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# Transduction in Photoreceptors with Bistable Pigments: Intermediate Processes*

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**Abstract.** The prolonged depolarizing after potential (PDA) in the R1–6 receptors of the fly was used to isolate intermediate processes in phototransduction which are not manifested directly in the voltage response. It is first demonstrated that a pigment shift by light from metarhodopsin to rhodopsin in four species of the flies: *Drosophila, Calliphora, Chrysomya* and *Musca* induces an independent antagonistic process to the PDA, which is manifested in a strong inhibitory effect on PDA induction and is called the anti-PDA.

By using mutants of *Drosophila* the existence of processes underlying the PDA were examined. The $norpA^{H52}$ and the *trp* mutant were used in which the voltage response of the photoreceptors could be reversibly abolished by elavated temperature and long intense light respectively. It is shown that the excitatory process underlying the PDA could be induced and depressed in conditions that block the voltage response of the photoreceptors, thus indicating the existance of intermediate processes which link the pigment activation by light to the PDA voltage response.

**Key words:** PDA – anti-PDA – *Drosophila* mutants – Phototransduction.

## Introduction

The receptor potential is a step in a cascade of events which are initiated by the absorption of photons by the visual pigment molecules. The nature of these events is largely unknown.

In this research we were interested in isolating processes which occur before the initiation of the photoreceptors voltage response but after the activation of the visual pigment. For this purpose we used a certain voltage response which is found in many invertebrates and called the prolonged depolarizing afterpotential (PDA). The PDA has several characteristics which make it very convenient for studying phototransduction (Hillman et al., 1977). The PDA is induced by an illumination that causes a net shift of pigment from rhodopsin (R) to its long lived photoproduct

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metarhodopsin (M) (Hochstein et al., 1973). It is manifested in a depolarization which in the dark decays spontaneously very slowly, long after the pigment shift from R to M took place. But it can also quickly be depressed at any time by shifting the pigment back from M to R (Nolte and Brown, 1972; Muijser et al., 1975; Minke et al., 1975a; Tsukahara et al., 1977).

A question arises whether the process underlying the PDA depression is just the reversal of the excitatory process underlying PDA induction or a separate independent antagoistic process. In the barnacle (Hochstein et al., 1973) and in the UV receptors of the *Limulus* median eye (Minke et al., 1973), where the PDA is usually not longer than 1 h, it had been demonstrated that a net M to R pigment shift, after the spontaneous decay of a maximal PDA, resulted in a period in which the induction of the PDA was strongly inhibited (Hochstein et al., 1973; Minke et al., 1973).

