electrode have been used; either fine tungsten wire was inserted into the cornea to a depth of about 40 μm, or a fine cotton wick, saturated with 0.9% NaCl, was placed on the surface of the cornea. Flies were then exposed to a constant current of air, and when heating was required the air current was preheated with a variable heat element. Temperature was recorded from a thermistor placed alongside the fly on the mounting block. The light stimulus was produced from a Wild microscope lamp using a 7 V, 21 W tungsten bulb positioned 30 cm from the animal. A heat filter was placed between the light source and the fly. Exposure was controlled by a manually operated iris.

Construction of shi^{ts1} Mosaics. Gynandromorphs were generated by crossing *y w shi^{ts1} f* males to females carrying the unstable ring-X chromosome (*In(1)w^{vC}*). (For a complete description of the properties of the ring-X chromosome, see ref. 6 and for the mutants, consult ref. 7.) Loss of the ring-X chromosome shortly after fertilization results in flies possessing both *y w shi^{ts1} f/In(1)w^{vC}* female tissue and *y w shi^{ts1} f*/0 male tissue. The X/0 genotype on the chitin was detected by expression of *yellow* on the body or *white* in the eye. The eye color mutant, *carnation* (*car*), was used for the production of mosaics for the phototactic study.

RESULTS

The ERG of a wild-type Oregon-R fly subjected to a flash of light of long duration (Fig. 1) consists of a fast positive "on"-transient, a sustained negative wave which remains for the entire period of illumination, a negative "off"-transient, and finally a diphasic decay of the sustained negative wave. With a variety of techniques (8–10), it has been shown that the main negative wave of the ERG originates from the photoreceptor (retinula) cells, while the "on"- and "off"-transients are produced by second order neurons in the lamina post-synaptic to the retinula cells.

As a temperature of 40° was used in some of these experiments, the effects of this temperature on the ERG of wild-type flies was checked. The only difference from the 22° ERG was an increase in the amplitude of the "on"- and "off"-transients.

The effect of temperature on the ERG of the *shi^{ts}* mutants

At 23°, flies carying any of the *shi^{ts}* alleles gave approximately normal responses. On heating, however, the effects varied somewhat from allele to allele. The alleles *shi^{ts2}* and *shi^{ts6}* will be considered first. The restrictive temperature used for these alleles was 35°.

As *shi^{ts2}* or *shi^{ts6}* flies were being heated to 35°, the "on"- and "off"-transients became very much reduced and even-

Abbreviations: ERG, electroretinogram; ts, temperature-sensitive.

* This is part XIX of the series "Temperature-sensitive mutations in *Drosophila melanogaster*." Part XVIII is ref. 11.

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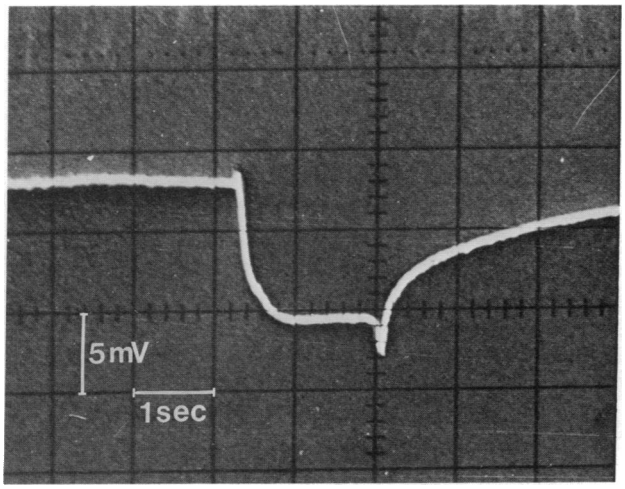


FIG. 1. D.C. recorded electroretinogram of wild-type Oregon-R at 23°.

tually disappeared (Fig. 2A). If the fly was then allowed to cool, the transients returned in a matter of seconds. In some preparations, at the time when the transients were lost, there appeared to be a delay in the production of the transients just before they disappeared entirely (Fig. 2B). One further point of note is that the fast decay of the receptor potential when the light is switched off is reduced at high temperature (compare Fig. 1 and Fig. 2A). This reduction in the fast decay is more pronounced with some of the other alleles (Fig. 3).

