Statocyst Hair Cell Activation of Identified Interneurons and Foot Contraction Motor Neurons in *Hermissenda*

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Crow, Terry and Lian-Ming Tian. Statocyst hair cell activation of identified interneurons and foot contraction motor neurons in *Hermissenda.* J Neurophysiol 92: 2874–2883, 2004. First published February 25, 2004; 10.1152/jn.00028.2004. Pavlovian conditioning of *Hermissenda* produces both light-elicited inhibition of normal positive phototaxis and conditioned stimulus (CS)-elicited foot contraction. Rotation, the unconditioned stimulus (US), elicits foot-shortening and reduced forward ciliary locomotion. The neural circuit supporting ciliary locomotion and its modulation by light is known in some detail. However, the neural circuits responsible for rotation-elicited foot-shortening and reduced forward ciliary locomotion are not known. Here we describe components of the neural circuit in *Hermissenda* that produce anterior foot contraction and ciliary activation mediated by statocyst hair cells. We have characterized in semi-intact preparations newly identified pedal ventral contraction motor neurons (VCMNs) and interneurons (Ib). Type Ib interneurons receive polysynaptic input from statocyst hair cells and project directly to VCMNs and cilia-activating motor neurons. Depolarization of VCMNs with extrinsic current in normal artificial seawater (ASW) and high-divalent cation ASW, and under conditions where central synaptic transmission was suppressed with 5 mM Ni2+/H11001, elicited a contraction of the ipsilateral anterior foot measured from videotape recordings. Mechanical displacement of the statocyst or depolarization of identified statocyst hair cells with extrinsic current elicited spikes and complex excitatory postsynaptic potentials (EPSPs) in type Ib interneurons and complex EPSPs and spikes recorded in VCMNs. Type Ib interneurons are electrically coupled and project to VCMNs and VP1 cilia-activating motor neurons located in the contralateral pedal ganglia. The results indicate that statocyst hair-cell-mediated anterior foot contraction and ciliary cilia modulate ciliary locomotion involve different interneuronal circuit components from the circuit previously identified as supporting light modulated ciliary locomotion.

INTRODUCTION

Information concerning the direction of gravity relative to body orientation is mediated by the statocysts in invertebrates. In *Hermissenda*, like other gastropod mollusks (Gallin and Wiedehold 1977; Janse 1982, 1983), the statocysts are paired spherical structures containing mechanoreceptor hair cells located around the perimeter of the sensory organ (Alkon 1975; Alkon and Bak 1973; Detwiler and Alkon 1973; Detwiler and Fuortes 1973). Locomotion, influenced by graviceptive input from statocyst hair cells that control reflexes and complex geotactic behaviors, has been studied in several invertebrate preparations (Alkon 1974; Arshavsky et al. 1985; Deliagina et al. 1998; Levi et al. 2004; Panchin et al. 1995; Wolff 1975)

Pavlovian conditioning of *Hermissenda* produces both light-elicited inhibition of normal positive phototaxis (Crow and Alkon 1978, 1980; Crow and Offenbach 1983) and conditioned stimulus (CS)-elicited foot contraction (Lederhendler et al. 1986). In the conditioning paradigm, the unconditioned stimulus (US), rotation, produces a clinging reflex consisting of foot-shortening (Lederhendler et al. 1986) and contraction of the body musculature (Alkon 1974) that is mediated by the statocysts (Alkon 1975). In addition, rotation reduces forward ciliary locomotion (Matzel et al. 1990).

