CONDITIONED MODIFICATION OF PHOTOTACTIC BEHAVIOR IN *HERMISSENDA*

II. Differential Adaptation of B-Photoreceptors¹

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Abstract

Changes in the response of B-type photoreceptors to illumination were examined in the isolated nervous systems of *Hermissenda* following the conditioning procedures described in the preceding paper. Analysis of the transient peak amplitude of the depolarizing generator potential at the onset of two of the three light intensities used in the behavioral studies did not reveal an enhanced photoresponse to the light. However, when the activity of the same B-photoreceptors was examined after 5 min of continuous light, there was a significant decrease in the light-adapted discharge rate and a decreased generator potential amplitude in conditioned animals as compared to the random controls. An examination of the light adapted photoresponse in preparations where spike generation and synaptic interactions were eliminated showed that the decreased photoresponse of conditioned animals was due to factors that are intrinsic to the B-photoreceptors. These results are consistent with previous work suggesting that conditioning produces substantial adaptation effects in B-photoreceptors (Crow, T. (1982) Soc. Neurosci. Abstr. 8: 824). Since the cellular changes qualitatively follow the behavioral changes and are observed at times and light intensities that are similar to those where the suppression of phototactic behavior is expressed, phototactic suppression may be directly related to the changes in the B-photoreceptors and may not require the previously proposed complex network interactions within the eyes of *Hermissenda*.

In the preceding paper (Crow, 1985) it was reported that conditioning in *Hermissenda* produces phototactic suppression over a broad range of light intensities. Since phototactic behavior is expressed under light-adapted conditions, it was proposed that the activity of light-adapted B-photoreceptors may be involved in the expression of suppressed phototactic behavior after conditioning. Previous cellular studies of conditioned *Hermissenda* have shown that conditioning results in changes in the photoresponse and input resistance of the B-type photoreceptors (Crow and Alkon, 1980a). Based upon an examination of the initial phase of the generator potential, it has been suggested that light produces an enhanced photoresponse in conditioned animals (Farley and Alkon, 1982; Alkon, 1983), and the enhanced photoresponse is related to the associative suppression of phototactic behavior (Farley and Alkon, 1982; West et al., 1982; Alkon, 1983). However, it is not known if the early or steady state phase of the photoresponse provides the necessary information to the nervous system that allows the change in behavior to be expressed since most of the cellular studies have only examined the initial phase of the light response or the response after the termination of light, referred

to as the long lasting depolarization (Alkon, 1983). Some progress has been made towards an understanding of the conductances responsible for the early transient part of the B-type generator potential (Alkon, 1979; Shoukimas and Alkon, 1983); however, the activity of B-type photoreceptors has not been investigated in conditioned animals under light-adapted conditions that actually correspond to conditions when phototactic behavior of *Hermissenda* is expressed and measured as reported in the preceding paper.

The purpose of the experiments described here was to examine the activity of B-type photoreceptors under light-adapted conditions that corresponded to the conditions reported in the previous behavioral experiments where the animals exhibited phototactic behavior. These results show that conditioning produces a diminished photoresponse that can be explained by cellular changes that are intrinsic to the B-photoreceptors. Thus, there appears to be a direct relationship between suppression of phototactic behavior and decreased responsiveness of the B-photoreceptors. Complex circuit interactions between B-type and A-type photoreceptors within the eyes of *Hermissenda* as previously proposed (Alkon, 1983) may not be necessary to explain the associative suppression of behavior.

Some of the results of these experiments have been reported in preliminary abstracts (Crow, 1983b, 1983c).

Materials and Methods

Adult *Hermissenda* were obtained from Sea Life Supply, Sand City, CA. The animals were maintained as described in the preceding paper

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in an artificial seawater aquarium at 14°C on a 12-hr light, 12-hr dark cycle.

Conditioning and measurement of phototactic behavior. The conditioning apparatus and behavioral training and testing procedures were described in the preceding paper and in detail in earlier publications (Crow and Alkon, 1978; Tyndale and Crow, 1979; Crow and Offenhach, 1983; Crow, 1983a). Animals used in the cellular neurophysiological studies received multiple session training consisting of 50 conditioning trials each day for 3 consecutive days. Random control groups were treated as described in the accompanying paper (Crow, 1985). Following training, behavior was examined 24 and 48 hr after the last conditioning session. Start latencies and the time that the animals remained in the central illuminated area in response to light of different intensities were recorded as described previously. Start latencies were taken to ensure that the behavior of *Hermisenda* after conditioning was consistent with changes in these two behavioral measures as reported previously (Crow and Offenhach, 1983).

