# Pharmacological Dissociation of Modulatory Effects of Serotonin in *Aplysia* Sensory Neurons

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**In the mollusk *Aplysia* the neurotransmitter serotonin (5HT) has a fundamental modulatory role in several forms of learning and memory that involve an increase in the efficacy of synaptic transmission between tail sensory neurons (SNs) and motor neurons. The classical 5HT antagonist cyproheptadine (CYP) permits dissociation of three forms of serotonergic modulation in these SNs: (i) CYP reversibly blocks spike-broadening induced either by exogenous application of 5HT or by sensitizing stimulation of a tail nerve. (ii) CYP does not block 5HT-induced or tail input-induced increases in SN somatic excitability. (iii) Concomitant with its block of spike-broadening, CYP reversibly blocks 5HT-induced facilitation of synaptic transmission from SNs. These results suggest that endogenously released 5HT may act at different receptor subtypes that are coupled to different forms of neuromodulation in tail SNs of *Aplysia*.**

THE MARINE MOLLUSK *APLYSIA* HAS been useful for understanding the cellular and molecular mechanisms underlying learning and memory (*1*). The cellular mechanisms of learning in *Aplysia* have been studied most thoroughly in two populations of sensory neurons (SNs) that mediate withdrawal reflexes either from stimulation of the tail (*2*) or the siphon (*3*). Several forms of learning involve heterosynaptic (presynaptic) facilitation of transmitter release from these SNs onto their follower cells (*1*). The neurotransmitter serotonin (5HT) is thought to have a fundamental modulatory role in these forms of learning. Consistent with this view is the observation that many of the effects of learning are mimicked by direct application of 5HT to the SNs. For example, in the tail SNs, 5HT mimics the effects of sensitization (a nonassociative form of reflex enhancement) by (i) increasing the excitability of the SNs (*4, 5*); (ii) increasing the duration and amplitude of their action potentials (*5, 6*); (iii) increasing adenosine 3′,5′-monophosphate (cAMP) concentrations within the neurons (*6, 7*); (iv) reducing a specific ionic ($K^+$) conductance (*5, 6*); and (v) enhancing transmitter release from the SNs onto their follower cells (*4, 6*).

To investigate the 5HT receptors that mediate these forms of modulation, we have used the classical 5HT antagonist cyproheptadine (CYP) to examine some of the 5HT-induced effects in the tail SNs. We first examined the effects of CYP on spike-broadening and on increased excitability induced by direct application of 5HT to the tail SNs. Bilateral clusters of SNs in the paired pleural ganglia from adult animals (100 to 300 g) were used. The cluster in one ganglion served as a control and was tested with 5HT (5 μM) alone; in independent experiments, the other cluster was treated with CYP (200 μM) (*8*) 10 min before, and during, 5HT application. At 5 μM, 5HT typically produced a ~20% increase in the duration of the action potential (Fig. 1A) (*9*). In the presence of CYP, however, 5HT-induced spike-broadening was blocked (Fig. 1, B and C). In control cells, 5HT produced a significant increase in spike duration (mean increase 19%; $P < 0.01$) (*10*), whereas in the presence of CYP, SNs showed no significant spike-broadening [−0.7%; not significant (NS)]. A between-group comparison showed that CYP caused a significant reduction of spike-broadening induced by exogenously applied 5HT ($P < 0.01$). CYP itself had no appreciable effect on membrane potential (*11*) or spike duration at 200 μM, nor did CYP interfere with the ability of the action potential to broaden in a 5HT-independent fashion (*12*).

In the same cells we also examined the increased excitability induced by 5HT. Constant-current pulses (200 ms, 0.5 nA) were used to elicit spikes in the SNs. 5HT produced a significant increase in spike number both alone (200%; $P < 0.02$) (Fig. 1, D and F) and in the presence of CYP (200%; $P < 0.02$) (Fig. 1, E and F). Thus, CYP did not block the 5HT-induced increase in SN excitability. These results raise the possibility that 5HT exerts its actions through different 5HT receptor subtypes (differentially sensitive to CYP); these subtypes appear to be coupled to different forms of neuromodulation in the tail SNs of *Aplysia* (*13*).

