The *nss* Mutation or Lanthanum Inhibits Light-induced Ca^{2+} Influx into Fly Photoreceptors

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ABSTRACT Ion-selective calcium microelectrodes were inserted into the compound eyes of the wild-type sheep blowfly *Lucilia* or into the retina of the no steady state (*nss*) mutant of *Lucilia*. These electrodes monitored light-induced changes in the extracellular concentration of calcium ($\Delta[Ca^{2+}]_o$) together with the extracellularly recorded receptor potential. Prolonged dim lights induced a steady reduction in $[Ca^{2+}]_o$ during light in the retina of normal *Lucilia*, while relatively little change in $[Ca^{2+}]_o$ was observed in the retina of the *nss* mutant. Prolonged intense light induced a multiphasic change in $[Ca^{2+}]_o$: the $[Ca^{2+}]_o$ signal became transient, reaching a minimum within 6 s after light onset, and then rose to a nearly steady-state phase below the dark concentration. When lights were turned off, a rapid increase in $[Ca^{2+}]_o$ was observed, reaching a peak above the dark level and then declining again to the dark level within 1 min. In analogy to similar studies conduced in the honeybee drone, we suggest that the reduction in $[Ca^{2+}]_o$ reflects light-induced Ca^{2+} influx into the photoreceptors, while the subsequent increase in $[Ca^{2+}]_o$ reflects the activation of the Na-Ca exchange which extrudes Ca^{2+} from the cells. In the *nss* mutant in response to intense prolonged light, the receptor potential declines to baseline during light while the Ca^{2+} signal is almost abolished, revealing only a short transient reduction in $[Ca^{2+}]_o$. Application of lanthanum (La^{3+}), but not nickel (Ni^{2+}), into the retinal extracellular space of normal *Lucilia* mimicked the effect of the *nss* mutation on the receptor potential, while complete elimination of the Ca^{2+} signal in a reversible manner was observed. The results suggest that La^{3+} and the *nss* mutation inhibit light-induced Ca^{2+} influx into the photoreceptor in a manner similar to the action of the *trp* mutation in *Drosophila*, which has been shown to block specifically a light-activated Ca^{2+} channel necessary to maintain light excitation.

INTRODUCTION

The compound eye of the fly has been an important model system for studies of invertebrate phototransduction, particularly because of the available phototransduc-

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tion-defective mutants (for reviews, see Pak, 1979, 1991; Selinger and Minke, 1988; Montell, 1989; Ranganathan, Harris, and Zuker, 1991*b*; Minke and Selinger, 1992*a*). In both the transient receptor potential (*trp*) mutant of *Drosophila* (Cosens and Manning, 1969; Minke, Wu, and Pak, 1975; Lo and Pak, 1981; Minke, 1982; Montell, Jones, Hafen, and Rubin, 1985; Montell and Rubin, 1989; Wong, Schaefer, Roop, La Mendola, Johnson-Seaton, and Shao, 1989) and the no steady state (*nss*) mutant of *Lucilia* (Howard, 1982, 1984; Barash, Suss, Stavenga, Rubinstein, and Minke, 1988; Suss, Barash, Stavenga, Stieve, Selinger, and Minke, 1989), the receptor potential, which appears normal in response to dim light, declines to baseline within a few seconds of illumination with intense light.

Several lines of evidence suggest that the *trp* and *nss* mutations affect the same gene product (Howard, 1982; Suss-Toby, Selinger, and Minke, 1991). The *trp* mutant lacks a protein whose sequence has been determined by Montell and Rubin (1989) and by Wong et al. (1989).

Recent studies (Hochstrate, 1989; Suss-Toby et al., 1991) have demonstrated that when lanthanum (La^{3+}), a blocker of Ca^{2+} binding proteins, is applied into the retinal extracellular space of the fly, the electrophysiological properties of the photoreceptors become very similar to those of the *trp* or *nss* mutant, while La^{3+} has very little effect on the light response of the *nss* mutant (Suss-Toby et al., 1991). The close similarity in the properties of the receptor potential of the La^{3+}-treated photoreceptor of the wild-type and of the *nss* mutants, together with existing evidence for the highly reduced intracellular Ca^{2+} ($[Ca^{2+}]_i$) level in *nss* photoreceptors, led Suss-Toby et al. (1991) to suggest that both La^{3+} and the mutation cause a severe reduction in $[Ca^{2+}]_i$. It was further suggested that this effect may arise from an inhibition of a Ca^{2+} channel/transporter protein located in the surface membrane that normally replenishes Ca^{2+} pools in the photoreceptors, and that this process is essential for a maintained light excitation.

Direct evidence that insect photoreceptors have a Ca^{2+} transport system to introduce Ca^{2+} from outside the cell during illumination comes from measurements of $[Ca^{2+}]_o$ in the extracellular space during and after illumination in the honeybee drone retina (Minke and Tsacopoulos, 1986; Ziegler and Walz, 1989) and the blowfly *Calliphora* (Sandler and Kirschfeld, 1988, 1991; Ziegler and Walz, 1989). A large influx of Ca^{2+} during light is observed accompanied by a large Na^+-dependent Ca^{2+} efflux during and after bright light via the Na-Ca exchanger. Additional evidence for influx of Ca^{2+} into *Drosophila* photoreceptors during light is provided by voltage clamp measurements of light-induced current using the whole cell recording technique (Hardie, 1991; Ranganathan, Harris, Stevens, and Zuker, 1991*a*). These studies indicate that the light-sensitive channels are primarily permeable to Ca^{2+} (Hardie, 1991; Ranganathan et al., 1991*b*).

