Sensory Regulation of Network Components Underlying

Ciliary Locomotion in *Hermissenda*

Terry Crow and Lian-Ming Tian

Department of Neurobiology and Anatomy
University of Texas Medical School
6431 Fannin Street
Houston, TX  77030

SENSORY MODULATION OF INTERNEURONS IN *HERMISSEND*A

Correspondence to:  T. Crow, Department of Neurobiology and Anatomy, University of Texas Medical School, 6431 Fannin Street, Houston, TX  77030

Email:  terry.crow@uth.tmc.edu

Phone: 713-500-5613

FAX: 713-500-0621
Ciliary locomotion in the nudibranch mollusk *Hermissenda* 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 I_i (off-cell) spike activity, excitation of type I_e (on-cell) spike activity, decreased spike activity in type III_i inhibitory interneurons, and increased spike activity of ciliary efferent neurons. Here we show that pairs of type I_i interneurons and pairs of type I_e interneurons are electrically coupled. Neither electrical coupling or synaptic connections were observed between I_e and I_i interneurons. Coupling is effective in synchronizing dark adapted spontaneous firing between pairs of I_e and pairs of I_i interneurons. Out-of-phase burst activity, occasionally observed in dark adapted and light adapted pairs of I_e and I_i interneurons, suggest that they receive synaptic input from a common presynaptic source or sources. Rhythmic activity is typically not a characteristic of either dark adapted, light adapted, or light evoked firing of type I interneurons. However, burst activity in I_e and I_i interneurons may be elicited by electrical stimulation of pedal nerves or generated at the off-set of light. Our results indicate that type I interneurons can support the generation of both rhythmic activity and changes in tonic firing depending upon 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 1993). The organization of CPGs in many invertebrate nervous systems may be multifunctional (Briggman and Kristan 2006; Jing et al. 2004; Kupfermann and Weiss 2001; Meynand et al. 1991; Morton and Chiel 1994; Popescu and Frost 2002; Weimann and Marder 1994). The different behaviors mediated by multifunctional neural networks may be closely related such as ingestion and egestion underlying feeding consummatory behavior (Morgan et al. 2002), swimming and crawling (Briggman and Kristan 2006), swimming and reflexive withdrawal (Getting and Dekin 1985). In contrast, the CPGs in the marine mollusks Pleurobranchaea and Tritonia support the generation of dissimilar behaviors; rhythmic escape swimming and nonrhythmic ciliary locomotion (Jing and Gillette 1999, 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 Hermissenda, interneurons that are part of the graviceptive and visual systems are involved in both muscular foot contractions and ciliary locomotion. The type I interneurons are components of the neural circuitry underlying ciliary locomotion (Akaike and Alkon 1980; Crow and Tian 2000, 2002a, 2002b, 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, 2002a). The visual system modulates ciliary locomotion by the effect of illumination on the activity of type Ie and II interneurons projecting through polysynaptic pathways to type IIIi 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 Ib 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 intrneurons in *Tritonia* and the As 1-4 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 IIi interneurons that receive synaptic input from the same photoreceptor are electrically coupled as are similar aggregates of Ie interneurons. Type Ie and IIi 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 multi-unit 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 IIi 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 *Hermissonda 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 1973; Alkon and Fuortes 1972; Frysztak and Crow 1993; Crow and Tian 2000). In isolated circumesophageal nervous systems, ciliary efferent neurons were identified based upon 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, 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 \(10^{-4}\text{W/cm}^2\) was attenuated with neutral density filters expressed in negative log units. Photoreceptors, interneurons and ciliary efferent neurons were impaled with microelectrodes filled with 4m 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 employed. 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.
Semi-intact preparations were prepared as described previously (Crow and Tian 2003a), by cooling the animals 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 foot was positioned ventral side up adjacent to the isolated circumesophageal nervous system. Ciliary movement was assessed indirectly by imaging the movement of small dried ink particles on the surface of the foot during depolarization of ciliary efferent neurons with extrinsic current.

