A Quantitative Comparison of the Effects of Intracellular Calcium Injection and Light Adaptation on the Photoresponse of *Limulus* Ventral Photoreceptors

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ABSTRACT Calcium ions were iontophoretically injected into ventral photoreceptors of *Limulus* by passing current between two intracellular pipettes. Changes in sensitivity and photoresponse time course were measured for both light adaptation and Ca^{++} injection. We found for some photoreceptors that there was no significant difference in the photoresponse time course for desensitization produced by light adaptation or by Ca^{++} injection. In other photoreceptors, the time delay of the photoresponse for Ca^{++} injection was slightly longer than for light adaptation. The variability of threshold response amplitude and time delay decreases when the photoreceptor is desensitized by either light adaptation or Ca^{++} injection. The peak amplitude versus log stimulus intensity relationships for controls, light adaptation, and Ca^{++} injection all could be described very closely by a single template curve shifted along the log intensity axis. A 40- to 50-fold change in sensitivity is associated with a 2-fold change in photoresponse time delay for both light adaptation and Ca^{++} injection.

INTRODUCTION

Light adaptation is the decrease in visual sensitivity that occurs when a photoreceptor is exposed to light. Concomitant with this decrease in visual sensitivity is a decrease in the time scale of the photoresponse. That is, the more light that adapted the photoreceptor, the less sensitive the receptor and the sooner the response occurs. Intracellular recordings from both vertebrate (Baylor and Hodgkin, 1974) and invertebrate (Fuortes and Hodgkin, 1964) photoreceptors have shown that there is a quantitative relationship between the changes in sensitivity and the time scale of the photoresponse that occur with light adaptation. It has been proposed (Lisman and Brown, 1972*a*) that a light-induced increase in intracellular Ca^{++} concentration is a factor controlling light adaptation in the ventral photoreceptors of *Limulus*, an invertebrate. Light adaptation of ventral photoreceptors causes a decrease in the amplitude and a decrease in the latency of the photoresponse to a constant intensity stimulus (Millecchia and Mauro, 1969; Brown and Lisman, 1975). Also, the injection of Ca^{++} into these photoreceptors causes a decrease in the amplitude and a

decrease in the latency of the photoresponse to a constant intensity stimulus (Lisman and Brown, 1972*b*; Brown and Lisman, 1975; Fein and Lisman, 1975). Thus, the intracellular injection of Ca^{++} qualitatively mimics the changes in sensitivity and latency that occur with light adaptation. We wondered whether an artificially induced rise in intracellular Ca^{++} would quantitatively mimic the changes in sensitivity and photoresponse time course that occur with light adaptation. We report here quantitative measurements of changes in sensitivity and photoresponse time course that occur with both light adaptation and the intracellular iontophoretic injection of Ca^{++}. We find, to a first approximation, that Ca^{++} injection produces changes in sensitivity and photoresponse time course that mimic the changes in sensitivity and photoresponse time course that occur with light adaptation. A brief account of this work has appeared previously (Charlton and Fein, 1976).

MATERIALS AND METHODS

The technique for preparing and the method of stimulating the ventral photoreceptors of *Limulus* have been described previously (Fein and DeVoe, 1973: Fein and Lisman, 1975; Fein and Charlton, 1975). Our methods are similar to those first described by Millecchia and Mauro (1969). In this study a single photoreceptor was impaled by two micropipettes, one filled with KCl, the other with a Ca^{++}-containing solution. Calcium was iontophoretically injected into the photoreceptor by passing current between the two intracellular pipettes. This procedure was necessary to insure that the large injection currents used in these experiments (up to 16 nA) did not pass across the cell membrane. Lisman and Brown (1972*b*) have shown that large currents (several nanoamperes) passing across the cell membrane will desensitize the photoreceptor. For all the experiments reported here, less than 0.5 nA of current passed across the cell membrane during the injections. The calcium-containing pipette (Ca^{++}-EGTA pipette) was filled with a solution containing 0.09 M $Ca(OH)_2$, 0.1 M Tris, and 0.1 M ethyleneglycol-bis-(β-amino-ethyl ether) *N,N'*-tetraacetic acid (Reuben et al., 1974; Lisman and Brown, 1972*b*; Fein and Lisman, 1975). These electrodes had resistances between 100 and 200 MΩ when measured in the artificial seawater (Fein and Charlton, 1975) that bathed the preparation. These Ca^{++}-EGTA-filled pipettes were used because current could be passed more reliably through them than through similar pipettes filled only with $CaCl_2$ (also see Lisman and Brown, 1972*b*). We checked, in three ways, that the results presented in this paper were the result of Ca^{++} passing out of these pipettes. We made K^+-EGTA electrodes by substituting 0.09 M KOH for the $Ca(OH)_2$. We found that cells injected from these pipettes did not show the effects illustrated in Figs. 1–3. That is, for large Ca^{++} injections (16 nA) from Ca^{++}-EGTA pipettes, we measured changes in sensitivity of about 3 log units (Fig. 3). Whereas for 16-nA injections from a K^+-EGTA electrode we measured desensitizations of under 0.2 log units. We also confirmed (Lisman and Brown, 1972*b*) that Ca^{++} injections from a pipette containing only $CaCl_2$ desensitized the photoreceptor over 2 log units. And we measured desensitizations of less than 0.2 log units for injections of up to 25 nA from a KCl-filled pipette. For these reasons we are confident that the results presented are due to Ca^{++} being iontophoretically injected into the photoreceptor out of the Ca^{++}-EGTA electrodes.