The suggestion of Minke and Selinger (1992*a*) and Suss-Toby et al. (1991) that the *trp* and *nss* mutations or La^{3+} abolish the activity of a light-activated Ca^{2+} channel/transporter protein located at the surface membrane is strongly supported by recent measurements in *Drosophila* photoreceptors. Using whole cell recordings from isolated *Drosophila* ommatidia, Hardie and Minke (1992) have recently demonstrated that the wild-type light response is mediated by at least two classes of light-sensitive channels. One type of channel, which is highly permeable to Ca^{2+}, is selectively

blocked by the *trp* mutation or external La^{3+}. This finding strongly suggests that the block of visual excitation and adaptation during light by the *trp* mutation and by La^{3+} results from blocking Ca^{2+} influx into the photoreceptors. To test directly, by an independent method, whether the *nss* mutation or La^{3+} blocks Ca^{2+} influx into fly photoreceptors, we applied Ca^{2+}-selective microelectrodes to normal *Lucilia* (untreated or treated with La^{3+}) and to the *nss* retina to measure changes in light-induced extracellular Ca^{2+} concentration. The photoreceptors are the only cells in the insect retina capable of absorbing or releasing Ca^{2+} in response to light (Coles and Orkand, 1985; Ziegler and Walz, 1989). The other class of cells located in the region of Ca^{2+} measurements is the glia (pigment) cells and they do not change their $[Ca^{2+}]_i$ during light (Coles and Orkand, 1985). Therefore, light-induced extracellular Ca^{2+} concentration changes reflect Ca^{2+} movements across the photoreceptors' membrane (see Discussion).

MATERIALS AND METHODS

Preparation

White-eyed *Lucilia cuprina* or its white-eyed *nss* mutant was used for the experiments. After the animals had been immobilized by means of wax, a piece of razor blade was used to cut a small triangular hole (sides 3–10 ommatidia long) in the cornea of the dorsal part of the compound eye, and the piece of cornea was carefully removed. The fly was grounded via a chloridized silver wire immersed in a drop of electrode gel on the same eye. Then the microelectrode was introduced through the corneal opening (Fig. 1).

Recordings and Stimulation

Intracellular receptor potentials were measured with standard glass micropipettes filled with 2 M KCl (electrical resistance 80–140 MΩ). The microelectrodes selective for Ca^{2+} were pulled from borosilicate double-barreled capillaries with filament (KBF 215075; Zak, Munich, Germany) and the tip was then broken off. The resulting tip measured 2.0–5.0 μm on the broad side of the electrode. The reference barrel was filled with 0.2 M NaCl solution. For silanization of the ion-selective barrel, a column of 5% trimethylchlorsilane solution in CCl_4, several hundred micrometers long, was sucked into its tip and pushed out again several times (silanization time 3–5 min). The ionophore (neutral carrier ETH 1001, calcium ionophore I-Cocktail A: Art.-Nr. 21048; Fluka, Neu-Ulm, Germany) then entered the pipette (70–700 μm), after which a layer of saline solution could be applied from the unprocessed end of the electrode. This solution consisted of 0.1 M $CaCl_2$. A bridge to the differential amplifier (J. Meyer, Munich, Germany) was formed by chlorided silver wires glued into the electrode with dental wax. These ion-selective microelectrodes were produced by a slight modification of the methods given by Lux and Neher (1973) and Oehme, Kessler, and Simon (1976). The electrical resistance of the active channel was between 3 and 25 GΩ. When immersed alternately in two calibration solutions, containing 200 mM NaCl plus either 1.6 or 1.1 mM $CaCl_2$, the electrical potential recorded by the Ca^{2+}-selective microelectrode reached 90% of its final value in <0.8 s. These electrodes were found to be highly selective for Ca^{2+}. For 12 Ca^{2+}-selective microelectrodes, the selectivity coefficients with respect to K^+ and Na^+ were determined by the "fixed interference" method (IUPAC Commission on Analytical Nomenclature, 1979; see also Sandler and Kirschfeld, 1991). The Ca^{2+} buffer solutions required for this calibration were basically the same as those used by Tsien and Rink (1980); as fixed ionic components they contained either 125 mM K^+ or 90 mM Na^+ plus 35 mM K^+, while the variable component had

a free Ca^{2+} concentration of 10^{-2}–10^{-8} M. The values of the potentiometric selectivity coefficients ($K_{CaK} = 10^{-5.10}$, $K_{CaNa} = 10^{-4.35}$) can be calculated from the concentration at the limit of detection (IUPAC Commission on Analytical Nomenclature, 1979; Ammann, 1986). The detection limit is obtained graphically from the intersection of the extrapolated parts of the linear portions of the response curves (Fig. 2). All the calibrations and experiments were carried out at room temperature (20–25°C) in a light-tight Faraday cage.

The neutral carrier ETH 1001 (calcium ionophore I; Fluka) is known to have a high Ca^{2+} selectivity. Selectivity coefficients have been determined for many ions such as H^+, K^+, Mg^{2+}, Na^+, Ba^{2+}, Li^+, Zn^{2+}, etc. (Oehme et al., 1976; Heinemann, Lux, and Gutnick, 1977; Lanter, Steiner, Ammann, and Simon, 1982), but as far as we know, not for Ni^{2+} or La^{3+}, which were applied to the fly eye in some of our experiments. Our determined selectivity coefficients for

