Serotonin regulates voltage-dependent currents in type $I_{e(A)}$ and $I_i$ interneurons of *Hermissenda*

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Submitted 16 June 2011; accepted in final form 27 July 2011

Jin NG, Crow T. Serotonin regulates voltage-dependent currents in type $I_{e(A)}$ and $I_i$ interneurons of *Hermissenda*. J Neurophysiol 106: 2557–2569, 2011. First published August 3, 2011; doi:10.1152/jn.00550.2011.—Serotonin (5-HT) has both direct and modulatory actions on central neurons contributing to behavioral arousal and cellular-synaptic plasticity in diverse species. In *Hermissenda*, 5-HT produces changes in intrinsic excitability of different types of identified interneurons in the circumesophageal nervous system. Using whole cell patch-clamp techniques we have examined membrane conductance changes produced by 5-HT that contribute to intrinsic excitability in two identified classes of interneurons, types $I_i$ and $I_{e(A)}$. Whole cell currents were examined before and after 5-HT application to the isolated nervous system. A 4-aminopyridine-sensitive transient outward $K^+$ current [$I_{K(V)}$], a tetraethylammonium-sensitive delayed rectifier $K^+$ current [$I_{K(A)}$], an inward rectifier $K^+$ current [$I_{K(IR)}$] and a hyperpolarization-activated current ($I_h$) were characterized. 5-HT decreased the amplitude of $I_{K(A)}$ and $I_{K(V)}$ in both type $I_i$ and $I_{e(A)}$ interneurons. However, differences in 5-HT’s effects on the activation-inactivation kinetics were observed in different types of interneurons. 5-HT produced a depolarizing shift in the activation curve of $I_{K(V)}$ and a hyperpolarizing shift in the inactivation curve of $I_{K(A)}$ in type $I_i$ interneurons. In contrast, 5-HT produced a depolarizing shift in the activation curve and a hyperpolarizing shift in the inactivation curve of both $I_{K(V)}$ and $I_{K(A)}$ in type $I_{e(A)}$ interneurons. In addition, 5-HT decreased the amplitude of $I_{K(IR)}$ in type $I_i$ interneurons and increased the amplitude of $I_h$ in type $I_{e(A)}$ interneurons. These results indicate that 5-HT-dependent changes in $I_{K(A)}, I_{K(V)}, I_{K(IR)}$, and $I_h$ contribute to multiple mechanisms that synergistically support modulation of increased intrinsic excitability associated with different functional classes of identified type 1 interneurons.

K$^+$ channels; intrinsic excitability

Serotonin (5-HT) is a well-conserved neurotransmitter that contributes to neural plasticity and the generation of complex behaviors through modulation of cellular excitability by both excitatory and inhibitory actions on neurons in the central and peripheral nervous systems of diverse species. The ionic basis for 5-HT-dependent excitability changes is complex, involving a number of potassium, sodium, and chloride conductances (for review see Bobker and Williams 1990). Central pattern generator-mediated motor activity related to feeding and locomotion in mammals has been shown to be modulated by the activity of brain stem serotonergic neurons (Jacobs and Fornal 1999; Veasey et al. 1995). In a number of invertebrate systems serotonergic neurons contribute to neural circuits that are involved in feeding (Jing and Gillette 1995; Jing et al. 2008; Yeoman et al. 1996), behavioral arousal (Jing et al. 2009; Jing and Gillette 2000; Katz et al. 2001), and various forms of locomotion and swimming (Arshavsky et al. 1985; Jing and Gillette 1995, 1999; Mackey and Carew 1983; McPherson and Blanchenship 1991; Newcomb and Katz 2009; Norekian and Satterlie 1996; Panchin et al. 1995; Parsons and Pinsker 1989; Satterlie and Norekian 1995). Consistent with its proposed role in arousal, 5-HT has also been shown to contribute to several examples of cellular plasticity and behavioral modifications in mammals (Bocchiaro and Feldman 2004; Mattson et al. 2004) and invertebrates (for reviews see Byrne and Kandel 1996; Sahley and Crow 1998).

In *Hermissenda*, 5-HT produces synaptic facilitation of type B to type A photoreceptor monosynaptic inhibitory postsynaptic potentials (IPSPs) (Frysztak and Crow 1997; Schuman and Clark 1994) and enhanced intrinsic excitability of identified sensory neurons (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). 5-HT is also effective as a nominal unconditioned stimulus (US) in a one-trial Pavlovian conditioning paradigm when applied to the exposed but otherwise intact nervous system and to the in vitro isolated nervous system (Crow and Forrester 1986; Crow and Siddiqui 1997; Crow et al. 1996, 1997). Previously published work has focused on an analysis of 5-HT effects on sensory neurons supporting behavioral plasticity (for review see Crow 2004). However, a second site of cellular and synaptic plasticity that has been investigated in *Hermissenda* is the two physiologically identified classes of type I interneurons in the cerebropleural ganglion (Crow and Tian 2002a). Consistent with the diversity of membrane conductances potentially contributing to 5-HT-dependent plasticity, a recent study using current-clamp techniques reported that 5-HT increased the peak amplitude of light-evoked complex excitatory postsynaptic potentials (EPSPs), enhanced intrinsic excitability, and increased spike activity of type $I_{e(A)}$ interneurons. In contrast, 5-HT reduced the amplitude of light-evoked EPSPs, increased intrinsic excitability, and depolarized the resting membrane potential of type $I_i$ interneurons (Jin et al. 2009). Serotonergic modulation of specific membrane conductances underlying excitability changes in the two classes of identified type I interneurons has not been examined.

