Serotonin-Immunoreactive CPT Interneurons in *Hermisenda*: Identification of Sensory Input and Motor Projections

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**INTRODUCTION**

In many invertebrates serotonin [5-hydroxytryptamine (5-HT)] plays a neuromodulatory role in the function of sensory and motor systems. For example, 5-HT has been shown to modulate rhythmic behaviors such as feeding (Jing and Gillette 2000; Weiss et al. 1981; Yeoman et al. 1996) and different forms of locomotion and swimming (Arshavsky et al. 1985, 1992; Jing and Gillette 1995, 1999; Katz and Frost 1995; Land and Crow 1985; Mackey and Carew 1983; McPherson and Blankenship 1991, 1992; Palovcik et al. 1982; Panchin et al. 1996; Parsons and Pinsker 1989; Satterlie and Norekian 1995, 1996). In *Aplysia*, identified serotonegic neurons are activated by aversive stimuli that produce behavioral arousal and sensitization of defensive reflexes (Mackey et al. 1989; Marinisco and Carew 2002; Marinisco et al. 2004; Wright et al. 1995). Serotonin immunoreactive (5-HT-IR) neurons have been identified in the cerebral ganglion of several opisthobranch molluscs (Croll 1987a; Croll et al. 2001; Fickbohn et al. 2001; Hawkins 1989; Jing and Gillette 1999; Katz and Frost 1995; Land and Crow 1985; Longley and Longley 1986; Panchin et al. 1995; Satterlie and Norekian 1995; Sudlow et al. 1998).

Because the various putative homologues of the cerebral 5-HT-IR neurons in different species are activated by aversive stimuli and project to neurons contributing to the generation of feeding and locomotor behavior, it has been proposed that they may serve as a general arousal system (Jing and Gillette 2000; Katz et al. 2001). In the marine mollusc *Hermisenda* several groupings of 5-HT-IR neuronal cell bodies have been identified in the circumesophageal nervous system (Auerbach et al. 1989; Croll 1987a; Land and Crow 1985). One cluster of three 5-HT-IR cell bodies, referred to as cerebropleural triplicae (CPT) neurons, have been identified on the dorsal surface of the cerebropleural ganglion (Land and Crow 1985) and are located in similar positions to 5-HT-IR neuronal cell bodies identified in other opisthobranch molluscs (for review see Katz et al. 2001). The 5-HT-IR CPT interneurons in *Hermisenda* were identified as potential candidates for synaptic connections with components of the visual system; photoreceptors and optic ganglion cells (Auerbach et al. 1989), and as a source for 5-HT-IR varicosities that encircle the optic nerve before entry into the cerebropleural ganglion (Land and Crow 1985). However, because double labeling of CPT neurons was not used in the initial studies, the synaptic projections of CPT neurons and their potential role in modulating sensory or motor neurons contributing to behavior have not been established.

In this study, using intracellular recordings, fluorescent labeling of neurons, and immunohistochemistry, we show that CPT interneurons are activated by cutaneous stimulation of the foot and illumination of photoreceptors. In addition, using double-labeling techniques we have found that identified CPT interneurons project to identified motor neurons in the contralateral pedal ganglion. In isolated nervous systems and semi-intact preparations we found that CPT interneurons form monosynaptic excitatory connections with identified VP2 motor neurons that were previously shown to innervate the anterior foot. However, CPT interneurons do not project to identified VP1 ciliary motor neurons or facilitate ciliary movement when stimulated with extrinsic current. Both ipsilateral and contralateral CPT interneurons exhibit reciprocal electrical coupling and synchronous inhibitory postsynaptic potentials (IPSPs). Our results indicate that CPT interneurons are polysensory and they contribute to reflexive foot movements. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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elicited by stimulation of sensory pathways. Some of the results reported here were previously presented in abstract form (Tian et al. 2004).

**METHODS**

**Animals**

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 (ASW) aquaria at 14°C on a 12-h light–dark cycle. All electrophysiological procedures were conducted during the light phase of the light–dark cycle. Circumesophageal nervous systems from 44 *Hermissenda* were used in the experiments. Nineteen nervous systems were processed for immunostaining after Lucifer yellow labeling of CPT interneurons. Electrophysiological experiments involved data collected from both physiologically–anatomically identified CPT interneurons and from double-labeled preparations. Therefore all electrophysiological experiments involved replications that were conducted using double-labeled preparations.