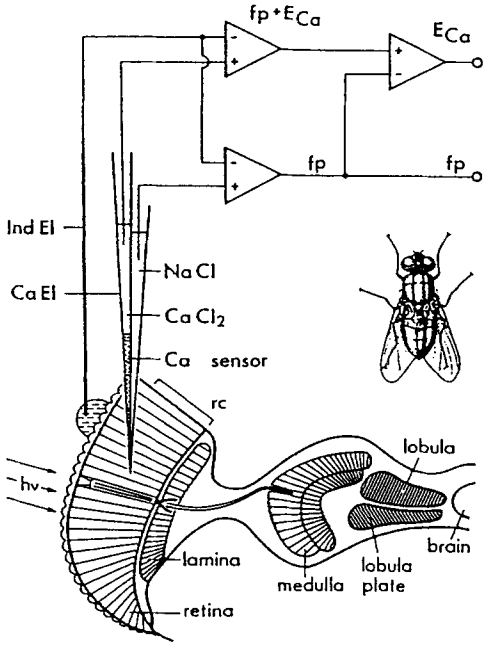

FIGURE 1. Diagrams of the experimental setup and of the fly eye. Arrangement of the apparatus and horizontal section through the optic ganglia. Diffuse white light stimulates part of the compound eye. From the retina the visual information passes to the next station, the optic ganglia (lamina, medulla, lobular plate). The indifferent electrode (*Ind El*) consists of a chlorided silver wire and makes contact with the eye by way of a droplet of electrode gel. The reference barrel of the extracellular Ca^{2+}-selective fluid microelectrode (*Ca El*) is filled with NaCl solution and measures the extracellular field potentials (*fp*). In the tip of the ion-selective barrel there is a Ca^{2+}-selective fluid membrane (*Ca sensor*) covered with a layer of $CaCl_2$ solution; this barrel records both the field potentials and, by potentiometry, the extracellular Ca^{2+} concentration (E_{Ca}). The electrodes are connected to electronic devices arranged so as to separate the two components (as indicated roughly by the diagram).

the two ions important for our study (K_{CaK}, K_{CaNa}) are in the same range as those measured by others (Oehme et al., 1976; Heinemann et al., 1977; Lanter et al., 1982). It is, therefore, reasonable to assume that the selectivity relative to ions that we have not measured is similar to those published by the above authors. In particular, this means that the values of the selectivity factors relative to Mg^{2+} and H^+ should be several orders of magnitude better than the tolerable values (Ammann, 1986). Since the selectivity of the carrier ETH 1001 is high with respect to all ions tested so far, we also assumed high selectivities relative to Ni^{2+} and La^{3+}. This expectation was corroborated by our determination of these selectivities using the separate solution method (IUPAC Commission on Analytical Nomenclature, 1976; Ammann, 1986). Testing three Ca electrodes and using solutions of 10 and 100 mM $CaCl_2$, $LaCl_3$, and $NiCl_2$, we yielded the following results: $\log K_{CaNi}$ is between −2.5 and −3.1 and $\log K_{CaLa}$ is about −2.4. The

concentrations of Ni^{2+} and La^{3+} used in our experiments were maximally 5 mM during an experiment and they were constant during the range of one stimulation period. We therefore conclude that the La^{3+} and Ni^{2+} used in our experiments did not affect the Ca^{2+} concentration measurements significantly.

Light stimuli. The light source was a xenon high-pressure lamp (XBO, 75 W). The white light emitted reached the compound eye in the shielded cage by way of a fiber optic and diffuser. The maximal luminous intensity at the eye surface was ~0.1 cd/cm², which is ~1.5 logarithmic intensity units above the intensity for a half-maximal response of the most common type of fly photoreceptors (type R1-6). The light intensity could be reduced by up to eight orders of magnitude by gray filters (MTO, Massy, France) mounted on two electronically controlled filter wheels.

Experimental setup, procedure, and evaluation. The outputs of the differential amplifier were connected to a storage oscilloscope (5111; Tektronix, Beaverton, OR), a chart recorder (Hewlett Packard 7402A), and a laboratory computer (Personal Computer AT10-4) (Fig. 1). For

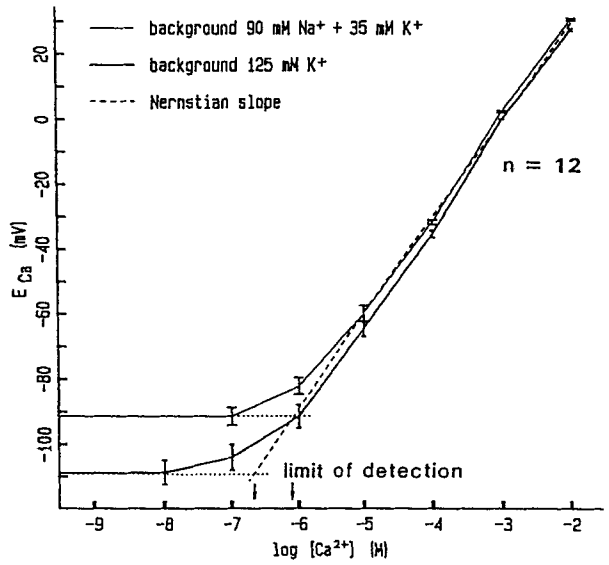

FIGURE 2. Calibration curve for the Ca^{2+}-selective microelectrodes. E_{Ca} is plotted against log[Ca^{2+}]. The curves indicate that the electrodes used are highly selective for Ca^{2+} and show the expected Nernstian slope in the range above $-\log[Ca^{2+}] = 6$, which is well below the concentration range measured in our experiments.

the measurements with ion-selective microelectrodes, low-pass filters were inserted before the chart recorder and the computer. Their corner frequencies were 100 Hz for the extracellular potentials and 10 Hz for the ion trace. A measurement card (DT 2801-A) and special-purpose software programmed in Turbo Pascal G. O. (Borland International, Munich, Germany) enabled the recorded signals to be fed to the computer for further analysis.

