Sensory Regulation of Network Components Underlying Ciliary Locomotion in *Hermisenda*

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Crow T, Tian L-M. Sensory regulation of network components underlying ciliary locomotion in *Hermisenda*. J Neurophysiol 100: 2496–2506, 2008. First published September 3, 2008; doi:10.1152/jn.90759.2008. Ciliary locomotion in the nudibranch mollusk *Hermisenda* is modulated by the visual and graviceptive systems. Components of the neural network mediating ciliary locomotion have been identified including aggregates of polysensory interneurons that receive monosynaptic input from identified photoreceptors and efferent neurons that activate cilia. Illumination produces an inhibition of type Ii (OFF-cell) spike activity, excitation of type Ie (ON-cell) spike activity, decreased spike activity in type Ii inhibitory interneurons, and increased spike activity of ciliary efferent neurons. Here we show that pairs of type Ii interneurons and pairs of type Ie interneurons are electrically coupled. Neither electrical coupling or synaptic connections were observed between Ie and Ii interneurons. Coupling is effective in synchronizing dark-adapted spontaneous firing between pairs of Ii and pairs of Ie interneurons. Out-of-phase burst activity, occasionally observed in dark-adapted and light-adapted pairs of Ii and Ie, interneurons, suggests that they receive synaptic input from a common presynaptic source or sources. Rhythmic activity is typically not a characteristic of dark-adapted, light-adapted, or light-evoked firing of type Ii interneurons. However, burst activity in Ii and Ie interneurons may be elicited by electrical stimulation of pedal nerves or generated at the onset of light. Our results indicate that type I interneurons can support the generation of both rhythmic activity and changes in tonic firing depending on sensory input. This suggests that the neural network supporting ciliary locomotion may be multifunctional. However, consistent with the nonmuscular and nonrhythmic characteristics of visually modulated ciliary locomotion, type I interneurons exhibit changes in tonic activity evoked by illumination.

**INTRODUCTION**

Motor activity underlying rhythmic movements such as respiration, locomotion, and feeding is produced by central pattern generators (CPGs) (for reviews, see Dickinson 2006; Marder and Calabrese 1996; Marder et al. 2005; Pearson 2000; Popescu and Frost 2002). Ciliary locomotion or crawling is a nonmuscular, nonrhythmic gliding form of movement expressed in a number of mollusks (Audesirk 1978a,b; Copeland 1919, 1922; Crow and Tian 2003a; Gainey 1976; Syed and Winlow 1989; Willows et al. 1997). Identified components of the CPGs in *Pleurobranchaea* and *Tritonia* express rhythmic neural activity during escape swimming and tonic firing during ciliary locomotion (Jing and Gillette 2000; Popescu and Frost 2002).

In *Hermisenda*, interneurons that are part of the graviceptive and visual systems are involved in both muscular and ciliary locomotion. The type Ii interneurons are components of the neural circuitry underlying ciliary locomotion (Akaike and Alkon 1980; Crow and Tian 2000, 2002a,b, 2003a). Each identified A and B photoreceptor in the eye forms a monosynaptic connection with an aggregate of two distinct type Ii interneurons (OFF-cells) and two type Ie interneurons (ON-cells) (Crow and Tian 2000, 2002b). The visual system modulates ciliary locomotion by the effect of illumination on the activity of type Ii and Ie interneurons projecting through polysynaptic pathways to type III inhibitory interneurons that, in turn, regulate the firing of ciliary efferent neurons (Crow and Tian 2003a). Excitation of ciliary efferent neurons is also provided by synaptic input from type Ie interneurons (Crow and Tian 2003a, 2004). In the dark, spike activity of Ie and Ii interneurons is typically not rhythmic and thus is consistent with the tonic firing of dorsal swim interneurons in *Tritonia* and the As1-4 neurons in *Pleurobranchaea* during ciliary crawling. However, it is not known if illumination of photoreceptors (light adaptation) simulating conditions underlying visually guided ciliary locomotion would generate rhythmic activity or alternatively changes in tonic spike activity of type I interneurons.

Here we show that aggregates of type Ii interneurons that receive synaptic input from the same photoreceptor are electrically coupled as are similar aggregates of Ie interneurons. Type Ii and Ie interneurons receive synaptic input from a common presynaptic source or sources that generates out-of-phase burst activity during dark and light-adapted conditions. Stimulation of identified pedal nerves that mimics activation of peripheral mechanoreceptors generates rhythmic bursting in type I interneurons. Suction electrode recordings of multiunit activity from identified pedal nerves that contain the axons of efferent neurons that innervate foot muscles and activate cilia exhibit both tonic and rhythmic firing during illumination. However, light adaptation produces an increase and decrease in...
the tonic firing of type Ie and Ii interneurons, respectively, inhibition of type IIIi inhibitory interneuron spike activity, and an increase in the tonic activity of ciliary efferent neurons. Consistent with the nonmuscular and nonrhythmic characteristics of visually modulated ciliary locomotion, our results show that type I interneurons express changes in tonic firing elicited by light. However, consistent with the proposal that the circuit may be multifunctional, synaptic input from other sensory systems may produce rhythmic activity in type I interneurons.

