Chemical Excitation and Inactivation in Photoreceptors of the Fly Mutants *trp* and *nss*

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ABSTRACT The *Drosophila* and *Lucilia* photoreceptor mutants, *trp* and *nss*, respond like wild-type flies to a short pulse of intense light or prolonged dim light; however, upon continuous intense illumination, the *trp* and *nss* mutants are unable to maintain persistent excitation. This defect manifests itself by a decline of the receptor potential toward baseline during prolonged intense illumination with little change in the shape or amplitude of the quantal responses to single photons (quantum bumps). Previous work on the *trp* and *nss* mutants suggests that a negative feedback loop may control the rate of bump production. Chemical agents affecting different steps of the phototransduction cascade were used in conjunction with light to identify a possible branching point of the feedback loop and molecular stages which are affected by the mutation. Fluoride ions, which in the dark both excite and adapt the photoreceptors of wild-type flies, neither excite nor adapt the photoreceptors of the *trp* and *nss* mutants. The hydrolysis-resistant analogue, GTPγS, which excites the photoreceptors of wild-type flies, resulting in noisy depolarization, markedly reduces the light response of both mutant flies. Intracellular recordings revealed, however, that the inhibitory effect of GTPγS on the *nss* mutant was accompanied neither by any significant depolarization nor by an increase in the noise, and thus was very different from the effect of a dim background light. The combination of inositol trisphosphate and diphosphoglycerate ($InsP_3$ + DPG), which efficiently excites the photoreceptors of wild-type *Lucilia*, also excites the photoreceptors of *nss Lucilia* mutant. The $InsP_3$+DPG together act synergistically with light to accelerate the decline of the response to light in the mutant flies. These results suggest that the fly phototransduction pathway involves a feedback regulatory loop, which branches subsequent to $InsP_3$ production and regulates guanine nucleotide-binding protein (G protein)–phospholipase C activity. A defect in this regulatory loop, which may cause an unusually low level of

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intracellular Ca^{2+}, severely reduces the triggering of bumps in the mutants during intense prolonged illumination.

INTRODUCTION

The transient receptor potential (*trp*) mutant of *Drosophila* (Cosens and Manning, 1969; Minke et al., 1975; Lo and Pak, 1981; Minke, 1982; Montell et al., 1985; Montell and Rubin, 1989) and the no steady-state (*nss*) mutant of the sheep blowfly *Lucilia* (Howard, 1984; Barash et al., 1988) can be very useful for dissecting invertebrate phototransduction (Pak, 1979). In these mutants the receptor potential, which appears normal in response to dim light, declines to baseline after within a few seconds of illumination with intense light which activates ≈10% of the photopigment molecules. The decline of the response is due to a reduction in the rate of occurrence of the quantum bumps (Minke et al., 1975; Barash et al., 1988) which sum up to produce the receptor potential (Dodge et al., 1968; Wu and Pak, 1978; Wong, 1978; Wong and Knight, 1980). The *trp* and *nss* mutations affect the triggering mechanism of the bump without affecting bump shape and amplitudes (*trp*: Minke et al., 1975; Minke, 1982; *nss*: Barash et al., 1988). The decline of the response is accompanied by a conductance decrease (Minke, 1982, Fig. 3; Howard, 1984, Fig. 2).

The observation that both *trp* and *nss* mutants have a nearly normal receptor potential in response to dim light or brief exposure to strong illumination indicates that all the molecular components needed to produce quantum bumps are present and functional in these mutants. However, the reduction to zero of the quantum-bump rate revealed that a maintained bump production during intense prolonged light depends on the *trp* protein which is missing in the mutant (Montell and Rubin, 1989). The role of this protein is still unknown. However, one possibility is that it may be a component of a light-activated negative feedback loop affecting the triggering mechanism of the bump (Barash et al., 1988). Alternatively, absence of the *trp* protein may cause in the mutant a temporary depletion of a critical factor (e.g., Ca^{2+}) needed for bump production (Stieve and Bruns, 1980; Bolsover and Brown, 1985). In order to find out what molecular stages are affected by the mutation, we compared, in the present study, the action of chemical agents known to excite invertebrate photoreceptors on mutant and normal photoreceptors.

A variety of chemical agents, such as hydrolysis-resistant GTP analogues, fluoride, vanadate, and metabolic inhibitors, are known to induce discrete voltage fluctuations (bumps) of the membrane potential in invertebrate photoreceptors in the dark (Fein and Corson, 1979, 1981; Payne, 1981, 1982; Bolsover and Brown, 1982; Corson and Fein, 1983; Minke and Stephenson, 1985; Stern et al., 1985). Metabolites generated by inositol phospholipid hydrolysis, such as 1,4,5 inositol trisphosphate ($InsP_3$), are very effective in exciting and adapting invertebrate photoreceptors (Fein et al., 1984; Brown et al., 1984; Payne et al., 1986*a*, *b*; Devary et al., 1987). F^- and the GTP analogues induce bumps similar in shape to those caused by light, but with amplitudes about five times smaller. This suggests a relatively small gain in the activation of the guanine nucleotide-binding protein (G protein) by rhodopsin (*Limulus*: Fein and Corson, 1979, 1981; Bolsover and Brown, 1982; *Musca*: Minke and Stephenson, 1985).

Two main mechanisms have been proposed to account for the effects of the various chemical agents: (*a*) activation of specific stages in the phototransduction cascade (Fein and Corson, 1981; Corson and Fein, 1983; Fein et al., 1984; Brown et al., 1984; Minke and Stephenson, 1985; Devary et al., 1987) and (*b*) blocking of inactivation stages, either directly or indirectly by depleting the ATP required for the inactivation reactions (Payne, 1981, 1982; Stern et al., 1985). The above two mechanisms are not mutually exclusive. According to the first mechanism, F^- and the GTP analogue may activate G protein, which is normally activated by the photopigment. The existence of such a light-activated G protein was demonstrated in the cephalopod retina (Calhoon et al., 1980; Saibil and Michel-Villaz, 1984; Vandenberg and Montal, 1984; Tsuda, 1987). In the fly eye the α-subunit of the G protein was identified as a 41-kD protein using ADP ribosylation by cholera toxin (Bentrop and Paulsen, 1986; Paulsen and Bentrop, 1986) or by photoaffinity labeling with azidoanilido-GTP and its quantity was determined by light-dependent [^{35}S]GTPγS binding (Devary et al., 1987). The relevance of the light-activated G protein to the physiological response was demonstrated by the finding that both light-stimulated GTPase activity in membrane preparations and the prolonged depolarizing afterpotential (PDA) in intact photoreceptors show a similar dependence on photopigment conversion by colored lights (Blumenfeld et al., 1985).

Relevant enzymes and cellular mechanisms indicate that the target for the G protein in the invertebrate photoreceptors is a phospholipase C (PLC) enzyme, the effector of the inositol lipid signaling system (Brown et al., 1984, 1987; Szuts et al., 1986; Devary et al., 1987; Baer and Saibil, 1988; Inoue et al., 1988; Bloomquist et al., 1988; Payne et al., 1988; Trowell, 1988; Wood et al., 1989). The evidence that a G protein activates the PLC was suggested by GDPβS inhibition of light excitation but not of the $InsP_3$-induced excitation in *Limulus* (Fein, 1986). It was also implicated by complete inhibition and large facilitation of light-activated PLC by GDPβS and GTPγS, respectively, and by a GDPβS-sensitive F^- activation of the PLC in the dark in studies of cell free membrane preparation of *Musca* eye (Devary et al., 1987; see also Wood et al., 1989). Using exogenous phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], Baer and Saibil (1988) showed in squid retina that light-activated production of $InsP_3$ requires GTP. Thus, it appears likely, that F^- and the metabolically stable GTP analogues induce bump production by activation of the G protein, consistent with the effects of these agents in other biological systems, i.e., vertebrate rods (Bigay et al., 1985) and hormone-regulated adenylate cyclase (Eckstein et al., 1979; Cassel and Selinger, 1977).

Fig. 1 summarizes the current view of the initial steps in the phototransduction cascade in invertebrates (Bolsover and Brown, 1985; Fein, 1986; Payne, 1986; Paulsen and Bentrop, 1986; Devary et al., 1987; Tsuda, 1987; Paulsen et al., 1987; Bloomquist et al., 1988; Payne et al., 1988). Photoexcited rhodopsin activates a G protein by facilitating GTP binding. The G protein then activates a phospholipase C (*PLC*) that generates inositol trisphosphate (*$InsP_3$*) which in turn acts as an internal messenger to release Ca^{2+} from the smooth endoplasmic reticulum (also called submicrovillar cisternae, *SMC*) (Brown and Rubin, 1984; Payne et al., 1986*b*). The suggested site of action of F^- and GTPγS is on the G protein (*G*) and that of exogenous $InsP_3$ is on the submicrovillar cisternae causing a release of Ca^{2+}. The hydrolysis of $InsP_3$ can be inhibited by 2,3-diphosphoglycerate (*DPG*), an $InsP_3$ phosphatase

inhibitor. The increase in Ca^{2+} feeds back to inhibit further Ca^{2+} release by $InsP_3$ (Payne et al., 1988). The identity of the second messenger for excitation is still in dispute as both $InsP_3$ (Fein et al., 1984; Brown et al., 1984; Devary et al., 1987) and cyclic GMP (Saibil, 1984; Johnson et al., 1986) have been implicated as second messengers of excitation in invertebrates.