Tail shock-induced sensitization in *Aplysia* is accompanied by spike-broadening in tail SNs. This effect on spike duration is mimicked by 5HT (*4–6*) (Fig. 1, A through C), suggesting that tail shock-induced sensitization is mediated, at least in part, by 5HT (*4*). We tested this hypothesis directly by examining the effects of CYP on spike-broadening induced by a sensitizing stimulus, electrical stimulation of tail nerve P9 (*14*). SNs in one pleural ganglion served as controls; those from the opposite side of the animal were treated with CYP (200 μM) 10

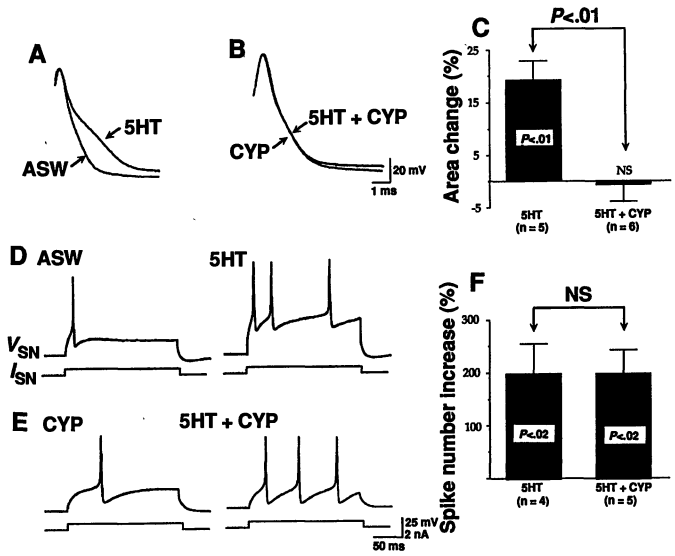


**Fig. 1.** (**A** through **C**) Effect of CYP on 5HT-induced spike-broadening. Action potentials were triggered with brief (2-ms) intracellular pulses (peak and descending limb of the spike are shown). (A) Spike in artificial seawater (ASW) and subsequently in 5HT (5 μm) are superimposed. (B) Superimposed spikes in CYP (200 μm) and 5HT + CYP. (C) Quantitative comparison (*10*). Data are expressed as mean percent change (± SEM) in spike area (*9*) compared to ASW. The number ($n$) of independent experiments (one SN per experiment) is in parentheses. (**D** through **F**) Effect of CYP on 5HT-induced excitability. Action potentials ($V_{SN}$) were triggered with a prolonged (200-ms), constant current, intracellular depolarizing pulse ($I_{SN}$). (D) Somatic excitability in ASW and 5HT. (E) Effect of CYP on the 5HT-induced excitability increase (the apparent slight reduction in net depolarization in 5HT + CYP compared to 5HT alone is due to a modest bridge imbalance in the 5HT-alone trace). Cells in (D) and (E) are the same as those shown in (A) and (B), respectively. (F) Quantitative comparison [method as in (C)].

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min before, and during, P9 stimulation. P9 stimulation significantly increased spike duration in control SNs (16%; $P < 0.05$) (Fig. 2, A and C). However, SNs receiving sensitizing stimulation in the presence of CYP showed no significant spike-broadening (3%; NS) (Fig. 2, B and C). Thus, spike-broadening induced in tail SNs either by exogenous application of 5HT (Fig. 1) or by sensitizing stimulation of the tail nerve (Fig. 2) was blocked by CYP, suggesting that sensitization is mediated at least in part by endogenously released 5HT.

