5-HT and GABA modulate intrinsic excitability of type I interneurons in *Hermissenda*

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Running Head: MODULATION OF EXCITABILITY IN TYPE I INTERNEURONS

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ABSTRACT

The sensory neurons (photoreceptors) in the visual system of *Hermissenda* are one site of plasticity produced by Pavlovian conditioning. A second site of plasticity produced by conditioning is the type I interneurons in the cerebropleural ganglia. Both photoreceptors and statocyst hair cells of the graviceptive system form monosynaptic connections with identified type I interneurons. Two proposed neurotransmitters in the graviceptive system, serotonin (5-HT) and γ-aminobutyric acid (GABA), have been shown to modify synaptic strength and intrinsic neuronal excitability in identified photoreceptors. However the potential role of 5-HT and GABA in plasticity of type I interneurons has not been investigated. Here we show that 5-HT increased the peak amplitude of light-evoked complex EPSPs, enhanced intrinsic excitability, and increased spike activity of identified type I<sub>e(A)</sub> interneurons. In contrast, 5-HT decreased spike activity and intrinsic excitability of type I<sub>e(B)</sub> interneurons. The classification of two categories of type I<sub>e</sub> interneurons was also supported by the observation that 5-HT produced opposite effects on whole-cell steady-state outward currents in type I<sub>e</sub> interneurons. Serotonin produced a reduction in the amplitude of light-evoked complex IPSPs, increased spontaneous spike activity, decreased intrinsic excitability, and depolarized the resting membrane potential of identified type I<sub>i</sub> interneurons. In contrast to the effects of 5-HT, GABA produced inhibition in both types of I<sub>e</sub> interneurons and type I<sub>i</sub> interneurons. These results show that 5-HT and GABA can modulate the intrinsic excitability of type I interneurons independent of the presynaptic effects of the same transmitters on excitability and synaptic efficacy of photoreceptors.

Key words: *Hermissenda*; hair cells; photoreceptors; interneurons; intrinsic excitability
INTRODUCTION

Both γ-aminobutyric acid (GABA) and serotonin (5-HT) are putative neurotransmitters in the graviceptive sensory system of *Hermissenda* (for Reviews see Blackwell 2006; Crow 2004)(Blackwell 2006; Crow 2004). The graviceptive sensory organ, the statocyst, contains endogenous GABA, and immunocytochemical procedures have localized GABA in statocyst hair cell axons and axon terminal processes (Alkon et al. 1993). Statocyst hair cells and photoreceptors form reciprocal monosynaptic inhibitory connections where caudal hair cells inhibit photoreceptors and cephalic hair cells are inhibited by type B photoreceptors (Alkon 1973). Stimulation of statocyst hair cells elicits a monosynaptic GABAergic IPSP recorded in type B photoreceptors (Alkon et al. 1993; Blackwell 2002; Rogers et al. 1994; Sakakibara et al. 1993), and enhanced excitability is produced by GABA application paired with depolarization of type B photoreceptors (Matzel and Alkon 1991). With regards to the second putative neurotransmitter of the graviceptive system, the terminal processes of serotonergic interneurons have been shown to form rings of varicosities surrounding photoreceptor axons in the optic nerve before entry into the cerebropleural ganglion (Land and Crow 1985). In addition, immunoreactive processes from 5-HT containing neurons project to a region near the eyes and photoreceptor synapses in the cerebropleural ganglion (Auerbach et al. 1989; Land and Crow 1985; Tian et al. 2006). Both GABA (Schultz and Clark 1997) and 5-HT produce synaptic facilitation of type B to type A photoreceptor monosynaptic IPSPs (Frysztak and Crow 1997; Schuman and Clark 1994), and changes in the intrinsic excitability of identified photoreceptors (Crow and Bridge 1985; Crow and Forrester 1991; Farley and Wu 1989; Matzel and Alkon 1991).
Aggregates of type I interneurons in the cerebropleural ganglion form a second site of synaptic convergence between the graviceptive system and visual system. Photoreceptors and statocyst hair cells form monosynaptic excitatory connections with type I_e interneurons and monosynaptic inhibitory connections with type I_i interneurons (Akaike and Alkon 1980; Crow and Tian 2000). Previous studies have shown that conditioning produces changes in intrinsic excitability in both photoreceptors and type I interneurons (Crow and Tian 2003; Crow and Alkon 1980). Since hair cells form synaptic connections with photoreceptors, type I interneurons, and 5-HT immunoreactive interneurons, the endogenous release of 5-HT and GABA could modulate excitability and synaptic strength in both photoreceptors and type I interneurons. To date, the potential effects of 5-HT and GABA on type I_e and type I_i interneurons have not been investigated. In the present study we examined the effect of 5-HT and GABA on complex PSPs associated with presynaptic input from photoreceptors and intrinsic excitability in identified type I interneurons.
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 ± 1°C on a 12 hr light/dark cycle. Electrophysiological data were collected during the light phase of the light/dark cycle.

