Neural Modification by Paired Sensory Stimuli

DANIEL L. ALKON

From the Section on Neural Systems, Laboratory of Biophysics, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20014, and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT With repetitive stimulation of two sensory pathways which are intact within the isolated nervous system of *Hermissenda*, features of a cellular conditioning paradigm were identified. Type A photoreceptors, unlike type B photoreceptors, produce fewer impulses in response to light following temporally specific pairing of light stimuli with rotation stimuli. Type A photoreceptor impulse waveforms are also specifically changed by such stimulus regimens. These findings can be explained, at least in part, by increased inhibition of type A cells by type B cells after stimulus pairing.

INTRODUCTION

Exposure to 3 h of light stimuli associated with 3 h of rotation stimuli decreases or eliminates for some hours the movement of the nudibranch mollusc, *Hermissenda crassicornis* toward a light source (Alkon, 1974*b*). Excitation of hair cells by light, normally caused by type A photoreceptor impulses, is reduced in animals exposed to such a regimen (Alkon, 1973*b*, 1975*a*, *b*).

In the work presented here, the light response of type A photoreceptors in isolated nervous system preparations is shown to be changed by temporally specific pairing of light and rotation. Responses of type B photoreceptors, however, are changed slightly, if at all, by such a stimulus regimen.

This approach was made possible by a technique which permits intracellular recording while delivering both visual and statocyst stimuli to the isolated nervous system of *Hermissenda* (Alkon, 1975*c*; Alkon, 1976). With this technique, type A photoreceptor, but not type B photoreceptor, impulse activity during steady-state light responses is abolished by rotation of the nervous system with a specific orientation with respect to the center of the rotating table. This response of type A photoreceptors was demonstrated to arise from synaptic inhibition by hair cells.

The change in type A photoreceptor light responses which will be shown to result from stimulus regimens of light and rotation is a persistent reduction of impulse activity as well as a modification of the impulse amplitude and waveform. The generator response to light is not specifically changed by the same stimulus regimen. The observed changes can account, at least in part, for the previously observed neural correlates of associative training of intact *Hermissenda*.

METHODS

Preparation

Hermissenda were provided by Dr. Rimmon Fay of the Pacific Biomarine Supply Co. (Venice, Calif.) and Mr. Michael Morris of the Peninsula Marine Biological Supply Co. (Monterey, Calif.). Animals were maintained with 6½ h of daily light (cf. Alkon, 1974*b*) in an "Instant Ocean" aquarium at 13°C. The circumesophageal nervous system of *Hermisenda* was dissected and isolated as previously described (cf. Alkon, 1973*a*; Alkon and Bak, 1973; Alkon, 1974*a*).

Type A photoreceptors (Alkon, 1973*a*) were penetrated by placement of the electrode tip in the ventral portion of the eye. That type A photoreceptor in the lateral portion of the eye was used. A silent nonspiking, unresponsive cell, presumably a pigment cell, was almost always penetrated before impalement of the type A photoreceptor.

Type B photoreceptors were penetrated by placement of the electrode tip in the dorsal posterior portion of the eye. Occasionally a silent cell was encountered before a successful type B impalement was made. Unless otherwise indicated all photoreceptors were dark-adapted at least 15 min before light stimuli were given.

Rotation of Circumesophageal Nervous System

The isolated circumesophageal nervous system is placed, immersed in a few drops of seawater, on a conventional microscope glass slide. Two strips of Vaseline, 1 cm long, are placed immediately above and below the preparation. Stainless steel pins are then laid across the connectives of the nervous system. The pins are in direct contact with the surface of the glass slide and are held this way because the ends of the pins are imbedded in the Vaseline strips. A ring of Vaseline (approximately 0.4 cm high and 3 cm in diameter) is then spread around the preparation pinned as described between the two Vaseline strips. A Lucite disc, 0.2 mm thick and 3.5 cm in diameter, is then placed on top of the Vaseline ring after it has been filled with seawater. Numerous holes (1.1 mm in diameter) permit passage of microelectrodes. The advantages of this means of mounting for rotation are discussed elsewhere (Alkon, 1975*c*).

The preparation, ventral side downward and mounted as described above, was placed on the turntable. The central axis of the cephalic end of the circumesophageal nervous system pointed toward the center of rotation.

Unless otherwise specified, rotation of the preparation (13 cm from the center of rotation) was effected by a Garrard turntable (Garrard Co., Swindon-Wiltshire, England, model Zero 92) which had been reinforced by a 0.5-in. aluminum annulus (Fig. 2). All the necessities for intracellular recording were mounted on the turntable including a Lucite stage, a Prior micromanipulator, and an amplifier. Electrical contacts for recording were made via six copper-copper slip rings (Airflyte, Bayonne, N. J.). The velocity of rotation was monitored by measuring the current induced in a stationary coil by a magnet fixed to the rotating table. (This is possible because the current induced is directly proportional to the velocity which which the magnet moves past the coil.) Rotation was begun when 70% of the 110-V electricity source was delivered to the turntable (in an "on" position) via a transformer.

Illumination

Unless otherwise specified, light was provided by 10 G. E. lamps (Type CM 332, 0.2 amp, 6 volt, 1 cd each) spaced evenly on a circle whose center was the center of rotation, 14 cm diameter and 45 cm immediately above the preparation.

Intracellular Recording

As in previous studies, the circumesophageal nervous system was incubated in a solution of a digestive enzyme prior to intracellular recording. In this study a somewhat milder treatment was used: 17 min of incubation in collagenase (type 1, Sigma Chemical Co., St. Louis, Mo.) solution (0.5–1.0 mg/cc).

Intracellular recordings were made with glass micropipettes filled with 4 M potassium acetate (resistance of 60–100 MΩ). The electrode was connected via a silver wire to the input stage of the high impedance amplifier. The reference electrode was a chloride/silver wire. A Wheatstone bridge circuit was used to pass current through the recording electrode. Current was monitored by recording the potential drop across a 10 MΩ resistor in series with the electrode. All experiments were performed at room temperature (approximately 22°C). Details concerning the amplifier used for potential recordings are given in Alkon (1975*c*).