The neural circuitry supporting light-elicited ciliary locomotion is now known is some detail (Crow and Tian 2000, 2002a, 2003a), although little is understood about the neural circuitry modulating statocyst-elicited foot contraction or graviceptive modulation of ciliary locomotion. In this study, we show that the neural circuitry supporting statocyst-mediated muscular foot contraction and graviceptive ciliary locomotion contain different interneuronal components from the circuitry responsible for light-modulated ciliary locomotion. We have characterized, in semi-intact preparations, newly identified pedal ventral contractile motor neurons (VCMNs) and interneurons (Ib). Type Ib interneurons receive polysynaptic input from statocyst hair cells and project directly to VCMNs and cilia activating motor neurons (VP1). Labeling of VCMNs revealed that the neurons contained a single axonal process that projected to the foot/body wall region through pedal nerve P2. Extracellular current depolarization of VCMNs, type Ib interneurons, or stimulation of statocyst hair cells with mechanical taps or direct mechanical displacement of the statocyst elicited contraction of the anterior foot in semi-intact preparations. Fluorescent labeling of type Ib interneurons revealed that axonal processes project to the contralateral pedal ganglion and that type Ib interneurons in each cerebropleural ganglia are electrically coupled. Type Ib interneurons were depolarized by mechanical stimulation of statocyst hair cells or by extrinsic current depolarization of hair cells in different regions of the statocyst. Extracellular current stimulation of rostral or lateral hair cells depolarized type Ib interneurons; however, caudal hair cells did not exhibit detectable synaptic connections with type Ib interneurons. Depolarization of type Ib interneurons with extrinsic current or statocyst hair cell stimulation elicited complex excitatory postsynaptic potentials (EPSPs) and spikes in VCMNs. In addition, we observed that depolarization of a single-type Ib interneuron with extrinsic current was sufficient to elicit contralateral anterior foot contraction. The analysis of the network mediating anterior foot contraction and graviceptive ciliary locomotion suggests that CS-elicited foot-shortening and statocyst-mediated reduced forward locomotion involve different interneuronal connections.
rons than the neural circuit controlling light-modulated ciliary locomotion. In addition, CS-elicited foot-shortening may involve different sites of interneuronal plasticity from those contributing to light-elicited inhibition of locomotion produced by Pavlovian conditioning.

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 aquarium at 14°C on a 12-h light-dark cycle. All electrophysiological procedures were conducted during the light phase of the light/dark cycle.

Intracellular recordings

Simultaneous intracellular recordings from statocyst hair cells and newly identified type Ib interneurons and type Ia interneurons and VCMNs were collected from partially split-foot semi-intact preparations. Anatomical and electrophysiological criteria were used to identify type Ib interneurons as described previously for type I and II interneurons (Crow and Tian 2000). Surgical desheathing of a small area of the cerebropleural and ventral pedal ganglion was conducted to expose the cell bodies of type Ib interneurons and VCMNs. In semi-intact preparations, pedal VCMNs were identified by verifying anterior foot contractions produced by depolarization with extrinsic current. Type Ib interneurons were physiologically identified by eliciting spikes with extrinsic current pulses and recording complex PSPs and spikes in VCMNs.

The partially desheathed circusesophageal 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 CaCl₂, and 55 MgCl₂, 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. Illumination of the preparation was provided by a tungsten-halogen incandescent lamp attached to a fiber optic bundle mounted underneath the recording chamber. Interneurons, hair cells, and pedal VCMNs were impaled with microelectrodes filled with 4 M KAc. Microelectrodes were connected to the two headstages of an Axoclamp 2A (Axon Instruments, Foster City, CA). Standard intracellular recording and stimulation techniques were employed. Extracellular recordings from pedal nerve P2 and stimulation of pedal nerve P1 were conducted with suction electrodes as previously described (Crow and Tian 2003a). Digitized data were analyzed and prepared for figures using Spike 2 software (Cambridge Electronic Design). Single spikes elicited by brief extrinsic current pulses and trains of action potentials elicited by current steps were applied in the dark through a bridge circuit. Evidence for monosynaptic connections between type Ib interneurons, VCMNs, and VP1 ciliary motor neurons was provided by EPSPs with short and relatively constant latencies and a one-to-one relationship between Ib action potentials and EPSPs recorded in normal ASW and in ASW containing high-divalent cations (3 × Ca²⁺ and 3 × Mg²⁺).

