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Light Paired with Serotonin *in vivo* Produces Both Short- and Long-Term Enhancement of Generator Potentials of Identified B-Photoreceptors in *Hermissenda*

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An *in vivo* conditioning procedure consisting of light paired with the direct application of 5-HT to the exposed but otherwise intact nervous system of *Hermissenda* produces a long-term modification of phototactic behavior. The long-term change in phototactic behavior produced by *in vivo* conditioning is dependent upon pairing light with 5-HT. In this paper, we investigate neural correlates of *in vivo* conditioning detected in 2 different identified photoreceptors. We found that *in vivo* conditioning produces a short-term and long-term enhancement of light-evoked generator potentials recorded from medial and lateral B-photoreceptors. We show that short-term enhancement is not dependent upon pairing light with 5-HT, is observed in both lateral and medial B-photoreceptors, and is expressed by a larger peak and plateau phase of light-evoked generator potentials. In contrast to short-term enhancement, we found that long-term enhancement is dependent upon pairing light with 5-HT, is detected in only lateral B-photoreceptors, and is expressed by a larger steady-state plateau phase of light-evoked generator potentials. We also present evidence that the direct action of 5-HT interacting with light- and/or voltage-dependent processes is sufficient to mimic the effects of *in vivo* conditioning on long-term enhancement. These results suggest that long-term enhancement may contribute to modified phototactic behavior in *Hermissenda* produced by 1-trial *in vivo* conditioning.

Phototaxis in *Hermissenda,* an example of visually guided behavior, can be modified by a 1-trial *in vivo* conditioning procedure. The conditioning trial consists of pairing light, the conditioned stimulus (CS), with the direct application of 5-HT to the exposed circumesophageal nervous system of otherwise intact *Hermissenda* (Crow and Forrester, 1986). While the cellular mechanisms for the modification of behavior are not known, it has been proposed that the effect of *in vivo* conditioning on phototactic behavior may be due, in part, to the action of 5-HT interacting with light- and/or voltage-dependent conductances in identified B-photoreceptors (Crow, 1988). In sensory systems of a number of invertebrate species, neuromodulators such as 5-HT have pronounced effects on both behavior and the excitability of identified target neurons (Adolph and Tuan, 1972; Barlow et al., 1977; Gershon, 1977; Corrent et al., 1978; Klein and Kandel, 1978; Gelperin, 1981; Mackey and Carew, 1983; Crow and Bridge, 1985; Jacklet and Acosta-Urquidi, 1985; Ocorr and Byrne, 1985; Acosta-Urquidi et al., 1989; Dixon and Atwood, 1989; Farley and Wu, 1989; Hawkins, 1989; Hawkins and Schacher, 1989; Mackey et al., 1989). Application of 5-HT to the visual system of *Hermissenda* enhances both the amplitude and the duration of light-evoked generator potentials recorded from type-B photoreceptors (Crow and Bridge, 1985; Farley and Auerbach, 1986; Sakakibara et al., 1987). Biochemical studies have demonstrated that 5-HT is endogenous to the cerebropleural and pedal ganglia of *Hermissenda* (Heldman and Alkon, 1978). Several serotonergic neurons that may provide a presynaptic source of serotonergic input to the visual system were identified in a recent immunohistochemical study of the circumesophageal nervous system. These results revealed 5-HT-immunoreactive fibers and varicosities in close proximity to the optic nerve and in the synaptic region in the neuropil near the photoreceptor synaptic terminals (Land and Crow, 1985).

In this paper, we have examined intrinsic modifications of light-evoked generator potentials recorded from identified B-photoreceptors following *in vivo* conditioning. We have found that *in vivo* conditioning produces both a short-term and a long-term enhancement of light-evoked generator potentials recorded from different identified type-B photoreceptors. Short-term enhancement examined 1 hr after conditioning was found in both medial and lateral B-photoreceptors and was not dependent upon pairing the CS with 5-HT. Long-term enhancement examined 24 hr after *in vivo* conditioning was found only in lateral B-photoreceptors and was dependent upon pairing the CS with 5-HT. We present evidence that long-term enhancement is expressed in lateral B-photoreceptors by a larger amplitude of the steady-state plateau phase of the generator potential without affecting the amplitude of the transient peak.

Materials and Methods

Animals. A total of 223 adult *Hermissenda crassicornis* was used in the experiments. *Hermissenda* were obtained from Sea Life Supply Co. (Sand City, CA). The animals were maintained in an artificial seawater (ASW) aquarium at 14 ± 0.5°C on a 12-hr light/12-hr dark cycle. Animals were fed small pieces of scallops each day during the experiments.

Surgery. Animals were anesthetized by a 0.25–0.5-ml injection of isotonic $MgCl_2$ and a small incision was made to expose the dorsal surface of the circumesophageal nervous system, as shown in Figure

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1A. Following the surgery, the animals were placed into a chamber containing 50 ml of ASW. The exposed nervous system was visualized in infrared illumination provided by a 45-W tungsten/halogen light source projected by a light guide through an infrared filter (Schott model RG-850). A dissecting microscope formed an image of the nervous system in the infrared light upon a Dage MTi videocamera connected to a videomonitor and time-lapse videorecorder.

In vivo *conditioning procedure.* The details of the *in vivo* conditioning procedure have been described in a previous publication (Crow and Forrester, 1986) and will be described only briefly in this report. The protocols for conditioning and control procedures are shown in the diagram of Figure 2. After exposure of the nervous system, the animals were placed in a chamber containing ASW and were dark adapted for 12 min (see Fig. 2). Immediately after the period of dark adaptation, the *paired group* received 1 trial of light (CS) paired with the application of 0.5 ml 5-HT. The 5-HT was applied to the region of the cerebropleural ganglion where 5-HT-immunoreactive varicosities and processes were identified in a previous immunocytochemistry study (Land and Crow, 1985). Serotonin was applied to the desired region of the nervous system by observing the image of the cerebropleural ganglion and injecting pipette on a videomonitor. The concentration of 5-HT was adjusted to yield a final concentration in the ASW bath of 0.1 mM. Animals remained in the light in the presence of 5-HT for 5 min, followed by an ASW rinse and an additional 12 min of dark adaptation. An *unpaired control group* received the 5-min CS, followed by 5 min of dark adaptation before the application of 5-HT (see Fig. 2). The 5-HT was applied in the dark (infrared illumination) for the unpaired control group and remained in the ASW bath for 5 min, followed by the application of normal ASW. Animals in the *backward control group* received 5-HT applied in the dark (infrared light), followed by normal ASW application and 5 min in the dark before the 5 min CS. The *normal control group* received only light without the application of 5-HT. The different treatment conditions were coded so that the subsequent cellular neurophysiological analysis was conducted without knowing whether an animal was from the paired, unpaired control, or backward control groups. The animals were not sutured after the treatment because the wound margins typically closed within several hours after surgery and appeared normal when examined 24 hr after the treatment, as shown in the example in Figure 1B.

