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Differential Effects of Serotonin, FMRFamide, and Small Cardioactive Peptide on Multiple, Distributed Processes Modulating Sensorimotor Synaptic Transmission in *Aplysia*

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At least two processes contribute to the modulation by 5-HT of the connections between sensory neurons and motor neurons in *Aplysia*. The first involves broadening of the presynaptic spike through modulation of 5-HT–sensitive K^+ channels that leads to elevated levels of intracellular Ca^{2+} and increased release of transmitter. A second process (or set of processes) apparently accounts for the amount of facilitation not produced by presynaptic spike broadening. This spike duration–independent (SDI) process is particularly prominent in depressed synapses. We used a protocol in which spikes were prebroadened into a range of durations in which further spike broadening by itself has little or no effect on facilitation of the EPSP. 5-HT produced pronounced facilitation in depressed synapses under these conditions. Another modulatory agent, small cardioactive peptide (SCP_b), also broadened spikes in sensory neurons but did not produce facilitation comparable to that produced by 5-HT. These results indicate that 5-HT activates the SDI process whereas SCP_b fails to do the same. A 5 min preexposure to the modulatory peptide FMRFamide inhibited 5-HT–induced activation of the SDI process, whereas a 1 min preexposure did not. Another process(es) that may modulate synaptic efficacy in sensorimotor synapses involves changes in the properties of the motor (follower) neuron, such as input resistance. FMRFamide decreased the input resistance of postsynaptic neurons. This action could contribute to the effects of FMRFamide when administered alone, but it did not appear to be responsible for the inhibitory action of FMRFamide on 5-HT–induced facilitation. Neither 5-HT nor SCP_b had a clear effect on input resistance. The actions of these three agents, therefore, seem to be differentially distributed among various pre- and postsynaptic processes involved in the modulation of synaptic transmission.

Sensitization and dishabituation are two simple forms of nonassociative learning studied extensively in defensive behaviors of the marine mollusk *Aplysia* (Pinsker et al., 1970; Carew et al., 1971; Rankin and Carew, 1987, 1988; Wright et al., 1991; for reviews, see Byrne, 1987; Carew, 1989). These behavioral modifications are due, at least in part, to presynaptic facilitation of the connections between sensory and motor neurons (Castellucci et al., 1970; Castellucci and Kandel, 1976). 5-HT, a neuromodulator believed to be released in response to sensitizing and dishabituating stimuli, induces presynaptic facilitation at these synapses (Glanzman et al., 1989; Mackey et al., 1989; Dale and Kandel, 1990; for reviews, see Kandel and Schwartz, 1982; Byrne, 1985, 1987; Carew and Sahley, 1986; Carew, 1987; Hawkins et al., 1987; Byrne et al., 1991). At least two processes are thought to contribute to the facilitation produced by 5-HT. The first is an increase in the duration of the action potential (spike) in the sensory neuron (Klein and Kandel, 1978), which is due, at least in part, to the cAMP-mediated closure of S-K^+ channels (Brunelli et al., 1976; Bernier et al., 1982; Siegelbaum et al., 1982; Belardetti and Siegelbaum, 1988). Recent experiments indicate that an apparently cAMP-independent regulation of membrane channels also contributes to spike broadening (Baxter and Byrne, 1990; Sugita et al., 1991). Spike broadening occurs during the 5-HT–induced facilitation of both nondepressed and depressed synapses, conditions that represent the cellular analogs of sensitization and dishabituation, respectively. In depressed synapses, however, facilitation produced by spike broadening alone cannot fully account for the increased amplitude of EPSPs (Gingrich and Byrne, 1985, 1987; Hochner et al., 1986; Gingrich et al., 1988). Thus, a second facilitatory process (or set of processes) of a spike duration–independent (SDI) nature has been proposed to explain the facilitation that can be produced in homosynaptically depressed sensorimotor connections. Although the precise nature of the SDI process has not been identified, it has been modeled as a mobilization of transmitter from a storage pool to a releasable pool (Gingrich and Byrne, 1985, 1987; Gingrich et al., 1988; see also Dale and Kandel, 1990).

