

Lanthanum Reduces the Excitation Efficiency in Fly Photoreceptors

EDITH SUSS-TOBY, ZVI SELINGER, and BARUCH MINKE

From the Departments of Physiology and Biological Chemistry, and The Minerva Center for Studies of Visual Transduction, The Hebrew University, Jerusalem 91010, Israel

ABSTRACT Lanthanum (La^{3+}), a known inhibitor of Ca^{2+} binding proteins, was applied to the extracellular space of fly retina. Shot noise analysis indicated that a combination of intense light and La^{3+} caused a large (down to zero) reduction in the rate of occurrence of the quantal responses to single photons (quantum bumps) which sum to produce the photoreceptor potential. Light in the presence of La^{3+} also increased the effective bump duration. These effects are very similar to the effects of the mutations *trp* of *Drosophila* and *nss* of *Lucilia* flies on the quantum bump rate and duration. La^{3+} applied to the *nss* mutant caused only a small reduction in the bump rate, suggesting that La^{3+} may affect the *nss* gene product which is deficient in the mutant. The close similarity in the properties of the receptor potential of the La^{3+} -treated photoreceptor of the wild type and of the *nss* mutant together with existing evidence for the highly reduced intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) level in *nss* photoreceptors suggest that both La^{3+} and the mutation cause a severe reduction in $[\text{Ca}^{2+}]_i$. This effect may arise from an inhibition of a Ca^{2+} transporter protein located in the surface membrane that normally replenishes Ca^{2+} pools in the photoreceptors, a process essential for light excitation.

INTRODUCTION

The inositol lipid signaling system appears to be an essential component of phototransduction in the microvillar photoreceptors of invertebrates (Fein et al., 1984; Brown et al., 1984; Payne, 1986; Devary et al., 1987; Tsuda, 1987; Selinger and Minke, 1988; Bloomquist et al., 1988). Of the several stages of the inositol-lipid signaling system, coordination of the release and entry of Ca^{2+} within and into cells is the least clear part (Chueh et al., 1987; Morris et al., 1987; Berridge and Irvine, 1989). Fly mutants, defective in Ca^{2+} mobilization, can be very useful for unraveling the molecular components involved in coordination of Ca^{2+} release and entry. Indirect evidence suggest that two fly mutations, i.e., the transient receptor potential (*trp*) of *Drosophila* and the no steady state (*nss*) of *Lucilia* primarily disrupt phototransduction subsequent to production of inositol triphosphate (InsP_3), and that the defect can arise from a temporary depletion of Ca^{2+} from the InsP_3 -sensitive Ca^{2+} pools (Suss et al., 1989). In both *trp* (Cosens and Manning, 1969; Minke et al., 1975; Lo and Pak, 1981; Minke, 1982; Montell et al., 1985; Montell and Rubin, 1989; Wong et al., 1989) and *nss* (Howard, 1982, 1984; Barash et al., 1988; Suss et al.,

Address reprint requests to Dr. Baruch Minke, Department of Physiology, The Hebrew University-Hadassah Medical School, Jerusalem 91010, Israel.

1989), the receptor potential, which appears normal in response to dim light, declines to baseline within a few seconds of illumination with intense light. The decline of the response is due to a reduction in the rate of occurrence of the quantal responses to single photons (quantum bumps), because there is no change in bump shape and amplitude when the response declines close to baseline during light (*trp*, Minke et al., 1975; *nss*, Barash et al., 1988). The quantum bumps are known to summate to produce the receptor potential (Dodge et al., 1968; Wong, 1978; Wu and Pak, 1978; Wong and Knight, 1980; Wong et al., 1982; for reviews see Stieve, 1986, and Payne, 1986). Several lines of evidence suggest that the *trp* and *nss* mutations affect the same gene product. Both mutants have a very similar phenotype (Howard, 1982) and are similarly affected by chemical agents that excite the photoreceptor cells (Suss et al., 1989). The *trp* mutant lacks a protein whose sequence has been determined by Montell and Rubin (1989) and by Wong et al. (1989). It encodes a 143-kD membrane protein composed of eight putative transmembrane segments with no sequence homology to any known protein (Montell and Rubin, 1989; Wong et al., 1989).

A recent study of Hochstrate (1989) has demonstrated that, when lanthanum (La^{3+}) is perfused into the retinal extracellular space of the blowfly *Calliphora*, the electrophysiological properties of the photoreceptors become very similar to those of the *trp* or *nss* mutants. Furthermore, because the decline of the response in the presence of La^{3+} is accompanied by a conductance decrease (as in the *trp* and *nss* mutants), it is unlikely that the decline is due to an increase in conductance to K^+ or Cl^- ions.

The striking similarity between the *trp* or *nss* phenotype and the La^{3+} -treated photoreceptors of normal *Calliphora* led Hochstrate to suggest that both La^{3+} and the *trp* mutation affect the same cellular processes in the photoreceptors, and that the *trp* gene product is normally located in the plasma membrane of the photoreceptors. The findings of Hochstrate (1989) raise a question concerning the mechanism of the decline to baseline of the light response in the presence of La^{3+} . The decline of the receptor potential in the presence of La^{3+} can arise from either a reduction in bump amplitude (light adaptation) or from a reduction in bump rate (a *trp*-like effect). We therefore applied shot noise analysis in the La^{3+} -treated wild type in order to calculate the rate of occurrence, the mean amplitude, and the effective duration of the quantal responses to single photons at various light intensities. We furthermore examined whether or not the application of La^{3+} to the photoreceptors of the *nss* mutant has effects similar to those found in wild-type flies. If La^{3+} and the mutation affect the same process, one would expect little or no effect of La^{3+} in the mutant. Lanthanum is a known inhibitor of Ca^{2+} transport processes. It inhibits influx of Ca^{2+} in cardiac cells (Wendt-Gallitelli and Isenberg, 1985; Nathan et al., 1988) or presynaptic terminals (Miledi, 1971). It also blocks Na-Ca exchange in vertebrate rods (Yau and Nakatani, 1985). The action of La^{3+} on the receptor potential of the fly may be elicited by affecting Ca^{2+} entry into the cell.

MATERIALS AND METHODS

Preparation

Intact white-eyed *Musca domestica*, *Lucilia cuprina*, and its white-eyed *nss* mutant (Howard, 1982, 1984), white-eyed *Drosophila* and white-eyed *Calliphora* 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. Intracellular recordings were carried out only in the larger flies. *Drosophila* flies were mounted in a manner similar to *Lucilia* except that only extracellular electroretinogram (ERG) measurements were recorded from the eye of the *Drosophila* by a low resistance (5 M Ω) Ringer's-filled pipette. The indifferent electrode, filled with Ringer's solution, was placed on the thorax. Ringer's solution composition was (in mM): 130 NaCl, 2 KCl, 2 CaCl₂, 5 MgCl₂, 10 HEPES buffer, pH 7. A third pipette (tip diameter of \approx 5 μ m) filled with Ringer's solution and containing 5 mM lanthanum was introduced into the small hole in the cornea close to the recording electrode. The lanthanum replaced an equimolar concentration of NaCl. Also, the pH was readjusted after the addition of lanthanum to the Ringer's. Lanthanum was injected into the extracellular space of the retina by a sequence of short (50 ms) pulses of pressure. Note that the normal circulation of body fluids within the intact fly replaces the fluid of the retina within \approx 1 h (Weyrauther et al., 1989). The injecting pipette was introduced into the retina only after control light responses were measured, to prevent the effect of possible leakage of La³⁺ from the pipette before injection. The given concentrations of lanthanum are those of solutions in the injecting pipette. At the beginning and the end of the experiment the size of the drops coming from the injecting pipette was examined. In all experiments the pressure injection into the retina was carried out in the dark.