The scheme of Fig. 1 represents the primary sequence of steps of phototransduction. The known regulatory loops of this cascade implicated from physiological

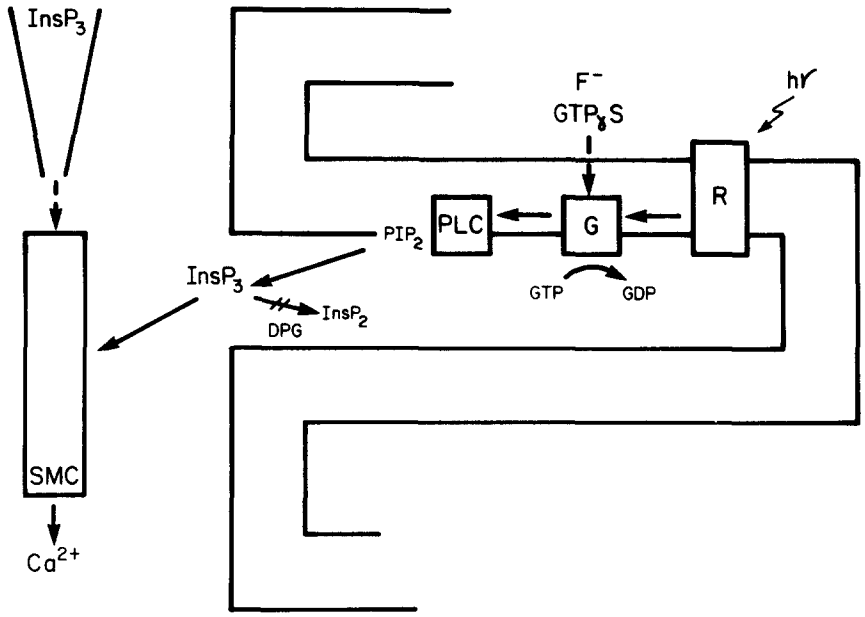

FIGURE 1. A model scheme which summarizes the current view of the initial steps in the phototransduction cascade in the microvilli of invertebrates. The cascade was proposed by Fein (1986), Payne (1986) Paulsen et al. (1987), and Devary et al. (1987). After absorption of a photon ($h\nu$) photoactivated rhodopsin (R) catalyses the exchange of GTP for GDP on a G protein (G). The activated G protein activates phospholipase C (PLC) which cleaves $InsP_3$ from phosphatidylinositol bisphosphate (PIP_2). $InsP_3$ then releases Ca^{2+} from submicrovillar cisternae (SMC). The $InsP_3$ is inactivated by an $InsP_3$ phosphatase which converts $InsP_3$ into $InsP_2$. This reaction can be blocked by 2,3-diphosphoglycerate (DPG) which is an $InsP_3$-phosphatase inhibitor. A possible site of action of F^- and GTPγS on the G protein is indicated. The site of action of exogenous $InsP_3$ on the submicrovillar cisternae is also indicated near a pipette filled with $InsP_3$. (The scheme is a modification of a similar scheme of Payne, 1986.)

experiments are the following: a positive feedforward loop which accelerates the response (Payne and Fein, 1986); a positive feedback loop which facilitates the response amplitude (Bolsover and Brown, 1985; Grzywacz et al., 1988) and increases the bump rate (Stieve and Bruns, 1980); a negative feedback loop which reduces the size but not the frequency of the bumps (Lisman and Brown, 1975; Grzywacz and Hillman, 1988; Payne et al., 1988); and a negative feedback loop which reduces the frequency but not the size of the bumps (Barash et al., 1988; see

reviews by Stieve, 1986; Payne, 1986; Payne et al., 1988). The role of regulatory loops in invertebrate phototransduction was recently described in a detailed quantitative study by Grzywacz et al. (1988).

To establish further the existence of a feedback regulatory loop which controls bump rate, we first tried to exclude the possibility that a mutation in the primary linear sequence may account for the *trp* and *nss* phenotype. If the *trp* or *nss* mutations affect a protein involved directly in the transduction pathway (see Discussion), and if the cascade is linear, then it should be possible to localize the *nss* (or *trp*) gene product in the main excitatory chain of reactions at a stage prior, subsequent or at the site of action of specific chemical agents such as GTPγS and $InsP_3$. In case that a linear cascade cannot account for the data and a feedback regulatory loop is required, as suggested by the results of Barash et al. (1988), it would be of interest to determine where in the cascade the pathway branches and what molecular stages are regulated by the feedback loop. To answer the above questions we compared the actions of F^-, GTPγS, and $InsP_3$+DPG on normal and mutant flies. The outcome of experiments using chemical excitation or a combined excitation by light and the above chemicals in the mutants should limit the possibilities for the sites of branching of the feedback loop and its target proteins.

MATERIALS AND METHODS

Intact white-eyed *Lucilia cuprina* and its white-eyed *nss* mutant (Howard, 1982, 1984) and white-eyed *Drosophila* and its white-eyed mutant trp^{CM} were used for the experiments. The white-eyed *Lucilia* and its *nss* mutant were obtained from Dr. G. G. Foster, CSIRO Division of Entomology, Canberra, Australia. The details of the experimental setup were described elsewhere (Barash et al., 1988). Flies were immobilized by cooling for 2 min and then mounted with wax on a rotating stage with dorsal side up. The upper part of the cornea was sliced off with a vibrating razor blade to expose a small hole in the dorsal part of the eye which was covered with petroleum jelly. The *Drosophila* flies were mounted in a manner similar to the *Lucilia* except that only extracellular electroretinogram (ERG) measurements were recorded from the eye of the *Drosophila* by a low-resistance (5 MΩ) Ringer's solution–filled pipette. The indifferent electrode, filled with Ringer's solution, was placed on the thorax. The composition of the Ringer's was (in millimolar): NaCl 140; KCl 2; $CaCl_2$ 2; $MgCl_2$ 5; HEPES 10, pH 7. In both *Drosophila* and *Lucilia* eyes a third pipette (tip diameter of ≈5 μm) filled with Ringer's solution, to which test compounds were added, was introduced into the small hole in the cornea close to the recording electrode. The test compounds replaced an equimolar concentration of NaCl to keep the osmolarity of the Ringer's solution constant. Also, the pH was readjusted after the addition of the test compound to the Ringer's. The test compound was injected into the extracellular space of the retina by a sequence of (usually 10) short (50 ms) pulses of pressure, resulting roughly in 1:10 dilution in *Lucilia* and about 1:2 in *Drosophila*. The dilution factor (calculated for the whole eye) was estimated by comparing the volume of pressure-induced drops (resulting from 100 pressure pulses) and the volume of the eye. Note that the internal perfusion of the eye in intact fly replaces the fluid of the retina within ≈1 h (Weyrauther et al., 1988). The injecting pipette was introduced into the retina only after control light-responses were measured, to prevent the effect of possible leakage from the pipette before injection. The given concentration of test compounds are those of solutions in the injecting pipette. At the end of the experiment the size of the drops coming from the injecting pipette was examined again. In some experiments the pressure injection into the retina was carried out in the dark and in other experiments it was applied during prolonged

maximal-intensity unfiltered (white) illumination. The energy of the white light (in conjunction with two heat filters, KG-3, Schott Glass Technology, Inc., Mainz, Federal Republic of Germany) was 30.5 mW/cm^2. The white illumination was used to facilitate the penetration of large hydrophilic molecules into the photoreceptors (Wilcox and Franceschini 1984*a*, *b*; Minke and Stephenson, 1985; Devary et al., 1987). After bright illumination combined with injection of test compound, the preparation was dark adapted for several (2–6) min. Guanosine-5′-*O*-(3-thiotriphosphate) (GTPγS) and guanylyl imidodiphosphate (GppNHp) were obtained from Boehringer (Mannheim, Federal Republic of Germany). Fluoride and 2,3-diphosphoglycerate (DPG) and 1,4,5-inositol trisphosphate ($InsP_3$) were obtained from Sigma Chemical Co. (St. Louis, MO).

In some experiments fast superfusion of the *Drosophila* isolated half eye was used in the same manner as described in detail by Minke (1982) and Minke and Stephenson (1985).

Calculation of Power Spectra

Intracellular recordings were performed on intact *Lucilia* flies and 2 M KCl-filled micropipettes of 100–150-MΩ resistance was used. The electrical responses were amplified ×100 and low-pass filtered by a differential amplifier (model 264A2, Tektronix, Inc., Beaverton, OR), with the 3-dB point at 1 KHz. The amplified and filtered responses were sampled from the steady-state phase of the response by a microcomputer (LSI model 11/23, Digital Equipment Corp., Maynard, MA) and stored on floppy discs. The rate of sampling in all the figures presented (Figs. 2–8) was 500 samples per second, which is sufficient for the bandwidth of the signal. In some experiments, to filter the background noise further and to examine for possible aliasing errors, the following procedure was used: the sampling rate was set at 2,000 (or 4,000) samples per second and the samples were grouped into sets, each of four (or eight) consecutive sampled points, and averaged. No significant differences were found between the power spectra calculated by the two sampling methods below 100 Hz. In some other experiments, with a sampling rate of 500/s, the bandwidth was limited to the range of 0–250 Hz by a custom-built, fourth-order, low-pass filter, and in this case, too, similar results (below 100 Hz) were obtained. Power spectra were calculated by fast Fourier transform from blocks of 1,024 points. The power spectra of several (usually 15) such consecutive nonoverlapping blocks were averaged. The averaged spectra were further smoothed by a moving n-points average, with $n < 31$. Peaks at 50, 100, and 150 Hz, which are artifacts, the first, second, and third harmonics of power line frequencies, were subtracted (before smoothing) from the power spectra, and the subtracted regions were interpolated between the nonsubtracted regions. The smoothed spectra reflect accurately the shape of the unsmoothed spectra and facilitate the comparison among various spectra.