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 I_e and pairs of type I_i interneurons (Crow and Tian 2000). As shown in the example in Fig. 2, pairs of type I_i and pairs of type I_e interneurons are electrically coupled. The analysis of the amplitude of electrotonic potentials elicited by current pulses from identified pairs of I_e and I_i interneurons revealed mean coupling ratios of .18 ± .02 and .16 ± .03 for reciprocally coupled type I_i interneurons (N=14) and .17 ± .03 and .15 ± .02 for pairs of identified type I_e interneurons (N=9). As shown in the example in Fig. 3, electrical coupling was not detected between any of the pairs of type I_e and I_i interneurons. A current that elicited a hyperpolarizing electrotonic potential in an I_i or I_e did not
evoke electrotonic potentials in an I_e or I_i respectively (Fig. 3A1-B1, 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 I_e and I_i interneurons were not observed as shown in the example of simultaneous recordings from I_i and I_e interneurons following current depolarization of type I_i or I_e interneurons respectively (Fig. 3A3-B3, A4-B4).

**Coupled type I_i and I_e interneurons exhibit synchronous spike activity**

Simultaneous recordings from pairs (N=6) of coupled type I_i interneurons revealed that they exhibit a synchronous discharge pattern recorded during dark adapted spontaneous spike activity (Fig. 4A1-A2). Consistent with this observation is the finding that coupled pairs of type I_e interneurons (N=5) also exhibit dark adapted spontaneous synchronous spike activity (Fig. 4B1-B2). Simultaneous recordings from two coupled I_e interneurons showed that current evoked spike activity in one type I_e interneuron resulted in an increase in spike activity recorded from the other coupled type I_e interneuron (Fig. 4C1-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.

**Type I 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 investigated 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). Since 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. 5C-D, illumination of the eyes produced a complex IPSP, inhibition of I_i interneuron spike activity and excitation of the type I_e interneuron. During the period of prolonged illumination, a burst of spikes occurred in the type I_i interneuron with a concomitant hyperpolarization and inhibition of the I_e interneuron. This pattern of activity in type I_i and I_e 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_i and I_e interneurons. As shown in the example of 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_i 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. 6C-D, simultaneous recordings from a pair of I_i and I_e interneurons revealed spontaneous IPSPs in the I_i interneuron that were one-for-one with 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_e and I_i interneurons. Previous research has shown that one source for monosynaptic input to type I 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 Ie interneuron (Fig. 6E-F) and monosynaptic IPSPs in a type Ii interneuron (Fig. 6G-H). The excitation and increased spike activity recorded in Ii interneurons and decreased spike activity of Ie interneurons observed during illumination (see Fig. 5) is inconsistent with the typical synaptic input to type Ii and Ie 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 Ii 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). An example of simultaneous recordings from an identified type Ii 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). Hyperpolarization of the type B photoreceptor with a current pulse during light (Fig. 7B2) resulted in a depolarization and spike activity recorded from the Ii interneuron (Fig. 7A2). Therefore oscillations of the generator potential and associated decreased spike activity that occurs between photoreceptors, may contribute to out-of-phase patterned activity detected in type Ie and Ii interneurons during illumination.

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.4-5). Type Ie
and Ii interneurons typically fire at a frequency near one spike/sec 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 if prolonged illumination would elicit repetitive patterns of burst activity in type I 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 sec intervals during the 5 min of illumination. Occasionally, patterned burst activity occurred in Ie and Ii interneurons in the dark and in light (see Fig. 5). However, light did not elicit repetitive or rhythmic burst activity in either Ii or Ie 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 I interneurons that typically exhibited regular tonic firing under dark adapted conditions. Taken collectively, while non-repetitive 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. Since 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 due to synaptic input from IIIi interneurons. Hyperpolarizing the IIIi interneuron in the dark to block spike generation revealed spontaneous EPSPs (Fig. 9C) that were inhibited during illumination of the photoreceptors (Fig. 9D). The source of the EPSPs is likely the synaptic input from type I interneurons, which is consistent with the previously identified circuitry (Crow and Tian 2003a). Simultaneous recordings from a IIIi interneuron and VP1 efferent neuron in the dark, during a 5 min period of light, and in the dark following light offset are shown in Fig. 9E-F. Consistent with the assessment of spike activity of type I interneurons during a 5 min period of illumination, light inhibited spike activity in the IIIi interneuron and increased the tonic spike activity of the VP1 ciliary efferent neuron. After the termination of illumination the spontaneous spike activity of the IIIi interneuron returned with a concomitant decrease in the firing of the VP1 efferent neuron (Fig. 9E-F).

