Polysensory Interneuronal Projections to Foot Contractile Pedal Neurons in *Hermissenda*

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Crow T, Tian L-M. Polysensory interneuronal projections to foot contractile pedal neurons in *Hermissenda*. J Neurophysiol 101: 824–833, 2009. First published December 10, 2008; doi:10.1152/jn.91079.2008. A Pavlovian-conditioning procedure may produce modifications in multiple behavioral responses. As an example, conditioning may result in the elicitation of a specific somatomotor conditioned response (CR) and, in addition, other motor and visceral CRs. In the mollusk *Hermissenda* conditioning produces two conditioned responses: foot-shortening and decreased locomotion. The neural circuitry supporting ciliary locomotion is well characterized, although the neural circuit underlying foot-shortening is poorly understood. Here we describe efferent neurons in the pedal ganglion that produce contraction or extension of specific regions of the foot in semi-intact preparations. Synaptic connections between polysensory type I, and type I, interneurons and identified foot contractile efferent neurons were examined. Type I, and type I, interneurons receive synaptic input from the visual, graviceptive, and somatosensory systems. Depolarization of type I, interneurons evoked spikes in identified tail and lateral foot contractile efferent neurons. Mechanical displacement of the statocyst evoked complex excitatory postsynaptic potentials (EPSPs) and spikes recorded from type I, and type I, interneurons and complex EPSPs and spikes in identified foot contractile efferent neurons. Depolarization of type I, interneurons in semi-intact preparations produced contraction and shortening along the rostrocaudal axis of the foot. Depolarization of I, interneurons in semi-intact preparations produced contraction of the anterior region of the foot. Taken collectively, the results suggest that type I, and type I, polysensory interneurons may contribute to the neural circuit underlying the foot-shortening CR in *Hermissenda*.

**INTRODUCTION**

Pavlovian-conditioning studies in diverse species have shown that learning involves the acquisition of multiple conditioned responses (CRs) produced by the same conditioning procedure (Ayers and Powell 2002; Black and Toledo 1972; Gantt 1960; Godsil et al. 2000; Konorski 1967; Powell 1999; Prokasy 1984; Schneiderman 1972; Weinberger and Diamond 1987). As an example, the acquisition of a somatomotor CR also results in the development of concomitant visceral CRs. The development of multiple responses with classical conditioning raises a number of issues concerning the CR complex. Do the different CRs develop independently or are there interactions between components of the underlying network? If acquisition rates are different for the multiple CRs, does learning in one response pathway contribute to the development of the other response? Are there mechanistic differences between the CRs with respect to initial acquisition or retention? The results of lesion studies suggest that the development of somatomotor CRs involves different areas of the brain than the visceral CRs that are concomitantly acquired (Buchanan and Powell 1982; Kao and Powell 1988; Lavond et al. 1984). However, the issue concerning independent or dependent development of multiple CRs is further complicated by the differential effects of trace versus delayed conditioning procedures (Green and Woodruff-Pak 2000; McLaughlin et al. 2002). Many of the questions concerning mechanistic processes associated with the synaptic interactions between conditioned stimulus (CS) and unconditioned stimulus (US) pathways supporting multiple CRs are effectively addressed in less complex nervous systems where the circuitry is more characterized.

In the nudibranch mollusk *Hermissenda crassicornis* Pavlovian conditioning results in the acquisition of two different CRs. The CS elicits foot-shortening and inhibition of forward locomotion (Crow and Alkon 1978; Lederhendler et al. 1986). An analysis of acquisition of the two CRs suggested that the responses are supported by distinct efferent pathways and develop independently (Matzel et al. 1990). The neural circuit contributing to turning behavior in *Hermissenda* has been examined (Goh and Alkon 1984) and recent studies have identified the neural circuit underlying ciliary locomotion (Crow and Tian 2000, 2002a,b, 2003a, 2004). However, little is known about the neural circuit mediating graviceptive elicited foot-shortening. In this study using semi-intact preparations, we have identified and characterized efferent neurons that innervate the tail and midlateral regions of the foot. We show that identified lateral foot contractile, tail contractile, and dorsal ciliary efferent neurons receive synaptic input from polysensory type I, interneurons. Depolarization of I, interneurons in semi-intact preparations evoked contraction along the rostrocaudal axis of the foot. We provide evidence that identified I, interneurons project to efferent neurons innervating the anterior foot. The I, interneurons are polysensory; receiving synaptic input from the graviceptive, visual, and somatosensory systems. The strong synaptic input from the graviceptive sensory system to I, and I, interneurons and their synaptic projections to efferent neurons innervating different regions of the foot suggest that type I, and type I, interneurons may contribute to the circuit generating the foot-shortening CR.