Light Stimulation

The light source consisted of a 100-W 12-V halogen lamp in conjunction with two Schott KG-3 heat filters, an OG-590 edge filter (Schott Glass Technology, Inc.), or a 550-nm interference filter (Ditric Optics, Inc., Marlboro, MA). The light intensity was attenuated by neutral-density filters (Ditric Optics, Inc.). The unattenuated green and orange light intensities at the level of the eye were 0.95 and 14 mW/cm^2, respectively. Green and orange lights were used to prevent the induction of the PDA (Barash et al., 1988). We also used a 150-J photographic flash (Broncolar, Bron Electronic, Allschwil, Switzerland) in conjunction with an OG-570 edge filter. Three orange flashes were sufficient to convert almost all the photopigment molecules existing in the metarhodopsin state back into the rhodopsin state. The light emitted from the light sources was conducted by a 4-mm diam light guide the end of which was placed ≈3 mm from the eye. Owing to the use of white-eyed flies the light diffused uniformly

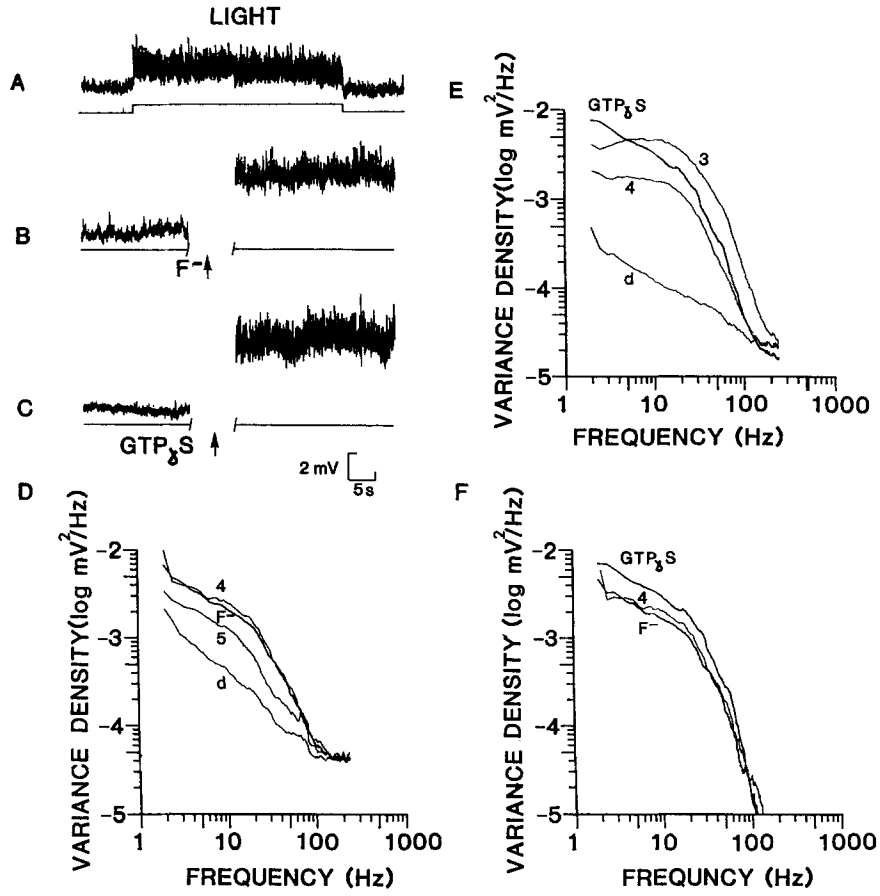

FIGURE 2. Both GTPγS and F^- induces noisy depolarization in normal *Lucilia*. The power spectra, calculated from the F^- and GTPγS-induced noise are similar to each other and to the power spectrum calculated from the noise induced by dim lights. All responses (*A–C*) are intracellular recordings. (*A*) A response to dim orange light (OG-590; log I_{max}/I = 4.0). (*B*) A trace recorded in the dark showing noisy depolarization induced by injections of F^- (20 mM in Ringer's solution) in the dark. The break in the solid line indicates a 13-min pause. During that pause F^- was injected by eight pulses of pressure each of 50-ms duration. Traces *A* and *B* were recorded from the same cell. (*C*) A noisy depolarization induced by GTPγS injections (40 mM in Ringer's solution). The injections were combined with 75 s of maximal-intensity white illumination given in three periods of 25 s interspaced by 25 min. The break in the solid line indicates a 70-min pause. During the white illuminations, GTPγS was injected by 47 pulses of pressure each of 50-ms duration. (*D* and *E*) Power spectra calculated from the (*D*) F^-- and (*E*) GTPγS-induced noise in two different flies. The other power spectra presented in *D* and *E* were calculated from the light-induced noise of the same flies. The relative intensities of the various light stimuli are indicated in log units. The bottom power spectra (*d*) were calculated from the dark noise which was considerably larger in *D* relative to *E*. (*F*) The power spectra of the dark noise were subtracted from the corresponding F^- and GTPγS spectra of *D* and *E*. The third power spectrum (*thin line*) was calculated from the light-induced noise of *D* (log I_{max}/I = 4.0), after subtraction of the power spectrum of the dark noise.

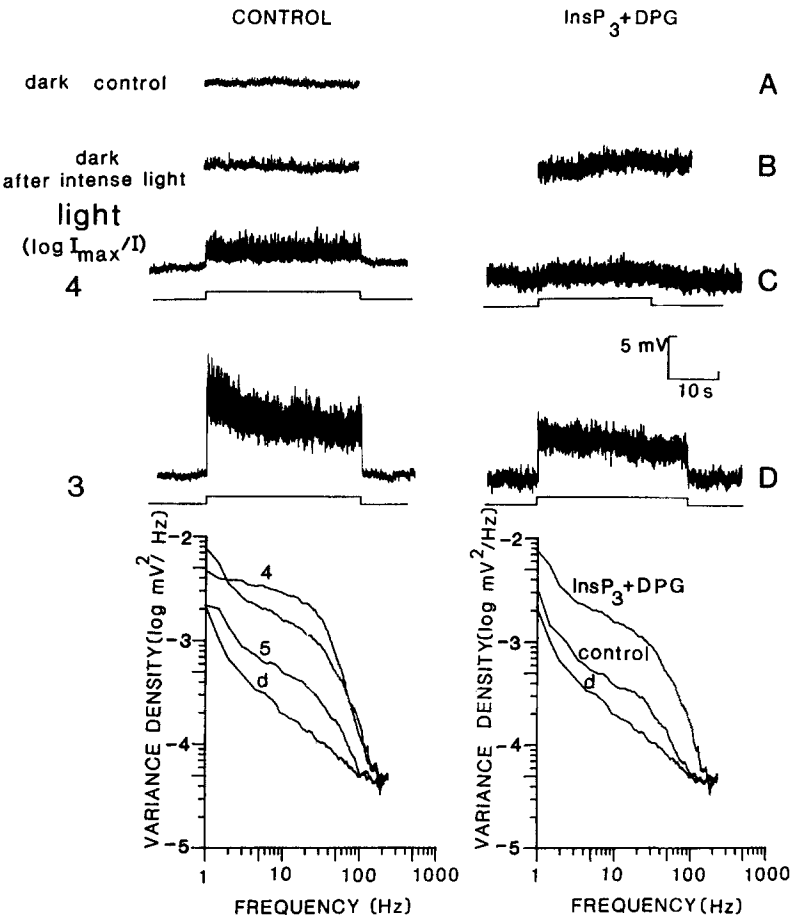

FIGURE 3. 1,4,5 $InsP_3$+DPG excite normal *Lucilia* photoreceptors in the dark. (*Left column*) Control intracellularly recorded receptor potentials in response to two dim orange (OG-590) lights with relative intensity as indicated (rows *C* and *D*). A trace showing the noise level in the dark-adapted cell (row *A*) and 5 min after intense (5 s) maximal-intensity white light (row *B*) is also presented. (*Right column*) Intracellularly recorded noise 5 min after injections of $InsP_3$ (1 mM) + DPG (50 mM) in Ringer's solution combined with the intense white light of 40-s duration in pulses of 5-s duration. There is a large increase in noise level in the dark as a result of the injections combined with illumination (row *B*). There was no significant response to dim orange light (log I_{max}/I = 5.0; not shown) only a small response to 10 times more intense light (log I_{max}/I = 4.0) was observed (row *C*). The response to another log unit brighter light (log I_{max}/I = 3.0) was larger in the control compared with that with $InsP_3$ + DPG, indicating adaptation by continuous $InsP_3$ + DPG action. The graph below (*left*) plots the power spectra calculated from the control responses to dim lights (log I_{max}/I = 5.0; log I_{max}/I = 4.0). The right graph plots the power spectra calculated from the noise of the dark-adapted cell of trace *A* (*d*); the dark control after intense white light (control) and from the noise in the dark after $InsP_3$+DPG injections 5 min after the bright white light was turned off (*$InsP_3$+DPG*). This trace together with trace *d* are also replotted on the left graph for comparison with the power spectra calculated from the light-induced noise of the control (*4, 5*). Since there was some small increase in background instrument noise after $InsP_3$+ DPG injection (before

all over the eye. The absolute light intensity reaching the eye in different experiments varied within the range of a factor of 3, as determined by measurements of the early receptor potential.

RESULTS

Pharmacological Studies in Normal Lucilia and the nss Mutant

Fluoride, GTPγS, and a combination of $InsP_3$+DGP excite Lucilia photoreceptors in the dark. Superfusion of a sliced *Musca* eye with F^- or GTPγS induces a noisy depolarization accompanied by desensitization of the photoreceptors. The power spectra of the photoreceptor noise induced by F^- and GTPγS are very similar to the power spectra calculated from noise of the responses to dim or medium intensity lights recorded from the same cells (Minke and Stephenson, 1985). Similar results were also obtained in the intact eye of the housefly *Musca* (Minke et al., 1988).