**Intracellular recordings**

Simultaneous intracellular recordings from CPT interneurons, type Ib interneurons, and lateral type B photoreceptors were collected from isolated circumesophageal nervous systems. Simultaneous intracellular recordings from CPT interneurons and identified VP1 and VP2 motor neurons were collected from isolated nervous systems and anterior split-foot semi-intact preparations as described previously (Crow and Tian 2004). Immunohistochemical and anatomical–electrophysiological criteria were used to identify CPT interneurons. The following criteria were used in the identification of CPT interneurons. First, before desheathing only cerebropleural ganglia were selected if the three CPT interneurons could be clearly identified on the line extending from the base of nerve 1, the rhinophore nerve (for description of circumesophageal nerves see MacFarland 1966; Russell 1929), to the anterior region of the cerebropleural commissure. Second, the cell bodies of the three CPT interneurons are adjacent to each other and are of medium size (about 30–35 μm). Third, recordings from CPT interneurons exhibited a high frequency of spontaneous IPSPs when the neurons were hyperpolarized to block spike activity (see Fig. 6). In addition, active CPT interneurons have a spontaneous firing rate of 0.1–1.6 spikes/s (mean = 0.59 ± 0.45) influenced by the level of dark adaptation. If all of the criteria could not be met, the preparation was discarded. Finally, in 19 preparations a CPT interneuron was labeled with Lucifer yellow followed by 5-HT immunostaining. Surgical desheathing of a small area of the dorsal cerebropleural and ventral pedal ganglion was conducted to expose the cell bodies of CPT interneurons and VP1 and VP2 motor neurons. In semi-intact preparations VP1 and VP2 pedal motor neurons were identified by verifying anterior foot contractions or ciliary movement produced by extrinsic current depolarization. Synaptic connections between CPT interneurons and VP2 motor neurons were physiologically verified by eliciting spikes in the interneurons with extrinsic current pulses and recording monosynaptic PSPs, complex PSPs, and spikes in VP2 motor neurons.

The partially desheathed circumesophageal nervous systems were pinned to a Sylgard (Dow Chemical) stage in a recording chamber filled with ASW of the following composition (in mM): 460 NaCl, 10 KCl, 10 CaCl₂, and 55 MgCl₂, buffered with 10 mM HEPES and brought to pH 7.46 with dilute NaOH. The ASW in the recording chamber was monitored by a thermistor and held at 15 ± 0.5°C. Illumination of the eyes was provided by a tungsten–halogen incandescent lamp attached to a fiber-optic bundle mounted underneath the recording chamber. Maximum light intensity was attenuated with neutral density filters expressed in negative log units. Interneurons and pedal motor neurons were impaled with microelectrodes filled with 4 M KAc or Lucifer yellow in 0.2 M LiCl. Microelectrodes were connected to the two headstages of an Axoclamp 2A (Axon Instruments, Foster City, CA). Standard intracellular recording and extrinsic current stimulation techniques were used. Digitized data were analyzed and prepared for figures using Spike2 software (Cambridge Electronic Design). Single spikes elicited by brief extrinsic current pulses and trains of action potentials elicited by current steps were applied in the dark through a bridge circuit. Evidence for monosynaptic connections between CPT interneurons and VP2 motor neurons was provided by excitatory postsynaptic potentials (EPSPs) with short and relatively constant latencies and a one-for-one relationship between CPT action potentials and EPSPs recorded in normal ASW and in ASW containing high-divalent cations (3 × Ca²⁺ and 3 × Mg²⁺).