**METHODS**

**Animals**

Adult *Hermissenda crassicornis* were used in the experiments. The animals were obtained from Sea Life Supply (Sand City, CA) and

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maintained in closed artificial seawater (ASW) aquaria 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 identified I, and I, interneurons and efferent neurons were collected from partially split-foot semi-intact preparations (see following text). Studies examining synaptic connections between the graviceptive system and interneurons and the visual system and interneurons included both isolated nervous systems and semi-intact preparations. Experiments involving recordings from efferent neurons were conducted in semi-intact preparations. Anatomical and electrophysiological criteria were used to identify type I, and type I, interneurons as described previously for type I and type II interneurons (Crow and Tian 2000). The location of I, and I, somas in the cerebropleural ganglion relative to reliable landmarks such as type I interneurons provided a general anatomical identification. Type I, and type I, interneurons were then physiologically identified by mechanically stimulating statocyst hair cells. Displacement of the statocyst elicited complex postsynaptic potentials (PSPs) and spikes in type I, and type I, interneurons and identified efferent neurons. The procedure for mechanical displacement of the statocyst is similar to the methods described in previously published reports (Alkon and Bak 1973; Detwiler and Alkon 1973). Briefly, mechanical stimulation of hair cells was produced by displacement of the statocyst provided by a glass probe attached to the end of a 7- to 11-μm displacement of the statocyst and oscillation of the statoconia within the lumen of the statocyst. Surgical desheathing of a small area of the cerebropleural and ventral and dorsal pedal ganglion was conducted to expose the cell bodies of interneurons and efferent neurons. In semi-intact preparations pedal efferent neurons were identified by verifying foot contractions produced by depolarization with extrinsic current.

The partially 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 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. Maximum light intensity (10^-4 W/cm²) was attenuated with neutral density filters expressed in negative log units. Interneurons and efferent neurons 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 used. 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. Electrophysiological data were digitized with a CED power 1401 (Cambridge Electronic Design [CED], Cambridge, UK) and stored on a computer hard drive. Digitized data were analyzed and prepared for figures using Spike2 software (CED).

**Semi-intact preparations**

Semi-intact anterior split-foot *Hermissenda* were prepared by cooling the animals in ASW to between 0 and 1°C, followed by isolation of the circumesophageal nervous system from the buccal crest and body, leaving intact pedal nerves P1 and P2. The partially split foot was positioned ventral side up adjacent to the isolated circumesophageal nervous system pinned to the elevated central stage in the recording chamber. For recordings from neurons on the ventral surface, the left pedal ganglion was rotated about 150° to provide for visualization of neuronal cell bodies. The exposed nervous system and 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. A Leica DFC280 Digital Camera (Leica Microsystems, Wetzlar, Germany) was used to record foot movement elicited by current stimulation of identified pedal efferent neurons. The camera was mounted on a stereomicroscope (Wild M5A) and connected to a computer. The camera was configured at 30 consecutive frames (multiple-image capture) with a cumulative interval of 50 ms. The exposure time was set at 500 ms. Pictures were computer processed with Image-Pro-Express (Version 5.1.0.12 for Windows 2000/XP Professional, Media Cybernetics).

**Interneuron labeling**

Identified I, interneurons were impaled with microelectrodes whose tips contained 2% Lucifer yellow in 0.2 M LiCl (electrode resistance 100–150 MΩ). A hyperpolarizing current (0.5–1 nA) was applied for 1 h to iontophorese Lucifer yellow. The nervous system remained in the recording chamber for an additional 1 h followed by overnight fixation with 4% paraformaldehyde in ASW. Nervous systems were rinsed three times (10-min interval) in 0.1 M phosphate-buffered saline, dehydrated in ethanol, cleared in methyl salicylate, mounted, and viewed under a fluorescent microscope. Images were collected using a laser scanning confocal microscope (BioRad Radiance 2100). The sampling steps were set at 2 μM and the z-stack of 65 sections was merged to generate the final image of labeled I, interneurons.