Intracellular recordings

Circumesophageal nervous systems were isolated in ASW (~14°C), and desheathed to expose the cell bodies of type I interneurons. The desheathed circumesophageal nervous systems were pinned to a silicone elastomer (Sylgard, Dow Chemical) stage in a recording chamber filled with ASW of the following composition (in mM): 460 NaCl, 10 KCl, 10 CaCl₂, 55 MgCl₂, buffered with 10 mM HEPES and brought to pH 7.46 with NaOH solution. Type I interneurons were identified using established anatomical and electrophysiological criteria as described previously (Akaike and Alkon 1980; Crow and Tian 2002a; 2000; 2002b). The ASW in the recording chamber was monitored by a thermistor and held at 14.5 ± 0.5°C. The illumination of the eyes was provided by a tungsten halogen incandescent lamp attached to a fiber optic bundle mounted underneath the recording chamber. Identified type I interneurons were impaled with microelectrodes filled with 4 M potassium acetate and connected to the head stage of an Axoclamp 2A (Axon Instruments, Foster City, CA). Electrode resistances varied between 70 and 130 MΩ. Extrinsic current pulses were applied through a bridge circuit.
Stimulation protocol

After 12 min of dark adaptation the amplitude of light-evoked complex PSPs was assessed before and after the bath administration of 5-HT or GABA. Complex PSPs were evoked by the presentation of a 10 sec period of illumination of the photoreceptors. The peak amplitude of light-evoked complex PSPs was determined by measuring the amplitude of the depolarization during the light step relative to the pre-light baseline membrane potential. This measure of complex PSP amplitude is consistent with previous reports when spike activity was blocked by hyperpolarizing type Ic interneurons below threshold for spike generation during the light step (Crow and Tian 2000, 2002a). Spike activity elicited during the 10-sec light step was compared to an equivalent period of spontaneous activity before light. Intrinsic excitability was assessed in the dark by presenting 1.8 sec depolarizing current pulses (0.1, 0.2 and 0.3nA) from a holding potential of -60mV maintained by injection of steady depolarizing or hyperpolarizing current. Current pulses were presented at 1 min intervals before and after the bath application of 5-HT or GABA delivered to the recording chamber by either perfusion or direct injection. Membrane potential was determined by measurement of the potential between spikes during a 10 sec pause in extrinsic current injection. The experimental protocol was repeated 3 times at 6 min intervals before and after the bath application of 5-HT or GABA. The final concentration of 5-HT and GABA in ASW was 10^{-4} M and 10^{-3} M, respectively. Digitized electrophysiological data were stored on a computer hard drive, and analyzed using Spike 2 software (Cambridge Electronic Design).
Whole-cell voltage-clamp recordings

Type Ie interneurons were identified by recording inward current underlying complex light-evoked EPSPs. After 12 min of dark adaptation, macroscopic whole-cell currents were recorded at 15 ± 0.5°C using an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Borosilicate glass pipettes (O.D., 1.5 mm; I.D., 1.17 mm) were pulled on a horizontal Flaming-Brown microelectrode puller (Model P80/PC, Sutter Instrument, San Rafael, CA) and fire-polished with a micro forge (MF-830, Narishige, Japan) to obtain tip diameters of 1-2 μm. Only cells in experiments with seal resistances > 1 GΩ were accepted for analysis. Records were filtered at 5 kHz with a lowpass Bessel filter and digitized at 10 kHz with a Digidata interface controlled by pClamp software, version 10.0.0.61 (Axon Instruments). Data analysis was performed with Clampfit (Axon Instruments) and Origin (Microcal software, Northampton, MA) software programs. The composition of bath solutions for recording outward currents was as follows (in mM): Choline-Chloride 450, KCl 10, MgCl₂ 50, CaCl₂ 0.5, HEPES 15, the pH value was adjusted with Tris to 7.46 at 20 ºC. The osmolarity of the bath solution was adjusted to 996-1005 mOsm. The composition of the pipette solution used for whole-cell recordings was (in mM): KCl 430, NaCl 20, MgCl₂ 2, EGTA 2, HEPES 50, Glutathione (reduced) 10, Mg-ATP 5, Na₂-GTP 1, the pH value is adjusted with KOH to 7.30 at 15 ºC. The osmolarity of the internal solution was adjusted to 970 mOsm. Whole-cell currents presented in I-V plots were adjusted for the correct junction potential (~7.6 mV). All chemicals were obtained from Sigma.
Statistical analysis

Descriptive statistics are expressed as means ± SE. Overall significant differences involving multiple groups were determined by a repeated measure ANOVA. Two group inferential statistical comparisons consisted of paired $t$-tests.

