Increased Intracellular Sodium Mimics Some but not All Aspects of Photoreceptor Adaptation in the Ventral Eye of *Limulus*

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ABSTRACT The effects of the intracellular iontophoretic injection of Na^+ ions have been quantitatively compared with adaptation in ventral photoreceptors of *Limulus*. We find that: (*a*) both light adaptation and sodium injection are associated with a decrease in the variability of the threshold response amplitude; (*b*) both light adaptation and sodium injection are associated with a decrease in the absolute value of the temporal dispersion of the threshold response time delay; (*c*) the same template curve adequately fits the intensity response relationships measured under light adaptation and Na^+ injection; (*d*) both light adaptation and Na^+ injection produce a fourfold decrease in response time delay for a desensitization of 3 log units; (*e*) the time course of light adaptation and dark adaptation is significantly faster than the onset of and recovery from desensitization produced by Na^+ injection; (*f*) unlike local illumination, Na^+ injection does not produce localized desensitization of the photoreceptor. These findings suggest that a rise in intracellular Na^+ concentration makes at most only a minor contribution (probably less than 5%) to the total adaptation of these receptors in the intensity range we have examined (up to 3 log units above absolute threshold). However, changes in intracellular Na^+ concentration may contribute to certain components of light and dark adaptation in these receptors.

INTRODUCTION

Intracellular recordings from a wide variety of invertebrate photoreceptors have shown that most of these receptors depolarize when illuminated (see review by Fuortes and O'Bryan, 1972). Voltage clamp studies of *Limulus* ventral photoreceptors (Millecchia and Mauro, 1969*b*) and barnacle photoreceptors (Brown et al., 1970) have shown that in these receptors, which depolarize upon illumination, there is an increased Na^+ conductance in light. In these cells, photoisomerization of rhodopsin leads to an increase in permeability to Na^+ ions (and to other ions as well) which in turn brings about an influx of Na^+ and membrane depolarization. The light-induced sodium influx causes the intracellular sodium concentration to rise (Brown, 1976) and metabolically activated processes (the sodium pump) then restore the intracellular sodium concentration to its dark (resting) level (Brown and Lisman, 1972; Koike et al., 1971).

Experiments on the depolarizing photoreceptors of *Limulus* ventral eye

(Lisman and Brown, 1972*b*) and the honey bee drone compound eye (Bader et al., 1976) have shown that, in these receptors, both light adaptation and intracellular iontophoretic injection of Na^+ ions produce a reversible decrease in the sensitivity of the photoreceptor. These findings raise the possibility that in these depolarizing photoreceptors, and possibly others as well, a light-induced rise in intracellular Na^+ concentration may contribute to light adaptation (Lisman and Brown, 1972*b*; Bader et al., 1976). However, when ventral photoreceptors are voltage clamped beyond the reversal potential for the light-induced current, the response still adapts to the stimulus (Millecchia and Mauro, 1969*b*). Therefore, a light-induced rise in intracellular Na^+ cannot account entirely for light adaptation (Lisman and Brown, 1972*b*). The experiments described in this paper were designed to evaluate quantitatively the role that intracellular changes in sodium concentration may have in both light and dark adaptation. We have addressed ourselves to two questions: (*a*) to what extent do the effects of the intracellular iontophoretic injection of sodium ions mimic light and dark adaptation? and (*b*) to what extent do intracellular changes in sodium concentration contribute to light and dark adaptation?

MATERIALS AND METHODS

The technique for preparing and the method of stimulating the ventral photoreceptors of *Limulus* have all been described previously (Fein and DeVoe, 1973; Fein and Lisman, 1975; Fein and Charlton, 1975*b*). When we began this study we planned to monitor the photoresponse by measuring the light-induced membrane depolarization. We had done this in a similar study where we examined the effects of the intracellular iontophoretic injection of Ca^{++} (Fein and Charlton, 1977*b*). We soon found that for Na^+ injections light-induced changes in membrane potential were not an appropriate measure of the photoresponse. This was because the injection of Na^+ into a ventral photoreceptor causes the cell to hyperpolarize (Brown and Lisman, 1972; Lisman and Brown, 1972*b*). This hyperpolarization has a number of effects on the photoresponse. First, the hyperpolarization causes the driving force for the light-induced current to increase (Millecchia and Mauro, 1969*b*). The photocurrent produced by a fixed light induced conductance change would thereby increase. Second, the hyperpolarization causes the input resistance to rise (due to membrane rectification [Millecchia and Mauro, 1969*b*]) and the membrane time constant thereby increases. Thus for a given light-induced current, the potential change would be larger and possibly slower. And finally, these photoreceptors have a spike-like potential which is potentiated by membrane hyperpolarization (Millecchia and Mauro, 1969*a*). All these factors, which are secondary to the membrane hyperpolarization, tend to confound the comparison of threshold responses. Therefore we decided to measure the photoresponse (light-induced current) with the cell voltage clamped to its resting (dark) potential. This procedure eliminates all the problems associated with the sodium-induced hyperpolarization.

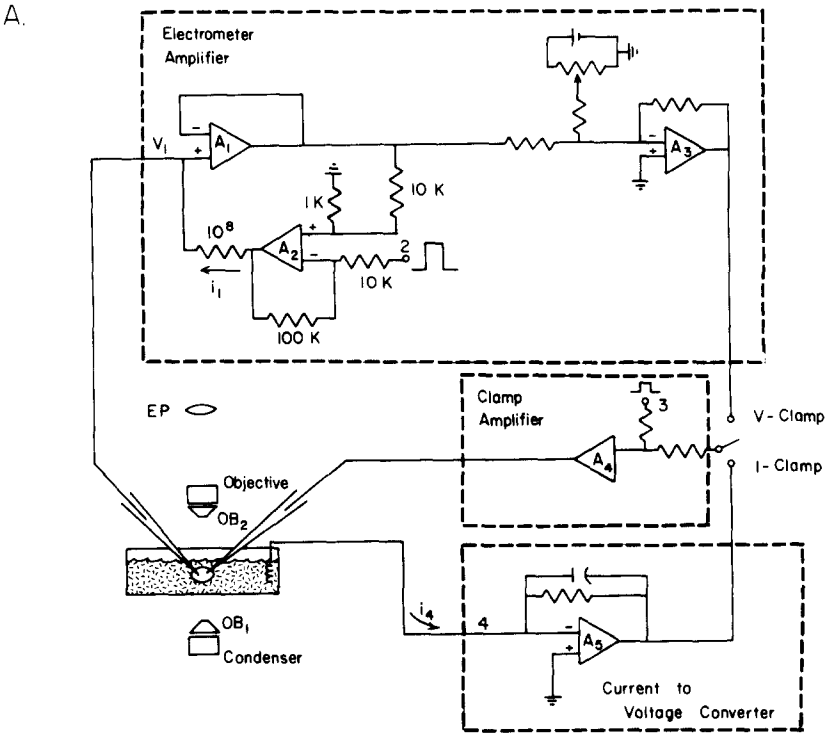
Under visual control, single photoreceptors were impaled with two pipettes, one containing KCl, the other NaCl. Both pipettes had resistances in the range of 15–20 MΩ measured in the artificial seawater (Fein and Charlton, 1975*b*) that bathed the preparation. These two pipettes were used for both injecting sodium into the photoreceptor and voltage clamping the photoreceptor. The amplifiers used for both injecting current and voltage clamping (Fig. 1 A) were of conventional design. The photoreceptor was voltage clamped when the switch in Fig. 1 A was in the voltage clamp position. The procedures followed for establishing that the photoreceptor was isopotential and that