In this study we examined specific membrane conductances modulated by 5-HT in identified type $I_i$ and $I_{e(A)}$ interneurons using whole cell voltage-clamp techniques. We found that 5-HT decreased the amplitude of 4-aminopyridine (4-AP)-sensitive transient outward $K^+$ current [$I_{K(A)}$] and tetraethylammonium (TEA)-sensitive delayed rectifier $K^+$ current [$I_{K(V)}$] in both identified type $I_i$ and $I_{e(A)}$ interneurons and decreased the amplitude of inward rectifier $K^+$ current [$I_{K(IR)}$] in type $I_i$ interneurons but increased the amplitude of hyperpolarization-
activated current $I_h$ in type IeA interneurons. In type I interneurons 5-HT produced a depolarizing shift in the activation curve of $I_{K(V)}$ and a hyperpolarizing shift in the inactivation curve of $I_{K(A)}$. In contrast, 5-HT produced a depolarizing shift in the activation curve and a hyperpolarizing shift in the inactivation curve of both $I_{K(V)}$ and $I_{K(A)}$ in type IeA interneurons. The results indicate that a multitude of mechanisms involving different alterations in the activation-inactivation kinetics of diverse K$^+$ conductances underlie decreases in $I_{K(A)}$ and $I_{K(V)}$ contributing to 5-HT-dependent changes in intrinsic excitability that are characteristic of different type I interneurons.

MATERIALS AND 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) aquarium at 14 ± 1°C on a 12:12-h light-dark cycle. Electrophysiological data were collected during the light phase of the light-dark cycle.

Isolation of central nervous system and identification of type I interneurons. 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. The ASW in the recording chamber was monitored by a thermistor, and the temperature of the bath solutions was maintained at 14.5 ± 0.5°C. Type I interneurons were identified by established anatomical and electrophysiological criteria, as described previously (Akaike and Alkon 1980; Crow and Tian 2000, 2002a, 2002b; Jin et al. 2009). Type Ia interneurons were identified by recording light-evoked inward currents that underlie light-evoked complex EPSPs, and type Ie interneurons were identified by recording light-evoked outward currents underlying light-evoked complex IPSPs. The illumination of the eyes was provided by a tungsten halogen incandescent lamp attached to a fiber-optic bundle mounted underneath the recording chamber.

Chemicals and solutions. All chemicals were obtained from Sigma Chemical (St. Louis, MO). Solutions used and their chemical compositions (in mM) were 1) ASW: 460 NaCl, 10 KCl, 10 CaCl$_2$, 5 MgCl$_2$, 10 HEPES (pH adjusted to 7.46 with NaOH; osmolality adjusted to 990–1,010 mosM); 2) external solution for whole cell recordings: 450 mM-N-methyl-D-glucamine (NMDG), 10 KCl, 55 MgCl$_2$, 15 HEPES (pH adjusted with HCl to 7.46 at 20°C; osmolarity of bath solution adjusted to 998–1,007 mosM); 3) pipette solution: 350 mM-NMDG, 150 KCl, 2 MgSO$_4$, 2 ethylene glycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 50 HEPES, 10 glutathione (reduced), 5 Mg-ATP, 1 Na$_2$-GTP (pH adjusted with HCl to 7.30 at 15°C; osmolarity of internal solution adjusted to 970 mosM). All external solutions were continuously perfused into the chamber. Stock solutions of 50 mM 4-AP and 400 mM TEA chloride were made and stored at −20°C. Aliquots of the 4-AP solutions were diluted in the recording solution. The bath TEA solution was made by a replacement of 100 mM NMDG of the bath solution with 100 mM TEA. The final concentration of 5-HT, 4-AP, and TEA in bath solutions was 0.1 mM, 5 mM, and 100 mM, respectively.

Whole cell voltage-clamp recordings. The whole cell currents were recorded at 15 ± 0.5°C with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Borosilicate glass pipettes (1.5-mm OD, 1.17-mm ID; Sutter Instruments) 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, Flaming-Brown microelectrode puller (model P80/PC, Sutter Instruments, San Rafael, CA)). The recording pipettes were filled with a 3 M-sodium acetate solution (pH adjusted to 7.2) containing 0.48 M NaCl, 14°C) and maintained in closed artificial seawater (ASW) aquaria at 14 ± 1°C on a 12:12-h light-dark cycle. Electrophysiological data were collected during the light phase of the light-dark cycle.

Electrical signals were amplified using a Dual Patch clamp Amplifier (model D100B, A-M Systems, Carlsborg, WA) and filtered at 3 kHz. Voltage and current were recorded at 10 kHz on a computer hard drive and analyzed with Clampfit (Axon Instruments), prism (GraphPad software), and Origin (Microcal Software, Northampton, MA) software programs.

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

RESULTS

Isolation of whole cell outward currents in identified type I interneurons. Isolation and characterization of different components of outward currents evoked from a hyperpolarizing potential of −80 mV to various levels of depolarization (see Fig. 1A, inset) were examined with 4-AP, TEA, and Ca$^{2+}$ or combinations of these agents applied to the external solution. Na$^{+}$ and Ca$^{2+}$ were removed by ionic substitution with NMDG. Outward currents evoked from a holding potential of −80 mV showed an early transient and a sustained component in both type Ia and Ie interneurons. A representative example of a family of outward currents elicited by 4-s voltage-clamp steps from −80 mV to +50 mV in 10-mV increments in an identified type Ia interneuron is shown in Fig. 1. The transient component was activated at step voltages more positive than −60 to −50 mV followed by a sustained component at step voltages more positive than −50 to −30 mV. The addition of 5 mM 4-AP to the external solution resulted in the removal of the fast inactivating component of the current and a reduction in the amplitude of the peak whole cell outward current (Fig. 1B). The sustained outward current remaining after the application of 4-AP was substantially reduced by the application of 100 mM TEA to the external solution (Fig. 1C). The residual current after the application of 4-AP and TEA was enhanced as external Ca$^{2+}$ was increased to 5 mM (Fig. 1D). The Ca$^{2+}$-dependent current had a delayed onset and exhibited no inactivation. These results indicate that three voltage-dependent outward currents are present in type I interneurons.