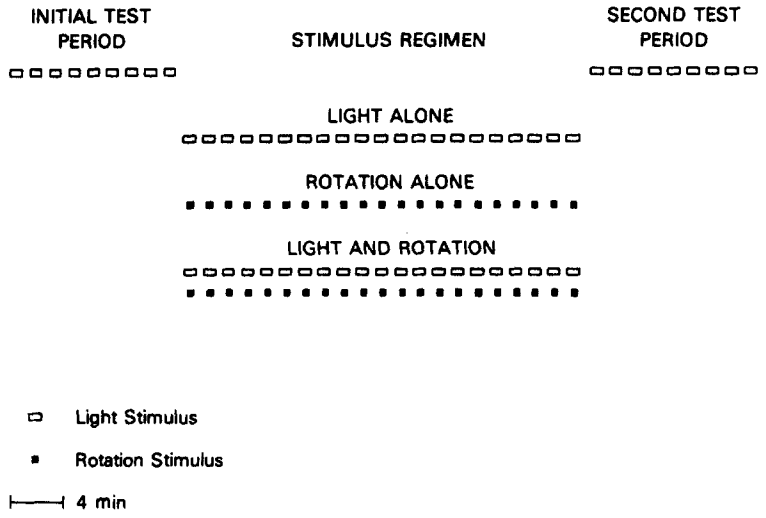

FIGURE 1. Cell treatment. Initial and second test periods include four to eight light steps, each 60 s, at 35 s intervals. Stimulus regimens, each 30 min, consist of light stimuli alone, rotation stimuli alone, or light with rotation stimuli. Other stimulus regimens used are not represented.

Cell Treatment

Photoreceptors were exposed to three distinct treatment intervals (Fig. 1): an initial test period, a stimulus regimen, and a second test period. Unless otherwise specified (see Temporal Specificity under Results) for the initial test period, lateral type A and type B photoreceptors were dark-adapted for 15 min before repeated 60 s light steps at 35 s intervals were begun. Most cells responded with an approximately constant number of impulses after the fourth to eighth light step (Fig. 2). Occasionally a few additional light steps were necessary before such a steady state was reached. The cells were exposed to between four and ten steps of light (at 35 s intervals) before beginning the stimulus regimen. A few photoreceptors were discarded which: (1) never achieved a steady state of impulse response; i.e., progressively fewer impulses occurred during the light response and/or responded with progressively smaller generator potential amplitudes; (2) re-

sponded with less than sixty impulses during the light response and a small generator potential.

The amplitude of the initial response of the photoreceptors following dark adaptation (using 1 cd lights arranged as described under Methods) ranged between 30 and 60 mV. This range approaches the maximum response range previously observed for photoreceptors (Alkon, 1973*a*, Alkon and Fuortes, 1972) using a quartz-iodide light source delivering 2×10^5 ergs cm^{-2} s^{-1} to the eyes.

The initial test period was followed by one of four possible physiologic stimulus regimens (Fig. 1): light stimuli alone, rotation stimuli alone, light with rotation stimuli, or a period of light stimuli followed by a period of rotation stimuli. The first three regimens lasted for 30 min. The last regimen consisted of a 30 min period of light stimuli followed by a 30 min period of rotation stimuli.

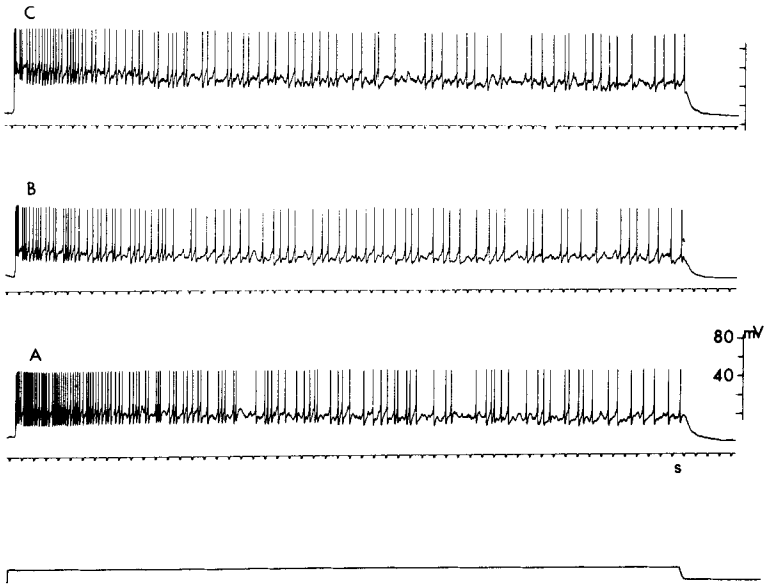

FIGURE 2. Responses to light stimuli alone. A, initial response of a type A photoreceptor to a 60-s light stimulus. B, response of photoreceptor to fourth light step delivered at 35 s intervals. C, response to light step after 30 min of light steps at 35 s intervals. Note the hyperpolarization following the response to the first light step. Note also that the impulse activity in response to the fourth light step is unchanged after 30 min, although the amplitude of the generator potential has increased.

The initial test period was also followed in other experiments, by one of two stimulus regimens: hyperpolarizing current pulses at 95 s intervals, or light stimuli with current pulses. These two regimens lasted for 20 min. Additional details concerning these and other stimulus regimens used will be included under the Results section.

The initial test period and stimulus regimen were followwed by a second test period. This consisted of delivering four or more light stimuli of exactly the same intensity duration and frequency used for the initial test period.

Analysis of Stimulus Regimen Effects

In order to evaluate quantitatively the effects of the stimulus regimens just described, impulse frequency ratios were determined for each cell. The denominator of each ratio was given by the number of photoreceptor impulses occurring in response to the last 10 s

of the light stimulus during the initial test period. This number was the average of two or more, obtained when the photoreceptor responded with an approximately constant number of impulses to each light step. The numerator of each ratio was given by the number of photoreceptor impulses obtained in exactly the same way, but after a stimulus regimen; i.e., during the second test period. Thus, a ratio measures the change in firing frequency of a photoreceptor in response to light during the two test periods, produced by a stimulus regimen.