Activation of the SDI process(es) of facilitation by 5-HT has been demonstrated in both cultured cells and intact ganglia in *Aplysia* (Hochner et al., 1986; Braha et al., 1990). Another modulatory agent, small cardioactive peptide (SCP_b), shares some actions with 5-HT in *Aplysia*. For example, SCP_b broadens spikes, facilitates nondepressed synapses, and elevates levels of cAMP (Abrams et al., 1984; Ocorr and Byrne, 1985; Schacher et al., 1990; Fitzgerald and Carew, 1991). SCP_b differs from 5-HT in some respects, however. For example, SCP_b neither modulates the delayed, voltage-dependent K^+ current ($I_{K,V}$) nor produces long-term facilitation, whereas 5-HT does both (Baxter and Byrne, 1989; Schacher et al., 1990). Also, separate receptors

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mediate the effects of these two agents on adenylate cyclase (Ocorr and Byrne, 1986). In addition, 5-HT produces facilitation in depressed synapses in culture, whereas SCP_b does not (Schacher et al., 1990). These differences suggest that SCP_b may be unable to modulate the SDI process.

Can the SDI process be inhibited? The peptide Phe-Met-Arg-Phe-NH_2 (FMRFamide) produces decreases in the duration of the presynaptic spike and the amplitude of the EPSP, effects that are due, at least in part, to its ability to increase the probability of opening of the S-K^+ channel (Abrams et al., 1984; Belardetti et al., 1987; Brezina et al., 1987; Mackey et al., 1987; Belardetti and Siegelbaum, 1988; Dale and Kandel, 1990; Critz et al., 1991; Ichinose and Byrne, 1991), and possibly to its ability to modulate the delayed K^+ current (Critz et al., 1991). The effect on the S-K^+ channel may be mediated by the direct action of a metabolite of arachidonic acid (Piomelli et al., 1987; Buttner et al., 1989). In addition, FMRFamide reverses the 5-HT–induced increase in the phosphorylation of proteins in sensory neurons (Sweatt et al., 1989). As FMRFamide generally seems to antagonize the actions of 5-HT, we reasoned that this peptide might also have an inhibitory effect on the SDI process of facilitation produced by 5-HT.

We assessed the effects of these agents on both the duration of action potentials in sensory neurons and the amplitude of the EPSPs that they produce in follower neurons. The LE cluster of the abdominal ganglion contains mechanosensory neurons that innervate the siphon skin (Byrne et al., 1974). We studied the monosynaptic connections that these cells made with follower neurons including the LFS cells, a group of motor neurons that innervate the siphon (Clark and Kandel, 1984; Frost et al., 1988). The connections between the LE cells and their follower neurons are important sites of plasticity in the siphon-gill withdrawal reflex in *Aplysia* (e.g., Kandel and Schwartz, 1982).

We used a protocol designed to minimize the contribution of spike broadening to facilitation, thereby revealing the effects of 5-HT and SCP_b on the SDI process(es) of facilitation. After bathing the ganglion in a high concentration of the K^+ channel blocker tetraethylammonium (TEA), action potentials evoked in the sensory neurons are broadened (prebroadened spikes) into a range of durations in which additional broadening of the spike (e.g., by 5-HT or SCP_b) elicits little or no increase in the amplitude of the EPSP (Gingrich and Byrne, 1985; Hochner et al., 1986; see also Goldsmith and Abrams, 1991). We also examined the effects of all three neuromodulators on the input resistance of follower neurons in order to detect changes in the postsynaptic component of this two-cell connection (see also Frost et al., 1988).

Our work confirms and extends a number of findings concerning the SDI process, and also provides the first demonstration of inhibition of this process. Unlike 5-HT, SCP_b failed to activate the SDI process in our preparation. FMRFamide inhibited the 5-HT–induced activation of the SDI process after a 5 min but not a 1 min preexposure. FMRFamide also decreased the input resistance of follower cells. This postsynaptic effect may act to supplement the presynaptic inhibitory effects of FMRFamide, further reducing the efficacy of the connection. Thus, 5-HT and FMRFamide modulate multiple processes controlling sensorimotor synaptic transmission, and FMRFamide appears to affect processes distributed across the presynaptic and postsynaptic components of this connection.