In order to assess the contribution of direct and indirect effects of 5-HT on type-B photoreceptors, the *in vivo* conditioning procedure was modified (shown in Fig. 8). After surgical exposure of the nervous system, 2 glass pipettes modified from patch-clamp electrodes were placed over each eye and proximal optic nerve. This procedure provided a tight seal between the pipette tip and base of the eye and optic nerve, as verified by injecting dye into the electrodes. The modification of the conditioning procedure consisted of pairing the CS with injection of 0.1 mM 5-HT into the electrodes containing the eyes. A control group received the 5-min CS, followed by 5 min of dark adaptation before injecting 5-HT into the electrode in the dark (infrared light).

Intracellular recording. Electrophysiological recordings were collected at 2 time periods following *in vivo* conditioning and control procedures. One hour or 24 hr after the termination of the treatments, the circumesophageal nervous system was removed from the animals and pinned to a stage in a recording chamber filled with ASW. Experiments were conducted in buffered ASW (10 mM HEPES; pH, 7.6) having the following composition (in mM): 460 NaCl, 10 KCl, 10 $CaCl_2$, and 55 $MgCl_2$. The ASW solution was brought to pH 7.6 at 15 ± 0.5°C with dilute NaOH. Identified type-B photoreceptors were isolated from synaptic interactions by cutting the optic nerve proximal to the cell body with a single-edge razor. The isolated nervous systems were incubated in a protease solution (Sigma Chemical Co.; 0.67 mg/ml, 5–6 min) to facilitate microelectrode penetration of identified B-photoreceptors. Glass microelectrodes used for intracellular recordings from isolated B-photoreceptors had resistances between 30 and 50 MΩ when filled with 4 M potassium acetate. The temperature of the ASW in the recording chamber was maintained at 14–15°C. Seawater temperature was monitored by a thermistor inserted into the recording chamber.

Light stimuli. Light steps (2 min) were delivered to the eyes by a tungsten/halogen source projecting through an optic fiber positioned above the preparation. The light was focused to a spot that covered the eye. The intensity of the light on the eye was 10×10^{-4} W/cm² at 510 nm, the wavelength used in all experiments. Photoreceptors were initially dark adapted for 15 min following electrode penetration before the delivery of the 2-min light step. After dark adaptation, light-evoked generator potentials were collected from identified isolated B-photoreceptors using standard intracellular recording procedures and microcomputer data acquisition and storage techniques. Short- and long-term enhancement is expressed in B-photoreceptors by increases in the amplitude of the peak and plateau phases of the generator potential. The transient peak represents the largest amplitude of the generator potential elicited at the onset of light measured from the dark-adapted resting potential. The plateau phase was determined by measuring the amplitude of generator potentials at the end of the 2-min light step. Both indices of enhancement are consistent with measurements of generator potential amplitude derived by integrating the voltage over time (Crow et al., 1991).

Four of the 5 photoreceptors within each eye of *Hermissenda* can be visually identified based upon their positions in the lateral, medial, anterior, and posterior part of the eye. For descriptive purposes, type-B photoreceptors located in the lateral-posterior and medial-posterior position in each eye will be referred to as lateral B-photoreceptors and medial B-photoreceptors, respectively. Different identified photoreceptors were examined following *in vivo* conditioning because it is not known if different B-photoreceptors within each eye represent a distinct and unique functional cell type or if they exhibit different potentials for the expression of short- and long-term enhancement.

Statistical analysis. A one-way analysis of variance was used to examine overall differences in the peak and plateau phases of generator potentials recorded from preparations that received paired, unpaired control, backward control, and normal control conditions. Data were analyzed separately for generator potentials collected at 1 hr (short-term) and 24 hr (long-term) after 1-trial conditioning. Following significant overall effects, paired comparisons involved the application of Dunnett's t statistic (Winer, 1962).

Results

The results of the analysis of neural correlates recorded from medial and lateral B-photoreceptors following *in vivo* conditioning are presented in 2 parts. In the first part, characteristics of light-evoked generator potentials recorded from isolated medial and lateral B-photoreceptors are described for paired, unpaired control, backward control, and normal control groups 1 hr after the conclusion of the different treatments. Increases in the amplitude of generator potentials detected 1 hr but not 24 hr after *in vivo* conditioning are referred to as short-term enhancement. In the second phase of the analysis, generator potentials recorded 24 hr following *in vivo* conditioning and control procedures are described. Increases in the amplitude of generator potentials recorded from identified photoreceptors 24 hr after conditioning are described as long-term enhancement.

