Facilitation of Monosynaptic and Complex PSPs in Type I Interneurons of Conditioned *Hermissenda*

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Synaptic plasticity and intrinsic changes in neuronal excitability are two mechanisms for Pavlovian conditioning. Pavlovian conditioning of *Hermissenda* produces synaptic facilitation of monosynaptic medial B–medial A IPSPs and intrinsic changes in excitability of type A and B cells in isolated and intact sensory neurons of the conditioned stimulus (CS) pathway. Recently two types of interneurons that receive either excitatory or inhibitory monosynaptic or polysynaptic input from photoreceptors have been identified. On the basis of morphological and electrophysiological criteria, the interneurons have been classified as type I_e, I_i (direct), and type II_e, II_i (indirect). We have now examined synaptic facilitation of monosynaptic PSPs in type I_e and I_i interneurons after conditioning and pseudoran-

dom control procedures. Here we report that CS-elicited spike activity is increased in type I_e interneurons and decreased in type I_i interneurons of conditioned animals relative to their respective baseline activity and pseudorandom control groups. Classical conditioning resulted in synaptic facilitation of type I_e and I_i monosynaptic PSPs elicited by lateral B spikes and enhancement of the amplitude of complex PSPs elicited by the CS. These results provide additional sites of plasticity in the neural circuit involved with the expression of learned behavior produced by Pavlovian conditioning of *Hermissenda*.

Key words: Hermissenda*; Pavlovian conditioning; synaptic facilitation; intrinsic excitability; interneuron plasticity; associative learning*

Synaptic plasticity has been a major focus of studies investigating potential mechanisms underlying Pavlovian conditioning (for review, see Beggs et al., 1999). However, there are numerous examples of intrinsic changes in neuronal excitability that can be detected in both mammals and invertebrates after Pavlovian conditioning procedures (Woody and Engel, 1972; Woody et al., 1976; Disterhoft et al., 1986; Moyer et al., 1996; Thompson et al., 1996) (for invertebrate review, see Sahley and Crow, 1998).

Conditioned *Hermissenda* express both enhanced excitability and synaptic facilitation in the pathway supporting the CS (Crow and Alkon, 1980; Alkon et al., 1982, 1985; Farley and Alkon, 1982; Crow, 1985, 1988; Goh et al., 1985; Matzel et al., 1990; Frysztak and Crow, 1993, 1994, 1997; Gandhi and Matzel, 2000). Pavlovian conditioning of *Hermissenda* produces conditioned stimulus (CS)-elicited foot shortening and CS-elicited suppression of locomotion (Crow and Alkon, 1978; Lederhendler et al., 1986). Enhanced excitability in identified neurons of conditioned *Hermissenda* is expressed by an increase in spike activity elicited by the CS or extrinsic current, an increase in the input resistance of type B photoreceptors, decreased spike frequency accommodation, an alteration in the amplitude of light-induced generator potentials, and a reduction in the peak amplitude of voltage-dependent (I_A, I_{Ca}) and Ca^{2+}-dependent ($I_{K,Ca}$) currents (Crow and Alkon, 1980; Alkon et al., 1982, 1985; Farley and Alkon, 1982; West et al., 1982; Crow, 1985; Collin et al., 1988; Frysztak and Crow, 1993).

Facilitation of the amplitude of monosynaptic IPSPs in identified photoreceptors has been observed in conditioned *Hermis-

senda* (Frysztak and Crow, 1994, 1997; Gandhi and Matzel, 2000). Moreover, 5-HT and GABA, two putative neurotransmitters in the unconditioned stimulus (US) pathway, produce synaptic facilitation of type B to type A monosynaptic IPSPs (Schuman and Clark, 1994; Schultz and Clark, 1995). Studies of signal transduction pathways have shown that protein kinase C (PKC) (Farley and Auerbach, 1986; Matzel et al., 1990; Crow et al., 1991; Farley and Schuman, 1991; Frysztak and Crow, 1997; Muzzio et al., 2001) and extracellular signal-regulated protein kinase (Crow et al., 1998) are activated by one-trial and multi-trial Pavlovian conditioning.

To date, the major focus of studies of mechanisms of Pavlovian conditioning in *Hermissenda* has been the photoreceptors, because the sensory neurons were the first to be identified as sites of plasticity (Crow and Alkon, 1980), and little was known regarding the circuitry supporting the generation of conditioned behavior. More recently, two types of interneurons in the CS pathway have been identified, and their projections to other regions of the CNS have been described (Crow and Tian, 2000, 2002).

Here we report synaptic facilitation of the monosynaptic connection between lateral type B photoreceptors and type I_e and I_i interneurons of conditioned animals. Type I_e interneurons expressed an increase in CS-evoked spike activity, enhancement of complex EPSP amplitude, and facilitation of monosynaptic EPSPs evoked by single, type B photoreceptor spikes. Type I_i interneurons exhibited a facilitation of spike-elicited monosynaptic IPSPs and CS-evoked complex IPSPs in conditioned animals as compared with pseudorandom controls.

MATERIALS AND METHODS

Animals. Adult *Hermissenda crassicornis* were used in the experiments. The animals were obtained from Sea Life Supply (Sand City, CA) and maintained in closed artificial seawater (ASW) aquaria at $14 \pm 1°C$ on a 12 hr light/dark cycle. Behavioral training, testing, and electrophysiolog-

ical procedures were performed during the light phase of the light/dark cycle.

