LIGHT-INDUCED CHANGES IN DYE-TREATED LOBSTER GIANT AXONS

JOHN POOLER

From the Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina 27706. Dr. Pooler's present address is the Department of Psychology, Duke University, Durham, North Carolina 27706.

ABSTRACT Single giant axons from the lobster circumesophageal connective were studied using the sucrose gap voltage-clamp technique. The axon area in the gap was bathed in acridine orange for several minutes and then rinsed for several minutes. Subsequent illumination resulted in progressive prolongation of electrically stimulated action potentials to durations of 150 msec. The prolongation was accompanied by an increase in threshold. Currents in voltage clamp were altered such that transient current inactivation was greatly slowed. The turn on of transient current was somewhat slowed, the voltage at which peak transient current could be obtained was shifted to more positive internal potentials, and transient current at all potentials was decreased. Steady-state current was similarly affected. Low calcium following illumination partially counteracted some of the changes, but not the slowing of inactivation. Low calcium increased the duration of prolonged action potentials. Selective alteration of parameters in the Hodgkin-Huxley equations brought about a qualitative match between computations and data.

INTRODUCTION

Neurophysiologists asking questions of ionic permeability mechanisms in excitable cells have utilized a number of techniques to alter normal cellular behavior. Among these techniques have been the application of drugs, toxins, and different ionic media. The resulting altered behaviors have been useful to neurophysiologists both because they may ultimately provide insights into mechanisms of permeability change and because they help define the range of excitation properties.

One less common method which can alter neuronal behavior is the illumination of giant axons which have been treated with dyes. This dye-illumination method was attempted successfully on excitable cells at least as early as 1919 by Adler (Blum, 1932) and became familiar to many neurophysiologists through the work of Chalazonitis and coworkers during the 1950's (Chalazonitis, 1954; Arvanitaki and Chalazonitis, 1961; Chalazonitis and Chagneux, 1961).

Chalazonitis found that illumination of dye-treated sepia axons resulted in de-

polarization, unstable oscillatory behavior, and then excitation. More recently, Lyudkovskaya and coworkers performed similar experiments on sepia and pacific squid. Lyudkovskaya found results very much like those obtained by Chalazonitis in one experiment (Lyudovskaya, 1961), but found very different results in two earlier experiments (Liudkovskaia and Kaiushin, 1959; Lyudkovskaya and Kayushin, 1960). In these earlier experiments he obtained no oscillations or excitation, but found rather drastic changes in the shape of electrically stimulated action potentials.

Both the Chalazonitis and Lyudkovskaya groups used conventional external recording techniques and were able to describe potential changes only, without presenting much evidence relevant to the events underlying them.

The present experiments were undertaken with the aim of describing the ionic basis for the altered axon behaviors resulting from dye-illumination by bringing the axon under potential and current control. Such control allows measurement of more excitation parameters than is possible with conventional external recording. Since squid and sepia were not available locally, lobsters were used in these studies. A secondary aim was to see whether the varying results of Chalazonitis and Lyudkovskaya were compatible with each other and with the description of ionic events obtained on lobster.

METHOD

The method employed was the sucrose gap voltage-clamp technique. The axon chamber and electronic measurement and control systems were very similar to those previously described by Julian, Moore, and Goldman (1962 *a*, *b*). An isolated giant axon from the lobster circumesophageal connective was placed in a Lucite chamber such that two sucrose streams divided the external bath into three pools. Current was injected into the left-hand pool, the central pool was connected to the virtual ground summing point of an operational amplifier, and the right-hand pool was connected to a high impedance preamplifier. Artificial sea water (ASW) normally flowed in the left and central pools, and depolarizing potassium-rich sea water (KSW) flowed in the right-hand pool. The normal temperature at the central pool was 7.5°C. Test solutions were flowed through the central pool in place of ASW. The test solutions used were ASW with acridine orange dissolved in it (dye-SW); ASW with acridine orange and 10^{-6} M tetrodotoxin (TTX) dissolved in it (dye-TTX SW); and sea water with one tenth the usual concentration of calcium (2.5 mM instead of 25 mM) and acridine orange dissolved in it (dye-low Ca^{++} SW). Unless otherwise noted the concentration of acridine orange was 0.01% or 0.015% (g/100 ml), and was of unknown purity.

The illumination system consisted of a 750 w projection bulb (code EDK) mounted within an air-cooled housing plus a lens system to focus the light onto the region of axon within the central pool of the chamber. An infrared heat filter (Corning Glass Works, Corning, N. Y., glass #4600) was attached to the bottom of the housing. White light was used for all experiments. When the light was not on, the axon remained in dim illumination. The temperature rise resulting from illumination was measured with a tiny thermistor set in the central pool gap and was found not to exceed 1°C during prolonged illumination.

Following the establishment of excitability, the normal experimental procedure was to flow dye-SW through the central pool for 3 min and then flow ASW through for another 2–3 min. This left the axon region in the gap slightly tinted and the central pool solution

almost free of color. No dye escaped into the side pools. Electrical measurements were then made on the axon and then again during and/or following 15–30 sec of illumination. In some initial control experiments and on a few other occasions the illumination period extended up to 1 min. Measurements of current, voltage, and voltage derivative were displayed on oscilloscopes and recorded on film. Current-voltage curves were obtained by subtracting estimated leakage current from total current at each potential and then plotting the peak transient and delayed steady-state currents as a function of voltage. Leakage was estimated by extrapolating its value from measured values for small voltage changes from the holding potential. Leakage current was observed to follow the form described by Moore et al. (1966).[1]

RESULTS

Control Experiments

The possible effects of dye alone or light alone were tested by keeping axons in voltage clamp for 4–8 min at a time and pulsing the membrane to the potential of maximum obtainable transient current every 2 sec. Transient current normally decayed slowly during these times,[2] but was not affected by illumination periods of up to 1 min or dye application periods of up to 3.7 min. No effect on action potentials could be seen from light or dye alone either. No attempt was made to determine the minimum dye exposure time necessary to sensitize the axon.

