5-HT and GABA Modulate Intrinsic Excitability of Type I Interneurons in \textit{Hermissenda}

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Jin NG, Tian L-M, Crow T. 5-HT and GABA modulate intrinsic excitability of type I interneurons in \textit{Hermissenda}. \textit{J Neurophysiol} 102: 2825–2833, 2009. First published August 26, 2009; doi:10.1152/jn.00477.2009. The sensory neurons (photoreceptors) in the visual system of \textit{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 \textgamma-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 excitatory postsynaptic potentials (EPSPs), enhanced intrinsic excitability, and increased spike activity of identified type Ie(A) interneurons. In contrast, 5-HT decreased spike activity and intrinsic excitability of type Ie(B) interneurons. The classification of two categories of type Ie interneurons was also supported by the observation that 5-HT produced opposite effects on whole cell steady-state outward currents in type Ie interneurons. Serotonin produced a reduction in the amplitude of light-evoked complex inhibitory PSPs (IPSPs), increased spontaneous spike activity, decreased intrinsic excitability, and depolarized the resting membrane potential of identified type I interneurons. In contrast to the effects of 5-HT, GABA produced inhibition in both types of Ie interneurons and type Ii 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.

INTRODUCTION

Both \textgamma-aminobutyric acid (GABA) and serotonin (5-HT) are putative neurotransmitters in the graviceptive sensory system of \textit{Hermissenda} (for reviews, see 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 inhibitory postsynaptic potential (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 regard 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 (Frystyk 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 Ii interneurons and monosynaptic inhibitory connections with type Ie 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 Alkon 1980; Crow and Tian 2003). Because 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 Ii and type Ie 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 \textit{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-h 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, and 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 2000, 2002a,b). 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-s 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 prelight baseline membrane potential. This measure of complex PSP amplitude is consistent with previous reports when spike activity was blocked by hyperpolarizing type I interneurons below threshold for spike generation during the light step (Crow and Tian 2000, 2002a). Spike activity elicited during the 10-s light step was compared with an equivalent period of spontaneous activity before light. Intrinsic excitability was assessed in the dark by presenting 1.8-s depolarizing current pulses (0.1, 0.2, and 0.3 nA) from a holding potential of −60 mV 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-s pause in extrinsic current injection. The experimental protocol was repeated three times at 6-min intervals before and after the bath application of 5-HT or GABA delivered to the recording chamber.

### Whole cell voltage-clamp recordings

Type I 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). Borosilicate glass pipettes (1.5 mm OD; 1.17 mm ID) were pulled on a horizontal Flaming-Brown microelectrode puller (Model P80/PC, Sutter Instrument, San Rafael, CA) and fire-polished with a microforge (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 low-pass 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): 450 choline-chloride, 10 KCl, 50 MgCl₂, 0.5 CaCl₂, and 15 HEPES, the pH value was adjusted with Tris to 7.46 at 20°C. The osmolarity of the bath solution was adjusted to 961–1005 mosM. The composition of the pipette solution used for whole cell recordings was as follows (in mM): 430 KCl, 20 NaCl, 2 MgCl₂, 2 EGTA, 50 HEPES, 10 glutathione (reduced), 5 Mg-ATP, and 1 Na₂GTP, the pH value is adjusted with KOH to 7.30 at 15°C. 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 I interneurons and monosynaptic IPSPs in type I 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 Ie(A) interneurons