Semi-Intact preparations

Semi-intact anterior split-foot Hermisenda were prepared by cooling the animals in ASW to between 0 and 1°C followed by isolation of the circusesophageal nervous system from the buccal crest and body, leaving one intact pedal nerve P2. Pedal nerve P2 contains the axons of motor neurons innervating the anterior region of the foot (Richards and Farley 1987). The partially split foot was positioned ventral side up adjacent to the isolated circusesophageal nervous system pinned to the elevated central stage in the recording chamber.

The left pedal ganglion was rotated ~150° to provide for visualization of neuronal cell bodies on the ventral surface. The exposed nervous system and anterior foot were imaged in visible light by a 45-W tungsten-halogen light source projected by a light guide to the central stage of the recording chamber. For measurements of foot contraction, the foot was visualized in infrared illumination provided by the insertion of an infrared filter (Schott model RG-850) in the light path. A dissecting microscope formed an image of the foot and nervous system in infrared light on a Dage MTi videocamera connected to a videomonitor and videorecorder. Foot contraction was assessed by video imaging of the anterior region of the foot during depolarization of pedal VCMNs or type Ib interneurons with extrinsic current (5 s, 0.5–1.5 nA). Anterior foot contraction was quantified by measuring foot displacement from prestimulus baseline positions on a transparency covering the video monitor screen. To rule out the possible contribution of indirect activation of central neurons to foot contraction, pedal VCMNs were depolarized and anterior foot contractions assessed with nervous systems exposed to ASW solutions containing 5 mM Ni²⁺ or high-divalent cations (3 × Ca²⁺ and 3 × Mg²⁺). For experiments involving suppression of central synaptic transmission, the nervous systems pinned to the elevated central stage were exposed to Ni²⁺-containing ASW by application inside a Vaseline ring that isolated the central ganglion from the normal ASW that contacted the foot and body chambers. The effectiveness of suppression of central synaptic transmission was shown by inhibition of the pedal VCMN complex EPSP elicited by stimulation of pedal nerve 1 in Ni²⁺-containing ASW.

Depolarization of statocyst hair cells was produced by a mechanical tap applied to the chamber or direct mechanical displacement of the statocyst produced by a glass probe. In some experiments, trains of action potentials and single spikes were evoked by extrinsic current pulses that depolarized hair cells in identified regions (rostral, lateral, caudal) of the statocyst. Spikes and complex and monosynaptic EPSPs were recorded simultaneously from type Ib interneurons and pedal VCMNs or hair cells and type Ib interneurons.

Cell labeling

Type Ib interneurons and pedal VCMNs were impaled with microelectrodes filled with 4% Lucifer yellow in 0.2 M LiCl or 5% 5(6)-carboxyfluorescein in 0.1 M KAc (molecular probes). The electrode tips were filled with the Lucifer yellow in LiCl and backfilled from the shank with 0.2 M LiCl. The connectivity of the Ib interneuron with the pedal VCMN was first established followed by iontophoresis of Lucifer yellow using a constant negative current (1.0 nA) for 20 min or 0.5 nA for 30 min with 5(6)-carboxyfluorescein labeling. Foot contraction elicited by extrinsic current stimulation of pedal VCMNs was verified before dye labeling of pedal VCMNs. After an additional 2 h to allow for diffusion of the Lucifer yellow, the ganglion was fixed with 4% paraformaldehyde in 0.2 M cacodylate buffer (pH 7.4) for 3–12 h followed by dehydration in an ascending ETOH series and cleared with methyl salicylate. 5(6)-carboxyfluorescein labeled neurons were visualized in normal ASW. The stained neurons were observed and photographed through a fluorescence microscope (Zeiss, Thornwood, NY).