Light Stimulation

The light source consisted of a 100-W 12-V halogen lamp in conjunction with two heat filters (model KG-3, Schott Glass Technology, Inc., Mainz, Germany) and a Schott model OG-590 filter that passed wavelength longer than \sim 590 nm. The light intensity was attenuated by neutral density filters (Ditric Optics, Inc., Marlboro, MA). The light from sources was conducted by a 4-mm-diam light guide to \approx 3 mm from the eye.

The intensity of unattenuated orange light at the level of the eye was 14 mW/cm². Orange light was used in order to prevent the induction of the prolonged depolarizing afterpotential (PDA; Barash et al., 1988).

Calculation of Power Spectra

Intracellular recordings from photoreceptors in intact flies were made with 2 M KCl-filled micropipettes (100–150 M Ω resistance). Amplified and filtered (1 kHz) responses were sampled from the steady-state phase of the response at 500 samples per second, which is sufficient for the bandwidth of the signal. In some experiments the following procedure was used: to filter the background noise further and to examine for possible aliasing errors the sampling rate was set at 2,000 (or 4,000) samples per second and samples of four or eight points were then grouped into sets. Each of the four (or eight) consecutive sampled points in a set were then averaged and gave 500 samples per second. No significant differences were found between the power spectra calculated by the two sampling methods below 100 Hz. In other experiments, with a sampling rate of 500/s, the bandwidth was limited to 250 Hz by a custom-built, fourth-order, low-pass filter; similar power spectra (below 100 Hz) were also obtained. Power spectra were calculated by fast Fourier transform from blocks of \sim 2-s duration (1,024 points). The power spectra of several such consecutive nonoverlapping blocks were averaged.

Peaks at 50, 100, and 150 Hz being the first, second, and third harmonics of power line frequencies were considered to be artifacts and were sometimes subtracted from the power spectra, and the spectra were interpolated between the nonsubtracted regions. The averaged

spectra were further smoothed by a moving n -points average, with $n < 31$. The smoothed spectra facilitate the comparison among various spectra without distortion of their general shape.

Calculations of the Mean Rate, Mean Amplitude, and Effective Duration of the Quantum Bumps

We applied noise analysis to the data to determine quantitatively the effects of La^{3+} application on the average bump parameters at various light intensities. The application of shot noise analysis to bump noise in photoreceptors has been well described by Dodge et al. (1968) and Knight (1972). Individual bumps are presumed to have a time course $B(t)$. We define effective shot-event amplitude a as:

$$a = \int_0^{\infty} B^2(t)dt / \int_0^{\infty} B(t)dt \quad (1)$$

and effective shot-event duration T as:

$$T = \frac{1}{a} \int_0^{\infty} B(t)dt \quad (2)$$

If the shot-events were rectangular in shape with height a and the duration T , then these equations would be self-evident. If bumps occur at a mean rate ν , the mean \bar{V} and the variance σ^2 of the receptor potential are given by Campbell's theorem (Rice, 1944):

$$\bar{V} = \nu a T \quad (3)$$

$$\sigma^2 = \nu a^2 T \quad (4)$$

where we assume that the receptor potential is a linear summation of bumps.

If the individual shot-events vary in height, the preceding equations still hold with the proviso that a represents the mean height (Rice, 1944). From the macroscopic features of the receptor potential, conversely, we can derive the parameters of the individual shot-event:

$$a = \sigma^2 / \bar{V} \quad (5)$$

$$\nu T = \bar{V}^2 / \sigma^2 \quad (6)$$

We did not correct the mean response amplitude (\bar{V}) for nonlinear summation of voltage responses (Martin, 1976) because changes in amplitudes of the plateau phase of the light responses were rather limited, especially after application of La^{3+} . Thus correction would increase the differences between the bump parameters before and after application of La^{3+} (see Fig. 4).

To calculate the effective bump duration T we used the theory and procedure of Wong and Knight (1980) and Wong et al. (1982). Wong and Knight (1980) assumed that the superimposed bumps have a common waveform $B(t)$ ¹ and that the bump shape can be described by an analytic form known as Γ distribution:

$$\Gamma(t; n, \tau) \equiv \frac{1}{n! \tau^n} (t/\tau)^n e^{-t/\tau} \quad (7)$$

¹ A detailed study conducted in the neuromuscular junction by Katz and Miledi (1972) demonstrates that the waveform of the shot event affects the results by a small factor only.

Eq. 7 yielded, upon Fourier transformation, the following:

$$|\tilde{B}(f)|^2 = |\tilde{\Gamma}(f; n, \tau)|^2 = \frac{1}{[1 + (2\pi\tau f)^2]^{n+1}} \quad (8)$$

where f is the frequency; n and τ can be evaluated by fitting Eq. 8 to the experimental power spectrum (see Fig. 3). The bump duration T is defined by the elementary integrals of Eq. 9;

$$T = \frac{\left[\int_0^\infty dt B(t) \right]^2}{\int_0^\infty dt B^2(t)} \quad (9)$$

which yields

$$T = \tau \cdot \frac{(n!)^2 2^{2n+1}}{(2n)!} \quad (10)$$

For details, see Wong and Knight (1980) and Schnakenberg and Wong (1986). In order to calculate the effective bump duration T (Eq. 10) the function $n!$ was interpolated by the Γ function (Davis, 1964). The results of the approximation were used to calculate T .

The application of Eqs. 5, 6, and 10 to calculate the mean bump parameters from the responses in the presence of La^{3+} requires that steady-state responses be used. Fig. 1 shows that this requirement is fulfilled in the last 20 s of a 35-s response to lights. Because each power spectrum was calculated from a segment of ~ 2 -s duration, slow variations in the steady-state amplitude did not affect the results significantly. This was verified by comparing averaged power spectra calculated from different time segments.

We used the right-hand side of Eq. 8 to approximate the power spectra calculated from the light responses. The approximation, used here on fly data (see also Johnson and Pak, 1986; Barash et al., 1988) provided good fits to the power spectra shown in Fig. 3. However, the power spectra of control responses to medium ($-\log I = 3$) and bright lights (not shown) showed attenuation at low frequencies due to light adaptation (see Dodge et al., 1968; Minke and Stephenson, 1985) and thus could not be fitted well by Eq. 8.