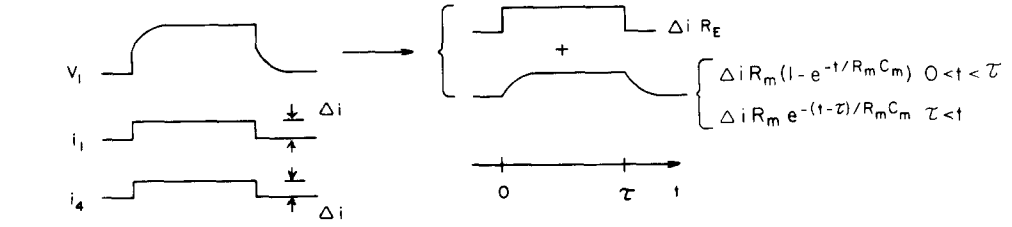
the clamp was working adequately are described in Fein and Charlton, 1977*a*. Na^+ was injected into the photoreceptor by passing current between the two intracellular pipettes. This was accomplished by connecting the switch in Fig. 1 A in the current clamp position (the clamp being set for zero membrane current). Amplifier A_2 in the electrometer amplifier (Fig. 1 A) was used to inject square pulses of current into the cell. The feedback pathway from amplifier A_1 to amplifier A_2 (Fig. 1 A) insured that these current pulses were not affected by differences or changes in pipette and membrane resistance. The current clamp circuit insured that whatever current was injected out of the electrometer pipette did not pass across the cell membrane (current clamped for zero membrane current). Fig 1 B, C illustrates how we checked that this circuit was working correctly. Fig. 1 B, C shows the input voltage of the electrometer (V_1) and the current (i_4) measured by the current-to-voltage converter when we injected square current pulses (i_1) through the electrometer pipette. Fig. 1 B illustrates the waveforms we measured when we injected current through the electrometer pipette and the other pipette was disconnected from the clamp amplifier. The electrometer measured a voltage drop across the pipette and the cell membrane. The current-to-voltage converter measured a current flowing across the membrane into the bath. When the other pipette was connected to the clamp amplifier the waveforms of Fig. 1 C were measured. The voltage drop across the membrane and the current flowing out across the cell membrane were absent (as illustrated in Fig. 1 C) or greatly reduced. For injection currents as large as 25 nA we never passed more than 0.5 nA across the cell membrane. The effects described in this paper are specific effects of injecting Na^+ into the photoreceptor; similar effects are not observed when K^+ is injected into the cell (Lisman and Brown, 1972*b*; Fein and Charlton, 1977*b*). Throughout this paper we display the photoresponse (inward membrane current) as an upward deflection of the trace. Injection currents for Na^+ ions are given as i_{Na^+}, where i_{Na^+} is the total current passing through the NaCl-filled electrode. Not all the current passing through the electrode would be expected to be carried by Na^+ ions, however.

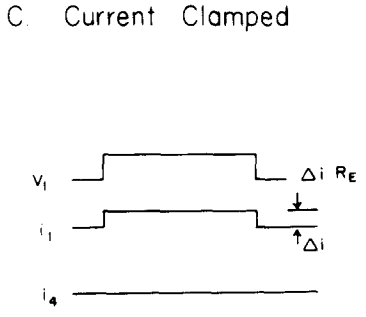
Light intensities I are given as $\log_{10} I/I_0$ where I_0 is the intensity of the unattenuated beam of white light which was used to stimulate the photoreceptor. The intensity of the unattenuated beam was found to be equivalent to 1.2×10^{15} 520 nm photons/cm^2-s (Fein and Charlton, 1977*a*). For uniform illumination of the photoreceptor (Figs. 2–5 and Fig. 8) the number of equivalent 520-nm photons incident on the photoreceptor for the unattenuated beam was found to be 6×10^{10}/s. The threshold for producing one quantal event on the average with a 20-ms flash of white light corresponds to a log intensity of −6.25 to −6.35 in Figs. 2–5 and Fig. 8 (uniform illumination of the photoreceptor) and to a log intensity of −4.45 to −4.55 (this is a lower bound) in Figs. 6 and 7 (stimulation with 10-μm diam spots of light).

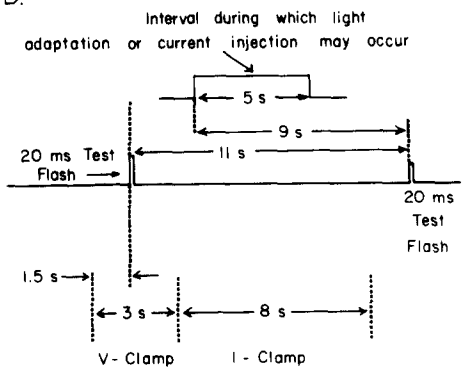
Fig. 1 D shows the timing sequence for the different events that occurred during an experiment. The photoreceptor was stimulated once every 11 s by a 20-ms test flash (chosen to be below the integration time of the photoreceptor) of variable intensity. The photoreceptor was voltage clamped to its resting (dark) potential for an interval that overlapped the time when the response to the test flash occurred. 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; (*c*) iontophoretically injected with Na^+ for a 5-s interval whose onset preceded the test flash by 9 s. The current clamp was turned on for an interval that overlapped the time when the Na^+ injection occurred.

Sometimes during the course of an experiment (a series of light adaptations, Na^+ injections, and recoveries) the resting potential would drift. This drift was in addition to the reversible hyperpolarization due to Na^+ injection. When this drift occurred the


A.
Electrometer Amplifier
V_1
A_1
A_3
1 K
10 K
10^8
A_2
2
10 K
i_1
100 K
EP
Clamp Amplifier
3
V - Clamp
I - Clamp
A_4
Objective
OB_2
OB_1
Condenser
i_4
4
A_5
Current to Voltage Converter


B. Unclamped
V_1
Δi
i_1
i_4
Δi
$\Delta i R_E$
+
$\Delta i R_m (1 - e^{-t/R_m C_m})$ $0 < t < \tau$
$\Delta i R_m e^{-(t-\tau)/R_m C_m}$ $\tau < t$
0
τ
t


C. Current Clamped
V_1
$\Delta i R_E$
i_1
Δi
i_4


D.
Interval during which light adaptation or current injection may occur
5 s
9 s
11 s
20 ms Test Flash
20 ms Test Flash
1.5 s
3 s
8 s
V - Clamp
I - Clamp

photoresponse was measured throughout the experiment with the photoreceptor clamped to its initial resting potential. The drift in resting potential never amounted to more than 15 mV and was typically under 5 mV.

RESULTS

Fig. 2 compares the changes in sensitivity and in the time course of the photoresponse produced by light adaptation and intracellular Na^+ injection. Control responses (solid lines, Fig. 2 A, B) were measured in the dark both before and after each light adaptation and sodium injection. Control responses were only measured after the cell had fully recovered from the desensitizing effects of light adaptation or sodium injection. The data of Fig. 2 were obtained as follows: (*a*) a set of control responses were measured for three intensities of the test flash differing by a factor of 2 (log intensity −5.5, −5.2, −4.9); (*b*) then the photoreceptor was repeatedly stimulated (for 5 s every 11 s, see Materials and Methods) by an adapting flash of log intensity −3.0, and the test flash intensity was adjusted (log intensity −3.1) to give a response equal in amplitude to the control response elicited by the dimmest test flash (log intensity −5.5). Two additional responses were obtained by doubling the test flash intensity twice (log intensity −2.8, −2.5). These three responses (log intensity of test flash −3.1, −2.8, −2.5) are given by the dots in Fig. 2 A and C; (*c*) the adapting light was turned off, the photoreceptor was allowed to recover, and a new set of control responses were measured; (*d*) then the photoreceptor was repeatedly injected with a 15-nA, 5-s square pulse (every 11 s, see Materials and Methods) from the Na^+-containing pipette. After 15 min of injection the response to a test flash of log intensity −3.1 was equal in amplitude to the response obtained with the same test flash during the previous light adaptation. Two additional responses were measured by doubling the test flash intensity twice (log intensity −2.8, −2.5). These three responses (log intensity of test flash −3.1, −2.8, −2.5)