Short-term enhancement. The statistical analysis of the amplitude of generator potentials evoked by light 1 hr after the conclusion of *in vivo* conditioning revealed that light responses were enhanced for both medial and lateral B-photoreceptors. Examples of light-evoked generator potentials from 4 different medial B-photoreceptors recorded after the paired, unpaired control, backward control, and normal control procedures are shown in Figure 3. The generator potentials recorded from the paired, unpaired control, and backward control groups were enhanced as compared to the example from a normal control group (Fig. 3D). The mean amplitude of the peak and plateau phases of light-evoked generator potentials for the paired, unpaired control, backward control, and normal control groups is shown in Table 1. Generator potential peak amplitudes recorded from groups that received light paired with 5-HT ($N = 17$), unpaired light and 5-HT ($N = 19$), the backward control procedure ($N = 6$), and normal controls ($N = 12$) showed significant overall effects ($F_{3,50} = 5.85$; $p < 0.01$). Comparing the different treatments to the normal controls revealed that the paired group ($t = 4.33$; $p < 0.005$), unpaired control group ($t = 2.72$; $p <$

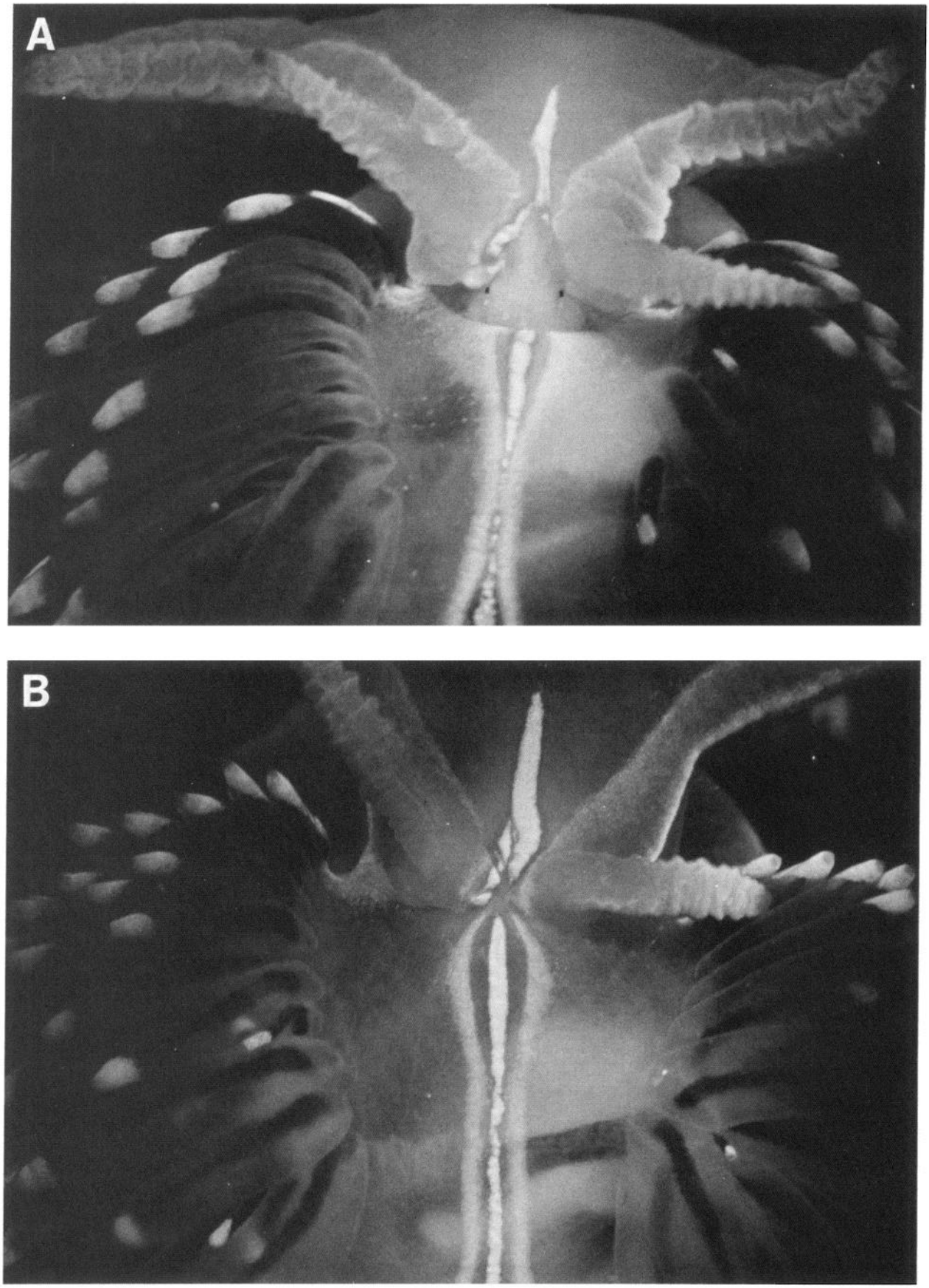

Figure 1. Photograph of the experimental preparation used to study short- and long-term enhancement following 1-trial *in vivo* conditioning. *A*, An example of a *Hermissenda* immediately following completion of a dorsal-lateral incision to expose the circumesophageal nervous system. The

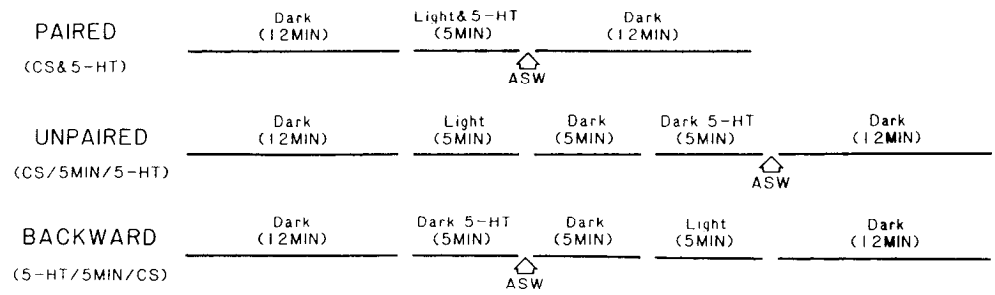

Figure 2. Experimental protocol for investigating 1-trial *in vivo* conditioning. Animals were assigned to 1 of 3 different treatment conditions. Following 12 min of dark adaptation, the paired light (CS) and 5-HT group received 1 5-min trial of light paired simultaneously with the injection of 5-HT onto the exposed nervous system, followed by a wash with normal ASW and 12 min in the dark. The unpaired control group received the light CS (5 min) followed by a 5-min period in the dark before applying 5-HT (5 min) to the nervous system in the dark (infrared illumination). The backward control group received the 5-HT (5 min) in the dark followed by a 5-min period of dark adaptation in normal ASW before presenting the light (CS).