When the heating was continued up to 40°, the base-line recording began to oscillate (Fig. 4A). The frequency of this oscillation was temperature-dependent and could be increased by further increasing the temperature (Fig. 4B). Upon cooling the fly, the sequence of events was the reverse of that noted when the fly was being heated. In contrast to these observations, no change in the ERG was seen when wild-type Oregon-R flies were heated in the identical manner.

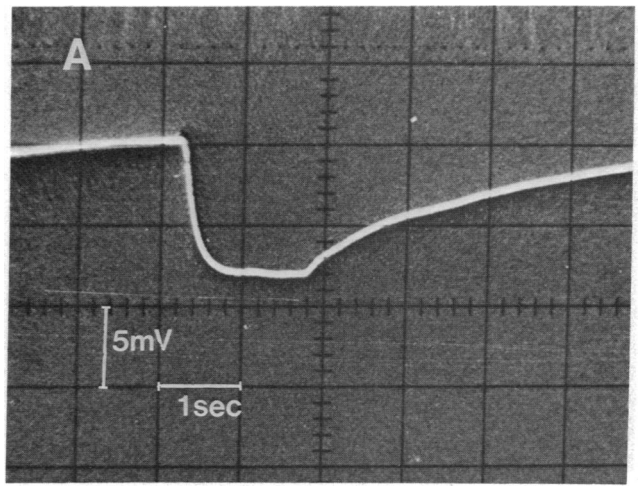
Flies carrying shi^{ts1} or shi^{ts3} behaved similarly to shi^{ts2} and shi^{ts6} upon heating. Furthermore, even at 23°, the "on"- and "off"-transients were much smaller than any recorded for the other shi^{ts} alleles or Oregon-R. This suggested that perhaps 23° was not completely permissive for these two alleles. Consequently, shi^{ts1} flies were cooled to 18°. This resulted in an increase in the amplitude of the transients, which indicates that 23° is not the true permissive temperature for these alleles.

The shi^{ts5} mutant exhibited the same effect as shi^{ts2} but required a temperature of 40° for the loss of the transients and no base-line oscillation was seen.

The remaining allele, shi^{ts4}, is known by its mutant phenotype to be the least debilitating, and even temperatures of 40° were insufficient to eliminate the transients, although they were reduced in amplitude.

In summary, all of the shi^{ts} alleles, with the exception of shi^{ts4}, lost both the "on"- and "off"-transients of the normal ERG, and all alleles showed some attenuation of the fast decay, with shi^{ts1} and shi^{ts3} having the most pronounced effect. Only shi^{ts4} and shi^{ts5} produced no base-line oscillation. The temperature needed to produce these effects varied for each of the alleles.

One other response of interest was noted on occasions when the reference electrode was placed under the scutellum into the thorax of the fly. Frequently under these recording conditions a series of spontaneous spikes was recorded (Fig. 5A) as the fly was being heated. As heating was continued, these spikes



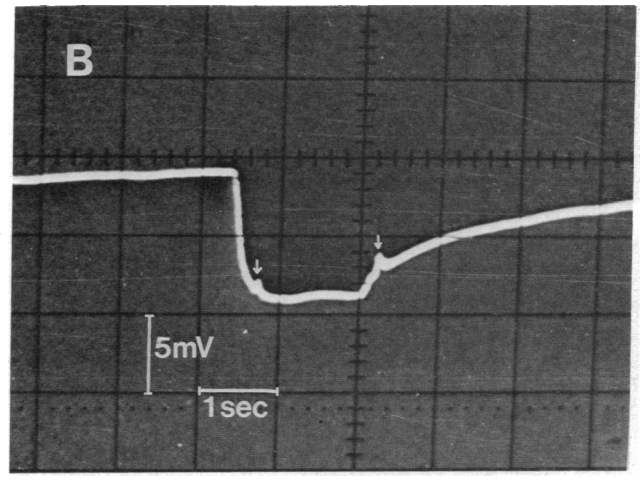


FIG. 2. (A) ERG of shi^{ts2} recorded at 35°. Note the loss of the "on"- and "off"-transients. (B) ERG of shi^{ts2} at the point when the transients were lost. Note the delay in the production of the transients (arrows).