Intracellular recording. Following behavioral assessment of phototactic suppression, the circumesophageal nervous system was removed from the animals and pinned to a stage in an artificial seawater-filled recording chamber. Experiments were conducted in either instant ocean or buffered artificial seawater (10 mM HEPES, pH 7.6) having the following composition (millimolar): 460, NaCl; 10, KCl; 10, CaCl₂; 55, MgCl₂. The solution was brought to pH 7.6 with dilute NaOH. The isolated nervous systems were incubated in a protease solution (Sigma Chemical Co.) (0.67 mg/ml, 5 to 6 min) to facilitate microelectrode penetration of the type B photoreceptors. Glass microelectrodes used for intracellular recordings had resistances between 60 and 80 megohms when filled with 4 M potassium acetate. fConventional methods were used for intracellular recording and stimulation. The temperature of the artificial seawater in the recording chamber was maintained at 14 ± 0.5°C. Seawater temperature was monitored by a thermistor inserted into the recording chamber.

Illumination was provided by a quartz-iodide incandescent lamp mounted on an optical bench. The light was focused to a spot that covered the eye and was attenuated as desired by inserting neutral density filters into the path of the collimated beam. Throughout this paper, light intensities are described in log units of attenuation, neutral densities (ND). The intensity of unattenuated light (referred to as 0 ND) was 9.2×10^{-4} W/cm² at 510 nm, the wavelength used in all experiments. Light-attenuated 2 and 4 log units are referred to as 2 ND and 4 ND, respectively. All cellular experiments were carried out using light stimuli that corresponded to the intensities and wavelength that were used in the behavioral experiments. The intensity of the light spot that was focused on the eye was measured with a photodiode and the output of the light source was adjusted to match the intensity used in the behavioral studies reported in the preceding paper.

Experimental procedure. A standard experimental protocol was used to examine the type B photoresponse following conditioning and behavioral testing that was similar to the conditions under which the animals were tested following behavior training. The photoreceptors were initially dark adapted for 20 to 25 min following electrode penetration of the cells. Photoreceptor responses were examined at three different levels of relative light intensity used in the behavioral studies: 4, 2, and 0 ND. The three intensities were presented in an ascending series for each of the B-type photoreceptors used in the analysis. Following measurements at the initial intensity (4 ND), the photoreceptors were dark adapted for 12 min, as in the behavioral studies, before presenting the next higher intensity. Electrophysiological data were recorded on a Gould 2400 pen recorder and included measurements of membrane potential and the frequency of firing during light. Measurement of membrane potential in the presence of spike activity was taken in the intervals between successive spikes or at the midpoint between the foot of the spike and the after-potential. Following the onset of light, measurements were taken of the peak amplitude of the generator potentials at a given light intensity followed by measures of membrane potential and spike frequency after 5 min of light adaptation. Interspike intervals (ISI) were measured for each cell during a 1 to 2-min period after 5 min of light adaptation. Mean discharge rate (mean ISI), ISI variance, and interspike interval histograms were generated by a computer. Spike frequencies under light-adapted conditions were measured as the inverse of the mean interval between successive action potentials. In experiments where spike generation and synaptic input were eliminated by axotomy, the amplitude of the peak generator

potential was measured at light onset and after 5 min of light adaptation.

Statistical analysis. A two-way analysis of variance was used to examine overall differences between conditioned and random control groups in response to the three different light intensities (Winer, 1962). Separate analyses were used to examine the light-adapted discharge rate, the transient peak amplitude of the generator potential, and the light-adapted generator potentials. Following significant overall effects, paired comparisons involved Newman-Keuls tests or in the case of significant interactions between treatment (random versus conditioned) and light intensity, the simple main effects were tested (Winer, 1962). In cases where the sample sizes were different, an unweighted means analysis of variance was used to assess overall effects (see Winer, 1962). Pearson's product-moment correlation coefficients (*r*) were computed after log transformations of the mean interspike intervals and interspike interval variance.

Results

The results of the cellular neurophysiological experiments performed on animals after conditioning are presented in two parts. First, neural correlates of conditioning in the type B photoreceptors of conditioned and random control animals are described for different periods of light adaptation, e.g., early transient peak amplitude and steady state light adaptation (5 min). Second, the light responses of B-type photoreceptors from conditioned and random controls were examined in preparations where synaptic activity was eliminated in order to assess the relative contribution of network interactions within the eye to the activity of the photoreceptors under light-adapted conditions.