Baseline test of phototactic behavior. The details of the conditioning procedure and methods for testing phototactic behavior have been described in detail in previous publications (Crow and Alkon, 1978; Crow and Offenbach, 1983; Crow, 1985) and will be described only briefly in this report. Animals were tested before training to determine baseline latencies to initiate locomotion in response to a test light. Animals that did not respond within a 20 min criterion period during the pretraining measurements were not used in the conditioning experiments. Previous research has shown that the increase in the time taken by the animals to locomote into a test light can be accounted for by an increase in the latency to initiate locomotion (Crow and Offenbach, 1983). Animals were placed into 228-mm-long glass tubes filled with artificial seawater. A foam plug inserted through an opening confined the animal to one end of the tube. The tubes were attached by spring clips to a modified turntable enclosed in an incubator maintained at 15°C. Animals were dark adapted for 12 min before phototactic behavior was tested. A light spot (10^{-4} watts/cm^2, white light) was projected onto the center of the turntable, illuminating a circular area 15–16 cm in diameter. The elapsed times to initiate locomotion in the presence of the test light were recorded when a *Hermissenda* moved between an infrared emitter and a phototransistor located at the starting end of each glass tube. When the infrared beam was interrupted, a free-running digital clock was turned off, and the time was recorded for later data analysis.

Conditioning procedure. After baseline testing, animals were randomly assigned to the conditioned group or pseudorandom control group. The conditioning phase consisted of 50 trials of the 10 sec CS (light) paired with the US (high speed rotation) (mean interspike interval = 2.5 min) presented each day for 2 consecutive days. The intensity of the CS was the same as the test light used to establish baseline responding for phototactic behavior during the pretest condition. The pseudorandom control group received a total of 100 trials of the CS and US (50 trials each day for 2 consecutive days), each programmed to occur randomly with respect to time and each other with the restriction that the CS and US could not overlap.

Post-acquisition test. All animals received behavioral testing identical to the pretraining (baseline) test measurement for phototaxis 24 hr after the second conditioning session. Animals that did not initiate locomotion in the presence of the CS within 20 min during the post-test received a maximum latency score. Assessment of conditioning was determined by computing suppression ratios that compared post-training phototactic behavior with pretraining test scores. The ratio is expressed as ($A/A + B$), where A represents pretraining scores and B represents post-training scores. Conditioned animals exhibited behavioral suppression that was similar in magnitude to previous reports (Crow and Alkon, 1978; Crow and Offenbach, 1983; Crow, 1985). After post-acquisition testing, all animals were coded so that the collection of electrophysiological data was conducted using completely blind experimental procedures.

Intracellular recordings. Intracellular recordings from identified lateral type B photoreceptors and type I interneurons were collected from isolated nervous systems. Anatomical and electrophysiological criteria were used to identify lateral B photoreceptors within the eyes as described previously (Alkon and Fuortes, 1972; Frysztak and Crow, 1994). The type I interneurons were localized to a region of the cerebropleural ganglion as noted in previous publications (Akaike and Alkon, 1980; Crow and Tian, 2000, 2002). For simultaneous recordings from photoreceptors and interneurons, the isolated nervous systems were incubated in a protease solution (Sigma type VIII; 0.67 mg/ml, 5 min) and rinsed with ASW before the surgical desheathing of a small area of the cerebropleural ganglion to expose the cell bodies of type I interneurons. Type I interneurons were identified on the basis of soma size, cell layer, location in the cerebropleural ganglion, light-elicited complex PSPs, and monosynaptic PSPs evoked by stimulation of identified photoreceptors.

The desheathed circumesophageal nervous systems were pinned to a silicone elastomer (Sylgard, Dow Chemical) stage in a recording chamber filled with ASW of the following composition (in mM): 460 NaCl, 10 KCl, 10 CaCl$_2$, 55 MgCl$_2$, buffered with 10 mM HEPES and brought to pH 7.46 with dilute NaOH. The ASW in the recording chamber was monitored by a thermistor and held at 15 ± 0.5°C. CS illumination of the eyes was provided by a tungsten halogen incandescent lamp attached to a fiber optic bundle mounted underneath the recording chamber. For simultaneous recordings, identified pairs of lateral B photoreceptors and type I interneurons were impaled with microelectrodes filled with 4 M KAc and connected to the two head stages of an Axoclamp 2A (Axon Instruments,
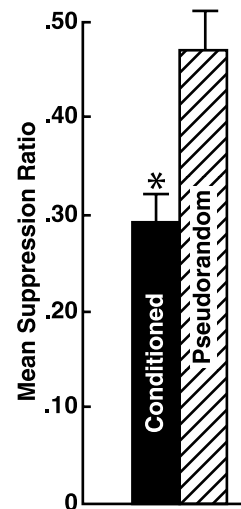

Figure 1. Mean suppression ratios ± SEM for conditioned ($n = 64$) and pseudorandom controls ($n = 54$). Conditioning produced statistically significant suppression of phototactic behavior compared with pseudorandom controls (*$p < 0.001$).

Foster City, CA). Electrode resistances varied between 60 and 90 MΩ. Standard intracellular recording and stimulation techniques were used. Electrophysiological data were collected on both videotape (Vetter Instruments) and a Gould chart recorder. Taped data were digitized and analyzed using Spike 2 software (Cambridge Electronic Design). Single spikes in identified lateral B photoreceptors were elicited by brief extrinsic current pulses applied in the dark. Evidence for monosynaptic connections between photoreceptors and interneurons was provided by PSPs with relatively constant latencies and a one-for-one relationship between photoreceptor action potentials and PSPs as described previously (Crow and Tian, 2000, 2002). For some experiments, the type I$_e$ interneurons were hyperpolarized to approximately −80 mV to block spike generation during the presentation of the CS, and the complex EPSP was recorded. Effects involving more than two groups were assessed with an ANOVA. Two-group comparisons involved *t* tests for independent groups.