FIGURE 1. (*Opposite*) A, Apparatus used for voltage clamping (V-clamp) and current clamping (*I*-clamp) *Limulus* ventral photoreceptors. Amplifiers A_1–A_5 are conventional commercial operational amplifiers. Amplifier A_2 was used to inject constant current pulses (i_1) through the micropipette. The feedback path between amplifier A_1 and A_2 insured that current i_1 was determined by the voltage at point 2 and the 10^8 Ω resistor. Any voltage drop across the pipette or cell membrane (V_1) was compensated for by the feedback from A_1 to A_2. The preparation was observed using the eyepiece (*EP*) and the objective (OB_2). The photoreceptor was stimulated by light projected onto it by the condenser (OB_1). The photostimulator is described in detail by Fein and Charlton (1975*b*). B, Input voltage (V_1) measured by the electrometer and the current (i_4) measured by the current-to-voltage converter when square current pulses (i_1) are passed through the electrometer pipette. These waveforms are measured when the other pipette is disconnected from the clamp amplifier. The voltage (V_1) measured by the electrometer is made up of two components: the voltage across the electrode, and the voltage across the cell membrane. C, V_1, i_4, and i_1 when the other electrode is connected to the clamp amplifier (as shown) and the clamp amplifier is connected for current clamping to zero membrane current (see text for further details). D, Timing diagram for events that take place during an experiment.

are given by the x symbols in Fig. 2 B, C; (*e*) the injection current was then turned off, the cell was allowed to recover, and a new set of controls were measured. The controls, shown by the solid lines in Fig. 2 A, B, were obtained after the light adaptation and before sodium injection. The time course of the sensitivity changes for Na^+ injection and light and dark adaptation will be discussed subsequently (see Fig. 5).

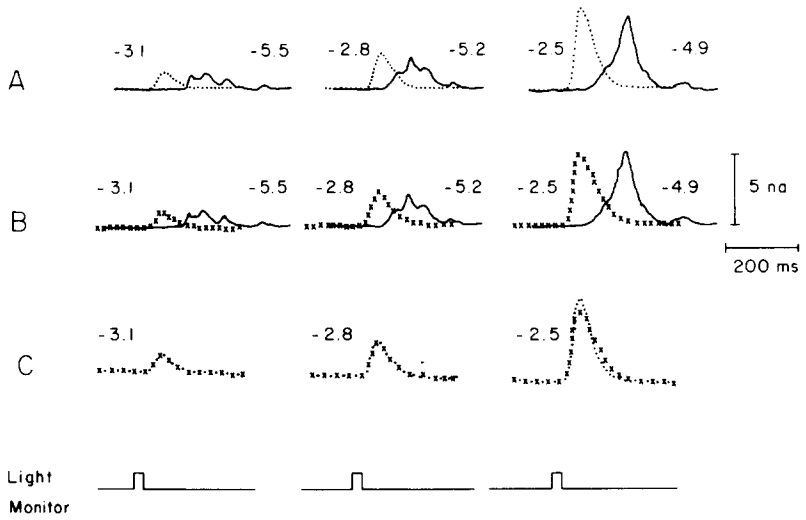


FIGURE 2. Comparison of changes in sensitivity and the time course of the photoresponse produced by light adaptation and intracellular Na^+ injection. The number next to each waveform gives the log intensity of the 20-ms test flash. The light monitor shows the time course of the test flash. A, Comparison of light-adapted responses (dots) with control responses (solid lines). The adapting light (log I = −3.0) desensitized the cell 2.4 log units and the light-adapted response occurred sooner than the control. B, Comparison of responses obtained after 15 min of Na^+ injection (×) with control responses. Injection of 15 nA from the Na^+ containing pipette desensitized the photoreceptor by 2.4 log units and the desensitized response occurred sooner than the control. C, Superposition of light-adapted responses (dots) from A with responses obtained during Na^+ injection (×) from B. The threshold for producing on the average one quantal event per 20-ms test flash corresponds to a log intensity of between −6.25 and −6.35 (see Materials and Methods, and Fein and Charlton, 1977*a*). See Results and Materials and Methods for details of experimental methods used in obtaining these data.

Fig. 2 A shows the changes in sensitivity and the time course of the photoresponse associated with light adaptation. The adapting light desensitized the photoreceptor by 2.4 log units and the desensitized response occurred sooner than the control. Fig. 2 B shows the changes in sensitivity and photoresponse time course associated with Na^+ injection. Injection of Na^+ for 15 min resulted in a 2.4 log unit decrease in sensitivity and the desensitized response occurred sooner than the control. In Fig. 2 C we compare the responses for light adaptation and Na^+ injection. For the two dimmest flashes (log intensity −3.1

and −2.8) the responses were essentially superimposable. For the brightest flash (log intensity −2.5) the falling phase of the light-adapted response was more rapid than that of the response obtained during Na^+ injection. This difference was consistently seen in all the photoreceptors we studied. We do not know what factors are responsible for this difference. Similar results were obtained when the Na^+ injection was carried out first and the light adaptation was equated to the Na^+ injection. The findings presented in Fig. 2 suggest that light adaptation and Na^+ injection desensitize the photoreceptor in a similar but not identical manner.

In the stimulus paradigm described for Fig. 2 the photoreceptor is stimulated with a more intense test flash during a light adaptation or a Na^+ 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 Na^+ injection. This possibility was checked by using the procedure described in Fein and Charlton (1977*b*). We found that during light adaptation or Na^+ injection the test flash did not significantly alter the adaptational state of the photoreceptor.

In Fig. 3 we quantitatively compare Na^+ injection and light adaptation at two different times during the Na^+ injection. A procedure similar to that described for Fig. 2 was followed and similar data were obtained. As a measure of the photoreceptor sensitivity we plot the log of the peak amplitude of the response to the 20-ms test flash against the log of the test flash intensity (Fig. 3 A). As a measure of the photoresponse time course we plot the log of the photoresponse time delay (time from stimulus onset until photoresponse first reaches 10% of peak amplitude) against the log of the test flash intensity (Fig. 3 B). Our results remain essentially unchanged for other definitions of time delay between 10% and 100% of peak amplitude. Fig. 3 A shows that a template curve with slope of 1 fits all the peak amplitude data reasonably well (Lisman and Brown, 1975 *a*; however, see Fein and Charlton, 1977*a*). Both light adaptation and Na^+ injection appear to desensitize the photoreceptor by causing the peak amplitude response curve to shift along the log stimulus intensity axis. Fig. 3 B shows that both light adaptation and Na^+ injection decrease the response time delay. Taken together Fig. 3 A and B show that for nearly equal desensitizations produced by either light adaptation or Na^+ injection the changes in time delay are nearly equal. The effects of both Na^+ injection and light adaptation were found to be completely reversible. Fig. 3A shows a decrease in the variability of threshold response amplitude associated with the desensitization produced by light adaptation and Na^+ injection. Similarly, Fig. 3 B shows a decrease in the absolute value of the variability in time delay associated with both light adaptation and Na^+ injection. Both these decreases in threshold response variability were found to be completely reversible. A possible basis for this decrease in threshold response variability will be considered in the Discussion.