**RESULTS**

Graviceptive stimulation produces reflexive contraction of foot muscles that results in a change in the overall configuration of the foot. A change in foot configuration underlies both the clinging response elicited by graviceptive stimulation and the foot-shortening CR evoked by the CS following Pavlovian conditioning (Lederhendler et al. 1986). Rotation applied as an unconditioned stimulus (US) in the Pavlovian-conditioning paradigm elicits a decrease in the length of the foot. The clinging response in *Hermissenda* is expressed when animals increase contact of the foot with the underlying substrate in response to seawater turbulence produced by agitation (Alkon 1974). In this study we have identified pedal neurons in semi-intact preparations that innervate contractions or extension of different regions of the foot and body wall that produce changes in foot configuration when stimulated with extrinsic current.

**Foot contractile pedal neurons**

The general anatomical organization of the pedal-motor system in *Hermissenda* consists of efferent neurons whose cell bodies are located on the dorsal and ventral surfaces of the pedal ganglia, with axonal projections in nerves P1 and P2. Previous research has shown that neurons with axons in P1 innervate the posterior three fourths of the foot, whereas neurons with axons in P2 innervate the anterior region of the foot (Richards and Farley 1987). In general, many dorsal pedal neurons have axons in P1 and ventral pedal neurons have axons projecting to the periphery in P2. In this study, several types of efferent neurons were identified that altered foot configuration when depolarized with extrinsic current. Depolarization of identified efferent neurons produced tail contraction (TC), lateral foot contraction (LFC), anterior foot contraction (VC),...
or tail extension (TE). In all, 75 semi-intact preparations were used in the studies. The surgical procedures used for the generation of semi-intact preparations involved cutting the pedal nerves innervating the right side of the foot, leaving intact left P1 and P2. This procedure resulted in an ipsilateral denervation and hemiplegia of the foot muscles on the right side and normal innervation of the left side of the foot. An example of tail contraction evoked by stimulation of a TC efferent neuron with a 5-s current pulse is shown in Fig. 1A. The slight turning of the tail region during contraction is the result of innervation of the muscles on the left side of the foot moving the flaccid denervated right-side tail region during current depolarization (Fig. 1A). The white tracing around the perimeter of the posterior region of the foot indicates foot position immediately before the onset of the current pulse. The contracted or shortened region of the tail is outlined in red and indicates the position of the tail at the end of the current pulse. The inset of Fig. 1A shows an example of depolarization of the TC neuron produced by the 5-s current step that resulted in the tail contraction shown in Fig. 1A. The white outline extending to the middle region of the foot indicates that the midregion of the foot did not contract during current stimulation of the TC neuron. An example of midfoot contraction produced by current depolarization of the LFC efferent neuron is shown in Fig. 1B. The white outline drawn on the ipsilateral foot indicates the position of the foot immediately before current depolarization of the LFC efferent neuron. The contracted midregion of the lateral foot is shown by the red line in the photograph taken at the end of the current pulse. Contraction of the lateral foot was restricted to the midregion and did not include the posterior region near the tail or the anterior part of the foot as shown in the photograph. The inset is an example of a recording from the LFC efferent neuron during current depolarization that produced the midfoot contraction shown in Fig. 1B. An example of tail extension evoked by stimulation of a TE efferent neuron is shown in Fig. 1C. In the photograph, the red outline shows the position of the posterior foot at the end of the depolarizing current pulse. The position of the tail before current stimulation of the TE neuron is indicated by the white line (Fig. 1C). The primary extension of the posterior foot was restricted to the ipsilateral region of the tail and did not involve the denervated contralateral side of the posterior tail. In addition, the ipsilateral midfoot and anterior region did not contract or extend during the current pulse (Fig. 1C). The inset shows an example of depolarization of the TE neuron that elicited the tail extension shown in Fig. 1C.

A diagram of the dorsal and ventral surfaces of the left pedal ganglion, indicating the location of identified efferent neurons generating the different contractions and extension of the foot, is shown in Fig. 1D. In addition, the location of identified ciliary efferent neurons and previously identified anterior foot contraction efferent neurons is shown in the diagram of the pedal ganglia.

