BRIEF COMMUNICATION

A QUANTITATIVE COMPARISON OF THE TIME-COURSE OF SENSITIVITY CHANGES PRODUCED BY CALCIUM INJECTION AND LIGHT ADAPTATION IN *LIMULUS* VENTRAL PHOTORECEPTORS

ALAN FEIN AND J. SHERWOOD CHARLTON, *Laboratory of Sensory Physiology, Marine Biological Laboratory, Woods Hole, Massachusetts 02543 U.S.A.*

ABSTRACT The time-course of light and dark adaptation was quantitatively compared with the time-course of the onset of and recovery from desensitization produced by intracellular calcium injection in *Limulus* ventral photoreceptors. The onset of light adaptation tended to be faster (by 60–90 s) than the onset of desensitization produced by intracellular Ca^{++} injection. The initial portion of the time-course of dark adaptation was faster (about 10–20 s) than the time-course of recovery from desensitization produced by intracellular Ca^{++} injection. The final portion of recovery from Ca^{++} injection had the same time-course as a comparable dark adaptation.

INTRODUCTION

Vertebrate and invertebrate photoreceptors share the ability to adapt to light and dark. It has been proposed that a rise in intracellular calcium $(Ca^{++})_i$ is a factor leading to light adaptation in the ventral photoreceptors of *Limulus*, an invertebrate (Lisman and Brown, 1972*a*). The experimental evidence in support of this proposal is that: (*a*) the intracellular injection of calcium ions causes a reversible decrease in the response to a constant intensity stimulus (Lisman and Brown, 1972*b*); (*b*) the intracellular injection of a calcium buffer tends to prevent light-induced changes in sensitivity (Lisman and Brown, 1975); (*c*) a light-induced rise of intracellular calcium has been detected directly with the photoprotein aequorin (Brown and Blinks, 1974); (*d*) local illumination and intracellular calcium ion injection locally desensitize the photoreceptor (Fein and Lisman, 1975); (*e*) the intracellular injection of calcium ions causes a reversible

Dr. Charlton's present address is: Department of Physiology, University of California, San Francisco, Calif. 94143.

shortening of the latency of the photoresponse to a constant intensity stimulus (Brown and Lisman, 1975); (*f*) both calcium ion injection and light adaptation produce quantitatively similar changes in photoreceptor sensitivity and photoresponse time course (Fein and Charlton, 1977*b*).

If the Ca^{++} hypothesis is correct, as the above evidence would seem to indicate, then the following should be true: the onset of desensitization during the injection of Ca^{++} should have the same time-course as light adaptation; and the recovery of sensitivity after a Ca^{++} injection should have the same time-course as dark adaptation. The object of the experiments described in this report was to test the above assertions quantitatively.

METHODS

The methods of dissecting, viewing, stimulating, recording, and injecting current into the photoreceptor have been described previously (Fein and DeVoe, 1973; Fein and Charlton, 1975; 1977*a*, *b*, and *c*). Our methods are similar to those first described by Millecchia and Mauro (1969) and Lisman and Brown (1972*b*). A difficulty arises in trying to test the above assertions because the intracellular injection of Ca^{++} causes a greater desensitization near the pipette tip than at more distant regions of the cell. (Fein and Lisman, 1975) Furthermore, the recovery of sensitivity after the Ca^{++} injection is slower in regions of the cell near the pipette tip than at more distant regions (Fein and Lisman, 1975). To make the adapting light similar to the Ca^{++} injection, the photoreceptor was stimulated with a spot light nominally 10 μm in diameter, located near the tip of the pipette. Both the Ca^{++}-containing electrode and the spots of light were located near a region of maximum sensitivity within the photoreceptor by the following procedure: Under visual control the photoreceptor was impaled with a KCl-filled electrode and then scanned with a nominally 10-μm diameter (light scatter made this approximately 20–30 μm) spot of light and a region of high sensitivity was located. The Ca^{++}-containing electrode was inserted into this region of the cell and the adapting and stimulus spots were positioned in the same region. This procedure helped to localize the effects of the Ca^{++} injection and the light adaptation to the same region of the photoreceptor by minimizing the effects of scattered light. However, the spot of light and the Ca^{++} injection are still geometrically different because the spot stimulates a volume of the cell, whereas Ca^{++} is injected from a point source. After an experiment was completed (Fig. 2), the voltage clamp response to a constant intensity spot was measured at different positions along the cell. The results of this measurement are shown in Fig. 1 A.