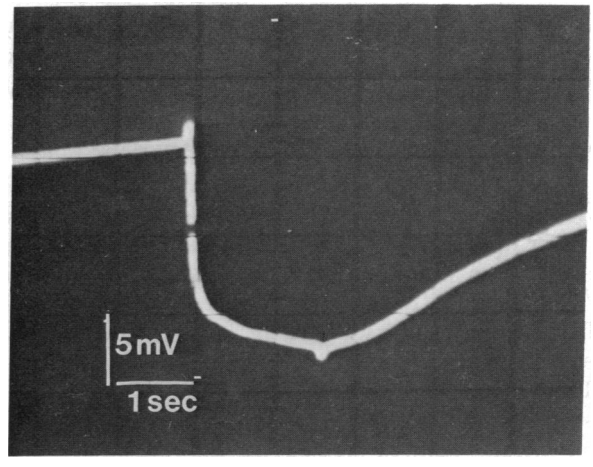


FIG. 3. ERG of shi^{ts1} at 28°. Note the absence of the fast decay after the light has been switched off.

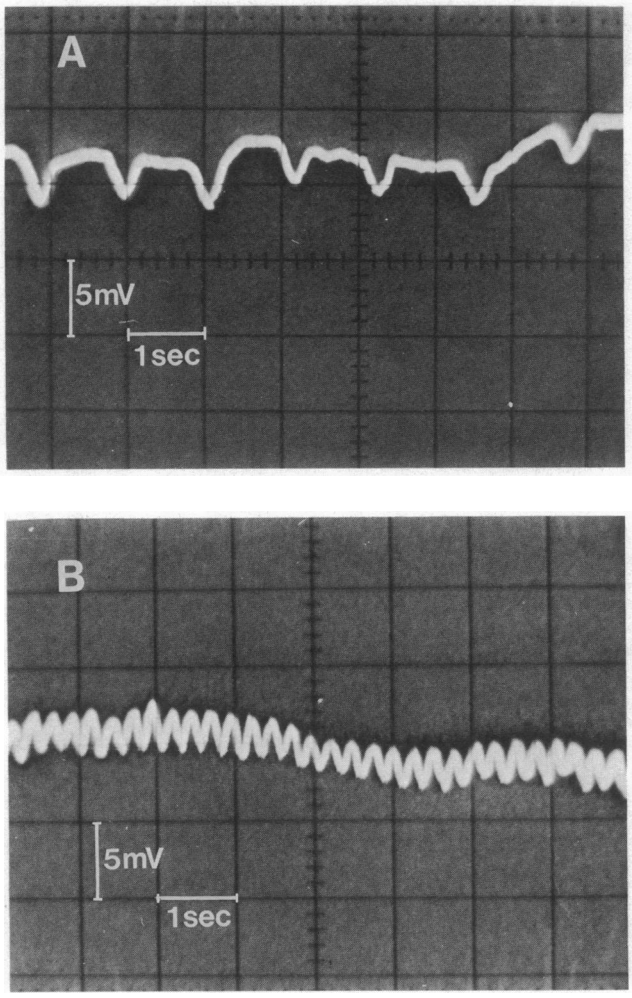


FIG. 4. (A) Base-line oscillation recorded from shi^{ts2} at 38°. (B) Increased frequency of the base-line oscillation on heating shi^{ts2} beyond 38°.