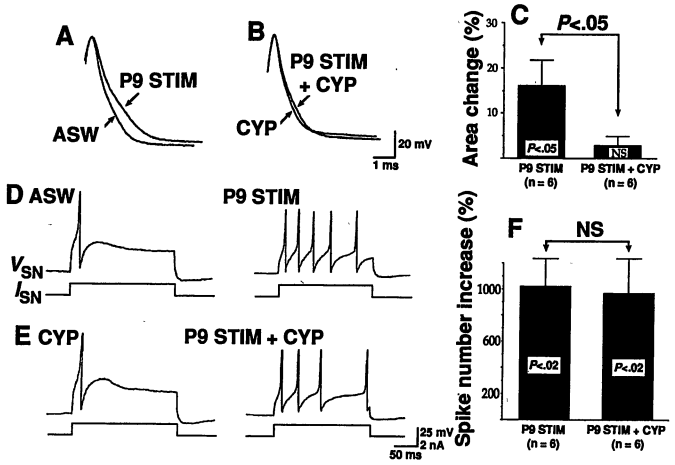


**Fig. 2.** (**A** through **C**) Effect of CYP on spike-broadening induced by tail nerve P9 stimulation. Data are expressed as in Fig. 1. (A) Spike-broadening induced by electrical stimulation of tail nerve P9 (P9 STIM). (B) CYP blocking of P9-induced spike-broadening. (C) Quantitative comparison. (**D**) Effect of P9 stimulation on excitability (tests were made within 60 s of P9 stimulation). (**E**) Effect of CYP on P9-induced excitability increase. (**F**) Quantitative comparison.