In Fig. 4 we present composite data from six photoreceptors for which we compared Na^+ injection and light adaptation. We have also included in Fig. 4 data from a previous study (Fein and Charlton, 1977*b*) where we compared Ca^{++} injection and light adaptation. In order to combine data from different photoreceptors injected with Na^+ we arbitrarily chose a 2-nA photocurrent as

the criterion response. Our results remain essentially unchanged for other values of the criterion response (see Fig. 3). In the Ca^{++} injection experiments the photoresponse was not measured under voltage clamp. In those experiments

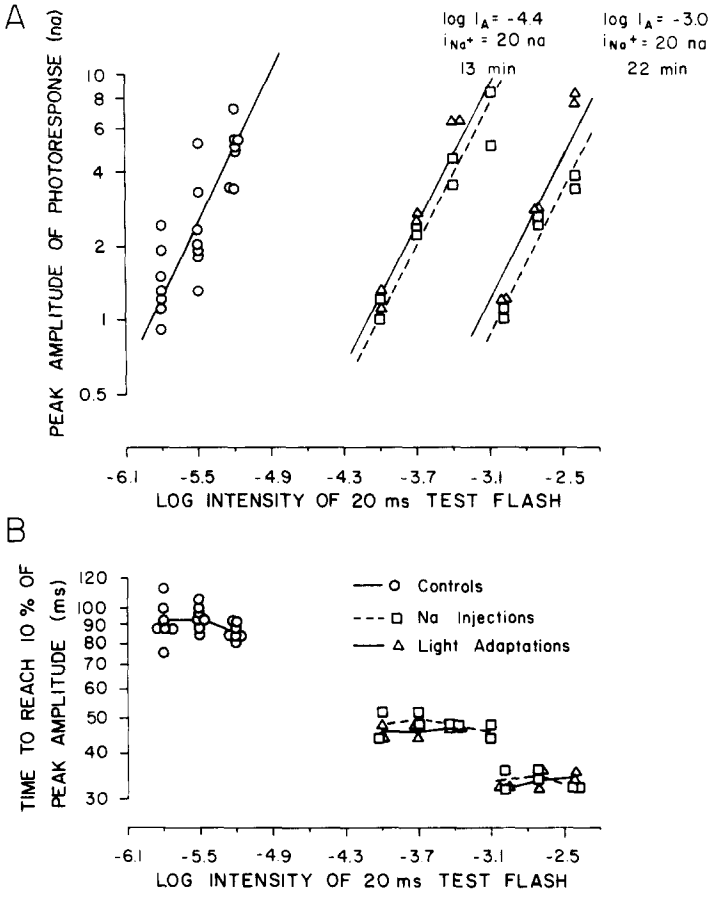


FIGURE 3. Comparison of light adaptation and Na^+ injection at two times during the Na^+ injection. A, Log-log plot of peak amplitude of photoresponse versus intensity of test flash. B, Log-log plot of response time delay versus intensity of test flash. For both A and B, log I_A gives the intensity of the adapting light, i_{Na^+} gives the current passing through the NaCl electrode, and the time in minutes gives the time after the onset of the Na^+ injection at which the Na^+ injection data were obtained. In both A and B, the controls are given by O, the light-adapted data by △, and the Na^+ injection data by □. These data were obtained from a photoreceptor different from that shown in Fig. 2. The same experimental methods as described in the text for Fig. 2 were used in obtaining these data.

we measured the photoresponse by monitoring the transmembrane depolarization. By choosing to compare a 10-mV response with a 2-nA response in Fig. 4 we have made the reasonable assumption that the input resistances of the photoreceptors are on the average 5 MΩ (Millecchia and Mauro, 1969*a*). Note in Fig. 4 that both the ordinate and abscissa are absolute (un-normalized)

scales. The results of Fig. 4 indicate that under the conditions of these experiments light adaptation, Na^+ injection and Ca^{++} injection produce changes in sensitivity and time delay that are to a first approximation quantitatively

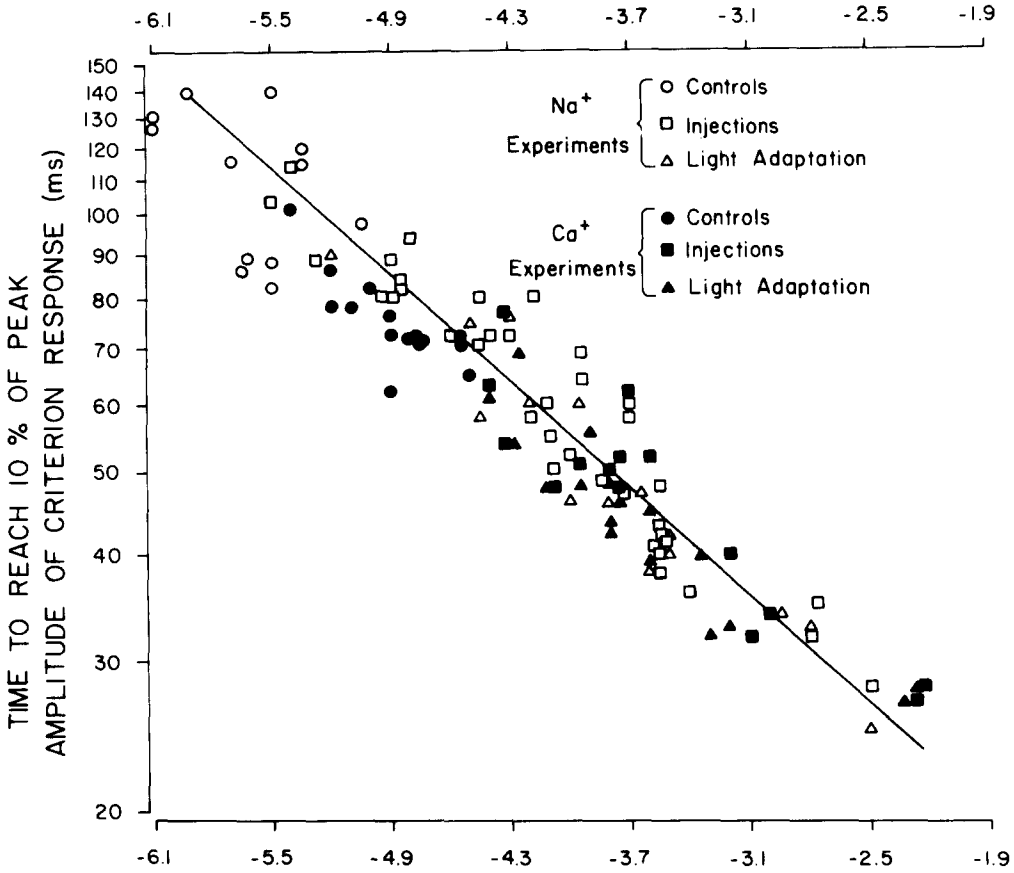


FIGURE 4. Log-log plot of the experimentally determined relationship between photoreceptor sensitivity and response time delay for light adaptation, Na^+ injection, and Ca^{++} injection. Voltage clamp data from this study (unfilled symbols) are combined with unclamped data from a previous study (filled symbols). For both types of data the response time delay is plotted on the same log scale. For the voltage clamp data (Na^+ experiments of this study) the log of the response time delay is plotted against the log of the test flash intensity needed to produce a 2 nA criterion response. For the unclamped data, Ca^{++} experiments of a previous study (Fein and Charlton, 1977*b*), the log of the response time delay is plotted against the log of the test flash intensity needed to produce a 10-mV criterion response. The straight line was drawn through the data points by eye. The data indicate that a 3 log unit decrease in sensitivity is associated with about a four-fold decrease in time delay. Composite data from 16 photoreceptors are plotted (6 for Na^+ experiments and 10 for Ca^{++} experiments). The experimental methods used in the Na^+ experiments (unfilled symbols) are the same as described in the text for Fig. 2. The experimental methods used in the Ca^{++} experiments (filled symbols) are given in Fein and Charlton (1977*b*).

equivalent. Also, there do not appear to be any systematic differences between the clamped and the unclamped data.