The ratios, for example, under $R + L$ of Table I compare firing frequencies during photoreceptor responses before and after 30 min of light and rotation stimuli. The ratios under L measure the change in firing frequency of photoreceptors produced by 30 min of light stimuli alone. The ratios under $R + D$ measure the effect on photoreceptor responses of 30 min of rotation stimuli alone.

RESULTS

Experiments were performed to examine the effect on photoreceptor light responses of the physiologic stimulus regimens described above. Other experiments involved intracellular injection of currents.

Responses to Light Stimuli Alone

The initial response of the type A photoreceptor to a 60 s light stimulus is a depolarizing generator potential (30–60 mV) with many superimposed impulses (Fig. 2 A). Immediately following the light step, the cell hyperpolarizes (5–10 mV) and the membrane potential does not return to the resting level for 40 to 60 s. The next light step begins after 35 s, before the resting level has been achieved. With successive light steps a new steady-state level of membrane potential (during the dark intervals) is reached, 10–15 mV negative with respect to the resting level. After four to eight light steps, i.e. the number of steps in the initial test period, the number of impulses occurring during the response of the type A photoreceptor to each light step is approximately constant (Fig. 2 B).

Following the initial test period, the light stimuli are continued for 30 min; i.e., throughout the stimulus regimen. The number of impulses occurring during the response of the type A photoreceptor to each subsequent light step following this stimulus regimen, during the second test period, was not significantly different from the number of impulses occurring during the initial test period (Fig. 2 C, *L*, Tables I and II). The amplitude of the generator potential, however, gradually increased over 20 to 30 min before it also reached a constant value (Fig. 2 A–C).

The effect of light stimuli on type B photoreceptors was similar with the exception that the hyperpolarization following the depolarizing generator potential was usually not present.

Responses to Rotation Stimuli Alone

Following the initial test period, the cells are exposed to continual darkness and rotation stimuli for 30 min. The onset of each rotation stimulus occurred at 95 s intervals. Maximum rpm was achieved within 15 s and lasted for 18 s. The initial response of the type A photoreceptor to rotation in darkness is a hyperpolarization of 2–8 mV (Fig. 3 A). This hyperpolarization decreased slightly with successive rotation stimuli (Fig. 3 B, C) before reaching an approximately constant

TABLE I

PHOTORECEPTOR RESPONSE RATIOS

Group	Ratios	*N*	Mean	SD	SEM	*t*	df	Sig.
Type A ratios—30 min regimen								
R+L	0.113	6	0.136	0.1163	0.0475	−18.19	5	$P < 0.001$
	0.239							
	0.0							
	0.290							
	0.018							
	0.156							
L	0.966	5	1.0236	0.3031	0.1356	0.174	4	NS
	0.612							
	1.44							
	1.15							
	0.95							
R+D	0.814	3	0.8980	0.27963	0.16144	−0.6318	2	NS
	1.21							
	0.67							
R/L	1.575	3	1.4583	0.5789	0.3342	1.370	2	NS
	0.830							
	1.970							
Cut *N*	0.9	3	0.9390	0.1165	0.06726	−0.9069	2	NS
R+L	1.07							
	0.847							
Type B ratios—30 min regimen								
R+L	0.57	3	0.710	0.12124	0.07	−4.143	2	NS
	0.78							
	0.78							
Type A ratios—20 min regimen								
R'+L' (out-of-phase)	0.423	3	0.5703	0.1286	0.07424	−5.788	2	$0.02 < P < 0.05$
	0.628							
	0.66							

R^n+L^n (in-phase)	0.752 0.627 0.18	3	0.5197	0.30073	0.17362	−2.767	2	NS
$U+L$	0.35 0.218 0.276 0.414*	4	0.31450	0.08555	0.04277	−16.03	3	$P<0.001$
$U+D$	1.18 0.615 1.68*	3	1.1583	0.53283	0.3076	0.51463	2	NS
Type A ratios—10 min regimen								
R^p+L^p (paired)	0.60 0.74 0.56 0.50	4	0.600	0.102	0.051	−7.843	3	$P<0.01$
R^u+L^u (unpaired)	0.9 0.93 1.09 0.82	4	0.935	0.113	0.057	−1.14	3	NS

* Values from the same cells. All other values are from individual cells. Not more than one cell was used in any nervous system preparation.
t, value from t test of the hypothesis that the mean is equal to one.
$R+L$ = rotation paired with light (Fig. 4).
$R+D$ = rotation in darkness.
R/L = rotating stimuli followed by light stimuli.
$U+L$ = negative current steps paired with light (Fig. 7).
$U+D$ = negative current steps in darkness.
R^p+L^p = rotation paired with light (Fig. 9).
R^u+L^u = rotation alternating with light (Fig. 9).
SEM = standard error of the mean.
Sig. = significance.
Cut N = cut nerve.

TABLE II

COMPARISON OF GROUP RATIOS*

Comparison	df	t	sig.
Type A ($R+L$) vs. Type B ($R+L$)	7	6.893	$P<0.001$
Type A ($R+L$) vs. Cut N ($R+L$)	7	9.757	$P<0.001$
Type A ($R+L$) vs. L	5.0	6.179	$0.001<P<0.002$
$R+D$ vs. L	6	0.582	NS
Type A ($R+L$) vs. $R+D$	2.4	4.528	$0.02<P<0.05$
R/L vs. L	6	1.431	NS
Type A ($R+L$) vs. R/L	2.1	3.917	NS
$U+L$ vs. L	4.8	4.988	$0.005<P<0.01$
$U+D$ vs. L	6	0.467	NS
($U+L$) vs. ($U+D$)	2.1	2.717	NS
$R'+L'$ vs. $R''+L''$	4	0.268	NS
(R^p+L^p) vs. (R^u+L^u)	6	4.83	$0.005<P<0.01$

* t tests were performed for independent groups testing the null hypothesis that the means of two compared groups are equal (see Table I for group designations). Group variances were tested for homogeneity. An approximation was used (cf. Brownlee, 1960) to allow for lack of homogeneity when it occurred.

magnitude. The number of impulses occurring during the response of the type A photoreceptor to subsequent light steps following this stimulus regimen, i.e., during the second test period, was not significantly different from the number of impulses occurring during the initial test period ($R + D$, Tables I and II).