**METHODS**

Adult *Hermissenda crassicornis* were used in the experiments. The animals were obtained from Sea Life Supply (Sand City, CA) and maintained in closed artificial seawater aquaria at 14 ± 1°C on a 12-h light-dark cycle. All electrophysiological procedures were conducted during the light phase of the light/dark cycle.

Simultaneous intracellular recordings from pairs of identified Ie and Ii interneurons or interneurons and ciliary efferent neurons were collected from isolated nervous systems. Recordings from ciliary efferent neurons were also collected from semi-intact preparations. For some experiments, simultaneous recordings from identified type B photoreceptors and type I interneurons were collected. Extracellular recordings were obtained from suction electrodes containing pedal nerves P1 or P2. Two types of protocols were used for the pedal nerve recordings. The first involved recording from horizontally oriented central nervous systems pinned on the SYLGARD stage as described below. The second procedure involved orienting the nervous systems vertically and stabilizing the preparations with insect pins in front and back of the nervous systems. Surgical desheathing of a small area of the cerebropleural and pedal ganglion was conducted to expose the cell bodies of interneurons and ciliary efferent neurons. As previously reported (Crow and Tian 2000), the criteria for identifying type Ie and Ii interneurons consisted of soma size, cell layer, location in the cerebropleural ganglion, and electrophysiological responses to light. Anatomical and electrophysiological criteria were used to identify type B photoreceptors, as described previously (Alkon 1973b; Alkon and Fuortes 1972; Crow and Tian 2000; Frysztk and Crow 1993). In isolated circumesophageal nervous systems, ciliary efferent neurons were identified based on soma size, position along the anterior-ventral edge of the pedal ganglion, electrophysiological responses to light stimulation of the photoreceptors, and extrinsic current stimulation of interneurons (Crow and Tian 2003a). In semi-intact preparations, ciliary efferent neurons were identified by recording ciliary movement evoked by depolarization of the neurons with extrinsic current (Crow and Tian 2003a).

The partially desheathed circumesophageal nervous systems were pinned to a SYLGARD (Dow Chemical) stage in a recording chamber filled with artificial seawater (ASW) of the following composition (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 eyes 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. Photoreceptors, interneurons, and ciliary 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 and extracellular recording and stimulation techniques were used. Electrophysiological data were digitized with a CED power 1401 (Cambridge Electronic Design) and stored on the computer hard drive. Digitized data were analyzed and plotted using Spike 2 software (Cambridge Electronic Design). Action potentials were elicited by depolarizing current steps applied in the dark through a bridge circuit. Depolarizing generator potentials were evoked by light steps of variable duration that followed appropriate periods of dark adaptation.

**RESULTS**

A neural network diagram is shown in Fig. 1 summarizing the synaptic connections between previously identified sensory neurons, interneurons, and newly identified reciprocal electrical coupling between interneurons that contribute to the circuit supporting ciliary locomotion.

**Reciprocal electrical coupling between identified pairs of type I interneurons**

Previous research has shown that each identified type A and B photoreceptor forms monosynaptic connections with specific pairs of type Ie and pairs of type Ii interneurons (Crow and Tian 2000). As shown in the example in Fig. 2, pairs of type Ie and pairs of type Ii interneurons are electrically coupled. The analysis of the amplitude of electrotonic potentials elicited by current pulses from identified pairs of Ii and Ie interneurons showed mean coupling ratios of 0.18 ± 0.02 and 0.16 ± 0.03 for reciprocally coupled type Ii interneurons (n = 14) and type Ie interneurons, respectively.

**FIG. 1.** Neural network supporting ciliary locomotion. Diagram of the sensory neurons, interneurons, efferent neurons, and synaptic connections. The synaptic connections from only 1 identified photoreceptor [lateral B (LB)] to the interneurons are shown. However, each photoreceptor within the eye forms monosynaptic connections with different aggregates of type I interneurons. Statocyst hair cells (HCs) also form monosynaptic connections with type I interneurons. Type IIIi interneurons form monosynaptic connections with VP1 ciliary efferent neurons. Filled circles, inhibitory synapses; open triangles, excitatory synapses; solid lines, monosynaptic connections; dashed lines, polysynaptic pathways.
larizing electrotonic potential in an Ii or Ie did not evoke electrotonic potentials in an Ie or Ii, respectively (Fig. 3, A1–B1 and A2–B2). Consistent with our previous findings, electrical coupling between pairs of type I interneurons that received synaptic input from different photoreceptors was also not observed (data not shown). In addition, synaptic connections between type Ia and Ii interneurons were not observed as shown in the example of simultaneous recordings from Ii and Ie interneurons following current depolarization of type Ii or Ie interneurons, respectively (Fig. 3, A3–B3 and A4–B4).