Fig. 2 shows an example of chemical excitation observed in the dark after F^- and GTPγS application to intact eyes of *Lucilia* (normal). Control light responses were first recorded in response to increasing intensities of orange lights (in steps of 0.3 log units). An example for one such light response is demonstrated in Fig. 2 *A*. Ringer's-filled injecting pipettes containing either 20 mM F^- (Fig. 2 *B*) or 40 mM GTPγS (Fig. 2 *C*) in two different flies were introduced into the retina. Trains of short (50 ms) pulses of pressure were used to inject into the retina F^- (in the dark) and GTPγS (during 75 s of maximal-intensity white lights). The GTPγS was injected during three periods (25 s each) of white illumination interspaced by ≈25-min periods. The resulting noisy depolarizations are demonstrated in Fig. 2 *B* (F^-) and 2 C (GTPγS). The F^--induced noise became evident ≈5 min after the injection while the GTPγS-induced noise became evident ≈15 min after the second injection. The power spectra (Fig. 2, *D* and *E*) calculated from the F^- (Fig. 2 *D*) and GTPγS-induced noise (Fig. 2 *E*) were similar to the power spectra calculated from the light-induced noise in the range between relative intensity *5* and *4*, for the F^--induced noise and in the range between relative intensity *4* and *3* for the GTPγS-induced noise. Traces *B* and *C* and graphs *D* and *E* show that the noise in the dark before injection was relatively large in the experiments with F^- injection. To compare the power spectra of the F^-- and GTPγS-induced noise, we subtracted the power spectra of the dark noise (*d* in Fig. 2 *D* and *E*) from the corresponding F^- and GTPγS spectra. The subtracted spectra are presented in Fig. 2 *F* together with the subtracted power spectrum of the light-induced noise ($\log I_{max}/I = 4.0$) of Fig. 2 *D*. The close similarity between the F^-, GTPγS and light spectra is consistent with similar findings described previously in *Musca* (Minke and Stephenson, 1985).

$InsP_3$+DPG, introduced into *Musca* photoreceptors by extracellular injection

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$InsP_3$ + DPG had an effect), this extra noise (which could be fitted to $1/f$ function, i.e., it showed a spectrum parallel to *d*) was subtracted from the trace $InsP_3$+DPG. The traces of the left column were recorded from one cell and those of the right column from another cell of the same fly. The numbers above the two power spectra (*left graph*) indicate the relative light intensity of the stimuli used to elicit the responses which were used to calculate the corresponding power spectra.

combined with bright illumination, strongly facilitated the response to light. Furthermore, it caused an extended noisy depolarization and a high rate of bumps in the dark (Devary et al., 1987). Similar phenomena were also found in normal *Lucilia* (Fig. 3) except that in *Lucilia* injections had to be accompanied by brighter and longer lights and the excitatory effect was less pronounced than in *Musca* (see below).

Fig. 3 demonstrates excitation and adaptation induced by application of $InsP_3$+DPG into intact *Lucilia* photoreceptors by extracellular injection combined with bright illumination. Fig. 3 (*left*) shows control responses to dim orange lights. It also shows the noise level in the dark adapted cell in the dark (row *A*) and 5 min after intense white light (row *B*, *left*). The power spectra of the noise in the dark (*d*) and of that calculated from the responses to dim lights ($\log I_{max}/I = 5$, $\log I_{max}/I = 4$; indicated by *5* and *4*) are typical of the dark noise and of dim lights, i.e., there is an increase in variance spectral density at all frequencies upon increase in light intensity (Fig. 3, *left* graph, curves *4* and *5*). Fig. 3 (*right*) shows excitation induced by $InsP_3$+DPG. Repeated injections combined with bright white illumination induced a persistent noisy depolarization in the dark (Fig. 3, row *B*, *right*) with a much larger noise level as compared with the control (row *B*, *left*). The left and right traces of row *B* were recorded after the same dark period. The increased noise level in the dark after $InsP_3$+DPG application was accompanied by adaptation as evidenced by a negligible response to illumination with very dim orange light ($\log I_{max}/I = 5.0$) during the $InsP_3$+DPG–induced noise (not shown). Increasing the intensity of the orange stimulus (to $\log I_{max}/I = 4.0$ and $\log I_{max}/I = 3.0$) gave significantly reduced responses compared with the control responses to the same stimuli (compare Fig. 3, left and right traces of rows *C* and *D*). These effects of $InsP_3$+DPG in the dark are typical for the effects of dim background light. The power spectrum calculated from the $InsP_3$+DPG–induced excitation in the dark shows an increase in variance spectral density of all frequencies relative to the control (*right graph*) and is similar to that calculated during dim light with relative intensities of $\log I_{max}/I = 5.0$ but with larger variance spectral density at all frequencies (*left graph*).

When the extracellularly injected $InsP_3$+DPG was combined with a flash, the response to light showed facilitation without adaptation (see Devary et al., 1987). Presumably in such conditions only a small amount of $InsP_3$+DPG entered the cell.

Application of F^- and GTPγS (but not $InsP_3$+DPG) produced individual bumps with calculated average amplitude of about five times smaller than the light-induced bumps. In the range of small mean depolarizations, the F^-- and GTPγS-induced noise was always accompanied by a larger depolarization as compared with the light response which induced similar noise (Fig. 2). This observation came from studies earlier in locust (Payne, 1982) and in *Musca* (Minke and Stephenson, 1985). It was explained by the presumed smaller amplitudes of the discrete events induced by F^- and GTPγS as compared with the amplitudes of the discrete events (bumps) induced by light.

The power spectra of the light-induced noise of normal *Lucilia* (Fig. 2, *D* and *E*; Fig. 3) changed in a typical manner with the increase in light intensity. These changes were described in more detail by Johnson and Pak (1986) in *Drosophila* and by Barash et al. (1988) in *Lucilia*.

Background light largely reduces the response to test light of the nss mutant and accelerates its decline. Fluoride, GTPγS, and $InsP_3$+DPG all induced a noisy depolarization in normal *Musca* and *Lucilia*, which is similar in several aspects to that induced by dim background light. It was therefore of interest to examine the effects of dim background lights on the response of the *nss* mutant to a constant light pulse. Fig. 4 shows response of the *nss* mutant to an orange test light pulse, which was intense enough to suppress the response to below baseline within a few seconds (*upper trace*). The lower two traces show the response to the same light intensity (in the same cell) when the test light was superimposed on background lights of two different intensities. The response to the test light declined much faster when superim-

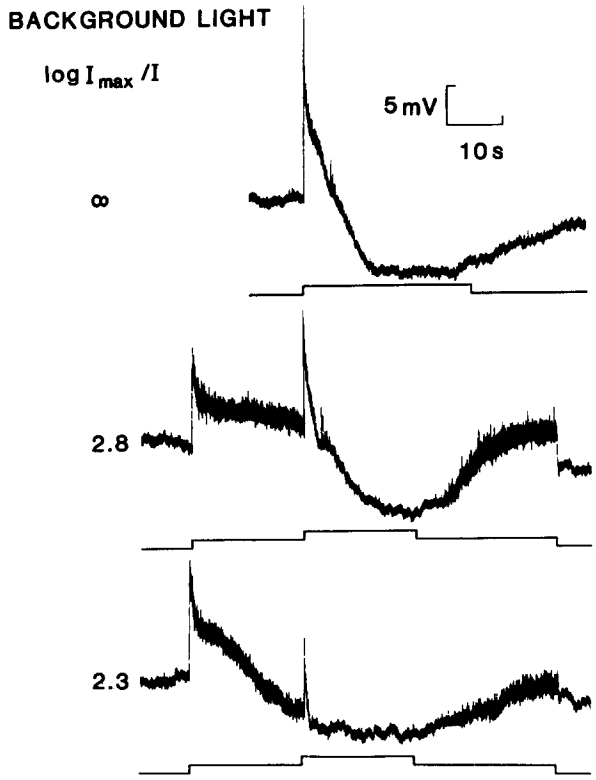

FIGURE 4. Background light reduces the *nss* response to a test light and accelerates its decline. Intracellular recordings from a *nss* photoreceptor in response to constant orange (OG-590 edge filter) test pulse with maximal-intensity attenuated by 1.5 log units. In the second and third traces the constant test pulse was superimposed on background light (OG-590 edge filter) with relative intensity as indicated. The onset of the background light induced a transient response followed by a noisy depolarization which was largely suppressed and even hyperpolarized by the test pulse, whereas the response of the test pulse was largely reduced in amplitude and declined much faster.

posed on background light. The decline was accelerated and the amplitude was much reduced when the intensity of the background light was increased three times (Fig. 4, *bottom*). The test light also suppressed the noise induced by the background light and hyperpolarized the cell. The noise induced by the background light recovered after the test light was turned off.

It can be argued that the decline of the response towards baseline during the test light (Fig. 4) is not due to a mutation-induced block of phototransduction but rather a result of an increase in K^+ or Cl^- conductance or a result of strong activation of the Na/K electrogenic pump. An increase in K^+ or Cl^- conductance is a very unlikely mechanism for the decline, since previous bridge measurements in both *trp*

mutant (Minke, 1982, Fig. 3) and *nss* mutant (Howard, 1984, Fig. 2) showed that the decline of the response is accompanied by a conductance decrease. Activation of the Na/K pump is probably responsible for the slow large hyperpolarization, since light-induced entry, of Na^+ into photoreceptors is known to activate the pump (Lisman and Brown, 1972). The hyperpolarization is accompanied by a decrease in noise (Fig. 4). In contrast, a hyperpolarization accompanied by large noise was usually observed in wild type flies after a flash of light (not shown), and thus it seems unlikely that activation of the electrogenic pump causes the decline of the *nss* response. The reduction in response amplitude and the speeding up of its decline during background light are induced much faster than expected from activation of the Na/K pump.