Responses to Light and Rotation Stimuli

Following the initial test period, the cells are exposed to light stimuli associated with rotation stimuli for 30 min. For these stimulus regimens, the rotation stimulus occurred simultaneously with the light stimulus. Each rotation stimulus was begun 10 s after the onset of the light stimulus and reached a maximum rpm after 15 s. This maximum was maintained for 18 s before deceleration was begun (cf. Fig. 4). The number of impulses occurring during the response of the type A photoreceptor to subsequent light steps following this stimulus regimen, i.e. during the second test period, was significantly less than the number of impulses occurring during the initial test period (Fig. 5, $R + L$, Tables I and II). This decrease in the number of impulses during the light response usually lasted for the duration of subsequent recording, one to 2 h. Persistent decreases were not reversed by periods of darkness of from 10 to 20 min. The firing frequency of type B photoreceptors during their responses to light was only slightly decreased by the same 30 min stimulus regimens just described for the type A photoreceptor (Fig. 6, Type B, $R + L$, Tables I and II). Most importantly, however, the effect of the same stimulus regimen caused an effect on type A photoreceptors significantly different from the effect on type B photoreceptors (Table II).

Other cells were exposed to light stimuli associated with rotation stimuli, but with the rotation stimulus not occurring simultaneously with the light stimulus. For these experiments maximum rpm was achieved 5 s after the end of each light step. The rotation and light stimuli were otherwise the same as described above. Such stimulus regimens lasting 20 min, also produced some decrease in impulse frequency during the light response of the type A photoreceptor

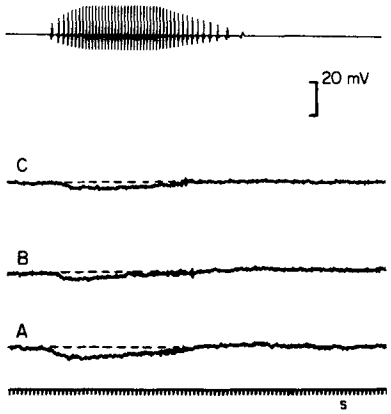

FIGURE 3. Responses to rotation stimuli alone. The initial response (A) of the type A photoreceptor to rotation is a hyperpolarization. This hyperpolarization decreases somewhat when the rotation is repeated at 95 s intervals. B, response to tenth rotation stimulus. C, response to seventeenth rotation stimulus. The amplitude of monitor signal (top trace) is proportional to the angular velocity of the turntable. The interval between monitor signals equals the period of the turntable's rotation.

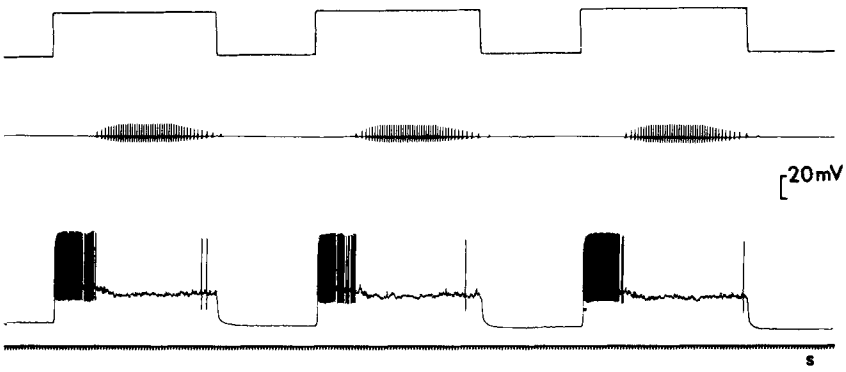

FIGURE 4. Responses to light and rotation stimuli. For this stimulus regimen, lasting 30 min, the rotation stimulus (middle trace) occurred simultaneously with the light stimulus (upper trace). During rotation impulse activity of the type A photoreceptor is eliminated. The amplitude of monitor signal (middle trace) is proportional to the angular velocity of the turntable. The interval between monitor signals equals the period of the turntable's rotation.

(Tables I and II). Such decreases persisted for 20 to 40 min before the level of firing in the initial test period was resumed. The changes produced by this regimen of light out-of-phase with rotation were not significantly different from changes produced when light was paired in phase with rotation, as above, for a comparable time period of 20 min.

It should be noted that no change in the photoreceptor response to light other than a decrease in firing frequency was caused by the regimen with light and