RESULTS

Conditioned phototactic suppression

A total of 118 animals were used in the experiments. The total for the conditioned groups was 64 animals, and 54 animals served as pseudorandom controls. All animals were tested 24 hr after conditioning followed by isolation of the nervous system and collection of electrophysiological recordings from identified type I interneurons or simultaneous recordings from pairs of lateral B photoreceptors and type I interneurons. The mean suppression ratios for the conditioned animals and pseudorandom controls are shown in Figure 1. The statistical analysis showed that 100 conditioning trials produced significant behavioral suppression ($\bar{X} = 0.29 \pm 0.03$) as compared with the group that received 100 pseudorandom presentations of the CS and US ($\bar{X} = 0.47 \pm 0.04$) ($t_{116} = 4.3; p < 0.001$).

CS-elicited increase in spike frequency of type I$_e$ interneurons

We have reported previously that identified A and B photoreceptors project to two aggregates of type I interneurons; one group is excited (type I$_e$) and the other inhibited (type I$_i$) by photoreceptor spikes (Crow and Tian, 2000). We initiated our analysis of Pavlovian conditioning on interneurons by examining CS-elicited changes in spike frequency in type I$_e$ interneurons and type I$_i$ interneurons from conditioned animals and pseudorandom controls. To assess potential changes in spontaneous activity pro-
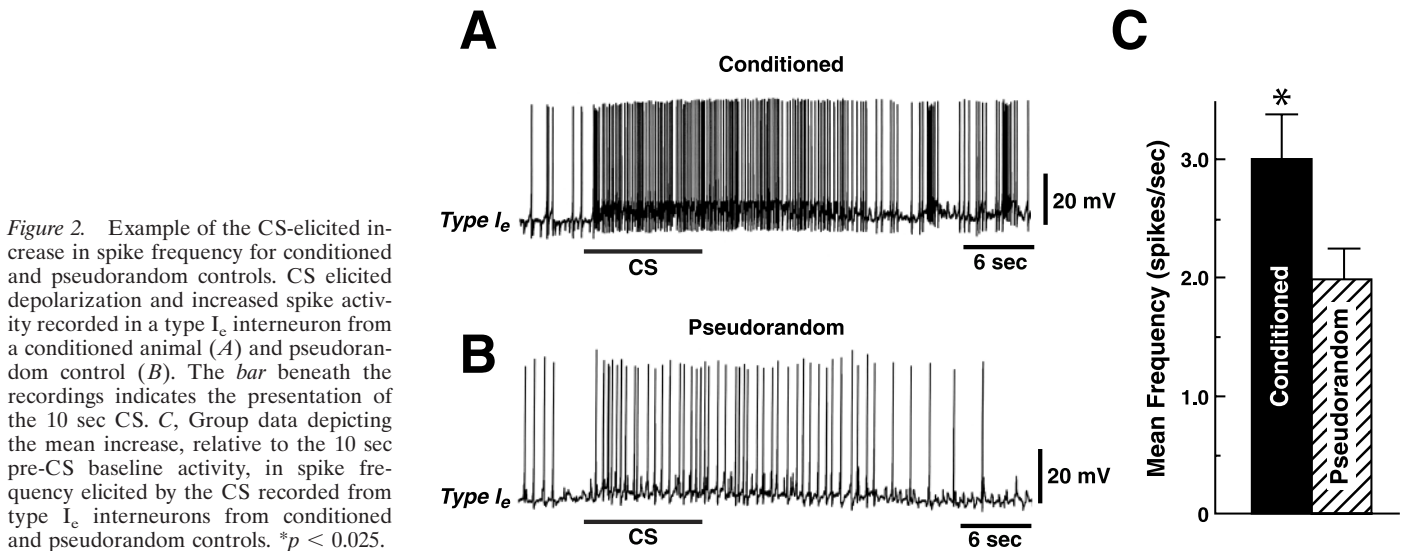
Figure 2. Example of the CS-elicited increase in spike frequency for conditioned and pseudorandom controls. CS elicited depolarization and increased spike activity recorded in a type I_e interneuron from a conditioned animal (*A*) and pseudorandom control (*B*). The *bar* beneath the recordings indicates the presentation of the 10 sec CS. *C*, Group data depicting the mean increase, relative to the 10 sec pre-CS baseline activity, in spike frequency elicited by the CS recorded from type I_e interneurons from conditioned and pseudorandom controls. *$p < 0.025$.

duced by conditioning, we examined spike activity in a 10 sec period immediately preceding the presentation of the CS. The results of the overall statistical analysis revealed no significant differences in spontaneous activity for type I_e or type I_i interneurons from the conditioned group or pseudorandom controls ($F_{(3,100)} = 0.72$; NS). Planned two-group comparisons of baseline spike activity between type I_i interneurons from conditioned and pseudorandom controls were also not significant ($t_{35} = 0.76$; NS). Comparisons of baseline spike activity between type I_e interneurons from conditioned and pseudorandom controls were also consistent with the overall analysis ($t_{65} = 0.72$; NS) as were comparisons in baseline spike activity between type I_i and I_e interneurons of conditioned animals ($t_{55} = 1.08$; NS) and pseudorandom controls ($t_{48} = 0.66$; NS). These results show that conditioning does not alter the spontaneous spike activity of type I interneurons.