Conditioned and random control animals were trained and tested as described in the accompanying paper. As a first step in the analysis of the effects of conditioning on the time that the animals remained in an illuminated area, intracellular recordings were made from B-type photoreceptors. Previous work (Crow and Alkon, 1980a) has shown that the primary sensory cells of the conditioned stimulus pathway (B-type photoreceptors) are modified by conditioning; thus, the memory for the associative experience may be encoded in the B-photoreceptors. The neurophysiological experiments were conducted in two phases. Initially, intracellular recordings were made from preparations that had training experiences known by the experimenter. In the second phase, the recordings were conducted using blind procedures. An analysis of the data from the two phases yielded similar results; therefore, the data were combined for the subsequent statistical analysis.

General characteristics of discharge in response to light

Since it is not known which part of the photoresponse is critical for the expression of this measure of phototactic behavior, the photoresponse was examined at light onset and after 5 min of steady illumination. The selection of 5 min of light adaptation was based upon an analysis of the start latencies of 244 conditioned animals. More than 70% of the conditioned animals started locomoting toward light after 5 min of illumination. An example of the early transient peak and light-adapted photoresponse to three different light intensities for B-type cells from a paired animal and random control is shown in Figure 1. Light of increasing intensity evoked depolarizing generator potentials of increasing amplitude with superimposed action potentials as has been shown previously for B-type photoreceptors (Alkon and Fuortes, 1972). An examination of the initial transient peak of the photoresponse revealed a striking similarity between the random control and paired preparation. This suggests that if the change in the B-photoresponse is causally related to phototactic suppression then some other phase of the response to light must contain the information necessary for the expression of the behavioral differences found after conditioning. In contrast to the early

Figure 1. Photoreponses evoked by three behaviorally relevant light intensities recorded in type B photoreceptors from a conditioned animal and a random control. The initial photoreponse to light is shown on the *left* and the photoreponses after 5 min of continuous light (light adapted) is shown on the *right*. The onset of light is indicated by the *event marker* beneath each voltage recording. *A*, photoreponse to bright light (0 attenuation). *B*, photoreponse to light-attenuated 2 ND units. *C*, photoreponse to light-attenuated 4 ND units. In *A*, *B*, and *C*, the B-photoreceptor from the conditioned animal (*P*, paired) is on the *top* and the random control (*R*) is on the *bottom* of each pair of recordings. The early transient peak of the generator potential is similar for both the conditioned example and random control, while under light-adapted conditions the activity of the B-photoreceptor from the conditioned animal is less than the random control at each of the three light intensities.

transient response, the photoreponse after 5 min of light adaptation showed clear differences between the random control and paired B-photoreceptors (see Fig. 1).

The light-adapted firing rate of the B-photoreceptor was less in the conditioned animals in response to the three light intensities as compared to the random control. The decrease in the light-adapted firing rate of conditioned animals was confirmed by examining the interspike interval histograms of the activity of cells shown in Figure 1. In response to light-attenuated 4 ND units, the distribution of B-cell spike intervals was shifted towards longer intervals for the cell from the conditioned animal (Fig. 2C). Similar results are shown for the interspike interval histograms of the B-photoreceptor from the conditioned animal in the presence of light-attenuated 2 ND units and in response to the brightest light (0 attenuation) (Fig. 2, *A* and *B*). In addition to the increase in spike intervals, conditioning also affected the pattern of spike discharge in response to bright illumination. One measure of histogram pattern is the dispersion of the ISI values about the mean as measured by the variance. In general the duration of the mean interspike interval (the inverse of discharge rate) was related to the variance of the interspike interval histogram. This relationship means that with decreases in the mean ISI duration (increase in the discharge rate) the variance also decreased and with increased mean ISI duration (decreased discharge rate) the variance increased.

An examination of the ISI histograms shown in Figure 2 shows that the mean ISI duration for both random and conditioned animals was in general related to the pattern (variance) of the ISI histograms. This was confirmed by a statistical analysis which showed that the variance was related to the

mean interspike interval duration in a log-log relationship for all but one of the light intensities tested. Table 1 shows the results of the regression analysis and the correlation coefficient between the mean ISI duration and variance for paired and random controls in response to the three light intensities. Statistically significant correlations between the mean ISI duration and variance were found for the random controls in response to the three light intensities. However, this relationship was not found for the conditioned group in response to the bright test light (0 attenuation) (see Table I). This finding suggests that the increased dispersion of the ISIs of conditioned animals in response to the bright test light may be due to factors other than the amplitude of the depolarizing generator potential. The effects of conditioning on the pattern of spike activity may be the result of alterations in the mutually inhibitory synaptic interactions between B-photoreceptors within each eye. Taken collectively, these results show that in general the histogram pattern (variance) is related to discharge rate rather than to conditioning effects with the exception of the response to the bright light. Figure 3 shows the mean discharge rate in response to the three different light intensities for each B-photoreceptor from the paired ($n = 10$) and random control groups ($n = 10$). The data show that the light adapted B-photoreceptors exhibited the highest discharge rate in response to light-attenuated 2 ND units.