**Coupled type Ii and Ie interneurons exhibit synchronous spike activity**

Simultaneous recordings from pairs (n = 6) of coupled type Ii interneurons showed that they exhibit a synchronous discharge pattern recorded during dark-adapted spontaneous spike activity (Fig. 4, A1 and A2). Consistent with this observation is the finding that coupled pairs of type Ie interneurons (n = 5) also exhibit dark adapted spontaneous synchronous spike activity (Fig. 4, B1 and B2). Simultaneous recordings from two coupled Ie interneurons showed that current evoked spike activity in one type Ie interneuron resulted in an increase in spike activity recorded from the other coupled type Ie interneuron (Fig. 4, C1 and C2). This finding suggested that the electrical coupling between specific pairs of type I interneurons contributes to the generation of synchronous spike activity detected under dark-adapted conditions.

**FIG. 3.** Type Ii and Ie interneurons are not electrically coupled. An example of a current elicited hyperpolarizing potential recorded in an identified Ii interneuron (A1) that did not evoke a potential in an identified Ie interneuron (B1). Current stimulation of the Ie interneuron (B2) did not elicit a potential in the Ii interneuron (A2). Type Ii and Ie interneurons are not synaptically connected. Spikes evoked by a 2-s current pulse applied to an Ii interneuron (A3) did not elicit postsynaptic potentials (PSPs) or spikes recorded from an identified Ie interneuron (B3). Spikes evoked by the current pulse in the Ii interneuron (B4) did not evoke PSPs or spikes recorded from the Ie interneuron (A4).

**FIG. 4.** Electrically coupled pairs of type Ii and pairs of Ie interneurons generate synchronous spike activity. Simultaneous recordings from a pair of electrically coupled identified type Ii interneurons (A1 and A2) exhibited spontaneous synchronous spike activity. Simultaneous recordings from a pair of electrically coupled type Ie interneurons (B1 and B2) also exhibited spontaneous synchronous spike activity. Electrical coupling contributes to synchronous spike activity. Simultaneous recordings from a pair of coupled-identified Ie interneurons showed that depolarizing current stimulation of 1 type Ii interneuron (C1) evoked a depolarization and increase in spike activity recorded in the 2nd Ie interneuron (C2).
Type 1 interneurons receive synaptic input from a common presynaptic source or sources

Previous work has indicated that, in the dark, type I interneurons exhibit a regular tonic firing of spikes with some variability in the discharge pattern examined during short epochs (Akaike and Alkon 1980; Crow and Tian 2000). We further studied type I spike activity in the dark-adapted and light-adapted state recorded during longer time periods. An example of dark-adapted spontaneous phasic spike activity recorded simultaneously from a type $I_e$ and $I_i$ interneuron is shown in Fig. 5. Irregular bursts of spike activity recorded in the type $I_e$ interneuron (Fig. 5A) were associated with decreased activity recorded from the type $I_i$ interneuron (Fig. 5B). Because type $I_e$ and $I_i$ interneurons are not synaptically connected, phasic activity as shown in Fig. 5 suggests a common synaptic input. Out-of-phase spike activity recorded from $I_e$ and $I_i$ interneurons may also occur during illumination. As shown in Fig. 5, C and D, illumination of the eyes produced a complex inhibitory postsynaptic potential (IPSP), inhibition of $I_e$ interneuron spike activity, and excitation of the type $I_i$ interneuron. During the period of prolonged illumination, a burst of spikes occurred in the type $I_e$ interneuron with a concomitant hyperpolarization and inhibition of the $I_i$ interneuron. This pattern of activity in type $I_e$ and $I_i$ interneurons under dark-adapted and light-adapted conditions is consistent with synaptic input from a common presynaptic source or sources.

We examined this further by recording PSPs from pairs of $I_e$ and $I_i$ interneurons. As shown in the example in Fig. 6, a burst of action potentials recorded from a type $I_e$ interneuron was associated with IPSPs and inhibition of spike activity recorded from a type $I_i$ interneuron. The IPSPs detected in the type $I_e$ interneuron (Fig. 6B) are in phase with the burst of spikes generated in the type $I_e$ interneuron shown in Fig. 6A. As shown in Fig. 6, C and D, simultaneous recordings from a pair of $I_e$ and $I_i$ interneurons showed spontaneous IPSPs in the $I_i$ interneuron that were one-for-one with excitatory PSPs (EPSPs) in the $I_e$ interneuron. However, as previously mentioned, synaptic connections between pairs of type $I_e$ and $I_i$ interneurons have not been observed, suggesting a common presynaptic source or sources must generate the spontaneous PSPs detected in $I_i$ and $I_e$ interneurons. Previous research has shown that one source for monosynaptic input to type $I_e$ interneurons is from identified photoreceptors (Akaike and Alkon 1980; Crow and Tian 2000). Consistent with the earlier reports, we found that synaptic input from a type B photoreceptor generated monosynaptic EPSPs in a type $I_e$ interneuron (Fig. 6, $E$ and $F$) and monosynaptic IPSPs in a type $I_i$ interneuron (Fig. 6, $G$ and $H$). The excitation and increased spike activity recorded in $I_e$ interneurons and decreased spike activity of $I_i$ interneurons observed during illumination (Fig. 5) is consistent with the typical synaptic input to type $I_e$ and $I_i$ interneurons from identified photoreceptors shown in Fig. 6. However, oscillations of the generator potentials in different photoreceptors during illumination could result in a transient inhibition of photoreceptor spike activity that may contribute to the excitation of type $I_e$ interneurons detected during light. Previous research has shown that out-of-phase oscillations may occur between two reciprocally inhibited type B photoreceptors in response to illumination (Alkon and Fuortes 1972). Examples of simultaneous recordings from an identified type $I_e$ interneuron and a lateral type B photoreceptor are shown in Fig. 7.
Light evoked a complex IPSP recorded from the type Ii interneuron (Fig. 7A1) and a depolarizing generator potential in the lateral type B photoreceptor (Fig. 7B1). Oscillations between photoreceptors may contribute to out-of-phase activity. Hyperpolarization of the lateral B photoreceptor during light (B2) resulted in a small depolarization and the generation of spikes recorded from the Ii interneuron (A2).