After the Ca^{++}-containing electrode and the stimulus and adapting spots were placed in the sensitive region of the cell, as described above, experiments were performed using the stimulus timings shown in Fig. 1 B. The procedure outlined in Fig. 1 B allowed us to measure the photoresponse under voltage clamp and then use the same intracellular electrodes for injecting Ca^{++} by passing current between the two intracellular electrodes. (Fein and Charlton, 1977*c*)

Light intensities are given as $\log_{10} I/I_0$ where I_0 is the intensity of the unattenuated beam of white light. The intensity of the unattenuated beam was found to be equivalent to 1.2×10^{15} 520 nm photons/cm^2-s (Fein and Charlton, 1977*a*)

RESULTS

Fig. 2 shows the data obtained from a typical experiment; similar data were obtained in 15 experiments from a total of 5 cells. In these other experiments the test flash

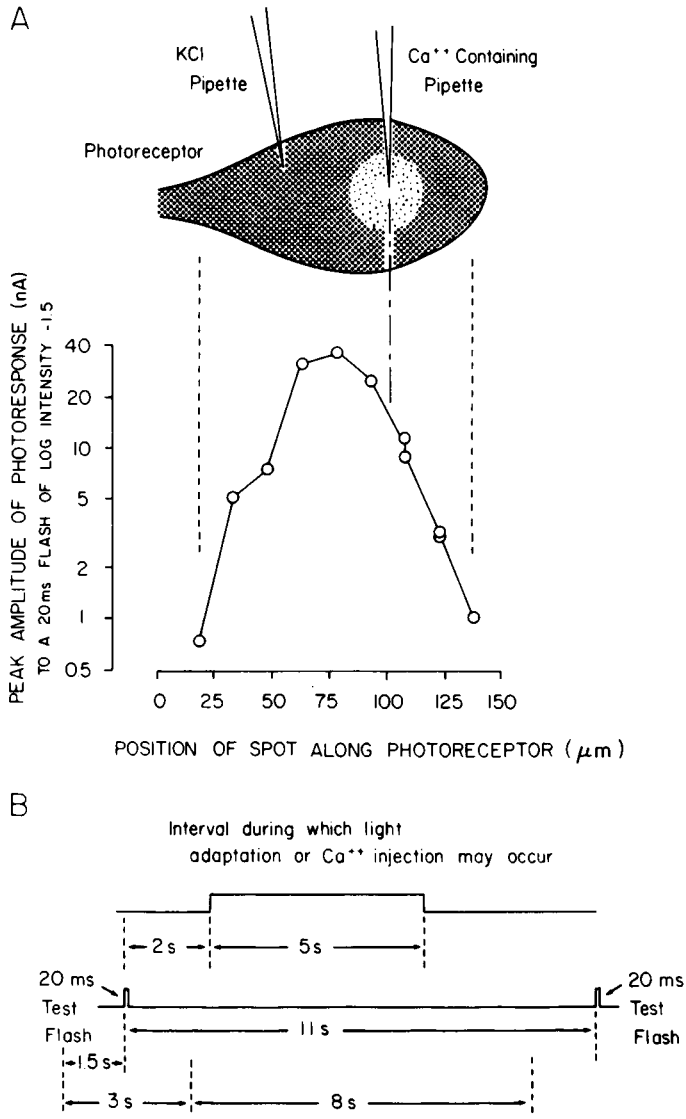

FIGURE 1 A. Photoreceptor sensitivity profile determined under voltage clamp (see Methods). B. Timing of events during an experiment (Figs. 2 and 3). The cell was stimulated once every 11 s by a 20-ms test flash of constant intensity. The test flash was a spot of light positioned at the Ca^{++} electrode, as described in Methods. During the time (3-s interval) when the response to the test flash occurred, the cell was voltage-clamped to its resting (dark) potential. Between the test flashes the receptor was either in darkness, light-adapted by a 5-s adapting flash, or injected with Ca^{++} by means of a 5-s constant current pulse. During the time (8-s interval) when the calcium injection might occur, the electronic circuitry was automatically switched from voltage clamp to current clamp. The current clamp circuit (set for zero membrane current) insured that the injection current passed between the two intracellular electrodes, rather than across the cell membrane. (Fein and Charlton, 1977*c*) In this figure and all subsequent figures the peak amplitude of the response to each test flash is plotted without any averaging or smoothing of the data.

