

Monosynaptic Connections Between Identified A and B Photoreceptors and Interneurons in *Hermisenda*: Evidence for Labeled-Lines

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Crow, Terry and Lian-Ming Tian. Monosynaptic connections between identified A and B photoreceptors and interneurons in *Hermisenda*: evidence for labeled-lines. *J Neurophysiol* 84: 367–375, 2000. The cellular and synaptic organization of the eye of the nudibranch mollusk *Hermisenda* is well-documented. The five photoreceptors within each eye are mutually inhibitory and can be classified into two types: A and B based on electrophysiological and anatomical criteria. Two of the three type B and two type A photoreceptors can be further identified according to their medial or lateral positions within each eye. In addition to reciprocal synaptic connections between photoreceptors, photoreceptors also project to second-order neurons in the cerebropleural ganglion. The second-order neurons receive convergent synaptic input from two additional sensory pathways; however, it has not been previously established if lateral A, lateral B, or medial B photoreceptors converge onto the same second-order neurons. To determine the specific synaptic organization of these components of the visual system, we have examined monosynaptic connections between identified lateral and medial type A and B photoreceptors and second-order cerebropleural (CP) interneurons. We found that monosynaptic connections between identified lateral A and lateral and medial B photoreceptors and CP interneurons follow a labeled-line principle. Illumination of the eyes or extrinsic depolarizing current applied to identified photoreceptors evoked excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs, respectively) in different CP interneurons. The PSPs in CP interneurons followed one-for-one spikes in the photoreceptors and could be elicited in artificial seawater solutions containing high divalent cations. Identified photoreceptors projected to more than one CP interneuron and expressed both excitatory and inhibitory connections with the different CP interneurons. In examples where a monosynaptic connection between a lateral B photoreceptor and a CP interneuron was identified, lateral A, medial A, or medial B photoreceptors did not project to the same CP interneuron. Moreover, when connections between medial B and CP interneurons were identified, lateral A, medial A, and lateral B connections were not found to project to the same CP interneuron. Similar results were obtained for a lateral A and CP interneuron connection. These results indicate that divergent labeled-lines exist between specific photoreceptors and second-order CP interneurons and potential convergence of synaptic input from primary and secondary elements of the visual system must occur at sites that are postsynaptic to the CP interneurons.

INTRODUCTION

Morphological and electrophysiological studies of the eyes of *Hermisenda* have resulted in a basic understanding of the

cellular and synaptic organization of the peripheral components of the visual system (Alkon 1973, 1975; Alkon and Fuortes 1972; Crow et al. 1979; Dennis 1967). The eyes are discrete structures that lie on the dorsal surface of the cerebropleural ganglion, and each contains two type A and three type B photoreceptors that can be distinguished using anatomical and electrophysiological criteria (Alkon 1973; Alkon and Fuortes 1972). Type B photoreceptors have been shown to excite and inhibit two classes of second-order neurons that surround the terminal photoreceptor processes within the cerebropleural ganglion (Akaike and Alkon 1980; Goh and Alkon 1984). The second-order neurons that are inhibited by B photoreceptors also received inhibition from both the hair cells of the statocyst and chemosensory pathways. The second type of second-order neuron that is excited by B photoreceptors is also depolarized by activation of either statocyst hair cells or chemosensory pathways (Akaike and Alkon 1980). Convergence from different sensory modalities onto cerebropleural (CP) interneurons is thus well-documented; however, with the exception of the medial A connection with interneurons, it is not known whether projections from other identified A and B photoreceptors converge onto the same interneurons or express divergence to different aggregates of interneurons. This is an issue of functional importance since the visual system of *Hermisenda* is one site of intrinsic modifications in cellular excitability and synaptic efficacy produced by Pavlovian conditioning (for reviews see Alkon 1989; Crow 1988; Sahley and Crow 1998). Both enhanced excitability and synaptic enhancement have been detected in identified photoreceptors within the eyes of conditioned animals (Fryszak and Crow 1994, 1997). Membrane conductances of identified type A and B photoreceptors have been characterized (Acosta-Urquidi and Crow 1985; Alkon 1989; Yamoah and Crow 1994, 1996; Yamoah et al. 1998), and specific conductances in identified photoreceptors have been shown to change with conditioning (Alkon et al. 1982, 1985; Farley and Han 1997). Taken collectively, studies of neural correlates of conditioning have shown that differences in the expression of cellular modifications are found between identified photoreceptors, indicating that not all cells have the same potential for supporting plasticity (Alkon et al. 1985; Crow 1985; Fryszak and Crow 1993, 1994, 1997).

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Moreover, it is not known whether differences in conditioning correlates observed at the level of the primary sensory neurons are maintained in the second-order neurons of the visual system and in other components of the pathway supporting the conditioned stimulus (CS).

In this report we provide evidence that monosynaptic connections between identified type A and B photoreceptors are to different aggregates of CP interneurons. In examples where a monosynaptic connection between a lateral type B photoreceptor and an interneuron was identified, lateral type A, medial type A, and B photoreceptors did not project to the same interneuron. Moreover, when connections between lateral type A or medial type B photoreceptors and CP interneurons were identified, other identified photoreceptor synaptic connections to the same interneuron were not detected. These results provide evidence for labeled-lines between identified type A and B photoreceptors and second-order interneurons in the CNS of *Hermisenda*.

METHODS

Animals

Adult *Hermisenda 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 $14 \pm 1^\circ\text{C}$ on a 12-h light-dark cycle. Animals were fed small pieces of scallop daily. All electrophysiological procedures were conducted during the light phase of the light/dark cycle.