The similarity in shape and amplitude of the bumps, recorded from the *nss* photoreceptor during dim light as compared to intense light when the response declined towards baseline (Barash et al., 1988), demonstrates that light adaptation is very weak or absent in the *nss* mutant and therefore cannot account for the effects of background light.

Similar experiments with various combinations of test light and background light revealed that in general, the decaying receptor potential of the *nss* mutant is very sensitive to the presence of dim background light which significantly accelerates its decay rate and reduces its peak amplitude. Accordingly, chemical excitation, equivalent to a dim background light, could provide a sensitive test as to the manner in which light and chemical excitation interact in the mutant photoreceptor. However, a high intracellular concentration of the chemicals capable of suppressing the response in a similar manner to intense prolonged light could not be obtained.

Differential action of F^-, GTPγS, and $InsP_3$+DPG on the response to light of the nss mutant. If the excitatory pathway of invertebrate phototransduction involves a linear cascade of enzymatic reactions and the *nss* mutant blocks phototransduction by inactivating one component in the cascade, then the following predictions can be derived: (*a*) If the defective *nss* gene product operates at a stage subsequent to the stage activated by a specific chemical agent, then this agent should produce a noisy depolarization and accelerate the decline of the response to a light pulse as shown in Fig. 4. Since only a limited amount of the chemical will enter the cell, no effect similar to that induced by intense light is expected. (*b*) However, if the *nss* gene product operates before the stage affected by the chemical excitation, then this agent should also induce noisy depolarization but should have no effect on the rate of decline of the response to a light pulse. Figs. 5, 6, and 7, show that neither of the above expectations is realized when F^- or GTPγS are applied. In contrast, $InsP_3$ did fulfill prediction *a*, i.e., it produced noise in the dark and accelerated the rate of decline of the *nss* response to light (Fig. 8).

Fig. 5 shows responses to illumination with two intensities of orange light before injection (control) and after injection of large quantities of 20 mM F^- in Ringer's solution (F^-). No significant differences were found between the responses before and after injection of F^-. Fluoride was applied to the eye during darkness. However, in two cases we applied the F^- during the intense white illumination and waited 5 min for dark adaptation. Even under these conditions, F^- had no effect. The same results were obtained in all the 19 cells in five different mutant flies that were exam-

ined. In both *Musca* and normal *Lucilia*, F^- was found to be the most potent agent in inducing noise. Accordingly, the amounts of F^- applied to the *nss* eye were by far (about five times) larger than the amounts needed to induce a significant noise and depolarization in the normal fly. Thus the *nss* mutation, like the *trp* mutation (see below), seems to abolish the ability of fluoride to excite or adapt intact fly photoreceptors.

An entirely different result was obtained when GTPγS was applied to the mutant. Fig. 6 shows the effects of injection of 40 mM GTPγS in Ringer's solution during repeated pulses (5 s) of maximal intensity white light (no effect of GTPγS was

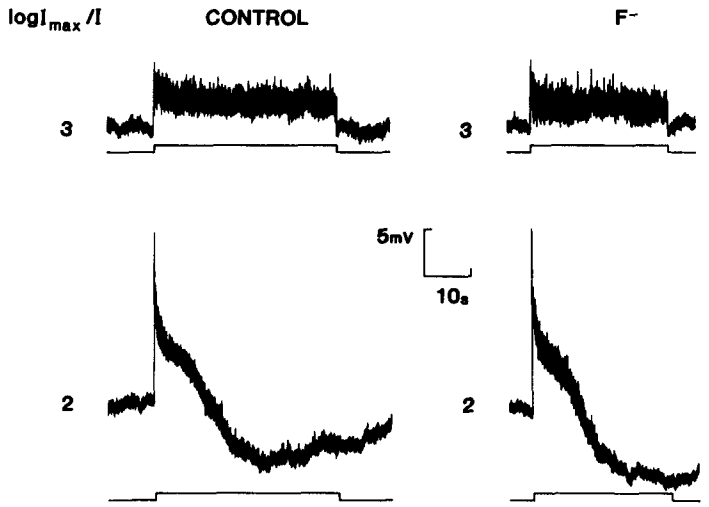

FIGURE 5. Fluoride does not excite the photoreceptors of the *nss* mutant. The left column shows control, intracellularly recorded, responses to orange (OG-590) lights. The right column shows responses to the same stimuli in the same eye but from a different cell after injections (35 pulses of pressure each of 50-ms duration) of F^- ions (20 mM in Ringer's solution) in the dark, during 50 min. The relatively large volume of injections made it difficult to record the right and the left traces from the same cell. However, it was found that responses of three different cells in the control recordings showed very similar responses; also penetrations to other seven cells in the same eye after F^- application gave results very similar to those shown.

observed in the dark). The upper trace shows the control response to an orange test pulse. Before injection, the maximal intensity white lights caused only temporary reduction in the response to the test light, which completely recovered 1 min after the white light was turned off (not shown). When the white light was given together with injection of GTPγS, a permanent reduction in the response to test lights was observed (in another cell of the same fly, Fig. 6, *middle trace*). Both the amplitudes of the responses to various intensities of test light and their noise level were severely reduced. This reduced noise level was manifested in calculations of power spectra (not shown). With further injections combined with bright white lights, further reductions in the responses of the same cell to the test pulse were observed (Fig. 6,

bottom trace). The reduction in response amplitude was not accompanied by speeding the decay of the response to baseline. Although these effects of GTPγS were not reversible up to 3 h, they clearly did not result from deterioration of the impaled cells since successful penetrations to six other cells in the same fly showed similar reduced responses but normal resting potentials (see also Fig. 7). Additional intense illuminations combined with injections resulted in an even larger suppression of the responses and no increase in noise level in the dark. The power spectra calculated from the noise in the dark at various times after GTPγS application had the same

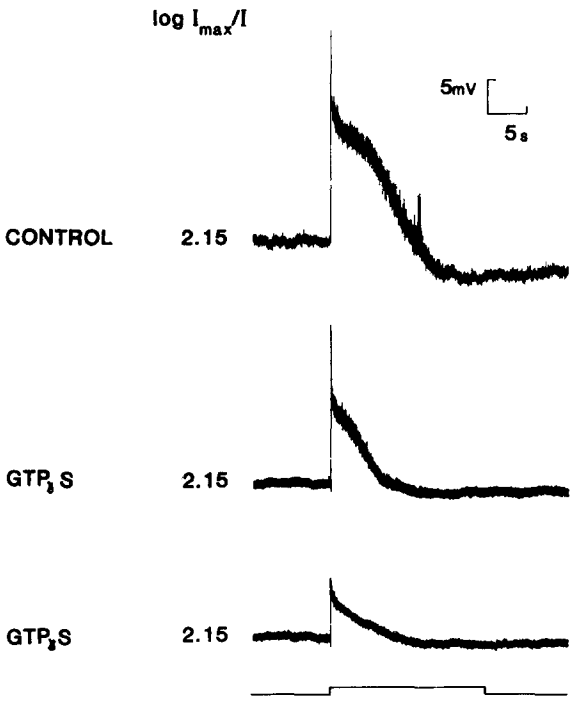

FIGURE 6. GTPγS largely reduces the light response without apparent increase in noise in the dark and with little change in the response waveform in the *nss* mutant. Intracellular recordings showing responses to the same constant orange test pulse before injection of GTPγS in one cell (control; three other cells in the same eye showed similar responses) and after 20 injections (50-ms pulse duration) of 40 mM GTPγS combined with 20 s of maximal-intensity white illumination (*middle*) and after additional 15 injections combined with the intense 15 s of white illumination (*bottom*) in another cell. The bottom two responses were recorded from a single cell after 21 min of dark adaptation. Six other cells with normal resting potentials were penetrated after the recordings of the bottom trace and gave very similar responses. 6 min after injection, which was combined with white illumination, the response (to the test light) reached a constant peak amplitude which was smaller than the amplitude obtained before injection. Additional test illumination in dark intervals of 2 min gave similar responses during periods of more than 20 min.

shape as the power spectra of the noise of the dark control (see Figs. 2 and 3, spectra *d*) in contrast to the GTPγS-induced noise in the dark in normal flies. The results indicate that the *nss* mutant has an altered response to GTPγS.

Fig. 7 shows the effect of GTPγS in another *nss* fly in which the whole experimental paradigm was performed in a single cell with a very good signal-to-noise ratio and in which no change in resting potential was observed during the experiment, indicating that the viability of that cell did not deteriorate. The noisy depolarization induced during the dim orange light (Fig. 7 *a*) was almost completely abolished after

20 s of bright white light accompanied by injections of GTPγS and followed by 3 min of dark adaption (Fig. 7 *d*). The response to the bright white light was also largely reduced after GTPγS application (Fig. 7, *b* and *e*). Interestingly, light was accompanied neither by any significant changes in resting potential nor an increase in the noise level in the dark. A background light which would have suppressed the light responses of the mutant to the same level observed in Figs. 6 and 7 should have been accompanied by a very pronounced noise and the light response should have