Representative recordings from a type Ie(A) interneuron after 12 min of dark adaptation and after 5-HT application are shown in Fig. 1. The 10-s period of illumination produced a complex EPSP and an increase in spike activity in the type Ie(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. Figure 1, inset, 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. 1, C and D, revealed that 5-HT significantly increased spontaneous (t₅ = 2.9; P < 0.05) and the light-evoked spike activity (t₅ = 2.8; P < 0.05) and increased the amplitude of the light-evoked complex EPSP (t₅ = 4.1; P < 0.01). 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 Ie(A) interneurons. This issue was examined by assessing intrinsic excitability of Ie(A) interneurons before and after the bath application of 5-HT. Spike activity evoked by 0.1-, 0.2-, or 0.3- nA 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 Ie(A) interneurons at all current levels that were applied (0.1 nA, t₅ = 7.1; P < 0.01; 0.2 nA, t₅ = 5.7; P < 0.01; 0.3 nA, t₅ = 4.1; P < 0.01). In addition, 5-HT produced a statistically significant depolarization of the membrane potential of type Ie(A) interneurons (t₅ = 7.1; P < 0.01; Fig. 2D).
Spike activity evoked by 0.1-, 0.2-, or 0.3-nA current injection was decreased in the presence of 5-HT (n = 7) (Fig. 4, A and B). The analysis of the group data (Fig. 4, C and D) showed that 5-HT significantly decreased intrinsic excitability of type Ie(B) interneurons as measured by current-evoked spike activity (0.1 nA, t<sub>6</sub> = 6.2; P < 0.01; 0.2 nA, t<sub>6</sub> = 5.7; P < 0.01; 0.3 nA, t<sub>6</sub> = 6.7; P < 0.01). In addition, 5-HT application resulted in a significant hyperpolarization of the membrane potential of type Ie(B) interneurons (t<sub>6</sub> = 3.3; P < 0.05; Fig. 4D).

**Dual effect of 5-HT on whole cell currents in type I<sub>e</sub> interneurons**

The results obtained from intracellular recordings of type I<sub>e</sub> interneurons showed that the bath application of 5-HT produced different effects on excitability, suggesting that there may be two types of I<sub>e</sub> interneurons that we have classified as I<sub>e(A)</sub> and I<sub>e(B)</sub>. To further investigate this classification, whole cell currents were examined in a sample of type I<sub>e</sub> interneurons (n = 8). To exclude the contamination of Na<sup>+</sup> and minimize Ca<sup>2+</sup> effects, Na-free and low-Ca<sup>2+</sup> (0.5 mM) bath solutions were prepared by ionic substitution of Na<sup>+</sup> and Ca<sup>2+</sup> 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<sub>e</sub> interneurons. Figure 5 illustrates a family of outward current traces evoked by voltage-clamp steps from −80 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 I<sub>e</sub> interneurons examined (n = 8). Overall significant decreases were found in the instantaneous current after 5-HT application.
The steady-state net outward current was significantly decreased in putative type Ie(A) interneurons ($t_5 = 4.7; P < 0.01$) and increased the spontaneous spike activity in type I interneurons ($t_5 = 4.1; P < 0.01$). It is likely that the excitatory effect of 5-HT on type I interneurons is not due to 5-HT-dependent excitatory effects on photoreceptors as described previously (Crow and Bridge 1985; Crow and Forrest 1991; Farley and Wu 1989). We examined this by assessing intrinsic excitability of Ii interneurons before and after the bath application of 5-HT. Because pronounced spike frequency accommodation occurred during the current step, we examined current elicited spike activity during the initial 1-s depolarization before and after 5-HT application. Spike activity evoked by 0.1-, 0.2-, or 0.3-nA current injection was increased in the presence of 5-HT (see Fig. 7). The analysis of the group data (Fig. 7, C and D) indicated that 5-HT significantly increased intrinsic excitability of type Ii interneurons at all levels of applied current (0.1 nA, $t_5 = 2.4; P < 0.05$; 0.2 nA, $t_5 = 3.6; P < 0.01$; 0.3 nA, $t_5 = 3.2; P < 0.025$). In addition, the membrane potential of type Ii interneurons was significantly depolarized by the 5-HT application ($t_5 = 5.0; P < 0.01$).

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

In all type Ie interneurons that were examined ($n = 7$), GABA produced an inhibitory effect. A representative Ie recording before and after GABA application is shown in Fig. 8. Consistent with other Ie 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. 8, C and D, revealed that GABA significantly decreased the amplitude of the light-evoked complex IPSPs, spike activity, intrinsic excitability, and membrane potential in type Ie interneurons.