Light evoked a complex IPSP recorded from the type Ii interneuron and lateral B photoreceptor during illumination. Light evoked a stereotyped depolarizing generator potential in the lateral B (B1) and complex IPSP in the Ii interneuron (A1). Oscillations between photoreceptors may contribute to out-of-phase activity. Hyperpolarization of the lateral B photoreceptor during light (B2) resulted in a small depolarization and the generation of spikes recorded from the Ii interneuron (A2).

**FIG. 7.** Simultaneous recordings from an identified Ii interneuron and lateral B photoreceptor during illumination. Light evoked a stereotyped depolarizing generator potential in the lateral B (B1) and complex IPSP in the Ii interneuron (A1). Oscillations between photoreceptors may contribute to out-of-phase activity. Hyperpolarization of the lateral B photoreceptor during light (B2) resulted in a small depolarization and the generation of spikes recorded from the Ii interneuron (A2).

Light results in an increase in the tonic spike activity of Ie interneurons and a decrease in the activity of Ii interneurons but not rhythmic burst activity

Spike activity in dark-adapted type Ie and Ii interneurons is characterized by tonic firing with occasional irregular burst activity that is not repetitive or rhythmic (see Figs. 4 and 5). Type Ie and Ii interneurons typically fire at a frequency near 1 spike/s under dark-adapted conditions. Brief light steps result in inhibition of firing of type Ii interneurons and depolarization with an increase in firing of type Ie interneurons. To examine whether prolonged illumination would elicit repetitive patterns of burst activity in type Ie interneurons, we recorded spike activity of Ii and Ie interneurons in the dark and during a 5-min period of light. Group summary data depicting the mean spike frequency at consecutive 1-min periods 3 min before light, during 5 min of light, and 3 min after light are shown in Fig. 8. Both Ie (n = 14) and Ii (n = 19) interneurons exhibited an initial transient change in spike activity that corresponded to the peak amplitude of the light-evoked generator potential of the photoreceptors (see Fig. 7B1). The transient peak was followed by a decrease in spike activity to a steady-state plateau level for type Ie interneurons and an increase from maximum inhibition to a steady-state plateau for type Ii interneurons. As shown by the group summary data, tonic firing is typically observed during light, as measured at 60-s intervals during the 5 min of illumination. Occasionally, patterned burst activity occurred in Ie and Ii interneurons in the dark and in light (Fig. 5). However, light did not elicit repetitive or rhythmic burst activity in either Ie or Ii interneurons. In the atypical cases where patterned burst activity was detected, the bursts occurred both before light onset and after the termination of light. However, light did not generate rhythmic burst activity in type Ie interneurons that typically exhibited regular tonic firing under dark-adapted conditions. Taken collectively, whereas nonrepetitive irregular burst activity may occur in both the dark and light, our results show that illumination of the eyes does not induce burst activity in either type Ie or Ii interneurons. Because the type I interneurons are components of the circuit modulating ciliary locomotion, it is unlikely that repetitive or rhythmic bursting is characteristic of the circuit supporting this form of locomotion.

**Light elicits an increase in tonic activity of ciliary efferent neurons and inhibition of IIIi interneuron spike activity**

Type IIIi interneurons form monosynaptic inhibitory connections with VP1 ciliary efferent neurons (Crow and Tian 2003a). One source for light modulation of ciliary efferent neurons is the pathway from type I interneurons to IIIi interneurons. Simultaneous recordings from an identified IIIi interneuron and VP1 efferent neuron are shown in Fig. 9. IPSPs recorded from the VP1 efferent neuron followed spikes in the IIIi interneuron one-for-one, suggesting that inhibition of VP1 efferent neurons is primarily caused by synaptic input from IIIi interneurons. Hyperpolarizing the IIIi interneuron in the dark to block spike generation showed spontaneous EPSPs (Fig. 9C).