Ib interneurons

Identified interneurons that project to both ventral contractile (VC) efferent neurons and VP1 ciliary efferent neurons have been characterized in previous studies of the neural circuitry underlying ciliary locomotion and anterior foot contraction (Crow and Tian 2004). Here we examined synaptic connections between identified type Ib interneurons and the recently identified efferent neurons producing contraction of different regions of the foot. Representative examples of simultaneous recordings from Ib interneurons and identified efferent neurons in semi-intact preparations are shown in Fig. 2. Type Ib interneurons project to several efferent neurons contributing to foot contraction and ciliary locomotion. Depolarizing current stimulation of an Ib interneuron elicited spikes recorded from an identified tail contractile (TC2) neuron (Fig. 2A). As shown in Fig. 2B, depolarization of an Ib interneuron evoked spikes recorded from a different identified tail contractile neuron (TC2) located in the rostral region of the pedal ganglion. Depolarization of an Ib interneuron also evoked spikes recorded from an identified lateral foot contractile (LFC) efferent neuron (Fig. 2C). Consistent with previous results showing

![Fig. 1](http://jn.physiology.org/)

**Fig. 1.** Photographs of representative semi-intact preparations before stimulation and at the termination of a 5-s depolarizing current pulse delivered to (A) a tail contractile efferent neuron (TC), (B) a lateral foot contractile efferent neuron (LFC), and (C) a tail extension efferent neuron (TE). The white outline indicates the position of the foot immediately before stimulation in A, B, and C. The red outline shows the position of the foot and the end of the current pulse in A, B, and C. The insets are recordings of spike activity in the efferent neurons immediately before and during current stimulation. In B, note that the anterior ipsilateral and posterior ipsilateral regions of the foot did not contract. In C, note that the anterior and middle regions of the foot did not contract or extend during the current pulse. D: dorsal and ventral surface maps of the pedal ganglion showing the locations of neuronal somata of ciliary efferent neurons (orange), tail contractile efferent neurons (yellow), lateral foot contractile efferent neurons (blue), anterior foot contractile efferent neurons (green), and tail extension efferent neurons (purple). The uncolored cells indicated in the drawings are useful landmarks for anatomical identification of efferent neurons. The large noncolor labeled cell in the left pedal ganglia is LP1 (Jerussi and Alkon 1981).
Synaptic connections between VP1 ciliary efferent neurons and Ib interneurons, Ib depolarization evoked a complex excitatory postsynaptic potential (EPSP) recorded from a ciliary efferent neuron located on the dorsal pedal ganglion (DC) (Fig. 2D). Type Ib and type Ie interneurons in the circuit supporting ciliary locomotion are polysensory. We have previously shown that type Ib interneurons receive synaptic input from ipsilateral rostral and lateral statocyst hair cells (Crow and Tian 2004). We further examined sensory projections to type Ib interneurons by recording EPSPs and spikes elicited during illumination of the eyes. Dark-adapted type Ib interneurons typically do not spontaneously generate spikes. As shown in the histograms of EPSPs in Fig. 3, light evoked an increase in EPSP frequency recorded from type Ib interneurons relative to dark-adapted baseline activity. EPSP frequency depended on light intensity. Unattenuated light evoked more Ib EPSPs (Fig. 3, A2 and B2) compared with light-attenuated 1 log unit (Fig. 3, A1 and B1).

The primary effect of light was to increase the frequency of EPSPs with amplitudes between 2 and 5 mV. An example is shown in the inset of Fig. 3B2. Unattenuated light (5 min) evoked an increase in the frequency of 2- to 5-mV EPSPs (mean = 0.38/s) compared with 5 min in the dark immediately before light (mean = 0.17/s). EPSPs with amplitudes >5 mV occurred less frequently in the light (mean = 0.007/s) and dark (mean = 0.02/s). Current depolarization of Ib interneurons sufficient to produce spikes in the dark revealed a modest increase in spike activity measured during 5-min periods of illumination (Fig. 3C). Analysis of the group data in Fig. 3D from Ib interneurons depolarized with steady current (n = 5) revealed that following 12 min of dark adaptation (mean spike frequency of type Ib interneurons between light-adapted and the two dark-adapted conditions [F(2,4) = 5.3; P < 0.03]. As a result, following the termination of illumination the average spike activity during a 5-min period in the dark was 0.04 ± 0.02 spikes/s. Statistical analysis revealed a significant overall difference in the mean spike activity of type Ib interneurons between light-adapted and the two dark-adapted conditions.
control to rule out potential synaptic input to Ib interneurons from extrinsic light responsive neurons or intrinsic light responses of Ib cells, we examined spike activity in the dark and in light during conditions of current depolarization in preparations where the eyes were ablated \((n = 5)\). Analysis of Ib spike activity from ablated preparations did not reveal statistically significant differences between dark-adapted conditions and illumination \((t_{42} = 0.42; \text{NS})\). These results suggest that type Ib interneurons receive synaptic input from the visual system unless synaptic input from other sensory systems provides sufficient depolarization to bring the cells to threshold for spike generation. Results of our analysis of synaptic input to Ib interneurons from the visual system and the identified connections between Ib interneurons and efferent neurons are consistent with results indicating that light typically does not elicit foot-shortening (Lederhendler et al. 1986).