0.025), and backward control group ($t = 2.59$; $p < 0.05$) were significantly different from normal controls.

The analysis of the plateau phase of the generator potential revealed that the normal controls were significantly different from the paired group ($t = 2.48$; $p < 0.05$), the unpaired control group ($t = 2.46$; $p < 0.05$), and the backward control group ($t = 2.45$; $p < 0.05$). However, the peak and plateau phases of generator potentials recorded from medial B-photoreceptors from the paired, unpaired control, and backward control groups were not significantly different from each other. These results indicate that short-term enhancement detected in medial B-photoreceptors is not dependent upon pairing light and 5-HT, and enhancement is expressed by an increase in both the peak and plateau phases of light-evoked generator potentials. We next examined generator potentials recorded from lateral B-photoreceptors evoked by light 1 hr following the application of light and 5-HT. Generator potentials recorded from lateral B-photoreceptors from the paired, unpaired, and backward control groups were enhanced as compared to normal controls. The mean amplitude of the peak and plateau phase of the generator potentials recorded from lateral B-photoreceptors is shown in Table 1. The statistical analysis revealed an overall difference between the paired ($N = 23$), unpaired control ($N = 22$), backward control ($N = 10$), and normal control groups ($N = 12$; $F_{3,63} = 2.98$; $p < 0.05$). Comparisons between the various treatment groups and normal controls showed that the plateau phases from paired ($t = 2.93$; $p < 0.01$), unpaired control ($t = 2.4$; $p < 0.05$), and backward control groups ($t = 2.32$; $p < 0.05$) were significantly different from normal controls, while none of the treatments differed from each other.

Consistent with the analysis of the plateau phase was the finding that the peak amplitude of the generator potential recorded from the paired ($t = 3.07$; $p < 0.005$), unpaired control ($t = 2.28$; $p < 0.05$), and backward control groups ($t = 2.1$; $p < 0.05$) were significantly larger as compared to normal controls. These results indicate that the presentation of light and 5-HT produces a non-pairing-specific enhancement of the peak and plateau phase of generator potentials in both lateral and medial type-B photoreceptors.

Long-term enhancement. Our previous research has shown that pairing light with the direct application of 5-HT to the exposed nervous system produced a pairing-specific change in phototactic behavior detected 24 hr after *in vivo* conditioning. To examine possible long-term neural correlates of *in vivo* conditioning, we recorded generator potentials from identified and isolated medial and lateral B-photoreceptors following treatment and control procedures.

Examples of light-evoked generator potentials recorded from medial B-photoreceptors of paired and unpaired control groups collected 1 hr and 24 hr following the conclusion of *in vivo* conditioning are shown in Figures 4 and 5. In contrast to the results of the analysis of generator potential amplitudes at 1 hr after conditioning, we found that *in vivo* conditioning does not produce long-term enhancement of generator potentials from medial B-photoreceptors recorded 24 hr after conditioning. This is supported by the statistical analysis, which did not reveal overall significant differences between the treatment and control groups. Group means for the peak and plateau phases of the generator potential recorded from medial B-photoreceptors are shown in Table 2. Paired comparisons showed that the paired ($N = 11$) and unpaired control groups ($N = 11$) were not significantly different from each other or normal controls ($N = 12$). These results show that *in vivo* conditioning produces only a short-term enhancement of generator potentials recorded from medial B-photoreceptors. The comparison between the peak amplitude of the generator potential recorded 1 hr and 24 hr after conditioning provides additional support for this conclusion. The peak amplitude of the generator potential was significantly smaller for the paired group 24 hr after conditioning as compared to response recorded 1 hr after conditioning ($t = 2.69$; $p < 0.025$). Similar results were obtained from the analysis of the plateau phase of the generator potential ($t = 2.36$; $p < 0.05$). The paired and unpaired control groups were not significantly different from each other or normal controls 24 hr after *in vivo* conditioning.

Examples of generator potentials recorded from 2 different lateral B-photoreceptors 24 hr after paired and unpaired light and 5-HT are shown in Figure 6. In contrast to the results of

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in vivo conditioning procedure consists of pairing light with the direct application of 5-HT to the exposed nervous system of otherwise intact animals. *B.* A photograph of the preparation as shown in *A* taken 24 hr after exposure of the nervous system, showing that the wound margins have closed and the animal appears normal. Intracellular recordings are collected from identified photoreceptors within the eyes of preparations that have recovered as indicated in *B.*