For all experiments we determined the current passing through the Ca^{++}-EGTA electrode. It is this current ($i_{Ca^{++}}$) that we give in Fig. 2. Not all this current, however, is carried by Ca^{++} (Reuben et al., 1974). Thus we cannot state the amount of Ca^{++} being injected in these experiments.

Light intensities (I) are given as $\log_{10} I/I_0$, where I_0 is the intensity of the unattenuated

beam of white light. The light stimuli uniformly illuminated the whole photoreceptor. The method of calibrating the light beam is given in Fein and Charlton (1977*a*). The intensity of the unattenuated beam was equivalent to 6.0×10^{10} 520 nm photons/s incident on the photoreceptor. The log intensity of a 20-ms flash of white light that would evoke on the average one quantal event (Fuortes and Yeandle, 1964) was found to be between −6.25 and −6.35.

The photoreceptor was stimulated once every 11 s by a 20-ms test flash. The duration of the 20-ms test flash was chosen to be below the integration time of the receptor. During the interval between test flashes the photoreceptor was either: (*a*) in darkness; (*b*) light adapted by a 5-s adapting flash whose onset preceded the test flash by 9 s; or (*c*) iontophoretically injected with Ca^{++} for a 5-s interval whose onset preceded the test flash by 9 s. The response to the test flash was measured in the steady state for each of the conditions described above. We did not systematically measure the time course for achieving the steady state. This was because we were varying either the test flash intensity, the adapting flash intensity, or the injection current to facilitate comparison of response waveforms for equal amplitude responses (see Fig. 1).

RESULTS

In Fig. 1 we compare the changes in photoresponse time course and sensitivity produced by light adaptation and Ca^{++} injection. In Fig. 1 A and B the waveforms given by the solid lines are the controls. Controls were measured in the dark both before and after each light adaptation and Ca^{++} injection. The photoreceptor was allowed to recover fully from each Ca^{++} injection or light adaptation before measuring the control responses. The data of Fig. 1 were obtained as follows. First, a set of control responses were measured for three intensities (differing by a factor of two) of the test flash. Next, the photoreceptor was repeatedly injected for 5 s every 11 s (see Materials and Methods) with a 16-nA square pulse of current from the Ca^{++}-containing pipette. When the sensitivity of the photoreceptor reached a steady state the intensity of the test flash was adjusted (log intensity −2.6) to give a response equal in amplitude to the control response that had been elicited by the dimmest test flash (log intensity −5.2). Two more responses were then obtained by doubling the test flash intensity twice (log intensity −2.3 and −2.0). (These three responses [log intensity −2.6, −2.3 and −2.0] are given by the dots in Fig. 1 A and C.) Then the Ca^{++} injection was turned off, the cell was allowed to recover, and another set of controls was measured. The photoreceptor was then light adapted by a 5-s adapting flash repeated every 11 s (see Materials and Methods). The intensity of the adapting flash was adjusted so that a test flash of log intensity −2.6 would elicit a response equal in amplitude to the response elicited by the same test flash during the Ca^{++} injection. Two additional responses were elicited by doubling the test flash intensity twice (log intensity −2.3 and −2.0). These three responses (log intensity −2.6, −2.3, and −2.0) are given by the x symbols in Fig. 1 B and C. Then the adapting flash was turned off, the photoreceptor was allowed to dark adapt, and another set of controls was measured. The control waveforms (solid lines) given in Fig. 1 A and B (log intensity −5.2, −4.9, −4.6) were those obtained between the Ca^{++} injection and light adaptation.