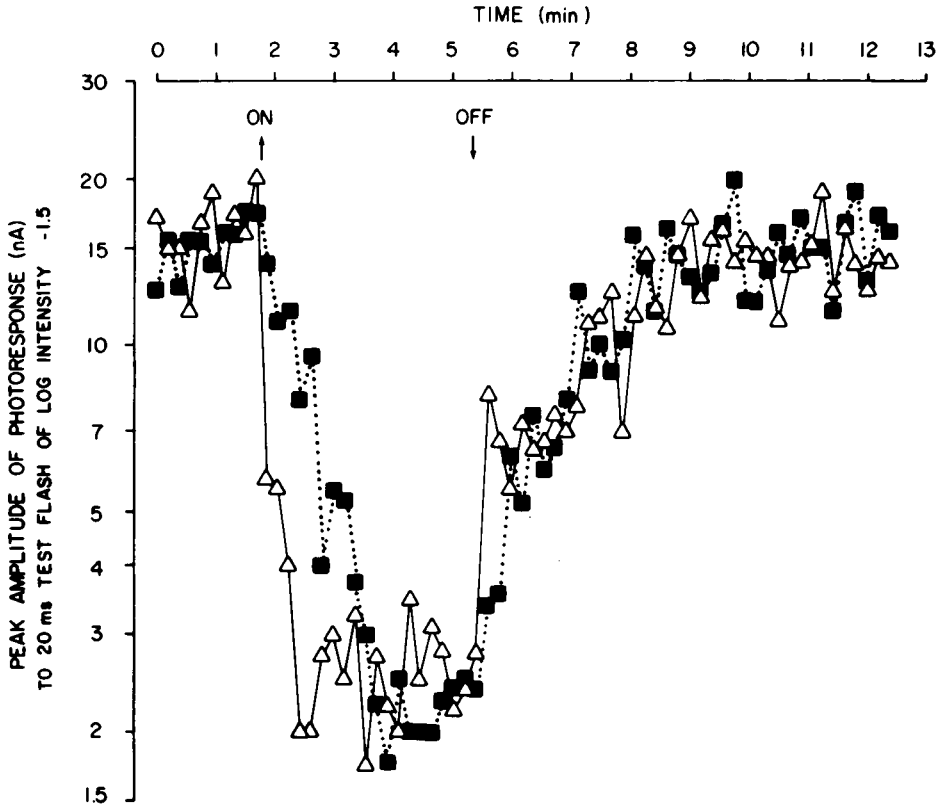

FIGURE 2 The effects of illumination and Ca^{++} injection. A comparison of the time-course of light and dark adaptation to the time-course of the onset of and recovery from desensitization produced by Ca^{++} injection. Same cell as Fig. 1 A. Both the adapting and stimulus flashes were nominally 10-μm diameter spots positioned near the tip of the Ca^{++}-containing electrode, as described in Methods. The adapting flash had a log intensity of -2.3 and the test flash had a log intensity of -1.5. See Fig. 1 B for the stimulus timing. The intensity of the adapting flash was adjusted to give approximately the same final desensitization as the Ca^{++} injection. Similar results are obtained if the effect of the Ca^{++} injection is equated to the effect of the light adaptation. The arrows labeled "ON" and "OFF" indicate when the trains of adapting flashes or Ca^{++} injections were initiated and terminated. (—△—) light adaption (log I_A = -2.3); (—■—) 2 nA Ca^{++} injection.

intensity was varied over a range of 1.5 log units and the intensity of the adapting light was varied over a range of 2 log units. We consistently found that light adaptation was more rapid than the onset of desensitization produced by a comparable Ca^{++} injection. On the other hand, the time-course of dark adaptation was consistently found to be similar to the time-course of recovery from the Ca^{++} injection. However, there was a tendency (as can be seen in Fig. 2) for the initial portion of the recovery after a Ca^{++} injection to lag behind the recovery during dark adaptation.

When faced with data like those in Fig. 2, one must decide whether the differences or the similarities between the effects of light and Ca^{++} injection are most important.