RESULTS

Axonal projections of pedal VCMNs

As previously reported, pedal motor neurons that innervate the anterior region of the foot project to postsynaptic targets through pedal nerve P2 (Richards and Farley 1987). A representative example of a 5(6)-carboxyfluorescein filled pedal VCMN is shown in the photograph of the ventral surface of a hemisected circusesophageal nervous system in Fig. 1A. A single labeled axonal process projecting from the pedal VCMN
Stimulation of pedal VCMNs with 5-s depolarizing current pulses (0.5–0.8 nA) elicited contraction of the ipsilateral anterior foot. Figure 3A1 shows an example of current-elicited spikes recorded from a pedal VCMN in normal ASW. The train of action potentials elicited in the VCMN was sufficient to evoke anterior foot contraction. An example of depolarizing current-elicited spikes recorded in the same pedal VCMN exposed to a high-divalent cation solution (3 × Ca^{2+} and 3 × Mg^{2+}) is shown in Fig. 3A2. The high-divalent cation ASW solution did not block foot contraction elicited by depolarization of the pedal VCMN, suggesting that VCMNs do not indirectly produce contraction through polysynaptic connec-

FIG. 3. Anterior foot contraction evoked by current stimulation of VCMNs. A1: intracellular recording from a pedal VCMN in ASW during depolarization with a 5-s 0.8-nA extrinsic current pulse. A2: depolarization of the same VCMN motor neuron in a high-divalent cation solution (3 × Ca^{2+} and 3 × Mg^{2+}). B: group data (means ± SE) summarizing anterior foot contraction evoked during the 5-s depolarization in artificial seawater (ASW) and high-divalent cation ASW (n = 5).
tions with central neurons. The group data (n = 5) for average foot contraction measured from video tape recordings are shown in Fig. 3B. For purposes of comparison, pedal VCMN spikes and anterior foot contractions shown in Fig. 3B were elicited by the same level of depolarizing current in normal ASW and high-divalent cation ASW. The smaller contractions detected in high-divalent cation ASW are probably due to the decrease in the number of spikes elicited by the current pulse after the application of the high-divalent ASW. Because multiple spikes are required to elicit foot contraction, summation of PSPs could produce sufficient activity in central polysynaptic pathways to contribute to foot movement. To address this issue, only the CNS was exposed to 5 mM Ni2+ ASW to suppress synaptic transmission (n = 4). As shown in Fig. 4A1, depolarization of a pedal VCMN elicited spikes sufficient to produce verified anterior foot contraction. The effectiveness of suppressing central synaptic transmission was established before examining foot contraction in Ni2+ ASW. Pedal nerve P1 contains processes of sensory neurons that project to postsynaptic targets within the circumesophageal nervous system (unpublished observations). Suction electrode stimulation of pedal nerve P1 evoked a complex EPSP recorded from the VCMN motor neuron that is mediated by activation of central polysynaptic pathways (Fig. 4A2). In the presence of 5 mM Ni2+ ASW, the same nerve P1 stimulation failed to elicit the complex EPSP recorded from the VCMN (Fig. 4A3). However, in 5 mM Ni2+ only applied to the circumesophageal nervous system, spikes elicited from the pedal VCMN by an extrinsic current pulse evoked anterior foot contraction (Fig. 4A4).

VCMNs receive synaptic projections from newly identified type Ib interneurons

Stimulation of statocyst hair cells by a mechanical tap or mechanical displacement of the statocyst resulted in a depolarization of newly identified type Ib interneurons and VCMN motor neurons (n = 10). As shown in Fig. 5, A and B, a mechanical tap (Fig. 5A1) elicited a single spike recorded in a type Ib interneuron and a small depolarization and spike recorded from a contralateral pedal VCMN. A stronger mechanical tap (Fig. 5A2) elicited several spikes recorded from the Ib interneuron and multiple EPSP recorded from the contralateral pedal VCMN hyperpolarized to −80 mV to block spike generation (Fig. 5B). These results also indicate that other neurons are activated by mechanical stimulation of the statocyst that project to pedal VCMNs because VCMN EPSPs occurred in the absence of spikes recorded from the Ib interneuron (see Fig. 5D). This observation most likely reflects the convergence of interneuronal projections to pedal VCMNs as well as multiple statocyst hair cell synaptic input to type Ib interneurons.