**FIG. 8.** Light evoked a change in the tonic activity of Ie and Ii interneurons. Graph of mean spike activity (±SE) of Ie and Ii interneurons 3 min before (dark-adapted), during 5 min of light (light-adapted), and 3 min after light offset plotted at 1-min intervals. Light did not evoke rhythmic firing of type Ie or Ii interneurons. Light attenuated −1.0 log unit.
activity may be produced by oscillation of type B photoreceptors, observed following the termination of a prolonged period of bright light. We have shown previously that stimulation of afferents in identified pedal nerves elicits synaptic potentials and changes in spike activity in components of the network underlying ciliary locomotion (unpublished observations). Here we examined the effect of activation of putative mechanosensory input to the neural network by stimulating pedal nerves 1 (P1) and 2 (P2) and recording changes in spike activity of type Ie and Ii interneurons. As shown in Fig. 10B, stimulation of P2 produced an increase in the frequency of EPSPs recorded in a type Ie interneuron hyperpolarized to block spike activity. In another example, stimulation of P2 produced burst activity in a type Ie interneuron (Fig. 10C). As shown in the examples in Fig. 11, a nerve shock delivered to P2 or P1 produced a pattern of rhythmic burst activity in type Ie (Fig. 11A) and Ii (Fig. 11B) interneurons that persisted for several minutes. The results of pedal nerve stimulation studies show that type Ie and Ii interneurons, which are major components of the ciliary locomotor network, can support the generation of rhythmic burst activity.

Patterned and tonic activity in interneurons and pedal nerves

Previous work has shown that efferent neurons that activate cilia on the foot surface and muscles of the foot and body wall that produce contraction have axons that project to postsynaptic targets through P1 and P2 (Crow and Tian 2003a; Hodgson and Crow 1992; Richards and Farley 1987). Identified ciliary efferent neurons increase their tonic firing in response to illumination of the eyes and provide a major contribution to patterned and tonic activity following the offset of light.

Pedal nerve stimulation produces rhythmic bursting activity

To test whether the Ie and Ii components of the ciliary circuit could support rhythmic burst activity, we examined spike activity following pedal nerve stimulation and at the offset of illumination. Both pedal nerve stimulation and the offset of light elicited burst activity in Ie and Ii interneurons. An example of a rhythmic burst pattern generated in a type Ie interneuron after the termination of light is shown in Fig. 10A. Burst
increased multiunit spike activity of pedal nerves recorded during light (Crow and Tian 2003a,b).

Previously published research has reported that multiunit recordings of pedal nerve activity evoked by light are less variable in vertically oriented nervous systems compared with horizontally oriented preparations (Richards and Farley 1987). Therefore both vertical \((n = 8)\) and horizontal \((n = 37)\) orientations were used in the experiments examining multiunit spike activity recorded from pedal nerves. A 16% increase from dark-adapted baseline multiunit activity was used to determine light responses of pedal nerve recordings. In different preparations, the peristimulus time (PST) histograms of multiunit activity recorded from P1 and P2 in the dark, during illumination, and after light offset showed diverse activity patterns. Several patterns of multiunit activity elicited by light were typically expressed in the recordings from P1 and P2. One pattern of multiunit activity was an increase in spike activity that occurred with a variable latency following light onset with one or a few variable peaks of activity expressed in the PST histogram. In most examples, the shape of the PST histograms of activity from P1 and P2 was similar. A second pattern of multiunit activity consisted of distinct bursts of spike activity expressed by multiple peaks in the PST histogram, indicating rhythmic firing. This pattern is similar to examples of multiunit recordings from pedal nerves reported in a previous study (Richards and Farley 1987). Evoked increases in multiunit activity during the 5-min light period were observed for 68% of the horizontally oriented preparations and 75% of the vertically oriented nervous systems. Categorizing the histograms as expressing rhythmic or nonrhythmic activity showed that 32% of the horizontally oriented preparations were rhythmic and 43% of the vertically oriented preparations showed rhythmic activity. However, burst activity could also be detected with horizontally oriented nervous systems, and in

vertically oriented preparations, PST histograms showing nonrhythmic increased tonic activity in light were also observed (see Fig. 14). The expression of light-evoked rhythmic activity may require synaptic input from the contralateral pedal ganglion. We examined this by leaving the pedal connective intact \((n = 8)\) in recordings from both horizontally and vertically oriented preparations. We found that rhythmic and nonrhythmic patterns of multiunit activity evoked by light in P1 and P2 were expressed with or without an intact pedal connective. Simultaneous suction electrode recordings from P1 and P2 and an identified type Ie interneuron in the dark, during a 5-min period of illumination and after light offset, are shown in Fig. 12, A–C. For this experiment, the nervous system was oriented horizontally. Interestingly, the peaks in the PST histograms of P1 and P2 multiunit activity corresponded to modest decreases in the discharge pattern of the type Ie interneuron indicated by the filled and opened circles in Fig. 12C. A segment of the recording on a faster time scale showed that decreased Ie spike activity occurred during the increase in the peak multiunit activity of P1 (Fig. 12, D and E). This result is consistent with previous work showing that type Ie spike activity can regulate ciliary efferent neuron firing (Crow and Tian 2003a). Therefore burst patterns in P1 and P2 multiunit recordings may reflect