**Semi-intact preparations**

Semi-intact anterior split-foot *Hermissenda* were prepared by cooling the animals in ASW to between 0 and 1°C followed by isolation of the circumesophageal nervous system from the buccal crest and body, leaving intact left pedal nerves P1 and P2. The anterior split foot was positioned ventral side up adjacent to the isolated circumesophageal nervous system pinned to the elevated central stage in the recording chamber. The left pedal ganglion was rotated approximately 150° to provide for visualization of neuronal cell bodies on the ventral surface. The exposed nervous system and foot were imaged in visible light by a 45-W tungsten–halogen light source projected by a light guide to the central stage of the recording chamber. For measurements of foot contraction or ciliary movement, the foot was visualized in infrared illumination provided by the insertion of an infrared filter (Schott model RG-850) in the light path. A dissecting microscope formed an image of the foot and nervous system in infrared light on a Dage MTi videocamera connected to a videomonitor. Potential CPT activation of cilia was assessed in infrared illumination by videomaging the movement of small dried ink particles on the foot during depolarization of identified CPT interneurons with extrinsic current. Anterior foot movement elicited by CPT depolarization (2, 5, or 10 s, 1 nA), VP2 motor neuron depolarization, or cutaneous stimulation of the foot was quantified by measuring foot displacement during stimulation from a prestimulus baseline position marked on a transparency attached to the videomonitor.

Responses of CPT interneurons to different mechanical stimuli were examined with von Frey hairs exerting different bending pressures. An ascending series of five von Frey hair stimuli was applied to the middle and tail region of the foot. No attempt was made to determine the precise receptive field for the three CPT interneurons. Cutaneous stimulation lasting about 0.5 s was accomplished by a brief stroke or flick of the von Frey hair applied with sufficient pressure to bend the hair. Bending pressure expressed in grams per square millimeter for the different von Frey hair stimuli was determined by measuring the force required for bending on an electronic analytical balance divided by the probe area. Response latencies of CPT interneurons to cutaneous stimulation were estimated by activating an electronic event marker for the data acquisition system when the experimenter initiated the “flicking” or “stroking” movement of the von Frey hair. When a stimulus artifact could be detected at high gain, the response latencies were estimated from the interval between the artifact and the spike. With these procedures CPT latencies in response to the two stiffest von Frey hairs were estimated to be between 290 and 360 ms.

**Immunostaining and interneuron labeling**

Putative 5-HT CPT interneurons were impaled with electrodes with tips filled with Lucifer yellow (4 or 8% in 0.2 M LiCl, electrode resistance 100–150 MΩ). After collecting electrophysiological data, a hyperpolarizing current (0.5–1 nA) was applied for 1 h for the iontophoresis of Lucifer yellow. The ganglia remained in the record-
Statistical analysis

Descriptive statistics involved group data presented as means ± SE. Overall significant differences were established with a two-way or one-way repeated-measures ANOVA followed by Neuman–Keuls post hoc two-group comparisons. For some comparisons, significant differences between two groups consisted of t-tests for correlated means.

RESULTS

Axonal projections of CPT interneurons

5-HT-IR CPT interneurons were identified in the cerebropleural ganglia using Lucifer yellow injection of a single neuron in conjunction with immunofluorescent labeling. A total of 19 preparations were studied after Lucifer yellow injections of CPT neurons to verify 5-HT immunoreactivity. Initially, anatomical localization and electrophysiological properties of CPT interneurons were used as criteria for identification before immunofluorescent labeling (see METHODS). All 5-HT-IR CPT interneurons projected a single axonal process from the cell body in the ipsilateral cerebropleural ganglia through the cerebropleural commissure to the cerebropleural–pedal commissure and into the contralateral pedal ganglion. A representative example of a Lucifer-labeled CPT interneuron is shown in Fig. 1A1. Because the axonal projection to the contralateral pedal ganglia was invariant, the photograph of the Lucifer-filled CPT interneuron in Fig. 1A1 shows only the cerebropleural ganglia (CPG) and the contralateral pedal ganglion (PG). The image from a confocal scan of the circumsophageal nervous system depicts a Lucifer-labeled CPT interneuron (green), indicated by the white bracket, whose axon enters the contralateral pedal ganglion (white arrow in Fig. 1A1). The Lucifer-labeled axon projected (white arrow, Fig. 2A3) below the base of the eye (labeled E in Fig. 1A3) before entering the pedal ganglion. Several groups of 5-HT-IR neurons were identified in the cerebropleural ganglia that were consistent with previous reports (Auerbach et al. 1989; Croll 1987a; Land and Crow 1985). Four areas of 5-HT-IR cell bodies were identified in the cerebropleural ganglia. Two 5-HT-IR giant cerebral cell bodies are indicated by the open arrows in Fig. 1A2. Several smaller-diameter 5-HT-IR cell bodies are located around the giant cerebral neuron cell bodies as previously noted (Auerbach et al. 1989; Croll 1987a; Land and Crow 1985). The 5-HT-IR CPT cell bodies are positioned near the cerebropleural commissure on the midline of the dorsal surface of the ganglia as indicated by the white bracket in Fig. 1A2. A pair of 5-HT-IR cell bodies are found medial and caudal to the CPT interneuron cell bodies and at the proximal edge of the contralateral pedal ganglion (small white arrow).
A posterior margin of the cerebropedal commissure. As previously reported (Auerbach et al. 1989; Croll 1987a; Land and Crow 1985), the majority of 5-HT-IR cell bodies are located in the pedal ganglia. The 5-HT-IR soma of asymmetrical neuron LP1 (Jerussi and Alkon 1981) is indicated by the solid arrow in Fig. 1A2. The confocal scan of three immunofluorescent-labeled CPT neurons is shown to the left of the white bracket in Fig. 1A2. Serotonin-IR cells, axons, and arborizations within the neuropil were observed throughout the cerebropedal and pedal ganglia. The merged confocal image of Fig. 1, A1 and A2 showed that the 5-HT-IR CPT interneuron and Lucifer yellow labeling were co-localized as shown by the labeled cell body within the grouping indicated by the white bracket (Fig. 1A3).