Intracellular receptor potentials recorded from dark-adapted photoreceptors were evaluated as long as the resting membrane potential was at least −50 mV. Stimulus duration was generally 0.2, 0.6, or 1.0 s, although when the recording was very stable 1-min stimuli were also used, as for the measurements of ion concentration (see below). These long stimuli were separated by intervals of at least 2 min, the others by 10–30 s. The eye of the fly is almost completely dark-adapted after 2 min. The extracellular calcium concentration ($[Ca^{2+}]_o$) was measured by first applying a tiny drop of Ringer to the corneal insertion site by means of a syringe mounted on a micromanipulator. The solution contained 140 mM NaCl, 4 mM KCl, 10 mM Tris (pH 7.4), and 1.4 mM $CaCl_2$. When the Ca^{2+}-selective microelectrode was withdrawn

from the retina into the droplet of Ringer, a potential difference was recorded from which the retinal $[Ca^{2+}]_o$ could be calculated.

To measure light-induced extracellular Ca^{2+} concentration changes, the eye was illuminated for 1 min. In these experiments the interstimulus interval was at least 2 min.

The Ca^{2+} concentrations were calculated from the Nernst equation. Because of the high selectivity of the Ca^{2+} electrodes there is no relevant error if one neglects physiologically induced K^+ and Na^+ concentration changes in the fly retina during light stimulation. Furthermore, the resulting errors due to changes of the liquid-junction potential between the reference electrolyte of the Ca^{2+} electrode and the retinal fluid are also small. Neglecting these factors overestimated the Ca_o decrease up to 3.5% for the light-induced plateau values and between 9 and 18% for the peak values, the so-called light minimum, from the maximal change measured (Sandler and Kirschfeld, 1991, and our Fig. 5).

RESULTS

The Dark Value of the Retinal $[Ca^{2+}]_o$ in the Normal Lucilia and the nss Mutant

The dark values of the retinal $[Ca^{2+}]_o$ were determined with Ca^{2+}-selective microelectrodes by placing a droplet of standard Ringer solution (1.4 mM $CaCl_2$) over the electrode insertion site and measuring the difference in the electrode potential as the tip is moved back and forth between retina and droplet. Without knowing the absolute $[Ca^{2+}]_o$ in the dark, only relative Ca^{2+} concentration changes (e.g., as in Fig. 5) can be calculated by means of the Nernst equation due to the light-induced potentiometric potential changes $[\Delta E_{Ca}]$ of the calcium electrode. In two normal *Lucilia* the $[Ca^{2+}]_o$ was determined to be 1.24 mM ± 0.19 mM (SD, $n = 8$) and 1.13 ± 0.42 mM ($n = 3$) and in two *nss* mutants the $[Ca^{2+}]_o$ was 1.38 ± 0.03 mM ($n = 3$) or 1.33 ± 0.17 mM ($n = 8$), respectively. These results indicate that the $[Ca^{2+}]_o$ in the dark is very similar for the *nss* mutants and the normal *Lucilia* or the blowfly *Calliphora* (Sandler and Kirschfeld, 1991), which is 1.4 mM on the average. We therefore considered the dark concentration of $[Ca^{2+}]_o$ in all our experiments to be 1.4 mM.

Light-induced $\Delta[Ca^{2+}]_o$ in Normal Lucilia and Inhibition of $[Ca^{2+}]_o$ Changes in the nss Mutant

In the retina of normal *Lucilia*, prolonged intense light elicited a transient reduction in $[Ca^{2+}]_o$ followed by a partial return of $[Ca^{2+}]_o$ toward baseline, indicating a maintained steady reduction in $[Ca^{2+}]_o$ during the light stimulus. After light off, a relatively rapid increase in $[Ca^{2+}]_o$ was observed, overshooting the resting level and returning to the original dark level within ~1 min (Fig. 3 *c*). The initial reduction in $[Ca^{2+}]_o$ at light onset probably reflects Ca^{2+} influx into the photoreceptors and the subsequent partial return to baseline probably reflects the activation of the Na-Ca exchanger, which partially balanced the Ca^{2+} influx (see Discussion). At the cessation of the light stimulus the light-induced Ca^{2+} influx stopped while the efflux, due to the Na-Ca exchanger, still operated, producing the fast increase in $[Ca^{2+}]_o$ and the subsequent decline to baseline in the dark. Fig. 3, *a* and *b* shows the typical intracellularly recorded receptor potential of *Lucilia* (*a*) (in another retina) and extracellular field potential (*b*) recorded at the same retinal location as trace *c* by the reference electrode. Fig. 3 (right) shows the corresponding light-induced responses

recorded from the retina of the *nss* mutant (*d–f*). Fig. 3 *f* shows that the relatively large changes in $[Ca^{2+}]_o$ observed in normal *Lucilia* were much reduced in the *nss* mutant and only a transient reduction in $[Ca^{2+}]_o$ was observed in the mutant. The magnitude of this transient reduction in $[Ca^{2+}]_o$ varied among different mutants (see Fig. 5 *B* for a summary). The intracellularly recorded receptor potential (Fig. 3 *d*) and the extracellular field potential show the typical decline of the *nss* receptor potential to baseline during the intense prolonged light.