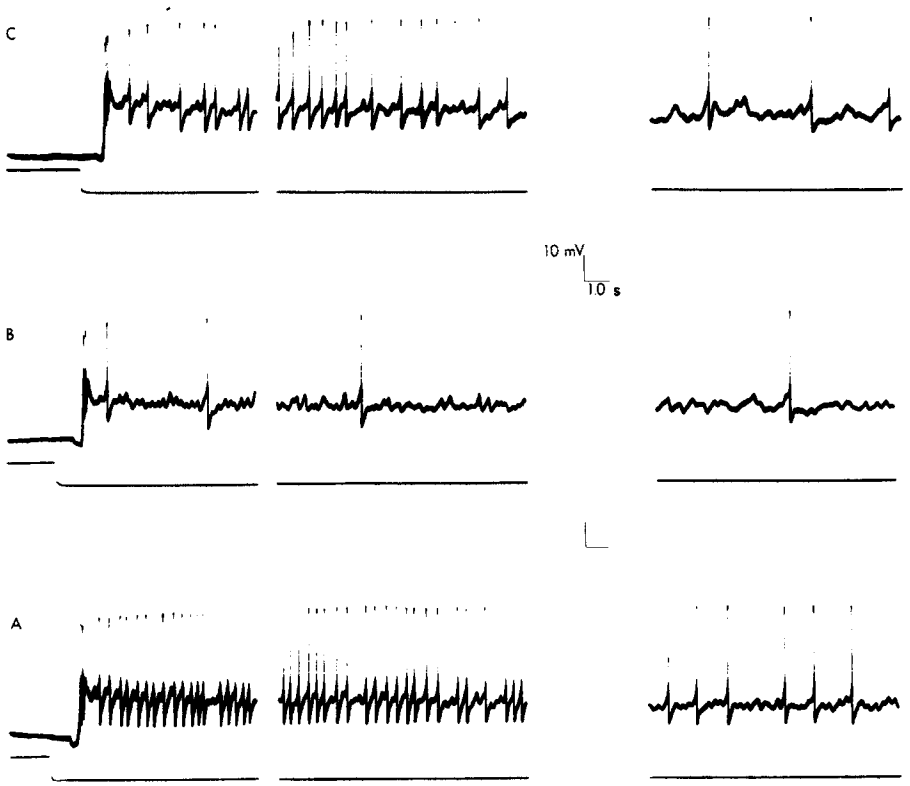

FIGURE 5. Effect of rotation and light stimulus regimen on responses of type A photoreceptor to light steps. A, steady-state response before stimulus regimen. B, response immediately after stimulus regimen. C, response 20 min after stimulus regimen. Bottom trace in each record indicates duration of the light stimulus. Note that the primary effect of stimulus regimen is a decrease of firing frequency during the type A response. This effect is reduced 20 min after stimulus regimen. Spike amplitudes in B are slightly smaller in comparison to spike amplitudes in A and C for approximately equal firing frequencies. First two frames in A, B, C interrupted by 1 s. Second and third frames interrupted by 20 s.

rotation stimuli. The afterhyperpolarization and gradual increase in generator potential amplitude observed for the regimen with light stimuli alone were not different for the regimen with light and rotation stimuli. For those cells in which the effect of light with rotation stimuli was reversible, only the firing frequency during the type A photoreceptor response to light was observed to first decrease and then to return to its previously constant level. In all, reversibility was observed for seven cells (Fig. 5).

Responses to Light Stimuli Followed by Rotation Stimuli

After the initial test period, the cells are exposed to 30 min of light stimuli and then 30 min of rotation stimuli. The frequency and duration of the rotation and light stimuli were identical to those given in the regimens already discussed. The number of impulses occurring during the response of the type A photoreceptor

to subsequent light steps following this stimulus regimen, i.e. during the second test period, was not significantly different from the number of impulses occurring during the initial test period (R/L, Table I).

Responses to Light and Rotation Stimuli after Statocyst Removal

Bilateral statocyst removal eliminates the type A photoreceptor hyperpolarizing response to rotation (Alkon, 1976). In addition, statocyst removal prevented any decrease of type A photoreceptor impulse frequency following stimulus regimens of light and rotation (Fig. 7, Cut N, $R + L$, Tables I and II).

Effect of Dark Periods

Stimulus regimens with 30 min of darkness were not used in the present study. The effect of 20 to 60 min of darkness, however, on the light responses of many type A and type B photoreceptors has been examined in past work. This effect

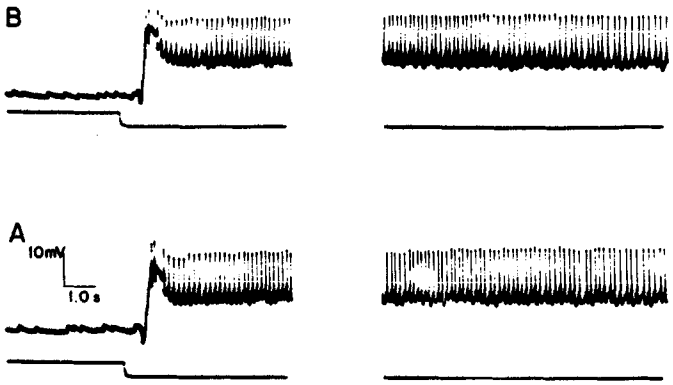

FIGURE 6. Effect of rotation and light stimulus regimen on responses of type B photoreceptor to light steps. A, steady-state response before stimulus regimen. B, response immediately after stimulus regimen. Note the absence of any significant decrease of firing frequency following stimulus pairing. Bottom trace in each record indicates duration of the light stimulus.

was always to initially produce a larger generator potential with some increase in the number of superimposed impulses in response to a light stimulus. For repeated light steps before and after dark adaptation, however, the number of impulses during each photoreceptor response approaches a constant value after four light steps (cf. Cell Treatment).

Temporal Specificity

For more careful examination of temporal specificity for the stimulus pairing, the intervals between light steps were increased to 2 min and the duration of rotation to 70 s. One group of type A cells was exposed to three stimulus pairs, the rotation reaching its maximum (here, generating 2.14 g) 4 s before the onset of the light stimulus and decreasing from its maximum at the end of the light stimulus (Fig. 9). A second group of cells was exposed to exactly the same three light and rotation stimuli but with the rotation beginning 20 s after each light

stimulus. Analysis of the paired vs. unpaired stimulus regimen effects was conducted as described for the other cell groups (i.e. comparing light responses during a test period following the stimulus regimen to responses during an initial test period.) For this analysis the light response immediately preceding the stimulus pairing provided the denominator. The response immediately following stimulus pairing provided the numerator. With such an analysis, the effects of the paired regimens were found to be significantly different from the effects of the unpaired regimens (cf. Tables I, II).

In the above experiments a "LED" high intensity lamp (Hewlett-Packard Co., Palo Alto, Calif.) was mounted on the turntable 2 cm. from the preparation. The lamp was controlled and powered through the slip rings referred to under Methods. The photoreceptor responses elicited were of magnitudes comparable to those with the other stimulus regimens. Also, for these experiments a somewhat different turntable was used. This consisted of an aluminum disc, 1.9 cm thick and 39.5 cm in diameter, powered by a $^1/_{20}$ hp motor (B and B Motor and Control Corp., New York, N. Y.). The preparation was placed 18 cm from the center of rotation with the orientation as already described.