Intracellular recordings

Intracellular recordings from identified medial or lateral type A or type B photoreceptors and CP interneurons were collected from isolated nervous systems. Anatomical and electrophysiological criteria were used to identify lateral or medial type A and B photoreceptors within the eyes as described previously (Alkon and Fuortes 1972; Fryszak and Crow 1994). We did not record from the intermediate type B photoreceptor since it cannot be unequivocally identified based on single electrode impalements. The CP interneurons were localized to a region of the cerebropleural ganglion as noted in a previous publication (Akaike and Alkon 1980). The isolated nervous systems were incubated in a protease solution (Sigma Type VIII; 0.67 mg/ml, 5 min) and rinsed with ASW prior to the surgical desheathing of a small area of the cerebropleural ganglion to expose the cell bodies of CP interneurons. Cerebropleural interneurons were identified based on soma size, cell layer and location in the cerebropleural ganglion, electrophysiological responses to light and extrinsic current stimulation, and synaptic input from identified type A and B 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 (mM): 460 NaCl, 10 KCl, 10 CaCl_2 , and 55 MgCl_2 , buffered with 10 mM HEPES and brought to pH 7.46 with dilute NaOH. Experiments were also conducted in high divalent cation ASW (30 mM CaCl_2 and 165 mM MgCl_2) that raised action potential threshold, thus suppressing spontaneous activity and reducing polysynaptic activation of CP neurons. The ASW in the recording chamber was monitored by a thermistor and held at $15 \pm 0.5^\circ\text{C}$. Illumination of the eyes was provided by a tungsten halogen incandescent lamp attached to a fiber optic bundle mounted underneath the recording chamber. Maximum light intensity was attenuated with neutral density filters expressed in negative log units. Identified pairs of A or B photoreceptors and CP interneurons were impaled with microelectrodes filled with 4M KAc and connected to the two headstages of an Axoclamp 2A (Axon Instruments, Foster

City, CA). Electrode resistances varied between 60 and 90 M Ω . Standard intracellular recording and stimulation techniques were employed. Electrophysiological data were collected on both videotape (Vetter Instruments) and a Gould chart recorder. Single spikes in identified A and B photoreceptors elicited by brief extrinsic current pulses and trains of action potentials elicited by current steps were applied in the dark through a bridge circuit. Depolarizing generator potentials in identified photoreceptors were evoked by light steps of different intensities following appropriate periods of dark adaptation. Evidence for monosynaptic connections between photoreceptors and CP interneurons was provided by postsynaptic potentials (PSPs) with relatively constant latencies and a one-for-one relationship between photoreceptor action potentials and PSPs in both normal ASW and in ASW solutions containing high divalent cations (3 times Ca^{2+} and 3 times Mg^{2+}). Additional supporting evidence for monosynaptic connections was provided by the linear regression analysis that examined the statistical relationship between light intensity and complex PSP amplitude in CP interneurons or between light intensity and spike and unitary PSP frequency in CP interneurons. Previous work has also shown that Co^{2+} -ASW eliminated PSPs recorded from CP interneurons (Goh and Alkon 1984). Consistent with this proposal for a chemical synapse is our observation that extrinsic current injections into CP interneurons did not produce voltage changes in identified photoreceptors, and electrotonic potentials below spike threshold produced by depolarization or hyperpolarization of identified photoreceptors did not result in detectable voltage changes in the CP interneurons. Taken together, these criteria indicate that the synaptic connections between identified photoreceptors and CP interneurons are chemical and monosynaptic.

RESULTS

CP interneuron excitation

In a preliminary study (data not shown) and a previous report (Goh and Alkon 1984), it was shown that Lucifer yellow-labeled CP interneurons did not send processes into pedal or cerebropleural nerves. In addition, we observed that stimulation of pedal nerves with extrinsic current through suction electrodes did not elicit antidromic spikes in CP interneurons. Collectively, these results are consistent with the identification of CP neurons as interneurons, a designation that is used in this report. Simultaneous intracellular recordings from identified type A or B photoreceptors and CP interneurons revealed that illumination of the eye elicited excitatory and inhibitory responses from different aggregates of CP cells. In the example shown in Fig. 1A, a light step (-1.0) elicited a depolarizing generator potential and increased spike frequency in a lateral B photoreceptor and a depolarization and increased spike frequency in the recording from the CP interneuron (Fig. 1A2). The maximum discharge of the CP interneuron corresponded temporally to the peak of the depolarizing generator potential shown in Fig. 1A1. In the example shown in Fig. 1B1 from a different preparation, the CP interneuron was hyperpolarized below threshold for spike generation. Before the presentation of the light step spontaneous spikes in the lateral B photoreceptor elicited simultaneous excitatory PSPs (EPSPs) in the CP interneuron with a one-for-one relationship. The light step elicited a 10- to 15-mV depolarization of the CP interneuron exceeding the spike-generating threshold at a peak potential that corresponded in time to the peak amplitude of the generator potential recorded from the lateral B photoreceptor (see Fig. 1B2). During the long-lasting depolarization of the photoreceptor following the light step and the subsequent re-