RESULTS

Previous work has shown that 5-HT modulates generator potentials and membrane conductances in type B photoreceptors (Acosta-Urquidi and Crow 1993; Crow and Bridge 1985; Crow and Forrester 1991; Farley and Wu 1989; Rogers and Matzel 1995; Yamoah and Crow 1995; 1996). Spikes in identified type A and B photoreceptors elicit monosynaptic EPSPs in type Ie interneurons and monosynaptic IPSPs in type Ii interneurons (Akaike and Alkon 1980; Crow and Tian 2000). Therefore, potential changes in type I interneurons produced by 5-HT and GABA may be induced by both presynaptic and postsynaptic processes.

Excitatory effect of 5-HT on light-evoked complex EPSPs, spike activity, intrinsic excitability and membrane potential in type $I_{e(A)}$ interneurons

Representative recordings from a type $I_{e(A)}$ interneuron after 12 min of dark adaptation and after 5-HT application are shown in Fig. 1. The 10 sec period of illumination produced a complex EPSP and an increase in spike activity in the type $I_{e(A)}$ interneuron (Fig.1A). As shown in Fig. 1B, bath application of 5-HT produced an increase in the amplitude of the light-evoked complex EPSP and an increase in spontaneous and light-evoked spike activity. The inset of Fig. 1 shows an example of the
enhancement of the complex light-evoked EPSP by 5-HT in a preparation without spontaneous spike activity. The analysis of the group summary data (n=6) shown in Fig. 1C and 1D revealed that 5-HT significantly increased spontaneous ($t_5 = 2.9; p< .05$) and the light-evoked spike activity ($t_5 = 2.8; p< .05$) and increased the amplitude of the light-evoked complex EPSP ($t_5 = 4.1; p< .01$). The excitatory effect of 5-HT may be due to the 5-HT-dependent excitatory effect on photoreceptors (Crow and Bridge 1985; Crow and Forrester 1991; Farley and Wu 1989), or direct effects on type I$_{e(A)}$ interneurons. This issue was examined by assessing intrinsic excitability of I$_{e(A)}$ interneurons before and after the bath application of 5-HT. Spike activity evoked by 0.1, 0.2 or 0.3nA current injection was increased in the presence of 5-HT (Fig. 2B). The analysis of the group summary data (n=6) (Fig. 2C) indicated that 5-HT significantly increased intrinsic excitability of type I$_{e(A)}$ interneurons at all current levels that were applied (0.1nA, $t_5 = 7.1; p< .01$; 0.2nA, $t_5 = 5.7; p< .01$; 0.3nA, $t_5 = 4.1; p< .01$). In addition, 5-HT produced a statistically significant depolarization of the membrane potential of type I$_{e(A)}$ interneurons ($t_5 = 7.1; p< .01$) (Fig. 2D).

Inhibitory effect of 5-HT on spike activity, intrinsic excitability and membrane potential of type I$_{e(B)}$ interneurons

In 7 of 13 type I$_e$ interneurons, the bath application of 5-HT produced inhibition of light-elicited spike activity. These cells were classified as type I$_{e(B)}$ interneurons. A representative example of spike activity elicited by a 10 sec light presentation in ASW and after 5-HT application is shown in Fig. 3. The bath application of 5-HT did not produce a significant change in the amplitude of light-evoked complex EPSPs (n=7) ($t_6 = .71; NS$) (Fig. 3C). However, 5-HT significantly decreased the spontaneous and light-
evoked spike activity of type $I_{el(B)}$ interneurons. The analysis of the group summary data shown in Fig. 3D revealed that 5-HT significantly reduced spontaneous ($t_6 = 3.6; p< .05$) and light-evoked spike activity ($t_6 = 2.9; p< .05$). We examined direct (intrinsic) vs indirect (presynaptic) effects of 5-HT by examining intrinsic excitability of $I_{el(B)}$ interneurons before and after the bath application of 5-HT. Spike activity evoked by 0.1, 0.2 or 0.3nA current injection was decreased in the presence of 5-HT (n=7) (Fig. 4A, 4B). The analysis of the group data (Fig. 4C, 4D) showed that 5-HT significantly decreased intrinsic excitability of type $I_{el(B)}$ interneurons as measured by current-evoked spike activity ($0.1nA, t_6 = 6.2; p< .01; 0.2nA, t_6 = 5.7; p< .01; 0.3nA, t_6 = 6.7; p< .01$). In addition, 5-HT application resulted in a significant hyperpolarization of the membrane potential of type $I_{el(B)}$ interneurons ($t_6 = 3.3; p< .05$) (Fig. 4D).