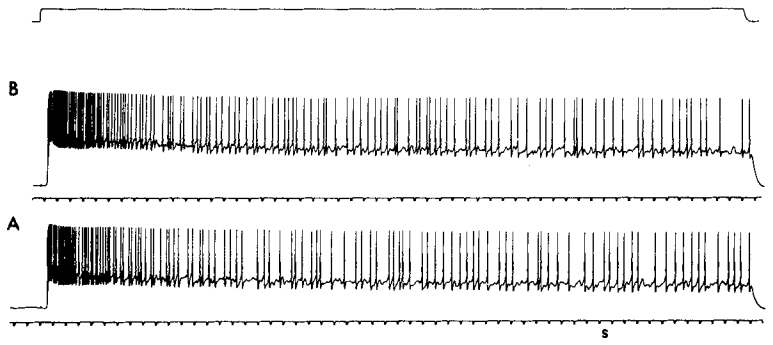

FIGURE 7. Effect of rotation and light stimulus regimen on responses of type A photoreceptor to light steps in preparation with statocysts removed. A, steady-state response before stimulus regimen. B, response immediately after stimulus regimen. Note the absence of any significant decrease of firing frequency following stimulus pairing. Top trace in each record indicates duration of the light stimulus.

Responses to Negative Current Pulses

Following an initial test period of 30 min, the cells are exposed to negative current pulses for 20 min. For these stimulus regimens, hyperpolarizing current injections of 1.0 nA lasted for 25 s. The onset of each pulse occurred at 95 s intervals. The number of type A impulses occurring in response to light following this stimulus regimen, i.e. during the second test period, increased somewhat over the comparable number during the initial test period ($U + D$, Table I).

Responses to Negative Current Pulses and Light Stimuli

Following an initial test period of 30 min, negative current pulses, as just described, are begun 20 s after the onset of each light step. Type A photorecep-

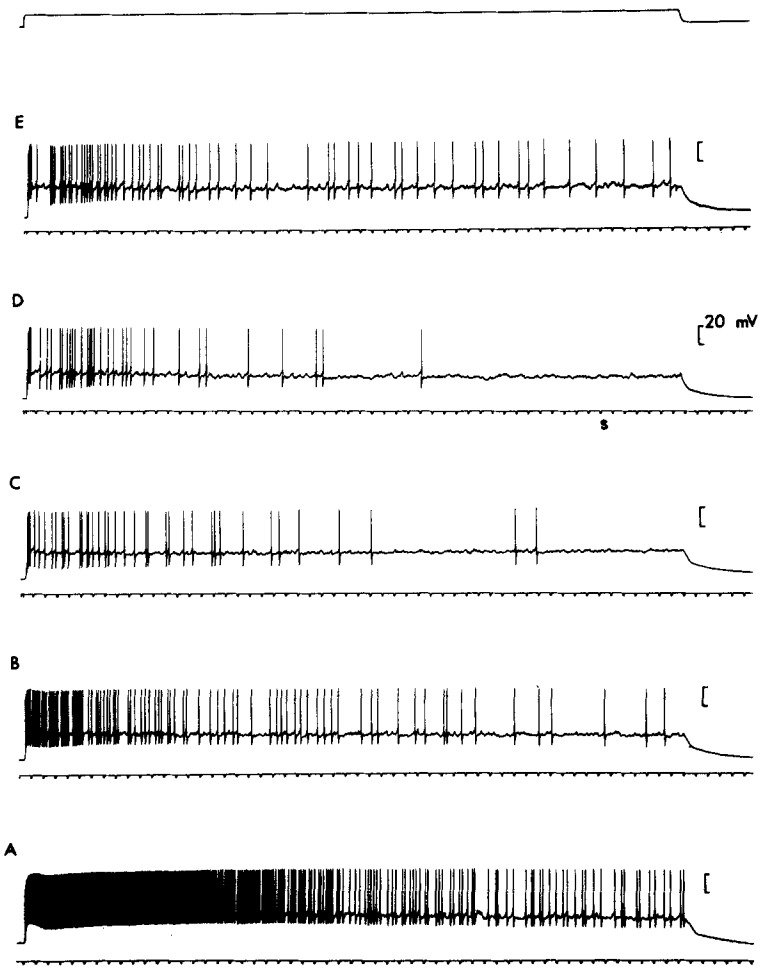

FIGURE 8. Effect of negative current (1.0 nA, 25-s pulses) and light stimulus regimen on responses of type A photoreceptor to light steps. A, response immediately after 15 min of dark adaptation. B, steady-state response before stimulus regimen (response to the sixth light step after dark adaptation). C, response immediately after 20 min of stimulus regimen. D, response 15 min after stimulus regimen. E, response 42 min after stimulus regimen. Top trace indicates duration of the light stimulus. Note that the primary effect of stimulus regimen is a decrease of firing frequency during the type A response. This effect is almost gone 42 min after stimulus regimen. Spike amplitudes in C and D are slightly smaller in comparison to spike amplitudes in A, B, and E for approximately equal firing frequencies.

tors respond to this current pulse during a light step with hyperpolarization accompanied by elimination of impulse activity. After 20 min of this stimulus regimen, the number of type A impulses occurring in response to light is less than that for the initial period ($U + L$, Tables I and II, Fig. 8). For one of the four cells exposed to current and light stimuli, it was possible to record the

reversal of the decrease in type A impulse frequency. This reversal was approximately complete 45 min after the stimulus regimen (Fig. 8).

Responses to Steady Current Injection

In several experiments a depolarizing current step, 0.6 nA, was injected throughout a light step during the second test period following a stimulus regimen of light and rotation. Although such current injections produced a transient increase of impulse frequency during the type A light response, subsequent responses contained substantially fewer impulses than were present prior to the current injection. This was true of longer current injections, i.e. during several light steps, as well.