Mechanosensory projections to CPT interneurons

The responses of CPT interneurons to mechanical stimuli were examined in seven semi-intact preparations, and four of the seven preparations involved double labeling to verify 5-HT-IR. Different regions of the ventral surface of the foot were stimulated with handheld von Frey hairs exerting different bending pressures. Representative examples of responses recorded from a CPT interneuron evoked by the application of an increasingly stiff series of von Frey hairs applied to the middle part of the foot are shown in Fig. 2A. Increasing the bending pressure produced a graded increase in the number of evoked spikes recorded from the CPT interneurons. Action potentials in CPT interneurons were not evoked by cutaneous stimulation with von Frey hairs exerting bending forces <0.11 g/mm². It is likely that CPT interneurons do not receive synaptic input from low-threshold mechanoreceptors because a control procedure involving the insertion of smaller-diameter von Frey hairs into the ASW near the foot did not elicit changes in the activity of the CPT interneurons that were significantly different from prestimulus baseline activity (t = 1.6; NS). The group data shown in the semilogarithmic plot in Fig. 2B summarizing data collected from stimulation of the middle region of the foot indicated that CPT responses to cutaneous stimulation were a graded function of von Frey hair pressure. Cutaneous stimulation of the middle part of the foot evoked a substantial increase in CPT spikes relative to prestimulus baseline activity that returned to baseline within seconds. The results of the two-way ANOVA revealed statistically significant effects of stimulus intensity [F(4,8) = 4.6; P < 0.03] and spike activity over time [F(6,12) = 7.2; P < 0.002]. In addition there was a significant interaction between stimulus intensity and time [F(24,48) = 5.8; P < 0.001]. The significant interaction can be accounted for by the increase in CPT spikes only after cutaneous stimulation for all of the von Frey hairs tested in the study. Similar results were detected after cutaneous stimulation of the tail with the application of the von Frey hairs. The results of the two-way ANOVA revealed statistically significant effects of stimulus intensity applied to the tail region [F(3,9) = 17.4; P < 0.001] and changes in spike activity over time [F(6,18) = 8.9; P < 0.001]. The statistically significant interaction [F(18,54) = 6.4; P < 0.001] can be accounted for by the large increase in CPT spikes elicited by cutaneous stimulation as compared with prestimulus baseline activity.

Foot movement elicited by CPT interneuron depolarization was examined in 11 anterior split-foot semi-intact preparations. In five preparations CPT interneurons were identified by immunostaining following the electrophysiological procedures. Extrinsic current depolarization of CPT interneurons elicited a burst of spikes sufficient to evoke movement of the anterior foot. Cutaneous stimulation of the middle region of the foot with a von Frey hair also produced movement of the anterior foot. The summary group data in the bar graph of Fig. 2C show mean anterior foot movement evoked by von Frey hair stimulation of the middle region of the foot (n = 5 preparations).
Activation of CPT interneurons with von Frey hair stimulation elicited anterior foot movement similar to foot movement evoked by depolarization of VP2 motor neurons (Crow and Tian 2003).