In summary: (*a*) Fig. 1 A illustrates the relationship between photoreceptor

sensitivity and response time course for desensitization produced by Ca^{++} injection; (*b*) Fig. 1 B illustrates the relationship between photoreceptor sensitivity and response time course for light adaptation; (*c*) Fig. 1 C compares the response time course for equal desensitizations (2.6 log units) produced by light adaptation and Ca^{++} injection. For this particular photoreceptor there was no

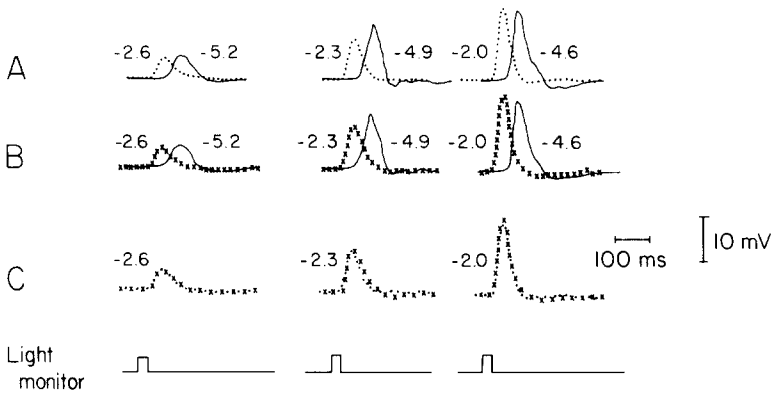

FIGURE 1. Changes in sensitivity and time course of photoresponse associated with light adaptation and intracellular iontophoretic injection of calcium ions. A, Comparison of calcium-desensitized and control responses. B, Comparison of light-adapted and control responses. C, Comparison of light-adapted and calcium-desensitized responses. The log intensity of the adapting light in B was −1.4. The numbers next to each response represent the log intensity of the 20-ms test flash. The injection current was 16 nA in A. Although this amount of current passed through the Ca^{++}-containing electrode, not all the current was carried by Ca^{++} ions (see Materials and Methods). As mentioned in the text, controls were measured before and after each light adaptation and Ca^{++} injection. The control responses shown in A and B were obtained between the Ca^{++} injection and light adaptation. See text for further details of the experimental conditions.

significant difference in the time course of the response for desensitization produced by either light adaptation or Ca^{++} injection (Fig. 1 C). Note also that the responses in Fig. 1 C are nearly superimposable for three intensities (log intensity −2.6, −2.3, −2.0) of the test flash even though the intensity of the adapting light had only been adjusted so that the response produced by the dimmest test flash (log intensity −2.6) would have equal amplitudes. The results presented in Fig. 1 remain unchanged if the light adaptation is carried out first and the Ca^{++} injection current is adjusted to produce a desensitization equal to that produced by the light adaptation.

In Fig. 2 we present data from another photoreceptor for which we compared the effects of light adaptation to the effects of Ca^{++} injection for three different values of injection current. The methods used in carrying out the experiments were identical to those described for Fig. 1, and similar data were obtained. However, it would be unwieldy to present all the response waveforms in Fig. 2. Therefore we condensed the data as follows. As a measure of sensitivity we plot the peak amplitude of the photoresponse elicited by the 20-ms test flash against the intensity of the same test flash. As a measure of the time course of the

photoresponse we plot the time interval from the onset of the test flash to the time when the response first reaches 10% of its peak amplitude (referred to as response time delay). Our findings remain essentially unchanged for other definitions of time delay (10–100% of peak amplitude). Note that for the