**I**s **interneurons**

In this study we have identified an additional interneuron type that receives synaptic input from the graviceptive system and has synaptic projections to identified efferent neurons. Recordings from newly identified type Is interneurons showed that they project to efferent neurons that innervate primarily the anterior region of the foot. Labeling of Is interneurons with Lucifer yellow \((n = 5\) preparations) revealed a bifurcation of the primary axonal process in the contralateral cerebropleural ganglion and two axonal processes that project to different regions of the contralateral pedal ganglion. A representative example of a Lucifer-labeled Is interneuron is shown in Fig. 4A. The fluorescent image from a confocal scan of the circumsophagel nervous system shows a labeled Is soma (green) and a bifurcating axonal process indicated by white arrowheads in the contralateral cerebropleural ganglion and pedal ganglion (Fig. 4A). We examined synaptic input to Is interneurons from different sensory systems. Type Is interneurons receive synaptic input from the graviceptive and somatosensory systems. As shown in Fig. 4B depolarization of hair cells by mechanical displacement of the statocyst evoked a complex EPSP recorded from an Is interneuron. A larger displacement of the statocyst in a different preparation evoked a train of spikes recorded from an Is interneuron (Fig. 4C). In a previous study identified polysensory interneurons in the cerebropleural ganglion were shown to receive somatosensory input from the foot and/or body wall (Tian et al. 2006). We examined whether polysensory Is interneurons also received somatosensory input from the foot by recording spike activity in Is cells evoked by cutaneous stimulation of the foot. As shown in Fig. 4D, flicking a von Frey hair with a pressure of 3.14 g/mm\(^2\) across the midregion of the foot evoked a depolarization and spike activity recorded in an Is interneuron. We next examined synaptic input to Is interneurons from the visual system. An example of EPSPs recorded from a dark-adapted Is interneuron and during illumination of the photoreceptors is shown in Fig. 5. Statocyst hair cell activation was used to assist in the identification of the Is interneurons (Fig. 5A). Stimulation of the eyes with light-attenuated 1 log unit increased the frequency of EPSPs with amplitudes between 1.5 and 3.2 mV (Fig. 5B, as indicated by the histogram and EPSP recordings from an Is interneuron shown in Fig. 5, C and D. The mean frequency of 1.5- to 3.2-mV EPSPs in the dark was, respectively, 0.15 and 0.27 spikes/s during the 5-min period of light. EPSPs with amplitudes >3.2 mV occurred less frequently in both light (mean = 0.07 spikes/s) and dark (mean = 0.05/s). The average spontaneous spike activity of Is interneurons in dark-adapted preparations was 0.002 ± 0.0007 spikes/s \((n = 18)\). Spontaneous spike activity recorded from Is interneurons during a 5-min period of illumination of the photoreceptors did not change significantly from dark-adapted activity. However, as shown in the example of Fig. 5E, current depolarization of type Is interneurons that exceeded spike threshold under dark-adapted conditions revealed a modest increase in spike activity detected during illumination. Analysis of the group summary data in Fig. 5F \((n = 5)\) collected during current depolarization showed a statistically significant increase in Is spike activity \((t_{d} = 6.7; P < 0.003)\) during illumination \((mean = 0.29 ± 0.02 spikes/s)\) relative to baseline spike activity recorded in the dark \((mean = 0.14 ± 0.02 spikes/s)\).
The position of the ipsilateral foot at the end of the current depolarization of Ib interneurons on contraction of the foot contractile efferent neurons we examined the effect of spikes recorded from a tail contractile (TC1) neuron (Fig. 6 recorded from a dark-adapted type Is interneuron, during 5 min of light-illumination of the photoreceptors. C