As shown in the examples in Figure 2, the CS elicited more spikes in the type I_e interneuron from the conditioned preparation (Fig. 2*A*) as compared with the pseudorandom control (Fig. 2*B*). The mean CS-elicited increase in spike frequency above the baseline computed from the spontaneous activity during a 10 sec period immediately preceding the CS presentation was 3.1 ± 0.4 for the conditioned group ($n = 16$) and 2.04 ± 0.3 for the pseudorandom controls ($n = 20$) (Fig. 2*C*). The statistical analysis revealed a significant difference in CS-elicited spike frequency of type I_e interneurons between conditioned preparations and pseudorandom controls ($t_{34} = 2.4$; $p < 0.025$). The results showed that conditioning produced an increase in CS-elicited spike activity in type I_e interneurons.

Enhancement of I_e complex EPSPs

The changes in synaptic input to type I_e interneurons after conditioning were examined by briefly hyperpolarizing the I_e interneurons to block action potentials and recording the complex EPSP elicited by the CS (Fig. 3). Representative examples of CS-elicited complex EPSPs recorded from I_e interneurons are shown for a conditioned preparation (Fig. 3*A*) and a pseudorandom control (Fig. 3*B*). The CS elicited a larger amplitude complex EPSP with an increased frequency of PSPs in the example from the conditioned preparation (Fig. 3*A,C*) as compared with the pseudorandom control (Fig. 3*B,D*). The mean peak amplitude of the complex EPSP recorded from conditioned animals

($n = 11$) was 28.5 mV $\pm$ 3.4 and 18.4 mV $\pm$ 2.1 for the pseudorandom control ($n = 11$) (Fig. 3*E*). The difference in peak complex EPSP amplitude between conditioned and pseudorandom controls was statistically significant ($t_{20} = 2.5$; $p < 0.01$).

Enhancement of I_i complex IPSPs

We examined the amplitude of CS-elicited complex IPSPs in type I_i interneurons and found that Pavlovian conditioning resulted in an enhancement of the peak amplitude of the complex IPSP elicited by the CS (Fig. 4). Representative examples of CS-elicited complex IPSPs recorded from I_i interneurons are shown for a conditioned preparation (Fig. 4*A*) and a pseudorandom control (Fig. 4*B*). The group data depicting the average peak amplitude of complex IPSPs for the conditioned and control preparations are shown in Figure 4*C*. The mean amplitude of the complex IPSP recorded from conditioned animals ($n = 18$) was 10.9 mV $\pm$ 1.0 and 6.9 $\pm$ 0.9 for the pseudorandom controls ($n = 12$). The results of the statistical analysis showed a significant difference in peak amplitude of complex IPSPs between conditioned and pseudorandom controls ($t_{28} = 2.6$; $p < 0.01$).

CS-elicited decrease in spike frequency of type I_i interneurons

The effectiveness of the CS in inhibiting spike activity of type I_i interneurons was examined by determining the number of spikes in type I_i interneurons generated during the CS presentation (Fig. 4*A*). As shown in the group data in Figure 4*D*, the presentation of the CS produced a greater decrease in spike activity relative to the 10 sec pre-CS baseline for inhibitory type I_i interneurons from conditioned animals ($n = 18$) ($\bar{X} = 84.7 \pm 4.6\%$) as compared with pseudorandom controls ($\bar{X} = 39.7 \pm 8.8\%$) ($n = 13$) ($t_{29} = 4.9$; $p < 0.001$). As would be expected by the finding of enhancement in the amplitude of complex IPSPs in type I_i interneurons, the CS was more effective in inhibiting activity in type I_i interneurons in conditioned animals relative to pseudorandom controls.

Input resistance of type I interneurons

The enhancement of the amplitude of complex PSPs detected in type I interneurons of conditioned animals could be produced by presynaptic mechanisms. However, the increase in complex PSP amplitude could also be caused by postsynaptic mechanisms such
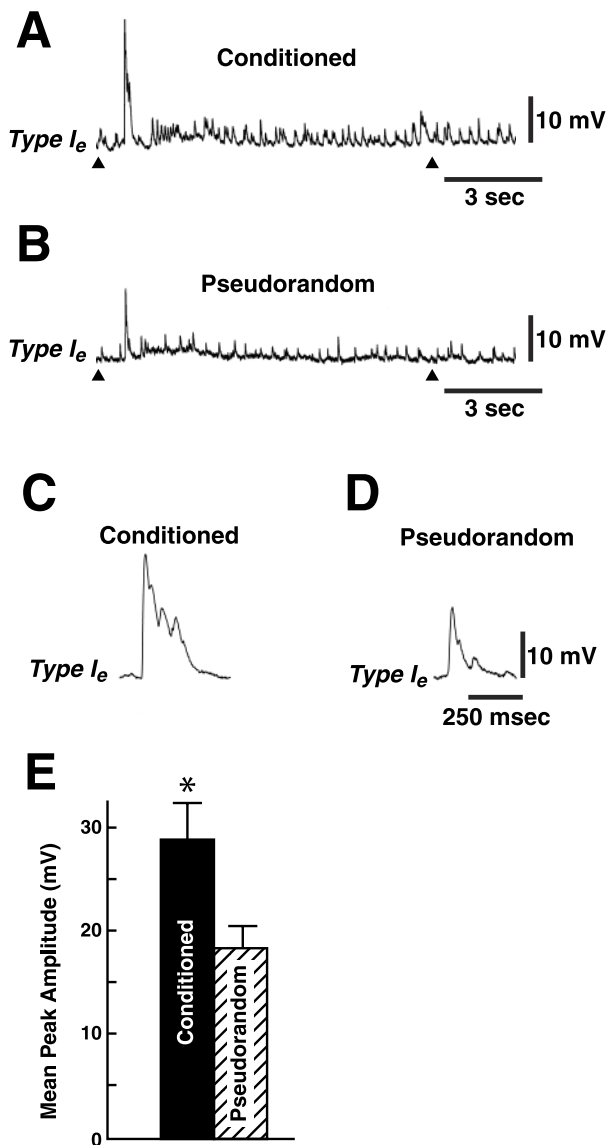
Figure 3. Conditioning enhances CS-elicited complex EPSP amplitude. CS-elicited complex EPSP produced from a type I_e interneuron from a conditioned animal (*A*) and a pseudorandom control (*B*). The *arrowhead* beneath each recording indicates the onset and offset of the 10 sec CS. In these experiments the I_e interneurons were briefly hyperpolarized to approximately −80 mV to block spike activity during the presentation of the CS. The initial component of the complex EPSP is shown on a faster time base in *C* and *D*. *E*, Group data depicting the mean (± SEM) peak amplitude of the complex EPSPs recorded from I_e interneurons in the conditioned group and pseudorandom controls. *$p < 0.01$.