Pedal nerve stimulation produces rhythmic bursting activity

To test if the Ic and Ii components of the ciliary circuit could support rhythmic burst activity we examined spike activity following pedal nerve stimulation and at the off-set of illumination. Both pedal nerve stimulation and the off-set of light elicited burst activity in Ic and
I_i interneurons. An example of a rhythmic burst pattern generated in a type I_e interneuron after the termination of light is shown in Fig. 10A. Burst 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 (Crow and Tian, 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 I_e and I_i interneurons. As shown in Fig. 10B, stimulation of P2 produced an increase in the frequency of EPSPs recorded in a type I_e interneuron hyperpolarized to block spike activity. In another example, stimulation of P2 produced burst activity in a type I_e interneuron (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 I_i (Fig. 11A) and I_e (Fig. 11B) interneurons that persisted for several minutes. The results of pedal nerve stimulation studies demonstrates that type I_e and I_i interneurons, that 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 pedal nerves 1 and 2 (Richards and Farley 1987; Hodgson and Crow 1991; Crow and Tian 2003a). Identified ciliary efferent neurons increase their tonic firing in response to illumination of the eyes, and provide a major contribution to increased multi-unit spike activity of pedal nerves recorded during light (Crow and Tian 2003a,b).
Previously published research has reported that multi-unit recordings of pedal nerve activity evoked by light are less variable in vertically oriented nervous systems as compared to horizontally oriented preparations (Richards and Farley 1987). Therefore, both vertical (N=8) and horizontal (N=37) orientations were used in the experiments examining multi-unit spike activity recorded from pedal nerves. A 16% increase from dark adapted baseline multi-unit activity was used to determine light responses of pedal nerve recordings. In different preparations, the peri-stimulus time (PST) histograms of multi-unit activity recorded from P1 and P2 in the dark, during illumination, and after light-off-set revealed diverse activity patterns. Several patterns of multi-unit activity elicited by light were typically expressed in the recordings from P1 and P2. One pattern of multi-unit activity was an increase in spike activity that occurred with a variable latency following light on-set 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 multi-unit 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 multi-unit recordings from pedal nerves reported in a previous study (Richards and Farley 1987). Evoked increases in multi-unit 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 non-rhythmic activity revealed 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 non-rhythmic 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 non-rhythmic patterns of multi-unit 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 off-set are shown in Fig. 12A-E. For this experiment the nervous system was oriented horizontally. Interestingly, the peaks in the PST histograms of P1 and P2 multi-unit 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 multi-unit activity of P1 (Fig. 12D-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 multi-unit recordings may reflect 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 multi-unit 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 multi-unit 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 15A1-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 (Alkon 1973a, 1973b; Akaike and Alkon 1980; Alkon and Fuortes 1972; Alkon et al. 1978; Crow and Tian, 2000, 2002a, 2002b, 2003a, 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 IIIi inhibitory interneurons through a polysynaptic pathway. The monosynaptic connection between IIIi 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 I_1 spike activity, decreased spike activity of type III_1 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 I_e spike activity evoked by light is less effective in exciting type III_1 interneurons than the disexcitation of III_1 interneurons produced by light inhibition of I_1 interneurons. Consistent with this view are the results of the present study showing that under steady-state light adapted conditions, type I_e spike activity is closer to dark adapted pre-light baseline activity than the relative decrease in I_1 spike activity from dark adapted baseline activity (see Fig. 8). The regulation of ciliary movement by the modulation of type III_1 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 systems. Type I interneurons are polysensory; receiving synaptic input from the graviceptive, somatosensory, visual, and chemosensory systems (Alkon et al. 1978; Akaike and Alkon 1980; Crow and Tian 2000). The regulation of ciliary locomotion by a “clutch”-like mechanism provided by III_1 interneurons is an efficient means of modulating spike activity in ciliary efferent neurons, since their membrane potential under baseline conditions is near the threshold for spike generation. Therefore, excitation or inhibition of spike activity in III_1 interneurons modulates spike activity of ciliary efferent neurons. A second pathway that modulates spike activity in ciliary efferent neurons is from the type I_b interneurons. Type I_b interneurons exhibit stronger synaptic activation from statocyst hair cells as compared to photoreceptor synaptic input (Crow and Tian 2004). Therefore, type I_b interneurons contribute more to the modulation of ciliary locomotion by activity from the graviceptive sensory system. Interestingly, type I_b interneurons form
monosynaptic connections with ventral contractile motor neurons and other identified motor neurons producing foot contraction (Crow and Tian 2004). Taken collectively, type Iδ interneurons may be outside the primary network for visually guided ciliary locomotion, but may contribute to the reconfiguration of the circuit supporting graviceptive control of locomotion and reflexive movements.