**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. 6, A and B). The analysis of the group summary data shown in Fig. 6, C and D, revealed that 5-HT significantly decreased the amplitude of light-evoked complex IPSPs ($t_5 = 4.7; P < 0.01$) and increased the spontaneous spike activity in type Ii interneurons ($t_5 = 4.1; P < 0.01$). It is likely that the excitatory effect of 5-HT on type Ii interneurons is not due to 5-HT-dependent excitatory effects on photoreceptors as described previously (Crow and Bridge 1985; Crow and Forrest 1991; Farley and Wu 1989). We examined this by assessing intrinsic excitability of Ii interneurons before and after the bath application of 5-HT. Because pronounced spike frequency accommodation occurred during the current step, we examined current elicited spike activity during the initial 1-s depolarization before and after 5-HT application. Spike activity evoked by 0.1-, 0.2-, or 0.3-nA current injection was increased in the presence of 5-HT (see Fig. 7). The analysis of the group data (Fig. 7, C and D) indicated that 5-HT significantly increased intrinsic excitability of type Ii interneurons at all levels of applied current (0.1 nA, $t_5 = 2.4; P < 0.05$; 0.2 nA, $t_5 = 3.6; P < 0.01$; 0.3 nA, $t_5 = 3.2; P < 0.025$). In addition, the membrane potential of type Ii interneurons was significantly depolarized by the 5-HT application ($t_5 = 5.0; P < 0.01$).

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

In all type Ie interneurons that were examined ($n = 7$), GABA produced an inhibitory effect. A representative Ie recording before and after GABA application is shown in Fig. 8. Consistent with other Ie 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. 8, C and D, revealed that GABA significantly decreased the amplitude of the light-evoked complex IPSPs, spike activity, intrinsic excitability, and membrane potential in type Ie interneurons.
complex EPSPs ($t_{6}/H11005 2.9; P/H11021 0.05$), spontaneous ($t_{6}/H11005 2.9; P/H11021 0.05$), and light-evoked spike activity ($t_{6}/H11005 5.6; P/H11021 0.01$). 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 Ie interneurons. This was examined by assessing intrinsic excitability of Ie interneurons before and after the bath application of GABA. Spike activity evoked by 0.1-, 0.2-, or 0.3-nA current injection was decreased in the presence of GABA (see Fig. 9). The analysis of the group data (Fig. 9, C and D) indicated that GABA significantly decreased the amplitude of light-evoked complex IPSPs ($t_{7} = 4.4; P < 0.01$) and decreased spontaneous spike activity in type Ie interneurons ($t_{7} = 3.3; P < 0.05$). 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 Ie interneurons. This was examined by assessing intrinsic excitability of Ie interneurons before and after the bath application of GABA.

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

A representative recording from a type Ii interneuron before (control) and after GABA application is shown in Fig. 10, A and B. 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. 10, C and D, revealed that GABA significantly decreased the amplitude of light-evoked complex IPSPs ($t_{7} = 4.4; P < 0.01$) and decreased spontaneous spike activity in type Ii interneurons ($t_{7} = 3.3; P < 0.05$). 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 Ii interneurons. This was examined by assessing intrinsic excitability of Ii interneurons before and after the bath application of GABA.
type II interneurons. GABA produced a significant hyperpolarization of intrinsic excitability of type II interneurons as measured by designations as $I_{e(A)}$ and $I_{e(B)}$, respectively. Additional support suggests that there are two types of $I_{e}$ interneurons that we have measured of excitability as a result of 5-HT application. This current evoked spike activity (0.1 nA, $I_{e}$ interneurons). Serotonin produces two effects on excitability of type II neurons.