Depolarization of Ib and Is interneurons in semi-intact preparations

Since type Ib interneurons have synaptic connections with foot contractile efferent neurons we examined the effect of current depolarization of Ib interneurons on contraction of the foot in semi-intact preparations (n = 12 preparations). Depolarization of contralateral type Ib interneurons produced contraction of the ipsilateral tail and lateral foot along the rostro-caudal axis of the foot. A representative example of the configuration of the foot immediately before current stimulation of an Ib interneuron is indicated by the white outline in Fig. 7A. The position of the ipsilateral foot at the end of the current pulse is indicated by the red outline in the photograph shown in Fig. 7A. The 5-s current depolarization of the Ib interneuron that evoked the foot contraction is shown in the inset of Fig. 7A. Current depolarization of Ib interneurons in semi-intact preparations (n = 6) evoked a small contraction of the anterior region of the foot. The black line in the photograph of Fig. 7B indicates the position of the ipsilateral anterior foot immediately before current stimulation of the Ib interneuron. Anterior foot position at the end of the current pulse is indicated by the red line in the photograph. The current depolarization of the Ib interneuron that evoked movement of the anterior foot is shown in the inset of Fig. 7B.

Visual input to foot contractile efferent neurons

Our results indicate that type Ib and type Is interneurons receive synaptic input from the visual system. Visual input to identified foot contractile efferent neurons was examined by measuring changes in spike activity of identified efferent neurons in semi-intact preparations during illumination of photoreceptors relative to dark-adapted baseline activity. Analysis of the group summary data for recordings in TC efferent neurons (n = 8) did not reveal statistically significant changes in spike activity during a 5-min presentation of light (mean = 0.87 ± 0.24 spikes/s) compared with a 5-min period in the dark (mean = 0.89 ± 0.25 spikes/s) immediately preceding the light (I7 = 0.9; NS). Although TC efferent neurons may not exhibit changes in spike activity expressed over a 5-min period of illumination, they may show transient “on” responses to illumination of the photoreceptors following dark-adapted conditions. We examined changes in spike activity of identified TC efferent neurons during two consecutive 5-s epochs following the onset of light compared with the average spike activity recorded during two 5-s periods in the dark immediately preceding light onset. Results of the ANOVA did not support statistically significant differences in spike activity relative to dark-adapted prelight baseline activity in either 5-s period following the onset of light-attenuated 1 log unit (n = 7) [F(2,6) = 1.6; NS] or bright light (0 attenuation) (n = 5) [F(2,4) = 1.8; NS].

Examination of light-evoked spike activity in identified VC efferent neurons (n = 7) during the first two 5-s epochs following the onset of light (mean = 0.40 ± 0.183 and 0.40 ±
0.15 spikes/s) compared with activity in the dark immediately before light (mean = 0.46 ± 0.18 spikes/s) also did not reveal statistically significant changes \( F(2,6) = 0.7; \) NS. In contrast to the results of illumination on the spike activity of TC and VC neurons, both TE efferent neurons and LFC efferent neurons exhibited increased spike activity evoked by illumination of the photoreceptors. As shown in the recording in Fig. 8A, light elicited an increase in the spike activity of an identified TE efferent neuron. Short-latency “on” responses were not characteristic of light-evoked activity of TE efferent neurons. Analysis of the group data shown in Fig. 8B revealed that mean spike activity of TE efferent neurons during light (mean = 1.5 ± 0.6 spikes/s) was significantly greater than the corresponding period in the dark (mean = 0.7 ± 0.5 spikes/s) \( (t_4 = 3.4; P < 0.025) \). In contrast to TE efferent neurons, recordings of spike activity in LFC efferent neurons \( (n = 10) \) exhibited “on” responses to illumination of the photoreceptors. Statistical analysis of the group data shown in Fig. 8C revealed an overall significant difference in spike activity in light compared with dark-adapted baseline activity \( F(2,6) = 4.3; P < 0.03 \). Paired comparisons (Tukey test) showed that the first 5-s epoch after light onset was not statistically different (mean = 1.3 ± 0.38 spikes/s) compared with the dark immediately preceding light (mean = 1.13 ± 0.4 spikes/s). However, the second 5-s epoch after light onset (mean = 1.7 ± 0.48 spikes/s) was significantly different from baseline \( (P < 0.05) \). In addition, the mean activity of LFC efferent neurons during a 2-min light presentation (Fig. 8D) (mean = 1.48 ± 0.4 spikes/s) was significantly greater than the equivalent period in the dark before the presentation of light (mean = 1.05 ± 0.4 spikes/s) \( (t_0 = 2.57; P < 0.025) \).