RESULTS

Derivation of the Quantum Bump Parameters from the Light-induced Noise in the Presence of La^{3+}

Effects of La^{3+} on the receptor potential. In fly photoreceptors, individual bumps can be observed only with very dim light. When the intensity of the light stimulus is increased slightly, the quantum bumps sum to produce a noisy depolarization (Fig. 1, CONTROL, upper three traces). With further increase in light intensity, an initial transient phase appears shortly after light onset and the receptor potential declines to a steady-state phase with reduced amplitude and noise (Fig. 1, CONTROL, $-\log I = 2.0$). Shot noise analysis in both *Limulus* and fly (Wong, 1978; Wu and Pak, 1978; Wong and Knight, 1980; Minke and Stephenson, 1985; Johnson and Pak, 1986) indicate that the rate of occurrence of the quantum bumps increases linearly with light intensity. The delayed decline of the initial response from its peak to a lower steady-state phase during light is related to the large reduction in the quantum bump amplitude in the process of light adaptation (Dodge et al., 1968; Wu and Pak,

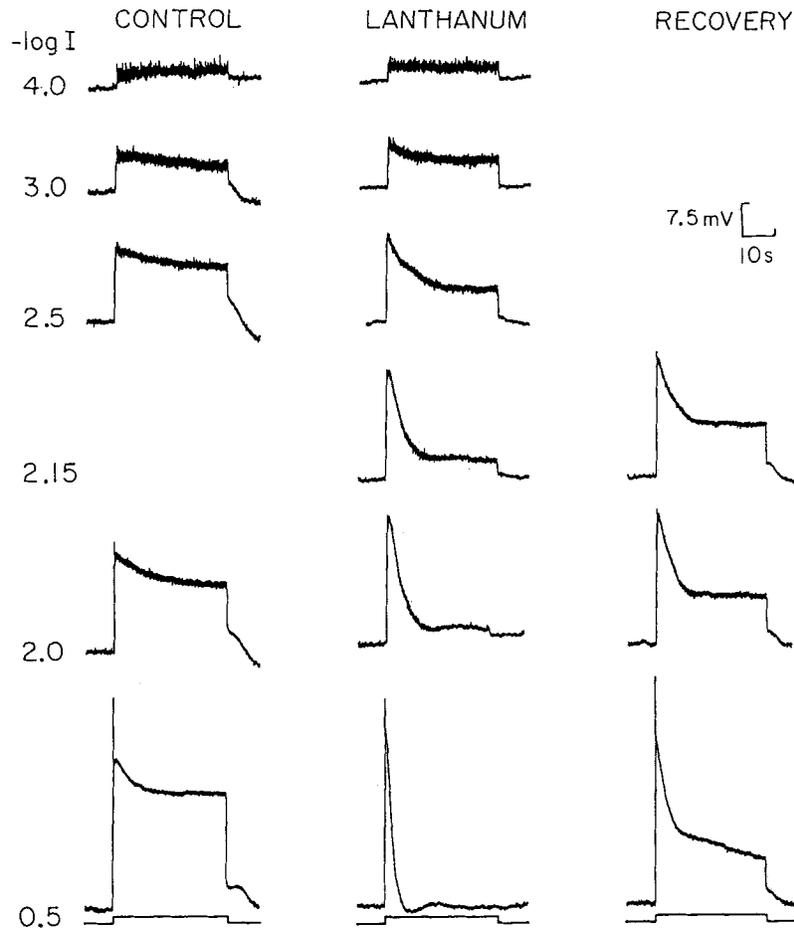


FIGURE 1. The effects of lanthanum on the receptor potentials of white-eyed *Musca* photoreceptors in response to increasing intensities of orange (Schott model OG 590 filter) lights as indicated. The left column shows control responses before application of La^{3+} ; the middle column shows the responses of the same cell in the presence of La^{3+} (injected by 20 pulses of 1 bar with 50-ms duration in the dark). The concentration of La^{3+} was 5 mM in Ringer solution. The right column shows partial recovery of the responses to intense lights 20 min after the injection of La^{3+} . Responses to intense lights best illustrate the effect of La^{3+} . The response to stimulus of $-\log I = 2.15$ was not recorded in the control experiments of this cell. However, recordings from other cells showed that the response to this intensity is similar in shape but slightly reduced in amplitude relative to the response to the stimulus intensity of $-\log I = 2.0$.

1978; Wong, 1980). The pronounced effect of light adaptation on the receptor potential of *Musca* is demonstrated in Fig. 1 (CONTROL, bottom trace).

Injection of 5 mM La^{3+} into the extracellular space of the intact *Musca* retina did not significantly affect the receptor potential at low light levels (LANTHANUM, $-\log I = 4.0$). However, the response to more intense light (Fig. 1, LANTHANUM, $-\log I = 2.5$) showed a slight decline of the plateau amplitude relative to the control.

The response to illumination with intensities above $-\log I = 3$ resulted in a pronounced reduction of the plateau amplitude in a narrow range of stimulus intensities, ($-\log I = 2.5$ – 2.0). Stimulation with bright light (Fig. 1, LANTHANUM, bottom trace) caused a rapid decline of the receptor potential to baseline during illumination. The right column of Fig. 1 (RECOVERY) shows that the effect of La^{3+} was partially reversible after 20 min (in the same cell), presumably because La^{3+} was partially removed from the extracellular space by the considerable flow of the hemolymph (Weyrauther et al., 1989).

Responses very similar to those of Fig. 1 were obtained in 18 *Musca* (housefly), 2 *Lucilia* (sheep blowfly), and 2 *Calliphora* (blowfly) by intracellular recordings and in 2 wild-type *Drosophila* (fruitfly) using electroretinogram (ERG) recordings (not shown). Quantitative analysis of the data (see below) was carried out on results from four *Musca* flies which had more stable light responses and the light-induced noise was largest. Owing to some variability in the sensitivity to light, the amplitude of the light responses and the strength of the effect of La^{3+} in different flies, the calculations were carried out separately for each cell without averaging. The responses and calculations presented in Figs. 1–4 are from the same cell which provided the most stable and longest recordings in a wide range of light intensities. The quantitative results obtained from this cell were confirmed in three other cells of three different *Musca* flies.