**DISCUSSION**

**Serotonin produces two effects on excitability of type $I_{e}$ interneurons**

In ~50% of the type $I_{e}$ 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 $I_{e}$ 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 $I_{e}$ interneurons that we have designated as $I_{e(A)}$ and $I_{e(B)}$, respectively. Additional support for the classification was obtained from whole cell voltage-clamp experiments. The sustained outward currents were decreased by 5-HT in four of eight type $I_{e}$ interneurons examined under whole cell voltage clamp. The remaining type $I_{e}$ interneurons exhibited an increase in sustained outward currents in the presence of 5-HT. In these experiments, most of the delayed rectifier K$^+$ current remained at steady-state conditions because A-type K$^+$ currents are typically inactivated after a 4-s voltage command step. It is well-documented that voltage-gated-delayed rectifier K$^+$ 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$^+$ current may contribute to the differential effect of 5-HT on type $I_{e}$ 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 (Brahut et al. 1990; Hochner and Kandel 1992; Sugita et al. 1992) and on the state of the synapse (Brahut 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 (Brahut 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 $I_{e(A)}$ and $I_{e(B)}$ interneurons.

Previous work has shown that photoreceptors form monosynaptic connections with type $I_{e}$ interneurons (Akaike and
Pavlovian conditioning in (Ehrlich et al. 1992; Sahley 1994) and may contribute to the enhancement of excitability in type Ie(A) interneurons. The increase in spontaneous spike activity in type Ie(A) interneurons may be due to a GABA-dependent decrease in excitability of photoreceptors and/or its direct inhibitory effect on type Ie(A) interneurons.

It is likely that several cellular processes, both pre- and postsynaptic, contribute to the enhancement of activity in type Ie(A) interneurons. The increase in spontaneous spike activity, light-evoked spikes, and light-evoked complex EPSPs may have both pre- 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. 1996, 2000; 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).

Effect of 5-HT on type Ie interneurons

In contrast to the differential effect of 5-HT on type Ie(A) and Ie(B) interneurons, 5-HT increased the spontaneous spike activity in type Ie 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 Siddiqi 1997; Grover et al. 1989). Therefore the 5-HT-dependent increase in the spontaneous spike activity in type Ie 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 Ie 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-HT2 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 Ie interneurons. Serotonin can also attenuate Ca2+-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 afterpotential is more pronounced in the type Ie(A) interneurons as compared with the type Ie interneurons, suggesting that the mechanism by which 5-HT attenuates accommodation may be different.

Effects of GABA on type Ie and Ii interneurons

As shown in Fig. 8 and 10, GABA produced a hyperpolarization of membrane potential in both type Ie and Ii interneurons. Previous work in Hermissenda photoreceptors reported that baclofen induced an increase in the amplitude of 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 hyperpolarization.

![FIG. 10. GABA decreases the amplitude of light-evoked complex IPSPs and spontaneous spike activity in the type Ie interneurons. Representative examples of light-evoked complex IPSPs in type Ie recordings after dark adaptation before (A) and after GABA application (B). The bar above the recordings indicates the presentation of the 10-s 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 Ie interneurons may be due to a GABA-dependent decrease in excitability of photoreceptors and/or its direct inhibitory effect on type Ie interneurons.](http://jn.physiology.org/)

![FIG. 11. Inhibitory effect of GABA on intrinsic excitability and membrane hyperpolarization in type Ie interneurons. Representative examples of spike activity evoked by 0.1-, 0.2-, or 0.3-nA 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 Ie interneurons.](http://jn.physiology.org/)
tions of both the type Ie and Ii interneurons. The application of GABA reduced the amplitude of the light-evoked complex EPSPs in type Ie interneurons and the light-evoked complex IPSPs in type Ii interneurons. Presynaptic GABA effects from photoreceptors may reduce the spontaneous and light-evoked spike activity while decreased intrinsic excitability of type Ie and type Ii interneurons is most likely postsynaptic. The inhibitory effect of GABA on the type Ie and Ii interneurons may be due to the activation of GABA receptors that 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 Ii neurons. Importantly, some effects of GABA on the type Ii interneurons may be different from those in B photoreceptors of *Hermissenda*. For example, GABA paired with intracellular depolarization 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 Ie 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.

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