5-HT modulation of tail currents in type II and IeA interneurons. 5-HT has been shown to modulate a number of voltage-dependent conductances in diverse systems (Bobker and Williams 1990; Lotshaw and Levitan 1987b). To rule out the possible contribution of Cl$^-$ to 5-HT-modulated whole cell currents in type II and IeA interneurons, we examined the reversal potentials ($E_{rev}$) of the tail currents before and after the application of 5-HT activated by 3-s depolarizations to +50 mV with test steps to potentials from −130 to +50 mV. The protocols are shown in the insets of Fig. 2A and Fig. 3A. For recording conditions (150 mM internal K$^+$ and 10 mM external K$^+$), tail currents were inward at potentials more negative than −80 mV and outward at potentials more positive than −60 mV in both type II and IeA interneurons. Representative current traces from an identified type II and an IeA interneuron are shown in Fig. 2, A and B, and Fig. 3, A and B. The
The current-voltage relationship revealed that the application of 5-HT decreased the amplitude of outward currents without significant changes in $E_{\text{rev}}$ in both type Ii (Fig. 2C) and type IeA (Fig. 3C) interneurons. The average $E_{\text{rev}}$ was $-71.7 \pm 1.7$ mV ($n = 7$) in type Ii interneurons and $-68.6 \pm 1.7$ mV ($n = 7$) in type IeA interneurons, which is similar to the equilibrium potential for K$^+$ ($-67$ mV) predicted by the Nernst equation.

The K$^+$ dependence of the net whole cell currents was further verified by determining the $E_{\text{rev}}$ of the tail currents at various external K$^+$ concentrations. Raising extracellular K$^+$ from 10 to 30 and 100 mM shifted $E_{\text{rev}}$ from $-67.7 \pm 1.7$ mV ($n = 7$) to $-39.3 \pm 1.3$ mV ($n = 6$) and $-10.3 \pm 1.5$ mV ($n = 5$) in type Ii interneurons (see Fig. 2C, inset) and from $-68.6 \pm 1.7$ mV ($n = 7$) to $-38.6 \pm 1.5$ mV ($n = 6$) and $-10.2 \pm 1.3$ mV ($n = 6$) in type IeA interneurons (see Fig. 3C, inset). The changes in $E_{\text{rev}}$ roughly followed the equilibrium potentials for K$^+$ predicted by the Nernst equation ($-40$ mV at 30 mM external K$^+$ and $-10$ mV at 100 mM external K$^+$). In contrast, alteration of both the bath and the pipette Cl$^-$ was independent of the direction of the shifts in $E_{\text{rev}}$ (data not shown). Therefore the whole cell currents are selectively permeable to K$^+$ before and after 5-HT application without the contamination of a Cl$^-$ conductance.

5-HT decreases $I_{K(V)}$ and $I_{K(A)}$ in type Ii and IeA interneurons. From a holding potential of $-80$ mV, 4-s step depolarizations to potentials between $-80$ and $+50$ mV (see insets of Fig. 4A and Fig. 5A) evoked outward currents with rapidly inactivating and sustained components in both type Ii (Fig. 4A) and IeA (Fig. 5A) interneurons. Because the rapidly inactivating and sustained currents share characteristic sensi-

![Fig. 1. Determination of different components of whole cell outward currents in type I interneurons. A: current traces evoked from an identified type IeA interneuron from a holding potential of $-80$ mV and stepped to $+50$ mV in 10-mV increments. B: application of 5 mM 4-aminopyridine (4-AP) reduced the amplitude of the peak current and abolished the fast activating and rapidly inactivating component of the currents shown in A. C: the remaining current (4-AP-insensitive current) was inhibited by the application of 5 mM 4-AP and 100 mM tetraethylammonium (TEA). D: increasing Ca$^{2+}$ to 5 mM resulted in the expression of a Ca$^{2+}$-dependent outward current.](image1)

![Fig. 2. Serotonin (5-HT) modulation of tail currents in type Ii interneurons. The current traces before (Ctrl; A) and after (B) 5-HT application from an identified type Ii interneuron show that 5-HT reduced both outward and inward components of the tail currents ($I_{\text{tail}}$). The current (I-V) relationship shown in C indicates that the reversal potential ($E_{\text{rev}}$) of the tail currents is unaltered before and after 5-HT application. Inset: data fit to the Goldman-Hodgkin-Katz equation. When the external K$^+$ concentration ($[K]^+$) was varied from 10 to 100 mM and the pipette K$^+$ concentration (150 mM) was maintained, $E_{\text{rev}}$ shifted from $-67.7 \pm 1.7$ mV ($n = 7$) in 10 mM to $-39.3 \pm 1.3$ mV in 30 mM ($n = 5$) and $-10.2 \pm 1.5$ mV in 100 mM ($n = 5$), as shown in C, inset. The results indicate that K$^+$ is the predominant charge carrier of the whole cell outward currents in type Ii interneurons.](image2)
revealed that 5-HT significantly decreased the amplitude of the peak ($F_{1.5} = 69.6, P < 0.0001$) and steady-state ($F_{1.5} = 22.3, P < 0.0001$) current in type I and type Iα interneurons (peak: $F_{1.5} = 72.2, P < 0.0001$; steady-state: $F_{1.5} = 338.2, P < 0.0001$). These results indicate that 5-HT reduces $I_{K(A)}$ and $I_{K(V)}$ in both type I and Iα interneurons. This was examined further by changing the holding potential from −80 mV to −40 mV. Step depolarizations from a holding potential of −40 mV following the protocol shown in the insets of Fig. 4B and Fig. 5B evoked $I_{K(V)}$. The currents lacked the rapidly inactivating component observed from more negative holding potentials after the application of 5-HT (Fig. 4E and Fig. 5E). We found that $I_{K(V)}$ in type I interneurons began to activate at potentials more positive than −30 mV, whereas $I_{K(A)}$ in type Iα interneurons started to activate at potentials more positive than −50 mV. The analysis of the group summary data ($n = 6$) shown in Fig. 4H and Fig. 5H revealed that 5-HT significantly decreased the amplitude of the steady-state currents in both type I ($F_{1.5} = 58.8, P < 0.0001$) and $I_{A}$ ($F_{1.5} = 172.6, P < 0.0001$) interneurons. To isolate the rapidly inactivating portion of the outward current, the sustained currents activated from a holding potential of −40 mV were subtracted from the mixed currents obtained from −80 mV. With this method, an average peak current–voltage relationship before (Fig. 4C and Fig. 5C) and after (Fig. 4F and Fig. 5F) 5-HT application was constructed. The analysis of the group summary data ($n = 6$) shown in Fig. 4I and Fig. 5I revealed that 5-HT significantly decreased the amplitude of the peak $I_{K(A)}$ current in type I ($F_{1.5} = 53.0, P < 0.0001$) and $I_{A}$ ($F_{1.5} = 39.66; P < 0.0001$) interneurons. These results indicate that 5-HT reduces both $I_{K(A)}$ and $I_{K(V)}$ in type I and Iα interneurons.