Figure 4. Complex IPSP amplitude elicited by the CS is enhanced in conditioned animals. CS-elicited complex IPSP recorded from a type I_i interneuron from a conditioned animal (*A*) and an example from a pseudorandom control (*B*). The *bar* beneath the recordings indicates the presentation of the 10 sec CS. The CS evoked a larger amplitude complex IPSP and greater inhibition of spike activity in the conditioned group (*A*) relative to controls (*B*). *C*, Group data (means ± SEM) for the peak amplitude of the complex IPSPs recorded from type I_i interneurons from the conditioned group and pseudorandom controls. *D*, Group data depicting the mean percentage decrease in spike activity recorded during the presentation of the CS relative to the pre-CS 10 sec period for the conditioned group and pseudorandom controls. *$p < 0.01$ for *C* and *$p < 0.001$ for *D*.

as a modification of intrinsic membrane conductances of type I interneurons. Therefore, a potential postsynaptic contribution to PSP enhancement may be expressed by an increase in the input resistance of type I interneurons. We examined this possibility by measuring the input resistance of type I interneurons from conditioned and pseudorandom controls using brief extrinsic hyperpolarizing current pulses at three levels (0.1, 0.2, 0.3 nA). No differences in input resistance between I_i and I_e interneurons from conditioned and pseudorandom controls were detected, so the groups were combined for the overall statistical comparison. Figure 5 *insets* show three superimposed electrotonic potentials elicited by hyperpolarizing current pulses from a holding poten-
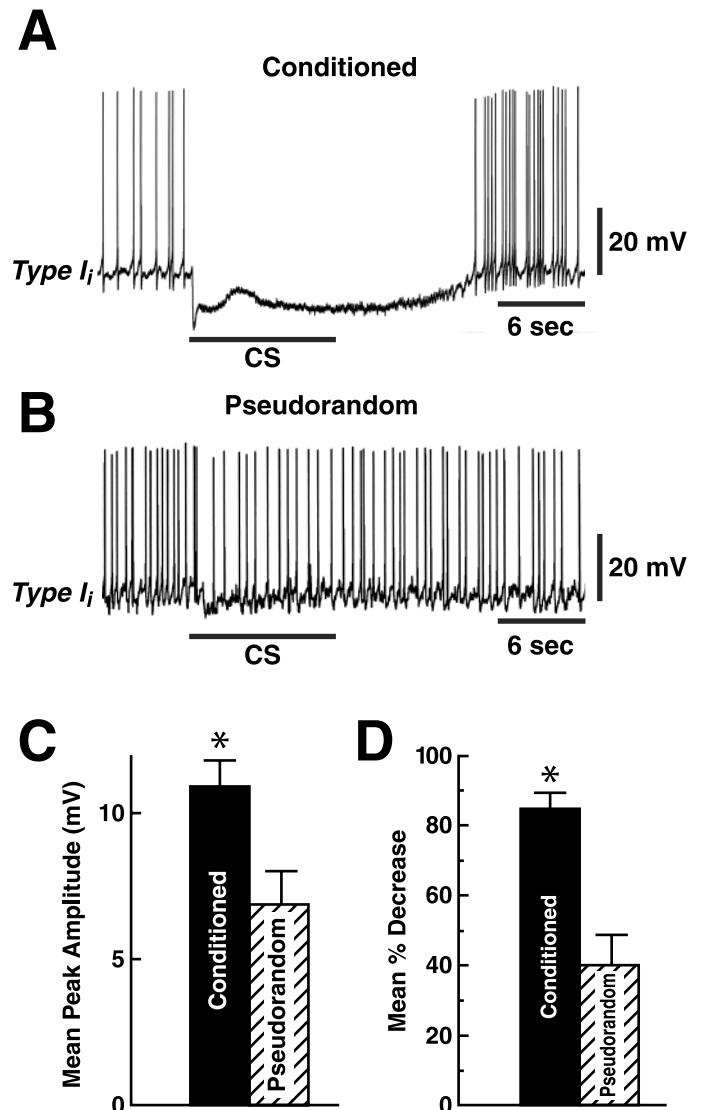
tial of −60 mV collected from an I_i interneuron in a conditioned preparation (bottom) and an I_i interneuron in a pseudorandom control (top). The slope of the I–V plots for the two examples shown in Figure 5 were almost identical. Examining R_{in} for all of the cells studied from the two groups revealed a mean R_{in} for all type I interneurons from conditioned animals of 117.9 ± 3.8 MΩ and 115.2 ± 3.7 MΩ for the pseudorandom controls. The modest difference between the two groups was not statistically significant ($t_{60} = 1.01$; NS). These results suggest that the enhancement of the amplitude of complex PSPs by conditioning is not likely the result of changes in the input resistance of type I interneurons
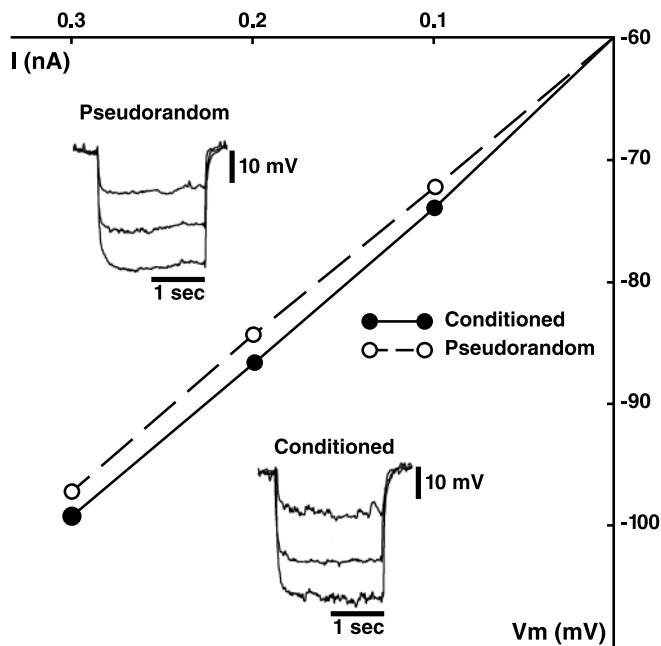
Figure 5. Current–voltage plot for a type I_i interneuron from a conditioned and pseudorandom control preparation. *Insets* show three superimposed electrotonic potentials elicited by hyperpolarizing current pulses of increasing magnitude (0.1, 0.2, 0.3 nA) from a holding potential of −60 mV for an example from a conditioned preparation (*bottom traces*) and pseudorandom control (*top traces*). The slopes of the two *I–V* plots were similar.