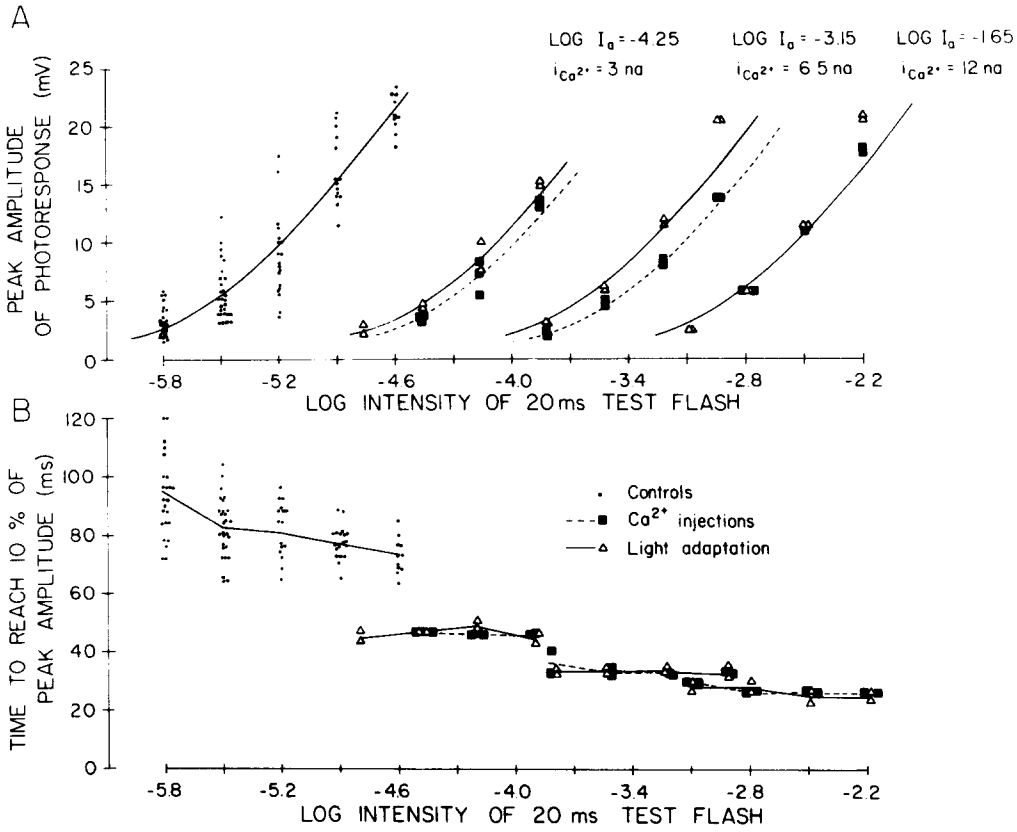

FIGURE 2. A, Peak amplitude of test flash response as a function of test flash intensity for different conditions of light adaptation and Ca^{++} injection. i_{Ca++} is the total current passing through the Ca^{++}-containing electrode. I_a is the intensity of the 5-s adapting flash. B, Response time delay as a function of test flash intensity for different conditions of light adaptation and Ca^{++} injection. In A, the same template curve has been shifted along the intensity axis and fitted by eye to the data. The template curve was established by combining data from controls and light-adapted and Ca^{++}-desensitized photoreceptors and finding a curve (by eye) that fit the composite data. The template curve remains essentially unchanged if only control data are used in establishing it.

controls there is much more variability in threshold response amplitude and time delay than there is for the threshold responses obtained when the receptor is desensitized by either light adaptation or Ca^{++} injection. This variability is believed to be due to variability in the amplitude, number, and time of occurrence of the quantal events which summate to give the response (Fuortes and Yeandle, 1964; Dodge et al., 1968). Thus, it appears that receptor desensi-

tization produced by Ca^{++} injection mimics desensitization produced by light adaptation in that both are associated with a decrease in threshold response variability. The peak amplitude versus log stimulus intensity relationships for controls, light adaptation, and Ca^{++} injection, plotted in Fig. 2 A, could all be described very closely by a single template curve (see legend to Fig. 2) shifted along the log intensity axis. That is, Ca^{++} injection appears to mimic light adaptation by maintaining the same response-intensity relationship. Furthermore, Fig. 2 B shows that for approximately equal desensitizations over a range of about 3 log units, Ca^{++} injection mimics the adapting light by inducing nearly equal time delays for these threshold responses.

As already mentioned in conjunction with Fig. 2, we observed that desensitization produced by both light adaptation and Ca^{++} injection is associated with a decrease in the variability of threshold response amplitude and time delay. This decrease in variability was readily apparent by observing many photoresponses on an oscilloscope. In Table I we present quantitative measurements of

TABLE I

CHANGES IN PHOTORESPONSE VARIABILITY PRODUCED BY LIGHT ADAPTATION AND Ca^{++} INJECTION

Experimental condition	Log intensity of 20-ms test flash	Peak amplitude of photoresponse		Response time delay*	
		Average	SD	Average	SD
		mV		*ms*	
Control	−5.6	7.42	2.38	122	18
3 nA Ca^{++} injection‡	−3.8	5.08	0.79	66	6
Control	−5.6	7.04	2.92	120	22
Light adaptation log I_a=−3.7	−3.8	5.96	0.58	54	5
Control	−5.6	6.69	2.84	123	21

Average and SD are calculated for a sample number of 12.

