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Drosophila Mutant with a Transducer Defect*

Baruch Minke

The Hebrew University, Hadassah Medical School
Jerusalem, Israel

Abstract. The *trp* is a conditional phototransduction mutant of *Drosophila*. Direct electrical measurements and shot noise analysis suggest that a prolonged intense light causes in the mutant a reduction in the quantum efficiency for quantum bump production that does not arise from bleaching of the visual pigment. This effect depends on the duration of the light and only weakly on its intensity. In the normal fly, an intense blue light that shifts the visual pigment from rhodopsin to metarhodopsin, induces an excitatory process manifested by a prolonged depolarizing after potential (PDA). In the mutant, the PDA has a small amplitude and bump noise is superimposed on the response. It can thus be shown that the excitatory process underlying the PDA is also present in those *trp* mutants where the PDA voltage response is small or absent. It is suggested that the absence of the PDA voltage response in the mutant is probably due to a defect in an intermediate process, which links the excitatory process to the membrane conductance change.

Key words: *Drosophila* mutant – Bump noise – PDA – Phototransduction.

A considerable amount of information is available today concerning the photochemical cycle of photoreceptor pigments and the ionic mechanism of the receptor potential. However, the intermediate processes, those that link the photopigment process to the photoreceptor membrane conductance changes, which in turn underlie the receptor potential, are largely unknown. Genetic dissection of the phototransduction process might prove a powerful tool for the investigation of these intermediate processes. Several single gene receptor potential mutants of *Drosophila* exist. The most interesting mutants for the study of phototransduction process are those which show normal receptor potentials under permissive conditions and abnormal receptor po-

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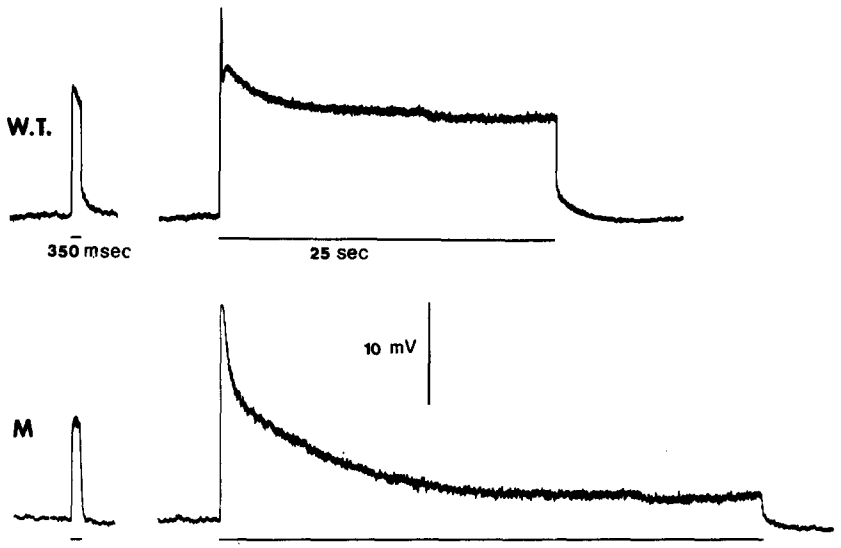

Fig. 1. Intracellularly recorded receptor potentials from peripheral retinular cells of a white-eyed normal fly (W.T.) and from a white-eyed *trp* mutant (M) to monochromatic 540 nm green light. The light intensity was 3.6×10^{14} photons cm^{-2} s^{-1} for the upper right trace and was attenuated by 0.5 log unit in the upper left trace and by 2.0 and 2.5 log units in the lower right and left traces respectively

tential under nonpermissive conditions without any obvious effect on the structure of the receptors. I would like to present a study on one of these mutants, which is a third chromosomal recessive mutant called *trp* (transient receptor potential mutant). I hope that this study will serve as an example of the possibilities that genetic dissection offers.