that can be detected at potentials more negative than −60 mV. However, changes in R_{in} of I_e interneurons detected at potentials more positive than the holding potential (−60 mV) could contribute to the CS-elicited increase in spike frequency of type I_e interneurons observed after conditioning. We did not examine R_{in} at more positive potentials because of problems with accurately measuring the amplitude of electrotonic potentials during spike generation.

Synaptic facilitation of I_e monosynaptic EPSPs

We examined changes in the monosynaptic component of the I_e interneuron synaptic input by eliciting single spikes in lateral type B photoreceptors and recording monosynaptic EPSPs from I_e interneurons. The dark-adapted membrane potential of type I_e interneurons from conditioned groups ($\bar{X} = 49.8 \pm 0.99$ mV) was not significantly different from pseudorandom controls ($\bar{X} = 50.2 \pm 1.1$ mV) ($t_{43} = 0.29$; NS). Figure 6 shows representative examples of I_e interneuron monosynaptic EPSPs elicited by a single lateral B spike from a conditioned animal (A2) and pseudorandom control (B2). The photoreceptors were briefly hyperpolarized to block spontaneous spike activity, and the membrane potential of I_e interneurons was held at −60 mV. As shown in the examples in Figure 6, conditioning resulted in a facilitation of the B spike-elicited monosynaptic EPSP amplitude in the I_e interneuron as compared with the pseudorandom control. The analysis of the group data for the amplitude of monosynaptic EPSPs from conditioned animals ($n = 6$) and pseudorandom controls ($n = 6$) revealed that conditioning produced a significant facilitation of EPSP amplitude ($t_{10} = 1.97$; $p < 0.05$). The summary data showing mean EPSP amplitude for the conditioned group and pseudorandom controls is shown in Figure 6C.

Synaptic facilitation of I_i monosynaptic IPSPs

In a previous study of conditioning, we showed facilitation of the medial type A monosynaptic IPSP elicited by a single spike in the medial type B photoreceptor (Frysztak and Crow, 1994). Type I interneurons are directly excited or inhibited by action potentials generated in identified A or B photoreceptors (Crow and Tian, 2000, 2002). Therefore, CS-elicited changes in the complex IPSP may be the result of facilitation of type I_i interneuron monosynaptic IPSPs. We examined the amplitude of monosynaptic IPSPs recorded from type I_i interneurons elicited by single spikes in lateral type B photoreceptors from conditioned and pseudorandom control animals. The statistical analysis of the dark-adapted membrane potential of type I_i interneurons revealed that there were no significant differences between conditioned groups ($\bar{X} = 50.4 \pm 1.04$ mV) and pseudorandom controls ($\bar{X} = 50.5 \pm 1.01$ mV) ($t_{59} = 0.03$; NS). Figure 7 shows representative examples of type I_i interneuron IPSPs elicited by a single B photoreceptor spike from a conditioned animal (A2) and a pseudorandom control (B2). The membrane potential of type I_i interneurons was held at −60 mV with the application of hyperpolarizing current. As shown in the two examples in Figure 7, conditioning resulted in a facilitation of the B spike-elicited monosynaptic IPSP in the I_i interneuron relative to the pseudorandom control. The analysis of the group data for the amplitude of monosynaptic IPSPs from conditioned animals ($n = 8$) and pseudorandom controls ($n = 9$) revealed that conditioning produced a significant facilitation of IPSP amplitude ($t_{15} = 3.1$; $p < 0.007$). As shown in the summary data in Figure 7C, mean IPSP amplitude was 7.8 mV for type I_i interneurons from the sample of conditioned animals and 4.5 mV for IPSPs recorded from the pseudorandom controls.