A diagram summarizing the synaptic connections between interneurons and efferent neurons identified in this study and previously identified components of the ciliary locomotion circuitry is shown in Fig. 9. As previously noted (Tian and Crow 2004) there is little overlap between the components of the graviceptive foot contraction circuit and the light-elicited ciliary locomotion circuit. However, type Ii interneurons that have synaptic connections with foot contractile efferent neurons also project to efferent neurons that activate cilia on the anterior and posterior foot.

**DISCUSSION**

*Neural circuit supporting ciliary locomotion*

Of the two behaviors that have been studied in *Hermisenda*, the neural circuit controlling ciliary locomotion and its modulation by the visual system is more extensively characterized (see Fig. 9). The neural circuit supporting ciliary locomotion consists of polysynaptic projections from type I interneurons to type IIIi inhibitory interneurons that form monosynaptic inhibitory connections with ciliary efferent neurons (Crow and Tian 2003a, 2004). Both type Ii and Ii interneurons excite type IIIi inhibitory interneurons, resulting in increased inhibition of ciliary efferent neuron spike activity. The visual system modulates ciliary locomotion by the monosynaptic connections from photoreceptors to aggregates of type I interneurons that result in light-evoked inhibition of Ii interneurons and light-
interneurons form monosynaptic connections with identified ciliary efferent neurons. However, I_b interneurons contribute to the activity of the graviceptive circuitry since statocyst hair cell activation generates both EPSPs and spikes in I_b interneurons and efferent neurons. Here we have shown that I_b interneurons project to efferent neurons that innervate different regions of the foot. Taken collectively, the results suggest that light modulation of ciliary locomotion and its modification by Pavlovian conditioning involves circuit components that may be distinct from the graviceptive system that contributes to foot contraction.

**I_b interneurons**

Type I_b interneurons are polysensory and receive synaptic input from the graviceptive, somatosensory, and visual systems. Bimorph stimulation of the statocyst evoked EPSPs and spikes recorded from identified I_b interneurons and cutaneous stimulation of the foot evoked EPSPs and spikes in I_b interneurons. Type I_b interneurons do not typically generate spikes in response to illumination, although light elicited an increase in EPSP frequency recorded from I_b interneurons. However, current depolarization of I_b interneurons sufficient to generate spikes revealed a modest increase in spike activity elicited during illumination compared with dark-adapted baseline activity. Here we show that type I_b interneurons also project to VC efferent neurons that innervate the anterior region of the foot and current stimulation of I_b interneurons in semi-intact preparations produced anterior foot contraction.

**Neural circuitry contributing to foot-shortening**

In the present study we have identified efferent neurons that innervate different areas of the foot that result in specific changes in foot configuration. The movements evoked by stimulation involve tail contraction, lateral foot contraction, contraction of the midfoot region, and tail extension. Efferent neurons innervating the anterior region of the foot have been previously identified (Crow and Tian 2003a, 2004). Interneuronal synaptic projections to efferent neurons have also been characterized in the present study. Current depolarization of I_b interneurons evoked spikes in I_c, LFC, and DC efferent neurons and contraction of the rostrocaudal axis of the foot, which is similar to hair cell–mediated foot-shortening.

The absence of light-evoked spike activity in I_b and I_c interneurons would suggest that foot contraction efferent neurons identified in this study may not respond to illumination. This observation is consistent with previous research showing that light does not elicit the clinging reflex (Alkon 1974) or evoke foot-shortening prior to conditioning (Lederhendler et al. 1986; Matzel et al. 1990). However, an identified putative motor neuron (MN1) was shown to increase spike activity in response to light stimulation of the eyes, activation of statocyst hair cells, interneuron depolarization, and current depolarization of type A photoreceptors (Goh and Alkon 1984). Current depolarization of MN1 also produced turning of the ipsilateral posterior foot in restrained semi-intact preparations (Goh and Alkon 1984). Taken collectively, the results of the Goh and Alkon (1984) study suggest that MN1 does not contribute to graviceptive-elicited foot-shortening. In addition, there are differences between MN1 and TC efferent neurons identified in

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**Legend for Diagram**

- **Filled circles**, inhibitory synapses; **open triangles**, excitatory synapses; **solid lines**, monosynaptic connections; **dashed lines**, polysynaptic or synaptic connections.