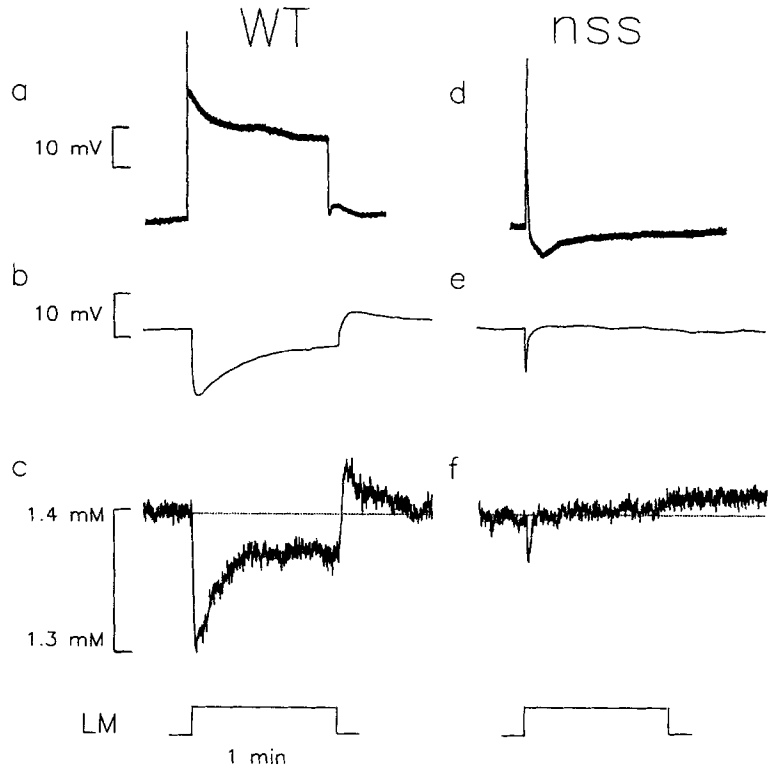

FIGURE 3. Light-induced Ca^{2+} concentration changes in the retina of white-eyed-*Lucilia* (*WT, left*) and the *nss* mutant (*right*). (*a* and *d*) Intracellular recordings. (*b* and *e*) Extracellular field potential (fp) measured with the reference barrel of the Ca^{2+}-selective electrode. (*c* and *f*) $\Delta[Ca^{2+}]_o$. The relative light intensity (I) was $-\log I = 1$. The dashed lines show the $[Ca^{2+}]$ level in the dark. The bottom pulses give the time scale and also symbolize the occurrence of the light stimulus (light monitor, *LM*). The figure shows that the *nss* mutation almost completely blocked the light-induced $\Delta[Ca^{2+}]_o$, leaving only a small transient reduction in $[Ca^{2+}]_o$.

Fig. 4 shows a comparison of changes in $[Ca^{2+}]_o$ and field potentials between normal *Lucilia* (left) and the *nss* mutant (right) as a function of increasing stimulus intensity (in relative-log scale). In normal *Lucilia* during dim lights only a reduction in $[Ca^{2+}]_o$ is observed. At the same light intensities only little change in $[Ca^{2+}]_o$ was observed in the *nss* mutant (see Fig. 5 *B* for a quantitative summary). With further increase in stimulus intensity the complex waveform of $\Delta[Ca^{2+}]_o$ (Fig. 3) was observed

in normal *Lucilia,* while only a short transient reduction in $[Ca^{2+}]_o$ was observed in the *nss* retina. Results similar to those of Fig. 4 were observed in all additional nine retinas of normal *Lucilia* and eight retinas of the *nss* mutant.

Fig. 5 summarizes the main feature of the Ca^{2+} signal and compares quantitatively the main differences in Ca^{2+} signals between normal and mutant flies. Fig. 5 *A* plots the three main phases of the Ca^{2+} signal as a function of light intensity: the peak reduction in Ca_o^{2+} (●); the plateau observed during light (○); and the peak increase in Ca^{2+} after the light was turned off (dark peak, △). Fig. 5, *A* and *B* were measured from normal *Lucilia* and the *nss* mutant, respectively. The figure shows a large difference between the Ca^{2+} signal measured in normal (*A*) and mutant flies (*B*). The initial reduction in $[Ca^{2+}]_o$ is much shorter in time course (Figs. 3 and 4) in *nss*

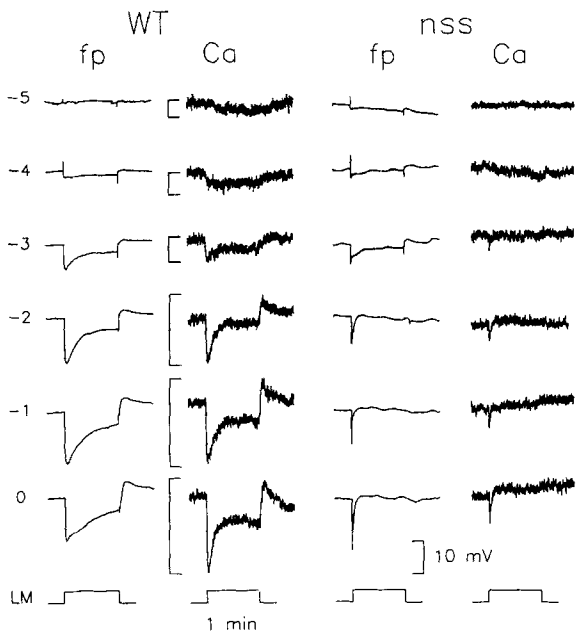

FIGURE 4. Light-induced $\Delta[Ca^{2+}]_o$ in white-eyed *Lucilia* (*left*) and the *nss* mutant (*right*) at various light intensities (indicated in −log scale on the left). *fp,* field potentials; *Ca,* Ca^{2+} concentration changes as in Fig. 3. The figure shows the typical light-induced Ca^{2+} signal in normal *Lucilia.* A relatively small transient reduction in $[Ca^{2+}]_o$ is observed in this particular *nss* mutant at bright lights. The calibration bars on the left of the Ca signal indicate $[Ca^{2+}]_o$ in the ranges of 1.4–1.38, 1.4–1.37, 1.4–1.36, 1.44–1.33, 1.44–1.30, and 1.42–1.29 mM for the light intensities of log*I* = −5, −4, −3, −2, −1, and 0, respectively. This scale applies to both WT and *nss.*