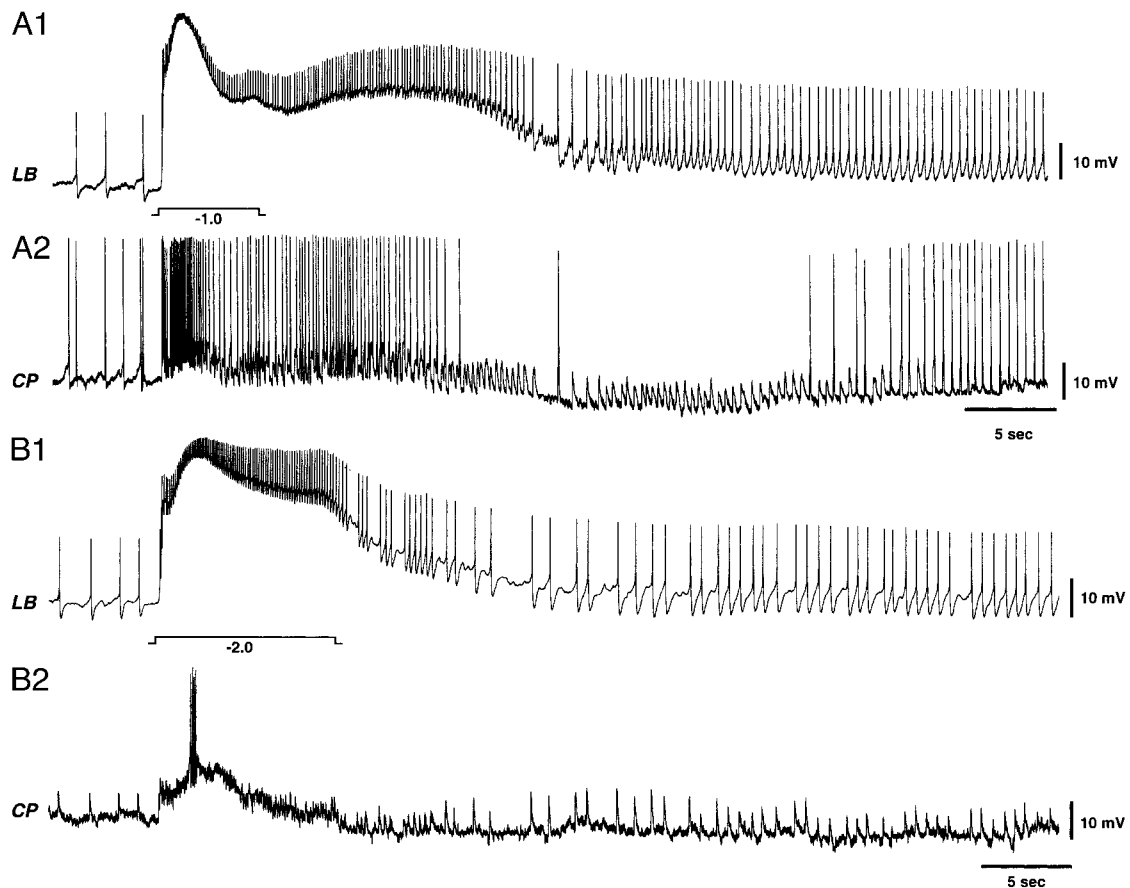


FIG. 1. Light response of a lateral B photoreceptor and cerebropurpur (CP) interneuron. Simultaneous recording from a lateral B photoreceptor (A1) and a CP interneuron (A2) in response to a 5-s light step attenuated -1.0 log unit. The maximum depolarization and increased spike discharge recorded from the CP interneuron corresponded in-time to the peak of the light-elicited generator potential. Simultaneous recording from a different lateral B photoreceptor (B1) and a CP interneuron (B2) hyperpolarized to block spontaneous spike generation ($mp = -77$ mV). The 10-s light step attenuated -2.0 log units elicited a depolarizing generator potential (B1) and a membrane depolarization of the CP interneuron that reached threshold for spike generation at the peak of the generator potential (B2). Excitatory postsynaptic potentials (EPSPs) in the interneuron (B2) followed photoreceptor action potentials one-for-one.

turn to baseline activity, EPSPs recorded from the CP interneuron followed one-for-one spikes recorded from the lateral B photoreceptor. Since action potentials recorded from the CP interneurons that were not hyperpolarized often masked the complex EPSP elicited by light, we measured either the number of unitary EPSPs or number of action potentials in CP interneurons during the first 5 s of illumination of the photoreceptors. The results of the linear regression analysis showed a significant correlation between light intensity and the number of spikes or EPSPs recorded from the CP interneurons ($r = 0.93$; $P < 0.02$, $n = 5$). The synaptic connections between the lateral B photoreceptor and CP interneurons were further examined by hyperpolarizing both neurons to block spontaneous spike activity. In the example shown in Fig. 2A1, a single spike elicited by an extrinsic current pulse in the lateral B photoreceptor evoked a unitary EPSP in the CP interneuron (Fig. 2A2). EPSPs recorded from CP interneurons followed spikes elicited by either light or extrinsic current with a relatively short and constant latency. In the example shown in Fig. 2B1, a 2-s depolarizing extrinsic current step applied to the lateral B photoreceptor elicited EPSPs in the CP interneuron (Fig. 2B2) that followed one-for-one the spikes in the B photoreceptor. These results are consistent with a direct or monosynaptic

connection between the lateral B photoreceptor and the CP interneuron. To provide additional evidence for a monosynaptic connection, simultaneous recordings were collected from preparations bathed in ASW containing high divalent cations (3 times Ca^{2+} and Mg^{2+}). As shown in Fig. 2C, in the high divalent cation solution a single spike elicited from a lateral B photoreceptor evoked a unitary EPSP in the CP interneuron (Fig. 2C2). In another example from a preparation maintained in a high divalent cation solution, a 1-s current step elicited several spikes from the B photoreceptor (Fig. 2D1) and EPSPs in the CP interneuron that followed the spikes one-for-one (see Fig. 2D2).

CP interneuron inhibition

An example of a simultaneous recording from a lateral B photoreceptor and CP interneuron showing an inhibitory response to light is shown in Fig. 3A. The CP interneuron exhibited a 12-mV hyperpolarization with a peak amplitude that corresponded in-time to the peak of the depolarizing generator potential recorded from the lateral B photoreceptor (Fig. 3A1). The CP interneuron in this example was spontaneously active and exhibited an inhibition of spike activity during

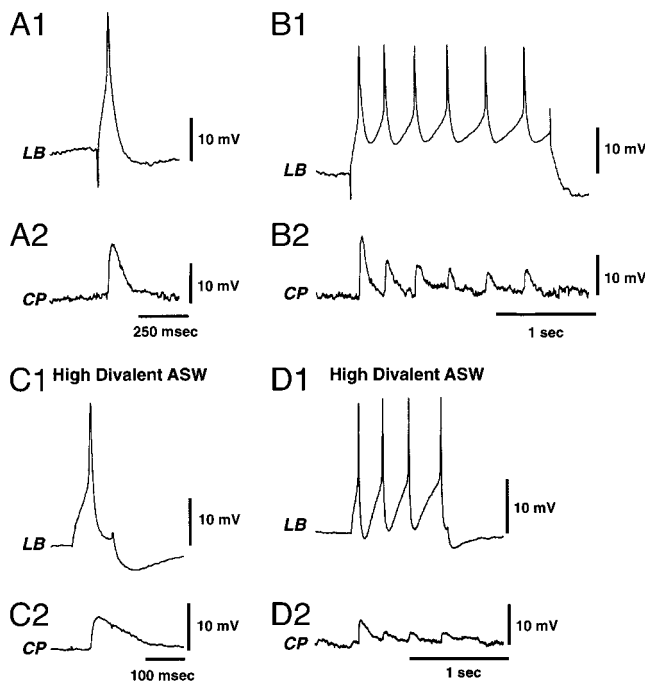


FIG. 2. Photoreceptor action potentials elicit monosynaptic EPSPs in CP interneurons. A single spike produced by a 0.4-nA, 50-ms depolarizing extrinsic current pulse in a lateral B photoreceptor hyperpolarized to -65 mV to block spontaneous firing (A1) elicited an EPSP recorded from a CP interneuron (A2). The CP interneuron was hyperpolarized to -78 mV to block spontaneous firing. A 0.2-nA, 2-s depolarizing extrinsic current pulse elicited a train of action potentials recorded from a lateral B photoreceptor (B1; mp = -65 mV) and EPSPs in the CP interneuron (B2) that followed the spikes one-for-one. In a high divalent cation solution (3 times Ca^{2+} , 3 times Mg^{2+}) a single spike elicited by a 50-ms, 0.5-nA pulse in the lateral B photoreceptor (C1) produced an EPSP recorded from the CP interneuron. Membrane potential of the photoreceptor was -61 mV and interneuron -63 mV. A 1-s, 0.2-nA extrinsic current step elicited several action potentials from the lateral B photoreceptor (D1) and EPSPs from the CP interneuron (D2) that followed spikes one-for-one. Preparation bathed in a high divalent cation solution as in C.