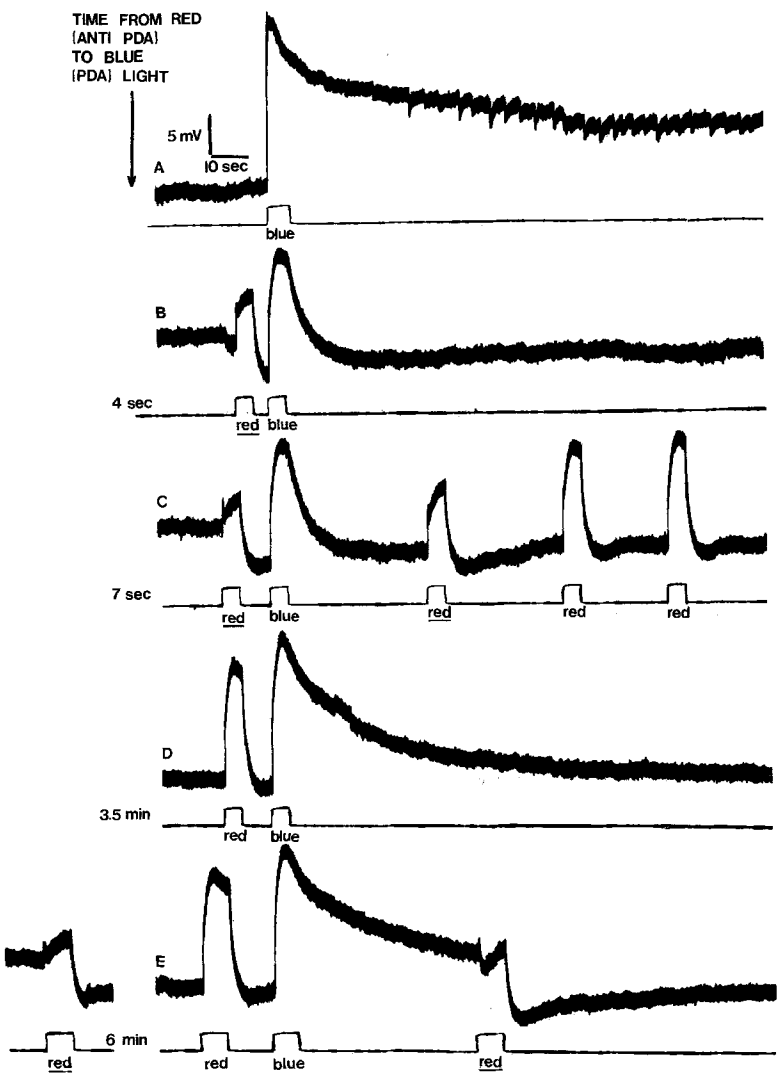


Fig. 1. (Legend see page 165)

The results indicated that an inhibitory process exists, called the anti-PDA process. The anti-PDA process is thus a separate antagonistic process which probably also causes PDA depression.

Several investigators have suggested recently that a pronounced anti-PDA process probably does not exist in the fly (Hamdorf and Razmjoo, 1977, in *Calliphora;* Tsukahara et al., 1977, in *Eristalis;* Wright and Cosens, 1977, in *Drosophila*). They proposed models which are based on direct and close relations between the pigment states and transitions and the photoreceptor voltage response.

We first examine if the anti-PDA does exist in four species of flies: *Drosophila, Calliphora, Chrysomya,* and *Musca.*

## Methods

The experiments were performed on white-eyed intact preparations of *Drosophila, Calliphora, Musca,* and *Chrysomya.* The preparation of the fly for electroretinogram (ERG) and intracellular recordings has been described elsewhere (Minke et al., 1975).

The details about the various illuminations that were used are given in the figure captions.

## Results and Discussion

### *The Existence of an Anti-PDA in the Fly*

It had been shown that a net M to R pigment shift during a maximal PDA, caused a PDA depression with no post stimulus effects. This was indicated by the ability to

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**Fig. 1.** Intracellular demonstration of induction of an anti-PDA in *Drosophila.* Intracellular recordings in a single white-eyed *Drosophila* R1–6 photoreceptor. Trace A shows the full PDA induced by a strong blue (Ditric "cut-off" filter, $\lambda < 460$ nm) stimulus in a cell adapted to white (12 V quartziodid lamp) and then red (Ditric "cut-on" filter, $\lambda > 580$ nm) light. The downward "bumps" during the latter part of the trace probably represent fly-movement artifacts. For all other traces, the blue PDA-inducing stimulus of the preceding trace served as the adaptation for the red anti-PDA-inducing stimulus. The PDA arising from this blue-adaptation was allowed to decline during the intracellular recording. (In this cell the full PDA was fortunately relatively short, about 1 h. This made possible intracellular measurement, which are extremely difficult to be maintained for longer than 1 h or so, and also ensured that after the decline of the PDA most of the pigment was still in the M state.) The cell was then exposed to a bright red anti-PDA-inducing light at various times before the blue PDA-inducing stimulus, as indicated. The duration of the PDA clearly increases with that time. In other words, the red-after-blue stimulus induces a process (the anti-PDA) which for some minutes decreasingly impedes the induction of a PDA. In traces B, C, and E the red anti-PDA-inducing stimulus depresses the baseline because the preceding PDA had not been fully declined. (The full PDA of trace A had declined for 25 min and the partial PDA's of traces B and D for about 5 min.) This means that in fact these anti-PDA's were not "full", being partly cancelled by the residual PDA's. Note that the anti-PDA-inducing stimuli for traces D and E are *not* those shown immediately preceding the blue stimuli. The anti-PDA-inducing red stimulus for trace D is the second red stimulus of trace C, while that of trace E is shown separately. In both these last traces, the red stimuli immediately preceding the blue stimuli should directly affect neither the PDA nor the anti-PDA but ensure that the light-adaptation state of the cell is similar in all of the last four traces. The word "red" is underlined for the M-to-R pigment-transferring and so anti-PDA-inducing stimuli

induce immediately additional PDA by additional R to M pigment shift (Hillman et al., 1972).

In order to demonstrate the existence of an anti-PDA it was essential to shift the pigment from the M to the R state *after* the decay of a maximal PDA, so that the anti-PDA will not be cancelled by an existing PDA.