**Dual effect of 5-HT on whole-cell currents in type $I_e$ interneurons**

The results obtained from intracellular recordings of type $I_e$ interneurons showed that the bath application of 5-HT produced different effects on excitability, suggesting that there may be two types of $I_e$ interneurons that we have classified as $I_{el(A)}$ and $I_{el(B)}$. To further investigate this classification, whole-cell currents were examined in a sample of type $I_e$ interneurons (n=8). To exclude the contamination of Na$^+$ and minimize Ca$^{2+}$ effects, Na-free and low Ca$^{2+}$ (0.5 mM) bath solutions were prepared by ionic substitution of Na$^+$ and Ca$^{2+}$ with choline. Choline substitution did not result in a change in holding current. Under these experimental conditions, macroscopic outward whole-cell currents were recorded from a sample of type $I_e$ interneurons. Figure 5 illustrates a family of outward current traces evoked by voltage-clamp steps from -80 mV to +50 mV in 10 mV increments from a holding potential of -80 mV. The transient component of the
outward current was activated at step voltages positive to -50 mV, followed by a sustained component activated at a voltage step positive to -20 mV. The net outward whole-cell currents were reversed at -53.1 ± 3.7 mV. The bath application of 5-HT decreased the peak amplitude of the initial transient currents in all type Ie interneurons examined (n=8). Overall significant decreases were found in the instantaneous current after 5-HT application for putative type Ie(A) ($F_{1,3}=32; p< .01$) and Ie(B) ($F_{1,3}=180; p< .001$) interneurons. However 5-HT produced opposite effects on the sustained steady-state outward currents in different type Ie interneurons as shown in Fig. 5. The steady-state net outward current was significantly decreased in putative type Ie(A) interneurons ($F_{1,3}=106.8; p< .002$) (Fig. 5A) and significantly increased in putative type Ie(B) interneurons ($F_{1,3}=62.9; p< .004$) (Fig. 5B). This suggests that the differential effect of 5-HT on intrinsic excitability of type Ie(A) and Ie(B) interneurons may be the result of the effect of 5-HT on the contribution of the delayed rectifier to the net outward current.

**Effect of 5-HT on light-evoked complex IPSPs, spike activity, intrinsic excitability and membrane potential in type Ii interneurons**

In all of the type Ii interneurons tested (n=6), the bath application of 5-HT produced excitation. A representative example of light-evoked spike activity in a type Ii interneuron in ASW and after 5-HT application is shown in Fig. 6. The bath application of 5-HT produced a decrease in the amplitude of light-evoked complex IPSPs, and an increase in spontaneous spike activity (Fig. 6A-B). The analysis of the group summary data shown in Fig. 6C and 6D revealed that 5-HT significantly decreased the amplitude of light-evoked complex IPSPs ($t_5 = 4.7; p< .01$) and increased the spontaneous spike activity in type Ii interneurons ($t_5 = 4.1; p< .01$). It is likely that the excitatory effect of 5-
HT on type I_i interneurons is not due to 5-HT-dependent excitatory effects on photoreceptors as described previously (Crow and Bridge 1985; Crow and Forrester 1991; Farley and Wu 1989). We examined this by assessing intrinsic excitability of I_i interneurons before and after the bath application of 5-HT. Since pronounced spike frequency accommodation occurred during the current step, we examined current elicited spike activity during the initial 1 sec depolarization before and after 5-HT application. Spike activity evoked by 0.1, 0.2 or 0.3nA current injection was increased in the presence of 5-HT (see Fig. 7). The analysis of the group data (Fig. 7C, 7D) indicated that 5-HT significantly increased intrinsic excitability of type I_i interneurons at all levels of applied current (0.1nA, $t_5 = 2.4; p< .05$; 0.2nA, $t_5 = 3.6; p< .01$; 0.3nA, $t_5 = 3.2; p< .025$). In addition, the membrane potential of type I_i interneurons was significantly depolarized by the 5-HT application ($t_5 = 5.0; p< .01$).