Preliminary reports of this work have appeared previously in abstract form (Pieroni and Byrne, 1989, 1990).

Materials and Methods

Aplysia californica (125–300 gm) were obtained from Alacrity Marine Biological Specimens (Redondo Beach, CA), Marine Specimens Unlimited (Pacific Palisades, CA), and Marinus, Inc. (Long Beach, CA). Animals were maintained in individual perforated cages at 15°C in aquaria filled with aerated artificial seawater (ASW; Instant Ocean, Aquarium Systems, Mentor, OH). Animals were exposed to a 12 hr light/12 hr dark cycle and were fed an amount of dried seaweed sufficient to maintain a constant body weight.

Prior to dissection, animals were anesthetized by injection of a volume of isotonic $MgCl_2$ equal to approximately one-half of their body volume. Abdominal ganglia were removed and rinsed in a cold mixture of artificial seawater (ASW; buffered to pH 7.6 with 10 mM Trizma, Sigma) and isotonic $MgCl_2$ (50:50, v/v) containing 0.5% glutaraldehyde (Sigma, St. Louis, MO) for 35 sec in order to reduce TEA- and 5-HT-induced contractions in the connective tissue sheath (Mirolli and Gorman, 1968; Byrne et al., 1979). Ganglia were then pinned to the floor of a Sylgard (Dow Corning, Midland, MI)-lined chamber (300 μl volume) and maintained in ASW/isotonic $MgCl_2$ (50:50, v/v) during dissection. Cells in the ventral left hemiganglion were exposed by surgical removal of the sheath, and subsequently the ASW/$MgCl_2$ solution was replaced with ASW. Sensory neurons in the LE cluster (Byrne et al., 1974) and follower neurons (usually LFS cells; Frost et al., 1988) were identified by their size, location, and electrophysiological properties. Sensory neurons with resting membrane potentials greater than −35 mV were considered acceptable, as were follower neurons with resting membrane potentials greater than −20 mV (in ASW containing TEA).

The static ASW bathing solution contained 100 mM TEA chloride (Eastman Kodak, Rochester, NY) and 240 μM TTX (Calbiochem, La Jolla, CA). Pretreatment of the ganglion with TEA blocks $I_{K,V}$ and the calcium-dependent K^+ current ($I_{K,Ca}$; Baxter and Byrne, 1989; Walsh and Byrne, 1989) and broadens the sensory neuron spikes into a range of spike durations in which additional broadening alone has little or no effect on release of transmitter (Gingrich and Byrne, 1985; Hochner et al., 1986; see also Goldsmith and Abrams, 1991). Under this condition, facilitatory changes in release occur relatively independently of spike broadening and therefore are due primarily to activation of a process(es) of a spike duration–independent nature (Gingrich and Byrne, 1985; Hochner et al., 1986). TTX was added to the bath in order to avoid multiple action potentials being triggered in the sensory neuron. TTX also reduced both spontaneous PSPs in the follower neuron and polysynaptic input (elicited by neurons interposed between the sensory and follower neurons) to the follower neuron. Serotonin (creatinine sulfate complex) was obtained from Sigma, and FMRFamide and SCP_b were obtained from Peninsula Laboratories (Belmont, CA). These agents were delivered to the static bath in 5 μl volumes, using the same solution as contained in the bath solution (100 mM TEA in ASW) as vehicle. The concentrations are similar to those used in other studies on sensory neurons (e.g., Abrams et al., 1984; Ocorr and Byrne, 1985, 1986; Brezina et al., 1987; Baxter and Byrne, 1989, 1990; Blumenfeld et al., 1990; Edmonds et al., 1990; Schacher et al., 1990). Two concentrations were used in experiments with SCP_b (4 and 25 μM), but the effects of these concentrations were indistinguishable and apparently maximal (a concentration of 50 μM, used in separate unpublished experiments, did not produce greater effects). Appropriate concentrations of stock solutions were made daily and kept on ice. Serotonin was protected from exposure to light. Experiments were conducted at room temperature (approximately 21°C).