The difficulty in demonstrating the existance of an anti-PDA in *Drosophila* (but not in the other three species) arises from the slow return of the pigment from the M to the R state in the dark with a time constant similar to the spontaneous decay of the maximal PDA, which is about 6 h (Pak and Lidington, 1974; Minke et al., 1975a; Wright and Cosens, 1977). We used two ways to overcome this difficulty. Firstly we used intracellular recordings in cells that show a PDA short compared to the time constant of M to R thermal transition, and secondly we used continuous PDA inducing blue light to keep the pigment in the M state.

In the barnacle the PDA is known to have variable durations in various cells (Hochstein et al., 1973; Lantz et al., 1977). We found that this is also true for the four species of flies mentioned above. In our experiments with *Drosophila* we used intracellular recordings in a cell that showed a maximal PDA of no longer than 1 h, so that when the PDA decayed spontaneously to one third of its amplitude (in about 25 min) relatively little M to R pigment shift had occurred. Figure 1 (trace A) illustrates the initial PDA of such a cell which was induced by a strong blue light after red adaptation. We found that *after* such a decay of the PDA, red light that put *all* the pigment back in the R state induced an inhibitory process that impeded PDA induction by a following blue light, for several minutes. This fact is demonstrated in traces B–E of Figure 1. The figure shows that when the dark time between the red light (that shifts the pigment from M to R state) and the blue (PDA inducing) light was short, very little PDA was induced. But when this dark time was increased (traces D and E) the PDA was much increased. The red lights which preceded the PDA inducing blue lights in traces D and E were given in order to show that the inhibition of the PDA in traces B and C did not arise simply from light adaptation. Since the first red pulses after blue adaptations (which are underlined) were strong enough to shift all the pigment to the R state the additional red pulses (in traces C–E) did not cause a *net* shift of pigment and therefore neither induced a PDA nor an anti-PDA. The figure shows clearly that M to R pigment shift by red light following blue adaptations not only depresses the PDA (trace E last stimulus) but also inhibits PDA induction for several minutes if this pigment shift occurred when no (or small) PDA is present.

The second line of evidence for the existence of the anti-PDA process in *Drosophila* came from electroretinogram (ERG) recordings which represent the average response of many photoreceptors. Since it took several hours for the average PDA which was recorded extracellularly to decay, we put the blue light on for several hours in order to keep the pigment in the M state. We found that the PDA decayed spontaneously in the presence of the strong blue light. The evidence for this fact is illustrated in Figure 2: The first red stimuli in traces B–D in Figure 2 did not change the baseline after the cessation of the red lights (for comparison see the last response to red light in trace D and the responses to the underlined red stimuli in Figure 1).