Effect of 5-HT on voltage-dependent activation and inactivation of $I_{K(A)}$ in type I and Iα interneurons. Differences currents were used to measure the activation kinetics of $I_{K(A)}$. Since it has been shown that $I_{K(A)}$ in some systems is sensitive to TEA (Kros and Crawford 1990), inactivation of $I_{K(A)}$ was assessed in the absence of TEA to prevent the possible complication of altered kinetics of the current by pharmacological blockers. The steady-state inactivation was investigated by the application of a 4-s conditioning pulse protocol to potentials between −80 and 0 mV followed by a test pulse step to 0 mV. The protocols are shown in the insets of Fig. 6. The steady-state current elicited at the test pulse was subtracted from the total current to generate the inactivation curve as previously described (Yamoh 1997). Representative current traces from an identified type I interneuron before and after 5-HT application are shown in Fig. 6, A and B, respectively. The analysis of the group summary data ($n = 6$) revealed that in type I interneurons 5-HT shifted the inactivation curve of $I_{K(A)}$ to a more hyperpolarized potential compared with the control conditions (Fig. 6C). The half-inactivation voltage ($V_{1/2}$) determined from a Boltzmann function fitted to the data was $−57.0 ± 0.5$ mV before and $−70.3 ± 1.3$ mV after 5-HT application. Therefore the application of 5-HT shifted $V_{1/2}$ in the negative direction by −13 mV in type I interneurons. However, the slope of the inactivation curve was not significantly affected by 5-HT. The maximum slope of inactivation ($K_{a}$) was $−5.8 ± 0.4$ mV for the control and $−6.4 ± 0.8$ mV for 5-HT (Fig. 6C). The activation curve of $I_{K(A)}$ fitted with a Boltzmann function was not altered by 5-HT application, with a half-activation voltage ($V_{a}$) $= 16.8 ± 1.3$ mV and a maximum slope of

![Diagram](http://jn.physiology.org/)

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Fig. 3. 5-HT modulation of tail currents in type Iα interneurons. The current traces before (A) and after (B) 5-HT application from an identified type Iα interneuron show that 5-HT reduced the outward component of tail currents but enhanced the inward component of the tail currents. The current-voltage relationship shown in C indicates that the $E_{rev}$ of the tail currents was unaltered by the application of 5-HT. Inset: fit of the data to the Goldman-Hodgkin-Katz equation. When the external $K^{+}$ concentration was varied at 10, 30, and 100 mM and the internal $K^{+}$ concentration was maintained at 150 mM, $E_{rev}$ shifted from $−68.6 ± 1.7$ mV ($n = 7$) in 10 mM to $−38.6 ± 1.5$ mV in 30 mM ($n = 6$) and $−10.2 ± 1.3$ mV in 100 mM ($n = 6$) as shown in C, inset. The results indicate that $K^{+}$ is the predominant charge carrier of the whole cell currents in type Iα interneurons.

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Fig. 4. 5-HT decreases $I_{K(V)}$ and $I_{K(A)}$ in type Ie interneurons. Representative current traces recorded from an identified type Ie interneuron before (A and B) and after (D and E) 5-HT application are shown. Insets: stimulus protocols. Application of 5-HT produced a reduction in the rapidly inactivating and sustained components of whole cell outward $K^+$ currents evoked by voltage steps from $-80$ to $+50$ mV from a holding potential ($V_{\text{holding}}$) of $-80$ mV (A and D). From a $V_{\text{holding}}$ of $-40$ mV, step depolarizations evoked a sustained current [$I_{K(A)}$] (B) that lacked the rapidly inactivating component [$I_{K(A)}$] observed from more negative $V_{\text{holding}}$. Application of 5-HT reduced $I_{K(V)}$ (E). Difference currents (C and F) obtained by subtracting the currents in B and E from those in A and D showed that the fast activating and rapidly inactivating current [$I_{K(A)}$] was reduced by the application of 5-HT. Group summary data are shown in G–I ($n = 6$).

Error bars indicate SE; where error bars are not visible, they are smaller than the symbols. pk, Peak; ss, steady state. *$P < 0.05$, **$P < 0.01$.