the period of illumination of the B photoreceptor. Occasionally, B photoreceptors will exhibit brief periods of membrane oscillation following the termination of light. As shown in Fig. 3A2, the CP interneuron was inhibited during the excitatory phase of the oscillation and resumed spontaneous activity during the hyperpolarizing phase of the B photoreceptor oscillation (Fig. 3A1). As shown previously for the excitatory connections between B photoreceptors and CP interneurons, IPSPs recorded from the CP interneuron followed one-for-one with spikes in the lateral B photoreceptor (see Fig. 3A). Consistent with the results for excitation, the regression analysis revealed a linear relationship and significant correlation between light intensity and the amplitude of the complex inhibitory PSP (IPSP) recorded in the CP interneuron ($r = 0.97$; $P < 0.005$, $n = 5$). Illumination of the eyes with unattenuated light (maximum intensity) resulted in a complex IPSP recorded from CP interneurons with the largest amplitude (mean, 14.3 mV). Light attenuated -4.0 log units resulted in a complex IPSP recorded from the CP interneurons with the smallest amplitude (mean, 8.0 mV).

In neurons hyperpolarized below the threshold for spontaneous spike generation, single spikes generated in lateral B photoreceptors elicited by extrinsic current evoked uni-

tary IPSPs in CP interneurons as shown in the example in Fig. 3B2. In response to an extrinsic depolarizing 2-s current step, CP interneurons exhibited unitary IPSPs that followed B photoreceptor spikes one-for-one (see Fig. 3C2). Synaptic inhibition from lateral B photoreceptors is sufficient to block spontaneous activity in CP interneurons as shown in Fig. 3D. A series of 2-s depolarizing current steps applied to the lateral B photoreceptor produced brief hyperpolarization and complete inhibition of spontaneous firing of the CP interneuron as shown in Fig. 3D2. The results of experiments employing illumination of the lateral B photoreceptors and stimulation with extrinsic current suggests that the inhibitory connection between the lateral B photoreceptor and CP interneuron is monosynaptic. This was examined further with preparations exposed to ASW containing high divalent cations (3 times Ca^{2+} and Mg^{2+}). As shown in Fig. 3E a single spike elicited by a brief current pulse in the lateral B photoreceptor evoked a unitary IPSP recorded from the CP interneuron in high Ca^{2+} - Mg^{2+} ASW (Fig. 3E2). The PSPs elicited by single spikes exhibited a relatively short and constant latency. These results provide additional evidence that the inhibitory connection between the lateral B photoreceptor and CP interneuron is monosynaptic. Simultaneous recordings from a total of 54 pairs of identified photoreceptors and CP interneurons revealed that 29 exhibited excitatory synaptic connections and 25 pairs expressed inhibitory photoreceptor-CP interneuron connections. In addition, recordings from 114 CP interneurons revealed that 62 cells exhibited excitatory responses to light stimulation and 52 cells inhibitory responses. Taken collectively, these results show that there are two populations of CP interneurons, one of which is excited by identified photoreceptor input and the other inhibited by monosynaptic input from identified photoreceptors.

Specificity of synaptic connections

We determined whether identified A and B photoreceptors projected to different populations of CP interneurons or, alternatively, whether there is a convergence of synaptic input onto the same CP interneuron from different identified photoreceptors. This was examined by first establishing a synaptic connection between an identified A or B photoreceptor and CP interneuron, and then testing for potential connections with other identified A or B photoreceptors. The results are summarized in the data shown in Table 1. The primary connection that was examined was between the lateral type B photoreceptor and CP interneurons. Of the 18 cases where the connection between the lateral B photoreceptor and CP interneuron was established and other potential connections were tested, there were no examples where the CP interneuron also received synaptic input from either the lateral type A, medial type B, or medial type A photoreceptor. An example of an inhibitory connection between a lateral B photoreceptor and CP interneuron is shown in Fig. 4A2. In this preparation, the same CP interneuron did not exhibit a PSP following the elicitation of a single spike in the medial B (Fig. 4B2), medial A (Fig. 4C2) or lateral A photoreceptor (Fig. 4D2). Consistent with this observation, an examination of excitatory connections between lateral B photoreceptors and CP interneurons did not reveal connec-