Based on other experiments (Fein and Charlton, 1977*c*) where we have compared the effects of light and Na^+ injection, we find the similarities in Fig. 2 striking.

In Fig. 3 we have juxtaposed a comparison between calcium injection and light adaptation with a comparison between sodium injection and light adaptation. Whereas compared to light it takes 60–90 s longer for the Ca^{++} to desensitize the cell (Figs. 2 and 3 A), it takes about 15 min longer for Na^+ to desensitize the cell (Fig. 3 B). And where the recovery from Ca^{++} injection initially lags about 10–20 s behind a comparable dark adaptation (Figs. 2 and 3 A), recovery from Na^+ injection can take nearly half an hour longer than a comparable dark adaptation (Fig. 3 B). The recovery from Ca^{++} injection only lags behind a comparable dark adaptation for about the first 30 s of the recovery, after which there is no significant difference between the two recoveries (Figs. 2 and 3 A). This should be compared with the Na^+ injection data of Fig. 3 B, for which the time-course of dark adaptation never overlaps the time course of recovery from Na^+ injection.

DISCUSSION

The purpose of this study was to compare quantitatively the time-course of sensitivity changes produced by Ca^{++} injection and light adaptation. The two time-courses were found to overlap only during the final portions of the recovery from the adapting light or Ca^{++} injection (Figs. 2 and 3 A). Otherwise, the onset of desensitization during Ca^{++} injection was slower than the time-course of light adaptation and the initial recovery from Ca^{++} injection was slower than a comparable dark adaptation. Therefore, our data can be taken as a direct confirmation of the Ca^{++} hypothesis for the interval during which the two time-courses overlap.

Before discussing the differences between the effects of light and Ca^{++} injection, we want to reiterate what we feel are striking similarities between the two. Fig. 3 indicates that the photoreceptor is desensitized about an order of magnitude more rapidly by Ca^{++} injection than by Na^+ injection. Also, the photoreceptor recovers more than an order of magnitude more rapidly from the Ca^{++} injection than from the sodium injection. When their effects are compared, the similarities between the effects of light and calcium injection become striking.

We suspect that two factors may contribute to the slower onset of desensitization during the Ca^{++} injection compared to the time-course of light adaptation (Figs. 2 and 3 A). First, whereas the Ca^{++} is injected by a series of constant current pulses, the photoresponses to the series of constant adapting flashes are not constant. The first of a series of adapting flashes produces a much larger response than subsequent adapting flashes. (Spiegler and Yeandle, 1974). Thus, the more rapid onset of light adaptation may partly reflect the above stated difference in the two methods of desensitizing the cell.

Second, the Ca^{++} is injected from a point source and it may diffuse slowly through the volume of the cell stimulated by the spot of light. The diffusion coefficient of Ca^{++} in the cytoplasm of *Limulus* ventral photoreceptors has not been measured