Effects of La^{3+} on the power spectrum. The power spectrum of the light-induced noise in steady-state conditions reflects the rate, as well as the amplitude and shape of the elementary voltage events that compose the noise (for details see Wong, 1978; Wong and Knight, 1980; Wong et al., 1982). The graphs in Fig. 2 are the power spectra calculated from the steady-state phases of the light responses of Fig. 1 before application of La^{3+} (CONTROL, left graphs) and from the light responses of the same cell in the presence of La^{3+} . The numbers above each curve are the light intensities (in relative $-\log$ scale) of the various stimuli. The power spectra were calculated from the noise of the receptor potential at the steady-state phase over a 20-s period. The power spectra before La^{3+} application depended in a characteristic way on light intensity (see Barash et al., 1988). The power spectra increased at all frequencies with increasing light-intensity in the dim light range (Fig. 2, left, $-\log I = 5$; $-\log I = 4$). With a further increase in light intensity, the power spectra decreased at low frequencies and increased at high frequencies. These characteristics are illustrated in the family of power spectra in two ways: (a) by the systematic reduction in the power spectra density in the low frequency range when the light intensity was increased ($-\log I = 3, 2.5, 1.8$) and (b) the power spectra calculated from responses to bright light systematically crossed the curves calculated from responses to dimmer light intensities. These changes in the power spectra with increased light intensity mainly reflected the changes in the amplitude and duration of the quantum bumps which became smaller and faster owing to light adaptation (Wong, 1978).

In the La^{3+} -treated eye the changes in power spectra with increasing light intensities were very different. The power spectra calculated from the responses to dim lights ($-\log I = 5, 4$, not shown) was roughly similar to that obtained before application of La^{3+} . However, with increases in stimulus intensity the high-frequency

component of the power spectra was systematically reduced (i.e., in the opposite direction relative to the control) with little reduction in variance density at low frequencies.

Fig. 3 presents several power spectra (which were taken from Fig. 2) in which the power spectrum calculated from the dark noise (d) was subtracted from the various power spectra which were calculated from the light-induced noise as indicated (in

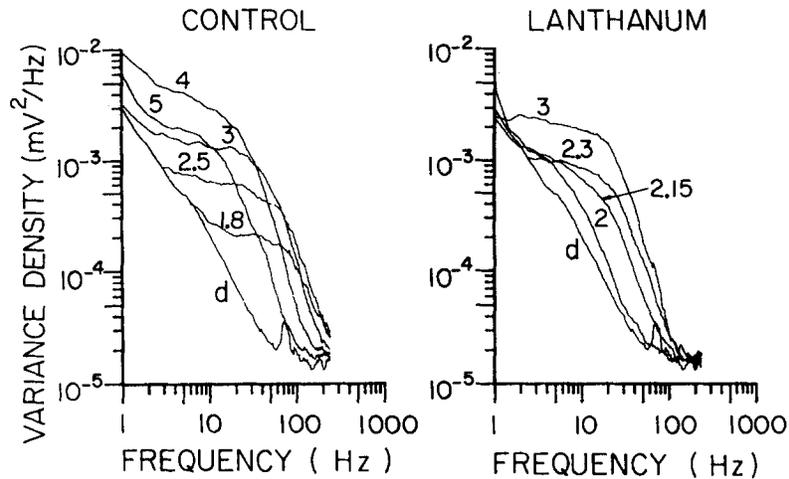


FIGURE 2. The graphs are a family of power spectra calculated from the steady-state phase of the receptor potentials shown in Fig. 1 from 15 to 35 s after light onset in response to increasing intensities of the orange lights before (*left*) and after application of La^{3+} (*right*). (*Left*) The bottom curve was calculated from the noise in the dark (*d*). The other curves were calculated from the light responses to increasing intensities of the orange lights as indicated above each curve. (*Right*) Power spectra calculated from the responses of the same cell after injection of 5 mM La^{3+} to light stimuli with relative intensities as indicated. All power spectra were calculated from responses of a single cell. The CONTROL part shows the typical effect of increasing the stimulus intensity on the power spectrum; namely, at dim light intensity ($-\log I = 4.5$). There is an increase in variance density at all frequencies with the increase in light intensity. With further increase in stimulus intensity, there is a reduction in the variance density at low frequencies and an increase at high frequencies. This reflects the fact that the bumps become smaller and faster with the increase in stimulus intensity. Lanthanum modified the family of power spectra at the critical narrow range of stimulus intensities where the *nss* phenotype is revealed as follows: The typical shift in the power spectra curves to higher frequencies in the CONTROL is modified to a shift in the opposite direction. In the presence of La^{3+} the curves are shifted to lower frequencies with the increase in light intensity.

relative $-\log$ scale) above each curve. In each pair the smooth curve is a plot of Eq. 8 (see Materials and Methods) that best fitted the experimental curve (noisier curve). The subtracted power spectra in Fig. 3 emphasize the results of Fig. 2, namely that application of La^{3+} changed the power spectrum calculated from a given light response to a power spectrum that would be obtained from a response to a dimmer stimulus intensity before application of La^{3+} . This is best illustrated in Fig. 3 A, which

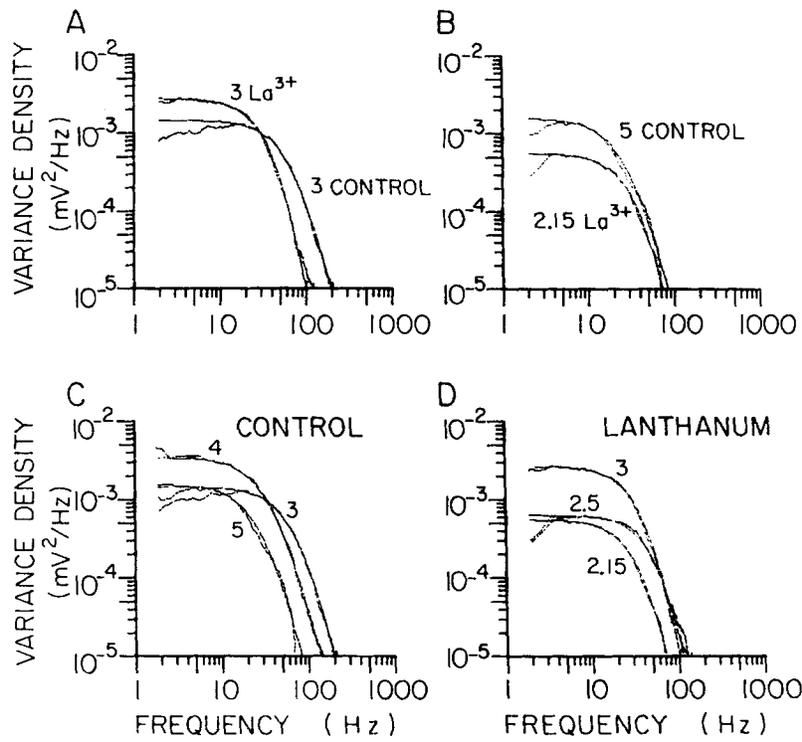


FIGURE 3. The power spectra calculated from responses to intense lights became similar to those calculated from dim lights in the presence of La^{3+} . The power spectra of Fig. 2 could be very well fitted by Eq. 8 (see Materials and Methods). The power spectrum calculated from the noise in the dark was subtracted from the power spectra calculated from the noise in the responses to lights of various intensities of the cell of Fig. 1. The relative intensities of the light stimuli are indicated in $-\log I$ scale above each curve. In each pair of curves the smooth curve is a plot of Eq. 8 that best fitted the experimental curve. The experimental power spectra showed a relatively large variability at low frequencies in different experiments, and therefore the deviation of the curve in this region from Eq. 8 is not significant except for intense lights in the control (A, 3 control). The parameters A , τ , and $n + 1$, which were used to plot Eq. 8 (see Materials and Methods), are listed in Table II. When these parameters were modified by $> \pm 5\%$ the fitted curves clearly deviated from the experimental one. (A) A comparison of the power spectrum calculated from the response to the same light intensity ($-\log I = 3$) before (CONTROL) and after injection of La^{3+} . (B) A comparison of the power spectrum calculated from a response to dim light ($-\log I = 5$), before application of La^{3+} to response to a much brighter light ($-\log I = 2.15$) after application of La^{3+} . (C) Power spectra calculated from responses to a wide range of light intensities (as indicated) before application of La^{3+} . (D) Power spectra calculated from responses to a narrow range of light intensities, in which the responses decline toward baseline, after application of La^{3+} .