Action Potentials

Illumination following dye exposure resulted in drastic changes in the shape of electrically-stimulated action potentials. The falling phase of the spike became progressively delayed such that a cardiac-like plateau formed between the rising and falling phases. At the same time the threshold for excitation went up and the resting potential decreased. These changes are illustrated in Fig. 1, which shows a series of seven superimposed electrically-stimulated action potentials. Stimulation occurred every 2 sec and illumination was continuous after the first spike. The maximum rates of rise and fall also decreased progressively during illumination. The changes in

[1] While Hodgkin and Huxley originally called the components of membrane current sodium, potassium, and leakage, the data in this paper is in terms of transient, steady state, and leakage. Transient current is a label to name the current which flows through the normally transiently open pathway and which is normally carried inward by sodium ions. The label also applies when the current is not inward and when it is not carried by sodium. Steady-state current is a label to name the current which flows through the normally slowly developing pathway and which is normally carried outward by potassium ions. The label applies when the current has not reached the steady state and also when the current is not outward and when it is not carried by potassium.

[2] It should be noted that isolated lobster axons in sucrose gap are difficult to keep excitable for extended periods of time (compared with squid axons). The functional time for any one area brought into the central pool did not exceed 30 min in these experiments. During this time the resting potential normally fell slowly and the threshold rose slowly. Poor condition axons decayed faster than this and it was frequently observed that apparently good condition axons suddenly depolarized by variable amounts. In such cases leakage current always increased too. These sudden depolarizations were unrelated to experimental conditions and were most likely a result of sudden changes in the sucrose gap configuration around the axons. Data are not included from axons in which it appeared that this occurred.

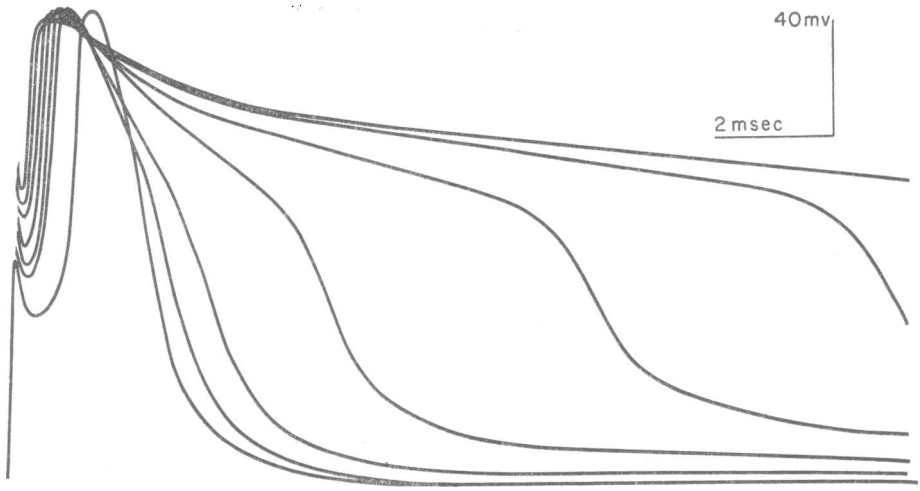

FIGURE 1 Membrane action potentials before and during illumination after exposure to acridine orange (tracings of oscilloscope photographs). Action potentials stimulated electrically every 2 sec. Illumination applied continuously after first action potential. The progressive increase in level of depolarization from the stimulus reflects a progressive fall in resting potential.

action potentials were essentially irreversible and persisted following the end of illumination for as many minutes as the axon remained excitable. The duration usually reached a few milliseconds during 20 sec of illumination, and in some cases the duration reached more than 150 msec. Table I summarizes the results obtained on eight axon areas. The average decrease in maximum rate of rise was 69% and the average decrease in maximum rate of fall was 57%.

TABLE I

MAXIMUM ACTION POTENTIAL RISING AND FALLING PHASES BEFORE AND AFTER ILLUMINATION IN ACRIDINE ORANGE COLORED AXONS

		Rising phase			Falling phase		
Illumination time	Dye concentration	Before light	After light	After/before	Before light	After light	After/before
sec	%	*v/sec*	*v/sec*		*v/sec*	*v/sec*	
60	0.001	520	120	0.23	95	56	0.59
60	0.001	464	104	0.22	80	48	0.60
20	0.015	705	160	0.23			
20	0.015	400	104	0.26	80	24	0.30
15	0.015	344	80	0.23	80	21	0.26
30	0.015	513	256	0.50	106	48	0.47
15	0.015	575	320	0.56	118	32	0.27
20	0.015	386	99	0.26	124	64	0.51
Average		488	155	0.31	98	42	0.43

The duration increase was always accompanied by the threshold increase. Illumination for much more than 30 sec, when the dye concentration was 0.015%, usually resulted in complete and permanent inexcitability. For axons in poor condition inexcitability was often produced before very much duration increase was produced. For axons in good condition maximum duration increase occurred after about 20 sec illumination. Illumination beyond 20 sec usually decreased the amount of elongation.

The effect of dye and illumination on resting potentials was not studied systematically because the sucrose gap method is not well suited for that purpose; it was observed, however, that the axon always depolarized during illumination by amounts up to 40 mv. This is in face of continual hyperpolarizing effect of sucrose.

Transient Current in Voltage Clamp

Illumination following dye exposure produced dramatic changes in transient current. At any one potential the transient current was markedly reduced in amplitude, the rate of turning on was decreased, and the rate of turning off was very much decreased. In a normal axon the transient current is inactivated by the time appreciable steady-state current flows, but after dye-illumination transient current continued to flow well into the time that steady-state current flowed, making it impossible to separate the two in time. This is illustrated in Fig. 2, which shows currents during a pulse to −10 mv before and after illumination. Notice in this figure that the post-illumination net membrane current after 8 msec is inward, while the pre-illumination current is outward after about 2.5 msec. The continued inward current results from a slowing in transient current inactivation and is the main reason for the production of prolonged action potentials.