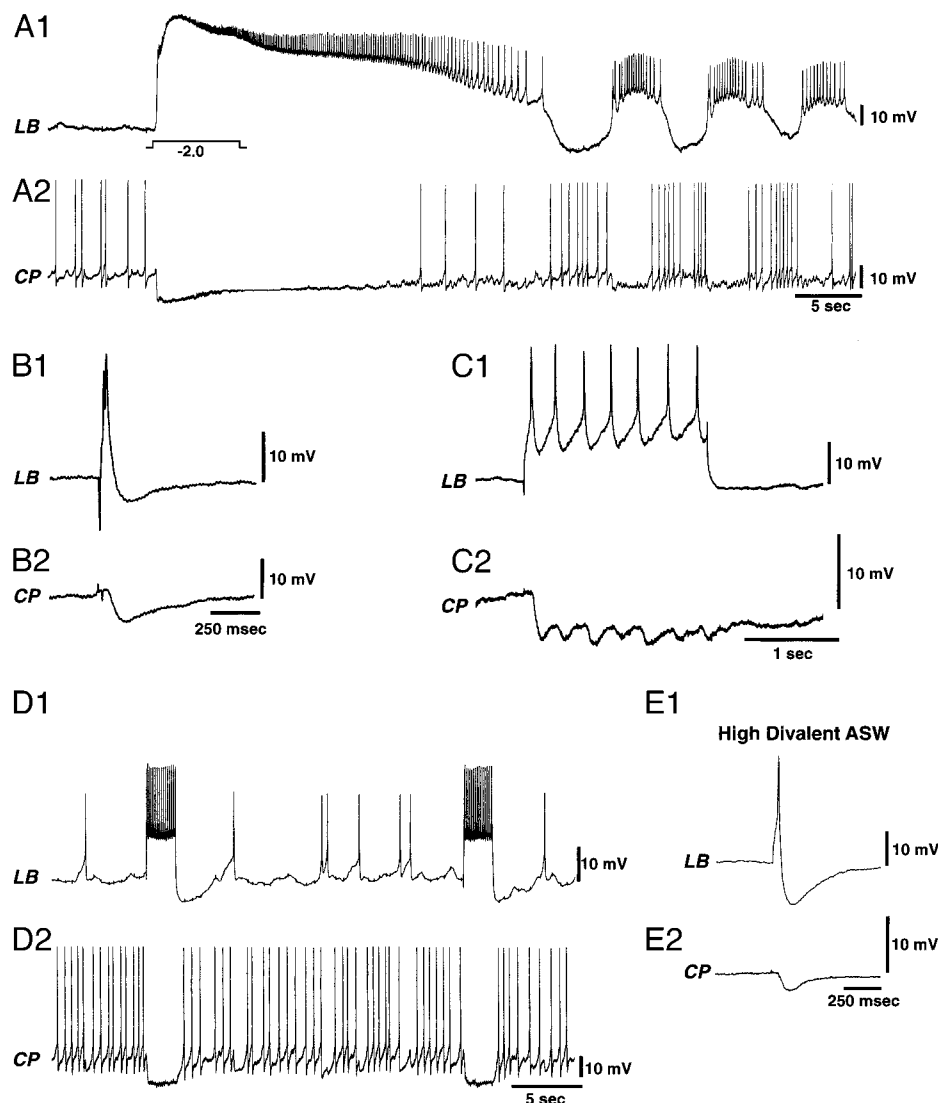


FIG. 3. Light elicited a depolarizing generator potential recorded from a lateral B photoreceptor and a hyperpolarization [complex inhibitory postsynaptic potential (IPSP)] recorded from a CP interneuron. Simultaneous recording from a lateral B photoreceptor (A1) and a CP interneuron (A2) in response to a 7-s light step attenuated -2.0 log units. The maximum hyperpolarization in the CP interneuron (A2) corresponded to the peak of the generator potential (A1). Large hyperpolarizing oscillations in the photoreceptor (A1) were associated with increased spike discharges in the CP interneuron (A2). A single spike elicited by a 20-ms, 0.8-nA extrinsic current pulse in the lateral B photoreceptor (B1) evoked an IPSP recorded from the CP interneuron (B2). A 2-s, 0.2-nA extrinsic current pulse elicited a train of action potentials in the lateral B photoreceptor (C1) and IPSPs in the CP interneuron (C2) that followed the spikes one-for-one. A burst of action potentials elicited by a 2-s, 0.4-nA extrinsic current pulse in the lateral B photoreceptor (D1) produced a hyperpolarization in the CP interneuron that blocked spontaneous activity (D2; $mp = -60$ mV). A single spike generated by a 0.5-nA, 50-ms extrinsic current pulse in the lateral B photoreceptor (E1) elicited an IPSP in the interneuron (E2) in a high divalent cation solution, indicating that the inhibitory synaptic connection between the lateral B photoreceptor and the CP interneuron is monosynaptic.

tions from other identified photoreceptors. Figure 5A shows an example for a lateral B photoreceptor-CP interneuron excitatory connection (Fig. 5A2) without input to the same

TABLE 1. Identified photoreceptor synaptic connections with CP interneurons

Established Connection With CP Interneuron		Test of Other Photoreceptor Connections With the Same CP Interneuron	
Photoreceptor	Number of connections	Photoreceptors	Connections/number tested
LB	18	MB	0/8
		LA	0/13
		MA	0/2
MB	2	LB	0/1
		LA	0/1
		MA	0/1
LA	1	MB	0/1
		LB	0/1
		MA	0/1

CP, cerebropleural; LB and MB, lateral and medial type B; LA and MA, lateral and medial type A.

CP interneuron from a medial B (Fig. 5B2) or a lateral A photoreceptor (Fig. 5C2). We also studied in a few examples the specificity of connections between lateral A and medial B photoreceptors and CP interneurons. As shown in the example where a connection between a lateral A photoreceptor and CP interneuron was established (Fig. 6A2), connections tested between a medial A (Fig. 6B2) and lateral B photoreceptor and CP interneuron (Fig. 6C2) were not observed. An examination of the group data ($n = 21$), where potential multiple connections could be examined in the same experiment, did not reveal one case of multiple photoreceptor convergence, i.e., only one identified photoreceptor projected to the same CP interneuron (see Table 1). To demonstrate that photoreceptors that did not exhibit connections with CP interneurons were healthy neurons and could support synaptic transmission, we examined connections between these photoreceptors and other identified photoreceptors, since the photoreceptors are mutually inhibitory. As shown in Fig. 7A2, a single spike elicited from a lateral B photoreceptor evoked an EPSP recorded in a CP interneuron. The same CP interneuron did not receive an input from a lateral A photoreceptor (Fig. 7B2). However, stimulation