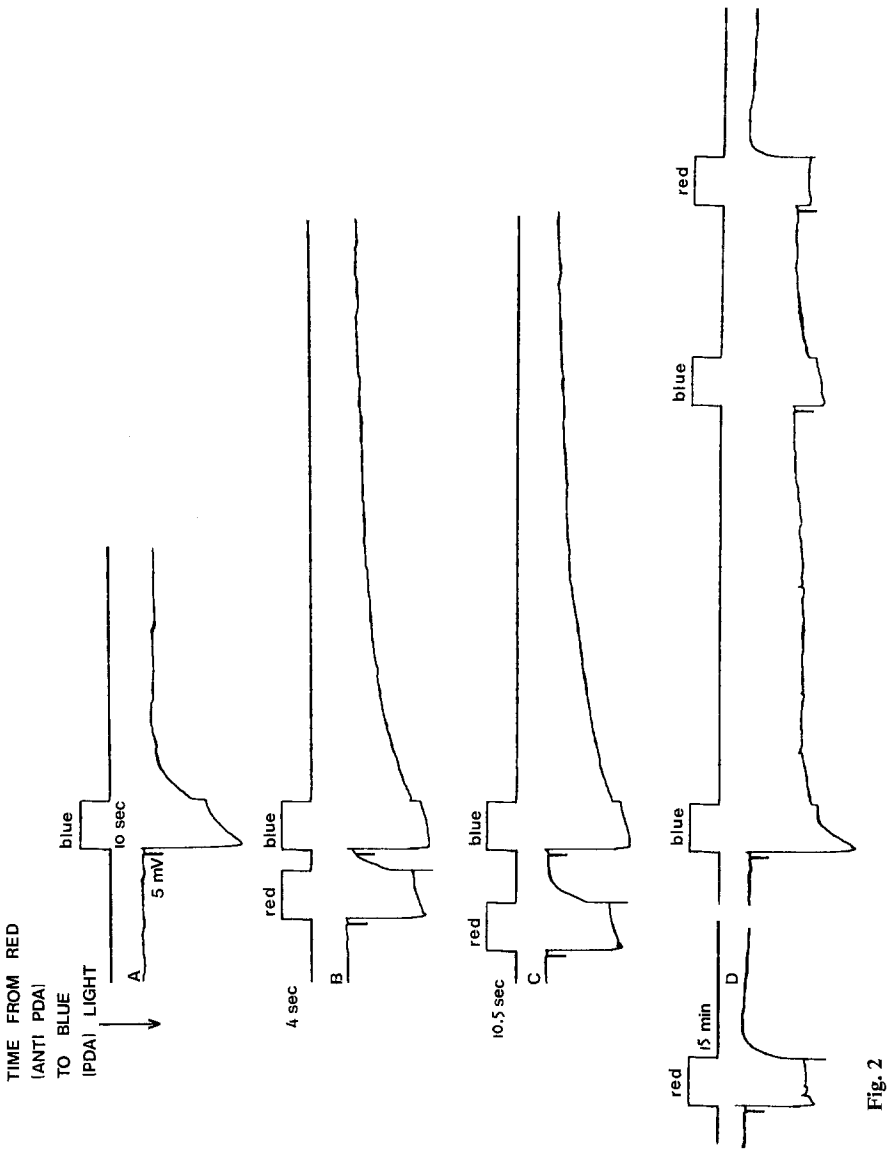


Fig. 2

**Fig. 2. A** The PDA declines without pigment return. These are ERG recordings from an intact white-eyed *Drosophila* at 22° C. Before trace A, the fly was exposed to the strong blue (PDA-inducing) light continuously for 5.5 h. Although this kept the pigment in the M state, the PDA had declined completely. This is seen in two ways: Firstly, the response to a blue stimulus 10 min after the continuous blue exposure (trace A) is the same as after red adaptation (traces B–D). (The near-absence of a PDA following the stimulus in trace A also confirms that most of the pigment is in state M. The small residual PDA may be due to the small amount of thermal return of pigment to R during the preceding 10 min of darkness.) Secondly, the anti-PDA-Inducing red stimuli of traces B–D do not appreciably "depress" (upward) the baseline. **B** ERG demonstration of anti-PDA. In traces B–D red stimuli are presented to the blue-adapted PDA-free eye at various times (as indicated) preceding the blue PDA-inducing stimuli. The PDA's of traces B and C are much shorter than that of trace D, indicating an anti-PDA of duration much less than 15 min and, judging by the substantial PDA's of traces B and C (and assuming that a full anti-PDA completely neutralizes a full PDA), perhaps no more than a few tens of seconds