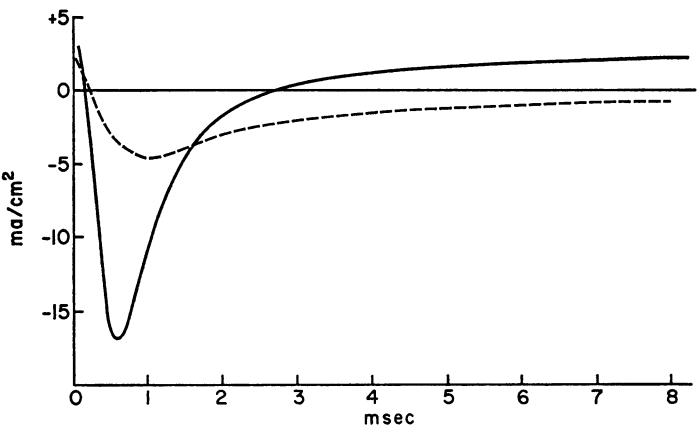

FIGURE 2 Current in voltage clamp before and after illumination after exposure to acridine orange (tracings of oscilloscope photographs). Axon held at −100 mv and pulsed to −10 mv. Solid curve before and dashed curve after illumination.

TABLE II

PARAMETERS OF TRANSIENT CURRENT BEFORE AND AFTER ILLUMINATION IN ACRIDINE ORANGE COLORED AXONS

		Maximum slope transient conductance			Time to peak I_{tr} at +20 mv			Membrane potential at peak I_{tr}		
Illumination time	Dye concentration	Before light	After light	After/before	Before light	After light	After/before	Before light	After light	After minus before
sec	%	*mmho/cm²*	*mmho/cm²*		*msec*	*msec*		*mv*	*mv*	*mv*
30	0.015	197	125	0.635	0.2	0.35	1.75	−20	0	+20
35	0.015	95	77	0.815				−20	−10	+10
35	0.015	75	37	0.49				−30	−14	+16
	0.02*	199	109	0.55	0.22	0.32	1.45	−17	−3	+14
25	0.01	178	52.5	0.294				−10	0	+10
25	0.01	136	77.5	0.57				− 5	+10	+15
30	0.01	315	153	0.486				0	+15	+15
25	0.01	107	52	0.485	0.27	0.9	3.34	−20	+5	+25
60	0.001	160	55	0.343				−20	0	+20
60	0.001	250	87	0.35				−13	0	+13
60	0.001	286	178	0.62				−10	+5	+15
15	0.015	152	81	0.53	0.22	0.4	1.8	−20	0	+20
20	0.015	238	93	0.39	0.28	0.55	2.0	−20	0	+20
20	0.015	170	91.5	0.54	0.22	0.5	2.3	−20	0	+20
20	0.015	190	100	0.53	0.22	0.55	2.5	−20	0	+20
20	0.015	163	71.6	0.44	0.25	0.6	2.4	−25	−10	+15
30	0.015	260	157	0.60	0.26	0.45	1.7	−10	+5	+15
35	0.015	109	64	0.50	0.28	0.5	1.8	−20	+3	+23
Average		182	92	0.51	0.24	0.51	2.1	−16.6	+0.3	+17

* Methylene blue.

The turn on of transient current was slowed, although not so much as inactivation. At a potential of +20 mv the time to peak transient current was usually more than doubled. The results on 10 axon areas are shown in Table II. The curve relating time to peak transient current vs. voltage, illustrated in Fig. 3, was shifted to more positive potentials by variable amounts up to 40 mv.

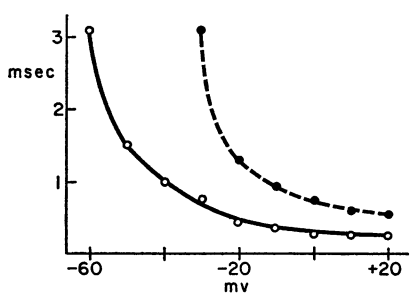

FIGURE 3 Time to peak transient current as a function of voltage before and after illumination after exposure to acridine orange. Solid curve is before and dashed curve is after illumination.

The current-voltage relation for peak transient current was also altered. The potential at which maximum transient current could be obtained under normal conditions was near −20 mv, while following dye and illumination it was about 0 mv. This shift is illustrated in Fig. 4 and tabulated in Table II.

Fig. 5 also shows the reduction of current at all potentials. The maximum positive slope conductance was typically decreased by about 50%. Table II summarizes the results on 18 axon areas.

At potentials near E_{tr} (the potential at which peak transient current reversed sign) it became difficult, and sometimes impossible, to read the transient current records. It appeared from all experiments, nevertheless, that E_{tr} was reduced by a few millivolts following illumination.

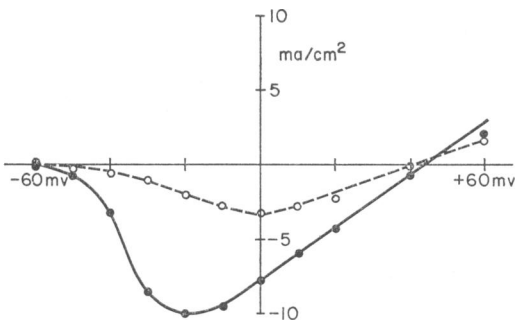

FIGURE 4 Peak transient current as a function of voltage before and after illumination after exposure to acridine orange. Solid curve is before and dashed curve is after illumination.

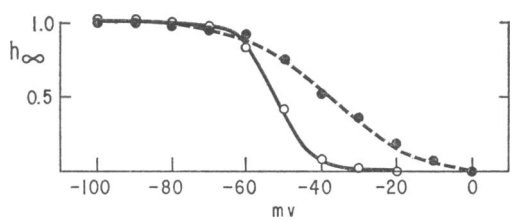

FIGURE 5 Transient current inactivation as a function of voltage before and after illumination after exposure to acridine orange. Open circles are data points before illumination and filled circles are data points after illumination. Smooth curves are equation 1 fitted to the data points.