In Fig. 5 we compare the onset of, and recovery from, desensitization produced by Na^+ injection to light and dark adaptation. The method of injecting Na^+ and light adapting the photoreceptor was the same as described for Fig. 2. The data in Fig. 5 (also see Fig. 8) clearly show that the onset of and

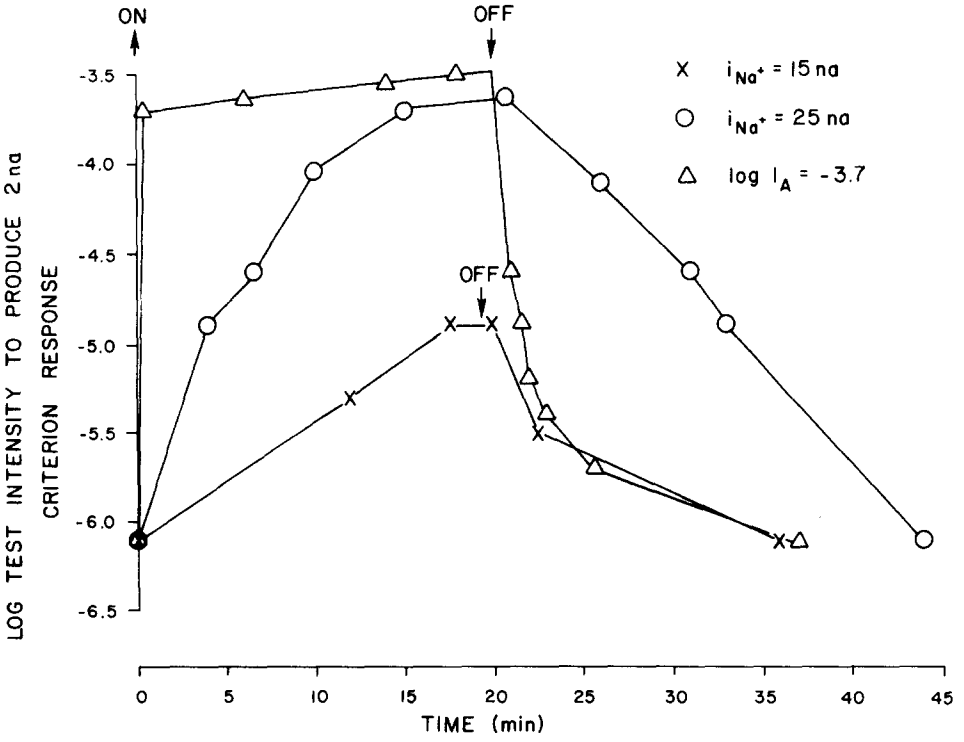


FIGURE 5. Comparison of the time course of light and dark adaptation with the onset of and recovery from desensitization produced by Na^+ injection. The log threshold (intensity of 20-ms test flash needed to produce a 2 nA criterion response) is plotted as a function of time for light and dark adaptation and Na^+ injection. The arrow labeled *ON* denotes the onset of the adapting light and the sodium injection. The arrows labeled *OFF* denote the time at which the adapting light and Na^+ injection were turned off. The current passing through the NaCl electrode is denoted by i_{Na^+} and I_A is the intensity of the adapting light. The same experimental methods as described in the text for Fig. 2 were used in obtaining these data.

recovery from desensitization for Na^+ injection are markedly slower than the time course of light and dark adaptation.

We have previously shown that local illumination of part of a ventral photoreceptor leads to a localized flow of membrane current (Fein and Charlton, 1975*a*). Furthermore, it has been shown that the light adaptation produced by local illumination is localized to the region of illumination (Fein, 1973;

Spiegler and Yeandle, 1974; Fein and Charlton, 1975*b*). Also, Fein and Lisman (1975) have shown that injection of calcium ions into ventral photoreceptors locally desensitized the receptor. And we (Fein and Charlton, 1977*a*) have shown that enhancement is spatially localized in these receptors. These findings led us to investigate whether Na^+ injection would locally desensitize these receptors.

Fig. 6 shows the data from an experiment where we tested for localized desensitization during Na^+ injection. Fig. 6 D is a schematized version of the photoreceptor showing the two stimulus spots (nominally 10 μm in diameter) and the location of the NaCl and KCl microelectrodes. We have previously described the photostimulator and experimental methods used in this type of experiment (Fein and Charlton, 1975*b*). Fig. 6 A, B shows that both regions 1 and 2 of the photoreceptor can be light adapted locally, whereas Fig. 6 C shows that Na^+ injection desensitizes region 1 and 2 equally. Fig. 6 shows that unlike

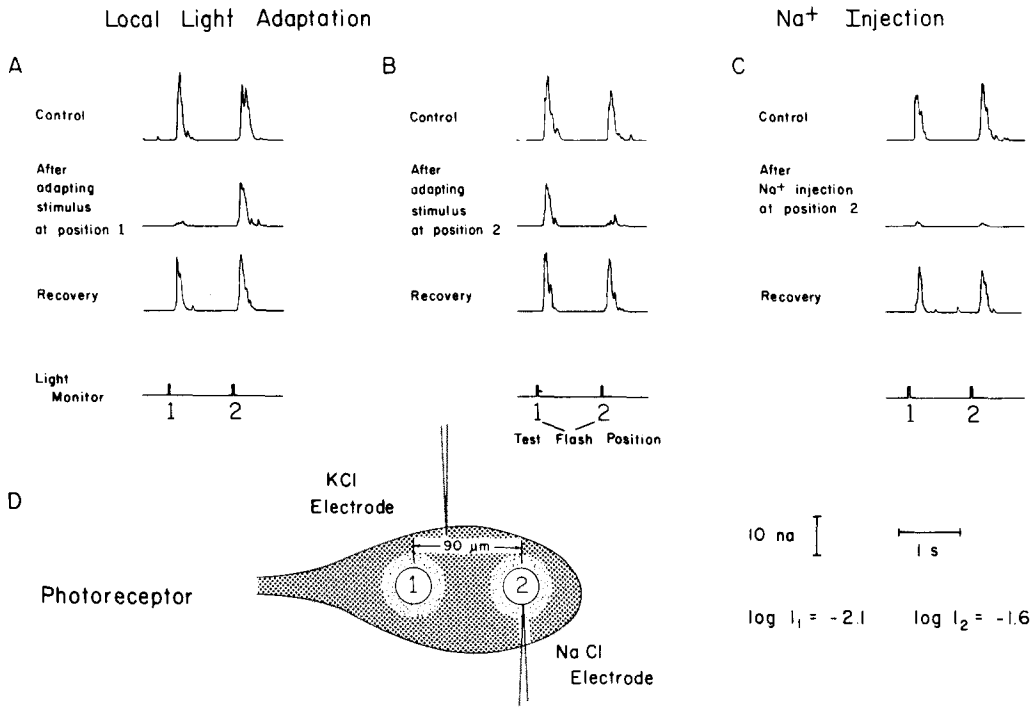


FIGURE 6. A comparison of local adaptation (A, B) and Na^+ injection (C). D, Schematized representation of the photoreceptor showing the position of the two stimulus spots labeled 1 and 2 (nominally 10 μm in diameter), and the position of the NaCl and KCl electrodes. I_1 and I_2 give the intensity of the 20-ms test flash located at positions 1 and 2, respectively. A and B show that both regions 1 and 2 of the photoreceptor can be adapted locally. The adapting spot of light had a log intensity of -2.1 in A and -1.6 in B. In both A and B the adapting light was on for 8 s and was turned off 2 s before the first test flash. C shows that a 15 nA Na^+ injection for about 9 min (see Fig. 7) equally desensitized both regions of the photoreceptor.

local illumination, the intracellular injection of Na^+ does not produce localized desensitization. In Fig. 7 we compare, for the two regions of the photoreceptor shown schematically in Fig. 6 D, the onset of and recovery from desensitization produced by Na^+ injection. The data of Figs. 6 and 7 are from the same photoreceptor. Fig 7 shows that we fail to find localized desensitization during the onset of and recovery from the Na^+ injection.