Analysis of light-adapted discharge rate

The analysis of the group means of the light-adapted discharge rate from the paired group and random controls in response to the three different behaviorally relevant light intensities is shown in Figure 4. Conditioning resulted in a

Figure 2. Interspike interval histograms of the B-photoreceptor activity in response to light for the photoreceptors shown in Figure 1. *A*, in response to bright light (0 attenuation). *B*, response to light-attenuated 2 ND units. *C*, response to light-attenuated 4 ND units. In *A*, *B*, and *C*, the example from the random control is on the top and the conditioned (paired) is on the bottom. The interval distribution for the B-photoreceptor from the paired animal is shifted to the right as compared to the random control in response to the three light intensities indicating a decrease in the spike discharge rate for the conditioned animal.

TABLE I

Summary of analysis of the correlation between mean interspike interval duration and variance for conditioned and random control groups

Behavioral Condition	Light Intensity	<i>r</i>	Significance Level
Paired (<i>n</i> = 10)	0	0.59	NS ^a
	-2.0	0.86	<i>p</i> < 0.01
	-4.0	0.97	<i>p</i> < 0.01
Random (<i>n</i> = 10)	0	0.72	<i>p</i> < 0.02
	-2.0	0.89	<i>p</i> < 0.01
	-4.0	0.84	<i>p</i> < 0.01

^a NS, not significant.

decrease relative to random controls in the light-adapted discharge rate of B-photoreceptors in response to the three different light intensities (Fig. 4). The depression in activity of the B-photoreceptors of conditioned animals (*n* = 10) was significantly different from the random controls (*n* = 10). This was shown by the results of the two-way analysis of variance where the main effect of conditioning was significant ($F_{1,18} = 6.40$; $p < 0.05$). The overall effect of light intensity on B-photoreceptor

discharge rate was significant for both the conditioned and random controls ($F_{2,36} = 16.82$; $p < 0.01$). A *post hoc* analysis (Newman-Keuls test) of the B-photoreceptor discharge rate showed that the discharge rate in response to light-attenuated 2 ND units was significantly higher than the light-adapted rate to 4 ND units ($p < 0.01$) and 0 attenuation ($p < 0.01$). However, light attenuation 4 ND units was not significantly different from 0 attenuation (see "Discussion"). These results show that (1) conditioning results in a significant decrease in the light-adapted discharge rate of B-photoreceptors in response to the three behaviorally relevant light intensities as compared to random controls and (2) light attenuated -2.0 log units produced the highest discharge rate for both the conditioned and random controls.

Analysis of photoresponse amplitude

Transient peak. The decrease in the light-adapted discharge rate of B-photoreceptors from conditioned animals could be the result of network interactions within the eye of *Hemissenda*. Since the B-photoreceptors are mutually inhibitory (Alkon and Fuortes, 1972), the decreased discharge rate could be due to an increase in synaptic inhibition. Alternatively, the decreased activity may be due to an intrinsic change in the B-photorecep-

Figure 3. Mean discharge rate computed from the interspike interval histograms of individual B-photoreceptors from the random control group ($n = 10$) and paired group ($n = 10$) in response to the three light intensities. The mean rate of each B-photoreceptor is displayed on the ordinate as spikes per sec. The discharge rates for each B-photoreceptor in response to light-attenuated 4 ND units, 2 ND units, and 0 attenuation are connected. A, discharge rates for paired group, B, discharge rates for random controls.

Figure 4. Group data for the paired ($n = 10$) and random control ($n = 10$) discharge rates in response to the three light intensities. The group mean is displayed on the ordinate as mean spikes per sec. Conditioning resulted in a significant overall decrease ($p < 0.05$) in the discharge rate relative to random controls in response to the three light intensities. The group data in this figure and subsequent figures are expressed as means $\pm$ SEM.

tors after conditioning that would result in a smaller generator potential. As a first step in examining these two hypotheses, the transient peak amplitude of the generator potential and light-adapted generator potential in response to the three different light intensities were measured for conditioned ($n = 10$) and random controls ($n = 12$) since it has been suggested that conditioning results in an alteration of the photoresponse amplitude. The results from the two groups and three light intensities are shown in Figure 5. Conditioning did not produce an overall enhancement of the peak photoresponse. This was shown by the results of the analysis of variance where the overall difference between the random control and paired group was not significant.