Effect of 5-HT on voltage-dependent activation and inactivation of $I_{K(V)}$ in type Ie and Ia interneurons. We next examined the voltage dependence of inactivation of $I_{K(V)}$ with the protocol shown in the inset of Fig. 8A. Membrane potential was held at conditioning potentials ranging from $-80$ to $+50$ mV in 10-mV increments for 4 s, followed by a test step to $+50$ mV. Representative current traces before and after 5-HT application from an identified Ie interneuron are shown in Fig. 8, A and B, respectively. The analysis of the group summary data ($n = 6$) is shown in Fig. 8C. $V_h$ before and after 5-HT, determined from a Boltzmann function fitted to the data, were $-19.0 \pm 3.4$ mV and $-23.4 \pm 4.4$ mV (Fig. 8C). $K_h$ was $-13.3 \pm 3.2$ mV for the control and $-11.9 \pm 4.1$ mV for 5-HT (Fig. 8C). The half-activation of the Boltzmann function fitted to the data was affected by the application of 5-HT ($V_a = 28.6 \pm 3.0$ mV) compared with the control ($V_a = 13.0 \pm 5.0$ mV) (Fig. 8C). However, the slope was not substantially altered by 5-HT application. $K_a$ was $25.4 \pm 1.5$ mV for the control and $28.7 \pm 3.9$ mV for the 5-HT condition (Fig. 8C). These results indicate that a 5-HT-dependent decrease in the amplitude of $I_{K(V)}$ in type Ie interneurons is due to a shift in the activation curve to a more depolarized potential.
The steady-state inactivation of $I_{K(V)}$ in type I$_{CA}$ interneurons was investigated with the protocol shown in the inset of Fig. 9A. Representative current traces before and after 5-HT application from an identified type I$_{CA}$ interneuron are shown in Fig. 9, A and B, respectively. The analysis of the group summary data ($n = 6$) shown in Fig. 9C showed that the 5-HT application produced a shift in the inactivation curve of $I_{K(V)}$. $V_h$ before and after 5-HT application, determined from a Boltzmann function fitted to the data, were $-11.2 \pm 2.9$ mV and $-24.0 \pm 2.4$ mV (Fig. 9C). Therefore the inactivation curve of $I_{K(V)}$ in type I$_{CA}$ interneurons was shifted $\sim 13$ mV more in the direction of the hyperpolarized potential by the application of 5-HT compared to the control without substantial changes in $K_h$ ($-19.4 \pm 3.2$ mV for the control and $-16.0 \pm 2.3$ mV for 5-HT) (Fig. 9C). The half-activation of the Boltzmann function fitted to the data was also affected by the application of 5-HT ($V_a = 29.1 \pm 5.5$ mV) compared with the control ($V_a = 16.9 \pm 6.6$ mV) (Fig. 9C). In contrast to the results obtained in type I$_I$ interneurons, $K_a$ was affected by 5-HT application in type I$_{CA}$ interneurons. $K_a$ was $31.5 \pm 4.7$ mV for the control and $23.8 \pm 2.7$ mV for the 5-HT condition (Fig. 9C). These results indicate that 5-HT produced a decrease in the amplitude of $I_{K(V)}$ in type I$_{CA}$ interneurons that may be due to both a shift in the inactivation curve to a more negative potential and a shift in the activation curve to a more positive potential.

**Effect of 5-HT on $I_{K(IR)}$ and $I_h$ in type I$_I$ and I$_{CA}$ interneurons.**

In a number of cell types inward rectifier currents contribute to the maintenance of the resting membrane potential, the regulation of action potential duration, and cellular excitability (see, e.g., Butt and Kalsi 2006; Isomoto et al. 1997; Yamoah et al. 1998). Here we found that 5-HT produced different effects on the inward rectifier component of the tail currents in type I$_I$ (see Fig. 2) and I$_{CA}$ (see Fig. 3) interneurons, suggesting that 5-HT may modulate inward rectifiers characteristic of $I_{K(IR)}$ and $I_h$ in type I interneurons. The effect of 5-HT on $I_{K(IR)}$ and $I_h$ was investigated in type I$_I$ and I$_{CA}$ interneurons by activating the currents with 3-s step hyperpolarizations to potentials between $-50$ and $-130$ mV from a holding potential of $-50$ mV (see insets of Fig. 10A and Fig. 11A). Representative current traces recorded from identified type I$_I$ and I$_{CA}$ interneurons before and after 5-HT application are shown in Fig. 10A and Fig. 11A, respectively. Hyperpolarization to potentials more negative than $-80$ mV induced a voltage-dependent and time-independent inward current that activated rapidly and exhibited little or no decay (Fig. 10A). These results indicate that $I_{K(IR)}$ is activated by membrane potentials more negative than the potassium $E_{rev}$, found in type I$_I$ interneurons. Application of 5-HT reduced $I_{K(IR)}$ in type I$_I$ interneurons (Fig. 10B), consistent with the effect of 5-HT on the inward component of tail currents detected in type I$_I$ interneurons (see Fig. 2). In contrast to type

Fig. 5. 5-HT decreases $I_{K(V)}$ and $I_{K(A)}$ in type I$_{CA}$ interneurons. Representative current traces recorded from an identified type I$_{CA}$ interneuron before (A and B) and after (D and E) 5-HT application are shown. Insets: stimulus protocols. Application of 5-HT produced a reduction in whole cell outward $K^+$ currents evoked by voltage depolarizations from $V_{holding}$ of $-80$ mV (A and D) and $-40$ mV (B and E). Difference currents (C and F) obtained by subtracting the currents in B and E from those in A and D showed that the fast activating and rapidly inactivating current [$I_{K(A)}$] was reduced by the application of 5-HT. Group summary data are shown in G–I ($n = 6$). Error bars depict SE; where error bars are not visible, they are smaller than the symbols. *$P < 0.05$, **$P < 0.01$. 

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Ii interneurons, in type IeA interneurons hyperpolarization to potentials more negative than \(-80\) mV induced a voltage- and time-dependent inward current that activated rapidly and showed a gradual increase in amplitude (Fig. 11A). These results indicate that type IeA interneurons express \(I_h\). Application of 5-HT increased \(I_h\) in type IeA interneurons (Fig. 11B), consistent with the effect of 5-HT on the inward component of tail currents recorded in type IeA interneurons (see Fig. 3). The analyses of the group summary data \((n = 6)\) shown in Fig. 10C and Fig. 11C indicate that 5-HT significantly decreased the amplitude of the steady-state component of \(I_{K(IR)}\) \((F_{1,5} = 304.4, P < 0.0001)\). These results explain the differential effects of 5-HT on tail currents detected in type Ii and IeA interneurons (see Fig. 2 and Fig. 3).

DISCUSSION

Serotonergic modulation of ionic conductances. There is an extensive literature on the modulation of different membrane conductances by 5-HT in various neuronal cell types. In this study, we examined the effects of 5-HT on various ionic currents in type Ii and IeA interneurons. The results showed that 5-HT modulated different ionic currents in these interneurons.