The permissive conditions for the receptor potential of the *trp* mutant are short intense illuminations or weak illuminations of any duration (Minke et al., 1975). This fact is illustrated in Figure 1 which compares the intracellularly recorded receptor potential of the normal white-eyed fly (upper row – W. T.) to the intracellularly recorded receptor potential of the white-eyed *trp* mutant (lower row – M). The response to the short stimulus looks similar in both the mutant and the normal fly. However, the mutant response to the longer (and three times stronger) green light differs from the normal response by a pronounced decay of the receptor potential to a low steady state level during illumination. The rate of the decay and the final steady state level attained vary among individual flies. The response often decays to the dark resting potential level. The rate of the decay of the mutant response speeds up in a light-adapted state and during an increased stimulus intensity. What causes the receptor potential of the *trp* mutant to decay during a prolonged stimulus? By using the unitary potentials (quantum bumps) which sum to produce the receptor potential (Wu and Pak, 1975) Minke et al. (1975) demonstrated directly, and by application of shot-noise analysis (Dodge et al., 1968), that the light stimulus causes a reduction in the quantum efficiency for quantum-bump production. Moreover this reduction cannot be attributed to bleaching of visual pigment. Thus, it seems that an intense, prolonged light becomes equivalent to weak light for the *trp* mutant. I have

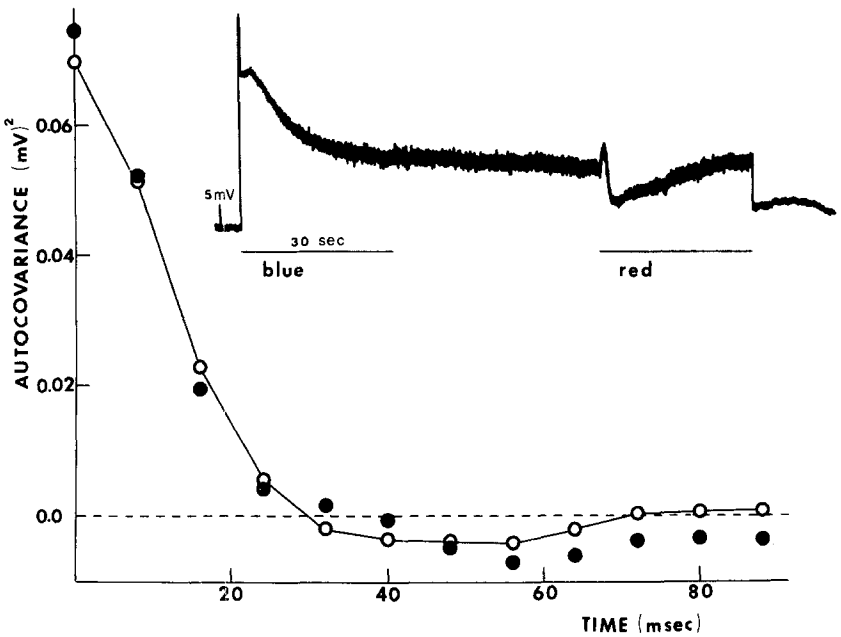

Fig. 2. Inset: An intracellularly recorded PDA response from the photoreceptor of the white-eyed mutant *trp*, induced by 480 nm blue light and depressed by 600 nm orange light stimuli. The light intensity was 4.0×10^{16} photons cm^{-2} s^{-1} and 2.5×10^{16} photons cm^{-2} s^{-1} for the blue and red stimuli respectively. Main figure: Autocovariance functions calculated from the noise superimposed on the saturated steady state phase of a receptor potential (○) and from a PDA noise (●) recorded from the same cell. The calculations were done from 750 points sampled at 8 ms. intervals. The ordinate is the autocovariance $c(\tau)$ in mV^2 and the abscissa is the time lag τ in ms. The responses were filtered with high frequency cutoff filter (half power cutoff at 30 Hz) before the analysis