Photoreceptor projections to CPT interneurons

Visual input to identified CPT interneurons was examined in both semi-intact and isolated circumsesophageal nervous systems. Illumination of dark-adapted photoreceptors (12 min) resulted in a modest increase in the spike discharge frequency of CPT interneurons relative to dark-adapted baseline activity. A representative example of a light-elicited increase in the activity of a CPT interneuron is shown in Fig. 3 A. In the example shown in Fig. 3 A the 10-s light step was attenuated 1 log unit (−1.0). Analysis of the group data shown in Fig. 3 B (n = 14) revealed that the mean difference in spike frequency recorded from CPT interneurons during illumination as compared with an equivalent period of baseline activity in the dark immediately before the presentation of light was statistically significant (t_{13} = 3.2; P < 0.007). Simultaneous recordings from identified photoreceptors and CPT interneurons hyperpolarized to block spike activity revealed a small complex EPSP associated with the depolarizing generator potential recorded from identified photoreceptors. Figure 4 shows a representative example of a simultaneous recording from an identified lateral type B photoreceptor and a CPT interneuron. A light step attenuated 1 log unit elicited a depolarizing generator potential with superimposed spikes (Fig. 4 A) characteristic of the light response of type B photoreceptors (Alkon and Fuortes 1972). The recording from the CPT interneuron hyperpolarized to −65 mV to block spike activity revealed a small complex EPSP (Fig. 4 B) associated with the light-elicited generator potential in the type B photoreceptor.

Electrical coupling between ipsilateral and contralateral CPT interneurons

CPT interneurons in the ipsilateral and contralateral cerebropleural ganglia are electrically coupled. An example shown in Fig. 5, A1–B1 and A2–B2 provides evidence for reciprocal coupling between two ipsilateral CPT interneurons. The mean coupling ratio was 0.05 ± 0.008 based on 10 observations from five different preparations. Simultaneous recordings from ipsilateral and contralateral CPT interneurons also revealed electrical coupling (mean = 0.04 ± 0.008, n = 6). Because all CPT neurons project a single axonal process to the contralateral pedal ganglia, the most likely site of coupling is in the cerebropleural commissure (see Fig. 1). Intracellular recordings from ipsilateral pairs of CPT interneurons (n = 5 preparations) and recordings from ipsilateral and contralateral pairs of CPT interneurons (n = 4 preparations) revealed synchronous IPSPs that were characteristic of their spontaneous activity, suggesting a common presynaptic source or sources (see Fig. 6, A–D).

I\textsubscript{b} synaptic connections with CPT interneurons

In addition to synaptic input from identified sensory systems, CPT interneurons also receive polysynaptic inhibitory input from identified ipsilateral type I\textsubscript{b} interneurons of the graviceptive sensory pathway. Previous research showed that type I\textsubscript{b} interneurons receive polysynaptic input from statocyst hair cells and project to foot contraction motor neurons and ciliary motor neurons (Crow and Tian 2004). As shown in Fig. 7, A and B, stimulation of a type I\textsubscript{b} interneuron with a 2-s depolarizing extrinsic current pulse (Fig. 7 A) inhibited spike activity recorded from a CPT interneuron (Fig. 7 B) during the period of stimulation and for 2 s after the termination of the current pulse. The group data (n = 8 preparations) showing the mean frequency of spike activity in CPT interneurons 6 s before I\textsubscript{b} stimulation, during extrinsic current stimulation of the I\textsubscript{b} interneuron, and 4 s after the termination of the current pulse are shown in Fig. 7 C. The results of the one-way...
ANOVA revealed significant overall differences in CPT spike frequency before, during, and after extrinsic current stimulation \( F \) (5,35) = 4.3; \( P \) < 0.004). Newman–Keuls multiple comparisons revealed that the activity of CPT interneurons during extrinsic current depolarization of Ib interneurons was significantly reduced as compared with prestimulus activity \( q \) = 2.9; \( P \) < 0.05). Extrinsic current depolarization of type Ib interneurons produced inhibition of CPT neurons that could be detected 2 s after the termination of the current pulse \( q \) = 3.8; \( P \) < 0.05). Activity of CPT neurons had returned to prestimulus baseline activity by 4 s after the termination of the current pulse.