\[ \text{Fig. 11. Nerve stimulation generates rhythmic burst activity in identified type I interneurons. Stimulation of P2 (0.2 mA, 500 Hz, 300-ms duration) resulted in rhythmic burst activity recorded from an identified type Ie interneuron (A). Stimulation of P1 (0.1 mA, 10 Hz, 1-s duration) produced rhythmic bursting in an identified type Ii interneuron (B). The bursts are indicated by the bars above the recordings. The arrows indicate the application of the current stimulus applied to P1 and P2.}\]

\[ \text{Fig. 12. Patterned multiunit activity recorded from P1 and P2 is associated with the transient increase and decrease in spike activity recorded from an identified Ie interneuron during light. Peristimulus time (PST) histograms of multiunit activity recorded from P2 (A) and P1 (B) showed burst activity in light that occurred during the transient decrease in light-evoked tonic activity recorded simultaneously from an identified type Ie interneuron (C). The open and closed circles indicate the decrease in type Ie tonic activity associated with the transient peaks in the PST histograms. Activity of the Ie interneuron associated with a PST histogram peak (open circle) displayed on a faster time scale (D and E). Light onset and offset (~1.0 log unit) indicated by the arrows beneath the recording in C.}\]
small changes in the tonic discharge of type I interneurons rather than the generation of rhythmic burst activity in premotor interneurons. An example of light-evoked activity with a delayed onset in the pedal nerve recordings collected from a vertically oriented preparation is shown in Fig. 13. The PST histograms show a delayed increase in tonic activity without evidence for the generation of rhythmic burst patterns. The delayed increase in tonic activity was observed in 24% of the preparations. The PST histograms shown in Fig. 14 are from an example of a short latency increase in the tonic spike activity recorded from P1 and P2 evoked by light in a vertically oriented nervous system. The short latency increase in light-evoked tonic activity was detected in 44% of the preparations. In general, the suction electrode recordings of the multiunit activity of P1 and P2 evoked by light in both the vertically oriented and horizontally oriented preparations exhibited activity consistent with the PST histograms. An example of burst activity in P1 and P2 recorded from a vertically oriented preparation is shown in Fig. 15. The peaks in the PST histograms elicited during light corresponded to small amplitude patterned activity detected in the suction electrode recordings of multiunit activity from P1 and P2. However, the light evoked increase in the frequency of larger amplitude spikes in P2 did not correspond to the peaks of the PST histograms in Fig. 15, A1 and B1.

**DISCUSSION**

**Network supporting Hermissenda ciliary locomotion**

Many of the components in the network supporting visually guided ciliary locomotion in *Hermissenda* have been identified and studied in semi-intact preparations. The synaptic interactions within and between the primary sensory neurons and second-order interneurons of the visual and graviceptive systems are well characterized (Akaike and Alkon 1980; Alkon 1973a,b; Alkon and Fuortes 1972; Alkon et al. 1978; Crow and Tian 2000, 2002a,b, 2004). Each eye contains three type B photoreceptors and two type A photoreceptors (Alkon and Fuortes 1972). Identified A and B photoreceptors form monosynaptic connections with aggregates of type Ie (ON-cells) and type Ii (OFF-cells) interneurons (Akaike and Alkon 1980; Crow and Tian 2000). Here we show that the two type Ie interneurons and type Ii interneurons that receive synaptic input from a single photoreceptor are electrically coupled. The reciprocal electrical coupling contributes to the synchronous firing of pairs of type Ie and Ii interneurons under both dark-adapted and light-adapted conditions. Visual and graviceptive synaptic input to type I interneurons regulates spike activity of type III inhibitory interneurons through a polysynaptic pathway. The monosynaptic connection between III inhibitory interneurons and ciliary efferent neurons regulates the spike activity of ciliary efferent neurons. Illumination of the eyes produces a complex IPSP and inhibition of Ii spike activity and decreased spike activity of type III inhibitory interneurons, which results in increased firing of ciliary efferent neurons and movement of the cilia on the foot (Crow and Tian 2003a). The complex EPSP and increase in type Ie spike activity evoked by light is less effective in exciting type III interneurons than the
Therefore excitation or inhibition of spike activity in IIIi efferent neurons, because their membrane potential under base- 
rions is an efficient means of modulating spike activity in ciliary 
graviceptive sensory system. Interestingly, type Ib interneurons 
and Tian 2004). Therefore type Ib interneurons contribute more 
hair cells compared with photoreceptor synaptic input (Crow 
interneurons exhibit stronger synaptic activation from statocyst 
rons. A second pathway that modulates spike activity in ciliary 
interneurons modulates spike activity of ciliary efferent neu-
rons during illumination (Fig. 12). One common type of mul-
tiunit activity produced by light inhibition of Ie interneurons is 
erease type IIIi interneuron activity. However, multiunit ac-
tivity recorded from pedal nerves during illumination 
show that light may evoke patterned activity (Figs. 12 and 
15). These examples of patterned multiunit activity recorded 
from pedal nerves are similar to bursting activity referred to as 
spindles in an earlier study (Richards and Farley 1987). We 
erved that burst activity recorded from pedal nerves may 
ccur in the dark before light onset and is one of several types of 
multiunit activity detected in pedal nerves during illumina-
tion. The intervals between spindle bursts in pedal nerves may 
be 1 min or longer, leaving their potential contribution to the 
generation of continuous gliding ciliary locomotion ambigu-
ous. Moreover, spindle burst activity may be correlated with 
the transient inhibition of the spike activity of type Ib interneu-
rons during illumination (Fig. 12). One common type of mul-
tiunit activity recorded from pedal nerves is an increase in tonic 
firing that occurred with a variable latency following light 