**Inhibitory effect of GABA on light-evoked complex EPSPs, spike activity, intrinsic excitability and membrane potential in type I_e interneurons**

In all type I_e interneurons that were examined (n=7), GABA produced an inhibitory effect. A representative I_e recording before and after GABA application is shown in Fig. 8. Consistent with other I_e recordings, light evoked a complex EPSP in the control recording (Fig. 8A). As shown in Fig. 8B, bath application of GABA produced a decrease in the amplitude of the light-evoked complex EPSP and a decrease in spontaneous and light-evoked spike activity. The analysis of group summary data shown in Fig. 8C and 8D revealed that GABA significantly decreased the amplitude of the light-evoked complex EPSPs ($t_6 = 2.9; p< .05$), spontaneous ($t_6 = 2.9; p< .05$), and light-evoked spike activity ($t_6 = 5.6; p< .01$). The inhibitory effects of GABA may be due to the
GABA-dependent inhibitory effect on photoreceptors (Alkon et al. 1993; Matzel et al. 1995; Rogers et al. 1994), or a direct effect of GABA on type I_e interneurons. This was examined by assessing intrinsic excitability of I_e interneurons before and after the bath application of GABA. Spike activity evoked by 0.1, 0.2 or 0.3nA current injection was decreased in the presence of GABA (see Fig. 9). The analysis of the group data (Fig. 9C, 9D) indicated that GABA significantly decreased intrinsic excitability of type I_e interneurons as measured by current evoked spike activity (0.1nA, $t_6 = 7.7; p< .01$; 0.2nA, $t_6 = 3.9; p< .01$; 0.3nA, $t_6 = 4.1; p< .01$). In addition, GABA significantly hyperpolarized type I_e interneurons ($t_6 = 3.7; p< .01$).

Effect of GABA on light-evoked complex IPSPs, spike activity, intrinsic excitability and membrane potential of type I_i interneurons

A representative recording from a type I_i interneuron before (control) and after GABA application is shown in Fig. 10A and 10B. The bath application of GABA decreased the amplitude of the light-evoked complex IPSP and decreased spontaneous spike activity (Fig. 10B). The analysis of group summary data shown in Fig. 10C and 10D revealed that GABA significantly decreased the amplitude of light-evoked complex IPSPs ($t_7 = 4.4; p< .01$) and decreased spontaneous spike activity in type I_i interneurons ($t_7 = 3.3; p< .05$). The results of GABA application shown here may be due to the GABA-dependent inhibitory effect on photoreceptors or a direct effect on the type I_i interneurons. This was examined by assessing intrinsic excitability of I_i interneurons before and after the bath application of GABA. Spike activity evoked by 0.1, 0.2 or 0.3nA current injection was decreased after the application of GABA (Fig. 11). The analysis of the group summary data (Fig. 11C, 11D) indicated that GABA significantly decreased
intrinsic excitability of type I<sub>i</sub> interneurons as measured by current evoked spike activity

(0.1nA, \( t_7 = 2.6; \) p< .05; 0.2nA, \( t_7 = 3.1; \) p< .05; 0.3nA, \( t_7 = 3.3; \) p< .05). In addition, GABA produced a significant hyperpolarization of type I<sub>i</sub> interneurons (\( t_7 = 2.7; \) p< .05).

296 DISCUSSION

297 *Serotonin produces two effects on excitability of type I<sub>e</sub> interneurons*