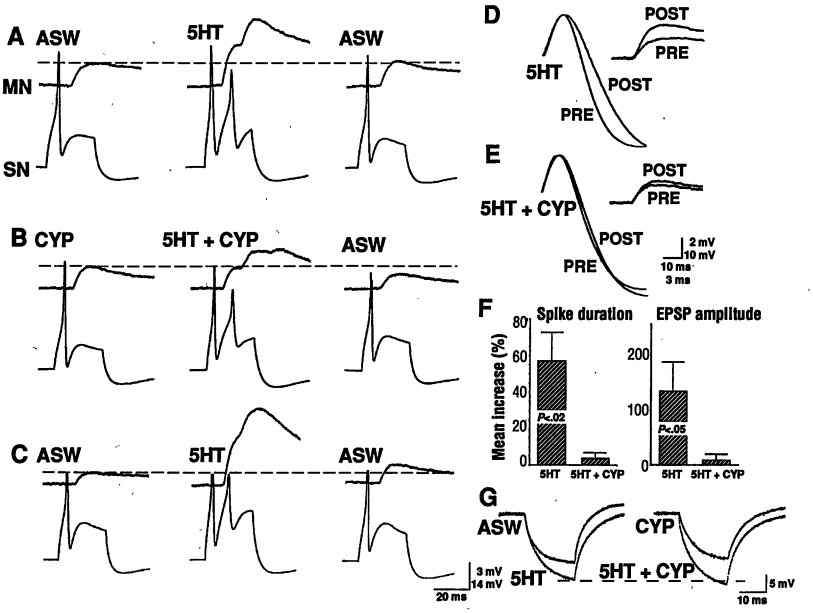


**Fig. 3.** Effect of CYP on EPSPs, monitored by intracellular recording from a tail motor neuron (MN) and SN. (**A**) A spike triggered in SN by a 30-ms depolarizing pulse evoked a monosynaptic EPSP in the MN. When 5HT (5 μm) was bath-applied, the EPSP was enhanced. 5HT increased SN excitability (producing a second spike), which evoked a second EPSP. After 10 min of wash, the EPSP returned to baseline. (**B**) After 10 min in CYP (first pair of traces), the EPSP was unchanged [compare with ASW in (A)]. When 5HT + CYP was bath-applied, synaptic facilitation was blocked, whereas the excitability increase in SN was unchanged. After washout, in ASW the EPSP is slightly reduced in amplitude. (**C**) Although the baseline (ASW) EPSP is now reduced [compared to (A)], 5HT still produced clear synaptic enhancement [Spikes are slightly clipped in (C)]. In (A) through (C), the dashed line indicates initial EPSP amplitude for that segment of the experiment. (**D**) Superimposed action potentials on an expanded time scale (PRE = ASW, POST = 5HT). Superimposed EPSPs from their corresponding action potentials are on a slower time scale to the right. [For calibration bars in (E), the top values are for EPSPs, and the bottom values are for spikes.] (**E**) Data presented as in (D). Superimposed action potentials and EPSPs are from the second phase (B) (PRE = CYP, POST = CYP + 5HT). (**F**) Quantitative comparison. (**G**) (Left) Superimposed electrotonic potentials in ASW (top trace) and then in 5HT (bottom trace) show the increase in input resistance (from 20.2 to 27.6 megohms) produced by 5HT in a SN. (Right) After 5HT washout and 10 min in CYP, the SN input resistance is slightly reduced compared to ASW (from 20.2 to 18.4 megohms, top trace), but in the presence of CYP, 5HT still produces the same increase in input resistance (dashed line for comparison) as in ASW (27.8 megohms, bottom trace). 5HT and CYP + 5HT also produced comparable depolarization of the SN (not shown). In the absence and presence of CYP, 5HT increased SN input resistance by a mean of 7.2 and 8.9 megohms, respectively ($n = 4$).

A further parallel between modulation of the tail SNs by 5HT and by tail nerve stimulation was that CYP did not block excitability increases: P9 stimulation produced a significant increase in spike number both under control conditions (1016%; $P < 0.02$) (Fig. 2, D and F) and in the presence of CYP (966%; $P < 0.02$) (Fig. 2, E and F) (*15*). Thus, in a manner consistent with our observations with exogenously applied 5HT (Fig. 1), CYP selectively blocked P9-induced spike-broadening without affecting P9-induced increases in SN excitability.

Serotonin also produces enhancement of synaptic transmission from tail SNs onto their follower cells (*4, 6*). To examine whether spike-broadening contributes to synaptic facilitation induced by 5HT (*16*), we analyzed monosynaptic excitatory postsynaptic potentials (EPSPs) between tail SNs and tail motor neurons (*17*). 5HT (5 μm) produced the expected increases in both SN excitability (Fig. 3A) and spike-broadening (Fig. 3D). In addition, 5HT substantially enhanced the EPSP elicited by stimulation of the SN (Fig. 3, A and D). These effects were reversible, returning to baseline after a 10-min washout of 5HT. CYP (200 μM) was then applied for 10 min; CYP itself had no appreciable effect on the resting potential of the SN or motor neuron nor any effect on synaptic transmission (Fig. 3B). In the presence of CYP, however, concomitant with the reduction of 5HT-induced spike-broadening (Fig. 3, E and F), there was a significant decrease in 5HT-induced facilitation of the EPSP (Fig. 3, B, E, and F). In a manner consistent with our earlier observations (Fig. 1, D through F), 5HT produced an increase in SN excitability in the presence of CYP. These effects were reversible, recovering in about 10 min (Fig. 3B). To ensure that the apparent CYP block of 5HT-induced synaptic facilitation was not due to the inability of 5HT to enhance the EPSP a second time, we repeated application of 5HT in the absence of CYP and observed clear spike-broadening and synaptic enhancement (Fig. 3C). A quantitative analysis ($n = 5$) revealed that 5HT alone produced significant EPSP facilitation (134%; $P < 0.05$) and significant spike-broadening (57%; $P < 0.02$), whereas 5HT plus CYP caused no EPSP facilitation (21%; NS) or spike-broadening (3.6%; NS) (Fig. 3F). Thus, when spike-broadening occurs in the SNs, enhancement of the EPSP is evident,