Type Ib interneurons receive synaptic input from identified hair cells

Extrinsic current stimulation of single hair cells provided evidence that depolarization of type Ib interneurons is mediated by stimulation of statocyst hair cells. Depolarization of a single statocyst hair cell (Fig. 6, A–D) was sufficient to elicit complex EPSPs and spikes recorded from type Ib interneurons (n = 14). As shown in Fig. 6A, extrinsic current depolarization of a hair cell located in the lateral statocyst evoked spikes recorded in a type Ib interneuron. Depolarization of a rostral hair cell (Fig. 6C) evoked a complex EPSP recorded from a type Ib interneuron hyperpolarized to −65 mV to block spike generation (Fig. 6D). The variable latency of type Ib interneuron EPSPs after
hair cell spikes (data not shown) and the absence of a one-for-one relationship between hair cell spikes and type I b EPSPs suggested that the synaptic connections between hair cells and type I b interneurons are polysynaptic. The procedures used in this study for mechanical stimulation of the statocyst that were sufficient to evoke spikes recorded in type I b interneurons and VCMNs also evoked a depolarizing generator potential with superimposed spikes in identified statocyst hair cells (n/H11002 4).

An example of a depolarizing generator potential with superimposed spikes recorded from a rostral statocyst hair cell is shown in Fig. 6E. Direct mechanical displacement of the statocyst used in this study produced depolarizing hair cell generator potentials of similar amplitude as previously reported (Alkon and Bak 1973).

Interestingly, the synaptic connections between statocyst hair cells and type I b interneurons are dependent on the position of the hair cell on the statocyst. As shown in the simultaneous intracellular recordings from identified hair cells and type I b interneurons in Fig. 7, A1–C2, hair cells located on the rostral and lateral region of the statocyst exhibited connections with type I b interneurons. Extrinsic current depolarization of rostral and lateral hair cells evoked complex PSPs in type I b interneurons hyperpolarized to block spike generation. In contrast, hair cells in the caudal region of the statocyst did not exhibit detectable connections with type I b interneurons (Fig. 7C2). The group summary data for hair cell connections with type I b interneurons is shown in Table 1. The majority (86%) of rostral and lateral hair cells examined (n = 14) projected to type I b interneurons. None of the caudal hair cells tested (n = 6) exhibited a connection with type I b interneurons. Taken collectively these results indicate that the response to a mechanical tap or direct mechanical displacement of the statocyst recorded in type I b interneurons is mediated by activation of hair cells located in specific positions on the statocyst.

### Table 1. Ipsilateral hair cell connections with type I b interneurons

<table>
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<tr>
<th>No. of Cells Tested</th>
<th>Type I b Synaptic Connections</th>
<th>Percentage</th>
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<tr>
<td>Rostral</td>
<td>7</td>
<td>6</td>
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<tr>
<td>Lateral</td>
<td>7</td>
<td>6</td>
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<td>Caudal</td>
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<tr>
<td>Total</td>
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Lucifer yellow labeling of type I b interneurons revealed that they project axonal processes to the contralateral pedal gan-
glion (n = 4, Fig. 8A, 1 and 2). As shown in the Lucifer-yellow-labeled type I_b interneuron in Fig. 8A1, the axonal processes from the type I_b interneuron crosses to the contralateral side in the cerebropleural commissure, courses below the eye, and terminates in the contralateral pedal ganglion (A, Fig. 8A2). As shown in Fig. 8, B and C, the labeled contralateral type I_b interneuron indicated in Fig. 8A, 1 and 2, exhibited a synaptic connection with a pedal VCMN. Depolarization of the type I_b interneuron with extrinsic current (Fig. 8B) evoked a complex EPSP recorded from the pedal VCMN (Fig. 8C1). Current depolarization of the same pedal VCMN shown in Fig. 8C1 evoked spikes and elicited ipsilateral anterior foot contraction (Fig. 8C2).

Simultaneous intracellular recordings from ipsilateral and contralateral type I_b interneurons in the cerebropleural ganglia revealed that they are electrically coupled. As shown in the photograph of two type I_b interneurons labeled with 5(6)-carboxyfluorescein in Fig. 9A, each I_b interneuron projects to the contralateral pedal ganglion as indicated by the white arrowheads in the pedal ganglia. The axonal processes cross in the cerebropleural commissure (A), a potential site for electrical coupling. Extrinsic current stimulation of one type I_b interneuron produced a hyperpolarizing electrotonic potential (B) and a smaller potential in the contralateral type I_b (C) indicating electrical coupling between the 2 type I_b interneurons. The likely site of the coupling is in the cerebropleural commissure indicated by ↓ in A.