In approximately 50 % of the type I<sub>e</sub> interneurons examined in this study, 5-HT produced an increase in spontaneous and light-evoked spike activity, an increase in the amplitude of light-evoked complex EPSPs, an enhancement of intrinsic excitability, and a depolarization of the membrane potential. The remaining type I<sub>e</sub> interneurons exhibited a decrease in the different measures of excitability as a result of 5-HT application. This suggests that there are two types of I<sub>e</sub> interneurons that we have designated as I<sub>e(A)</sub> and I<sub>e(B)</sub>, respectively. Additional support for the classification was obtained from whole-cell voltage-clamp experiments. The sustained outward currents were decreased by 5-HT in 4 of 8 type I<sub>e</sub> interneurons examined under whole-cell voltage-clamp. The remaining type I<sub>e</sub> interneurons exhibited an increase in sustained outward currents in the presence of 5-HT. In these experiments most of the delayed rectifier K<sup>+</sup> current remained at steady-state conditions since A-type K<sup>+</sup> currents are typically inactivated after a 4 sec voltage command step. It is well-documented that voltage-gated delayed rectifier K<sup>+</sup> currents play an important role in maintaining membrane potential and regulating electrical excitability in neurons as well as many other kinds of cells (Gutman et al. 2005). Thus, the differential effects of 5-HT on the delayed rectifier K<sup>+</sup> current may contribute to the
differential effect of 5-HT on type Ie interneuron excitability. Similar differential effects of 5-HT were observed in two-electrode voltage-clamp studies of *Hermissenda* photoreceptors (Acosta-Urquidi and Crow 1993). The question could be raised concerning the coexistence of two different 5-HT receptors. There is evidence to suggest that two pharmacologically and physiologically distinct 5-HT receptors are expressed in the *Leech*, and 5-HT terminals are capable of selectively activating only one of the receptors (Drapeau and Sanchez-Armass 1988). Although it is possible that two distinct 5-HT receptors might be differentially regulated by functionally distinct serotonergic terminals, under the present experimental conditions, the bath administration of 5-HT cannot specifically regulate presynaptic terminals and synaptic receptors. It has been shown that PKA and PKC are differentially recruited depending on the duration of 5-HT application (Braha et al. 1990; Hochner and Kandel 1992; Sugita et al. 1992) and on the state of the synapse (Braha et al. 1990; Ghirardi et al. 1992; Goldsmith and Abrams 1991). For example, facilitation of depressed synapses is blocked by inhibitors of PKC but not PKA, whereas the converse has been shown for nondepressed synapses (Braha et al. 1990; Ghirardi et al. 1992; Goldsmith and Abrams 1991). This state- and time-dependence of PKA and PKC recruitment by 5-HT remains unexplained (Byrne and Kandel 1996). Alternatively, it is possible that 5-HT activates two different receptor types expressed in the different type Ie(A) and Ie(B) interneurons.

Previous work has shown that photoreceptors form monosynaptic connections with type Ie interneurons (Akaike and Alkon 1980; Crow and Tian 2000). Since 5-HT has been shown to enhance photoreceptor excitability (Crow and Bridge 1985; Farley and Wu 1989), the increase in spontaneous and light-evoked spike activity, and the amplitude
of the complex light-evoked EPSPs detected following 5-HT application in type \( I_e \) interneurons may be presynaptic. However, the present results also show that 5-HT produces an intrinsic enhancement of excitability in type \( I_{e(A)} \) interneurons.

It is likely that several cellular processes, both pre- and postsynaptic, contribute to the enhancement of activity in type \( I_e \) interneurons. The increase in spontaneous spike activity, light-evoked spikes, and light-evoked complex EPSPs may have both presynaptic and postsynaptic contributions.

It has been shown that 5-HT as a modulatory neurotransmitter is critical for associative learning of *Leech* shortening (Ehrlich et al. 1992; Sahley 1994) and may contribute to Pavlovian conditioning in *Hermissenda* (Crow 2004). Potentiation of excitability is an important mechanism for encoding and storing information during learning and memory in both vertebrates and invertebrates (Alkon et al. 1985; Antonov et al. 2001; Burrell et al. 2001; Cleary et al. 1998; Crow and Alkon 1980; Gainutdinov et al. 1998; Moyer et al. 2000; Moyer et al. 1996; Oh et al. 2003; Saar et al. 1998; Stackman et al. 2002; Straub and Benjamin 2001; Thompson et al. 1996), and dysfunctions in the modulation of excitability may contribute to age-related deficits in learning and memory (Moyer et al. 2000; Wu et al. 2002).

**The effect of 5-HT on type \( I_i \) interneurons**

In contrast to the differential effect of 5-HT on type \( I_{e(A)} \) and \( I_{e(B)} \) interneurons, 5-HT increased the spontaneous spike activity in type \( I_i \) interneurons. Previous work has shown that 5-HT increases intrinsic excitability and potentiates the amplitude of generator potentials in *Hermissenda* photoreceptors (Crow and Bridge 1985; Crow and
Therefore the 5-HT-dependent increase in the spontaneous spike activity in type I$_i$ interneurons may be due to a 5-HT-induced increase in the activity of photoreceptors. In addition, 5-HT may also contribute to increased spike activity of the type I$_i$ interneurons by enhanced excitability and membrane potential shifts. This is supported by the observation that 5-HT significantly increases intrinsic excitability. It has been reported that 5-HT$_2$ receptor activation facilitates a persistent Na$^+$ current in spinal motoneurons of rats (Harvey et al. 2006). It is also likely that closure of K$^+$ channels may also contribute to the increase in excitability because 5-HT depolarizes the membrane potential in the type I$_i$ interneurons. Serotonin can also attenuate Ca$^{2+}$-activated K$^+$ currents (Yamoah and Crow 1995) and delayed rectifier K$^+$ currents as well as A type K$^+$ currents in *Hermissenda* photoreceptors (Acosta-Urquidi and Crow 1993).