We consistently find that the desensitizing effect of Na^+ injection is delayed

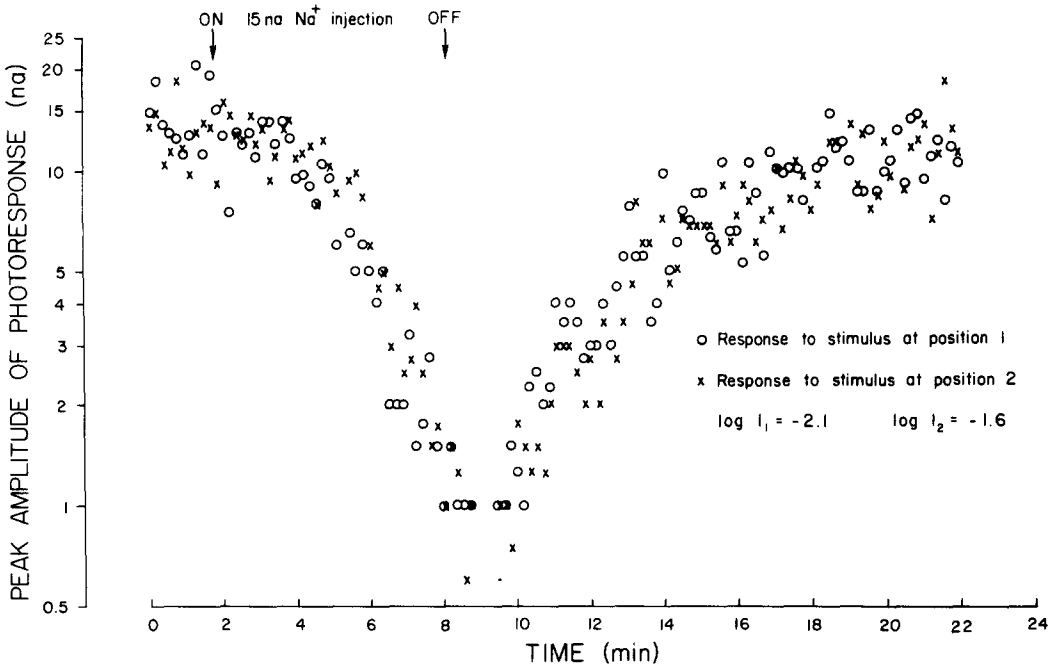


FIGURE 7. Onset of and recovery from desensitization produced by Na^+ injection (as measured at two spatially separated regions of a photoreceptor). These data are from the same cell as shown in Fig. 6 and the experimental conditions are as shown in part D of that figure. The arrows indicate when the 15 nA Na^+ injection was turned on and off. I_1 and I_2 are intensities of the test flashes at positions 1 and 2 on the photoreceptor. Note that there is no localized desensitization at any time during the onset of or recovery from the desensitization produced by the Na^+ injection.

after the onset of the injection; in the experiment shown in Fig. 7, this delay was about 2 min (also see Fig. 8). This time delay could indicate that the Na^+ concentration must attain some critical value before desensitization occurs.

DISCUSSION

A. *Sodium Injection, Light Adaptation, and Dark Adaptation*

It has previously been shown that the intracellular injection of Na^+ reversibly decreased the response to a constant intensity stimulus for *Limulus* ventral photoreceptors (Lisman and Brown, 1972*b*). The results of our study extend

the findings of these investigations. Specifically, we have shown the following: (*a*) both light adaptation and the intracellular injection of sodium are associated with a decrease in the variability of the threshold response amplitude (Fig. 3 A); (*b*) both light adaptation and the intracellular injection of Na^+ are associated with a decrease in the absolute value of the variability in threshold response time delay (Fig. 3 B); (*c*) a template curve with a slope of 1 (Fig. 3 A) fits all the data (controls, light adaptation, Na^+ injection) reasonably well in the response range of 0–10 nA; (*d*) both light adaptation and sodium injections produce similar changes in response time delay for desensitizations as great as 3 log units (Figs. 2–4). This last result suggests that, except for some small differences in the falling phase of the photoresponse (see Fig. 2 C), the intracellular injection of sodium quantitatively mimics the changes in sensitivity and time delay produced by light adaptation.

These findings can be interpreted in terms of the "bumps" (quantal events) that are believed to make up the photoresponse in *Limulus* receptors (Fuortes and Yeandle, 1964; Adolph, 1964; Dodge et al., 1968; Millecchia and Mauro, 1969*a*; Spiegler and Yeandle, 1974). Dodge et al. (1968) have proposed that: (*a*) the photoresponse arises from a superposition of bumps which are triggered by the absorption of light; (*b*) the average size of the bumps decreases with increased illumination and is the major mechanism of light adaptation. The results presented in Figs. 2–4 can be interpreted in terms of the above-stated ideas if the following are true. (*i*) Both Na^+ injection and light adaptation cause a reversible decrease in the size of a bump, thereby reversibly decreasing sensitivity and the variability in response amplitude (Fig. 3 A). The variability for a constant amplitude response is decreased when the cell is desensitized because the desensitized response is made up of a greater number of smaller bumps (Dodge et al., 1968). (*ii*) Both Na^+ injection and light adaptation are associated with a reversible decrease in the time delay and the absolute temporal dispersion of bump occurrence, thereby reversibly decreasing response delay and the absolute value of time delay variability (Fig. 3 B). (*iii*) The rate of bump production is to a first approximation a linear function of light intensity, therefore a template curve with a slope of 1 fits all the peak amplitude data reasonably well (Fig. 3 A); however, see Fein and Charlton, 1977*a*. (*iv*) Both Na^+ injection and light adaptation produce similar changes in bump amplitude and bump time delay for desensitization up to three log units (Figs. 2–4).

We have suggested (on the basis of the previous work of Dodge et al., 1968) that the variability in response amplitude is decreased when the cell is desensitized because the response to a brighter flash is made up of a greater number of smaller bumps. If a photoreceptor is tested with a constant intensity test flash during desensitization the average number of bumps elicited by the test flash should remain constant provided the light adaptation or Na^+ injection does not affect the quantum efficiency of bump production (Dodge et al., 1968). Then during desensitization by light or Na^+ injection the response to a constant intensity stimulus should only reflect changes in the average size of a bump. If this assertion is true, the absolute variation in response amplitude to constant intensity stimulus should decrease during desensitization but the percentage variation in response amplitude should remain essentially un-

changed. The percentage variability, in the sense we are using it, is a dimensionless quantity and is equivalent to taking the ratio of the standard deviation to the mean. We have tested the above assertion and the results are presented in Fig. 8. In Fig. 8 A the peak amplitude of the response to a constant intensity stimulus is plotted on a linear scale as a function of time for equal desensitizations produced by light adaptation or Na^+ injection. In Fig. 8 B the same data as in Fig. 8 A are presented by use of a logarithmic scale to plot the amplitude. It can be seen in Fig. 8 A that during desensitization produced either by light adaptation or Na^+ injection the absolute variability in response amplitude is decreased as suggested above. When the same data are plotted on a logarithmic scale as in Fig. 8 B, it can be seen that the percentage variation in response amplitude remains essentially unchanged throughout the desensitization, as suggested above. The findings presented in Fig. 8 are consistent with the idea that both light adaptation and Na^+ injection desensitize the photoreceptor by similarly affecting the bumps that are believed to underlie the photoresponse.