FIG. 15. Light-evoked patterned activity recorded from P1 and P2. PST histograms of multiunit activity recorded from P2 (A1) and P1 (B1) during dark adaptation, 5 min of light, and after light offset. Corresponding simultaneous suction electrode recordings from P2 (A2) and P1 (B2). Peaks in the histograms are associated with patterns of P1 suction electrode recordings indicated by the bars beneath the recording. Nervous system was oriented vertically. Light onset and offset indicated by the 2 arrows above the histograms. Light attenuated −1.0 log unit.

disexcitation of IIIi interneurons produced by light inhibition of 
Ie interneurons. Consistent with this view are the results of this 
study showing that, under steady-state light-adapted conditions, 
type Ie spike activity is closer to dark-adapted prelight 
baseline activity than the relative decrease in Ie spike activity 
from dark-adapted baseline activity (Fig. 8). The regulation of 
ciliary movement by the modulation of type IIIi inhibitory 
interneuron activity is an effective mechanism for controlling 
locomotion in an active preparation. Gliding locomotion in 
Hermisenda is influenced by stimulation of all sensory sys-
tems. Type I interneurons are polysensory, receiving synapt ic 
input from the graviceptive, somatosensory, visual, and che-
mosensory systems (Akaike and Alkon 1980; Alkon et al. 
1978; Crow and Tian 2000). The regulation of ciliary locomo-
tion by a “clutch”-like mechanism provided by IIIi interneu-
rons is an efficient means of modulating spike activity in ciliary 
efferent neurons, because their membrane potential under base-
line conditions is near the threshold for spike generation. 
Therefore excitation or inhibition of spike activity in IIIi 
interneurons modulates spike activity of ciliary efferent neu-
rons. A second pathway that modulates spike activity in ciliary 
efferent neurons is from the type Ie interneurons. Type Ie 
interneurons exhibit stronger synaptic activation from statocyst 
hair cells compared with photoreceptor synaptic input (Crow 
and Tian 2004). Therefore type Ie interneurons contribute more 
to the modulation of ciliary locomotion by activity from the 
graviceptive sensory system. Interestingly, type Ie interneurons 
form monosynaptic connections with ventral contractile motor 
neurons and other identified motor neurons producing foot 

Multi-functional neural networks

Multi-functional neural networks are commonly found to 
support related behaviors that use the same or similar muscles. 
In the Aplysia feeding system, increased activity of radula 
closer motor neurons may result in either an ingestive or 
egestive motor pattern, depending on stimulation of two com-
mand-like neurons in the CPG (Morgan et al. 2002). The 
coordination of the same sets of longitudinal and circular 
muscles in the leech result in swimming and crawling that 
are generated by both multi-functional and dedicated CPGs 
(Briggsman and Kristan 2006). In the leech, ~93% of the 
eurons in the swimming and crawling networks overlap. 
Multi-functional networks may also control different effector 
systems. There are a number of examples of multi-functional 
networks supporting muscular and ciliary activity. In Pleuro-
branchaea and Tritonia, the CPG for swimming also supports 
cilia-mediated locomotion (Jing and Gillette 2000; Popescu 
and Frost 2002). In these species, swimming is a brief rhythmic 
activity and crawling is a nonrhythmic tonic activity. The 
dorsal swim interneurons and swim motor neurons in Tritonia 
and As1-4 neurons and locomotor G pedal neurons in Pleuro-
branchaea fire rhythmically during swimming, and tonically 
during crawling. In addition, the As1-4 neurons in Pleurobran-
chaea exert a general activation of an arousal network and 
contribute to avoidance turning (Jing and Gillette 1999, 2000). 
The network mediating ciliary locomotion in Lymnaea may be 
involved in both respiration and ciliary locomotion (Syed and 
Winlow 1989). Consistent with the non-rhythmic tonic activity 
of networks supporting crawling in other systems, our results 
show that the spike activity of Ie and Ii interneurons and ciliary 
efferent neurons during illumination is tonic and not rhythmic. 
Moreover, prolonged illumination (5 min) does not result in the 
generation of rhythmic bursting or patterned activity in type I 
or type IIIi interneurons. Taken collectively, these results indi-
cate that light modulates ciliary locomotion by tonically 
altering the spike activity of type Ie and Ii interneurons and the 
subsequent disinhibition of ciliary efferent neurons by de-
creased type IIIi interneuron activity. However, multiunit ac-
tivity recorded from the pedal nerves during illumination 
showed that light may evoke patterned activity (Figs. 12 and 
15). These examples of patterned multiunit activity recorded 
from pedal nerves are similar to bursting activity referred to as 
spindles in an earlier study (Richards and Farley 1987). We 
erved that burst activity recorded from pedal nerves may 
ccur in the dark before light onset and is one of several types of 
multiunit activity detected in pedal nerves during illumina-
tion. The intervals between spindle bursts in pedal nerves may 
be 1 min or longer, leaving their potential contribution to the 
generation of continuous gliding ciliary locomotion ambigu-
ous. Moreover, spindle burst activity may be correlated with 
the transient inhibition of the spike activity of type Ie interneu-
rons during illumination (Fig. 12). One common type of mul-
tiunit activity recorded from pedal nerves is an increase in tonic 
firing that occurred with a variable latency following light 