The effect of 5-HT on the reduction of the hyperpolarizing after-potential is more pronounced in the type I$_e(A)$ interneurons as compared to the type I$_i$ interneurons, suggesting that the mechanism by which 5-HT attenuates accommodation may be different.

**The effects of GABA on type I$_e$ and I$_i$ interneurons**

As shown in Fig. 8 and 10, here we show that GABA produced a hyperpolarization of membrane potential in both type I$_e$ and I$_i$ interneurons. Previous work in *Hermissenda* photoreceptors reported that Baclofen induces an increase in amplitude of the nonvoltage and voltage-dependent conductances, which contribute to the slow hyperpolarization that can be blocked by TEA (Matzel et al. 1995). These conductances may also be activated by GABA and contribute to the GABA-induced hyperpolarizations of both the type I$_e$ and I$_i$ interneurons. The application of GABA
reduced the amplitude of the light-evoked complex EPSPs in type I_e interneurons and the light-evoked complex IPSPs in type I_i interneurons. Presynaptic GABA effects from photoreceptors may reduce the spontaneous and light-evoked spike activity while decreased intrinsic excitability of type I_e and type I_i interneurons is most likely postsynaptic. The inhibitory effect of GABA on the type I_e and I_i interneurons may be due to the activation of GABA receptors which results in a hyperpolarization and decreased spike activity of type B photoreceptors (Alkon et al. 1993; Matzel et al. 1995; Rogers et al. 1994) and decreased activity in the type I_e and I_i interneurons. Importantly, some effects of GABA on the type I interneurons may be different from those in B photoreceptors of *Hermisenda*. For example, GABA paired with intracellular depolarizations can induce enhanced excitability of type B photoreceptors (Matzel and Alkon 1991), a cellular change associated with Pavlovian conditioning (Crow and Alkon 1980; Farley 1987a; b; Farley and Alkon 1982; West et al. 1982). However, in the type I_e interneurons, the complex EPSP evoked by illumination in the presence of GABA induces not an increase, but a decrease in intrinsic excitability. Further study may be needed to unveil the mechanism of the discrepancy.
Fig. 1. 5-HT enhances the amplitude of light-evoked complex EPSPs and increases spontaneous and light-evoked spike activity in type I_e(A) interneurons. A representative recording from a dark-adapted type I_e(A) interneuron before (A) and after 5-HT application (B). Inset shows an example of light-evoked complex EPSPs before and after 5-HT application in a type I_e(A) interneuron that did not exhibit spontaneous spike activity. The bar above the recordings indicates the presentation of the 10 sec light. Group summary data of 5-HT-induced increase in the amplitude of light-evoked complex EPSPs (Mean depolarization), spontaneous and light-evoked spike activity are shown in C and D, respectively. * p < 0.05, **p < 0.01.

Fig. 2. Excitatory effect of 5-HT on intrinsic excitability and membrane potential (MP) in type I_e(A) interneurons. Representative examples of spike activity evoked by 0.1, 0.2 or 0.3nA current injection before (Ctrl.) (A) and after 5-HT (B). The group summary data for mean spike activity and MP are shown in C and D. *p < 0.01. The 5-HT-dependent increase in excitability and membrane depolarization suggests a direct excitatory effect of 5-HT on type I_e(A) interneurons.

Fig. 3. 5-HT decreases the amplitude of light-evoked complex EPSPs and spike activity in type I_e(B) interneurons. A representative recording from a dark-adapted type I_e(B) interneuron before (A) and after 5-HT application (B). The bar above the recordings indicates the presentation of the 10 sec light. The group summary data of 5-HT effects on light-evoked complex EPSPs, spontaneous and light-evoked spike activity are shown in C and D. * p < 0.05.
Fig. 4. 5-HT decreases intrinsic excitability and hyperpolarizes the MP in type Ie(B) interneurons. A representative example of spike activity evoked by 0.1, 0.2 or 0.3nA current injections before (A) and after 5-HT (B). The group summary data for 5-HT effects on spike activity and MP are shown in C and D. *p < 0.05, **p < 0.01. The 5-HT-induced decrease in excitability and membrane hyperpolarization suggest a direct inhibitory effect on type Ie(B) interneurons.