The findings discussed above point out some very striking similarities between the effects of light adaptation and sodium injection. However, there are also some very striking differences. We consistently found that the time course of light adaptation and dark adaptation was faster than the onset of and recovery from desensitization produced by Na^+ injection (see Figs. 5 and 8). This finding suggests that a rise in intracellular Na^+ concentration does not make a large quantitative contribution to adaptation in these receptors. Specifically, during the first 5 min of dark adaptation in Fig. 5 the threshold (△) drops nearly 2.3 log units, whereas in the same period during recovery from the 25-nA Na^+ injection the threshold (○) only dropped 0.5 log units. Therefore, on the basis of the 25-nA Na^+ injection, recovery from intracellular sodium accumulation could only account for <2% of the recovery of threshold during the first 5 min of dark adaptation in Fig. 5. If one argues similarly, the 15 nA Na^+ injection (×) suggests that recovery from intracellular Na^+ accumulation could account for at most 5% of the recovery of threshold during the first 5 min of dark adaptation in Fig. 5. Thus it would appear that over 95% of the recovery of threshold that occurs during the first few minutes of dark adaptation is not caused by a decrease in intracellular Na^+ accumulation.

The time course of light adaptation cannot be simply compared to the desensitization produced by a series of constant Na^+ injections. This is because the photoresponse to each of a series of constant adapting flashes is not constant. The first of the series of adapting flashes produces a much larger response than subsequent flashes (Spiegler and Yeandle, 1974). Therefore we do not draw any quantitative conclusions based on the difference between the time course of light adaptation and the onset of desensitization produced by Na^+ injection.

B. *Sodium Injection and Local Adaptation*

Local illumination of ventral photoreceptors leads to a local influx of Na^+ (Fein and Charlton, 1975*a*) and to local adaptation (Fein, 1973; Spiegler and Yeandle, 1974; Fein and Charlton, 1975*b*). This may suggest that a local rise in intracellular Na^+ concentration gives rise to local adaptation. The data in Figs. 6 and 7

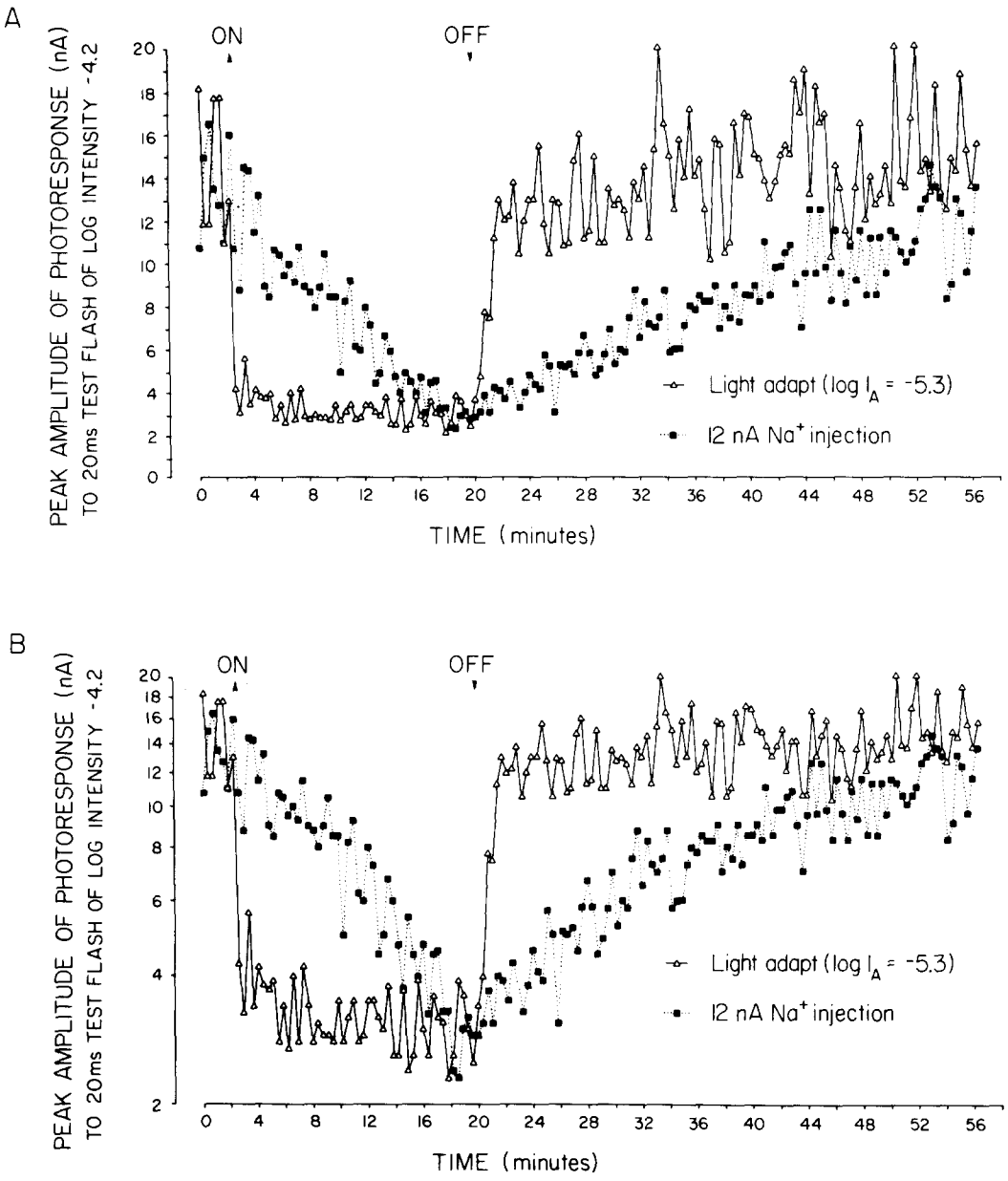


FIGURE 8. The effects of light adaptation and Na^+ injection on photoresponse variability for a constant intensity test flash. A, The peak amplitude of the response to a constant intensity (log I_t = −4.2) test flash plotted on a linear scale as a function of time for equal desensitizations produced by light adaptation or Na^+ injection. B, The same data as in A replotted with a logarithmic scale for the peak amplitude. The arrows labeled *ON* denote the onset of the adapting light and the sodium injection. The arrows labeled *OFF* denote the time at which the adapting light and Na^+ injection were turned off.

indicate that this is not the case. That is, the local injection of Na^+ does not locally desensitize the photoreceptor. We had previously speculated (Fein and Charlton, 1975*b*) that a local influx of Na^+ ions would not sustain an intracellular Na^+ gradient, on the basis of the measured mobility of intracellular Na^+ in other tissues. Therefore we concluded that a localized change in intracellular Na^+ concentration could not account for local adaptation (Fein and Charlton, 1975*b*). The results presented in Figs. 6 and 7 indicate that this conclusion was correct. Therefore, the results of Figs. 6 and 7 can be interpreted as follows: (*a*) injection of Na^+ out of the intracellular pipette gives rise to increased concentration of Na^+ near the tip of the pipette; (*b*) diffusion of Na^+ will cause the Na^+ concentration to equilibrate throughout the cell body; this will occur with a half-time of several seconds (Fein and Charlton, 1975*b*); (*c*) when the intracellular Na^+ concentration throughout the cell body reaches some critical value, the sensitivity of the photoreceptor begins to fall simultaneously throughout the cell body (Figs. 7 and 8); (*d*) when the Na^+ injection is turned off, the photoreceptor begins to recover sensitivity as the sodium pump (Brown and Lisman, 1972) begins to reduce the intracellular sodium concentration. Some effect of local illumination other than local accumulation of Na^+ must account for local adaptation: possibly a local accumulation of Ca^{++} ions (Fein and Lisman, 1975).