DISCUSSION
Overview
Previous studies of Pavlovian conditioning of *Hermissenda* have identified several sites of plasticity involving modifications in excitability of identified type B and A photoreceptors. Voltage-clamp experiments of type B photoreceptors isolated by axotomy after conditioning revealed that voltage (I_A, I_{Ca}) and Ca^{2+}-dependent ($I_{K,Ca}$) currents are reduced (Alkon et al., 1982, 1985; Collin et al., 1988). Taken collectively, the net effect of modifications in the different intrinsic membrane conductances of type B photoreceptors could explain both the CS-elicited enhancement of the generator potential and CS-evoked increased spike activity detected after conditioning. Anatomical studies of type B photoreceptors indicate that there are three spatially segregated compartments (Eakin et al., 1967; Stensaas et al., 1969; Crow et al., 1979; Senft et al., 1982). Phototransduction occurs in the soma-rhabdomeric compartment, spike generation occurs in the distal axon, and synaptic interactions occur in the axon terminal regions within the cerebropleural neuropil (Alkon and Fuortes, 1972; Crow et al., 1979). Therefore, the decrease in K^+ conductances could contribute both directly and indirectly to enhanced excitability by increasing the amplitude of CS-elicited generator potentials and increasing CS-elicited spike activity in the spike-generating zone by modification of conductances that influence the interspike interval. More recently, synaptic facilitation of the monosynaptic connection between type B and type A photoreceptors has been identified after Pavlovian conditioning (Frysztak and Crow, 1994, 1997; Gandhi and Matzel, 2000). Thus, Pavlovian conditioning of *Hermissenda* results in changes in both PSPs and cellular excitability in identified neurons of the CS pathway.
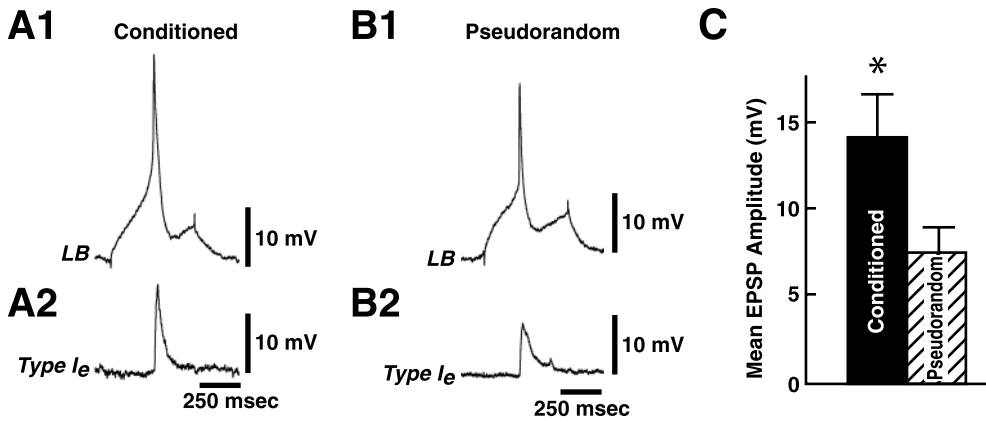
Figure 6. Conditioning results in facilitation of I_e monosynaptic EPSPs. A single lateral B spike generated by an extrinsic current pulse (*A1*) elicited a monosynaptic EPSP in a type I_e interneuron (*A2*) from a conditioned preparation. A single lateral B spike in *B1* elicited a smaller EPSP in a type I_e interneuron (*B2*) from a pseudorandom control animal. *C*, Group data (mean ± SEM) for the amplitude of I_e interneuron monosynaptic EPSPs recorded from conditioned animals ($n = 6$) and pseudorandom controls ($n = 6$). *$p < 0.05$.