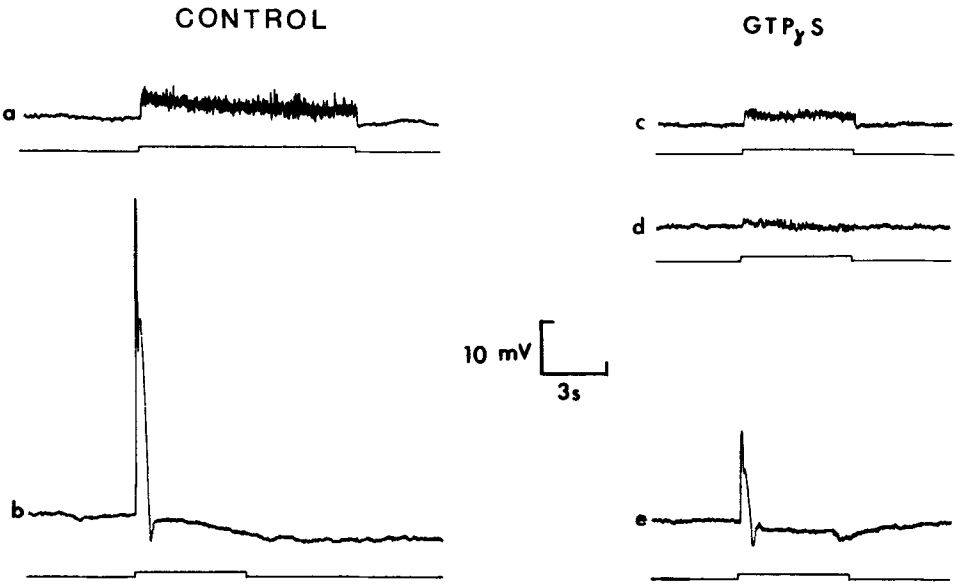

FIGURE 7. Intracellular recordings from *nss* fly showing responses to dim orange light pulses with relative intensity of log $I_{max}/I = 3.0$ (*a, c, d*) and to maximal-intensity white light pulses (*b, e*). All records are from the same cell which showed a very stable resting potential level and a good signal-to-noise ratio. GTPγS (40 mM) was injected by a series of five pulses of pressure each of 50-ms duration. Trace *c* was recorded 6 min after three series of injections combined with three white pulses of 5-s duration. Trace *d* was recorded 3 min after a 5-s white light pulse was given without any additional injection. Additional stimulations with relative intensity of log $I_{max}/I = 3.0$, in intervals of 3 min during 12 min, showed responses similar to trace *d* (not shown). Trace *e* was recorded in response to the same white stimulus as trace *b* (control) 16 min after trace *d* was recorded. No apparent change in resting potential was observed during the whole experiment and a resting potential of 56 mV was measured when the electrode was withdrawn from the cell, 7 min after trace *e* was recorded.

declined in Fig. 6 (*bottom*) much faster (compare Fig. 4). Also, if the combination of GTPγS and bright light suppresses responsivity permanently, like intense prolonged light, a response to dim light (Fig. 7, *c* and *d*) is not expected to be observed, contrary to our observations. The phenomenon of response suppression without apparent increase in noise was observed in all five flies tested. The effects of GTPγS observed in a single cell (Fig. 7) was later verified in four other cells from the same eye which all showed similar reduced responses and noise but normal resting poten-

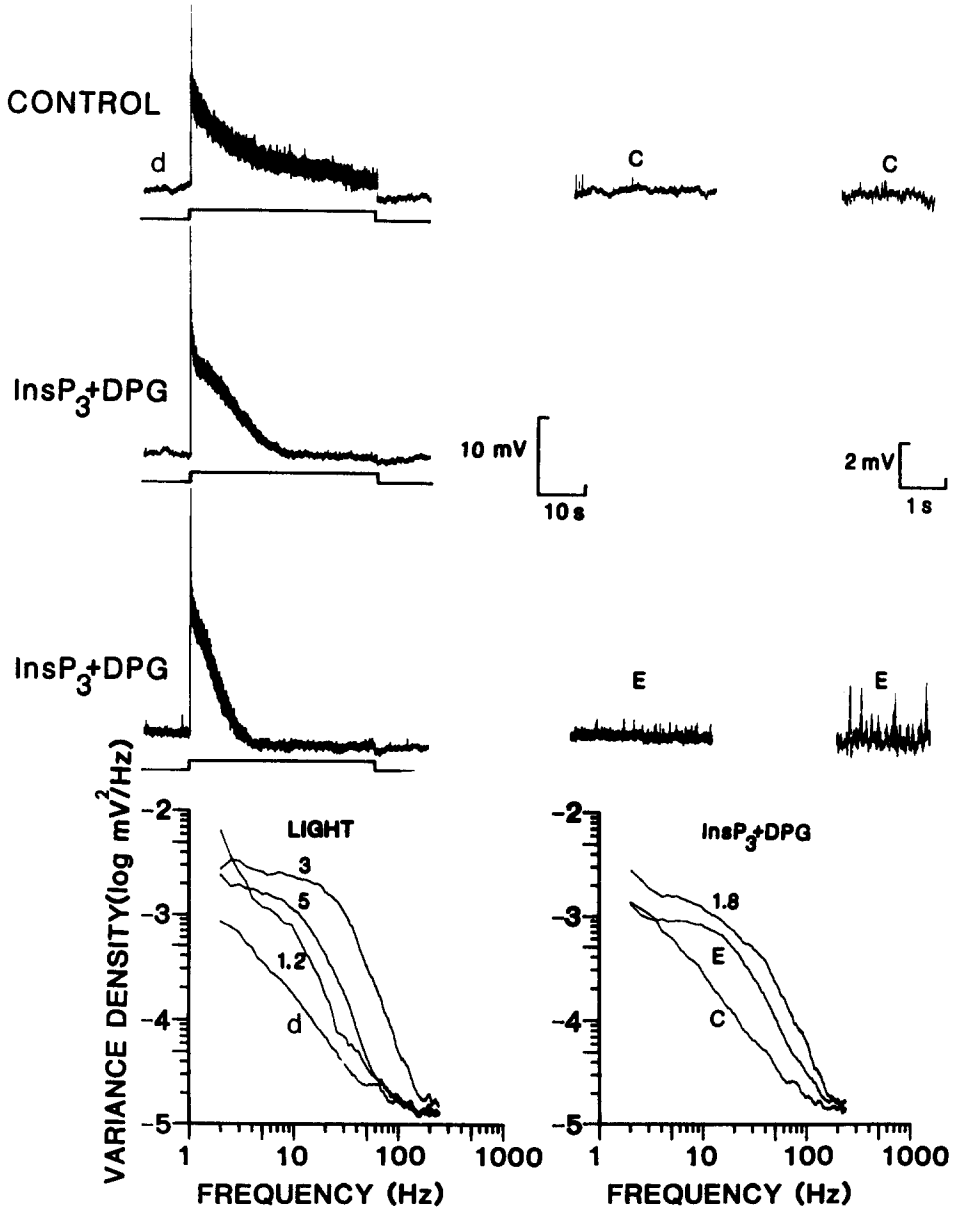

FIGURE 8. $InsP_3$+ DPG facilitates the light response of the *nss* mutant, accelerates its decline to baseline, and induces noise in the dark. (*Left column, upper three traces*) Intracellular recordings from a dark-adapted (3 min) photoreceptor of the *nss* mutant before injection (*control*) showing a response to an orange light pulse (OG-590, $\log I_{max}/I = 1.8$) and to the same orange light after $InsP_3$ (1 mM) and DPG (50 mM) were injected by 10 pulses of pressure (of 50 ms) combined with 20 s of maximal-intensity white light (*second trace*). The third trace shows the response of the same cell to the same stimulus after additional 18 pulses of $InsP_3$+DPG combined with maximal-intensity orange and white illuminations (1 min and 10 s, respectively) were applied. The second and third traces were recorded after 3- and 5-

tial. The results of Figs. 5, 6, and 7 indicate that the G protein and possibly its target protein PLC do not function normally in the mutant.

In contrast to either F^- and GTPγS, $InsP_3$+DPG did mimic the main effects of dim background light on the response to light of the mutant. Application of 1 mM $InsP_3$ + 50 mM DPG induced a significant increase of the dark noise (Fig. 8 *E*) and largely accelerated the decline of the responses to light (Fig. 8, *$InsP_3$+DPG*). The maintained noise during light, which was evident in the control responses, was largely reduced during the response to the same light in the presence of $InsP_3$+DPG. The rate of decline of the response increased when additional amounts of $InsP_3$+DPG were applied to the eye (compare the second and third traces on the left). Unlike the effect of background light, $InsP_3$+DPG facilitated the initial transient phase of the response to a test light by increasing its amplitude. The facilitation of the responses to the test light (Fig. 8, *left column, middle two traces*) was also observed in normal *Lucilia* when small amounts of $InsP_3$+DPG were applied (not shown). Similar facilitation of the light response of *Musca* by $InsP_3$+DPG was described by Devary et al. (1987).

The increase in noise after $InsP_3$+DPG application in the mutant was quantified by calculations of the power spectrum of the noise in the dark (Fig. 8, *bottom right graph, E*). A significant increase in variance spectral density at all frequencies (*E*) relative to the control (*C*) was observed. The left graph shows the power spectra calculated from the light-induced noise (at three different intensities as indicated) before $InsP_3$+DPG application. These power spectra are typical for the *nss* response to light (see Barash et al., 1988). The power spectra calculated from the $InsP_3$+DPG–induced noise in the dark (*E*) and from the noise of the steady-state phase of the response to light ($\log I_{max}/I = 1.8$, control, *upper left trace*) have similar shapes. The somewhat weaker effect of $InsP_3$+DPG in the mutant (Fig. 8) relative to the normal fly (Fig. 3) may arise from a smaller amount of the chemicals which penetrated the photoreceptors, perhaps due to the transient nature of the response to light.