An additional set of experiments was performed in order to detect any changes in the transient current inactivation (h_∞) curve. These were carried out using the standard double pulse technique described by Hodgkin and Huxley (1952*a*). The membrane was held hyperpolarized between pulses, pulsed to various depolarized levels by a conditioning pulse of 100 msec duration, and pulsed by a test pulse to the potential at which maximum transient current could be obtained. Runs were made just before and then following 15–30 sec of illumination. After illumination the test pulse was adjusted to the new potential of maximum transient current. The results are illustrated in Fig. 5 and tabulated in Table III. Fig. 5 shows that the curve was flattened somewhat and about 30 mv additional depolarization beyond the normal case was needed to bring about complete inactivation. This was partly a result of a shift of the curve along the voltage axis, and partly the result of flattening. The fact

that inactivation was complete following a 100 msec depolarizing pulse indicates that the apparent continued transient current illustrated in Fig. 2 is a slowly decaying and not a truly steady value.

The inactivation curve (Fig. 5) may be described by equation 1 (Chandler, Hodgkin, and Meves, 1965).

$$h_\infty = \frac{1}{1 + \exp \frac{(V_m - V_h)}{k}} \tag{1}$$

where V_m = membrane potential, V_h = membrane potential at which $h_\infty = 0.5$, and k = slope factor.

TABLE III

EXPERIMENTAL VALUES FOR V_h AND k BEFORE AND AFTER ILLUMINATION IN ACRIDINE ORANGE COLORED AXONS

		V_h			k		
Illumination time	Dye concentration	Before light	After light	After minus before	Before light	After light	After/before
sec	%	*mv*	*mv*	*mv*	*mv*	*mv*	
20	0.015	−57	−50	+7	6.75	9.75	1.44
30	0.015	−53	−43	+10	3.75	12.0	3.2
20	0.015	−51	−43	+8	5.5	13.5	2.45
30	0.015	−39	−35	+4	4.5	10.25	2.28
30	0.015	−50	−44	+6	6.5	11.0	1.69
30	0.015	−35	−34	+1	4.75	11.5	2.42
20	0.015	−61	−55	+6	8.25	13.75	1.67
30	0.015	−42	−36	+6	8.0	13.5	1.69
30	0.015	−55	−29	+26	5.25	15.0	2.86
15	0.015	−55	−48	+7	4.75	13.5	2.84
30	0.015	−52	−38	+14	4.82	11.0	2.28
30	0.015	−54	−35	+19	4.5	12.2	2.71
Average		−50.3	−40.8	+9.5	5.73	12.55	2.3

In Fig. 5 this equation was fitted to the data points. V_h was shifted 14 mv in the positive direction and k was increased by a factor of 2.28 in this case. Table III shows for all experiments that the average shift of V_h was +9.5 mv and the average increase in k was a factor of 2.3.

In order to ensure that inactivation reached its final value during the conditioning pulse, several runs were made with a conditioning pulse duration of more than 1 sec. This did not change the results.

Steady-State Current in Voltage Clamp

It had been observed during the study of transient current that steady-state current was reduced following dye and illumination. Systematic observations were difficult,

TABLE IV

PARAMETERS OF STEADY-STATE CURRENT BEFORE AND AFTER ILLUMINATION IN TTX TREATED ACRIDINE ORANGE COLORED AXONS

Illumination time	Dye concentration	Maximum slope steady-state conductance			Time to ½ maximum I_{ss} at +60 mv			Membrane potential at intercept of linear slope conductance with zero current axis		
		Before light	After light	After/ before	Before light	After light	After/ before	Before light	After light	After minus before
sec	%	*mmho/ cm²*	*mmho/ cm²*		*msec*	*msec*		*mv*	*mv*	*mv*
30	0.013	66	34	0.52	1.2	1.9	1.6	−34	−22	+12
30	0.013	25	13	0.52	1.6	1.9	1.2	−14	−12	+2
30	0.013	64	42	0.65				−23	+12	+35
30	0.013	197	143	0.73	0.9	1.9	2.1	−36	−19	+17
30	0.01	246	234	0.95				−46	−44	+2
30	0.01	111	113	1.01				−53	−37	+16
30	0.01	49	43	0.88				−65	−60	+5
30	0.015	38	33	0.87	1.4	4.2	3.0	−56	−12	+44
60	0.015	44	29	0.66	1.2	2.6	2.2	−72	−57	+15
30	0.015	54	24	0.45	1.5	5.1	3.4	−58	−34	+24
20	0.015	47	37	0.79				−32	+8	+40
20	0.015	40	26	0.65				−36	−28	+6
Average		82	64	0.72	1.3	2.9	2.2	−44	−25	+18

however, because steady-state current turned on before transient current was inactivated. This difficulty was obviated by blocking transient current with a relatively high concentration (10^{-6} M) of TTX. TTX has been shown to block transient current without affecting steady-state current (Narahashi, Moore, and Scott, 1964; Moore et al., 1967). In these experiments TTX was applied together with the dye before illumination. This concentration blocked transient current completely and allowed measurements of steady-state current free from transient current.

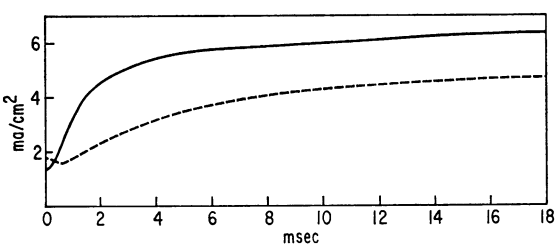

FIGURE 6 Current in voltage clamp before and during illumination after exposure to acridine orange and TTX (tracings of oscilloscope photographs). Axon held at −100 mv and pulsed to +60 mv. Solid curve is before and dashed curve is during illumination.

Following dye and illumination, steady-state current was reduced in magnitude and its time course of activation was slowed. A decrease in maximum slope conductance of 28% was found and the time to half maximum current at a potential of +60 mv was increased by a factor of 2.2. Table IV summarizes the results on 12 axon areas. Fig. 6 shows currents at +60 mv before and after illumination after exposure to dye-TTX SW. The decrease in maximum current and rate of activation are typical.

The current-voltage relation for steady-state current was shifted to more positive potentials by 18 mv. This change is shown in Fig. 7.

The effects of illumination on dye-treated axons appear to affect steady-state current in a manner similar to the effect on transient current.