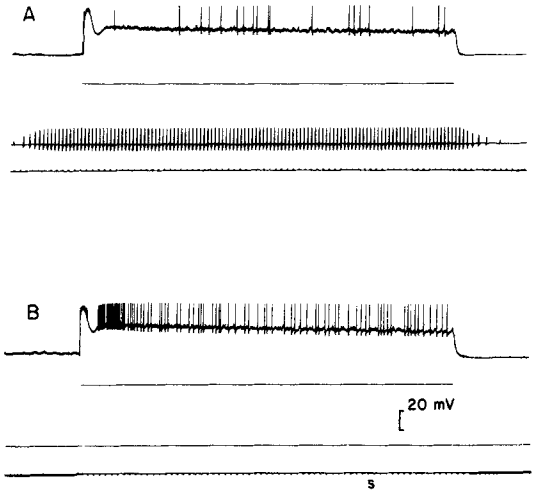

FIGURE 9. Responses of type A photoreceptor to light and rotation stimuli. For this stimulus regimen, used to demonstrate temporal specificity, intervals between light steps were 2 min and the duration of each rotation stimulus was 70 s. Each light step was either (A) paired with the rotation stimulus (generating 2.14 *g* at maximum) or (B) not paired, preceding by 20 s the onset of each rotation stimulus. Trace beneath each record indicates duration of light stimulus. The amplitude of monitor signal (trace above time record) is proportional to the angular velocity of the turntable. The interval between monitor signals equals the period of the turntable's rotation.

Hyperpolarizing current steps of 0.5 to 1.0 nA, for 1 to 10 min, during darkness or during light stimuli, never reversed the decrease of impulses described for type A photoreceptors which had been exposed to stimulus regimens of light with rotation.

Responses to Single Positive Pulses during Stimulus Regimens

The only change observed thus far which was specific to stimulus regimens of light with rotation was a decrease in firing frequency during the type A photoreceptor's response to light. Waveforms and amplitudes of type A photoreceptor impulses were also examined before, during, and after the stimulus regimens described above. The waveforms of four cells were studied for each set of

conditions. A clear reduction in the impulse afterpotential occurred following light steps alone (Fig. 10*a, b, c*). Following stimulus regimens of light with rotation, there was both a prolongation of the impulse recovery phase and a reduction of impulse amplitude (Fig. 10*d–f*). These latter changes disappeared when the type A photoreceptor resumed the firing frequency observed prior to the stimulus regimen (Fig. 10*g*). The impulse waveform and amplitude were unaffected by a stimulus regimen of rotation alone or light alone.

DISCUSSION

Neural Modification

The response of a cell, the type A photoreceptor, to one stimulus, light, can be significantly modified by temporally specific pairing of this stimulus with a second distinct sensory stimulus, rotation. Thus, the modification is not produced by repeated light stimuli followed by repeated rotation stimuli. Nor is it produced by repeated light stimuli alone or repeated rotation stimuli alone. Furthermore, with longer intervals between light stimuli, paired but not unpaired light and rotation were effective (Fig. 9). Finally, the effect of the paired stimulus regimen on type B photoreceptors was significantly different from the effect on type A photoreceptors.

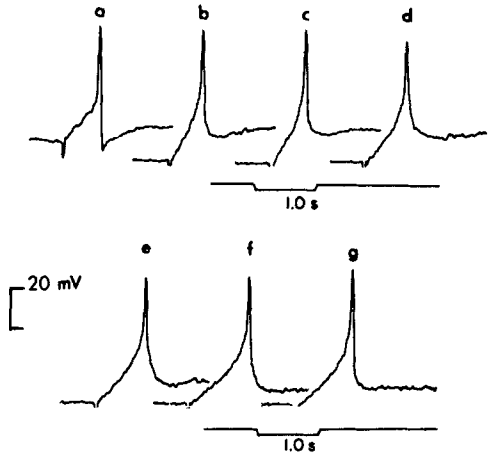

FIGURE 10. Type A photoreceptor impulses before and after stimulus regimen of light and rotation. By injection of positive current pulses (0.25 nA, 4.0 s for *a–d;* 0.2 nA, 4.0 s for *e–g*) through a balanced bridge circuit, single impulses were elicited in dark intervals between light steps. *a*, after 15 min dark adaptation, *b*, after eight light steps, *c*, after sixteen light steps, *d*, after six light steps following 20 min of rotation and light stimulus regimen, *e*, after seven light steps following stimulus regimen, *f*, after sixteen light steps following stimulus regimen, *g*, after thirty light steps following stimulus regimen. Note that the impulse afterpotential decreases with repeated light steps (*a–c*). Impulse amplitude decreases and falling phase slows following stimulus regimen (*d*). Reversal of these last two effects occurs approximately 45 min after stimulus regimen (*g*). Record trace before each pulse indicates level of membrane potential.

The type A photoreceptor modification, then, results from a stimulus paradigm identical to paradigms used for conditioning of animal behaviors. An outstanding difference between the cellular model of conditioning reported here and behavioral conditioning is the duration of the effects. A much more long-lasting effect would be necessary for a complete parallelism of the neural and behavioral phenomena.

It remains to be seen whether or not the cellular model will provide insight into the mechanisms of behavioral conditioning in invertebrates (cf. Bruner and Tauc, 1966; Clark, 1966; Horridge, 1968; Mpitsos, 1975) and vertebrates. In order to explore this possibility long-term behavioral experiments with intact *Hermissenda* and their subsequently isolated nervous systems might utilize stimulus paradigms from the present study.

The stimulus regimens used here for the isolated sensory pathways were chosen, at least in part, for their similarity to the associative training previously given intact *Hermissenda* (Alkon, 1974*b*, 1975*a*). In animals exposed to associative training, it was previously found that the depolarizing response of hair cells to illumination of the ipsilateral eye, and thus to type A photoreceptor input, was diminished or absent (Alkon, 1975*a*). The present study suggests that one cause for this neural correlate is a reduction of type A impulses triggered by photoreceptor generator potentials following stimulus regimens of light paired with rotation.

Mechanisms of Modification

The paradigm demonstrating temporal specificity (Fig. 9) suggests a process responsible for the observed neural modification. With this paradigm, rotation, and thus hair cell inhibition, when paired with light, almost abolishes the type A photoreceptor activity. After three such pairings fewer impulses are produced in response to light alone. Excluding other neural influences, such a result would be paradoxical since with many fewer type A impulses during the stimulus pairs much less impulse accommodation due to sodium inactivation and/or potassium accumulation would be expected. Close observation of type A photoreceptor recordings reveals that inhibitory postsynaptic potentials occur throughout the pairing of rotation with light (Alkon, 1976). This is consistent with the observation (Alkon, 1976) that type B photoreceptor impulse activity during a light response is not significantly reduced by rotation. It could be hypothesized, therefore, that hair cell abolition of the type A photoreceptor impulses disinhibits the type B photoreceptors, particularly at their synaptic endings, allowing much more effective and prolonged inhibition of the type A cell itself. This is quite possible, since at least one type A cell in each eye has reciprocal inhibitory interactions with the three type B photoreceptors (Alkon and Fuortes, 1972). The prolonged decrease of type A impulse activity during its light response would be due, then, to prolonged inhibition (from type B cells) caused by stimulus pairing.