Presynaptic, calcium-mediated (in TTX) spikes were initiated in sensory neurons by suprathreshold depolarizing constant current pulses (10–17 nA, 1.5 msec) at 60 sec intervals. Follower neurons were hyperpolarized (via a second microelectrode) by approximately 30 mV from the resting membrane potential to prevent spiking during evoked EPSPs. In some experiments, input resistance of follower neurons was assessed by delivering brief (1.5 sec) hyperpolarizing current pulses at 60 sec intervals (30 sec before each stimulation). In the figures, spikes and EPSPs labeled *initial* were generated approximately 10 min (rest period) after a synaptic connection was established. Data were digitized and stored on a computer, and also recorded with a Gould pen recorder. The peak amplitude of the monosynaptic EPSPs and the half-amplitude spike duration (duration from peak of the spike to 50% of its repolarization to the resting membrane potential) were calculated by a computer program. Input resistances were calculated from records made on the Gould recorder.

Data (normalized to initial values) are presented as mean percentage

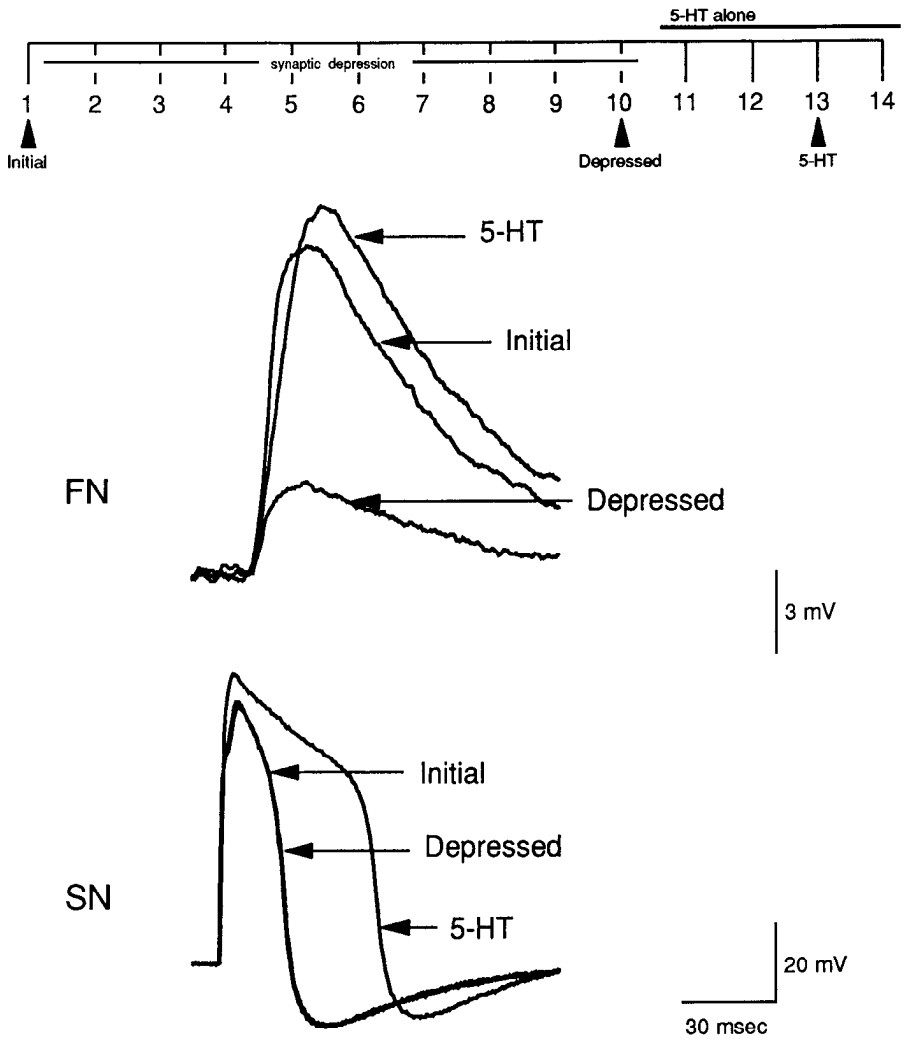

Figure 1. Changes in the durations of action potentials in a sensory neuron (*SN*) and in the amplitudes of EPSPs in a follower neuron (*FN*) during homosynaptic depression and application of 5-HT: representative traces. Spikes were elicited by 1.5 msec constant current pulses. Repeated spikes in the *SN* (at 60 sec intervals) led to depression of EPSPs in the *FN*, but little or no change in spike duration. Application of 5-HT (25 μM) increased the duration of the prebroadened spike and produced pronounced facilitation of the depressed EPSP. The *time line* (in min) at the *top* summarizes the experimental protocol, and *arrowheads* indicate which traces are presented.

change ± SEM of the amplitude of the EPSP, the duration of the spike, or the input resistance (unless noted). In some figures, data from pairs of consecutive spikes and EPSPs are averaged (referred to as "blocks of stimuli" or "blocks" in figures and text). For multiple comparisons of the data presented in Figures 2, 4, 7, 9, and 10, a two-factor [treatment and time (blocks)] analysis of variance (ANOVA) with repeated measures was used to test for overall significance. Post hoc tests were used when the F statistic was significant for treatment and/or interaction tests. The Dunnett's test (two-tailed, post hoc) for multiple comparisons of the control mean to experimental means was used