CPT interneuron projections to identified pedal motor neurons

Projections of identified CPT interneurons to pedal motor neurons were examined in semi-intact and isolated circum-ophagedial nervous systems. Recently the neural circuit supporting ciliary locomotion and anterior foot contraction in *Hermisenda* has been identified (Crow and Tian 2000, 2002, 2003). We initially examined possible connections between CPT interneurons and VP2 foot contraction motor neurons. As shown in Fig. 8A, a train of spikes evoked by an extrinsic current pulse in an identified CPT interneuron elicited EPSPs in a contralateral VP2 motor neuron hyperpolarized to \(-65\) mV to block spike activity (Fig. 8B). The EPSPs recorded from the VP2 motor neuron followed CPT spikes one for one (Fig. 8B). A burst of spikes elicited in a CPT interneuron (Fig. 8C) produced a summation of EPSPs and a large depolarization of the VP2 motor neuron (Fig. 8D). In contrast, we did not detect synaptic connections between CPT interneurons and identified VP1 ciliary motor neurons. As shown in Fig. 8E, a spike evoked from an identified CPT interneuron did not elicit a PSP in an identified VP1 ciliary motor neuron (Fig. 8F). A current pulse evoked a burst of spikes recorded from an identified CPT interneuron (Fig. 8G), but did not elicit PSPs recorded from an identified VP1 ciliary motor neuron (Fig. 8H). The synaptic connection between CPT interneurons and VP2 motor neurons is most likely monosynaptic. As shown in the superimposed spikes and PSPs in Fig. 8, three consecutive spikes elicited in a CPT interneuron (Fig. 8I) evoked EPSPs with relatively short and constant latencies recorded from a VP2 motor neuron (Fig. 8J). In normal ASW a single spike evoked in a CPT interneuron (Fig. 8K1) elicited a short latency EPSP detected in a VP2 motor neuron (Fig. 8L1). In high-divalent cation ASW the VP2 EPSP (Fig. 8L2) could still be elicited by a single spike in the same identified CPT interneuron (Fig. 8K2).
In summary, CPT interneurons form monosynaptic connections with VP2 motor neurons, although none of the CPT interneurons (n = 10 preparations) exhibited synaptic connections with VP1 ciliary motor neurons under the experimental conditions that were examined in recordings from isolated nervous systems. Consistent with the electrophysiological data collected from isolated nervous systems were observations in semi-intact preparations showing that extrinsic current stimulation of CPT interneurons did not facilitate or elicit ciliary activity (n = 11 preparations). However, stimulation of CPT interneurons with extrinsic current or cutaneous stimulation of the foot elicited anterior foot contraction in semi-intact preparations (n = 11 preparations).

**DISCUSSION**

In this report we used immunohistochemistry and fluorescent labeling to identify 5-HT-IR CPT interneurons. We found that identified CPT interneurons are depolarized by cutaneous stimulation of the foot and illumination of the eyes. In semi-intact preparations we showed that identified CPT interneurons form monosynaptic connections with identified contralateral VP2 pedal motor neurons, but not to identified VP1 ciliary motor neurons. In addition we have found that ipsilateral and contralateral CPT interneurons are reciprocally electrically coupled and are inhibited by depolarization of type I, interneurons that were previously shown to project to ciliary motor neurons and foot contraction motor neurons.

**5-HT-IR cell bodies in the Hermisenda circumesophageal nervous system**

Previous studies of the circumesophageal nervous system using immunohistochemistry identified several areas of 5-HT-IR cell bodies in the cerebropleural and pedal ganglia (Auerbach et al. 1989; Croll 1987a; Land and Crow 1985). The clusters of three medium- to small-sized cell bodies close to the midline near the cerebropleural commissure were of special interest because of the potential synaptic interaction with components of the visual system. Lucifer yellow labeling of the S/E optic ganglion cell, caudal hair cells, and type B photoreceptors in conjunction with 5-HT immunostaining revealed that they are not 5-HT-IR, although 5-HT-IR axonal processes are present in the same general area of the neuropil where the terminal processes of photoreceptors, optic ganglion cells, and hair cells are found (Auerbach et al. 1989). Sections of the immunostained nervous system revealed 5-HT-IR fine processes and varicosities encircling the optic nerve as it projected into the cerebropleural ganglion and in the neuropil of the cerebropleural ganglion (Land and Crow 1985). However, sources of the 5-HT-IR processes and varicosities in the earlier studies were not established.