further investigated the effect of adapting light on the recovery of sensitivity of the mutant response. I have found that after exposing the fly to a short adapting light (short compared to the decay time of the response during light) the mutant shows dark adaptation kinetics similar to a normal fly. However, a long adapting light (long enough to suppress the response) reduces the sensitivity to light. The reduction is more than two orders of magnitude below the sensitivity level obtained by a short adapting light of the same, or even of a larger amount (intensity × duration) of light. The major component of the recovery of sensitivity lasts a few minutes. It probably represents the recovery of the cell from the reduction in the quantum efficiency of bump production. This reduction in quantum efficiency seems to depend on the *duration* of the adapting light for a wide range of light intensities, while dark adaptation in normal flies depends on the *amount* of the adapting light over a similar range of light intensities.

In *Drosophila* as well as in many invertebrates, the absorption spectrum of the visual pigment, rhodopsin, differs substantially from its stable photoproduct, metarhodopsin. Under these conditions, the wavelength of an intense stimulus light can be so chosen that the light is absorbed primarily by rhodopsin, resulting in a substantial shift of pigment to metarhodopsin. This shift of pigment induces an excita-

tory process that is manifested by a prolonged depolarizing after potential (PDA) which far outlasts the stimulus, so that the photoreceptor behaves as if the light were still on (for several hours in *Drosophila*). Photoconverting the pigment back from metarhodopsin to rhodopsin induces an inhibitory process and results in depression or prevention of the PDA (Hochstein et al., 1973). Induction and depression of the PDA in the *trp* mutant are shown in Figure 2. This is an intracellular recording from a photoreceptor which exhibits a relatively large steady state response. The cell was first stimulated with red light shifting all the pigment to the rhodopsin state. The cell was then stimulated with blue light which shifts a considerable fraction of the pigment to the metarhodopsin state. At the cessation of the blue light, the cell remains depolarized due to the induction of a low amplitude PDA. Increased voltage noise can be seen superimposed on the response. This increased noise level remains during the entire PDA period. The red stimulus that shifts the pigment back from metarhodopsin depresses the PDA and reduces the noise to the level before PDA induction. The lower graph shows the autocovariance function calculated from the noise recorded in the dark during the PDA (●) and from the response at the steady state level during the light (○). The similarity between the curves calculated from the PDA noise and the light-coincident noise suggests that the PDA, like the stimulus coincident response, is a superposition of quantum bumps. PDA induction seems to be equivalent to an induction of quantum bumps which proceeds in the dark for hours (in *Drosophila*) after the cessation of the light. I suggest that the excitatory process is induced in packets, bumps, and that the quantum bumps are induced by the excitatory process and not directly by photons.

In normal white-eyed *Drosophila*, PDA induction by a blue light following red adaptation prevents further depolarizing responses for many minutes (Minke et al., 1975), presumably due to saturation of a step in the transduction process. If red light follows the blue light, the PDA is depressed and the responsiveness of the cell is restored immediately. Following prolonged white illumination, on the other hand, the photoreceptor response recovers after a few minutes in the dark even in those *trp* mutants whose steady-state response approaches baseline. A strong blue light of long duration also elicits from the mutant a response with a low steady-state level. After the blue illumination, however, the responsiveness of the cell does not recover for many minutes in the dark unless a red light is first given. Thus, in the *trp* mutant, the absence of the depolarizing voltage response (PDA) following a long blue stimulus does not seem to indicate that the excitatory process underlying the PDA has been abolished. On the contrary, the inability of the photoreceptors to respond to further stimulation suggests that the excitatory process is very much there and has been saturated by the PDA inducing blue illumination, even though the PDA voltage response is not.