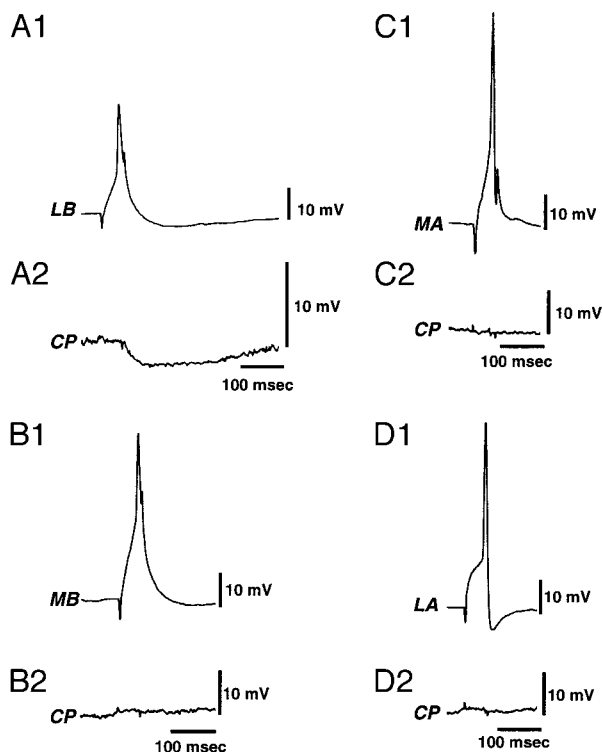


FIG. 4. A CP interneuron that received an inhibitory synaptic connection from a lateral B photoreceptor did not receive detectable synaptic input from a medial B, medial A, or lateral A photoreceptor. Simultaneous recording from a lateral B photoreceptor and a CP interneuron. A 0.4-nA, 50-ms current pulse generated a single spike in the lateral B photoreceptor (A1) and elicited an IPSP in the CP interneuron (A2). In contrast, a single spike generated in a medial B photoreceptor (B1) by a 0.5-nA, 50-ms pulse did not elicit a PSP recorded from the same CP interneuron (B2), or a single spike generated by a 0.6-nA, 50-ms pulse in a medial A photoreceptor (C1) did not elicit a PSP from the same CP interneuron (C2), or a single spike-generated by a 0.7-nA, 50-ms pulse in a lateral A photoreceptor (D1) did not elicit a PSP from the same CP interneuron (D2). CP interneuron mp = -60 mV.

of the lateral A photoreceptor elicited an IPSP recorded from the lateral B photoreceptor (Fig. 7C2). Taken collectively, these results indicate that the connections between lateral type B photoreceptors and CP interneurons follow a labeled-line principle, and recordings from other identified A and B photoreceptors are consistent with this type of organization.

Divergent projections to CP interneurons

It is well-documented that the different identified photoreceptors are mutually inhibitory (Alkon and Fuortes 1972; Crow

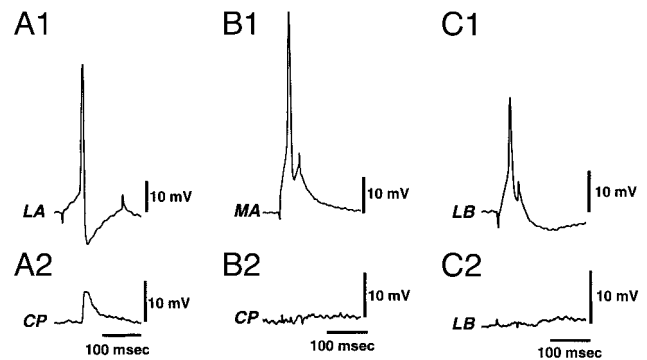


FIG. 5. A CP interneuron that received excitatory synaptic input from a lateral A photoreceptor did not receive synaptic input from a medial A or lateral B photoreceptor. Simultaneous recording from a lateral A photoreceptor and CP interneuron. A single spike generated in the lateral A photoreceptor by a 0.4-nA, 150-ms pulse (A1) elicited an EPSP in the CP interneuron (A2). However, a single spike generated by a 0.7-nA, 50-ms pulse in the medial A photoreceptor (B1) did not elicit a PSP in the same interneuron (B2), or a single spike generated by a 0.4-nA, 50-ms pulse in a lateral B photoreceptor (C1) did not elicit a PSP in the same CP interneuron (C2). CP interneuron mp = -54 mV.

et al. 1979). Therefore an identified photoreceptor can exhibit a dual synaptic function, producing an EPSP in a CP interneuron and expressing an inhibitory connection to another photoreceptor as shown in Fig. 7 and proposed by Akaike and Alkon (1980). However, dual synaptic projections to different CP interneurons has not been previously established. After identifying a synaptic connection between a lateral B photoreceptor and CP interneuron, we examined other CP interneurons for a potential connection with the same lateral B photoreceptor. In all examples where this was examined ($n = 8$), the lateral B photoreceptor projected to two or more CP interneurons. The connections between a single lateral B photoreceptor and multiple CP interneurons ranged between two and four. An example of an excitatory and inhibitory synaptic connection with two different CP interneurons is shown in Fig. 8. The single spike in the lateral B photoreceptor elicited an EPSP in the CP interneuron (Fig. 8A2) and an IPSP in a different CP interneuron (Fig. 8B2). In another preparation, an example of multiple connections from a lateral B photoreceptor to CP interneurons recorded in a high divalent cation solution is shown in Fig. 8, C2 and D2. A single spike in the lateral B photoreceptor elicited an EPSP in one CP interneuron (Fig. 8C2) and an IPSP in a different CP interneuron (Figs. 8D2). The group data showed that approximately half of the connections were excitatory (10/19) and half were inhibitory (9/19), which is similar

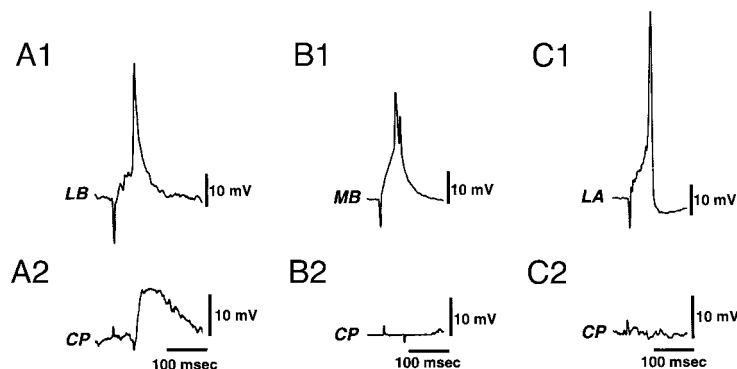


FIG. 6. A CP interneuron that received an excitatory synaptic connection from a lateral B photoreceptor did not receive detectable synaptic input from a medial B or lateral A photoreceptor. Simultaneous recording from a lateral B photoreceptor and a CP interneuron. A single spike generated in the lateral B by a 0.4-nA, 50-ms pulse (A1) elicited an EPSP in the CP interneuron (A2). In contrast, a single spike generated in a medial B photoreceptor (B1) by a 0.3-nA, 50-ms pulse did not elicit a PSP recorded from the same CP interneuron (B2), or a single spike generated by a 0.5-nA, 50-ms pulse in a lateral A photoreceptor (C1) did not elicit a PSP recorded from the same CP interneuron (C2). CP interneuron mp = -63 mV.