**Multifunctional neural networks**

Multifunctional 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 upon stimulation of two command-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 multifunctional and dedicated CPGs (Briggman and Kristan 2006). In the leech approximately 93% of the neurons in the swimming and crawling networks overlap. Multifunctional networks may also control different effector systems. There are a number of examples of multifunctional networks supporting muscular and ciliary activity. In *Pleurobranchaea* 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 *Pleurobranchaea* fire rhythmically during swimming, and tonically during crawling. In addition, the As1-4 neurons in *Pleurobranchaea* exert a general activation of an arousal network and contribute to avoidance turning (Jing and Gillette 2000, 2003). The network mediating ciliary
locomotion in *Lymnaea* may be involved in both respiration and ciliary locomotion (Syed and Winlow 1989). Consistent with the nonrhythmic tonic activity of networks supporting crawling in other systems, our results show that the spike activity of I_e and I_i interneurons and ciliary efferent neurons during illumination is tonic, not rhythmic. Moreover, prolonged illumination (5 min) does not result in the generation of rhythmic bursting or patterned activity in type I or type III_i interneurons. Taken collectively, these results indicate that light modulates ciliary locomotion by tonically altering the spike activity of type I_e and I_i interneurons and the subsequent disinhibition of ciliary efferent neurons by decreased type III_i interneuron activity. However, multi-unit activity recorded from the pedal nerves during illumination showed that light may evoke patterned activity (See Figs. 12 and 15). These examples of patterned multi-unit activity recorded from pedal nerves are similar to bursting activity referred to as spindles in an earlier study (Richard and Farley 1987). We observed that burst activity recorded from pedal nerves may occur in the dark before light onset, and is one of several types of multi-unit activity detected in pedal nerves during illumination. 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 ambiguous. Moreover, spindle burst activity may be correlated with the transient inhibition of the spike activity of type I_e interneurons during illumination (see Fig. 12). One common type of multi-unit activity recorded from pedal nerves is an increase in tonic firing that occurred with a variable latency following light onset (see Figs. 13-14). 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 multi-unit 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 revealed IPSPs that occurred synchronously, suggesting that they may be generated from a common presynaptic source or sources. Synaptic activation of the two efferent neurons may be synergistic since 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 I_b interneurons project to foot contraction efferent neurons and ciliary efferent neurons. Graviceptive input to I_b interneurons is sufficient to generate foot contractions and ciliary activity. In contrast, visual input to I_b interneurons is typically not sufficient to increase spike activity unless the interneurons are spontaneously active. However, visual input during graviceptive activated depolarization of I_b 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-elicited foot-shortening and light-elicited inhibition of ciliary locomotion (Lederhendler et al. 1986; Crow and Alkon 1978). Pavlovian conditioning can reconfigure the ciliary circuit that typically supports the positive phototaxis to produce light-elicited inhibition of ciliary locomotion (Crow 2004; Crow and Tian 2003b; 2006).