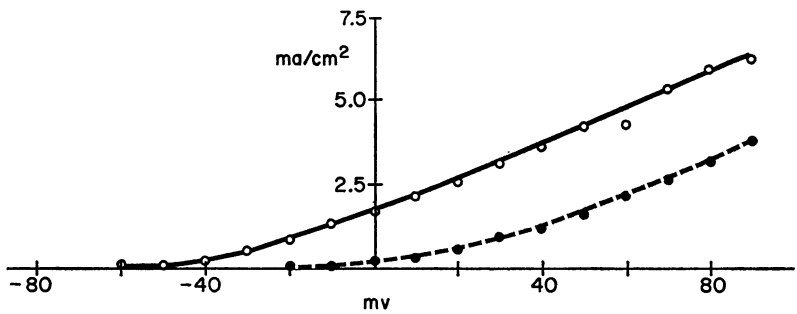

FIGURE 7 Maximum steady-state current as a function of voltage before and after illumination after exposure to acridine orange and TTX. Solid curve is before and dashed curve is after illumination.

Low Calcium

The effects of dye-illumination are similar in many respects to those produced by an increased calcium concentration in the bathing medium (Frankenhaeuser and Hodgkin, 1957). It seemed likely, therefore, that a decreased calcium concentration after dye-illumination might tend to offset some of the changes resulting from light; this is precisely what was found.

For these experiments, in which only one input to the central pool was available, it was necessary to apply dye and the low calcium medium together. The procedure followed was to bathe the axon in dye-low Ca^{++} SW for several minutes, then wash in ASW for several minutes, illuminate, then without further illumination bathe in dye-low Ca^{++} SW again, and finally bathe in ASW again.

Following illumination, the previously described changes in behavior were noted. Subsequent exposure of the altered axon to dye-low Ca^{++} SW without further illumination partially counteracted some of the light-induced changes. In particular, the voltage at which maximum transient current could be obtained, which had been shifted to more positive potentials by light, was shifted back to within 5 mv of the pre-illumination level. The time to reach peak transient current at +20 mv, which

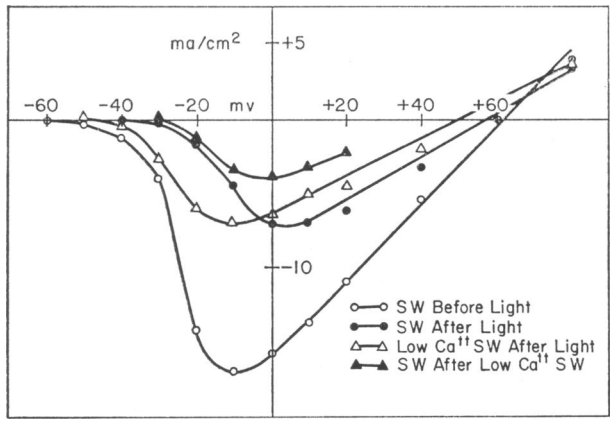

FIGURE 8 Transient current in voltage clamp after acridine orange—before illumination, after illumination, in low Ca^{++}, and in ASW.

had been doubled by illumination, was decreased by about 10% of the doubled value in the low Ca^{++}.

Fig. 8 shows transient current vs. voltage curves in ASW before and after illumination, then in dye-low Ca^{++} SW, and then in ASW again. Notice that exposure to dye-low Ca^{++} SW shifts the voltage of maximum transient current, but does not change the magnitude of maximum obtainable current. The decrease in current in the final ASW exposure probably reflects deterioration of the preparation.

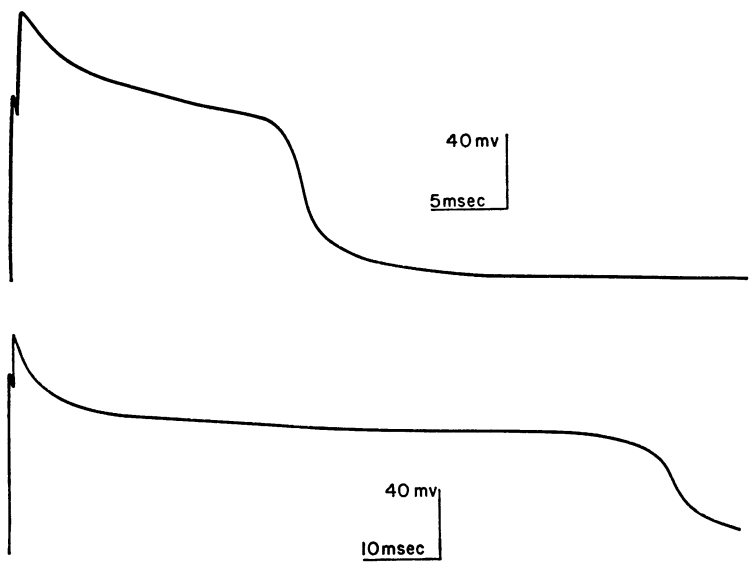

FIGURE 9 Membrane action potentials after exposure to acridine orange and illumination—before and in low Ca^{++} (tracings of oscilloscope photographs). Upper curve is before and lower curve is in low Ca^{++}.

It was also found that dye-low Ca^{++} SW produced an additional prolongation of the already prolonged action potentials. This is illustrated in Fig. 9 (notice the two time scales in this figure), which shows action potentials after illumination in ASW, and then in dye-low Ca^{++} SW. The low calcium effect appeared to be reversible, but was difficult to explore because the axons tended to deteriorate in the time necessary to complete all the solution changes.

In a few cases (see Tables I and II), a dye concentration of 0.001 was used and the illumination time was increased to 60 sec. Qualitatively, it appeared that decreases in concentration were compensated for by increases in illumination time.

Other Dyes

Some preliminary studies with other dyes such as eosin, methylene blue, and neutral red indicate that the effects with these dyes are identical to those obtained with acridine orange. One of these cases is included in Table II.

DISCUSSION

In the present experiments dye-illumination resulted in three kinds of effects: (*a*) decrease in over-all conductance to transient and steady-state current, (*b*) shift of the voltage-dependent parameters for transient and steady-state conductance to more positive internal potentials, (*c*) slowing of the time course for transient current inactivation.