Fig. 6. In type Ii interneurons voltage-dependent inactivation of \(I_{K(A)}\) was shifted to a more negative potential by 5-HT. Representative current traces before (A) and after (B) 5-HT application recorded from an identified type Ii interneuron are shown. Insets in A and B show the stimulus protocols used to generate the inactivation curves. The activation and inactivation currents were reduced by the application of 5-HT (see A and B). C: normalized peak currents \((I_{\text{peak}})/I_{\text{max}}\) at 0 mV relative to the noninactivating component evoked by the test potential are plotted as a function of the conditioning potential (ranging from \(-80\) to 0 mV) and fitted with a Boltzmann function. The half-inactivation voltage \(V_{\text{h}}\) of the steady-state inactivation curve was shifted by 5-HT application from \(-57.0 \pm 0.5\) mV to \(-70.3 \pm 1.3\) mV without substantial changes in maximum slope of inactivation \(K_{\text{h}}\) \((n = 6)\). Normalized activation currents obtained from the current subtraction procedure shown in Fig. 4, C and F, are plotted as a function of the conditioning potential (ranging from \(-80\) to +50 mV) and fitted with a Boltzmann function. The half-activation voltage \(V_{\text{a}}\) and maximum slope of activation \(K_{\text{a}}\) of the steady-state activation curve were not substantially altered by 5-HT application \((n = 6)\).

Fig. 7. Modulation of the voltage-dependent activation and inactivation of \(I_{K(A)}\) by 5-HT application in type IeA interneurons. Representative current traces recorded from an identified type IeA interneuron before (A) and after (B) 5-HT application are shown. The inset in A shows the protocol used to generate the inactivation curves. C: normalized peak currents \((I_{\text{peak}})/I_{\text{max}}\) at 0 mV relative to the noninactivating component evoked by the test potential are plotted as a function of the conditioning potential (ranging from \(-80\) to 0 mV) and fitted with a Boltzmann function. The half-inactivation voltage \(V_{\text{h}}\) of the steady-state inactivation curve was shifted by 5-HT application from \(-57.0 \pm 0.5\) mV to \(-70.3 \pm 0.8\) mV by 5-HT application without substantial changes in \(K_{\text{h}}\) \((n = 6)\). Normalized activation currents obtained from the current subtraction shown in Fig. 5, C and F, are plotted as a function of the conditioning potential (ranging from \(-50\) to +50 mV) and fitted with a Boltzmann function. The half-activation voltage \(V_{\text{a}}\) and maximum slope of activation \(K_{\text{a}}\) of the steady-state activation curve were not substantially altered by 5-HT application \((n = 6)\).
conductances by 5-HT. 5-HT-dependent excitability changes have been shown to involve sodium (Tsutsui et al. 2008), calcium (Hsiao et al. 2005), potassium (Deng et al. 2007; Tsutsui et al. 2008), nonselective cation channels (Gasparini and DiFrancesco 1999), and chloride conductances (for review see Bobker and Williams 1990). In the present study external Na\(^+\) and Ca\(^{2+}\) were replaced with the membrane-impermeant substance NMDG to exclude Na\(^+\) and Ca\(^{2+}\) conductances.

Under these experimental conditions we found that type I\(_{\text{ii}}\) and I\(_{\text{eA}}\) interneurons contain a transient 4-AP-sensitive A-type-like current \([I_{\text{K(A)}}]\), a TEA-sensitive sustained outward rectifying K\(^+\) current \([I_{\text{K(V)}}]\), and two inward rectifier currents \([I_{\text{K(IR)}}]\ and \([I_{\text{h}}]\). In addition, 5-HT has been shown to modulate Cl\(^-\) conductances in some nervous systems (Bobker and Williams 1990; Lotshaw and Levitan 1987b). However, in type I\(_{\text{ii}}\) and I\(_{\text{eA}}\) interneurons Cl\(^-\) conductances were not affected by 5-HT since \(E_{\text{rev}}\) was shifted close to the value of the equilibrium potential for K\(^+\) as the external [K\(^+\)] was changed and the alteration of external [Cl\(^-\)] did not produce a significant change in the amplitude or the \(E_{\text{rev}}\) of the whole cell currents.

**Fig. 8.** Alteration of voltage-dependent activation and inactivation of \(I_{\text{K(V)}}\) by 5-HT application in type I\(_{\text{ii}}\) interneurons. Representative current traces before (A) and after (B) 5-HT application recorded from an identified type I\(_{\text{ii}}\) interneuron are shown. The inset in A shows the stimulus protocol used to generate the inactivation curve. C: normalized currents \((I/I_{\text{max}})\) at +50 mV evoked by the test potential are plotted as a function of the conditioning potential (ranging from −80 to +50 mV) and fitted with a Boltzmann function. The \(V_{\text{i}}\) of the steady-state inactivation curve was shifted by 5-HT application from −19.0 ± 3.4 mV to −23.4 ± 4.4 mV without substantial changes in \(K_{\text{h}}\) (\(n = 6\)). Normalized activation currents obtained from Fig. 4, B and E, are plotted as a function of the conditioning potential (ranging from −80 to +50 mV) and fitted with a Boltzmann function. The \(V_{\text{a}}\) of the steady-state activation curve was shifted by 5-HT application from 13.0 ± 5.0 mV to 28.6 ± 3.0 mV without substantial changes in \(K_{\text{a}}\) (\(n = 6\)).

**Fig. 9.** Modulation of voltage-dependent activation and inactivation of \(I_{\text{K(V)}}\) by 5-HT application in type I\(_{\text{eA}}\) interneurons. Representative current traces before (A) and after (B) 5-HT application recorded from an identified type I\(_{\text{eA}}\) interneuron are shown. The inset in A shows the stimulus protocol used to generate the inactivation curve. C: normalized currents \((I/I_{\text{max}})\) at +50 mV evoked by the test potential are plotted as a function of the conditioning potential (ranging from −80 to +50 mV) and fitted with a Boltzmann function. The \(V_{\text{i}}\) of the steady-state inactivation curve was shifted by 5-HT application from −11.2 ± 2.9 mV to −24.0 ± 2.4 mV without substantial changes in \(K_{\text{h}}\) (\(n = 6\)). Normalized activation currents obtained from Fig. 5, B and E, are plotted as a function of the conditioning potential (ranging from −80 to +50 mV) and fitted with a Boltzmann function. The \(V_{\text{a}}\) and \(K_{\text{a}}\) of the steady-state activation curve were shifted by 5-HT application from 16.9 ± 6.6 mV to 29.1 ± 5.5 mV and from 31.5 ± 4.7 mV to 23.8 ± 2.7 mV (\(n = 6\)).
before and after 5-HT application. Moreover, the kinetics of the currents based on activation and inactivation curves are consistent with \( K_{\text{H11001}} \) as the predominant charge carrier of the whole cell currents modulated by 5-HT application in type Ii and IeA interneurons.