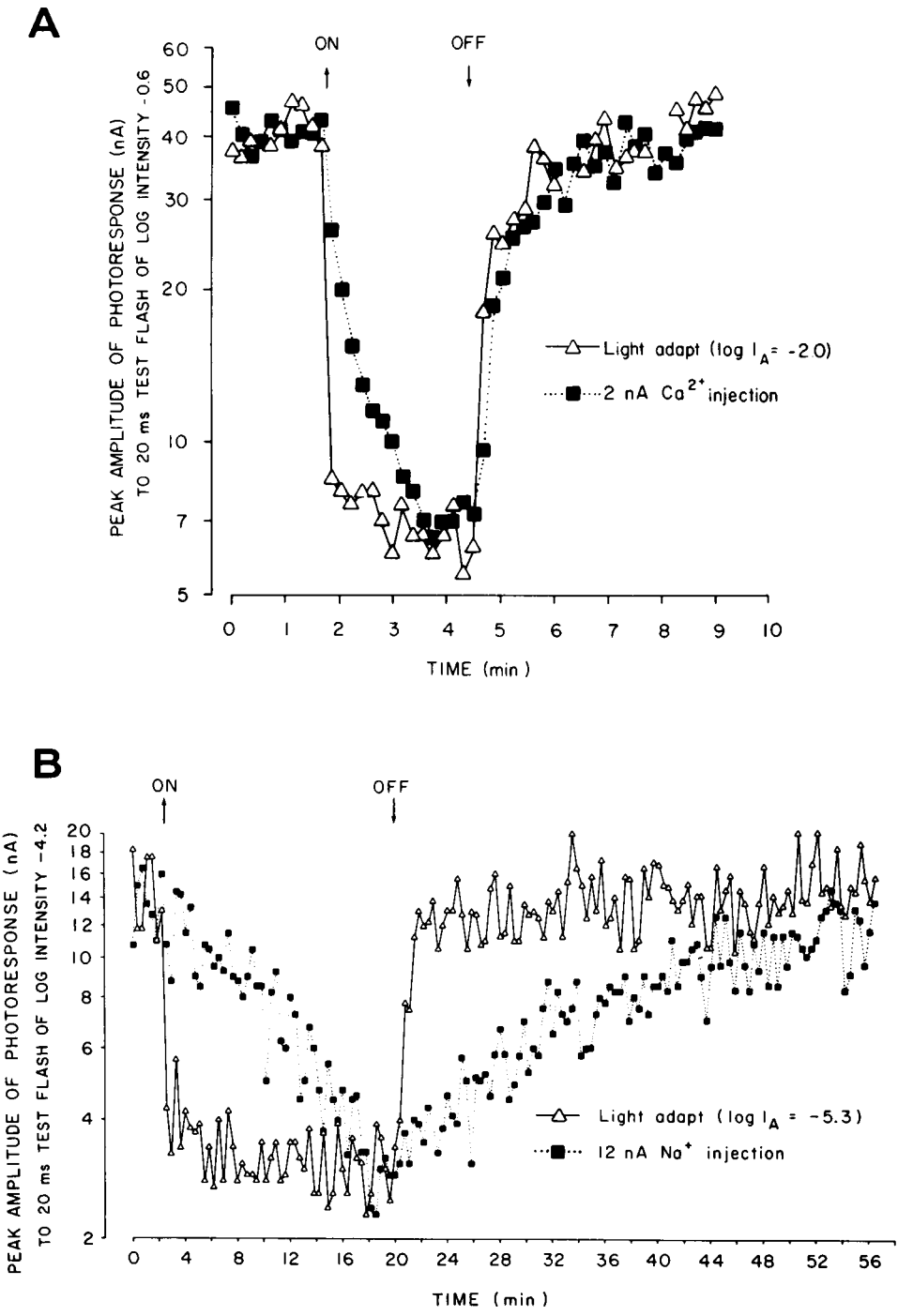
A
ON
OFF
PEAK AMPLITUDE OF PHOTORESPONSE (nA)
TO 20 ms TEST FLASH OF LOG INTENSITY -0.6
60
50
40
30
20
10
7
5
Light adapt (log I_A = -2.0)
2 nA Ca^{2+} injection
0 1 2 3 4 5 6 7 8 9 10
TIME (min)
B
ON
OFF
PEAK AMPLITUDE OF PHOTORESPONSE (nA)
TO 20 ms TEST FLASH OF LOG INTENSITY -4.2
20
18
16
14
12
10
8
6
4
2
Light adapt (log I_A = -5.3)
12 nA Na^+ injection
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56
TIME (min)

FIGURE 3

directly. However, Fein and Lisman (1975) have compared the effects of injected Ca^{++} at two regions of the photoreceptor one at the injection site, the other 60 μm distant from the injection site. They found that for a 5 nA Ca^{++} injection it took 80 s longer for the Ca^{++} injection to produce the same level of desensitization at the distant region, as compared to the region at the injection site. In their experiments the Ca^{++} was repeatedly injected with constant current pulses throughout the measurement; therefore not all the Ca^{++} had 80 s to diffuse. We have chosen half the time interval (40 s) as the average time the Ca^{++} had to diffuse. Therefore their data suggest that it takes about 40 s for the Ca^{++} to diffuse the 60 μm between the two regions of the cell. With these numbers we can estimate the diffusion coefficient D, using the following equation in which r is the average distance the Ca^{++} diffuses in time t: $r^2 = 6Dt$. This equation assumes free diffusion in three dimensions for infinite isotropic media (Crank, 1956). Using the above equation, we calculate that the diffusion coefficient of Ca^{++} in the cytoplasm of the photoreceptor is 1.5×10^{-7} cm^2 s^{-1}. This value for the diffusion coefficient of Ca^{++} in cytoplasm is in excellent agreement with the value of 1.4×10^{-7} obtained for myoplasm (Kushmerick and Podolsky, 1969). Obviously the assumption of free diffusion in three dimensions with infinite isotropic media is a great oversimplification of the actual situation. However, if for example the geometry of the cell were to effectively limit the diffusion of Ca^{++} to two dimensions, the effect on the above equation would be to make the constant 4 instead of 6. The estimate for D would then become 2.3×10^{-7}, still in reasonable agreement with the value for myoplasm.