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 P2 elicited burst activity recorded from both type I_e and I_i interneurons (see Fig. 11). Rhythmic activity was also observed after the termination of illumination (see Fig. 10). In addition, occasional spontaneous burst activity recorded in type I_e and I_i 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 As1-4 neurons in *Pleurobranchaea* (Jin and Gillette 2003). 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. The present 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.
FIGURE LEGENDS

Fig. 1. Neural network supporting ciliary locomotion. Diagram of the sensory neurons, interneurons, efferent neurons and synaptic connections. The synaptic connections from only one identified photoreceptor [lateral B (LB)] to the interneurons are shown in the figure. However, each photoreceptor within the eye forms monosynaptic connections with different aggregates of type I interneurons. Statocyst hair cells (HC) also form monosynaptic connections with type I interneurons. Type IIII interneurons form monosynaptic connections with VP1 ciliary efferent neurons. Filled circles denote inhibitory synapses, open triangles indicate excitatory synapses. Solid lines, monosynaptic connections, dashed lines polysynaptic pathways.

Fig. 2. Specific aggregates of type I interneurons exhibit reciprocal electrical coupling. Simultaneous recording from two identified type Ii interneurons. Current stimulation of one identified type Ii interneuron elicited a hyperpolarizing electrotonic potential (B1) and a smaller amplitude potential recorded from the second identified Ii interneuron (A1). Current stimulation of the second Ii interneuron elicited a hyperpolarizing electrotonic potential (A2) and a smaller amplitude potential recorded from the first Ii interneuron (B2). Aggregate pairs of type Ie interneurons are also reciprocally coupled. Current stimulation of an identified Ie interneuron elicited a hyperpolarizing potential (C1) and a smaller amplitude potential recorded from the second identified Ie interneuron (D1). Current stimulation of the second Ie interneuron (D2) elicited a smaller amplitude potential recorded from the first Ie interneuron (C2).

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 I_c interneuron (B1). Current stimulation of the I_c interneuron (B2) did not elicit a potential in the I_i interneuron (A2). Type I_i and I_c interneurons are not synaptically connected. Spikes evoked by a 2 sec current pulse applied to an I_i interneuron (A3) did not elicit PSPs or spikes recorded from an identified I_i interneuron (B3). Spikes evoked by the current pulse in the I_c interneuron (B4) did not evoke PSPs or spikes recorded from the I_i interneuron (A4).

Fig. 4. Electrically coupled pairs of type I_i and pairs of I_c interneurons generate synchronous spike activity. Simultaneous recordings from a pair of electrically coupled identified type I_i interneurons (A1 and A2) exhibited spontaneous synchronous spike activity. Simultaneous recordings from a pair of electrically coupled type I_c 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 I_c interneurons showed that depolarizing current stimulation of one type I_c interneuron (C1) evoked a depolarization and increase in spike activity recorded in the second I_c interneuron (C2).

Fig. 5. Type I_c and I_i interneurons may exhibit irregular spontaneous phasic activity. Simultaneous recordings from identified I_c and I_i interneurons showed that bursts of activity in the I_c indicated by the bars above the recording (A) were associated with inhibition of spike activity in the I_i interneuron (B). Out-of-phase activity may also occur during illumination of the eyes. Simultaneous recordings from identified I_i and I_c interneurons during light showed burst activity in the I_i (C) may be associated with inhibition of spike activity recorded in the I_c interneuron (D). Light attenuated -1.0 log unit.
Fig. 6. Type I_e and I_i interneurons receive synaptic input from a common presynaptic source or sources. Simultaneous recordings from identified I_e and I_i interneurons showed that a burst of spikes in the I_e (A) was accompanied by a complex IPSP and single IPSPs recorded from the I_i interneuron (B). Simultaneous recordings from a different pair of I_i and I_e interneurons showed that spontaneous IPSPs in the I_i (C) occurred concomitantly with the generation of EPSPs in the type I_e (D). The monosynaptic connection between a photoreceptor and type I_i interneuron may contribute to out-of-phase activity. Simultaneous recordings from an I_e interneuron (E) and lateral type B photoreceptor (F) and I_i interneuron (G) and lateral B photoreceptor (H) show PSPs in interneurons follow photoreceptor spikes one-for-one.

Fig. 7. Simultaneous recordings from an identified I_i interneuron and lateral B photoreceptor during illumination. Light evoked a stereotyped depolarizing generator potential in the lateral B (B1) and complex IPSP in the I_i 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 I_i interneuron (A2).