Fig. 5. Differential effects of 5-HT on whole-cell outward currents in identified type Ie interneurons. Inset: Type Ie interneurons were identified by detecting an increase in the amplitude of whole-cell inward currents and an enhancement in spike activity at the holding potential of -60 mV in response to illumination of the eyes. Representative current traces for control (Ctrl.) and after 5-HT application are shown in the top and middle panels in A and B. The outward currents began to active at -50 mV from a holding potential of -80 mV. Averaged instantaneous (is) and steady-state (ss) current density-voltage relationships of the net outward currents at the holding potential of -80 mV are shown in the bottom panels. The initial transient outward current was inhibited by the bath application of 5-HT in all type Ie interneurons examined. However, 5-HT produced a decrease in the steady-state outward current in 50% of type Ie interneurons tested (A) and an increase in the steady-state net outward current in the remaining type Ie interneurons (B), consistent with the type Ie(A) and Ie(B) classification obtained from the intracellular recordings (see Fig. 1-4). * p < 0.01 for all paired comparisons.

Fig. 6. 5-HT decreases the amplitude of light-evoked complex IPSPs, and increases spontaneous spike activity of type Ii interneurons. A representative type Ii recording from a dark-adapted type Ii interneuron (A) and after 5-HT application (B). The bar...
above the recordings indicates the presentation of the 10 sec light. The group summary
data of 5-HT-dependent effects on light-evoked complex IPSPs, and spontaneous spike
activity are shown in C and D. \( *p < 0.01 \).

Fig. 7. The effect of 5-HT on intrinsic excitability and MP in the type I\(_i\) interneurons.
Representative examples of spike activity evoked by 0.1, 0.2 or 0.3nA current injection
before (Ctrl.) (A) and after 5-HT application (B). The group summary data of 5-HT
effects on spike activity during the first 1 sec depolarizing current injection are shown in
C. The group data for 5-HT effects on MP are shown in D. \( *p < 0.05 \) and \( **p < 0.01 \).

Fig. 8. The inhibitory effect of GABA on light-evoked complex EPSPs and spike activity
in type I\(_e\) interneurons.  Representative examples of type I\(_e\) light-evoked complex EPSPs
after dark adaptation before (A) and after GABA application (B). The bar above the
recordings indicates the presentation of the 10 sec light. The group summary data of
GABA-induced effects on light-evoked complex EPSPs, spontaneous and light-evoked
spike activity are shown in C and D. \( * p < 0.05 \) and \( **p < 0.01 \). The inhibition of the
light-evoked activity of type I\(_e\) interneurons may be due to a GABA-dependent decrease
in excitability of photoreceptors, and/or its direct inhibitory effect on type I\(_e\) interneurons.

Fig. 9. The inhibitory effect of GABA on intrinsic excitability and MP of type I\(_e\)
terneurons.  Representative examples of spike activity evoked by 0.1, 0.2 or 0.3nA
current injections before (A) and after 5-HT application (B). The group summary data on
the effects of GABA on spike activity and MP are shown in C and D. \( *p < 0.01 \). The
GABA-induced decrease in excitability and hyperpolarization of the MP suggest a direct
inhibitory effect of GABA on type I\(_e\) interneurons.
Fig. 10. GABA decreases the amplitude of light-evoked complex IPSPs and spontaneous spike activity in the type I interneurons. Representative examples of light-evoked complex IPSPs in type I recordings after dark adaptation before (A) and after GABA application (B). The bar above the recordings indicates the presentation of the 10 sec light. The group summary data of GABA-induced effects on light-evoked complex IPSPs and spontaneous spike activity are shown in C and D. * $p < 0.05$, **$p < 0.01$. The inhibition of the light-evoked activity in the type I interneurons may be due to a GABA-dependent decrease in excitability of photoreceptors, and/or its direct inhibitory effect on type I interneurons.

Fig. 11. Inhibitory effect of GABA on intrinsic excitability and membrane hyperpolarization in type I interneurons. Representative examples of spike activity evoked by 0.1, 0.2 or 0.3nA current injections before (A) and after 5-HT application (B). The group summary data for spike activity and membrane hyperpolarization are shown in C and D. *$p < 0.05$. The GABA-induced decrease in excitability and membrane hyperpolarization suggest a direct inhibitory effect on type I interneurons.
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Fig. 1
Fig. 2
Fig. 3
**Fig. 4**

**A**

Ctrl.

I_{ Cs(0) }

**B**

5-HT

I_{ Cs(0) }

[Graph showing the effect of 5-HT on ionic currents with currents at 0.1nA, 0.2nA, and 0.3nA]

**C**

[Bar graph showing mean spike rates for Ctrl. and 5-HT at three different currents]

**D**

[Graph showing mean RMP for Ctrl. and 5-HT]
Fig. 5
Fig. 6
Fig. 7