compares the power spectrum calculated from the response to the same light intensity ($-\log I = 3$) in the same cell before application and in the presence of La^{3+} . Fig. 3 A shows that in the presence of La^{3+} there is an increase in amplitude of the power spectrum curve at lower frequencies and a decrease in the amplitude of the

power spectrum curve at high frequencies (i.e., there is a shift in the curve to lower frequencies).

Effects of La³⁺ on the quantum bump parameters. The effective bump duration T , the mean steady-state response \bar{V} and its variance σ^2 were used to calculate the mean bump rate ν and the mean bump amplitude a before and during the effect of La³⁺ (see Materials and Methods). The derived bump parameters for the cell of Figs. 1 and 2 are presented in Tables I and II for the control and the La³⁺-treated eye. The same analysis was carried out for three cells from other *Musca* flies with similar results.

Tables I and II and Fig. 4, which plots part of the data of the tables, show a systematic shortening of the effective bump duration T , with the increase in stimulus intensity both before (●) and after application of La³⁺ (○) over the range of dim light intensities (up to $-\log I = 2.5$). The effective bump duration was somewhat

TABLE I
Calculated Bump Parameters as a Function of Light Intensity before and during
Application of Lanthanum

$-\log I$	Effective bump duration, T		Mean bump rate, ν (1/s)		Mean bump amplitude, a	
	Control	La ³⁺	Control	La ³⁺	Control	La ³⁺
	<i>ms</i>		<i>1/s</i>		<i>mV</i>	
5	25.36		2.0×10^2		0.1936	
4	19.70	29.86	1.9×10^3	9.0×10^2	0.1073	0.1190
3	12.08	17.76	6.9×10^3	7.2×10^3	0.0650	0.0480
2.5	6.27	13.20	1.0×10^4	2.6×10^4	0.0206	0.0218
2.15		20.48		7.2×10^3		0.0288
2	5.33	41.58	1.8×10^5	3.5×10^5	0.0153	0.0238
1.8	4.57		3.5×10^5		0.0099	

T was calculated by using Eq. 10 with the parameters A , τ , $n + 1$, which were determined by fitting Eq. 8 to the various power spectra (see below). a and ν were calculated by using Eqs. 5 and 6. The mean bump rate ν increased linearly with light intensity (44.7°-slope ≈ 1 in log-log scale). The values of the various parameters at various light intensities are given in Table II.

shorter before than after application of La³⁺ at a similar stimulus intensity as found in the wild-type compared to the *nss* mutant (Barash et al., 1988). The main difference in T before and after application of La³⁺ was apparent above the medium light intensities, which caused a decline of the response toward baseline during illumination (Table I $-\log I = 2.5$, $-\log I = 2.15$, and $-\log I = 2.0$). While T before La³⁺ application continued to decrease, reaching a limiting value of ~ 5 ms at high intensities, the effective bump duration after application of La³⁺ reached a minimum of ~ 13 ms at a light intensity at which the La³⁺ effect clearly appeared and then increased to values similar to the bump duration observed with dim lights ($-\log I = 2.5$, $-\log I = 2.0$; Fig. 4 B). The results from *nss* mutant compared to wild type (Barash et al., 1988) are similar to those from La³⁺ treatment compared to controls.

The shortening of the bump duration and the reduction of the bump amplitude with the increasing stimulus intensity is a well-known effect of light adaptation

TABLE II
Parameter at Various Light Intensities

-logI	A		τ		n + 1		σ ²		V̄	
	Control	La ³⁺	Control	La ³⁺	Control	La ³⁺	Control	La ³⁺	Control	La ³⁺
	log mV ² /Hz		ms				mV ²		mV	
5	-2.20		5.90		2.20		0.1965		4.03	
4	-2.48	-2.34	4.87	4.80	2.03	3.82	0.4326	0.3740	5.42	6.17
3	-2.85	-2.71	2.41	3.06	2.21	3.42	0.3532	0.2970	13.03	7.50
2.5	-3.25	-3.20	1.35	2.25	2.45	3.48	0.2689	0.1638		
2.15		-3.26		3.80		3.05		0.1227		4.25
2	-3.41	-3.52	1.24	6.40	2.20	4.10	0.2300	0.0833	15.00	3.50
1.8	-3.71		0.92		2.70		0.1594		16.00	

(Dodge et al., 1968; Wong, 1978; Stieve, 1986). Table I and Fig. 4 B thus show that there is some effect of light adaptation on the bump of the La³⁺-treated eye at dim light intensities that only partially revealed the *nss* phenotype. However, these manifestations of light adaptation are reduced at light intensities that cause the decline of the response close to baseline.

La³⁺ Had Only Little Effect on the Light Response of the nss Mutant

If the *nss* mutation and La³⁺ affect the same membrane protein (as may be hypothesized from the findings of Hochstrate [1989] and the present results), then one would expect no effect or at most a little effect of La³⁺ on the light response of the *nss* mutant. Figs. 5 and 6 shows that this expectation was fulfilled.

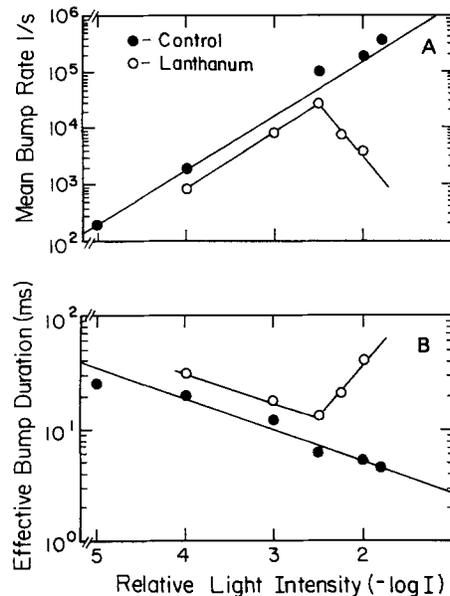


FIGURE 4. A plot of the calculated bump parameters as a function of light intensity before and after application of La³⁺. (A) A log-log plot of the mean bump rate before (●) and after (○) application of 5 mM La³⁺. The mean bump rate increased linearly (with a slope of 1) over the whole range of stimulus intensity before application of La³⁺. The mean bump rate increased and then decreased after application of La³⁺. (B) A log-log plot of the effective bump duration before (●) and after (○) application of La³⁺. Before La³⁺ application the effective bump duration decreased monotonically with the increase in light intensity. After La³⁺ application the effective bump duration decreased at dim light intensities but increased at the same high light intensities in which the mean bump rate decreased (A).