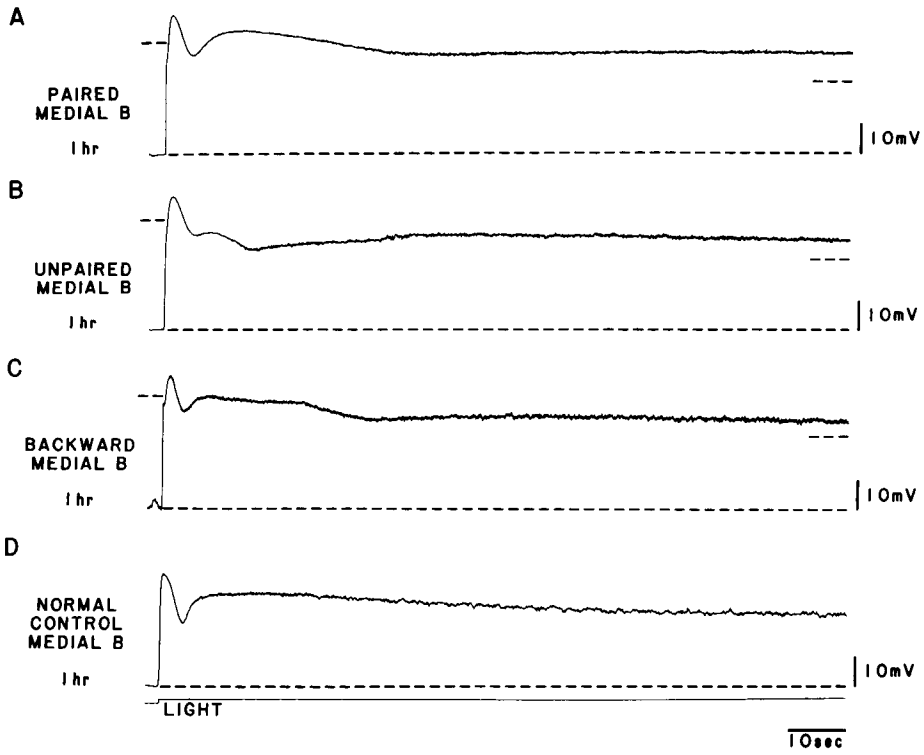

Figure 3. Short-term enhancement of light-evoked generator potentials recorded from surgically isolated medial B-photoreceptors 1 hr after *in vivo* conditioning. *A*, Intracellular recording of a generator potential from an isolated medial B-photoreceptor evoked by a 2-min light step 1 hr following the presentation of light paired with 5-HT. *B*, Intracellular recording of a light-evoked generator potential from a medial B-photoreceptor 1 hr after unpaired light and 5-HT. *C*, Generator potential evoked by a 2-min light step recorded from a medial B-photoreceptor 1 hr following the backward control procedure. *D*, Generator potential of a medial B-photoreceptor recorded from a normal control preparation. The amplitudes of the generator potentials shown in the examples in *A–C* are enhanced as compared to the normal control shown in *D*. The *short dashed lines* in the recordings in *A–C* represent the peak and plateau amplitude of the normal control shown in *D*.

the analysis of light-evoked generator potentials from medial B-photoreceptors, lateral B-photoreceptors exhibited a long-term enhancement. In addition, unlike short-term enhancement, long-term enhancement detected in lateral B-photoreceptors is specific to pairing light and 5-HT (Fig. 7). The statistical analysis of the generator potential peak revealed that there were no overall significant differences. The paired ($N = 26$), unpaired control ($N = 23$), and backward control groups ($N = 5$) were not significantly different from each other or normal controls ($N = 12$). However, the analysis of the plateau phase measured at the end of the 2-min light step revealed an overall significant difference ($F_{3,62} = 5.93$; $p < 0.01$). Paired comparisons showed that the plateau phase of the paired group was significantly larger than the normal control ($t = 3.66$; $p < 0.005$), unpaired control ($t = 3.32$; $p < 0.005$), and backward control group ($t = 2.82$; $p < 0.01$), while the unpaired and backward controls were not significantly different from normal controls. These results show that long-term enhancement is expressed only in lateral B-photoreceptors by a larger amplitude of the plateau phase of the generator potential (Fig. 7).

In summary, the results indicate that *in vivo* conditioning produces 2 effects in identified photoreceptors within the eyes of *Hermissenda*. One effect is a short-term enhancement of generator potentials detected in both lateral and medial B-photoreceptors. Short-term enhancement does not depend upon pairing light with 5-HT because paired, unpaired control, and

Table 1. Short-term enhancement of generator potentials

	Medial type-B photoreceptors		Lateral type-B photoreceptors	
Experimental condition	Peak	Plateau	Peak	Plateau
Paired	44.3 ± 1.20* ($n = 17$)	29.9 ± 0.66*	43.3 ± 0.71* ($n = 23$)	29.3 ± 0.72*
Unpaired control	43.1 ± 0.84* ($n = 19$)	28.5 ± 0.49*	42.9 ± 0.78* ($n = 22$)	28.9 ± 1.08*
Backward control	42.6 ± 1.09* ($n = 6$)	28.5 ± 0.51*	42.5 ± 1.2* ($n = 10$)	28.7 ± 0.93*
Normal control	38.2 ± 0.35 ($n = 12$)	25.5 ± 1.03	39.0 ± 1.1 ($n = 12$)	24.7 ± 0.99

This table shows the mean amplitude (mV ± SEM) of the generator potential peak and plateau phase evoked by a 2-min light step recorded from medial and lateral B-photoreceptors 1 hr after the experimental and control procedures described in Materials and Methods.

* Indicates comparisons that are significantly different from normal controls as described in Results (n = number of photoreceptors).

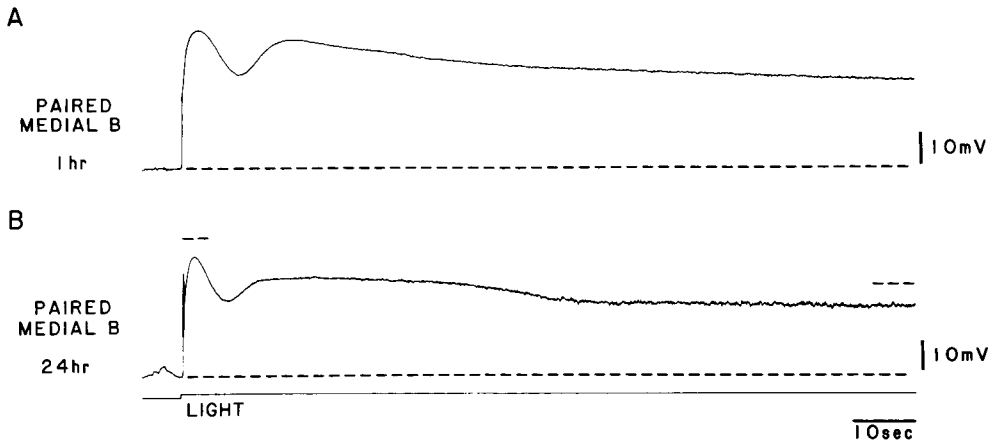

Figure 4. Medial B-photoreceptors express short-term enhancement, but not long-term enhancement, 24 hr following *in vivo* conditioning. *A,* Generator potential from a medial B-photoreceptor evoked by the light step 1 hr following the presentation of light paired with 5-HT. *B,* Generator potential recorded from a medial B-photoreceptor 24 hr following paired light and 5-HT. The *short dashed lines* in the recording in *B* represent the peak and plateau amplitude of the generator potential shown in *A*. The generator potential shown in *A* is enhanced as compared to the example shown in *B*. These results show that medial B-photoreceptors do not exhibit long-term enhancement.

backward control results were similar. The second effect, which was observed only in lateral B-photoreceptors, is a long-term enhancement of the generator potential that depends upon pairing light with 5-HT. The long-term enhancement is expressed by an increase in the amplitude of the steady-state plateau phase of the generator potential.