* Time interval from the onset of the test flash to the time when the response first reaches 10% of peak amplitude.

‡ 3 nA is the total current passing through the Ca^{++}-containing electrode.

threshold response variability for controls, light adaptation, and Ca^{++} injection. The results presented in Table I clearly demonstrate that both light adaptation and Ca^{++} injection are associated with decreases in the variability of the threshold response time delay and amplitude.

In the stimulus paradigm described for Fig. 1 and used throughout this study the photoreceptor is stimulated with a more intense test flash during a light adaptation or a Ca^{++} injection than during a control run. This raises the possibility that the test flash might significantly alter the adaptational state of the photoreceptor produced by light adaptation or Ca^{++} injection. This possibility was checked by turning the test flash off for a minute or two during a light adaptation or Ca^{++} injection. We then compared the test flash response measured just before turning the test flash off to the first response measured after turning the test flash on again. We found that the change in test flash response produced by this procedure could not be distinguished from the

inherent variability of the test flash response described in Fig. 2 and Table I. We therefore conclude that during light adaptation or Ca^{++} injection the test flash did not significantly alter the adaptational state of the photoreceptor.

In Fig. 3 we present composite data from 10 photoreceptors. In order to combine data from different photoreceptors we arbitrarily chose 5 mV as a criterion response for every receptor. Our results remain essentially unchanged for other values of criterion response (see Fig. 2). Note that both the ordinate and the abscissa in Fig. 3 are plotted on absolute scales. The data have not been

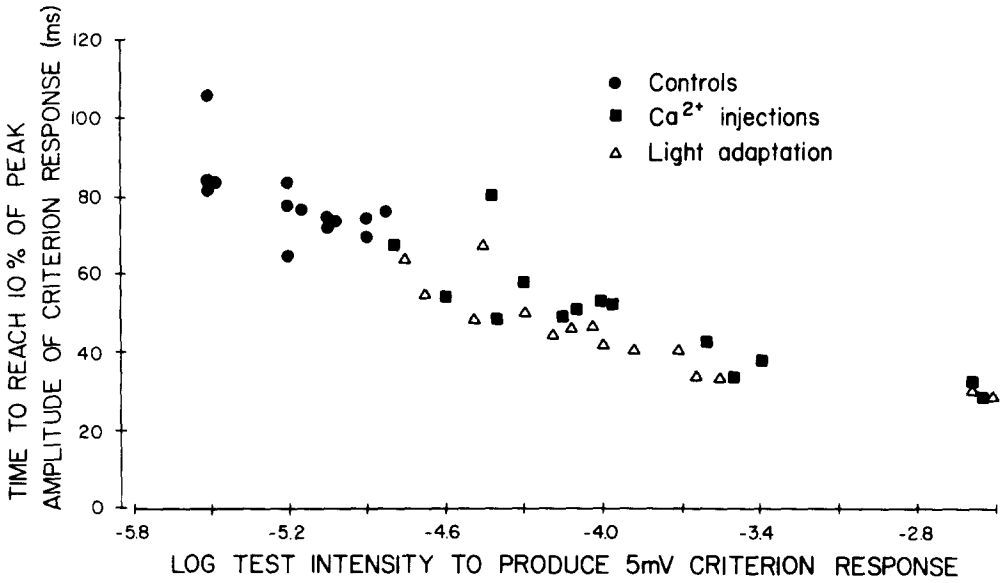

FIGURE 3. Comparison of changes in sensitivity and time delay produced by light adaptation and Ca^{++} injection. Data were obtained from 10 different photoreceptors. Note that both the dependent and the independent variables are plotted on absolute scales. The data have not been normalized in any way. Data from different photoreceptors have been combined by defining an absolute value (5 mV) for the criterion response.

normalized in any way. These results show that in this sample of 10 photoreceptors Ca^{++} injection appears to mimic the relationship between sensitivity and time delay produced by light adaptation. There is a small difference between Ca^{++} injection and light adaptation which is apparent in Fig. 3. On the average, the time delays for Ca^{++} injection tend to be slightly longer than those for light adaptation. This difference was not present in all photoreceptors tested, as is indicated in Fig. 1. We attribute this discrepancy to the difference in the spatial distribution of the two stimuli. Ca^{++} is injected from a point source in the photoreceptor and causes nonuniform desensitization of the photoreceptor (Fein and Lisman, 1975), whereas the test flash and adapting flash uniformly illuminate the whole photoreceptor. Nevertheless, to a first approximation Ca^{++} injection mimics the relationship between sensitivity and time delay produced by light adaptation.