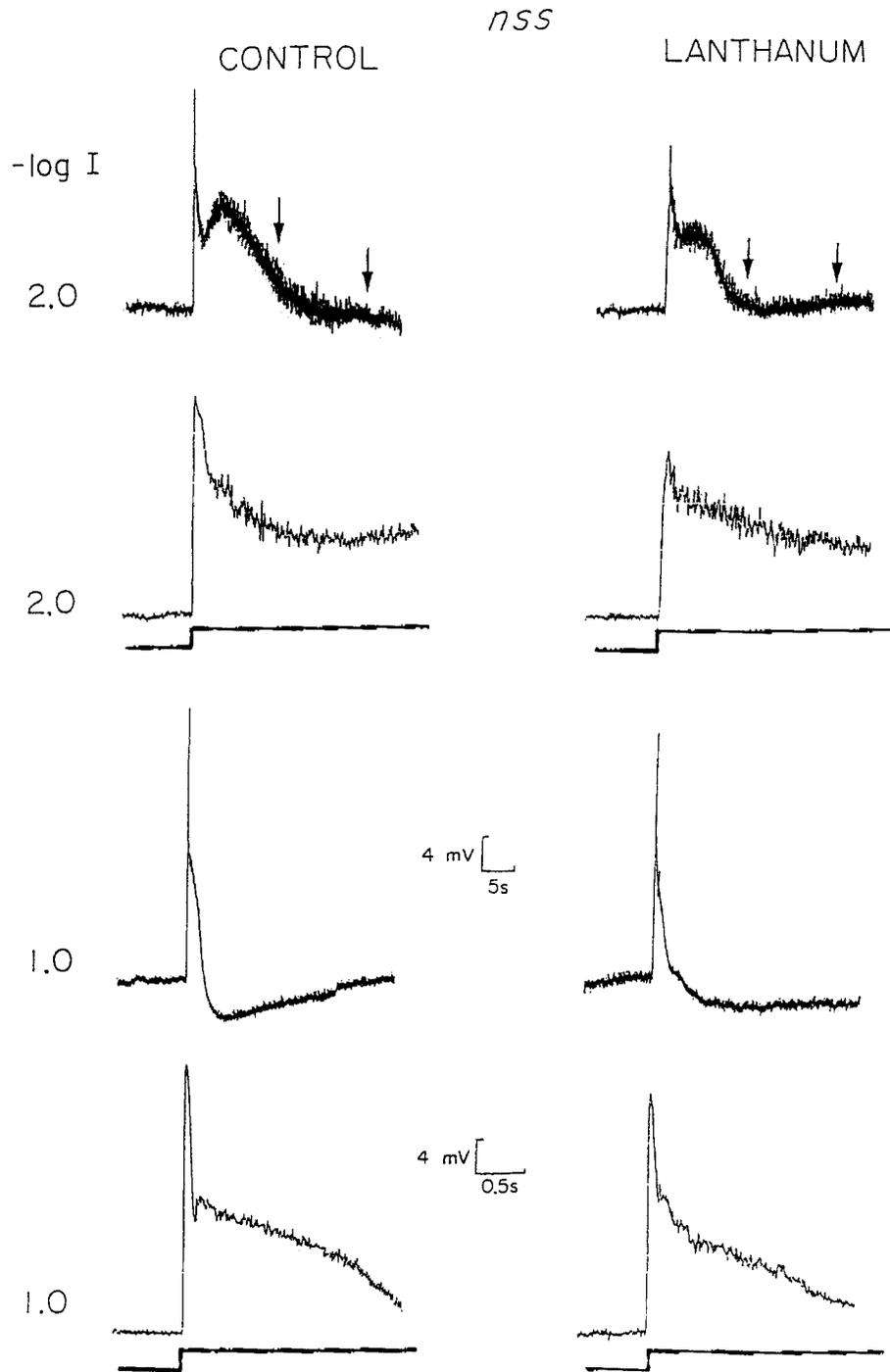


FIGURE 5.

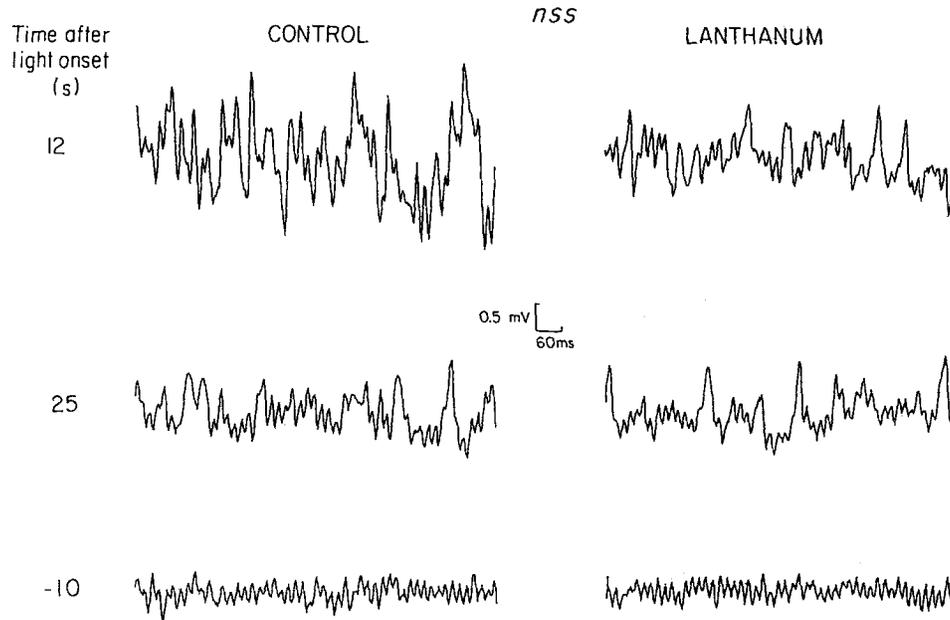


FIGURE 6. There is a relatively small reduction (of about twofold) in quantum bump rate during light in the La^{3+} -treated fly relative to the bump rate of the control in the *nss* mutant. The figure shows amplified segments of the light responses indicated by arrows in Fig. 5. The bottom traces show the noise in the dark (mainly instrumental noise). The lower amplitude of the noise during La^{3+} at shorter time from light onset (*upper right trace*) reflects the slightly faster decline of the response towards baseline relative to control. At longer times after light onset, both responses declined to a similar voltage level and individual bumps, occurring at approximately twice the rate, can be observed in control relative to the La^{3+} -treated eye.