However, the interaction between the conditioning or ran-

Figure 5. Mean photoresponse amplitude of the B-photoreceptors from the paired ($n = 10$) and random control groups ($n = 12$) in response to three light intensities (4 ND, 2 ND, 0). The group mean of the early or initial transient (peak) response to the onset of light is shown by the solid line. The light-adapted response of the same B-photoreceptors is shown by the dashed line. Conditioning results in an overall decrease in the light-adapted photoresponse amplitude ($p < 0.01$) as compared to random controls. The peak response at light onset was not significantly different overall for the paired group as compared to the random controls with the exception of the response to light-attenuated 4 ND units.

dom control procedure and light intensity was significant ($F_{2,55} = 4.19$; $p < 0.05$). This means that the conditioned group was significantly different from the controls in response to at least one of the three light intensities. This was confirmed by examining differences between the groups at each light intensity. This analysis revealed that the conditioned group exhibited an enhanced photoresponse (transient peak amplitude) to light-attenuated 4 ND units ($p < 0.025$), but not to light-attenuated 2 ND units or 0 attenuation. Increasing the light intensity produced a significantly larger generator potential for both the random controls and conditioned animals ($F_{2,55} = 306.90$; $p < 0.01$). Overall these results show that (1) conditioning results in an enhanced photoresponse (transient peak) only in the presence of the dim (4 ND) light and (2) the behavioral differences between conditioned and random controls observed under light-adapted conditions cannot be explained on the basis of the peak amplitude of the generator potential since differences between random controls and conditioned animals were not found in these experiments.

Light adapted. The photoresponses of the same B-photoreceptors from random controls and conditioned animals were examined after 5 min of light adaptation (Fig. 5). The amplitude of the light-adapted photoresponse of conditioned animals was smaller compared to random controls in response to the same three light intensities. The results of the analysis of variance showed overall significant differences between the conditioned

group and random controls at the three different light intensities ($F_{1,55} = 5.55$; $p < 0.05$). The Newman-Keuls test showed that the light-adapted generator potential of conditioned animals was significantly smaller than the random controls in response to bright light (0 ND) ($p < 0.05$) and light-attenuated 2 ND units ($p < 0.05$). Increasing light intensity increased the light-adapted generator potential amplitude for both random controls and conditioned animals ($F_{2,55} = 29.93$; $p < 0.01$). These results show that conditioning produces a decrease in the light-adapted photoresponse over a range of light intensities.

Analysis of photoresponse amplitude in axotomized preparations

The presence of action potentials and synaptic potentials in the recordings from intact isolated nervous systems makes the precise measurement of generator potential amplitude difficult. In addition, synaptic interactions between the B-photoreceptors may contribute to the light-adapted photoresponses examined in this study. In order to eliminate action potentials and synaptic input to the B-photoreceptors, the optic nerve was cut, thus isolating each photoreceptor from the areas of synaptic input and spike generation. This procedure results in an isolated cell that is capable of normal generator potentials as described previously (Alkon and Fuortes, 1972). An example of photoresponses from a paired animal and a random control after axotomy is shown in Figure 6. The initial peak amplitude of the generator potentials evoked by light for the example of a B-photoreceptor from the paired group and random control group are virtually identical (see Fig. 6). Overall the results from the study of the amplitude of the peak of the generator potential of isolated B-photoreceptors are consistent with the results of the recordings from nervous systems with intact optic nerves (see Figs. 1 and 5). In contrast to the results of the initial peak response, after 5 min of light adaptation (continuous illumination), the photoresponses of the B-photoreceptor from the conditioned group were substantially smaller than the photoresponses of the random controls (see Fig. 6).

Group data from the axotomized conditioned and random control preparations are shown in Figure 7. The results of the statistical analysis of the initial peak photoresponse showed that there were no overall significant differences between the conditioned animals and the random controls. Since the previous analysis of the peak responses from intact photoreceptors had shown that dim light (4 ND units) resulted in differences

between groups even though the overall effect was not significant, a paired comparison was conducted to examine possible differences in response to dim light. The results confirmed the earlier observation from intact B-photoreceptors indicating a significantly larger peak response to dim light (4 ND) for the conditioned animals ($p < 0.05$). The significant decrease in the light-adapted photoresponses of conditioned animals was indicated by the results of the analysis of variance. Significant overall effects were found between the conditioned group and random controls ($F_{1,34} = 8.53$, $p < 0.01$). Increasing light intensity resulted in larger light-adapted generator potentials for both groups ($F_{2,34} = 15.14$, $p < 0.01$). Paired comparisons revealed that conditioning resulted in significantly smaller light-adapted photoresponse amplitudes to light-attenuated 2 ND units ($p < 0.05$) and unattenuated light (0 ND) ($p < 0.01$). These results suggest that while synaptic interactions may make some contribution to the light-adapted discharge rate of B-photoreceptors the decrease in amplitude of the light-adapted generator potential of conditioned *Hermisenda* can be explained by changes that are intrinsic to the B-photoreceptors rather than by synaptic interactions.