relative to normal flies. The plateau and dark peak phases are (nearly) absent in the *nss* response, indicating that the bulk of Ca^{2+} influx during prolonged light is absent in the *nss* mutant. The main variability found in the various recordings made in normal *Lucilia* were manifested in the rate and the degree of the return in $[Ca^{2+}]_o$ toward the dark baseline (see, for example, Fig. 6 *A*). In some retinas there was no return of $[Ca^{2+}]_o$ to baseline and the reduction in $[Ca^{2+}]_o$ during light had a square shape.

Figs. 4 and 5 thus demonstrate that the *nss* mutation largely reduced the light-induced changes in $[Ca^{2+}]_o$ observed in normal flies; in particular, the sustained $[Ca^{2+}]_o$ reduction is absent in the *nss* mutant.

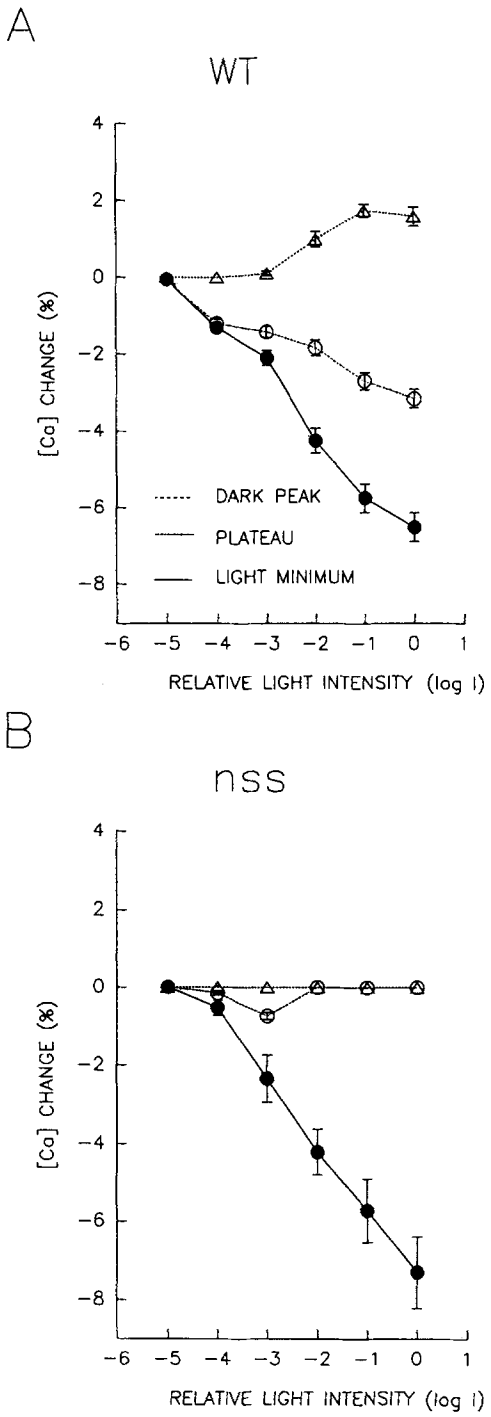

FIGURE 5. The various phases of extracellular Ca^{2+} concentration changes in white-eyed *Lucilia* (*A*) and in the *nss* mutant (*B*) as a function of the relative light intensity. Three parameters of $\Delta[Ca^{2+}]_o$ are plotted: the peak amplitude of the light-induced reduction in $[Ca^{2+}]_o$ (●), the concentration change after 1 min of illumination (○, *plateau*), and the peak amplitude in $[Ca^{2+}]_o$ observed after the light stimulus ends (△, *dark peak*). Vertical bars show the standard deviation of the mean. Nine and eight curves were used for the graphs of WT and the *nss*, respectively.

La^{3+} but Not Nickel (Ni^{2+}) Reversibly Abolished the Changes in $[Ca^{2+}]_o$

Injection of 5 mM La^{3+} in Ringer solution into the retinal extracellular space by repeated short (~0.5 s) pulses of pressure applied during 1 min resulted in abolishment of $\Delta[Ca^{2+}]_o$ even to maximal intensity light stimuli (Fig. 6 *A*, lower line). It also resulted in a large reduction of the steady-state phases of the field potential, leaving mainly a transient response (Fig. 6 *A*, upper line) similar to that observed intracellularly (Suss-Toby et al., 1991).