Fig. 8 demonstrates that excitation by $InsP_3$+DPG and by light acts synergistically to accelerate the decline of the mutant response to light. If the phototransduction

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min dark periods, respectively. The acceleration in the decay rate of the response is typical for the effect of prolonged dim background illumination on the response to a test pulse of medium intensity (see Fig. 4). The facilitation in the amplitude of the initial transient after $InsP_3$+DPG application, is typical for a weak effect of these agents. The middle column (traces *C*) shows recordings of noise in the dark 1 min after the cessation of maximal-intensity white light in the control (*C*) and 1 min after the 10 s of white light combined with $InsP_3$+DPG were applied (*E*). The right column shows enlarged segments of the traces in the middle column (the right calibration corresponds to these traces). Power spectra were calculated from voltage traces, recorded in the dark (*C* and *E*), from the dark-adapted cell before injection (*d*), and from a series of control orange light responses with relative intensities as indicated in log units (*left graph*). The right graph shows that there is a significant increase in variance spectral density in the dark after $InsP_3$+DPG injection (*E*) relative to the dark control (*C*) and the $InsP_3$+DPG–induced noise has a power spectrum with a shape similar to that of a control light response with an intensity sufficient to cause a decline of the response close to baseline ($\log I_{max}/I = 1.8$; *upper left trace*).

cascade is linear, then this result indicates that $InsP_3$ operates prior to the *nss* gene product, thus contradicting the results with F^- and GTPγS in the mutant. These seemingly contradictory results cannot be reconciled by a linear cascade (see Discussion).

Pharmacological Studies in Normal Drosophila and the trp Mutant

One of the shortcomings in using the *nss* mutant in a combined genetic and pharmacological dissection of phototransduction is the minimal genetic tools and knowledge available for this mutant (see Howard, 1982) in comparison with the *trp* mutant of *Drosophila* (Montell et al., 1985; Montell and Rubin, 1989). Owing to the detailed knowledge which is already available, about the *trp* gene product of *Drosophila* (see Discussion), it is desirable to repeat the *Lucilia* and *nss* mutant experiments of the previous subsection in normal *Drosophila* and the *trp* mutant in order to verify a pharmacological similarity between the *nss* and *trp* mutants. Unfortunately, in spite of considerable efforts, we were unable to repeat those experiments using intracellular recordings in *Drosophila*. Nevertheless, to demonstrate a probable pharmacological similarity between the *nss* and *trp* mutants we examined the effects of F^- and GTP analogues on the *trp* mutant using ERG recordings. However, because chemical excitation in the dark is weak (see Fig. 8 *E*), we were unable to demonstrate the effects of $InsP_3$+DPG in the *trp* mutant in the ERG. Also, the increase in decay rate of the light response during the expected $InsP_3$+DPG–induced excitation could not be demonstrated since it was probably masked by the much (about five times) slower decline to baseline of the ERG response compared with the response observed by intracellular recordings.

Differential action of F^- on the light response of the normal Drosophila and the trp mutant. Pressure injection of 5 mM F^- into the eye of normal *Drosophila* in the dark had an effect on the ERG response to a short intense orange test flash, which is similar to the effect of background light. This is illustrated in Fig. 9 (*left*), which compares the response to a short intense orange test flash in three different conditions: (*a*) in a dark-adapted eye (*upper responses*); (*b*) during background light (*middle responses*); and (*c*) in a dark-adapted eye into which 5 mM F^- was injected. The baseline in both the light-adapted eye and the F^--injected eye was shifted negatively with respect to the baseline of the dark-adapted state (indicating depolarization of the photoreceptors), and the response to the bright orange test flash became smaller and faster (Fig. 9, *left arrowheads with dashed lines*).[1] Both the baseline shift and the reduction in amplitude of the light response was partially reversed 41 min after injection of F^- (Fig. 9, *left bottom trace*).

The same experimental setup with the same injecting electrode was used to inject F^- into the retina of the *trp* mutant (Fig. 9, *right*). In the *trp* mutant about the same amount of F^- injection had no significant effect (compare the response to the test flash before [Fig. 9, *upper right response*] and after injection). The middle trace of Fig. 9 (*right*) shows the *trp* response to background light (which decays to baseline during light) and the effect of background light in diminishing the response to the

[1] The slow decline of the response to the flash (Fig. 9, *upper row*) arises from the slow response of the pigment (glia) cells (Minke, 1982).

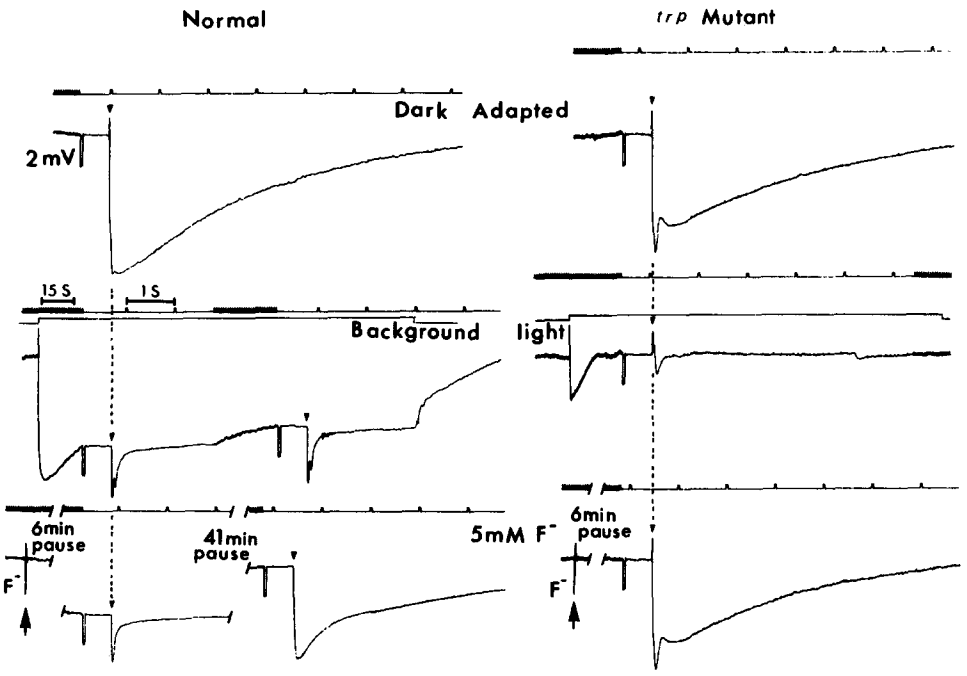

FIGURE 9. A comparison between the effect of fluoride (applied in the dark) on the ERG recorded in the dark and in response to light flashes, in intact normal (*left*) and *trp* mutant of *Drosophila* (*right*). The upper row shows the response to short (1.5 ms) maximal-intensity (see Materials and Methods) orange flashes (OG-570 edge filter) in the dark-adapted flies. The second row shows the response to the same flashes when superimposed on orange background light (OG-590 edge filter) with maximal intensity attenuated by 1.5 log units. In the normal fly, the onset of background light (log $I_{max}/I = 1.0$) induced a maintained corneal negative deflection in the ERG indicating depolarization in the photoreceptors. The response to the test flash became smaller due to light adaptation and due to a reduction in the driving force of the membrane potential. The initial "on transient" was also abolished. In the mutant, the onset of background light induced a receptor potential which declined to baseline during illumination. The response to the test light became much reduced and slower although there was no reduction in membrane driving force. These effects were typical for the *trp* mutant. The bottom line shows the effect of injecting Ringer's solution containing 5 mM F^- into the retina in the dark. In the normal fly application of F^- ions caused a negative deflection in baseline, indicating depolarization in the photoreceptors which slowly declined with time. The response to the test flash became smaller, similar to the response superimposed on background light. The response to the test flash largely recovered 41 min after injection. In the *trp* mutant similar injections of F^-, with the same electrode and in the same experimental setup, had no significant effect.

flash. In the majority of the experiments in which pressure injection was used in *Drosophila*, we injected alternately to normal and mutant *Drosophila* with the same injecting pipette and with the same duration and pressure to make sure that approximately similar amounts of F^- were injected in the two cases.

In all of the 12 mutants and 14 normal fruitflies that were examined, we obtained

results similar to those shown in Fig. 9. A similar result was obtained when we raised the F^- concentration to 20 mM (two flies).

To make sure that F^- ions had access to the photoreceptors in the eye of the mutant we superfused the sliced isolated heads of *trp* mutants with oxygenated Ringer's solution containing various concentrations of F^- in the range between 1 and 10 mM and obtained essentially similar results. Fluoride had only an effect of metabolic inhibitor (not shown).

GTPγS suppresses the ERG response of the normal Drosophila and the trp mutant. An experimental paradigm similar to that used to examine the action of F^- on normal *Drosophila* and the *trp* mutant was also used to test the effects of GTPγS. An example of these experiments is demonstrated in Fig. 10. Injection of 40 mM GTPγS combined with bright illumination, which presumably introduces the chemical into the cell, had a very pronounced effect on the ERG response. Fig. 10 shows the response of the dark-adapted eye to the constant orange test flash and the response to the same flash when superimposed on background light in both normal and *trp* fly (Fig. 10, *upper two rows;* compare Fig. 9). The third line in Fig. 10 presents an additional control. It shows the response to the same test flash after 6 min of darkness, which enabled the eye to recover from prior illumination for 2 min with maximal-intensity white light. The response recovered completely in the dark 6 min after the prolonged intense illumination was turned off. A similar intense light was used to facilitate the penetration of GTPγS into the photoreceptors.

In the *trp* mutant the GTPγS injection, combined with 2 min of bright illumination, caused a very large positive shift in baseline during illumination, which slowly returned towards baseline (not shown). We observed large variabilities in the levels of the baseline which finally reached a steady-state level a few minutes after the bright light was turned off. Accordingly, the level of the baseline after injection and illumination in the *trp* mutant could not be used as a reliable measure of depolarization in the photoreceptors. In normal *Drosophila* similar fluctuations in baseline with a similar experimental paradigm were not observed. The bottom line in Fig. 10 shows that injection of GTPγS combined with illumination caused a similar effect on the ERG response in the mutant and the normal fly (Fig. 10 *d*, *bottom line*). In both cases, the response to the constant test flash became smaller. Shortly after the cessation of the bright light, which was combined with injection, the response was abolished temporarily in the *trp* fly. However, 6 min after the adapting light was turned off, both responses of normal and mutant flies reached a constant smaller amplitude (Fig. 10 *d*). With further injections accompanied by bright illumination the responses to the test flashes reached a stable value, after the 6-min dark interval, which was significantly smaller (Fig. 10 *e*) than that reached after the first injection (Fig. 10 *d*). When the procedure was repeated, the response became systematically smaller (Fig. 10 *f*) until it was completely abolished. The same phenomena were observed in all six mutants and eight normal flies that were tested. The same experimental paradigm was repeated with GppNHp (40 mM) injected instead of GTPγS in seven mutants and nine normal flies. Very similar results were obtained.