Assessment of direct effects. Long-term enhancement may be the result of either a direct effect of 5-HT upon lateral B-photoreceptors or indirect effects mediated by neurons presynaptic to the photoreceptors that are activated by 5-HT. In order to examine this issue, we paired light with the direct application of 5-HT to the eyes using the procedures shown in the diagram in Figure 8. Significant long-term enhancement of the plateau phase of the generator potential was observed for the group that received the CS paired with injection of 5-HT into the pipettes ($N = 6$), as compared to an unpaired control group ($N = 7$; $t_{11} = 3.04$; $p < 0.01$). Group means of the plateau phase for the paired and unpaired control groups are shown in Figure 9. The enhanced plateau phase of the generator potential from the paired group as compared to the mean amplitude of the plateau phase for the unpaired control group indicates that direct application of 5-HT to lateral B-photoreceptors when paired with the CS is sufficient to produce a significant long-term enhancement of generator potentials.

Discussion

One trial in vivo *conditioning: relationship to behavior*

We have found that *in vivo* conditioning produces both short- and long-term enhancement of generator potentials in different identified photoreceptors within the visual system of *Hermissenda.* Because the enhanced generator potentials are expressed after conditioning in isolated B-photoreceptors, the changes are intrinsic and, once induced, are not due to an alteration in synaptic input from neurons that are presynaptic to the photoreceptors. This finding suggests that long-term enhancement may contribute to the modification of phototactic behavior produced by *in vivo* conditioning because postsynaptic neurons that contribute to the locomotor circuit would be expected to receive enhanced synaptic input from photoreceptors. However, the precise postsynaptic action of enhancement in the visual system has not been determined. In an earlier report, we presented evidence that the exogenous application of 5-HT, when paired with the CS, can effectively serve as an unconditioned stimulus (US) to produce long-term phototactic suppression (Crow and Forrester, 1986). Because the component of phototactic behavior that is modified by *in vivo* conditioning has been shown previously to exhibit long-term phototactic suppression following 3 d of behavioral conditioning, it is attractive to suggest that a common mechanism may underlie both examples of conditioning. In fact, it has been suggested that 5-HT may be the transmitter in the US pathway. Consistent with this view are recent reports of the involvement of 5-HT in *in vitro* conditioning of the isolated nervous system (Grover et al., 1989) and evidence for serotonergic modulation of membrane conductances of B-photoreceptors (Crow and Bridge, 1985; Farley and Wu, 1989; Acosta-Urquidi and Crow, 1990). Additional support for this hypothesis comes from cellular studies, where there is now a considerable amount of evidence indicating that classical conditioning produces cellular correlates that are intrinsic to the type-B photoreceptors in *Hermissenda* (for a recent review, see Crow, 1988). While it is attractive to suggest that 5-HT plays a role in classical conditioning of *Hermissenda,* there are reported differences in the cellular correlates produced by 1-trial *in vivo* conditioning and the conditioning procedure consisting

Table 2. Generator potential amplitude 24 hr following *in vivo* conditioning

Experimental condition	Medial type-B photoreceptors		Lateral type-B photoreceptors
	Peak	Plateau	Peak
Paired	40.7 ± 1.7 ($n = 11$)	25.1 ± 2.06	40.1 ± 1.01 ($n = 26$)
Unpaired control	40.5 ± 0.91 ($n = 11$)	26.0 ± 2.2	39.9 ± 0.58 ($n = 23$)
Backward control	39.5 ± 2.0 ($n = 2$)	25.6 ± 1.8	40.7 ± 2.4 ($n = 5$)
Normal control	39.2 ± 0.53 ($n = 12$)	25.9 ± 1.2	40.2 ± 1.8 ($n = 12$)

This table shows the mean amplitude (mV ± SEM) of generator potential peak and plateau phase evoked by a 2-min light step recorded from medial B-photoreceptors and peak phase recorded from lateral B-photoreceptors 24 hr after the experimental and control procedures described in Materials and Methods. The mean amplitude of the plateau phase for lateral B-photoreceptors is shown in Figure 7. None of the comparisons were significantly different from normal controls as described in Results (n = number of photoreceptors).

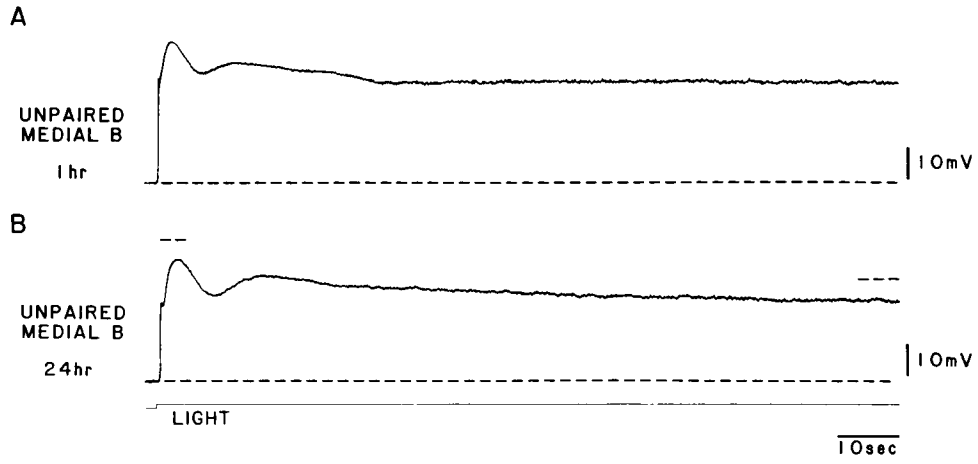

Figure 5. Medial B-photoreceptors exhibit short-term enhancement, but not long-term enhancement, 24 hr after unpaired light and 5-HT. *A*, Generator potential from a medial B-photoreceptor evoked by the light step 1 hr after the presentation of unpaired light and 5-HT. *B*, Generator potential recorded from a medial B-photoreceptor 24 hr after unpaired light and 5-HT. The *short dashed lines* in the recording in *B* represent the peak and plateau amplitude of the generator potential shown in *A*. The generator potential is enhanced at 1 hr but not at 24 hr.