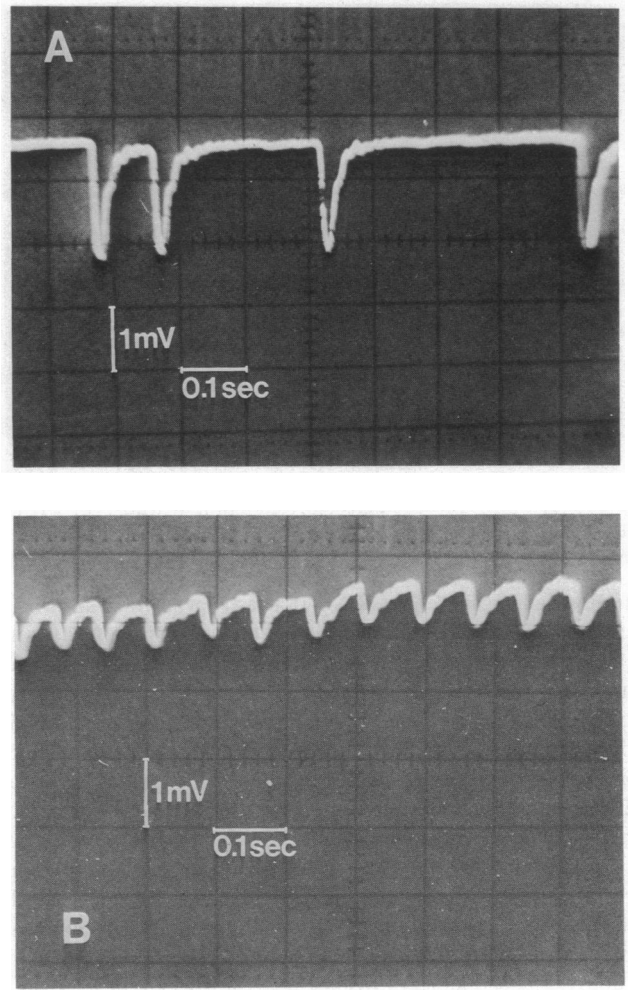


FIG. 5. (A) Spontaneous firing of thoracic muscle of shi^{ts1} at 23°. (B) Increase in spontaneous firing accompanied by a reduction in amplitude of the action potential from thoracic muscles of shi^{ts1} at 29°.

increased in frequency but decreased in amplitude (Fig. 5B) and finally disappeared. On closer examination of this phenomenon, it could be shown that the reference electrode had penetrated a thoracic muscle and that it was this muscle which was firing spontaneously. Furthermore, low frequency muscle activity could be observed in shi^{ts1} flies at 23° but was absent when these flies were cooled to 18°. Other workers have also shown that heating shi^{ts1} mutants induces the spontaneous firing of flight muscles (J. Levine, personal communication).

Phototactic behavior of $shi^{ts1}/+ : shi^{ts1}/0$ mosaics

Since shi^{ts1} flies are paralyzed at 30°, their phototactic behavior at the restrictive temperature could not be determined. However, it is possible to recover gynandromorphs in which the loss of an unstable ring-X chromosome produces both $shi^{ts1}/+$ and $shi^{ts1}/0$ cells (6). Tests of some of these mosaic individuals that could walk at 30° indicated that the shi^{ts1} allele does induce blindness at the restrictive temperature.

Three mosaic flies were recovered which possessed mutant tissue encompassing only one eye. In diffuse light, at 30°, all three mosaics behaved normally by showing no preferential orientation or direction of movement. However, when a point light source was used to illuminate the vial containing these flies, they were seen to walk towards the light source, following a helical path. Furthermore, when a rotating drum consisting of black and white vertical stripes was placed between the flies and the light source, the flies turned in the direction of rotation of the drum only until the mutant eye faced the light. In contrast, wild-type flies turned at least until they had their backs towards the light. Taken together, these two results indicate that while motor activity was retained in these flies at the restrictive temperature, their visual response was aberrant. Such mosaics have been generated only for the shi^{ts1} allele and so the suggestion that all shi^{ts} alleles induce blindness is conjectural at present.