We can calculate the average distance the Ca^{++} ions would diffuse during the time it takes the Ca^{++} injection to desensitize the cell to the same level attained by light adaptation. We found for five cells that it takes from 60 to 90 s longer for the Ca^{++} injection to desensitize the cell (see Figs. 2 and 3 A). However, not all the Ca^{++} is injected at the beginning of the 60–90-s time interval. The Ca^{++} is repeatedly injected with constant current pulses throughout the interval of time. Therefore, as in the calculations above, we have chosen half the time interval (30–45 s) as the time on which to base the calculation of the average distance the Ca^{++} diffuses. Using the equation given above, together with the data from Fein and Lisman (1975) discussed above, we calculate that Ca^{++} would diffuse on the average 50–60 μm in 30–45 s. Note that the

FIGURE 3 The time-course of photoreceptor desensitization produced by Ca^{++} injection and Na^+ injection. A. Comparison of the time-course of light and dark adaptation to the time-course of the onset of and recovery from desensitization produced by Ca^{++} injection. The methods used in obtaining the data were the same as those described for Figs. 1 and 2. B. Comparison of the time-course of light and dark adaptation to the time-course of the onset of and recovery from desensitization produced by Na^+ injection. The data in this figure comes from Fein and Charlton (1977*c*) and the experimental details are given there. Note, though, that the adapting and test flashes illuminated the photoreceptor uniformly. It was not necessary to use spots of light in the Na^+ injection experiment because Na^+ injection does not locally desensitize the photoreceptor (Fein and Charlton, 1977*c*). The data in A and B were obtained from different cells. The data in A were obtained from a different cell than in Figs. 1 and 2.

diffusion coefficient estimated above does not enter into this calculation. Ventral photoreceptors are approximately ellipsoidal in shape with minor and major diameters of about 50 and 100 μm. (Clark et al, 1969; Stell and Ravitz, 1970). In our experiments the light path is parallel to a minor diameter of the photoreceptor (Fig. 1 A). Therefore, the injected Ca^{++} would have to diffuse the distance (50 μm) of the minor diameter in order to affect the same regions of the cell being stimulated by light. This is because the tip of the Ca^{++}-containing electrode is not deep within the interior of the cell but is just below the plasma membrane. The above calculations indicate that the slower onset of desensitization produced by Ca^{++} injection could be due to the time it takes the Ca^{++} to diffuse through the cytoplasm of the photoreceptor.

When the Ca^{++} injection is turned off, the cell initially recovers more slowly than the comparable dark adaptation. (Figs. 2 and 3 A) We attribute this to the diffusion of previously injected calcium from the region of the pipette tip into more distant regions of the cell. After the injected calcium has had time to redistribute subsequent to turning of the injection, the time-course of recovery becomes the same for both Ca^{++} injection and dark adaptation. In support of this interpretation, we have sometimes observed that the sensitivity continues to fall, before recovering, after the Ca^{++} injection is turned off. This would appear to indicate that the previously injected Ca^{++} continues to diffuse from the vicinity of electrode tip into the region stimulated by the spot of light.

We have attributed all the differences between the effects of Ca^{++} injection and light adaptation to the rate of diffusion of Ca^{++} in cytoplasm, coupled with the difference in geometry between stimulating with light and injecting from a microelectrode. Using the diffusion coefficient of Ca^{++} in the cytoplasm of these photoreceptors (see Discussion above) we have shown that we can quantitatively account for the difference in the time-course between desensitization produced by light adaptation and Ca^{++} injection. Therefore, we conclude that all the findings presented in this paper are consistent with the Ca^{++} hypothesis (Lisman and Brown, 1972*a*).

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