I have discussed in this report two phenomena, both observed in the mutant following PDA induction: (1) the presence of shot-noise and (2) the inability of the photoreceptors to respond to light stimulus following a prolonged blue illumination, even though the prolonged voltage response normally associated with the PDA is small or absent. These two phenomena suggest that (1) the PDA voltage response is not induced directly by photons but through the excitatory process and (2) the excitatory process in turn acts on the membrane conductance through an intermediate process. This conclusion is schematically illustrated in Figure 3. According to

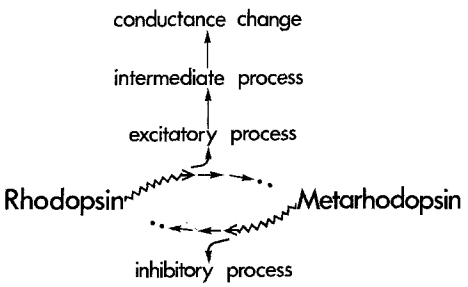

Fig. 3. The model needed to explain the observations discussed in the text

this scheme the transition of pigments from rhodopsin to metarhodopsin induces the excitatory process and the transition from metarhodopsin to rhodopsin induces the inhibitory process. The effect of the inhibitory process is only to neutralize the excitatory process. The role of the excitatory process is to open the membrane channels via another intermediate process. I suggest that in the mutant, as in the normal fly, the saturation of the excitatory process is responsible for the inability of the photoreceptor cell to respond to stimulation during maximal PDA. The absence of the PDA voltage response in the mutant is probably due to a defect in the intermediate process, which links the excitatory process to the membrane conductance change. Thus in the *trp* mutant the excitatory process remains saturated during PDA, thus preventing further response induction, even though the PDA voltage response is small or absent.

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Discussion
F. Conti, Camogli, Italy

Some comments concerning the basic reference on invertebrate photoreceptors noise quoted by Dr. Minke [Dodge, Knight and Toyoda, Science **160**, 88 (1968)] seem wortwhile to be made.

1. The relationship between the noise autocovariance and the amplitude squared of the linear frequency response, Equation 2, is true only if the responses to flashes of dim light have the same time course as the individual spontaneous bumps, an assumption which is not justified. Vice versa, the verification of Equation 2 is not sufficient to support the hypothesis of the bumps superposition mechanism. However, for dim light intensities such mechanism is suggested by direct observations of the bumps statistics.

2. The estimate of α for 0 log light attenuation in Figure 3 is erroneously small by one order of magnitude. Consequently, the statement that "The steady state bumps size decreases continuously approximately as the inverse square root of the light intensity" is incorrect. Indeed, for bright lights α approaches a finite value of about 10^{-5} times the dark conductance, G_0.

3. Assuming $G_0 \sim 10^{-7}\ \Omega$, the limiting value for α is about $10^{-12}\ \Omega^{-1}$. Such value is comparable or smaller than what expected for any commonly found ionic channel in a biological membrane. In such conditions the noise due to open-close kinetics of individual ionic channels may be expected to overcome the bump noise modelled by Dodge et al. (1968).

Discussion
U. Thurm, Münster, Federal Republic of Germany

One may try to interpret the change in the receptor potential responses in the *trp* mutant on the line of two membrane conductivity change mechanisms which have different time courses: a purely phasic conductivity increase decaying during a continuing stimulus by some kind of inactivation to the resting conductance (not to the resting state) and, secondly, by a slowly activated tonic conductivity increase. The defect in the mutant may be one in the responsiveness of the tonic mechanism. We have some evidence for two different membrane response mechanisms in epidermal mechanoreceptors of insects, especially of flies. The reversal potential during the phasic response is different from that during the tonic response (Thurm, 1974, in "Mechanoreception" Ed. by J. Schwartzkopff, Abhandlg. Rhein. Westf. Akademie der Wissenschaften, p. 355). In bees the phasic depolarization changed its response ratio with respect to a slowly arising tonic depolarization as the state of the cell changed [Thurm, Z. vergl. Physiol. **48**, 131 (1964)].