The waveform of the field potential (and the intracellularly recorded receptor potential) was largely recovered 260 min after the injection. The reduction in $[Ca^{2+}]_o$

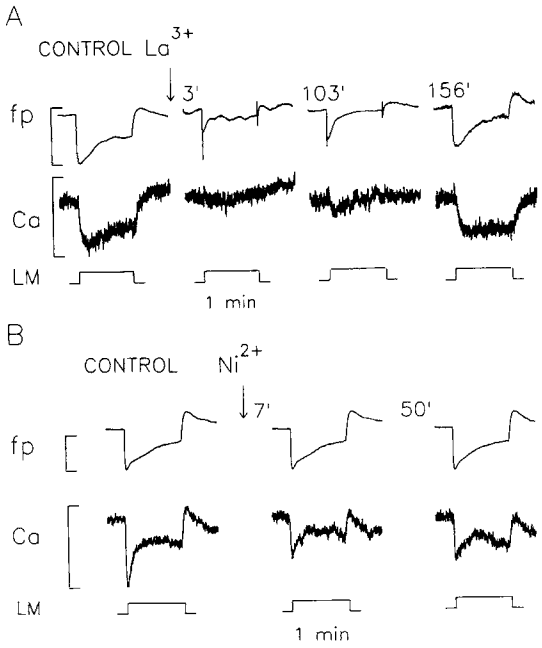

FIGURE 6. (*A*) Lanthanum ions reversibly abolish the light-induced Ca^{2+} signal and make the receptor potential mainly transient. (*Upper row*) Field potentials (*fp*); (*lower row*) $[Ca^{2+}]_o$. The Ca^{2+} signal in the control was abolished within 3 min after injection of 5 mM La^{3+} (in Ringer solution) into the retinal extracellular space. The fp became mainly transient (with a small steady-state level) after La^{3+} application. The responses largely recovered after 262 min. Numbers between traces indicate pauses between the recorded traces. The calibration bar at the left of the Ca signal indicates $[Ca^{2+}]_o$ in the range of 1.43–1.33 mM for *A* and 1.42–1.29 mM for *B*. The calibration at the left of the field potentials indicate 10 mV for both *A* and *B*. (*B*) Nickel ions (5 mM) do not abolish the light-induced Ca^{2+} signal. (*Upper row*) Field potential; (*lower row*) $[Ca^{2+}]_o$. Unlike La^{3+}, Ni^{2+} ions reduced but did not abolish the Ca^{2+} signal. The relative light intensity was $-\log I = 0$ for both *A* and *B*.

also largely recovered 260 min after the injection. The recovery from the effect of La^{3+} presumably resulted from the wash of La^{3+} from the retina to the body by the hemolymph flow, which replaces the extracellular fluids within 90 min (Weyrauther, Roebrock, and Stavenga, 1989). Similar measurements with similar results were observed from seven additional retinas of normal *Lucilia*. As a control to Fig. 6 *A*, Ringer solution (without La^{3+}) was injected into the retinas and no effects were observed.

To examine the specificity of La^{3+} action, another Ca^{2+} channel blocker, nickel (Ni^{2+}, 5 mM), was injected into the retina (Fig. 6 *B*). The effect of Ni^{2+} was to reduce

(but not abolish) the Ca^{2+} signal in an irreversible manner. Results similar to Fig. 6 *B* were obtained in three other retinas. Fig. 6 shows that extracellular La^{3+} reversibly abolishes the light-induced $[Ca^{2+}]_o$ changes in the fly retina; however, another Ca^{2+} channel blocker (Ni^{2+}) was much less efficient in blocking the Ca^{2+} signal.

DISCUSSION

Interpretation of the Changes in $[Ca^{2+}]_o$ during and after Illumination

Ca^{2+}-selective microelectrodes have been an important tool for studying directly light-induced changes in intracellular Ca^{2+} in *Limulus* (Levy and Fein, 1985) and barnacle photoreceptors (Brown, Rydqvist, and Moser, 1988). Ca^{2+} is a key factor in the mechanism of light and dark adaptation in *Limulus* (Lisman and Brown, 1972), in barnacle (Brown, Hagiwara, Koike, and Meech, 1970), and in the drone retina (Bader, Baumann, and Bertrand, 1976). Ca-selective microelectrodes have been the only tool for studying $[Ca^{2+}]$ changes in the extracellular space. The relatively small volume of the extracellular space of insect retina makes $[Ca^{2+}]_o$ a very sensitive means of measuring $[Ca^{2+}]_o$ changes in vivo.

A large number of detailed studies have been conducted in which light-induced ion concentration changes in the extracellular space of the insect eye (Coles and Tsacopoulos, 1979; Tsacopoulos, Orkand, Coles, Levy, and Poitry, 1983; Orkand, Dietzel, and Coles, 1984; see Sandler and Kirschfeld, 1991, for a summary), allowing a consistent and reliable interpretation of the change in $[Ca^{2+}]_o$ in the retina. There are two main processes that could distort the relationship between changes in $[Ca^{2+}]_o$ and movement of Ca^{2+} across the photoreceptor's membrane: (*a*) Ca^{2+} movement into or from cells other than the photoreceptors. As mentioned above, the only other class of cells located at the region of the Ca^{2+} measurements are the glia (pigment) cells, and there is no evidence of significant light-induced $[Ca^{2+}]$ changes in the glia of the drone retina (Coles and Orkand, 1985). There is no reason to assume that the glia cells of the fly are different from those of the drone in this respect. (*b*) Shrinkage of the extracellular space during or after light. Changes in the volume of the extracellular space of the drone retina during light were measured by Orkand et al. (1984) and in great detail by Ziegler and Walz (1989). On the average, 16% shrinkage of the extracellular space was found in the detailed study. The extracellular space of fly retina is considerably larger than that of the drone and therefore smaller changes, if any, in extracellular volume are expected. Even if there is some shrinkage of the extracellular space during light in fly retina, such a shrinkage, which is fairly constant during illumination (Ziegler and Walz, 1989), would tend to reduce the amplitude of observed light-induced transient reduction in $[Ca^{2+}]_o$ (i.e., to underestimate the light-induced reduction in $[Ca^{2+}]_o$) and to somewhat distort the proportions among the amplitudes of the three phases which characterize the $[Ca^{2+}]_o$ signal (see Fig. 5). Shrinkage of the extracellular space cannot explain the large difference between $\Delta[Ca^{2+}]_o$ in the normal and the mutant *Lucilia;* on the contrary, it is expected to reduce the difference between the Ca^{2+} signal of normal and mutant fly due to the larger light response in normal fly (the main factor expected to cause shrinkage of the extracellular space). We therefore conclude that in analogy to previous measurements of $\Delta[Ca^{2+}]_o$ in insect retina, a reduction in $[Ca^{2+}]_o$ in *Lucilia* is a result of Ca^{2+}

influx into the photoreceptors, and that an increase in $[Ca^{2+}]_o$ results from either cessation of Ca^{2+} influx or from Ca^{2+} efflux or both.