Serotonin has different effects on \( I_{\text{K(A)}} \) and \( I_{\text{K(V)}} \) in interneurons and sensory neurons. It is well known that shifts in activation and inactivation curves of \( I_{\text{K(A)}} \) and \( I_{\text{K(V)}} \) would lead to a change in the "window current" observed between the steady-state activation and inactivation curves that may contribute to a change in cellular excitability. Here we found that 5-HT reduced the amplitude of \( I_{\text{K(A)}} \) and shifted the \( V_h \) of both \( I_{\text{K(A)}} \) and \( I_{\text{K(V)}} \) to a more negative potential and the \( V_a \) of \( I_{\text{K(A)}} \) and \( I_{\text{K(V)}} \) to a more positive potential. These changes may explain the previously observed increase in the peak amplitude of light-evoked complex EPSPs, enhanced intrinsic excitability, and increased spike activity of identified type IeA interneurons (Jin et al. 2009).

The effects of 5-HT on shifts in the activation and inactivation curves of \( I_{\text{K(A)}} \) and \( I_{\text{K(V)}} \) of sensory neurons are different from our observations of interneurons. As an example, in

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**Fig. 10.** 5-HT reduces the amplitude of the steady-state \( I_{\text{K(IR)}} \) in type Ii interneurons. A: in control solutions type Ii interneurons exhibited an inward rectifier time-independent current evoked by hyperpolarizing steps from \(-50\) to \(-130\) mV from a holding potential of \(-50\) mV. B: application of 5-HT reduced the magnitude of the steady-state \( I_{\text{K(IR)}} \). C: voltage-current relationship before (Ctrl) and after 5-HT (5-HT) application (\( n = 6 \)). *\( P < 0.05 \), **\( P < 0.01 \).

**Fig. 11.** 5-HT increases the steady-state \( I_{\text{K}} \) in type IeA interneurons. A: hyperpolarizing voltage step from a holding potential of \(-50\) mV evoked a time-dependent \( I_{\text{K}} \). B: application of 5-HT enhanced \( I_{\text{K}} \). C: voltage-current relationship before and after 5-HT application (\( n = 6 \)). **\( P < 0.01 \).
Manduca sexta olfactory neurons 5-HT was shown to produce a decrease in the maximal conductance of $I_{K(V)}$ without affecting its voltage dependence (Kloppenburg et al. 1999). The authors proposed that the changes may contribute to increased excitability and modulation of the sensitivity of olfactory neurons. In Drosophila photoreceptors the application of 5-HT shifts the state-steady inactivation curves of both $I_{K(A)}$ and $I_{K(V)}$ to more depolarized potentials, which may lead to an increase in the “window current” and contribute to modulating the sensitivity of photoreceptors to light (Hevers and Hardie 1995). These observations are in agreement with the findings that 5-HT plays important roles in switching insect photoreceptors from a high-acuity, low-sensitivity day state to a low-acuity, high-sensitivity night state (Weckstrom and Laughlin 1995). In Hermissenda photoreceptors the application of 5-HT paired with the presentation of light produces a depolarized shift in the steady-state activation curve of $I_{K(A)}$ without altering the inactivation curve. The shift in the activation curve would reduce the “window current” and contribute to enhanced excitability underlying Pavlovian conditioning (Yamoah et al. 2005).

Serotonin effects on $I_{K(A)}$ and $I_{K(V)}$ are different in type I and $I_{A}$ interneurons. An interesting feature of our analysis is the finding that the effects of 5-HT on the activation-inactivation kinetics of $I_{K(A)}$ and $I_{K(V)}$ are different in different types of interneurons. The application of 5-HT produced a depolarizing shift in the activation curve of $I_{K(V)}$ and a hyperpolarizing shift in the inactivation curve of $I_{K(A)}$ in type I interneurons. The changes observed in type I interneurons suggest that the depolarizing shift in the activation curve of $I_{K(V)}$ produced by 5-HT may contribute to decreasing the amplitude of light-evoked IPSPs, whereas the shifts in activation-inactivation curves of $I_{K(A)}$ and $I_{K(V)}$ may contribute to enhanced excitability. In contrast, 5-HT produced a depolarizing shift in the activation curve and a hyperpolarizing shift in the inactivation curve of both $I_{K(V)}$ and $I_{K(A)}$ in type $I_{A}$ interneurons. These changes may contribute to increased EPSP amplitude and increased spike activity in type $I_{A}$ interneurons.

Although the current-voltage relationship of $I_{K(V)}$ in type I interneurons is similar to that in type $I_{A}$ interneurons, there are important differences. First, $I_{K(V)}$ was activated at potentials about $-30 \text{ mV}$ in type I interneurons, whereas $I_{K(V)}$ was activated at potentials about $-50 \text{ mV}$ in type $I_{A}$ interneurons. This observation implies the presence of different kinds of delayed rectifier $K^{+}$ conductances in type I interneurons. The decreased $I_{K(V)}$ produced by 5-HT in type I interneurons may not contribute significantly to maintaining the resting membrane potential ($-60$ to $-50 \text{ mV}$) but may contribute to enhanced excitability at membrane potentials more positive than $-30 \text{ mV}$ and increased spike activity, consistent with the physiological functions of $I_{K(V)}$ in auditory interneurons (Grissmer et al. 1994), whereas the decreased $I_{K(V)}$ produced by 5-HT may contribute to depolarizing the resting membrane potential and increased excitability in type $I_{A}$ interneurons. The role of $I_{K(V)}$ in maintaining membrane potential and in regulating excitability in neurons as well as other kinds of cells has been described in detail elsewhere (Gutman et al. 2005). Second, $I_{K(V)}$ in type I and $I_{A}$ interneurons differs in the dynamics of 5-HT block. In type I interneurons, the application of 5-HT increased the remaining inactivation current at voltages more positive than $-15 \text{ mV}$ but decreased the remaining inactivation current in type $I_{A}$ interneurons. In agreement with a previous study (Jin et al. 2009), the differential effect of 5-HT on the inactivation of $I_{K(V)}$ is likely to play a role in decreased spike activity in type I interneurons and increased spike activity in type $I_{A}$ interneurons at depolarized membrane potentials between $-15$ and $+50 \text{ mV}$. Previous research has shown that type I interneurons may exhibit spontaneous and light-evoked phasic spike activity (Crow and Tian 2008). The differential effects of 5-HT on the inactivation of $K^{+}$ currents could contribute to phasic spike activity. Overall, these findings indicate that the effects of 5-HT on the kinetics of $I_{K(A)}$ and $I_{K(V)}$ are different in type I and $I_{A}$ interneurons.