Fig. 5 (CONTROL) shows the typical light responses of a *Lucilia nss* photoreceptor to medium ($-\log I = 2.0$) and intense ($-\log I = 1.0$) lights in slow and fast time scales. The right column of Fig. 5 (LANTHANUM) shows the light responses to the same stimuli recorded from the same eye after injection of 10 mM La^{3+} to the extracellular space of the *nss* retina. A reduction in the initial response amplitude (initial transient phase) and a small acceleration in the decay rate of the response were observed. The response width at 10% height was 8.5 s in control and 6 s in La^{3+} . In each of four *nss* flies tested, we found similar results. In one extreme case the bump noise during the light response was significantly reduced and the initial

FIGURE 5 (*opposite*). Lanthanum had only little effect on the decay rate of the light response of the *nss* mutant. Receptor potentials of the *nss* mutant of *Lucilia* in response to two intensities of orange (OG - 590 filter) lights as indicated before (left) and after (in the same cell, right) application of 10 mM La^{3+} in slow and fast time scales. The responses are typical for the *nss* mutant. The only affects of La^{3+} were a reduction of the amplitude of the fast initial transient phase of the light-response and a slight acceleration of the decay rate of the response. The arrows indicate time segments which are expanded in Fig. 6.

transient phase was almost abolished after treatment with La^{3+} but the decay rate was only little affected (not shown).

Unlike the La^{3+} -treated wild-type fly, we could not apply shot noise analysis to the *nss* responses, owing to the large hyperpolarization (of still unknown origin) which usually accompanies the *nss* response to light (Fig. 5, $-\log I = 1.0$). In the *nss* mutant the mean response amplitude \bar{V} cannot be measured reliably because of this hyperpolarization, which distorts \bar{V} (see Barash et al., 1988, and Fig. 5). We, therefore, compared only qualitatively the responses of *nss* before and during La^{3+} treatment. Fig. 6 compares the bump rate of the *nss* response before and during La^{3+} at two time segments after light onset (Fig. 5, arrows) in a fast time scale. At short (12 s) time from light onset, individual bumps could not be resolved, and a reduced noise and smaller amplitude of the receptor potential in the La^{3+} -treated eye were observed (upper line, right). The instrumental noise in the dark is shown for a comparison (bottom). At a longer time from light onset (25 s), there is a clear reduction in bump rate in both the La^{3+} -treated eye and the control. The rate of bumps after La^{3+} application seems to be reduced to about half relative to the control. Fig. 6 thus shows that La^{3+} caused some reduction in bump rate during light but its effect is small compared to the effect of La^{3+} on the bump rate in wild-type flies (see Table I and Fig. 4).

In summary, the main effect of La^{3+} on the light response of the *nss* mutant was to reduce the amplitude of the initial transient (Fig. 5). This initial transient was somewhat reduced, but in a significant and reproducible manner. This change indicated that La^{3+} did reach the membrane of the photoreceptor. The concentration of La^{3+} in the experiments with *nss* was doubled relative to wild type to make sure that La^{3+} reached the cells.

DISCUSSION

La³⁺ Reduces the Excitation Efficiency in Wild-Type Flies

The effect of La^{3+} on the mean bump rate ν was consistent with a reduction in excitation efficiency as found in the *nss* mutant. Both before and after application of La^{3+} (Table I and Fig. 4A) the mean bump rate increased linearly with the increase in light intensity up to medium intensity. However, after application of La^{3+} , ν reached a peak at $-\log I = 2.5$ and then decreased with the increase in light intensity (Fig. 4A, \circ). At a stimulus intensity of $-\log I = 2$ the rate of bump production was ~ 50 times smaller in the La^{3+} -treated eye than in the control. Table I and Fig. 4A thus demonstrated that in a narrow range of stimulus intensities (between $-\log I = 2.5$ and $-\log I = 2.0$), where the steady-state response was largely reduced, a sharp decline in mean bump rate also occurred.

The effect of La^{3+} on the mean bump amplitude a at various light intensities was somewhat different from that found in the *trp* or *nss* mutation. In the mutants, similar bump amplitude was found during dim light and three orders of magnitude brighter lights (Barash et al., 1988). After application of La^{3+} there was a reduction in the mean bump amplitude with an increase in light intensity up to the light intensity in which the disparity between the control and the La^{3+} -treated eye was evident in both mean rate and effective duration (Table I). With further increase in light intensity,

the mean bump amplitude reached a constant value, unlike the mean bump amplitude before La^{3+} application which continued to decline in brighter light (Table I).

These results indicate that La^{3+} modified the properties of the light response in the wild-type fly in a very similar manner to the modifications induced by the *trp* and *nss* mutations in terms of the bump parameters. Namely, it induced a reduction in the rate of occurrence of the quantum bumps with little change in bump shape. The only difference between the effect of La^{3+} and the mutation was a constant reduction in bump amplitude at intense lights which did not recover during illumination, in the La^{3+} -treated eye. The reduction in the mean bump amplitude in the presence of La^{3+} probably reflects the fact that La^{3+} affects more processes than the *trp* or *nss* mutations. The sequencing of the *trp* gene product (Montell and Rubin, 1989; Wong et al., 1989) indicates that the *trp* protein is not a Na-Ca exchanger. Lanthanum, on the other hand, is known to block the Na-Ca exchanger and this effect can result in a reduced bump amplitude.

La³⁺ May Have a Specific Effect on Fly Photoreceptors

The result of the present study is consistent with the recent study in *Calliphora* which concluded that La^{3+} mimics *trp* in *Calliphora* photoreceptors (Hochstrate, 1989). The low concentration of La^{3+} (i.e., 1–100 μM) and the quick onset and offset (in the presence of EGTA) of the La^{3+} effects is consistent with an effect of La^{3+} on the plasma membrane via the extracellular surface (Nathan et al., 1988; Hochstrate, 1989). In the present experiments the concentration of La^{3+} in the vicinity of the recorded cell was unknown owing to the dilution of the small injected drops in the eye. Since the onset of the effect of La^{3+} was fast, we repeatedly injected La^{3+} with very short pulses of pressure until the effect was observed. In this way we ensured that a suitable concentration range for a specific effect, presumably similar to that found by Hochstrate (1989), was obtained.

The small effect of La^{3+} in accelerating the decline of the *nss* response during intense illumination (Fig. 5) and its relatively small effect on the apparent bump rate (Fig. 6) is consistent with the hypothesis that La^{3+} and the *nss* mutation affect the same process, or possibly the same protein. On the one hand, if a nonspecific effect of La^{3+} on the receptor potential would be manifested by the conversion of a sustained response into a transient one, the transient response of the mutant is expected to become even shorter, or perhaps completely eliminated by La^{3+} . However, this expectation was contrary to our observations (Fig. 5). On the other hand, if La^{3+} affects a specific membrane protein that is missing in the mutant, no effect on the main phenotype of the mutant (i.e., the decay rate of the response) is expected, as found in our experiments. Nevertheless, the *nss*-like phenotype could be generated by a defect in any of several mechanisms, each of which involves several proteins. If so, a mutation in the gene encoding any one of these proteins could cause a *nss*-like phenotype.