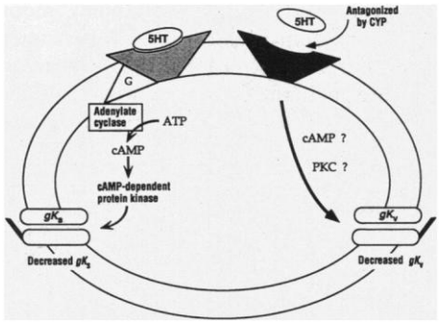


**Fig. 4.** Model of 5HT actions in tail SNs. Two ionic conductances ($g$) modulated by 5HT are illustrated: (i) a decrease of $gK_s$, which is mediated by cAMP (*5–7*), and (ii) a decrease of $gK_v$, which may be mediated by PKC (*5, 20, 21*) and perhaps cAMP (*22*). Our data extend this model by suggesting that each pathway may be coupled to a different 5HT receptor subtype to give rise to different forms of neuromodulation (the CYP-insensitive 5HT effects were increased excitability and increased input resistance, and the CYP-sensitive effects were spike-broadening and synaptic facilitation; see text). Net SN facilitation by 5HT is thus brought about by the combined actions of these two pathways.

whereas when spike-broadening is blocked by CYP, synaptic enhancement is also blocked. These results add support to the hypothesis that 5HT-induced spike-broadening (*16, 18*) contributes significantly to enhanced synaptic transmission from SNs (*19*).

Work in tail SNs has shown that 5HT, through cAMP, causes a reduction in the 5HT-sensitive $K^+$ current $I_{ks}$, which is accompanied by spike-broadening, increased excitability, increased input resistance, and synaptic facilitation (*5, 6*). More recently it has been shown that reduction of another $K^+$ current, the delayed rectifying current $I_{kv}$, contributes significantly to 5HT-induced spike-broadening in the tail SNs (*5, 20*). 5HT-induced spike-broadening through reduction of $I_{kv}$ is thought to involve activation of one or more kinase systems [perhaps protein kinase C (PKC) (*21*) as well as cAMP (*22*)]. Our data permit the extension of a model describing these 5HT actions in tail SNs (Fig. 4) in two ways. First, a CYP-insensitive subtype of 5HT receptor may mediate reduction of $I_{ks}$. Consistent with this hypothesis is the observation that 5HT-induced increased excitability, depolarization, and increased input resistance [all of which are thought to be mediated by modulation of $I_{ks}$ (*4, 6*)] are not blocked by CYP (Fig. 1, D through F and Fig. 3C) (*23*). Further, a second class of 5HT receptor (CYP-sensitive) may mediate reduction of $I_{kv}$ (*24*). Second, our data show that CYP, which blocks spike-broadening (presumably primarily through a reduction of $I_{kv}$), also blocks 5HT-induced synaptic facilitation without blocking increased excitability or increased input resistance (presumably because $I_{ks}$ modulation is still intact). Thus, at least in the tail SNs, 5HT modulation of $I_{kv}$ may have a proportionately greater role in synaptic facilitation than 5HT modulation of $I_{ks}$.

Cyproheptadine could be useful for addressing questions of general significance, such as the long-standing issue concerning the relation between short-term and long-term memory (*25*). 5HT produces both short- and long-term synaptic changes in tail SNs that accompany, respectively, short- and long-term memory for sensitization (*4, 26, 27*). Because short-term synaptic changes are blocked by CYP (Fig. 3), one can now ask whether short-term changes must occur before long-term changes can be produced, that is, whether short-term and long-term memory occur in series or in parallel.