The prolonged action potentials may be seen as primarily a result of a slowing in transient current inactivation. While the observed decrease in steady-state conductance should contribute to action potential prolongation, the magnitude of decrease would only slightly slow the falling phase of the spike. On the other hand, the continued flow of transient current would keep the membrane at a depolarized level for as long as transient current conductance remained high.

The development of the slowing in transient current inactivation was always accompanied by the decrease in maximum transient current conductance, which tended to make the membrane inexcitable. With axons in exceptionally good condition an action potential duration exceeding 100 msec could be produced, but with the usual leaky axons the extent of duration increase was most often limited to a few milliseconds.

The high calcium-like effects of dye-illumination are quite striking. The shifts in the conductance-voltage relations and the turn on kinetics for both transient and steady-state current were in the order of almost 20 mv in the depolarizing direction. This is equivalent to the shift which would result from raising external calcium by a factor of more than ten (Frankenhaeuser and Hodgkin, 1957; Julian, Moore, and Goldman, 1962*b*; Blaustein and Goldman, 1966). The fact that a tenfold lowering of external calcium following illumination partially counteracted these shifts confirms the high calcium-like effects of dye-illumination.

The large scale depressions of over-all conductance and large increase in the time

constant for inactivation cannot be duplicated by high calcium and were not counteracted by the tenfold lowering of calcium. This demonstrates that dye-illumination has additional effects and suggests that it may act at several sites on the membrane. Narahashi and Haas (1968) have proposed that agents which inhibit inactivation may act on the inside of the membrane and those which decrease transient conductance may act at the outside. An interesting experiment in this regard would be to apply the dye to the inside of the membrane with internal perfusion.

The irreversibility of effects suggests a permanent change in the membrane structures responsible for ionic conductance mechanisms, as opposed to Chalazonitis' (1964) proposal of parallel photoconductive or photovoltaic events which in turn lead to changes in the voltage-sensitive structures through changes in membrane potential. This is further suggested by the ability of the altered membrane to interact with calcium changes in the period following illumination.

Possibly relevant to this point are some studies by Forgacs and Stein (1965) on permselective artificial membranes in which they obtained a slight photoconductivity upon illumination of naturally-colored membranes, but no photoconductivity on illumination of dye-treated uncolored membranes.

The present results are similar in many respects to the results of Lyudkovskaya's early experiments (Liudkovskaia and Kaiushin, 1959; Lyudkovskaya and Kayushin, 1960), but appear to differ quite markedly from his later results (Lyudovskaya, 1961) and from those of Chalazonitis (Chalazonitis, 1954; Arvanitaki and Chalazonitis, 1961; Chalazonitis and Chagneux, 1961). Grouped together, the somewhat confusing results of Chalazonitis and Lyudkovskaya fall into two types: (*a*) light-induced changes in the shape of electrically-stimulated action potentials without direct excitation by light, and (*b*) light-induced oscillatory potentials and multiple spikes without the necessity of electrical stimulation. Changes in the shape of electrically-stimulated action potentials were found by Lyudkovskaya on squid and on sepia when both were bathed in sea water. These results appear to reflect the same kind of light-induced changes that were obtained on lobster in the present experiments. Oscillatory potentials and multiple spikes without electrical stimulation were obtained by Lyudkovskaya on sepia and by Chalazonitis on sepia when the axons were pretreated with calcium chelating agents such as sodium citrate, which were applied in order to increase the excitability of the preparation (Chalazonitis, 1954; Lyudovskaya, 1961). Although the second kind of result appears to be different from those obtained on lobster, consideration of the combined effects of light and decalcification indicates that illumination was probably having the same effect on the membrane in both cases. Partially decalcified sepia and squid axons are quite unstable and tend to oscillate and/or fire repetitively (Arvanitaki, 1939; Brink and Bronk, 1941; Brink, Bronk, and Larabee, 1946; Frankenhaeuser and Hodgkin, 1957; Huxley, 1959). Any depolarizing action on such membranes tends to increase oscillations which grow into spikes. This is precisely what Chalazonitis found, whether the depolarization was produced by illumination without electrical stimulation or by

long-duration current pulses. Lyudkovskaya also found this effect with illumination as well as rather complex interactions between light and short-duration electrical stimuli. If the difference between the two kinds of results is not accounted for by decalcification then one is left wondering how Lyudkovskaya could obtain two such differing effects under otherwise identical conditions on the same preparation. Decalcification of axons from the lobster circumesophageal connective rarely leads to oscillations and repetitive activity (Dalton, 1958); in sucrose gap the hyperpolarization would tend to prevent their occurrence, as would the low membrane resistance (Tomita and Wright, 1965).

Chalazonitis also found that illumination beyond the point necessary to elicit oscillations and spikes gave rise to a depolarized plateau; this is consistent with the lobster data. Thus the apparently divergent results obtained by Chalazonitis, Lyudkovskaya, and the author may all reflect a common action at the membrane, if one takes into account the complicating effects of decalcification.

Chalazonitis and Lyudkovskaya also found that photodepolarization and oscillations were reversible if the illumination times were not more than a few seconds. They may have seen reversible effects on sepia which went unnoticed on the leaky high-threshold lobster axons and which became overridden by the irreversible effects at longer illumination times. It might be informative to illuminate a sensitive axon (not in sucrose gap) with a very high intensity flash and compare results following a lower intensity and much longer illumination.

None of the changes in ionic behavior described in this paper are unique: there are many ways in which the present behavior can be duplicated qualitatively. The depressions of the over-all transient and steady-state conductance are similar to the depressions following treatment with several anesthetic drugs (e.g. Blaustein, 1968). The shifts of the voltage-dependent parameters are similar to the shifts following addition of calcium or several other polyvalent cations (e.g. Blaustein and Goldman, 1968). And there are several very different means of effecting an increase in the time constant for inactivation, including perfusion with low ionic strength solutions (Adelman, Dyro, and Senft, 1965), treatment with some venoms and poisons (Koppenhöffer and Schmidt, 1968; Narahashi and Haas, 1968; Hille, 1968), and perfusion with sodium fluoride (Chandler and Meves, 1966) or cesium (Adelman and Senft, 1966).