**Depolarization of I_b interneurons is sufficient to elicit anterior foot contraction**

Depolarization of type I_b interneurons with a 2-s extrinsic current pulse elicited multiple spikes recorded from both type I_b interneurons and pedal VCMNs (Fig. 10, A1–B1). Hyperpolarizing the VCMN below threshold for spike generation (−65 mV) during the period of type I_b depolarization revealed a complex EPSP recorded from the VCMN (Fig. 10, A2–B2). As shown in Fig. 10, C and D, depolarization of a contralateral type I_b interneuron with a 5-s 1.5-nA current pulse produced a train of spikes recorded from the VCMN that was sufficient to elicit anterior foot contraction. The mean contraction (± SE) produced by extrinsic current depolarization of type I_b interneurons (n = 3) is shown in the summary group data (Fig. 10E).

**Multiple projections of type I_b interneurons**

Previously we showed that the primary pathway for activation of ciliary motor neurons is from type I interneuronal connections with type III, inhibitory interneurons (Crow and Tian 2003a). A second pathway that can modulate VP1 ciliary motor neuron spike activity has been identified (Crow and Tian 2003a). The second pathway involves activation of type II_b
interneurons that is sufficient to depolarize VP1 and VP3 ciliary motor neurons. Components of the ciliary motor system modulated by visual input are different from the neural system supporting foot contraction because type Ie and Ii interneurons do not project to pedal VCMNs (see Fig. 13). In this report, we provide evidence that statocyst hair cell depolarization of VP1 ciliary motor neurons and photoreceptor activation of ciliary motor neurons is primarily mediated by two different pathways.

Simultaneous recordings (n = 9) from pairs of type Ib interneurons and VP1 ciliary motor neurons or pedal VCMNs suggested that the synaptic connection is monosynaptic. As shown in Fig. 12A a single spike in a type Ib interneuron evoked a short and relatively constant latency EPSP in a VP1 ciliary motor neuron (Fig. 12B1) that also followed Ib spikes one-for-one as indicated by the superimposed EPSPs recorded in high-divalent ASW (Fig. 12B2). A single current elicited spike recorded in a type Ib interneuron (Fig. 12C1) evoked a short and relatively constant latency EPSP in a VCMN (Fig. 12D1) that followed Ib spikes one-for-one in high-divalent ASW as indicated by the superimposed EPSPs in Fig. 12D2.

DISCUSSION

In this report, we have identified anterior foot contraction motor neurons (VCMNs) and characterized synaptic input to VCMNs from statocyst hair cells and type Ib interneurons. Depolarization of type Ib interneurons with extrinsic current or mechanical stimulation of statocyst hair cells evoked complex EPSPs and spikes in VCMNs sufficient to elicit contraction of the anterior foot. Direct mechanical stimulation of the statocyst was sufficient to evoke a depolarizing generator potential with superimposed spikes in statocyst hair cells as previously re-
that result in either contraction or elongation of the posterior region of the foot when depolarized with extrinsic current. In addition, depolarization of a single contralateral type Ib interneuron produces both ciliary movement and posterior foot contraction in semi-intact preparations with only pedal nerve P1 intact (unpublished observations). These results suggest that type Ib interneurons project to contractile and ciliary motor neurons innervating all regions of the foot.