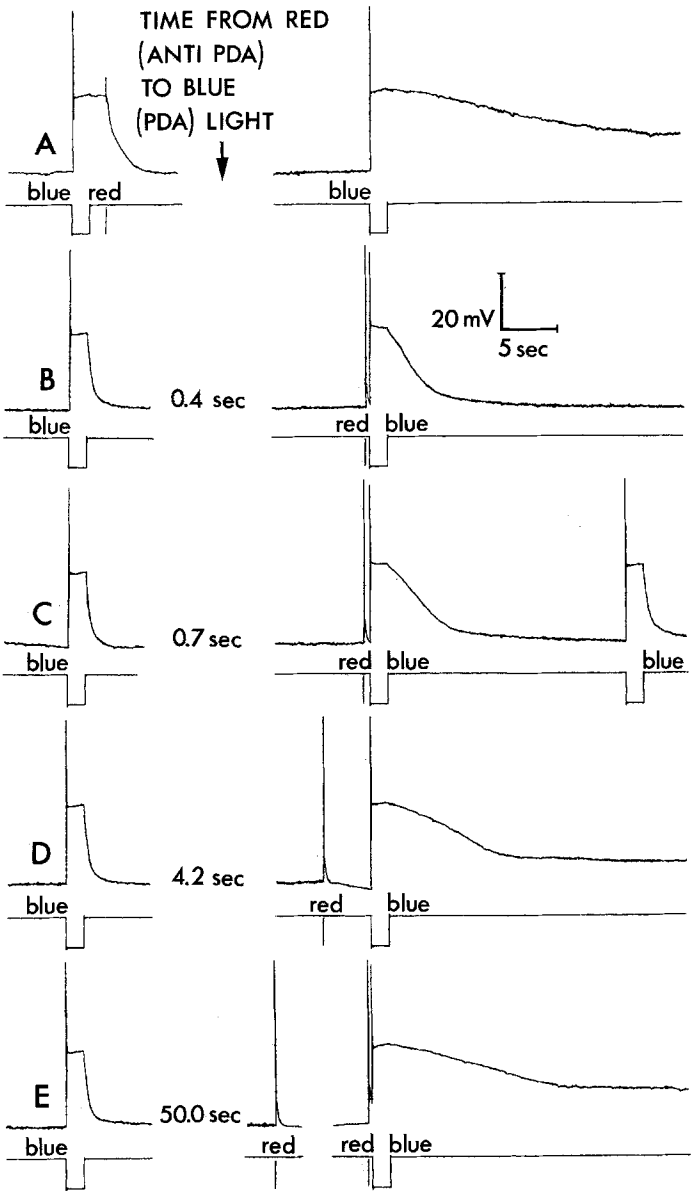


**Fig. 3.** Intracellular demonstrations of induction of an anti-PDA in *Calliphora*. Intracellular recordings in a single white-eyed *Calliphora* R1–6 photoreceptors. Trace A (right column) shows the full PDA induced by strong monochromatic blue (457 nm interference filter-Schott-depal) in a cell adapted to strong red flash (Braun type F900 in conjunctions with 590 Schott OG cut on and KG 1 heat filter). One red flash was strong enough to shift all the pigment from M to R state as indicated in trace A (on the left) by the complete PDA depression of a full PDA. One blue pulse was enough to shift maximal pigment to the M state as indicated by the lack of PDA in all the responses to the blue pulses in the left column traces B–E. After the decline of the maximal PDA (in a few minutes) the cell was always blue adapted at the beginning of each trace (left column traces B–E). Then the cell was exposed to the strong red flash at various times before the PDA inducing stimulus as indicated. The duration of the PDA clearly increases with that time. In trace E an additional red flash was given close to the PDA inducing blue light in order to ensure that the state of light adaptation of the cell is similar in traces B, C, and E

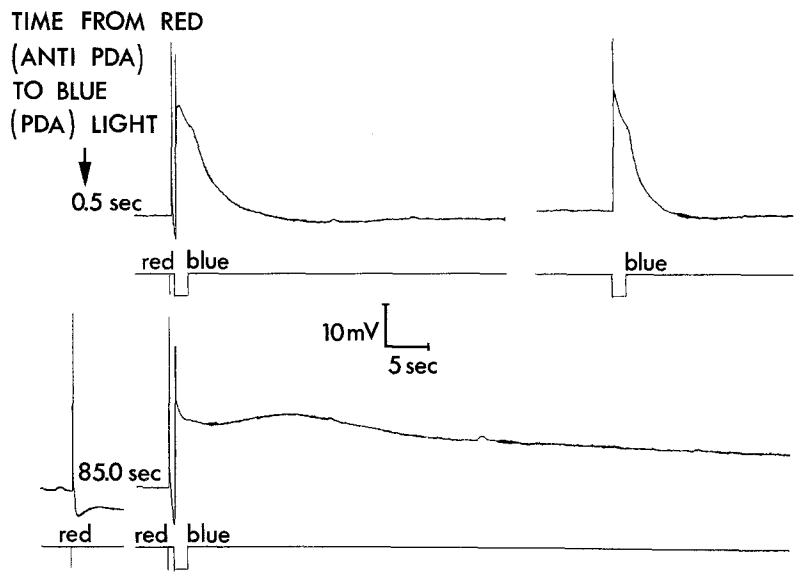


**Fig. 4.** Intracellular recordings in white-eyed *Musca* which demonstrate the existance of anti-PDA in this species. The experimental conditions are similar to those of traces B—E in Figure 3.

After the complete decay of the PDA the blue light gave a response without or with only a short PDA as demonstrated in Figure 2A. A red light that shifted the pigment back from M to R (Fig. 2B, C) inhibited PDA induction by a following blue light for several minutes in a manner similar to the intracellular experiment. The PDA in traces B and C (as compared to trace D) probably arised from the partial decay of the anti-PDA *during* the relatively long anti-PDA and PDA inducing red and blue lights respectively.