Figure 7. Conditioning results in facilitation of I_i monosynaptic IPSPs. A single lateral B photoreceptor spike generated by an extrinsic current pulse (*A1*) elicited a monosynaptic IPSP recorded in a type I_i interneuron (*A2*) from a conditioned animal. A single lateral B spike generated by a current pulse (*B1*) elicited a smaller IPSP recorded in a type I_i interneuron (*B2*) from a pseudorandom control animal. *C*, Group data (means ± SEM) for the amplitude of type I interneuron IPSPs recorded from conditioned animals ($n = 8$) and pseudorandom controls ($n = 9$). *$p < 0.007$.
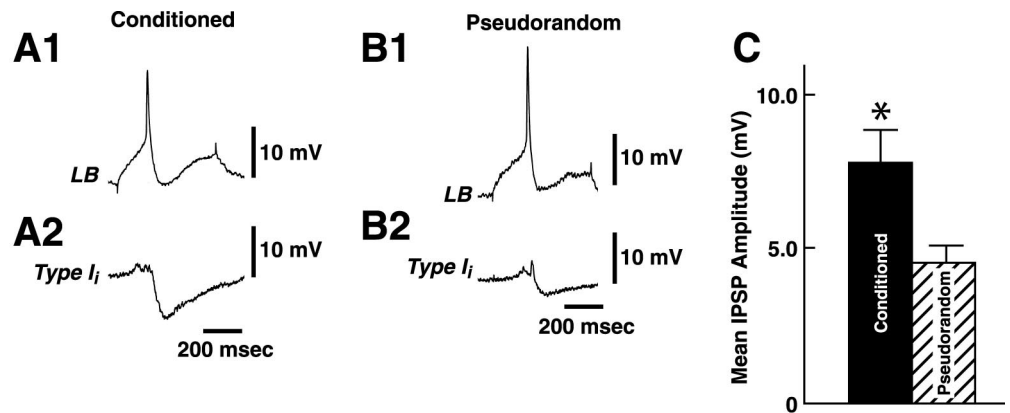
Synaptic facilitation

Progress in the identification of neurons in the circuitry generating visually influenced mucociliary locomotion has provided additional sites for conditioning-dependent plasticity. We have identified two aggregates of interneurons: one receiving monosynaptic input from identified type A and B photoreceptors (type I_e and I_i) and the other receiving polysynaptic input from identified photoreceptors (type II_e and II_i) (Crow and Tian, 2000, 2002). Here we have shown that conditioning results in facilitation of monosynaptic EPSPs and IPSPs elicited by single spikes in lateral B photoreceptors and CS-evoked enhancement of the complex EPSP and IPSP in type I_e and I_i interneurons relative to pseudorandom controls. Our results suggest that the facilitation of monosynaptic EPSPs and IPSPs and the enhancement of complex EPSPs and IPSPs may be caused by a presynaptic source because there were no detectable differences between conditioned and pseudorandom controls with respect to either input resistance or membrane potential of type I interneurons. However, other exclusively postsynaptic mechanisms could contribute to facilitation of spike-elicited monosynaptic PSPs and CS-evoked complex PSPs (Chitwood et al., 2001).

The enhanced amplitude of complex IPSPs in conditioned animals produced a statistically significant decrease in the CS-elicited spike activity of type I_i interneurons relative to the pre-CS baseline. The analysis revealed that the CS-elicited complex IPSP was more than twice as effective in inhibiting spike activity in type I_i interneurons as compared with pseudorandom controls (Fig. 4). In addition, our electrophysiological analysis revealed that the CS elicited significantly more spikes in type I_e interneurons of conditioned animals relative to interneuron recordings from pseudorandom controls. Consistent with the finding of an increase in CS-elicited spike frequency in I_e interneurons with conditioning was our observation that complex EPSP amplitude was enhanced in conditioned animals. In addition, monosynaptic EPSPs in type I_e interneurons elicited by single spikes in lateral B photoreceptors exhibited synaptic facilitation. However, the dark-adapted resting membrane potential and input resistance of type I interneurons were not modified by conditioning. Taken collectively, the results would be consistent with the hypothesis that enhancement of complex PSP amplitude detected in conditioned animals may be produced by both facilitation of monosynaptic PSPs and CS-elicited increases in spike activity in identified photoreceptors that are presynaptic to type I_e and I_i interneurons.

General role of interneurons in behavioral plasticity

The cellular and synaptic analysis of Pavlovian conditioning in *Hermissenda* indicates that the neural circuitry supporting conditioning is much more complex than envisioned initially (Goh and Alkon, 1984; Goh et al., 1985). It has now been shown that several identified sites in the pathway supporting the CS express both synaptic plasticity and intrinsic changes in cellular excitability. Conditioning results in an enhancement of the CS-elicited generator potential, enhancement of type A and B spike activity elicited by the CS and extrinsic current, facilitation of the B spike monosynaptic IPSP in type A photoreceptors, and facilitation of monosynaptic PSPs in type I interneurons. These results suggest that a cellular and synaptic analysis at a neural systems level will be required to produce a reasonably complete description of the neural basis for the generation of conditioned behavior. Because

there are no direct synaptic connections between photoreceptors and pedal motor neurons supporting locomotion (Crow et al., 1979), the contribution of type I and type II interneurons and their postsynaptic targets to light-elicited locomotion may be essential in understanding how conditioning is expressed in behavior.

Work with other invertebrate species has identified interneurons as important sites of plasticity. For example, olfactory learning and olfactory discrimination in *Limax* involve a network of olfactory interneurons (Kleinfeld et al., 1994; Gelperin and Flores, 1997; Ermentrout et al., 2001). In *Aplysia*, interneurons have been implicated as sites of plasticity in habituation and sensitization, examples of non-associative learning (Cleary et al., 1995). Moreover, intrinsic changes in excitability of an interneuron (S cell) in the leech have been detected during non-associative learning of the shortening reflex (Burrell et al., 2001), and Retzius cells express a correlate of CS–US predictability (Sahley and Crow, 1998). In addition, modifications in interneurons in the feeding circuit of *Lymnaea* have been identified after conditioning (for review, see Benjamin et al., 2000).

The results of the present report and previously identified sites of plasticity in the CS pathway suggest that changes in both excitability and synaptic strength may occur at multiple loci within components of the neural circuit supporting conditioning. Our investigation of conditioning in *Hermissenda* will now focus on postsynaptic targets of type I_e and I_i interneurons.

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