While the analysis of the peak and light-adapted generator potentials of B-photoreceptors in cut nerve preparations confirms the initial observations from intact photoreceptors, there are some differences in the measurements. These differences can be attributed to either the synaptic interactions between the intact B-photoreceptors or to the difficulty in precisely measuring the generator potential in recordings that contain action potentials.

Discussion

The results of these experiments support two major conclusions regarding the relationship between cellular correlates of conditioning in B-photoreceptors and the associative suppression of phototactic behavior in *Hermisenda*. First, when the activity of B-photoreceptors is examined under conditions of illumination that closely resemble the conditions under which the behavior is expressed, conditioning produced a diminished photoresponse. Second, the relationship between light intensity and behavior qualitatively follows the diminished photoresponse to some of the light intensities that were examined, and the diminished response to light can in part be explained by changes that are intrinsic to the B-photoreceptors. These conclusions will be examined in more detail in each of the following sections.

Figure 6. Generator potentials evoked by bright light (0 attenuation) recorded from B-photoreceptors in a cut nerve preparation. The onset of light is indicated by the event marker beneath each voltage recording. Example from a conditioned animal (P, paired) on the top and a random control (R) (bottom). The left side shows the light onset (initial transient response) and the right side shows the light-adapted response (5 min of continuous illumination). The dashed line beneath each voltage recording indicates the dark-adapted resting membrane potential and the plateau phase of the early photoresponse. The example from the conditioned animal exhibits a smaller light-adapted generator potential (greater adaptation) compared to the random control even though the initial peak is similar to the random control.

Figure 7. Mean generator potential amplitude of B-photoreceptors in cut nerve preparations from paired and random controls in response to three light intensities. The solid line represents peak photoresponses at the onset of light and the dashed line shows the light-adapted response (5 min of continuous illumination). The initial peak photoresponses are similar for the paired group and random controls with the exception of the response to light-attenuated 4 ND units. However, the light-adapted photoresponse is overall significantly smaller for the paired group as compared to the random controls ($p < 0.01$). Conditioning results in a smaller light-adapted generator potential as compared to the random controls. This shows that the diminished photoresponse is due to an intrinsic property of the conditioned B-photoreceptors.

Photoresponse amplitude. If the memory of the associative experience is stored in the photoreceptors, then the question can be raised concerning the part or phase of the B-photoreceptor response (transient peak, plateau, steady state) that contains the information necessary for the expression of modified phototactic behavior. The results of this study demonstrate that the early transient peak of the generator potential in response to bright and moderately bright light is not significantly altered by conditioning. In fact, the early peak response to bright light (0 attenuation) was virtually identical for the paired group and random controls examined in this study (see Fig. 5). These results are consistent with the initial observations of generator potential amplitude in conditioned animals in response to brief light flashes reported by Crow and Alkon (1980a). West et al. (1982) also reported that in response to bright light the peak initial transient of the B-photoreceptors photoresponse was not significantly different for paired as compared to random controls. In contrast, more recent observations have reported significant differences in the peak photoresponse between paired and random controls in the presence of 15 mM 4-aminopyridine (Farley and Alkon, 1983). However, the differences between the paired group and random controls in the Farley and Alkon study (1983) may be the result of a smaller generator potential in the control group rather than a potentiation or enhancement

of the peak photoresponse amplitude of conditioned animals. Additional evidence to support this conclusion comes from studies of normal untrained animals (T. Crow, unpublished observations), which show that the peak amplitude of the generator potential is virtually identical to values for the conditioned and random controls reported in this study. These results show that the conditioning procedure does not enhance the transient peak of the generator potential in response to bright (0 attenuation) and moderately bright illumination (2 ND). The present results do demonstrate that the transient peak of the B-photoreceptor to dim light (4 ND) is enhanced by conditioning (see Fig. 5). This result is consistent with an earlier report showing an increase in B-photoreceptor spike activity of conditioned animals evoked by dim illumination (Crow and Alkon, 1980b). However, under light-adapted conditions, the same B-photoreceptors of conditioned animals exhibit a diminished photoresponse in the presence of dim (4 ND) illumination. This indicates that if the B-activity is involved in phototactic behavior, then the activity of B-photoreceptors under light-adapted conditions shows a closer correspondence to the behavior than photoresponse activity in the first few hundred milliseconds (transient peak amplitude) of the generator potential. Taken together, these results indicate that the information provided by the B-photoreceptors that may be related to the expression of phototactic behavior is not contained in the early (transient peak) part of the generator potential.