**Differences between visual and graviceptive modulation of ciliary locomotion**

Our evidence indicates that anterior foot contraction and statocyst-mediated ciliary locomotion involve different interneurons than the neural circuit mediating visually influenced ciliary locomotion (see Fig. 13). Here we report that type Ib interneurons project to both VCMNs and VP1 ciliary motor neurons. However, statocyst-mediated ciliary activation is the result of direct type Ib depolarization of VP1 ciliary motor neurons while light results in a disinhibition of VP1 ciliary motor neurons through decreased activity of type III interneurons (Crow and Tian 2003a). We have shown previously that type Ib, IIc, and IIi interneurons provide convergent synaptic input to type III inhibitory interneurons responsible for inhibition of VP1 and VP3 cilia-activating motor neurons (Crow and Tian 2003a). In addition, type IIb, IIc, and IIi interneurons that mediate light modulation of VP1 and VP3 neural activity do not project to VCMNs (Crow and Tian 2003a). However, motor neuron VP2 receives synaptic input from the same type III interneuron that projects to VP1 and VP3 ciliary motor neurons and thus may be involved in a behavior that is complimentary to ciliary locomotion. Depolarization of motor neuron VP2 elicited a slow lateral movement of the anterior foot and ventral tentacle that may increase the contact area between the ventral foot and the underlying substrate during ciliary locomotion (Crow and Tian 2003a). Therefore the different

**Neural circuitry contributing to foot-shortening**

Foot-shortening elicted by the CS in conditioned animals and by the US involves a larger contraction of the posterior foot and smaller contraction of the anterior foot near the ventral tentacle (Lederhendler et al. 1986). Previously it was shown that extrinsic current depolarization of a pedal motor neuron (MN1) produced a contraction of the middle region of the ipsilateral foot and a subsequent turning of the posterior foot toward the ipsilateral direction (Goh and Alkon 1984). In addition, it has been shown that light does not elicit the clinging reflex (Alkon 1974) or foot-shortening before conditioning (Lederhendler et al. 1986). Because light was shown to depolarize MN1 (Goh and Alkon 1984), it is unlikely that MN1 is a contractile motor neuron innervating the posterior region of the foot. We have observed in semi-intact preparations that stimulation of a neuron on the dorsal surface of the pedal ganglion produces vigorous contraction of the ipsilateral posterior foot (unpublished observations). Moreover the pedal neuron, like the VCMNs described in this report, is not depolarized by light. We have now identified a number of putative pedal motor neurons with axons projecting in pedal nerve 1

**FIG. 12.** Type Ib interneurons directly excite pedal VCMNs and VP1 ciliary activating motor neurons. Simultaneous recordings from type Ib, interneurons and VP1 ciliary motor neurons or pedal VCMNs in a high-divalent cation solution (3 × Ca$^{2+}$ and 3 × Mg$^{2+}$). A1: a single spike generated in a type Ib interneuron by a current pulse evoked a monosynaptic EPSP recorded from a VP1 motor neuron (B1). The latency between the type Ib spike and VP1 is short, relatively constant, and 1-for-1 as shown by 3 consecutive superimposed spikes (A2) and evoked superimposed EPSPs (B2). C1: a single spike generated in a type Ib interneuron by a current pulse evoked a monosynaptic EPSP recorded from a VCMN (D1). EPSPs recorded from the VCMN followed spikes in the type Ib interneuron 1-for-1 with a brief and relatively constant latency as indicated by 3 consecutive superimposed spikes (C2) and superimposed EPSPs recorded from the VCMN (D2).
motor acts mediated by VP1, VP3, and VP2 motor neurons are not necessarily incompatible, and coactivation may synergistically facilitate ciliary locomotion.

**Different behaviors may be controlled by the same neural circuit**

It is well documented that motor systems can support the generation or expression of multiple motor acts (for reviews, see Getting 1989; Kupfermann and Weiss 2001; Marder and Calabrese 1996; Pearson 1993). Representative examples of different behaviors controlled by the same neural network have been identified in *Tritonia* (Popescu and Frost 2002), *Pleurobranchaea* (Jing and Gillette 2000), and *Lymnaea* (Syed and Winlow 1989). Escape locomotion evoked by aversive or noxious stimuli is a closely related locomotor behavior that may be mediated by the same neural network as ciliary locomotion in *Hermissenda*. Ciliary movement is a nonrhythmic, nonmuscular gliding form of locomotion in contrast to escape locomotion which is muscular and rhythmic, involving the generation of foot contractions. However, the two behaviors could be complimentary because increased ciliary activity generated in phase with rhythmic contractions of the pedal musculature during contact of the foot with the substrate would be expected to enhance the speed of escape locomotion. The neural circuit that supports ciliary locomotion in *Hermissenda* could be reconfigured to generate rhythmic activity in VP1, VP2, VP3, and VCMNs. Light inhibits type Ii interneurons and excites type Ii interneurons and both interneuronal types project to type III inhibitory interneurons (Crow and Tian 2003a). Intrinsic rhythmic bursting of type Ii and Ii interneurons could generate periods of excitation and inhibition in type III interneurons that would produce phasic excitation of contractile and ciliary motor neurons. Therefore the neural network supporting ciliary locomotion and its regulation by light could be reconfigured by appropriate sensory input to generate the rhythmic pedal motor program underlying a different behavior, escape locomotion.