Figure 3 also demonstrates the anti-PDA phenomenon in *Calliphora*. As in *Drosophila* following the decay of a maximal PDA which usually extends for several minutes (trace A), a M to R pigment shift by a short (2 ms) intense red flash, which was shortly followed by PDA inducing blue light, gave a relatively small PDA (traces B and C). However if the dark time between the red and the blue lights was increased to 50 s (trace E) a considerably larger PDA was induced. The second red flash close to the PDA inducing blue light (trace E) only light adapted the cell. Results similar to those of Figure 3 were obtained in all 26 R1—6 cells in seven different flies that were investigated.

We found similar results, by intracellular recordings from white-eyed *Chrysomya* and *Musca*. The results from *Musca* are illustrated in Figure 4.

The above experiments indicate that pigment transitions from the M to R states induce an independent process antagonistic to the PDA which we call the anti-PDA process due to its similarity to the anti-PDA found in other preparations (Hochstein et al., 1973; Minke et al., 1973).

*The Excitatory Process Underlying the PDA is not Manifested Directly in a Voltage Response*

In an attempt to find additional processes which are not manifested directly in a voltage response (like the anti-PDA) but link the pigment shift to the voltage response we used two mutants of *Drosophila*. We used the *norpA*$^{H52}$ (Deland and Pak, 1973; Pak et al., 1976) and the *trp* (Cosens and Manning, 1969; Minke et al., 1975b; Minke, 1977) mutants in which the voltage response can be reversibly abolished by elevated temperature and long strong light respectively. In these experiments we shifted the pigment from R to M and from M to R by blue and red lights respectively in conditions, in which the voltage response of the photoreceptors had been depressed, and examined if the processes underlying PDA induction and depression were still induced under such conditions. Such a strategy has previously been used by Wong et al. (1976), who abolished the voltage response of *Drosophila* photoreceptors by anoxia and showed that the excitatory process underlying the PDA can be induced and depressed without apparent voltage response.

Figure 5 shows intracellular recordings from a single photoreceptor in a white-eyed *trp* mutant. In trace A we show how light can strongly depress the response. The prolonged strong red light depressed the receptor potential close to the baseline. A short time of 25 s in the dark was enough to allow the recovery of the response, as shown in trace B. In both responses, the red light was slightly of a too short duration to cause a complete suppression of the response to the baseline, which, however, occurred for red lights of a duration of 20 s or more. In trace D we demonstrate that blue light that shifts R to M and induces a PDA of several hours in a normal fly failed to induce a PDA voltage response which is longer than several seconds. The response instead decayed to the baseline in the same way as in a response to a continuous 20 s red stimulation. The *striking difference between* the influence of the *red* and the *blue* lights is the *failure of the cell to depolarize in response to further stimulation,* for many minutes, *after the blue,* but not after the red light. However, if the blue stimulus was followed by a red light that shifts the pigment back from M to R, the cell's responsiveness is restored within a few seconds as shown in traces C and E. We examined whether the voltage response to the blue light was necessary for inducing the extended suppression of the cell's responsiveness. We performed this experiment by shortening the dark time between long red and blue stimuli. We found that as this dark time was shortened the response to the blue light became shorter and smaller as indicated in trace B. (When the dark time was reduced to zero, no response to the blue light was obtained.) Nevertheless, the responsiveness of the cell was depressed exactly as in trace D and it could be restored by a strong red light as shown in trace C.

The above observations (Fig. 5D) show that in the mutant the R to M pigment shift induced a PDA which, in similar manner to the effect of continuous illumination, depressed the voltage response of the photoreceptor and continued to suppress the response as long as the excitatory process underlying the PDA was present. There are two indications that the excitatory process was induced in these conditions and in saturating amounts:

a) Abolition of further depolarizing voltage responses to blue light after maximal PDA induction is a typical phenomenon in normal *Drosophila*. It probably arises