Light-adapted discharge rate. The analysis of the light-adapted discharge rate of B-photoreceptors shows that conditioning results in a decrease in photoreceptor activity (see Figs. 1 to 4). This decrease in discharge rate could be due to synaptic interactions between photoreceptors within the eyes of *Hermissenda*. It was previously shown that the type B photoreceptors within each eye are mutually inhibitory (Alkon and Fuortes, 1972). Therefore, the decrease in discharge rate could be caused by an increase in the frequency of IPSPs, an enhancement of IPSP amplitude or both. An increase in the frequency of IPSPs would indicate that the discharge rate of at least one light-adapted B-photoreceptor is enhanced by conditioning. This possibility is not supported by the data collected from the B-photoreceptors following conditioning. An examination of the light-adapted generator potentials of B-photoreceptors following axotomy showed that the light-adapted photoresponse was smaller in conditioned animals. Since axotomy eliminates synaptic interactions between the photoreceptors, the diminished photoresponse must be due at least in part to changes that are intrinsic to the photoreceptors of conditioned animals.

B-Photoreceptor activity and phototactic behavior. It has been suggested that complex network interactions between the type B photoreceptor, type A photoreceptor, interneurons, and motor neurons can account for long lasting suppression of phototactic behavior (Alkon, 1983). According to this proposal, enhanced B-photoreceptor excitatory responses of conditioned animals inhibit type A photoreceptors and thus decrease the frequency of excitatory synaptic input from A-type photoreceptors to interneurons and motor neurons involved in turning the animal toward light (Alkon, 1983). Thus, if the motor neurons involved in turning the animal toward light are inhibited, there should be a suppression of positive phototactic behavior.

Evidence has been presented showing a decrease in light-evoked type A photoreceptor activity with conditioning (Farley and Alkon, 1982) and a decrease in light-evoked activity in a motor neuron associated with turning (Goh and Alkon, 1984). However, it is not clear that only the decrease in type A photoreceptor activity is directed related to the expression of phototactic suppression. Furthermore, it has yet to be established that the animals' inability to turn toward light is the

critical event either in the delay to locomote towards light or the decrease in the time that conditioned animals remain in the light (see "Discussion" in preceding paper). In fact, the behavioral evidence (Crow, 1985) would argue against such a proposal. Conditioned animals remain in light less after entering the illuminated area although the number of entries is not different from preconditioning base line tests. If conditioned animals are unable to turn in the presence of light, then one might argue that the animals would remain in light longer.

An alternative to the network hypothesis involving B-photoreceptor inhibition of A-photoreceptors as the critical event is provided by the results of this report. If phototactic behavior is controlled by the output of the B-photoreceptors, then an increase in photoreceptor activity, such as produced by an increase in illumination, would increase positive phototactic behavior. This is consistent with the behavioral observations showing that light intensity controls phototactic behavior (see preceding paper). Thus, if conditioning results in a decrease in the photoresponse to a particular light intensity, then phototactic behavior would decrease in response to the same light intensity after conditioning. However, any cellular explanation of phototactic suppression must account for the behavioral change found in response to a broad range of light intensities. The decreased photoresponse found in conditioned animals in this study fits the behavioral results for phototactic suppression in response to light-attenuated 4 and 2 ND units, although it cannot explain the behavioral suppression in response to bright light (0 attenuation) (see Fig. 4), since the discharge rate is not significantly different between 4 ND and 0 attenuation and phototactic behavior is different. This means that it is very unlikely that an alteration in a single B-photoreceptor in one eye can explain phototactic suppression.

However, the summation of activity between the B-type and A-type photoreceptors may provide a possible explanation since the type A photoreceptors are sensitive to bright light and thus would respond to light-attenuated 2 ND units and 0 attenuation, but not to light-attenuated 4 ND units under these periods of dark adaptation. If, in addition, the type A photoreceptors of conditioned animals exhibited an intrinsic decreased discharge rate in response to bright light, then the decreased excitatory output of the visual system of *Hermisenda* would follow phototactic suppression over a broad range of light intensities. Another possibility is that the B-photoreceptors are light adapted in less intense illumination while the animals are at the starting end of the tubes before initiating locomotor responses; thus, the animals never actually experience a bright light (0 attenuation) immediately after the 12 min of dark adaptation. These alternatives are currently under investigation.

Neural correlates of conditioning in B-photoreceptors. How is this newly identified correlate of conditioning in the B-photoreceptors related to previously investigated correlates of conditioning in *Hermisenda*? In the initial report implicating the involvement of the B-photoreceptors in conditioning, it was found that in conditioned animals the spontaneous activity of dark-adapted B-photoreceptors was increased, the dark-adapted input resistance was significantly increased, and the B-photoreceptors of dark-adapted conditioned animals were more depolarized than random controls (Crow and Alkon, 1980a). In addition the amplitude of the hyperpolarizing dip and depolarizing tail of the generator potential in response to brief light flashes were significantly increased in conditioned animals (Crow and Alkon, 1980a).