**Conditioning produces modifications in multiple response systems**

Pairings of light and rotation produce conditioning in two different behavioral response systems; ciliary locomotion and foot-shortening. The identification of the neural circuits responsible for ciliary locomotion and foot contraction is consistent with behavioral studies suggesting that CS-elicited foot-shortening and light-elicited inhibition of phototactic behavior may develop independently (Matzel et al. 1990). An alternative hypothesis is that light inhibition of phototaxis is a secondary consequence of CS-elicited foot-shortening. However, it is now clear that conditioning produces light-elicited inhibition of ciliary motor neurons (Crow and Tian 2003b).

In the leech, a conditioning procedure consisting of a tactile CS and shock US modifies two different behaviors, stepping and shortening (Sahley and Ready 1988). Classical conditioning in vertebrates exhibits even more complexity of conditioning in multiple response systems (for example see Black and de Toledo 1972). Moreover, both conditioned foot contraction and conditioned inhibition of phototaxis involve the development or emergence of a new response to the CS, not the potentiation, through US presentations of an already existing response to the CS referred to as reflex potentiation (for review, see Sahley and Crow 1998). Consistent with these observations is the finding that light does not depolarize VCMNs or elicit foot-shortening prior to conditioning. In both conditioned responses, there is a transfer of functional aspects of the response-evoking properties of the US to the CS (Crow and Alkon 1978; Lederhendler et al. 1986; Matzel et al. 1990). This aspect of conditioning probably accounts for the increased complexity and independence of the circuits supporting the CS and US and the multiple sites of CS-US interactions within the neural network.

**Specificity of hair cell projections to interneurons**

Our results showing that caudal hair cells do not project to type Ii interneurons suggest that during conditioning, orientation in the direction of the rotational force vector would elicit foot contraction. Rotation or gravity causes the statocyst to press against motile cilia of hair cells in front of the centrifugal or gravitational force vector, resulting in a depolarizing generator potential and an increase in spike activity (Alkon 1975). Since *Hermissenda* exhibit a negative geotactic response, stimulation of rostral hair cells by a downward orientation would produce foot contraction and initiate turning to reorient in a vertical direction, opposite the gravitational force vector.

The synaptic organization of primary and secondary components of the visual pathway and statocyst pathway of *Hermissenda* has been characterized and described in considerable detail (Akaike and Alkon 1980; Alkon 1973a,b; Alkon and Bak 1973; Alkon and Fuortes 1972; Alkon et al. 1978; Crow and Tian 2000; 2002a; 2003a; Crow et al. 1979; Dennis 1967). The convergence sites providing for synaptic interactions between the CS and US pathways have been identified (Akaike and Alkon 1980; Alkon 1973b; Alkon et al. 1978; Crow and Tian 2000, 2002a,b, 2003a). More recently most of the components of the network supporting ciliary locomotion have now been identified (Crow and Tian 2000, 2003a). In addition, modulation of ciliary motor neurons by light before and after conditioning has been described and analyzed (Crow and Tian 2003b). The results presented here provide basic elements for the neural circuit supporting statocyst hair-cell-mediated foot contraction and statocyst-mediated ciliary locomotion and in addition, potential loci for cellular and synaptic plasticity associated with the generation of CS-elicited foot-shortening after Pavlovian conditioning.

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