La³⁺ and the nss Mutation Affect Ca²⁺ Mobilization

Several lines of indirect evidence suggest that the *trp* or *nss* mutations may lead to abnormally low level of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$). Intracellular screening pigment

migration, which depends on an increase in $[Ca^{2+}]_i$ (Kirschfeld and Vogt, 1980) is relatively small and transient in *trp* (Lo and Pak, 1981) and does not occur in the *nss* mutant (Howard, 1984). It has also been shown that background light or a short adapting light pulse, which shortens the response latency due to an increase in $[Ca^{2+}]_i$ in *Limulus* ventral photoreceptors (Brown and Lisman, 1975), has the opposite effect (i.e., increase in latency) in the *trp* or *nss* mutants (and in the La^{3+} -treated wild type). In addition, light adaptation, acting via an increase in $[Ca^{2+}]_i$, which makes the bumps smaller and faster, does not occur in the *trp* or *nss* mutant photoreceptors when the response to a prolonged stimulus declines close to baseline (Barash et al., 1988). A recent study on the spatial spread of adaptation and the *nss*-induced inactivation indicates that light adaptation is replaced by inactivation in the *nss* photoreceptors (Minke and Payne, 1991).

Taking together the close similarity in the parameters mentioned above between the phenotypes of the *trp* or *nss* and the La^{3+} -treated eye, the apparent low $[Ca^{2+}]_i$ in the mutants and the known action of La^{3+} in variety of tissues in blocking Ca^{2+} transport processes, strongly suggest that La^{3+} and the *trp* or *nss* mutations both inhibit Ca^{2+} entry into the photoreceptors. The observation of a relatively small fast delayed decline of the initial response from its peak to a lower response level in *trp* or *nss* flies and the decrease in bump duration in both the mutants and the La^{3+} -treated wild type at medium levels of light intensities (Barash et al., 1988, and Fig. 4) indicate that there is a small effect of light adaptation in *nss* and La^{3+} -treated wild type, thereby suggesting that some increase in $[Ca^{2+}]_i$ level does occur in the mutant during light. How inhibition of Ca^{2+} entry into the photoreceptor leads to a reduction in excitation efficiency up to elimination of the light response is still an open question. Nevertheless, we take the *trp* or *nss* and the La^{3+} data as additional evidence that Ca^{2+} is required for excitation as already suggested by several studies (Stieve and Bruns, 1980; Bolsover and Brown, 1985; Payne et al., 1986).

A clue to the correlation between Ca^{2+} transport and depletion of $InsP_3$ -sensitive Ca^{2+} pools may be derived from other Ca^{2+} transport systems in some neurons, smooth muscle cell-lines reported by Chueh et al. (1987) and Morris et al. (1987) and parotid acinar cells (Takemura et al., 1989; for reviews see Berridge and Irvine, 1989; Berridge, 1990; Irvine, 1990). It has been suggested by the above and several other studies that there are Ca^{2+} -transport mechanisms which are activated by a reduction in Ca^{2+} in the $InsP_3$ -sensitive Ca^{2+} pools and that this reduction mobilizes Ca^{2+} from the extracellular space to the intracellular pools via transport of Ca^{2+} to the cytosol. If similar mechanisms operate in the fly and if a step in this mechanism is inhibited by the *trp* or *nss* mutation or La^{3+} , then the decline of the light response in the mutants may arise from a depletion of the $InsP_3$ -sensitive Ca^{2+} pools that failed to be replenished. The recovery of the transient light response in the dark may occur via alternative, less efficient Ca^{2+} entry mechanisms. It is interesting to note that the suggested hypothetical mechanism causing the transient response in the La^{3+} -treated eye is valid only if the internal Ca^{2+} pools have a limited capacity and therefore Ca^{2+} needs to be constantly replenished from the outside during intense light. In the *Limulus* ventral photoreceptors where most Ca^{2+} comes from huge internal stores, application of 100 μM La^{3+} in (1 mM Ca^{2+}) artificial seawater to the extracellular fluid did not cause a transient response similar to that of the fly (Z.-X. Zhang and A. Fein, personal communication). A more detailed study indicated that for the ventral

photoreceptor cells of *Limulus*, addition of 1 mM La^{3+} to artificial seawater that contains 10 mM Ca^{2+} causes a decrement in the sustained responses to steady, dim lights accompanied by an increased latency of transient responses; however, neither the early transient responses to dim or bright lights nor the sustained responses to bright lights are much affected (J. E. Brown, personal communication). In the barnacle lateral ocelli where the intracellular Ca^{2+} pools are presumably very small and the increase in $[\text{Ca}^{2+}]_i$ during light comes primarily from the extracellular space (Brown and Blinks, 1974), 1 mM La^{3+} in (0.1 mM Ca^{2+}) artificial seawater abolished reversibly the light response (U. Werner, E. Suss-Toby, and B. Minke, unpublished observations). The striking difference in the effect of La^{3+} on three different invertebrate species can be explained by assuming that La^{3+} blocks the light-sensitive channels but the three species have different types of channels. An alternative and more attractive explanation is to assume that the above three species have similar light-sensitive channels but very different sizes of internal Ca^{2+} pools, and that La^{3+} blocks the replenishment of these internal pools from the extracellular space while Ca^{2+} is required for excitation.

Direct evidence that insect photoreceptors require Ca^{2+} transport from outside the cell after illumination came from measurements of $[\text{Ca}^{2+}]_o$ in the extracellular space during and after light in the honeybee drone retina (Minke and Tsacopoulos, 1986; Ziegler and Walz, 1989) and the blowfly *Calliphora* (Sandler and Kirschfeld, 1988; Ziegler and Walz, 1989). It was found that a large influx of Ca^{2+} during light was accompanied by around fourfold larger Na^+ -dependent Ca^{2+} efflux during and after bright light via the Na-Ca exchanger. This Ca^{2+} deficit needs to be replenished. Evidence for such a replenishment came from the relatively fast reduction of the increased $[\text{Ca}^{2+}]_o$ (Minke and Tsacopoulos, 1986). This reduction in $[\text{Ca}^{2+}]_o$ was ~ 17 times faster than that measured as diffusion of an artificially elevated $[\text{Ca}^{2+}]_o$ level without illumination (Ziegler and Walz, 1989). Since no other cells in the retina besides the photoreceptors have been found to have the ability to absorb Ca^{2+} , the reduction in $[\text{Ca}^{2+}]_o$, most likely, reflects an uptake by the photoreceptor cells.

In summary, very specific and complex Ca^{2+} mobilization processes are apparently an integral part of light excitation and adaptation in insect photoreceptors. A major component of this Ca^{2+} transport may be blocked by La^{3+} and the *trp* or *nss* mutations.

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