Injection of GTP analogues combined with bright illumination thus induced a very pronounced reduction in the response to light of the *trp* mutant. It was not clear, however, from these experiments, whether the reduction in the amplitude of

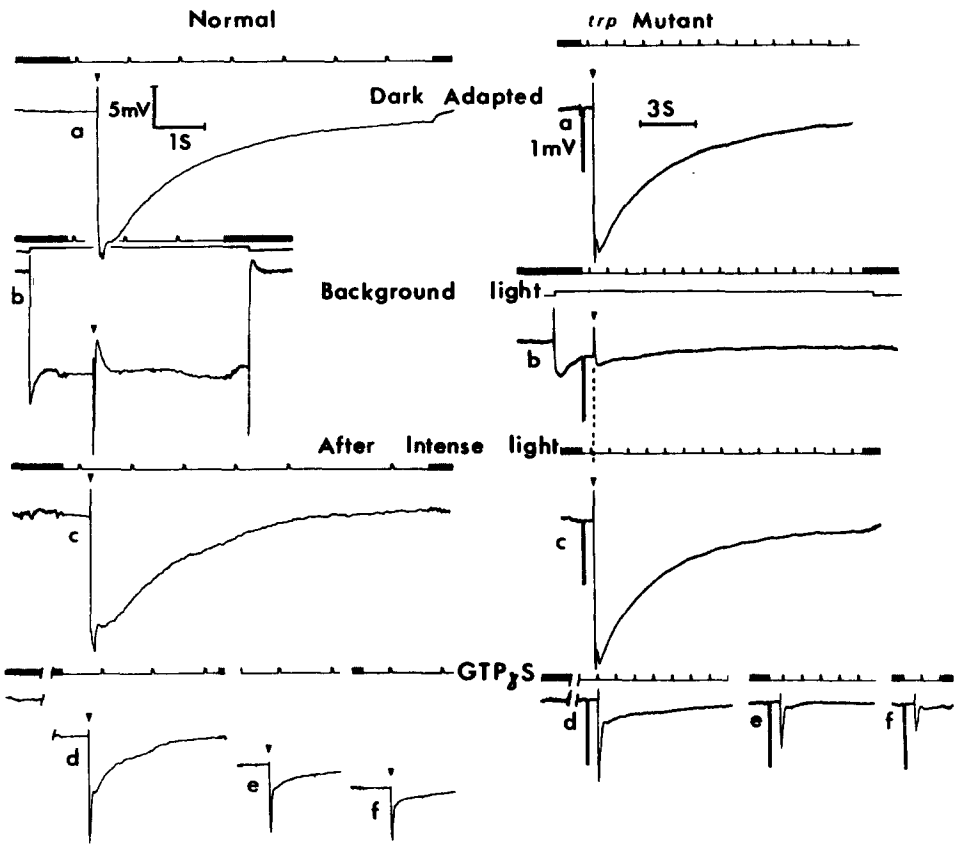

FIGURE 10. A comparison between the effect of GTPγS on the ERG recorded in the dark and in response to bright test flash in intact normal (*left*) and *trp* fly (*right*). The experimental paradigm is similar to that of Fig. 9 except that 2 min of maximal-intensity white light pulses were given four times during the GTPγS injection. The third line is a control showing that responses to the test flash, before injection, and after complete recovery (6 min after the 2 min of bright illumination was turned off). The bottom line shows that a combination of injection and bright illumination resulted in the normal fly in a corneal negative shift in baseline (indicating depolarization in the photoreceptors), and the response to the test flash became smaller and faster (with reduced amplitude of the slow response of the pigment cells) in both normal and mutant fly. Those effects became even more pronounced when additional injections combined with 2 min of illumination were given (*d–f*). In each record (*d–f*) injection combined with 2 min of maximal-intensity white illumination were applied. The given traces were recorded after an additional 6-min dark period. The figure shows that unlike F^- ions (Fig. 9), which do not affect the mutant but have an effect on the wild type, GTPγS affected both the mutant and normal fly by reducing the amplitude of the responses to the constant test flashes. In the case of the mutant the baseline level after injection cannot be considered a reliable measure of depolarization (see text).

the response to light was due to chemically induced excitation of the photoreceptors or to inhibition of the light response without apparent excitation, as found by the intracellular recordings during similar experiments in the *nss* mutant. The change of shape of the light response after GTPγS application could arise from the lack of response in the pigment cells which are only activated by a receptor potential with a large amplitude.

The pharmacological experiments using F^- and GTP analogues in the *trp* mutant, are consistent with the suggestion that the *trp* has a phenotype very similar to that of the *nss* mutant both in the characteristics of the light response and in its pharmacological properties.

DISCUSSION

Several lines of evidence suggest that the *trp* and *nss* mutations affect the same gene product. Both mutants have similar responses to light, are similarly affected by chemical agents that excite photoreceptor cells, and contain mutations that map to similar chromosomal locations (Howard, 1982). Nevertheless, it cannot be excluded that two different genes may cause the same phenotypic expression.

There are at least two main ways to interpret the results of the present study: (*a*) The *trp* and *nss* mutations encode for a protein without enzymatic activity which is related indirectly to phototransduction, thus having pleiotropic effects on several components of the cascade. This can be a protein that controls the level of a critical factor which is directly involved in the cascade. Elimination of the *trp* or *nss* protein by mutations may cause a temporary shortage in this critical factor during prolonged intense illumination. (*b*) The mutations eliminate a protein which is directly involved in phototransduction but in a feedback regulatory loop.

The result of the present study is not consistent with a linear phototransduction cascade. The outcome of the experiments using combined excitation by light and $InsP_3$+DPG in the mutant limits the possibilities for the site of branching of the feedback loop to stages subsequent to $InsP_3$ production. This is because light and weak excitation by $InsP_3$+DPG acted synergistically to accelerate the decay rate of the *nss* response to light (Fig. 8).

The lack of an effect of F^-, which is expected to activate the G protein (Bigay et al., 1985), and the inhibition of further response to light by GTPγS without producing noise, suggest that the G protein and possibly its target protein, the PLC, do not function properly in the *nss* mutant. The effect of GTPγS is difficult to interpret in the *trp* mutant, since the use of ERG recordings cannot differentiate between a reduction in response to a test light owing to persistent excitation by GTPγS or to another still unknown action, similar to that observed in the *nss* mutant. We consider it rather unlikely that GTPγS functions differently in the *trp* and *nss* mutants. The clear-cut and reproducible elimination of the light response following the application of GTPγS (and GppNHp in *trp*) clearly indicates that GTPγS did enter the photoreceptors. There is also no reason to doubt that F^- had any difficulties in penetrating cells since it is capable of producing metabolic inhibition in a perfused *trp* preparation (not shown).

The *trp* gene (and possibly the *nss* gene) does not code for a G protein. Montell and Rubin (1989) recently found that the *trp* mutation eliminates in three different

alleles a 143-kD eye-specific membrane protein from the photoreceptor cells. This missing protein is too large to be a G protein and its amino acid sequence does not resemble any known protein. The G protein of the fly eye, on the other hand, belongs to the G protein family (Blumenfeld et al., 1985; Bentrop and Paulsen, 1986; Paulsen and Bentrop, 1986; Devary et al., 1987; Yarfitz et al., 1988).

The *trp* protein may be a component of a feedback loop which regulates the G protein activity or its interaction with the PLC. The impaired function of the G protein–PLC complex may also arise from a depletion of a critical factor (e.g., Ca^{2+}) needed for G protein–PLC action during intense illumination.

Another possible interpretation of the effects of the GTP analogs in the mutants is that they inhibit a release of Ca^{2+} from already largely depleted $InsP_3$-sensitive Ca^{2+} stores. Such a mechanism was found in neuronal and smooth muscle cell lines (Chueh et al., 1987; and see below).

The *trp* and the *nss* mutants do not show the normal increase in $[Ca^{2+}]_{in}$ during illumination, as is evident by the transient (*trp;* Lo and Pak, 1981) or lack of pigment migration (*nss;* Howard, 1984). Also, the *trp* (Minke, 1982) and *nss* (Barash et al., 1988) mutants show an unusual small effect of light adaptation. Since high Ca^{2+} levels shorten the response latency (Lisman and Brown, 1975) and since the response latency is abnormally long during background light in the *trp* and *nss* mutants, the defect in the mutants most likely involves Ca^{2+}. Accordingly, the synergistic action of light and $InsP_3$+DPG in the *nss* mutant may arise from a depletion of $InsP_3$-sensitive Ca^{2+} stores.

Stieve and Bruns (1980), Bolsover and Brown (1985), Stieve (1986), Payne and Fein (1986), and Payne et al. (1986*b*, 1988) already attributed important roles for Ca^{2+} in excitation of *Limulus* ventral photoreceptors. We therefore speculate that the *trp* (and *nss*) gene product is a new type of Ca^{2+} transporter protein which is light-regulated and constitute the main pathway for transporting Ca^{2+} from the extracellular space during illumination. We assume that some level of intracellular Ca^{2+} is required for a maintained bump production during light (Bolsover and Brown, 1985). The transient light response of the mutants may arise from a temporary depletion of the $InsP_3$-sensitive Ca^{2+} stores resulting in pleiotrophic affect on several components of the cascade.

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