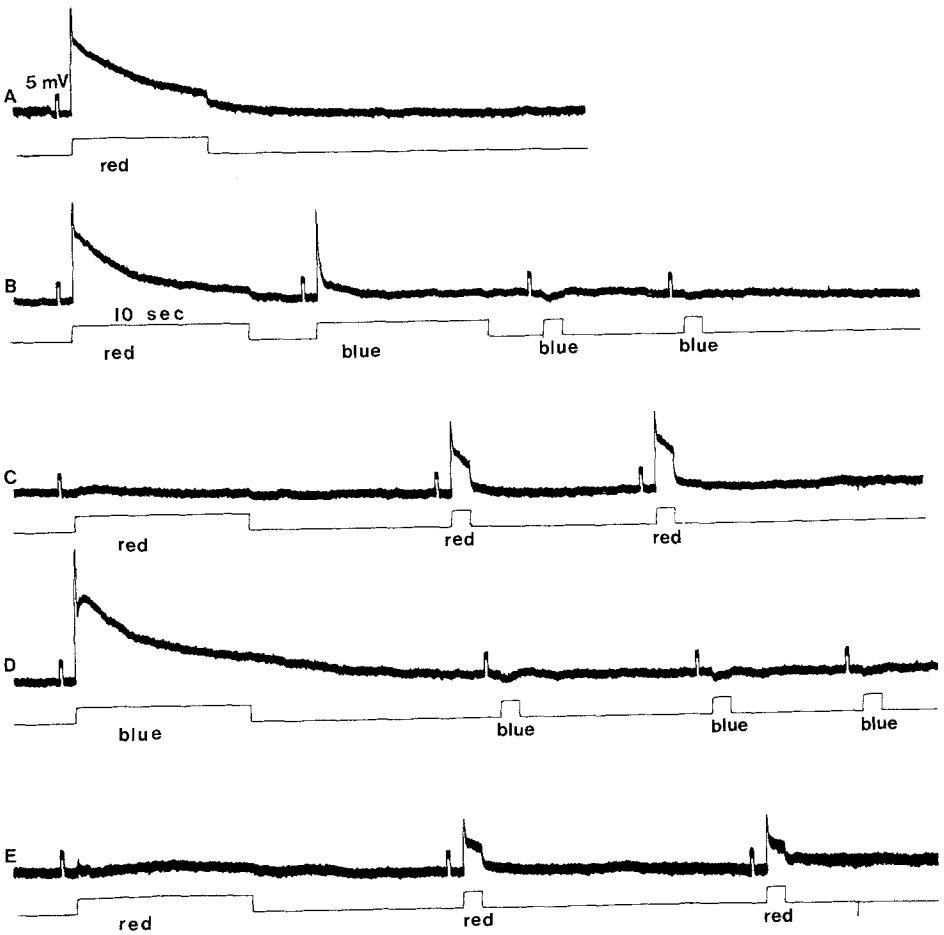


**Fig. 5.** Intracellular recordings obtained from a peripheral retinula cell of a white-eyed *trp* mutant of *Drosophila*. All responses are from a single cell which was illuminated with either red (Ditric "cut-on" filter with $\lambda > 580$ nm) or blue (Ditric "cut-off" filter with $\lambda < 460$ nm) lights. 1 s of red and 3 s of blue lights were needed to bring the pigment system to photoequilibrium. Trace A shows the response to a prolonged red light. The small voltage step at the cessation of the light indicates that the light was not long enough to suppress the response completely. Trace B shows that if a blue light is given close to a long red light only a short response is observed (compared to the first blue light in trace D, which shows response to the *same light* but with longer dark interval). Nevertheless the pigment shift from R to M which was induced by the blue light abolished the cell responsiveness for many minutes as indicated by the lack of depolarizing responses to further blue stimulation of the same maximal intensity during the following blue pulses in traces B and D. Traces C and E show that red light following blue adaptation gives very little or no response but removes the suppression of cell responsiveness as indicated by the response to further red pulses. In trace D one can see that the low amplitude PDA that is induced by the long blue light and observed at the cessation of the light, continues to reduce the cell response in the dark until it reaches the baseline. The responses to the red pulses in trace E are smaller than those of trace C due to the deterioration of the cell with time. The Figure shows that the excitatory process underlying the PDA can be induced and depressed without apparent voltage response