We have previously shown that local light adaptation can induce more than a 20-fold difference of sensitivity over a distance of 80 μm (Table I, Fein and Charlton, 1975*b*). On the basis of the arguments given above we can conclude that this 20-fold difference in sensitivity is not due to a local accumulation of Na^+. Therefore, no more than 5% of the light adaptation produced by local illumination is caused by an increase of intracellular Na^+.

C. *Relationship between Sensitivity and Time Delay*

Fuortes and Hodgkin (1964) were the first to point out that for different levels of light adaptation a quantitative relationship exists between the sensitivity and time to peak of the photoresponse in *Limulus* lateral eye. The results presented in Fig. 4 indicate that such a relationship exists for ventral photoreceptors as well. The straight line drawn through the data points in Fig. 4 indicates that a 3 log unit decrease in sensitivity is associated with about a fourfold decrease in time delay. Brown and Lisman (1975) have shown that both light adaptation and the intracellular injection of Ca^{++} cause a decrease in the latency of the photoresponse of *Limulus* ventral photoreceptors. The data in Fig. 4 indicate that the intracellular injection of both sodium and calcium produces a decrease in sensitivity and a time delay that are quantitatively similar to those produced by light adaptation. This finding suggests that changes in both intracellular Na^+ and Ca^{++} concentration cause their effects by somehow acting at a point or points in the transduction process close, or identical, to those at which light acts.

It might be thought that any process that desensitizes the photoreceptor causes changes in sensitivity and the time course of the photoresponse that are similar to light adaptation. However, this is not the case. Lisman and Brown (1975*b*) have shown that the intracellular injection of a Ca^{++} buffer (EGTA) desensitizes the photoreceptor but slows the rate of rise of the photoresponse.

Also, Lantz and Mauro (1977) have shown that anoxia, DNP, and 100% CO_2 cause a reversible decrease in photoreceptor sensitivity that is associated with a slowing of the photoresponse. Therefore, the correspondence shown in Fig. 4 between the decrease in sensitivity and time delay produced by light adaptation, Ca^{++} injection, and Na^+ injection appears to be specific and not shared by every process that may desensitize the photoreceptor.

D. *Mechanism of the Na^+ Effect*

Lisman and Brown (1972*b*) have suggested that the desensitizing effect of intracellular Na^+ injection in *Limulus* ventral photoreceptors is not direct. They have shown that in Ringer's solution containing less than 0.1 mM Ca^{++} there is almost no decrease in photoresponse during intracellular Na^+ injection. They have also shown that the intracellular injection of Ca^{++} also desensitized the photoreceptor. On the basis of these findings they proposed that a rise in intracellular Na^+ leads to an increase in intracellular Ca^{++} and thereby to desensitization of the photoreceptor (Lisman and Brown, 1972*b*). Also, Waloga et al. (1976) have shown that intracellular Na^+ injection leads to a rise in intracellular Ca^{++}. The results presented in Figs. 2–4 are consistent with the above proposal.

Lisman and Brown (1972*a*) have proposed that a rise in intracellular Ca^{++} is a factor leading to light adaptation in *Limulus* ventral photoreceptors. The results presented in Fig. 4 are consistent with this hypothesis. The Ca^{++} injection data of Fig. 4 are more fully discussed elsewhere (Fein and Charlton, 1977*b*).

E. *Summary and Conclusion*

We had two questions in mind while carrying out these experiments. First, to what extent does the intracellular iontophoretic injection of Na^+ mimic adaptation? And second, to what extent do changes in intracellular Na^+ concentration contribute to adaptation?

In answer to the first question, the results of Figs. 2–4 indicate that Na^+ injection quantitatively mimics changes in sensitivity, photoresponse time course, and response variability associated with light adaptation. Also, the same template curve adequately fits the intensity response relationships measured for light adaptation and Na^+ injection (Fig. 3 A). On the other hand, the results of Figs. 5 and 8 indicate that the time course of light and dark adaptation is faster than the onset of and recovery from desensitization produced by Na^+ injection. Moreover, Figs. 6 and 7 show that, unlike local illumination, Na^+ injection does not produce local adaptation. Thus Na^+ injection mimics certain aspects of adaptation while failing to mimic others.

Previous studies have indicated that an increase in intracellular Na^+ cannot account entirely for light adaptation (Millecchia and Mauro, 1969 *b*; Lisman and Brown, 1972*b*; Lisman, 1976). Therefore we have answered, in quantitative terms, our second question. The results of Fig. 5 indicate that recovery from intracellular Na^+ accumulation can account for at most 5% of dark adaptation. Similarly, the results of Figs. 6 and 7, together with the results of Fein and Charlton (1975 *b*) (see Discussion, section B), indicate that at most 5% of the

light adaptation produced by local illumination can be accounted for by a rise in intracellular Na^+ concentration. These conclusions hold only for the range of light intensities considered in these experiments (~3 log units above absolute threshold).

F. *Speculation: the Role of Intracellular Na^+ in Light and Dark Adaptation*

We have concluded that changes in intracellular Na^+ concentration make at most a small quantitative contribution to the total adaptation observed in *Limulus* ventral photoreceptors over the first 3 log units of adaptation above bump threshold. However, we do not mean to imply that a rise in intracellular Na^+ concentration does not make any contribution to adaptation.

Previous studies (Fein and DeVoe, 1973) and the results presented in Fig. 5 indicate that there is an initial fast component and a later slow component of dark adaptation. We have also found that with prolonged intense adaptation (log I = 0, duration 10–20 min) the slow component of dark adaptation can be greater than 1 log unit (unpublished observation). Furthermore, in Fig. 5 there is a slow increase in threshold during the 20 min of light adaptation. Also, in our study of local adaptation (Fein and Charlton, 1975 *b*) we found that there was a component of adaptation (that increased with adapting intensity) which was not localized and which could not be accounted for by light scatter. Thus there appear to be a slow component and a nonlocalized component of adaptation that increase with the intensity of the adapting stimulus. These components are not very prominent at the threshold elevations (3 log units) we have investigated in this study (Figs. 5–7). Brighter adapting lights and thereby greater elevations of threshold are needed to bring out these components. We speculate that part or all of these components of adaptation may be associated with changes in intracellular Na^+ concentration.

The slow component of light adaptation (Fig. 5) may be due to the intracellular accumulation of Na^+, and the time course of the slow component of dark adaptation (Fig. 5) may possibly reflect the rate at which accumulated Na^+ is pumped out of the cell. Similarly, the nonlocalized component of light adaptation that is not due to light scatter may reflect a rise in intracellular sodium throughout the cell. The involvement of Na^+ in these components of adaptation is currently being investigated.

It is appropriate to ask what are the possible connections between the internal concentrations of sodium and calcium, and how they may be related to adaptation. As summarized by Fein and Charlton (1977 *b*), all the available evidence is consistent with the suggestion that a rise in intracellular Ca^{++} is a factor controlling adaptation (Lisman and Brown, 1972 *a*). Also, as discussed in part D, the desensitization of the photoreceptor produced by intracellular Na^+ injection appears to be due to a rise in intracellular Ca^{++} (Lisman and Brown, 1972 *b*; Waloga et al., 1976). We have suggested above that certain components of adaptation may be due to a rise in intracellular Na^+.

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