of multiple-session training. Multiple-session training consists of 50 trials of the CS paired with the US (high-speed rotation) each day, for 3 consecutive days (Crow and Alkon, 1978). First, it has been reported that 150 trials of multiple-session training produces a reduction in the amplitude of the steady-state plateau phase of light-evoked generator potentials measured after 5 min of illumination (Crow, 1985). We suggested that long-term phototactic suppression produced by conditioning may be explained by the CS eliciting a smaller generator potential (Crow, 1985). Second, it was recently reported that only medial B-photoreceptors exhibit enhanced generator potentials and a concomitant reduction in 2 K^+ currents detected 24 hr after 150 trials of multiple-session training (Alkon et al., 1985). These findings suggested that phototactic suppression was due to enhanced B-photoreceptor inhibition of type-A photoreceptor output. Thus, light-dependent excitation of medial type-A photoreceptors would be diminished by enhanced type-B inhibition of medial A-photoreceptors that project via interneurons to motor neurons (Alkon, 1984; Alkon et al., 1985). However, neural correlates of conditioning detected in putative motor neurons are inconsistent with this interpretation. It was reported that, after classical conditioning, the activity recorded from identified pedal neurons elicited by the CS (light) was reduced below the frequency of spontaneous activity recorded in the dark (Crow, 1981). Because type-A photoreceptors do not typically discharge action potentials spontaneously in the dark, their activity during illumination cannot be less than their activity in the dark. In addition, previous research has shown that behavior in response to the CS is suppressed by conditioning, while locomotion in the dark is not significantly changed after conditioning (Crow and Offenbach, 1983). These results suggest that B-photoreceptors may project to interneurons that inhibit pedal motor neurons or project to spontaneously active interneurons that are inhibited by type-B photoreceptor synaptic input. Moreover, different identified type-B photoreceptors may project to different postsynaptic neurons with different motor projections. Thus, differences in cellular correlates produced by the 2 procedures may indicate that a complex behavioral response such as visually guided locomotion can be modified by different cellular mechanisms contributing to the same behavioral outcome. Taken collectively, the present findings may indicate that 1-trial *in vivo* conditioning produces phototactic suppression by a mechanism quite different from the one produced by multiple-session conditioning. However, because little is known about cellular correlates produced by a single conditioning session, it may be premature to conclude that the mechanisms are, in fact, different. While there are clear differences in outcomes, there are some interesting similarities between single-session training and 1-trial *in vivo* conditioning (see next section). A satisfactory explanation for phototactic suppression requires an understand-

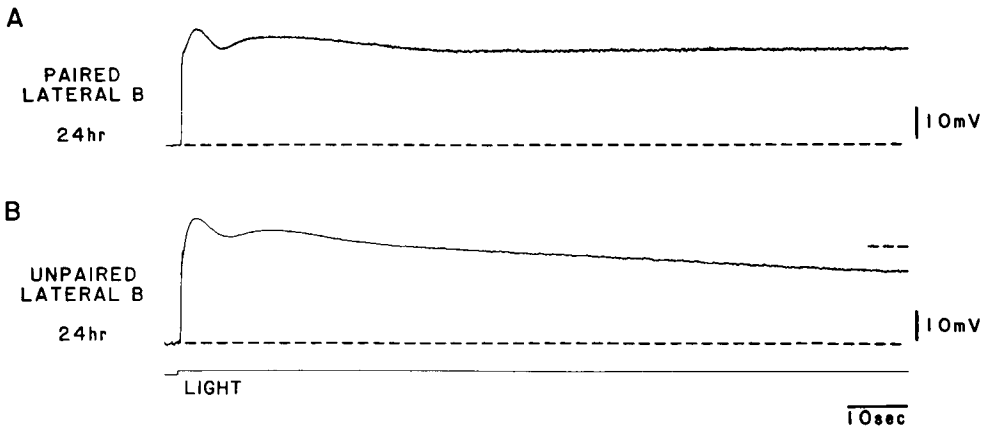

Figure 6. Long-term enhancement of light-evoked generator potentials recorded from surgically isolated lateral B-photoreceptors 24 hr following *in vivo* conditioning. *A*, Generator potential from an isolated lateral B-photoreceptor evoked by light 24 hr following the presentation of light paired with 5-HT. *B*, Generator potential recorded from a lateral B-photoreceptor 24 hr following unpaired light and 5-HT. The generator potential in *A* exhibits an enhanced plateau as compared to the example shown in *B*. The peak of the generator potential recorded from lateral B-photoreceptors does not show long-term enhancement. The *short dashed line* in *B* represents the amplitude of the generator potential plateau shown in *A*.

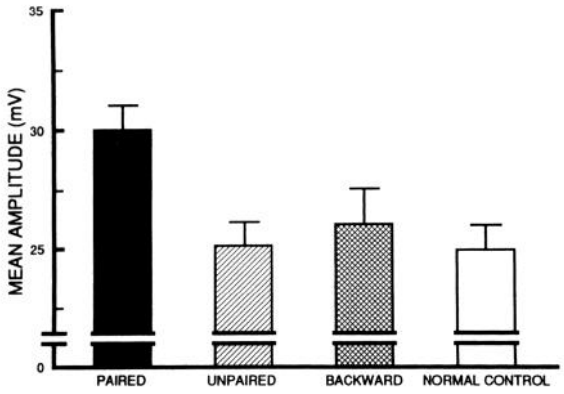

Figure 7. Group data showing the mean amplitude (mV ± SEM) of the generator potential plateau phase recorded from lateral B-photoreceptors for the groups that received the CS paired with 5-HT (N = 26), the unpaired CS and 5-HT control (N = 23), the backward control group (N = 5), and normal control treatment (N = 12). The plateau phase of the generator potential is significantly enhanced for the paired light and 5-HT group as compared to the different control groups.

ing of how the activity of photoreceptors within the visual system can influence the activity of the neural network responsible for locomotion in *Hermissenda.* To date, the interactions between photoreceptors and postsynaptic neurons in the circuit generating locomotion are not known; however, such studies have been initiated (Crow, 1981; Goh and Alkon, 1984; Hodgson and Crow, 1987; Richards and Farley, 1987).