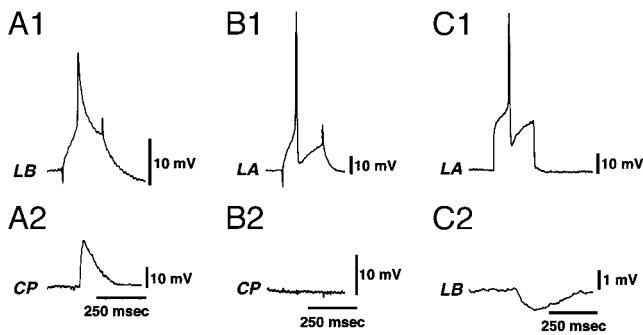


FIG. 7. A lateral A photoreceptor that did not exhibit a detectable connection to a CP interneuron that expressed a synaptic connection with a lateral B photoreceptor, did project to an identified photoreceptor. Simultaneous recording from a lateral B or lateral A photoreceptor and a CP interneuron. A single spike generated in the lateral B photoreceptor by a 0.5-nA, 200-ms current pulse (A1) elicited an EPSP from a CP interneuron (A2). A single spike generated by a 0.4-nA, 200-ms pulse in a lateral A photoreceptor (B1) did not elicit a PSP from the same interneuron (B2). However, a spike generated by a 0.3-nA, 200-ms pulse in the lateral A photoreceptor (C1) did elicit an IPSP recorded from the lateral B photoreceptor (C2). CP interneuron mp = -59 mV and B photoreceptor mp = -63 mV.

to the percentage of excitatory and inhibitory connections found with the total sample.

DISCUSSION

Synaptic organization of the visual system

The synaptic organization of the *Hermisenda* eye is well-documented (Alkon 1975; Alkon and Fuortes 1975). The type A and B photoreceptors are mutually inhibitory, exhibiting an example of lateral inhibition. The mutually inhibitory synaptic connections between photoreceptors are monosynaptic based on both electrophysiological and morphological criteria (Alkon and Fuortes 1972; Crow et al. 1979). In addition to inhibition from neighboring photoreceptors, both statocyst hair cells and chemosensory synaptic input to the photoreceptors has been shown to be inhibitory (Alkon 1973; Alkon et al. 1978). However, at the level of second-order visual neurons, different aggregates of cells are excited and inhibited by activation of statocyst hair cells and chemosensory neurons (Akaike and Alkon 1980). While the photoreceptors within the eyes are mutually inhibitory, there are differences in the strength of the synaptic connections between identified type A and B photoreceptors. Stimulation of the medial B photoreceptor with an extrinsic current step produced strong inhibition of spontaneous firing of the medial A photoreceptor in contrast to the modest effect of both lateral B inhibition of lateral A spontaneous activity (Fryszak and Crow 1993) and lateral A inhibition of lateral B photoreceptors (see Fig. 7). Previous work has shown that the intermediate and medial B photoreceptors produced stronger inhibition of medial type A photoreceptors than lateral B photoreceptors (Goh and Alkon 1984).

Labeled-lines

While there is convergence from different sensory systems onto the same second-order neurons (Akaike and Alkon 1980), the issue of convergent or divergent synaptic input to second-order visual neurons from all the identified photoreceptors has not been previously addressed in *Hermisenda*. In the initial

report of the identification of second-order visual neurons it was proposed, but not demonstrated, that interneurons may receive synaptic input from more than one photoreceptor (Akaike and Alkon 1980). In their study, a connection between an unidentified type B photoreceptor and second-order neuron was established by both extrinsic current stimulation and illumination of the eye. It was proposed in one example, using light stimulation of the eye, that a small IPSP recorded in the B photoreceptor corresponded to a small EPSP in the CP interneuron, and thus both PSPs could be produced by a common presynaptic source (photoreceptor). However, they did not present recordings from the putative presynaptic source (photoreceptor) that would account for either the IPSPs or EPSPs. Indeed, most early studies that examined the electrophysiology of type A and B photoreceptors did not appreciate the functional significance of further classification of both types into lateral and medial photoreceptors with the exception of Goh and Alkon (1984). They showed that stimulation of medial type A photoreceptors elicited PSPs in CP interneurons that projected to a putative motor neuron, and neither lateral type A nor unidentified type B photoreceptors produced PSPs in the same interneurons (Goh and Alkon 1984). In this report

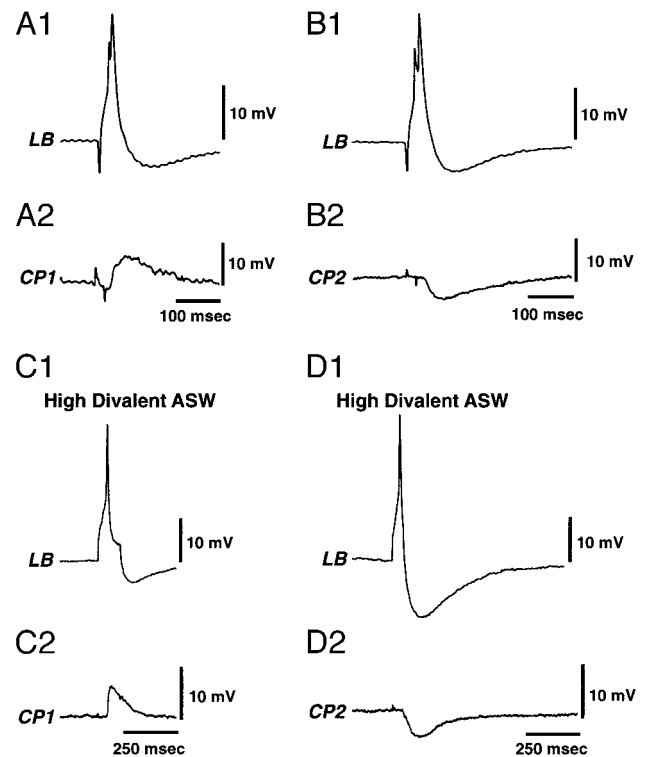


FIG. 8. An identified photoreceptor exhibits divergent projections to aggregates of CP interneurons. Simultaneous recording from a lateral B photoreceptor and a CP interneuron. A single spike generated by a 1-nA, 20-ms pulse in a lateral B photoreceptor (A1) elicited an EPSP in the CP interneuron (A2). A spike generated by a 1-nA, 20-ms pulse in the same photoreceptor (B1) elicited an IPSP from a different CP interneuron (B2). The divergent connections from single identified photoreceptors to CP interneurons are monosynaptic. Simultaneous recording from a lateral B photoreceptor and a CP interneuron in high divalent cation artificial seawater (ASW). A spike evoked by a 0.5-nA, 50-ms pulse from a lateral B photoreceptor bathed in a high divalent cation solution (C1) elicited an EPSP in the CP interneuron (C2). A spike generated by a 0.5-nA, 50-ms pulse in the same lateral B photoreceptor bathed in a high divalent cation solution (D1) elicited an IPSP in a different CP interneuron (D2). CP interneuron mp = -62 mV (A2), -61 mV (B2), -63 mV (C2), and -56 mV (D2).

we have evidence that identified lateral type B photoreceptors have monosynaptic connections to multiple CP interneurons that do not receive synaptic input from other identified A and B photoreceptors. Consistent with this observation is the finding that lateral A photoreceptors project to CP interneurons that do not receive synaptic input from either medial A, lateral B, or medial B photoreceptors. The few examples of medial B connections to CP interneurons in this study are in agreement with our observations of lateral B and lateral A photoreceptors. Thus our results indicate that identified A and B photoreceptors project to different aggregates of second-order visual interneurons, consistent with a labeled-line organizational principle.