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2. Tail (VC) neurons are located in bilateral clusters in the pleural ganglia [E. T. Walters, J. H. Byrne, T. J. Carew, E. R. Kandel, *J. Neurophysiol.* **50**, 1522 (1983)].
3. Siphon (LE) SNs are located in the abdominal ganglion [V. Castellucci, H. Pinsker, I. Kupfermann, E. R. Kandel, *Science* **167**, 1745 (1970)]. For a general review, see E. R. Kandel and J. H. Schwartz, *ibid.* **218**, 433 (1982).
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8. In a dose-response analysis of CYP and 5HT (A. R. Mercer and T. J. Carew, in preparation), 200 μm of CYP displayed minimal agonist activity but maximal inhibition of 5HT-induced spike-broadening (range tested, 0.5 to 500 μm CYP). At higher concentrations (500 μm) CYP effects were more variable and included occasional spike-broadening and a shift in the membrane potential.
9. A low concentration of 5HT (5 μm) was used to minimize desensitization with repeated 5HT application. Action potentials were triggered with a very brief (2-ms) depolarizing pulse to permit accurate assessment of spike duration, which we measured by integrating the area under the action potential [with the software program SPIKE (Hilal Associates, Englewood Cliffs, NJ)]. The voltage area integrated was from the peak of the spike to the point at which the descending limb of the spike returned to baseline. Data were expressed in mV ms.
10. Between-group and within-group (difference score) comparisons were made with $t$ tests for independent and correlated means, respectively.
11. CYP alone (200 μm) produced a small (<10%) reduction in input resistance (Fig. 3G) and a modest hyperpolarization (1 to 2 mV) of the SNs.
12. Repeated activation of the SN with intracellular current pulses caused substantial spike-broadening [due to cumulative inactivation of $I_{kv}$ (*5*)]. Spike-broadening produced in this fashion was unaffected by 200 μm CYP.
13. CYP interacts with both $5HT_1$ and $5HT_2$ receptor subtypes but has a greater affinity for $5HT_2$ receptors [S. J. Peroutka, R. M. Lebovitz, S. H. Snyder, *Science* **212**, 827 (1981)]. Although our use of CYP has permitted pharmacological dissociation of 5HT effects, studies using more selective blockers will be required to confirm the identity of 5HT receptor subtypes in *Aplysia*.
14. P9 was stimulated with a 3-s train of 5-ms pulses (10 pulses per second, 20 to 40 V). These stimulus parameters did not trigger an antidromic action potential in the SNs.
15. P9-induced increases in excitability were optimal with a stimulus pattern of three 1-s trains of shock (10 pulses per second, 20 to 30 V) with a 0.5-s intertrain interval (at this shock level no antidromic spikes were triggered in the SNs). With these parameters, action potentials often continued long after the offset of the 200-ms depolarizing pulses that were injected, which accounts for the large percent increases in Fig. 2F.
16. In LE SNs, 5HT-induced presynaptic facilitation predominantly involves spike-broadening at nondepressed synapses, but a second (non–spike-broadening) process has a major role in 5HT-induced facilitation at depressed synapses [B. Hochner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8794 (1986); K. J. Gingrich and J. H. Byrne, *J. Neurophysiol.* **53**, 652 (1985)]. By analogy to LE SNs, we examined nondepressed synapses (*17*).
17. We examined the EPSP by injecting brief depolarizing pulses into the SN at a low rate of activation (interstimulus interval, 100 s) to minimize the synaptic decrement.
18. We correlated reduction by CYP of 5HT-induced spike-broadening, measured in the SN cell body, with reduction of 5HT-induced facilitation of EPSP amplitude. Thus, these experiments do not directly show that spike-broadening in SN terminals is blocked by CYP. CYP, in addition to blocking somatic spike-broadening, may exert other effects that could contribute to reduction of 5HT-induced synaptic enhancement.
19. A direct prediction from these results is that CYP should significantly reduce synaptic facilitation produced by stimulation of the tail nerve P9 (Fig. 2).
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23. At resting potential, 5HT-induced depolarization and increased input resistance would be due to reduction of $I_{ks}$, which, as first described in LE sensory neurons by S. A. Siegelbaum, J. S. Camardo, and E. R. Kandel [*Nature* **299**, 413 (1982)], is a background current (*5, 6*), and would not be due to reduction of $I_{kv}$, which is not active at rest (*5*).
24. In principle, a single CYP-sensitive receptor could mediate all of these effects of 5HT. For example, CYP could reduce the concentration of a 5HT-coupled second messenger (for example, cAMP) to below the threshold for some of its effects (such as spike-broadening) but not below the threshold for other effects (such as increased excitability). Although such differential second messenger threshold effects have not been demonstrated in *Aplysia*, this hypothesis cannot be ruled out by our data.
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