The ultimate importance of these various studies of altered behaviors lies in what they imply about molecular mechanisms, and it is tempting to speculate on the molecular basis for dye-illumination. The present experiments, however, are of a somewhat preliminary sort and were intended only to describe ionic events during dye-illumination. Since there are still unanswered questions about ionic events and since there are so many obvious realizable experiments yielding information relevant to molecular events (e.g. action spectrum, pH dependence, etc.), it seems premature to speculate further on molecular mechanisms without more data. It is hoped that

future information revealed by the dye-illumination method in conjunction with the present data may offer new implications not given by previous experiments with drugs and altered ionic media.

COMPUTATIONS

One very convenient means of classifying changes induced in nerves by experimental conditions is to describe the changes in the Hodgkin-Huxley (H-H) equation parameters necessary to simulate the experimentally-induced behavior (Hodgkin and Huxley, 1952*b*). The present experiments constitute a good example.

A slightly modified version of an analog computer program of the H-H equations written by J. W. Moore was used for the present computations on an Electronic Associates TR-48 computer (Electronic Associates, Inc., Long Branch, N. J.). Three changes were made in H-H parameters to reduce some of the differences between the H-H computations and present lobster data. The value for $\bar{g}_K$ was de-

TABLE V

PARAMETERS IN H-H EQUATION COMPUTATIONS

	Squid (original H-H)	Lobster control	Lobster after light	Lobster after light in low Ca^{++}
$\bar{g}_{Na}$, *mmho/cm²*	120	120	37.7	37.7
$\bar{g}_K$, *mmho/cm²*	36	14.1	9.2	9.2
$\bar{g}_l$, *mmho/cm²*	0.3	4.52	4.52	4.52
V_1, *mv*	−10.6	0	0	0
τ_n multiplier	1	1	1.8	1.8
τ_h multiplier	1	1	100	100
α and β shift, *mv*	0	0	−30	−15

creased and the value of $\bar{g}_l$ was increased so that the ratios of leakage, steady-state, and transient conductances equaled the values in the lobster data. And V_l was changed to 0 mv (using the H-H convention for voltage) so that the leakage current would be zero at the resting and holding potential of 0 mv. These changes were not an attempt to rewrite the equations for lobster, but served to bring about a closer match between computations and data.[3]

After making the above three changes, curves of voltage-clamp current and current-clamp voltage were plotted. These curves served as the controls. Then changes were made in some H-H parameters to simulate the data of light-induced effects. The data to be duplicated were the following: decrease of maximum transient current slope conductance by 49%; decrease of maximum steady-state slope conduct-

[3] In the normal H-H equations this results in a reversal of transient current 3 mv below the reversal potential for sodium current, and with $\bar{g}_{Na}$ reduced by 75%, the reversal is 12 mv below the sodium current reversal.

ance by 28%; shift of potential of maximum transient current to more depolarized levels by 18 mv; increase in time to reach half maximum steady-state current at −125 mv by a factor of 2.2; increase in time to reach peak transient current at −75 mv by a factor of 2; shift of potential at the intercept of the linear portion of the steady-state current vs. voltage curve to more depolarized levels by 18 mv; increase in threshold for action potentials; and elongation of action potentials to qualitatively resemble those in the lobster data. H-H parameters were adjusted on an "informed guesswork" basis so as to match the above changes. The specific changes in the H-H parameters necessary to result in the above changes are given in Table V.

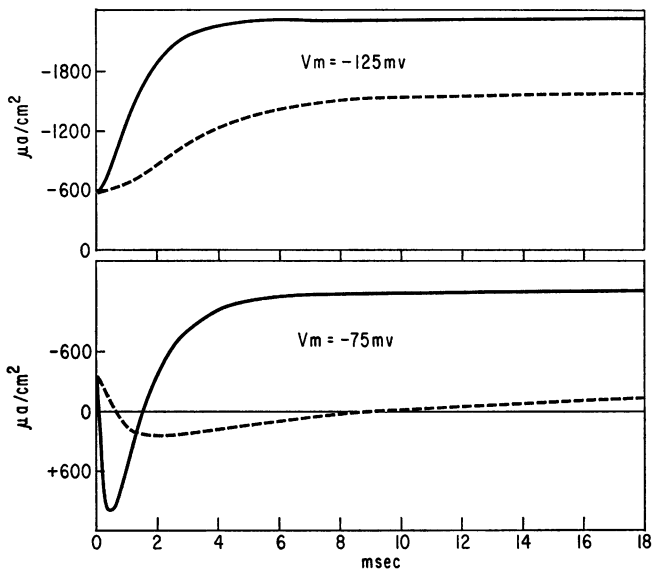

FIGURE 10 Computed voltage clamp currents. Upper figure at −125 mv with simulated TTX application. Lower figure at −75 mv. Solid curves are normals and dashed curves are simulated after dye and illumination.

The results of these changes may be seen in Figs. 10 and 11. Fig. 10 shows I_m before and after simulated light-induced changes at −75 mv and −125 mv (using H-H convention for voltage). At −125 mv $\bar{g}_{Na}$ was set to zero to simulate a condition of TTX application. The curves in Fig. 10 should be compared with the curves in Figs. 2 and 6, which show the lobster data. The data and computations show a relatively good qualitative agreement.

The upper curves in Fig. 11 show H-H action potentials before and after simulated light-induced changes. The lower curve in Fig. 11 shows an H-H action potential after simulated light-induced changes and with a simulated 10 times reduction in external calcium. The reduction in external calcium was programmed as Huxley (1959) did, by shifting the α's and β's for m, n, and h towards more negative internal potentials. In this case the shift was 15 mv. This prolonged the already prolonged

action potentials even more, as it did on the lobster axon. The computed action potentials in Fig. 11 bear a close resemblance to the lobster data shown in Fig. 8. The 15 mv low calcium shift is in addition to the 30 mv shift in the other direction, so that the net shift is 15 mv in the depolarizing direction.