J Neurophysiol • VOL 100 • NOVEMBER 2008 • www.jn.org
onset (Figs. 13 and 14). Interestingly, the time to initiate locomotion in response to light also shows a variable onset latency in different animals. It is possible that the examples of patterned burst activity reflected in multiunit recordings from pedal nerves during light may be from neurons that are not part of the primary network generating ciliary locomotion. Our results suggest that the patterned activity reflected in multi-unit recordings during light is from yet to be identified sources outside the primary network supporting ciliary locomotion.

**Multifunctional neural network in *Hermissenda***

Interneurons contributing to the ciliary locomotor network in *Hermissenda* project to different types of efferent neurons. Type I and type II interneurons can alter spike activity of both ciliary efferent neurons and VP2 pedal neurons. However, synaptic connections with tail and lateral foot contraction efferent neurons have not been established. Current depolarization of VP2 pedal neurons evokes a lateral movement of the anterior foot and ventral tentacle (Crow and Tian 2003a). Simultaneous recordings from pairs of identified VP2 neurons and ciliary efferent neurons showed IPSPs that occurred synchronously, suggesting that they may be generated from a common presynaptic source or sources. Synchronous activation of the two efferent neurons may be synergistic because increased VP2 activity could result in increased contact between the foot and underlying substrate during ciliary locomotion. Both the graviceptive and visual systems are involved in the modulation of locomotion and the generation of local contractions of foot muscles (Crow and Tian 2004; Lederhendler et al. 1986). Our initial observations suggested that the neural circuitry supporting foot contraction and visually influenced ciliary locomotion involved little overlap (Crow and Tian 2004). However, type Ib interneurons project to foot contraction efferent neurons and ciliary efferent neurons. Graviceptive input to Ib interneurons is sufficient to generate foot contractions and ciliary activity. In contrast, visual input to Ib interneurons is typically not sufficient to increase spike activity unless the interneurons are spontaneously active. However, visual input during graviceptive activated depolarization of Ib interneurons could contribute to increased spike activity and provide a pathway for the visual system to modulate foot contractions. Neural circuits supporting different responses may reorganize or reconfigure by modifying effective synaptic connections or by neurons entering a circuit (Morton and Chiel 1994). Graviceptive activity would thus provide for visual activation of circuit interneurons innervating foot muscles and potentially contribute to behavioral plasticity. In *Hermissenda*, conditioning produces CS-elicted foot-shortening and light-elicted inhibition of ciliary locomotion (Crow and Alkon 1978; Lederhendler et al. 1986). Pavlovian conditioning can reconfigure the ciliary circuit that typically supports the positive phototaxis to produce light-elicted inhibition of ciliary locomotion (Crow 2004; Crow and Tian 2003b).

Our results show that light does not result in the generation of rhythmic patterned activity in the ciliary locomotor network. However, other sensory input to the circuit may result in the generation of rhythmic burst activity. Stimulation of P1 and P2 elicited burst activity recorded from both type Ic and Ii interneurons (Fig. 11). Rhythmic activity was also observed after the termination of illumination (Fig. 10). In addition, occasional spontaneous burst activity recorded in type Ic and Ii interneurons exhibited out-of-phase firing. One form of escape locomotion in *Hermissenda* involves vigorous alternating lateral muscular movements evoked by nociceptive stimulation. Rhythmic activity of the reconfigured ciliary circuit could support the generation of alternating muscular activity. In addition, phasic burst activity in type I interneurons could contribute to avoidance turning as shown for the Asl-4 neurons in *Pleurobranchaea* (Jing and Gillette 1999). Taken together, this evidence indicates that the network can generate both tonic and rhythmic spike activity, which would be consistent with a multifunctional neural circuit. These results and previous studies support the view that the network contributes to a number of different behaviors such as the generation of ciliary locomotion in the dark, inhibition of light-dependent forward locomotion produced by graviceptive stimulation, visually guided locomotion, and the generation of muscular foot movements.

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**References**


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