In the initial study, the neural correlates indicating an increase in photoreceptor excitability were found shortly after the conclusion of behavioral training. Subsequent studies have examined long term correlates in the B-photoreceptors 1 to 2 days following behavioral conditioning (Crow and Alkon,

1980b; Crow, 1982, 1983b, 1983c; West et al., 1982; Farley and Alkon, 1982). These results showed that the increased dark-adapted input resistance and enhanced tail of the generator potential persisted several days after conditioning while the increased discharge rate of dark-adapted photoreceptors and the tonic depolarization found immediately after conditioning were not long term (West et al., 1982; Farley and Alkon, 1982). It was also suggested that conditioned animals exhibited an enhanced response to light based upon an increase in light-evoked discharge rate of B-photoreceptors during the terminal 25 sec of a 30-sec light step (Farley and Alkon, 1982). The reported significant differences ($p < 0.10$) were found only in response to two of the four light intensities that were tested in the horizontal orientation 24 hr after conditioning in the Farley and Alkon study.

An examination of the discharge rate of the last 25 sec of the initial 30 sec of the B-photoreceptors light response in the present report did not reveal significant differences between the conditioned group and random controls. The differences reported by Farley and Alkon (1982) may be due to the low discharge rate for the cells in the random control group rather than an enhancement of light-evoked activity produced by conditioning. It is also possible that the inconsistencies reflect actual differences in the photoresponses of the three B-photoreceptors (medial, central, lateral) within each eye.

How are the various correlates of conditioning that have been investigated in the B-photoreceptors related to the expression of phototactic suppression? All of the previously published cellular correlates of conditioning in B-photoreceptors have been based upon an analysis of the early phase of the photoresponse. However, the behavior that is examined after conditioning is generated after the animals have been light adapted for many minutes. Therefore it is difficult to argue that cellular events occurring during the early phase of the photoresponse can explain behavioral changes that occur on a much longer time scale. None of the previous studies of cellular correlates have examined changes under steady state light-adapted conditions that correspond to the conditions under which the behavior is expressed. Indeed, the results of this report show that the early phase of the photoresponse evoked by dim light (4 ND units of attenuation) is enhanced by conditioning (see Fig. 5). However, the same B-photoreceptors exhibited a diminished response after 5 min of light adaptation. The enhanced response to dim light found in this study is in general consistent with the increased excitability of B-photoreceptors observed in the dark-adapted state reported by Crow and Alkon (1980a) and the increased light evoked discharge rate evoked by dim light reported by Crow and Alkon (1980b). However, the present results show that the peak amplitude of the photoresponse was not enhanced at brighter light intensities. Taken together, these results suggest that the diminished photoresponse of conditioned animals measured under light-adapted conditions exhibits a closer correspondence to phototactic suppression than previously studied correlates in B-photoreceptors.

Mechanisms of associative modifications in B-photoreceptors. It was recently suggested that the previously reported increase in the dark-adapted input resistance of conditioned animals can be explained by a decrease in a transient outward K^+ current (I_A) (Alkon et al., 1982), and that this reduction in I_A would enhance the photoresponse. The quantitative contribution that the A-current makes in modulating the photoresponse is unclear. However, a recent modeling study that incorporated the reduction in the A-current produced by conditioning (30% reduction) showed that the reduction in the magnitude of the A-conductance had little effect upon the initial generator potential (Shoukimas and Alkon, 1983). This finding is in agreement with the results of voltage recordings presented in this report which show that the peak of the generator potential is

not significantly affected by conditioning. The possible effect of a reduction in I_A on other phases of the generator potential (plateau, steady state) has not been systematically explored in *Hermisenda*. However, it is unlikely that the reduction in I_A could account for the diminished photoresponse found under light-adapted conditions since the reduction of an outward K^+ current would be expected to increase the photoresponse, not reduce its amplitude.

In addition, recent evidence has shown that conditioning produces an increase in light-evoked photoreceptor desensitization that cannot be mimicked by blocking I_A (Crow, 1982, 1983b). Photoreceptors from conditioned animals also exhibited a delay in the recovery of sensitivity following adapting light (T. Crow, in preparation). These results suggest that the regulation of intracellular Ca^{2+} may play a role in the conditioning effects of B-photoreceptors. A rise in intracellular Ca^{2+} may result in a diminished photoresponse by either increasing an outward K^+ current (Ca^{2+} -dependent K^+ current) or perhaps by reducing the light-activated conductance. These proposals should result in a decrease in the steady state voltage response to illumination.

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