ERG recordings from mosaic $shi^{ts1}/+ : shi^{ts1}/0$ flies

All mosaic flies were tested for their ERG response at 23° and 29°. In this case, the temperature-dependent loss of the "on"- and "off"-transients was taken to indicate a mutant response. Table 1 shows the classes of eye that could be produced. It can be seen that some of the flies that had a wild-type eye color, thereby suggesting that the retinula cells were wild-type in genotype, did give a mutant response. It is assumed that in these cases the dividing line between mutant and wild-type cells ran between the retina and the lamina. Thus, although

TABLE 1. *ERG analysis of mosaic flies at 32°*: number of flies of the possible phenotypes*

Eye	ERG	
	Wild-type	Mutant
Wild-type	444	25
Mutant	0	183

* Only those flies that have either a completely mutant or wild-type eye have been included in this analysis.

the retinula cells have a normal response, the cells in the lamina that give rise to the transients are mutant and inactive at the restrictive temperature. It might also be expected that the converse mosaic patterns should arise with equal frequency; that is, mutant eyes that give rise to a wild-type ERG even at the restrictive temperature. However, as can be seen from Table 1, this class of mosaic fly is not represented in the sample analyzed. The absence of this class suggests that when an eye is mutant it may be incapable of transmitting the relevant information to those neurons that are normal and capable of producing the transients. In other words, it would appear that the shi^{ts1} mutation affects not only the neurons giving rise to the transients, but also the receptor cells that transmit the necessary information to those neurons.

DISCUSSION

This study describes temperature-induced changes in the ERG of mutants of *Drosophila melanogaster* which were initially isolated as temperature-sensitive paralysis mutants. The fact that the visual response in these mutants is defective at higher temperatures eliminates the possibility that only motor neurons and/or muscles are aberrant. Rather, it would appear that the defect is general throughout the nervous system. The ability of the retinula cell to respond to light at higher temperature, as measured by the receptor potential, indicates that the defect does not result in the disruption of all cell membranes to produce a loss of excitability.

With the exception of shi^{ts4}, all of the shi^{ts} alleles lose the "on"- and "off"-transients of the ERG at higher temperatures. The problem is whether the loss of the transients is due solely to the inactivation of those neurons that generate the transients is due solely to the inactivation of those neurons that generate the transients, or to the inability of the retinula cells to transmit the relevant signal to those neurons. The observation in the ERG analysis that wild-type eyes in a mosaic can lose the transients at higher temperature shows that there is a definite post-synaptic effect, for even if the retinula cells are normal, mutant higher order neurons are unable to respond. The absence of any mutant eyes that have a normal response at high temperature suggests that, even if the transient-producing neurons are wild-type, they fail to receive any signal from the retinulae. In summary then, the shi^{ts} mutations appear to have both a pre- and post-synaptic effect. This result eliminates the possibility that only transmitter production, transmitter release, or transmitter–receptor interactions are the sites of malfunction in these mutants, for if one of these processes were defective, it would be seen as either a pre- or a post-synaptic effect, but not both.

Other mutants reported to have altered ERG responses have all been detected or recovered on the basis of their altered phototaxis. The fact that most, if not all, of these mutants exhibit no other gross behavioral aberrations, suggests that the mutations specifically affect the visual pathway. It is of interest, therefore, that the shi^{ts} mutants that exhibit the nonspecific phenotype of high-temperature-induced paralysis also show temperature-induced alterations in the ERG. This would suggest that the shi^{ts} mutants are defective in some component that is present throughout the nervous system and is not restricted to either the motor or visual system. In a recent study, it was shown that the shi^{ts} mutants exhibit a temperature-dependent resistance to tetrodotoxin (11). This toxin is known to specifically inhibit the regenerative sodium channel of the action potential mechanism (12). It remains to be seen whether the resistance to the toxin and the changes in the ERG responses of the shi^{ts} mutants can be unified into a coherent model for the action of the shi^{ts} gene product on the nervous system.

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