Extracellular $[Ca^{2+}]_o$ measurements in the honeybee drone retina have shown that intense prolonged light induces Ca^{2+} influx followed by a much larger Ca^{2+} efflux leading to a net Ca^{2+} efflux. Those two phases of Ca^{2+} change could be separated by removing Na^+ from the extracellular space resulting in a reversible abolishment of the Ca^{2+} efflux but not the influx, thus indicating that the efflux arises from a Na-Ca exchange (Minke and Tsacopoulos, 1986; Ziegler and Walz, 1989). In the drone the Ca^{2+} influx is observed only during illumination, while the Ca^{2+} efflux continues for several minutes after light is turned off. Figs. 3 and 4 show that similar phases of $\Delta[Ca^{2+}]_o$ were observed in the fly retina, but the phase of Ca^{2+} efflux (Fig. 5, open circles) is much smaller relative to that observed in the drone retina. Fig. 5 *A*, which plots the peak amplitude of the three phases as a function of relative light intensity, shows a dependence of these three phases on light intensity that is very similar to those obtained in the fly *Calliphora* (Sandler and Kirschfeld, 1988, 1991). We interpret the waveform of the $[Ca^{2+}]_o$ signal of *Lucilia* in the same manner as is interpreted in the drone retina: the initial fast negative phase reflects Ca^{2+} influx via the light-sensitive channel, and the subsequent increase in $[Ca^{2+}]_o$ results from the increase in $[Ca^{2+}]_i$, which activates a Na-Ca exchanger extruding Ca^{2+} from the photoreceptor to the extracellular space. In *Lucilia,* this Ca^{2+} efflux has a smaller magnitude relative to the Ca^{2+} influx, resulting in a continuous net Ca^{2+} influx during light. After the cessation of the light stimulus, the light-induced influx stops and the observed "off" response reflects the net Ca^{2+} efflux due to the Na-Ca exchange which continues to operate for ~1 min in the dark.

The nss Mutation and La^{3+} Inhibit Ca^{2+} Mobilization

The small but significant Ca^{2+} influx observed in normal *Lucilia* during dim lights ($-\log I$ = 5, 4, 3) was largely reduced by the *nss* mutation (Figs. 4 and 5). Even during intense lights, which induced a relatively large Ca^{2+} influx in normal *Lucilia,* only a relatively short Ca^{2+} influx was observed in the *nss* mutant without the off response, indicating that the *nss* mutation blocks the main pathway for Ca^{2+} entry into the photoreceptors during prolonged illumination. The observation of a small transient influx of Ca^{2+} in the *nss* mutant during intense lights fits nicely with the observation of Hardie and Minke (1992) that the light-activated non-*trp* channels of *Drosophila,* which produce mainly the transient phase of the light response, have a small but significant permeability to Ca^{2+} during intense light. This observation also fits with the findings that (*a*) in the *trp* and *nss* mutants a small degree of Ca^{2+}-mediated light adaptation exists during intense lights (Minke, 1982; Suss-Toby et al., 1991), and (*b*) that in the *trp* mutant a small and transient Ca^{2+}-mediated screening pigment migration (Lo and Pak, 1981) can still be observed.

The effect of La^{3+} on the $[Ca^{2+}]_o$ signal gives strong evidence that La^{3+} blocks Ca^{2+} entry and thus strongly supports the suggestion that La^{3+} affects fly photoreceptors by blocking Ca^{2+} influx (Suss-Toby et al., 1991; Hardie and Minke, 1992).

Inositide-mediated Ca^{2+} Influx

This study provides additional critical evidence to support recently accumulating evidence that fly photoreceptors are endowed with a light-activated Ca^{2+} channel that

can be blocked by the *trp* or *nss* mutation and by La^{3+}. The fact that blocking this channel leads to a reduction in intracellular Ca^{2+}, as evidenced indirectly, and to complete elimination of the receptor potential during light, strongly suggests that light-induced transport of Ca^{2+} from the extracellular space via the *trp* channels is necessary to maintain excitation in fly photoreceptors.

The no receptor potential A (*norpA*) mutant of *Drosophila* which encodes for phospholipase C (Bloomquist, Shortridge, Schneuwly, Pedrew, Montell, Steller, Rubin, and Pak, 1988) provides critical evidence that light-activated inositol lipid signaling cascade (Devary, Heichal, Blumenfeld, Cassel, Suss, Barash, Rubinstein, Minke, and Selinger, 1987) is necessary for the operation of fly phototransduction because severe alleles of *norpA* mutation totally abolish the light response (Minke and Selinger, 1992*b*). It therefore appears that the *nss* or *trp* mutations block specifically an inositide-mediated Ca^{2+} entry into the photoreceptors. There is a general interest in this process, not only in photoreceptors but in all inositol lipid signaling systems (for review, see Berridge, 1990; Irvine, 1990, 1991; Putney, 1990). The *nss* (and *trp*) mutants are likely to provide invaluable information for this important and still unknown mechanism.

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