**Diagram Description**

- **Foot contraction** and **Ciliary locomotion**
- **I_b** and **I_c** interneurons, **I_e** and **I_f** interneurons, **I_e** and **I_f** interneurons, **VCL**, and **LFC** efferent neurons.
- Synaptic connections indicated by lines: monosynaptic connections (solid lines), polysynaptic connections (dashed lines).
- **Lateral foot contractile (LFC)**, **tail contractile (TC)**, **Ventral contractile (VC)**, **foot shortening (FS)**, **foot contractions (FC)**, **tail contractions (TC)**.
- **Statocyst hair cells (HCs)** and **photoreceptor within the eye** form monosynaptic connections.

**Figure 9. Neural circuit supporting ciliary locomotion and foot contraction.**

Diagram of the sensory neurons, interneurons, efferent neurons, and synaptic connections. The synaptic connections from only one identified photoreceptor (lateral B [LB]) to the type I interneurons is shown. However, each photoreceptor within the eye forms monosynaptic connections with different aggregates of type I interneurons (Crow and Tian 2000). Statocyst hair cells (HCs) form monosynaptic connections with type I interneurons (Akaike and Alkon 1980). The synaptic connection between type III, inhibitory interneurons and ciliary efferent neurons (VP1) are monosynaptic. Type I_e and type I_e polysensory interneurons receive input from the visual and graviceptive sensory systems and project to identified foot contractile efferent neurons. Ventral contractile (VC), lateral foot contractile (LFC), tail contractile (TC), and ciliary efferent neurons on the dorsal surface of the pedal ganglia (DC). Filled circles, inhibitory synapses; open triangles, excitatory synapses; solid lines, monosynaptic connections; dashed lines, polysynaptic or synaptic connections where potential monosynaptic inputs have not been tested.
this study regarding their response to illumination. Statistically significant changes in spike frequency of MN1 elicited by light in nonconditioned animals occurred primarily during the first 5 s of illumination (Goh et al. 1985). We found that TC efferent neurons exhibited a modest, but nonstatistically significant, increase in spike activity during the first 5 s of illumination (see RESULTS). However, spike activity had returned to dark-adapted baseline levels during the second 5 s of light, which is also consistent with the previous report of MN1 spike activity (Goh et al. 1985). Moreover, average spike activity recorded from identified TC efferent neurons during 5 min of light was not significantly different from dark-adapted baseline activity collected 5 min immediately before light or 5 min of dark-adaptation after the termination of light. In addition, we did not observe reliable evidence for post-illumination spike bursting of TC efferent neurons, as was reported in the previous study of MN1 (Goh and Alkon 1984). Our findings indicate that stimulation of the graviceptive system makes the major contribution to contraction of different regions of the foot through polysynaptic interneuronal projects to foot contractile efferent neurons. With the exception of TE and LFC efferent neurons, light does not elicit foot contractions or produce significant changes in the spike activity of TC or VC efferent neurons. However, both TE and LFC efferent neurons may contribute to light-elicited increased foot length observed before conditioning (Lederhendler et al. 1986). Our analysis of the foot contraction circuitry is consistent with the behavioral observations showing that light does not elicit foot-shortening prior to conditioning (Lederhendler et al. 1986; Matzel et al. 1990).

Circuitry supporting the CR complex

The acquisition of multiple conditioned responses produced by pairings of a single CS with a single US is characteristic of many Pavlovian-conditioning preparations. Somatomotor CRs such as eyeblink, nictitating membrane, or leg flexion, are accompanied by concomitant conditioned visceral changes involving heart rate, blood pressure, skin conductance, and pupillary diameter. Visceral CRs are typically rapidly acquired by pairings of a single CS with a single US is characteristic of Circuitry supporting the CR complex (Lederhendler et al. 1986; Matzel et al. 1990). Our analysis of the foot contraction circuitry is consistent with the behavioral observations showing that light does not elicit foot-shortening prior to conditioning (Lederhendler et al. 1986; Matzel et al. 1990).

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References