One-trial in vivo *conditioning and single-session training*

It is of interest to note some of the similarities between 1-trial *in vivo* conditioning and studies of single-session conditioning in *Hermissenda.* First, we reported that 5 and 10 conditioning trials resulted in significant short-term nonassociative changes in phototactic behavior (Crow, 1983). These results indicated that the nonassociative contribution to phototactic behavior was expressed soon after training and decremented rapidly following the conclusion of both single-session and multiple-session training. Our present results show that immediate or short-term enhancement is not dependent upon pairing (nonassociative), and that the pairing-dependent enhancement is expressed at 24 hr, a time when the short-term nonassociative enhancement has decremented. These studies suggested that, because associative and nonassociative components of phototactic suppression follow different time courses, the underlying mechanisms may be independent; that is, the mechanism for the associative effect may not be an elaboration of the nonassociative mechanism. It is interesting to note that the nonassociative correlate of *in vivo* conditioning is expressed before the long-term associative correlate. However, we have evidence that long-term enhancement is independent of short-term nonassociative enhancement (see next section).

Short- and long-term enhancement: possible mechanisms

The suggestion that short- and long-term enhancement may involve different mechanisms is supported by our recent cellular studies. First, we have shown that long-term enhancement depends upon protein synthesis, while short-term enhancement does not (Crow and Forrester, 1990). Second, we have examined the role of activation of protein kinases in the induction and expression of short- and long-term enhancement (Crow and

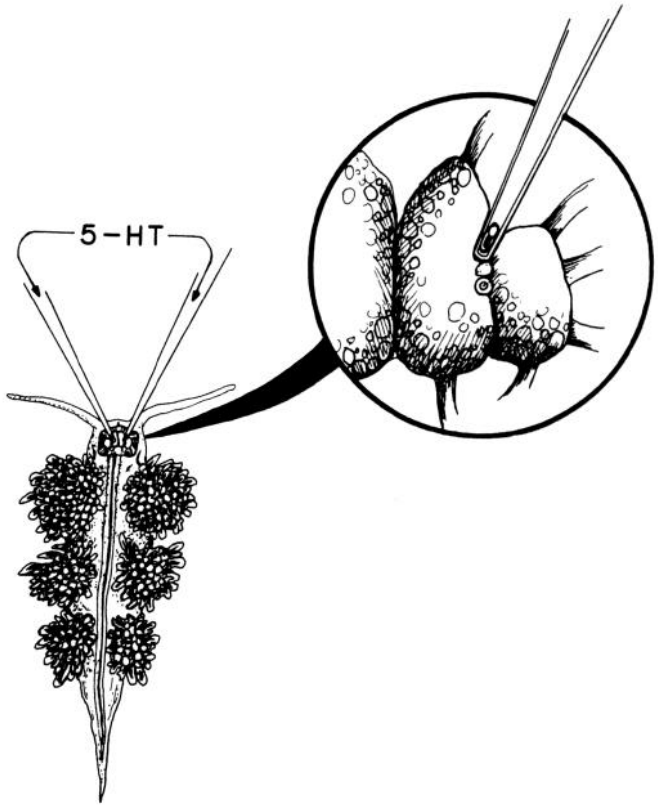

Figure 8. Drawing of modification of the *in vivo* conditioning procedure designed to restrict stimulation with light and 5-HT to the visual system. Two glass pipettes, prepared from patch-clamp electrodes and mounted in micromanipulators, were placed over each eye of a surgically prepared animal. Illumination of the eyes was paired with the injection of 0.1 mM 5-HT into each pipette. An unpaired control group received 5 min of light and a 5-min period of dark adaptation before the 5-HT injection in the dark (infrared illumination; see protocol in Fig. 2).

Forrester, 1988; Forrester and Crow, 1988, 1989; Crow et al., 1991). Numerous studies have implicated the Ca^{2+}/phospholipid-dependent kinase protein kinase C (PKC) in cellular regulation and synaptic and cellular plasticity (Berridge, 1986; Farley

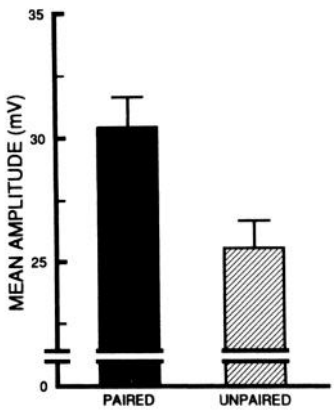

Figure 9. Group data showing the mean amplitude (mV ± SEM) of the generator potential plateau phase recorded from lateral B-photoreceptors for the groups that received the CS paired with 5-HT (N = 6) and the unpaired CS and 5-HT (N = 7). The paired group showed significant enhancement ($p < 0.01$) as compared to the unpaired control group. The direct application of 5-HT to the lateral B-photoreceptors when paired with the CS is sufficient to produce long-term enhancement.

and Auerbach, 1986; Neary et al., 1986; Alkon et al., 1988; Crow, 1988; Nishizuka, 1988; Bank et al., 1989). We found that short- and long-term enhancement can be induced by pairing the CS with activation of PKC with diacylglycerol analogs or phorbol esters (Crow and Forrester, 1988). Furthermore, the induction of short-term enhancement is dependent upon activation of PKC because kinase inhibitors and downregulation of PKC block enhancement (Forrester and Crow, 1988; Crow et al., 1991). Interestingly, the conditions that are sufficient to block the induction of short-term enhancement do not block long-term enhancement (Forrester and Crow, 1988). Taken collectively, the results described above and our present results showing that short- and long-term enhancement are expressed in different identified B-photoreceptors suggest that long-term enhancement may depend upon a parallel signaling system that is independent of the processes responsible for the induction of short-term enhancement. These results have implications for models of short- and long-term memory, suggesting that the mechanisms for long-term memory may be independent from short-term memory.

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