Serotonin modulates different inward rectifier currents in type $I_{A}$ and $I_{I}$ interneurons. There are a number of examples of different types of inward rectifier currents that are expressed in seemingly homogeneous cells. In frog saccular hair cells, spherical cells have a Na+/K+-selective inward rectifier current ($I_{h}$), and the more cylindrically shaped cells have both $I_{h}$ and a K+-selective inward rectifier current ($I_{K(IR)}$) (Holt and Eatock 1995). In Hermissenda photoreceptors type A cells express $I_{h}$, while type B cells express $I_{K(IR)}$ (Yamoah et al. 1998). Here we found that two different classes of type I interneurons express two different kinds of inward rectifier currents with different modulating effects of 5-HT. The properties of the inward rectifier current in type I interneurons share features consistent with $I_{K(IR)}$ due to the time-independent activation kinetics. The properties of the inward rectifier current in type $I_{A}$ interneurons express features consistent with $I_{h}$ due to the time-dependent activation kinetics, a gradual slow time course of activation that is distinguishable from the faster activation of $I_{K(IR)}$. $I_{h}$ in type $I_{A}$ interneurons exhibits time-dependent activation similar to the properties of $I_{h}$ identified in Hermissenda photoreceptors (Yamoah et al. 1998), leech heart interneurons (Angstadt and Calabrese 1989), and neonatal rat motoneurons (Larkman et al. 1995). 5-HT decreases $I_{K(IR)}$ in type I interneurons and increases $I_{h}$ in type $I_{A}$ interneurons. The $I_{h}$ in type $I_{A}$ interneurons contributes to the regulation of intrinsic excitability since the $E_{rev}$ of $I_{h}$ is approximately $-30$ to $-40 \text{ mV}$ under physiological conditions, is more positive than the resting potential. Therefore, the increase in $I_{h}$ produced by 5-HT would result in membrane depolarization. In contrast, in type I interneurons the decrease of $I_{K(IR)}$ produced by 5-HT may increase excitability because the $E_{rev}$ of $I_{K(IR)}$ is more negative than $-70 \text{ mV}$ under physiological conditions and thus would result in membrane depolarization. Consistent with our results, there are a number of reports showing that 5-HT modulates inward rectifier currents. In identified Aplysia neurons 5-HT increases $I_{K(IR)}$ (Benson and Levent 1983; Lotshaw and Levent 1987a; Taussig et al. 1989), and in mammalian neurons 5-HT increases $I_{h}$ (for review see Bobker and Williams 1989).

Serotonergic modulation of intrinsic excitability. Enhanced intrinsic excitability is one mechanism supporting 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 Benjamini 2001; Thompson et al. 1996). 5-HT-dependent enhanced excitability contributes to learning in Aplysia (Byrne and Kandel 1996; Byrne et al. 1993; Hawk-
ins et al. 1993), leech (Ehrlich et al. 1992; Sahley 1994), and Hermissenda (Crow 2004). In Hermissenda, 5-HT facilitates the monosynaptic PSP between identified photoreceptors (Frysztaf and Crow 1994, 1997; Schuman and Clark 1994) and modulates membrane conductances in identified sensory neurons (photoreceptors) that are sites of cellular and synaptic plasticity associated with Pavlovian conditioning (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). Type I interneurons in the cerebrolateral ganglia of Hermissenda are a second site of cellular and synaptic plasticity produced by Pavlovian conditioning (Crow and Tian 2002a). We previously reported that in type I_A and I_i interneurons 5-HT produces a membrane depolarization, an increase in spontaneous and light-elicited spike activity, and enhanced intrinsic excitability (Jin et al. 2009).

Here we show that the ionic mechanisms of enhanced excitability produced by 5-HT in type I_A and I_i interneurons are composed of 1) a reduction in the amplitude of I_K(A) and I_K(V) 2) a shift in the activation and inactivation curves of I_K(A) and I_K(V) that produces a decrease in the “window current,” 3) a reduction of I_h(IR) in type I_i interneurons, and 4) an increase in I_h in type I_A interneurons.

Visually influenced locomotion in Hermissenda is regulated by the activity of type I_i and I_i interneurons operating through type III inhibitory interneurons that form monosynaptic connections with ciliary efferent neurons (Crow 2004; Crow and Tian 2000, 2002a, 2002b, 2003, 2004). It has been proposed that 5-HT operates as a neurotransmitter in the US pathway by projections to both photoreceptors and interneurons (Crow 2004). Interestingly, the different effects of 5-HT on the activation-inactivation kinetics of I_K(A) and I_K(V) in I_i and I_i interneurons and on I_h(IR) and I_h in I_i and I_i interneurons provide for a synergistic effect on the activation of type III interneurons. Taken collectively, the characteristics of I_K(A), I_K(V), I_h(IR), and I_h(5-HT) that express distinct effects of 5-HT on activation-inactivation kinetics and membrane conductances are appropriate for the different synaptic properties of I_i and I_i interneurons. The modifications of I_K(A), I_K(V), I_h(IR), and I_h(5-HT) by 5-HT may contribute to neural plasticity in the circuit supporting the generation of ciliary locomotion through integrated modulation of cellular excitability in type I_i and I_i interneurons.

GRANTS

This work was supported by National Institute of Mental Health Grants MH-40860 and MH-58698 to T. Crow.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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