Fig. 8. Light evoked a change in the tonic activity of I_e and I_i interneurons. Graph of mean spike activity (± SEM) of I_e and I_i interneurons 3 min before (dark adapted), during 5 min of light (light adapted), and 3 min after light off-set plotted at 1 min intervals. Light did not evoke rhythmic firing of type I_e or I_i interneurons. Light attenuated -1.0 log unit.
Fig. 9. Light inhibits spike activity of type III\textsubscript{i} interneurons and increased tonic firing of VP1 ciliary efferent neurons. A: spontaneous spike activity in the dark recorded from an identified III\textsubscript{i} interneuron. B: IPSPs recorded simultaneously from a VP1 ciliary efferent neuron follows the spikes one-for-one. C: Recording from the same III\textsubscript{i} interneuron in the dark hyperpolarized to block spikes revealed spontaneous EPSPs. D: Illumination of the eyes inhibited the spontaneous EPSPs recorded from the III\textsubscript{i} interneuron. E-F: Simultaneous recordings from an III\textsubscript{i} interneuron and VP1 efferent neuron in the dark, during 5 min of illumination, and after light off-set. Illumination inhibited tonic spike activity of the III\textsubscript{i} interneuron and increased the tonic spike activity of the VP1 efferent neuron. After the termination of light spontaneous spike activity in the III\textsubscript{i} interneuron returned with a concomitant decrease in VP1 spike activity. Light on-set and off-set indicated by the two arrows above the recording in E. Light attenuated -1.0 log unit.

Fig. 10. The off-set of light may evoke rhythmic firing. Recording from an identified I\textsubscript{e} interneuron (A) showed tonic firing near the end of the period of illumination and rhythmic burst activity following the termination of light indicated by the arrow. Pedal nerve stimulation evoked burst activity in a type I\textsubscript{e} interneuron. Stimulation of P2 (0.2 mA, 50 Hz, 300 ms duration) in a preparation hyperpolarized to block spontaneous spike activity of an identified I\textsubscript{e} interneuron revealed an increase in EPSP frequency following nerve stimulation (B). Recording from an identified type I\textsubscript{e} interneuron (C) showed burst activity following current stimulation of P2 (0.02 mA, 50 Hz, 100 ms duration).
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 I_i interneuron (A). Stimulation of P1 (0.1 mA, 10 Hz, 1s duration) produced rhythmic bursting in an identified type I_e 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.

Fig. 12. Patterned multi-unit activity recorded from P1 and P2 is associated with the transient increase and decrease in spike activity recorded from an identified I_e interneuron during light. Peri-stimulus time (PST) histograms of multi-unit 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 I_e interneuron (C). The open and closed circles indicate the decrease in type I_e tonic activity associated with the transient peaks in the PST histograms. Activity of the I_e interneuron associated with a PST histogram peak (open circle) displayed on a faster time scale (D-E). Light on-set and off-set (-1.0 log unit) indicated by the arrows beneath the recording in (C).

Fig. 13. Light evoked a delayed increase in multi-unit activity recorded from P1 and P2. PST histograms of increased multi-unit activity evoked by light recorded from P2 (A1) and P1 (B1). Corresponding suction electrode recordings from P2 (A2) and P1 (B2) during dark adaptation, 5 min of light, and after light off-set. The nervous system was oriented vertically. Light on-set and off-set indicated by the two arrows above the histograms. Light attenuated -1.0 log unit.
Fig. 14. Short-latency on-set of light-evoked activity recorded from P1 and P2. PST histograms of multi-unit activity recorded from P2 (A1) and P1 (B1) during dark adaptation, 5 min of light, and after light off-set. Corresponding suction electrode recordings from P2 (A2) and P1 (B2). The nervous system was oriented vertically. Light on-set and off-set indicated by the two arrows above the histograms. Light attenuated -1.0 log unit.

Fig. 15. Light-evoked patterned activity recorded from P1 and P2. PST histograms of multi-unit activity recorded from P2 (A1) and P1 (B1) during dark adaptation, 5 min of light, and after light off-set. 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 on-set and off-set indicated by the two arrows above the histograms. Light attenuated -1.0 log unit.
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