This hypothesis would explain the temporal specificity required. Hair cell inhibition of the type A cell in darkness would have no effect because type A cells are not spontaneously active and the type B photoreceptors are firing with a relatively low spontaneous frequency. The hypothesis would also account for the

fact that pairing of steady hyperpolarizing currents in the type A cell with light produces the same effect (Fig. 8) as pairing of light and rotation. Finally, the hypothesis is consistent with the finding that type B cell activity is not significantly reduced by stimulus pairing.

Prolonged inhibition of the type A cell should be accompanied by some hyperpolarization of its terminal endings which both send and receive synaptic signals. Such hyperpolarization, although effective in reducing impulse conduction and/or initiation, may be relatively small as recorded in the photoreceptor soma. Hyperpolarization did in fact accompany neural modification following stimulus pairing. The magnitude of this hyperpolarization (average value: 4.83 mV ± 5.33 SD) however, was not significantly larger than the hyperpolarization following stimulus regimens of light alone (average value: 2.42 mV ± 2.0 SD). Using the techniques of the present report accurate measurement of small potential differences in the type A cell may not have always been possible during prolonged intracellular recordings. Stimulus regimens of rotation alone were not followed by any prolonged hyperpolarization.

How might the stated hypothesis be further tested? For it to be correct we might expect that similar changes in the lateral type A photoreceptor could be produced by sufficient impulse activity of ipsilateral type B photoreceptor(s). This could be accomplished by simultaneous recording from type A and B photoreceptors of the same eye. Experiments of this type and others supporting the above hypothesis have been performed and will be the subject of a forthcoming report (Alkon and Akaike, data to be published).

In conclusion, the photoreceptor modification produced by stimulus pairing may result from an enhanced and thus prolonged inhibition of type A cells by type B cells. This enhancement would occur because the type B cell is released from type A cell inhibition by the effect of rotation on type A impulse activity. Thus, the inhibitory effect of hair cells due to rotation would bias the network of the five photoreceptors in each eye during a light stimulus. If repeated, this bias would be prolonged and type A impulse activity reduced during subsequent responses to light.

SUMMARY

(a) Exposure of the isolated *Hermissenda* nervous system to repeated pairing of a light stimulus with a rotation stimulus causes a persistent reduction in the firing frequency of the type A photoreceptor during its response to light.

(b) This reduction of impulse frequency does not occur with repeated presentation of either the light stimulus or the rotation stimulus alone, nor does it occur when a period of light stimuli is followed by a period of rotation stimuli.

(c) With 2 min intervals between light steps, the stimulus pairing is temporally specific.

(d) The neural specificity of this stimulus pairing is demonstrated by the difference of its effect on type A and type B photoreceptors.

(e) In an isolated nervous system, therefore, a relatively simple neural network, involving the photoreceptors and hair cells of *Hermissenda* can provide a cellular model of conditioning.

(f) Negative current injections, only when paired with the intermittent light stimulus, produced the same changes in type A photoreceptor activity which were observed with pairing of light and rotation stimuli.

(g) Regimens of light with rotation stimuli also cause a slowing of the type A impulse falling phase and reduction of impulse amplitude.

Received for publication 31 July 1975.

BIBLIOGRAPHY

ALKON, D. L. 1973*a*. Neural organization of a molluscan visual system. *J. Gen. Physiol.* **61**:444–461.

ALKON, D. L. 1973*b*. Intersensory interactions in *Hermissenda*. *J. Gen. Physiol.* **62**:185–202.

ALKON, D. L. 1974*a*. Sensory interaction in the nudibranch mollusk *Hermissenda crassicornis*. *Fed. Proc.* **33**(4):1083–1090.

ALKON, D. L. 1974*b*. Associative training of *Hermissenda*. *J. Gen. Physiol.* **64**:70–84.

ALKON, D. L. 1975*a*. Neural correlates of associate training in *Hermissenda*. *J. Gen. Physiol.* **65**:46–56.

ALKON, D. L. 1975*b*. A dual synaptic effect on hair cells in *Hermissenda*. *J. Gen. Physiol.* **65**:385–397.

ALKON, D. L. 1975*c*. Responses of hair cells to statocyst rotation. *J. Gen. Physiol.* **66**:507–530.

ALKON, D. L. 1976. Signal transformation with pairing of sensory stimuli. *J. Gen. Physiol.* **67**:197–212.

ALKON, D. L., and A. BAK. 1973. Hair cell generator potentials. *J. Gen. Physiol.* **61**:619–637.

ALKON, D. L., and M. G. F. FUORTES. 1972. Responses of photoreceptors in *Hermissenda*. *J. Gen. Physiol.* **60**:631–649.

BROWNLEE, K. A. 1960. Statistical Theory and Methodology in Science and Engineering. John Wiley and Sons, Inc., New York N. Y. 235–239.

BRUNER, J., and L. TAUC. 1966. Long-lasting phenomena in the molluscan nervous system. S.E.B. Symposium No. 20. Nervous and Hormonal Mechanisms of Integration. C. M. Hughes, editor. Academic Press, Inc., New York. 475–476.

CLARK, R. B. 1966. The integrative action of a worm's brain. S.E.B. Symposium No. 20. Nervous and Hormonal Mechanisms of Integration. C. M. Hughes, editor. Academic Press, Inc., New York. 345–380.

HORRIDGE, G. A. 1968. Interneurons. Their Origin, Action, Specificity, Growth and Plasticity. W. H. Freeman & Co., San Francisco.

MPITSOS, G. J., and S. D. COLLINS. 1975. Learning: Rapid aversive conditioning in the gastropod mollusk *Pleuorbranchaea*. *Science (Wash. D. C.)*. **188**:954–956.