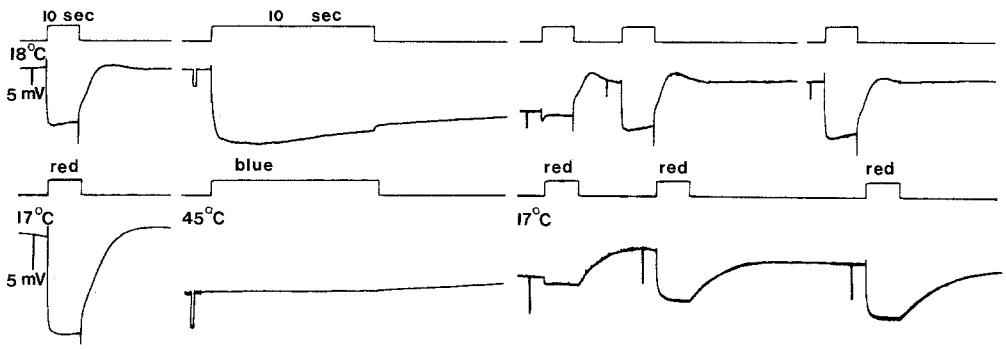


**Fig. 6.** Extracellular ERG recordings from a white-eyed *norpA*$^{H52}$ mutant of *Drosophila*. First row: the first red (Ditric "cut-on" filter) light, given at 18° C elicited a typical response that decayed to baseline at the cessation of the light. 1 min later a blue light induced a prolonged negative response (PDA). An additional red light, 1 min later, depressed the PDA at the cessation of the light back to baseline. Further red lights gave normal responses. Second row: A stimulation paradigm similar to that of the upper row, in another fly. The first red light elicited a normal response at 17° C. Then the temperature was elevated to 45° C within 1 min. This elevated temperature caused a negative shift in baseline. During the 20 s of elevated temperature a PDA inducing blue (Ditric "cut-off") light was given, but no response to this stimulus is observed. The temperature was returned to 17° C within 1 min. However, the baseline remained in the shifted negative direction. A second red light induced PDA depression which brought the baseline close to the origin. Further red lights gave responses smaller and slower than normal and without the second order neurons responses (the "on" and "off" transients) due to some irreversible damage that was done by the high temperature. The figure shows that the excitatory process underlying the PDA can be induced in conditions that block the responsiveness of the photoreceptors

from saturation of a step in phototransduction. Even the small hyperpolarization during the shorter strong blue stimuli in traces B and D (which were consistently observed) are typically found superimposed on a full PDA in normal *Drosophila* (Minke et al., 1975a).

b) Traces C and E show that red light, although giving little response, restored the cell's responsiveness to further stimulation in a short time. This phenomenon is always observed when a full PDA in a normal fly is depressed by red light.

We therefore conclude that the excitatory process underlying the PDA can be induced and depressed by a pigment shift even in conditions that do not allow the voltage response of the cell to appear due to a defect in some gene product.

Fly ERG reflects fairly well the steady state response of the photoreceptors (Goldsmith and Bernard, 1974). Therefore the observations of Figure 5 were replicated in ERG recordings. We also used ERG recordings whenever stable recordings at elavated temperature were necessary. In the following experiment we used the same strategy illustrated in Figure 5 by using another mutant in which the receptor potential can be reversibly suppressed by elevated temperature.

In Figure 6 we show ERG recordings of the *norpA*$^{H52}$ mutant. The upper row shows ERG responses to red and blue stimuli at a temperature of 18° C. The blue light induced a PDA. This PDA was depressed by the following red light, and further red stimuli elicited responses similar to the first one in the row. In the lower line the first red light gave a normal response at 17° C. Then the temperature of the

fly was elevated (within 1 min) to 45° C for 20 s. We usually found that the baseline became negative during the elevated temperature. The figure shows that the elevated temperature abolished the voltage response of the photoreceptors as indicated by the absence of a voltage response to the strong PDA inducing blue light. The temperature was then reduced to 17° C within 1 min. Normally, in the dark, such a temperature change was followed by a return of the voltage to baseline, but the figure shows that in this case very little return occured. That this failure to repolarize is due to the appearance of a PDA is evident in the response to further red light that induced a rapid repolarization.

As in the *trp* mutant the excitatory process underlying the PDA was induced in conditions when the voltage response of the cell was blocked. Photometric measurements showed that it was possible to shift R to M and M to R normally in both $norpA^{H52}$ and *trp* mutants in the conditions that the responsiveness of the eye was abolished (Minke et al., 1975b; Pak et al., 1976). Therefore, it is clear that the defect, in both mutants, takes place in a stage that links the excitatory process, underlying the PDA, to the membrane conductance change.

These results show that it is possible to block the transduction process at specific intermediate stages. It is not yet known whether anoxia (Wong et al., 1976) and the defective *trp* and $norpA^{H52}$ gene products block the transduction cascade at the same level or at three different levels. It is very likely that the $norpA^{H52}$ and the *trp* gene products, which are produced on different chromosomes, also differ from each other since they are expressed in two different phenotypes when they are defective. Therefore, we suggest that two different stages in the transduction process are affected by the two mutations.

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