**CPT interneurons may be homologues of 5-HT neurons in other opisthobranchs**

In many marine invertebrates the central nervous systems may be highly conserved in closely related species (for discussion see Katz and Harris-Warrick 1999). For example, opisthobranch molluscs have very similar central ganglia containing a number of putative homologous neurons that have been identified in different species based on anatomical, pharmacological, and physiological evidence (Croll 1987b; Dickinson 1979; Dorsett 1974; Katz et al. 2001; Pentreath et al. 1982). Many opisthobranch molluscs have several clusters of 5-HT-IR neurons in the medial region of the cerebral ganglion (Fiekbohm et al. 2001; Panchin et al. 1995; Satterlie and Norekian 1995; Satterlie et al. 1995; Sudlow et al. 1998). As an example, the dorsal swim interneurons (DSIs) in *Tritonia* are 5-HT-IR (Katz et al. 1994; McClellan et al. 1994), are components of the central pattern generator underlying swimming (Getting 1981), and excite motor neurons contributing to ciliary locomotion (Audesirk 1978; Popescu and Frost 2002). Putative homologues of the 5-HT-IR DSIs have been identified in *Pleurobranchaea* (Jing and Gillette 1995, 1999), *Clione* (Satterlie et al. 1995), *Phestilla* (Croll et al. 2001), and *Aplysia*...
Role of CPT interneurons in motor activity of Hermannseda

Lucifer yellow fills of DSIs in Tritonia showed a single axonal process that projected to the contralateral pedal ganglion (Getting et al. 1980). Electrophysiological studies have shown that DSIs make monosynaptic connections with contralateral pedal flexion neurons (Getting et al. 1980) and ciliary motor neurons (Audesirk 1978; Popescu and Frost 2002). Our results have shown that extrinsic current stimulation of identified CPT interneurons does not elicit detectable PSPs recorded from identified VP1 ciliary motor neurons. In addition, stimulation of CPT interneurons in semi-intact preparations did not facilitate or elicit ciliary activity.

In Hermannseda, the neural circuitry supporting light-elicited ciliary locomotion is different from statocyst hair-cell-mediated foot contraction and graviceptive ciliary locomotion (Crow and Tian 2004). Light modulates the activity of ciliary motor neurons by hyperpolarizing type I interneurons (“off” cells) and depolarizing type II interneurons (“on” cells). The integration of synaptic input from type I interneurons results in a net decrease in the excitation of type III, inhibitory interneurons during light that produces a decrease in the frequency of IPSPs in identified VP1 ciliary motor neurons (Crow and Tian 2003). The reduced inhibition of VP1 ciliary motor neurons results in an increase in their spike activity during light with a concomitant facilitation of ciliary activity. Therefore light modulation of ciliary activity is a requirement in Hermannseda that may not be found in other species and may explain why CPT interneurons are not part of the circuit supporting ciliary locomotion. Indeed, the sensitivity of photoreceptors may not be sufficient to detect sunlight in Tritonia’s natural habitat (Chase 1974). In contrast, the visual system of Hermannseda supports two very specific light-dependent behaviors; a positive phototaxis and a shadow reflex (Crow 2004). Consistent with this hypothesis is the finding that type IIb interneurons, which are part of the circuitry supporting statocyst-mediated foot contraction and graviceptive ciliary locomotion, inhibit CPT interneurons. In addition, illumination of the photoreceptors produces only a modest increase in the spike activity of CPT interneurons in contrast to the effect of light on type I interneurons in the ciliary locomotor circuit. However, 5-HT may play a role in the control of ciliary and nonciliary locomotion in Hermannseda by the activity of other clusters of cerebral 5-HT-IR neurons.

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