Divergence of photoreceptor synaptic connections

Studies of relatively simple nervous systems have provided the opportunity to examine the synaptic actions mediated by different branches of a single neuron to different postsynaptic target neurons (for representative examples see Blitz and Nusbaum 1999; Kandel et al. 1967). In a number of examples in these systems, the same neurotransmitter produces both inhibition and excitation in different postsynaptic targets (Kandel et al. 1967; Strumwasser 1962; Tauc and Gerschenfeld 1961). In *Aplysia*, action potentials in interneuron L10 produces a synchronous EPSP in follower cells R15 and an IPSP in follower cell L3 (for review see Kandel and Gardner 1972). Previous work in *Hermissenda* reported two different synaptic actions of different branches of a B photoreceptor (Akaike and Alkon 1980); an action potential in a type B photoreceptor evoked an EPSP in a central visual neuron and an IPSP in neighboring photoreceptors. The dual synaptic actions of photoreceptor branches is likely mediated by acetylcholine, the only transmitter identified in the eyes (Heldman et al. 1979). In this paper we show that the divergence of synaptic input from photoreceptors to second-order interneurons is more extensive. A type B photoreceptor has been shown to project to as many as four CP interneurons in addition to the multiple inhibitory synaptic connections to different photoreceptors within the *Hermissenda* eye (see Figs. 7 and 8). This finding can explain the observation of Goh and Alkon (1984), that PSPs recorded from pedal neuron MN1 during stimulation of the medial A photoreceptor and CP interneuron occurred with greater frequency than the number of spikes in the CP interneuron, suggesting the contribution of other interneurons. The analysis of the synaptic actions mediated by different branches of a single identified photoreceptor has revealed that approximately half are excitatory connections to CP interneurons, and half are inhibitory.

Functional implications of divergent photoreceptor projections

Cellular neurophysiological studies of the visual system of conditioned *Hermissenda* have identified several examples of modifications in excitability extrinsic to both type A and B photoreceptors (Alkon et al. 1982, 1985; Crow 1985; Crow and Alkon 1980; Farley and Alkon 1982; Farley et al. 1990), and changes in synaptic efficacy between identified photoreceptor synapses (Fryszak and Crow 1994, 1997; Gandhi and Matzel 1999). There is now considerable evidence that specific identified photoreceptors express different types of cellular corre-

lates following conditioning. Although not specifically identified as medial B photoreceptors, Alkon et al. (1985) proposed that medial B photoreceptors exhibited an increase in the amplitude of light-elicited generator potentials (West et al. 1982) and an increase in light-elicited spike frequency following conditioning (Farley and Alkon 1982). Moreover, in conditioned animals, lateral B photoreceptors express a decreased excitability to the CS, which may be due to both intrinsic conductance changes and enhancement of synaptic inhibition from neighboring photoreceptors (Crow 1985). In addition, lateral A photoreceptors of conditioned animals exhibit an increase in excitability to both the CS and extrinsic current, while medial A photoreceptors do not express enhanced excitability but do show enhancement of the medial B to medial A synaptic connection (Fryszak and Crow 1993, 1994, 1997). A previous examination of a partial neural circuit from photoreceptors to a putative motor neuron focused on medial type A synaptic projections to CP interneurons (Goh and Alkon 1984). This study showed that stimulation of medial A photoreceptors with light or extrinsic current produced excitation of CP interneurons and depolarization of a putative motor neuron (MN1). It was further proposed that type B inhibition of the medial A photoreceptor during light stimulation would result in a decrease in excitatory synaptic input to MN1, which potentially could affect the orientation of animals toward a light stimulus. While lateral type A and unidentified type B photoreceptors did not elicit PSPs in the CP interneurons that projected to MN1 (Goh and Alkon 1984), projections from other CP interneurons to MN1 were not examined. In addition, projections from other identified photoreceptors to CP interneurons and putative motor neurons were also not established. Moreover, the synaptic input to MN1 from other CP interneurons or different aggregates of CP interneurons has not been thoroughly investigated.

We previously reported that the lateral type A photoreceptor is the only A photoreceptor type that encodes for the pairing specificity of the CS and unconditioned stimulus (US) (Fryszak and Crow 1993). Therefore the CP interneurons that receive synaptic input from the lateral A photoreceptors would have information concerning pairing specificity. However, neural correlates of conditioning detected in putative motor neurons are not consistent with the hypothesis that type A photoreceptor activity is the only critical event in phototactic suppression (Hodgson and Crow 1992). Studies have shown that CS-elicited activity recorded from pedal neurons or multiunit activity recorded from pedal nerves is reduced below the frequency of spontaneous activity recorded in the dark (Crow 1981; Hodgson and Crow 1991, 1992; Richards and Farley 1987). Because type A photoreceptors do not discharge action potentials spontaneously in the dark, their activity in response to light cannot be less than their activity in the dark. Taken collectively, the evidence suggests that both identified type B and type A photoreceptors may contribute to alterations in the activity of target neurons contributing to the circuitry controlling muco-ciliary locomotion.

The divergence of identified photoreceptor projections to specific interneuronal aggregates may provide for the opportunity to maintain the differential expression of correlates of conditioning in the different A and B photoreceptors at the level of second-order neurons. Such an organization would allow plasticity intrinsic to some elements of the visual system to be processed at postsynaptic targets separate and parallel

from elements of the visual system responsible for normal light intensity discriminations. We are currently investigating the postsynaptic targets of the CP interneurons to determine whether this divergent organization is maintained at the level of motor neurons supporting foot contraction and/or mucociliary locomotion.

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