DISCUSSION

Lisman and Brown (1972*a*) have proposed that a light-induced increase of intracellular free calcium is a factor controlling light adaptation in *Limulus* ventral photoreceptors. 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 Ca_i has been detected directly with the photoprotein aequorin (Brown and Blinks, 1974); (*a*) 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 shortening of the latency of the photoresponse to a constant intensity stimulus (Brown and Lisman, 1975).

All the evidence cited above is qualitative. It was our intention to test the calcium hypothesis in a more quantitative manner. The results of our study are as follows: (*a*) both light adaptation and calcium injection are associated with a decrease in the variability of the threshold response amplitude (Fig. 2 A and Table I); (*b*) both light adaptation and calcium injection are associated with a decrease in the variability of the threshold response time delay (Fig. 2 B and Table I); (*c*) the peak amplitude versus log stimulus intensity relationships for controls, light adaptation, and Ca^{++} injection could all be described very closely by a single template curve shifted along the log intensity axis (Fig. 2 A); (*d*) for some photoreceptors there was no significant difference in the time course of the response for desensitization produced by either light adaptation or Ca^{++} injection (Fig. 1 and Fig. 2 B); (*e*) in other photoreceptors the time delay of the photoresponses for Ca^{++} injection was slightly longer than for light adaptation (Fig. 3). This was attributed to the difference in the spatial distribution in the two stimuli (see Results); (*f*) a 40–50-fold change in sensitivity is associated with a 2-fold change in time delay for both light adaptation and Ca^{++} injection (Fig. 3).

The results of this study can be interpreted in terms of the "quantum bumps" (discrete waves of depolarization) which are believed to make up the photoresponse of *Limulus* receptors (Fuortes and Yeandle, 1964; Adolph, 1964; Millecchia and Mauro, 1969; Yeandle and Spiegler, 1973). Dodge et al. (1968) have proposed that: (i) the photoresponse arises from a superposition of bumps which are triggered by the absorption of light; (ii) the average size of the bumps decreases markedly with increasing illumination of the cell, and is the major mechanism of light adaptation.

In order to explain the results of Fig. 1 one need only assume that both light adaptation and Ca^{++} injection cause the average size of a bump to decrease by 2.6 log units and the time delay of the bumps to decrease by a factor of about three. The results of Fig. 2 A and Table I can be explained by assuming that the decrease in the average size of the bump accounts for the decrease in the variability of threshold response amplitude observed with light adaptation and Ca^{++} injection. The variability of the threshold response about a given mean amplitude is decreased when the cell is desensitized because at threshold the

desensitized response is made up of a greater number of smaller bumps (Dodge et al., 1968). This suggestion is supported by the findings presented in Fein and Charlton (1977*b*) where it was shown for a constant intensity test flash that the percent variation in response amplitude remains essentially unchanged when the cell is desensitized. The decrease in variability of threshold response time delay (Fig. 2 B and Table I) suggests that both light adaptation and Ca^{++} injection may decrease the dispersion in time of bump occurrence. The results of Fig. 3 indicate that for the sample of photoreceptors we have studied, both light adaptation and Ca^{++} injection bring about similar changes in bump amplitude and time course.

Fuortes and Hodgkin (1964) were the first to point out that for light adaptation a quantitative relationship exists between photoreceptor sensitivity and the time to peak of the photoresponse. Our results show that in *Limulus* ventral photoreceptors a quantitative relationship holds between sensitivity and response time delay for both light adaptation and Ca^{++} injection.

It might be thought that any process which brings about a decrease in the sensitivity of the photoreceptor also causes a decrease in the time delay of the photoresponse. This is not the case. Lisman and Brown (1975) found that the pressure injection of Ca-EGTA buffers produces a desensitization of the cell together with a slowing of the response rise time. Also, Lantz and Mauro (1977) have shown that treatment with anoxia, DNP, or CO_2 causes a desensitization associated with an increase of the time delay of the photoresponse.

Our results indicate that both light adaptation and Ca^{++} injection have quantitatively similar effects on the photoresponse of the cell. This suggests that both Ca^{++} injection and light adaptation act at a similar point in the transduction process. As such, our results are consistent with the Ca^{++} hypothesis of Lisman and Brown (1972*a*).

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