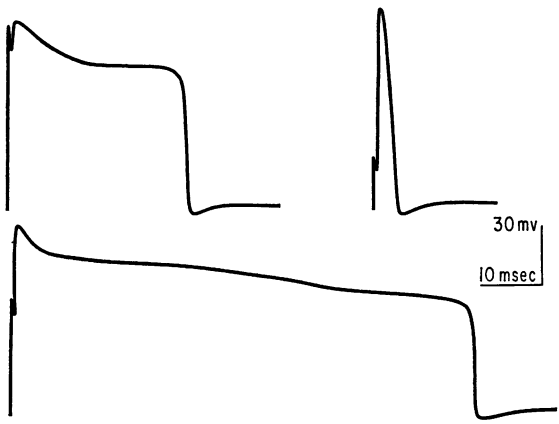

FIGURE 11 Computed action potentials. Upper right—normal. Upper left—simulated after dye and illumination. Lower—simulated after dye and light in low calcium.

Considering the difference between the normal H-H equations and normal lobster data, it is remarkable that the H-H equations could simulate so closely the light-induced effects. And had the computations been done before the low calcium experiments, they could have been used to predict the results. The low calcium shift in combination with the simulated light-induced effects produced simulated action potentials which were considerably more prolonged than those from light-induced effects alone. This result follows directly from the equations but was not anticipated at the time of the experiments until it was actually observed.

This research was carried out while the author was fullfiling the requirements for the Ph.D. degree at Duke University and while he was a postdoctoral trainee in the Duke University Research Training Program in the Sciences Related to the Nervous System. It was supported by funds from U.S.P.H.S. Fellowship A1-F1-GM-332-01A1 and from N.I.M.H. Training Grant 08394. The author wishes to thank Dr. J. W. Moore for suggesting the research area and for guidance throughout. He also thanks Drs. M. Tarr and T. Narahashi for critical readings of the manuscript.
A preliminary report of this work appeared in the Biophysical Society Abstracts, 1968.

Received for publication 15 March 1968 and in revised form 4 June 1968.

REFERENCES

ADELMAN, W. J., JR., F. M. DYRO, and J. P. SENFT. 1965. *J. Cellular Physiol.* **66**(Suppl. 2):55.
ADELMAN, W. J., JR., and J. P. SENFT. 1966. *J. Gen. Physiol.* **50**:279.
ARVANITAKI, A. 1939. *Arch. Intern. Physiol. Biochim.* **49**:209.
ARVANITAKI, A., and N. CHALAZONITIS. 1961. *In* Nervous Inhibition. E. Florey, editor. Pergamon Press, New York. 194.
BLAUSTEIN, M. P. 1968. *J. Gen. Physiol.* **51**:293.
BLAUSTEIN, M. P., and D. E. GOLDMAN. 1966. *J. Gen. Physiol.* **49**:1043.

BLAUSTEIN, M. P., and D. E. GOLDMAN. 1968. *J. Gen. Physiol.* **51**:279.
BLUM, H. F. 1932. *Physiol. Rev.* **12**:23.
BRINK, F., and D. W. Bronk. 1941. *Am. J. Physiol.* **133**:222.
BRINK, F., D. W. BRONK, and M. G. LARABEE. 1946. *Ann. N. Y. Acad. Sci.* **47**:457.
CHALAZONITIS, N. 1954. *Thèse Sciences, Paris.* 1.
CHALAZONITIS, N. 1964. *Photochem. Photobiol.* **3**:539.
CHALAZONITIS, N., and R. CHAGNEUX. 1961. *Bull. Inst. Oceanog.* **58(1223)**:1.
CHANDLER, W. K., A. L. HODGKIN, and H. MEVES. 1965. *J. Physiol. (London).* **180**:821.
CHANDLER, W. K., and H. MEVES. 1966. *J. Physiol. (London).* **186**:121 P.
DALTON, J. C. 1958. *J. Gen. Physiol.* **41**:529.
FORGACS, C., and G. STEIN. 1965. *J. Phys. Chem.* **69**:4221.
FRANKENHAEUSER, B., and A. L. HODGKIN. 1957. *J. Physiol. (London).* **137**:217.
HILLE, B. 1968. *J. Gen. Physiol.* **51**:199.
HODGKIN, A. L., and A. F. HUXLEY. 1952*a*. *J. Physiol. (London).* **116**:497.
HODGKIN, A. L., and A. F. HUXLEY. 1952*b*. *J. Physiol. (London).* **117**:500.
HUXLEY, A. F. 1959. *Ann. N. Y. Acad. Sci.* **81**:221.
JULIAN, F. J., J. W. MOORE, and D. E. GOLDMAN. 1962*a*. *J. Gen. Physiol.* **45**:1195.
JULIAN, F. J., J. W. MOORE, and D. E. GOLDMAN. 1962*b*. *J. Gen. Physiol.* **45**:1217.
KOPPENHÖFER, E., and H. SCHMIDT. 1968. *Experientia.* **24**:41.
LIUDKOVSKAIA, R. G., and L. P. KAIUSHIN. 1959. *Biophysics.* **4(4)**:23.
LYUDKOVSKAYA, R. G., and L. P. KAYUSHIN. 1960. *Biophysics.* **5(6)**:754.
LYUDOVSKAYA, R. G. 1961. *Biophysics.* **6(3)**:36.
MOORE, J. W., N. ANDERSON, M. BLAUSTEIN, M. TAKATA, J. Y. LETTVIN, W. F. PICKARD, T. BERNSTEIN, and J. POOLER. 1966. *Ann. N. Y. Acad. Sci.* **137**(Art. 2):818.
MOORE, J. W., M. P. BLAUSTEIN, N. C. ANDERSON, and T. NARAHASHI. 1967. *J. Gen. Physiol.* **50**: 1401.
NARAHASHI, T., and H. G. HAAS. 1968. *J. Gen. Physiol.* **51**:177.
NARAHASHI, T., J. W. MOORE, and W. R. SCOTT. 1964. *J. Gen. Physiol.* **47**:965.
TOMITA, T., and E. B. WRIGHT. 1965. *J. Cellular Physiol.* **65**:195.