for comparisons *between* treatment groups, and the Tukey test (two-tailed, post hoc) was used for comparisons *within* treatment groups. Values of $p < 0.05$ were considered significant. Critical values for the $F_{\text{numerator DF, error DF}}$ statistic, the $q'_{\text{error DF}, p}$ error term (Dunnett's), and the $q_{\text{error DF}, k}$ error term (Tukey) are from Zar (1984). Data are plotted in a minute-by-minute format (amplitude of the EPSP vs duration of the spike) in Figures 5 and 8.

Results

In a series of experiments that will be discussed in detail below, the effects of three different treatments on both the duration of action potentials and the amplitude of evoked EPSPs in depressed synapses were examined. One treatment, 5-HT alone, was applied in order to produce activation of the SDI process of facilitation. Another treatment, SCP_b, was examined for its ability to activate this process. The third treatment examined the inhibitory effect of a 5 min preexposure to FMRFamide on the 5-HT–induced activation of the SDI process. In order to compare the effects of these three treatments on the EPSP, it was necessary to use a two-factor [treatment and time (blocks of stimuli)] ANOVA with repeated measures. This overall statistical analysis indicated that there was a significant effect on the amplitude of the EPSP ($F_{2,34} = 22.78$, $p < 0.001$ for treatment; $F_{6,102} = 13.84$, $p < 0.001$ for treatment–time interaction). A second two-factor ANOVA compared the effects of the three treatments on spike duration and indicated that there was a nonsignificant treatment effect ($F_{2,34} = 3.53$) and a significant interaction effect ($F_{6,102} = 3.37$, $p < 0.01$). The effects of the three treatments on both amplitude of the EPSP and spike duration were further analyzed in post hoc statistical comparisons, both between treatment groups (comparing each of the experimental groups to 5-HT alone) and within treatment groups [comparing different times (blocks of stimuli)]. These analyses are presented in detail below.

5-HT activates the SDI process of facilitation

Spikes were initiated in sensory neurons once every 60 sec. Ten spikes (in a few cases, nine spikes) were initiated to depress the synapse prior to the application of 5-HT. Such repetitive stimulation resulted in marked homosynaptic depression (Figs. 1; 2*A*, blocks I, II). The average amplitude of the initial EPSP was 10.0 ± 3.2 mV (mean ± SEM; $n = 13$). Synaptic depression reduced the EPSP to 33.1 ± 3.0% of its initial amplitude (Fig.

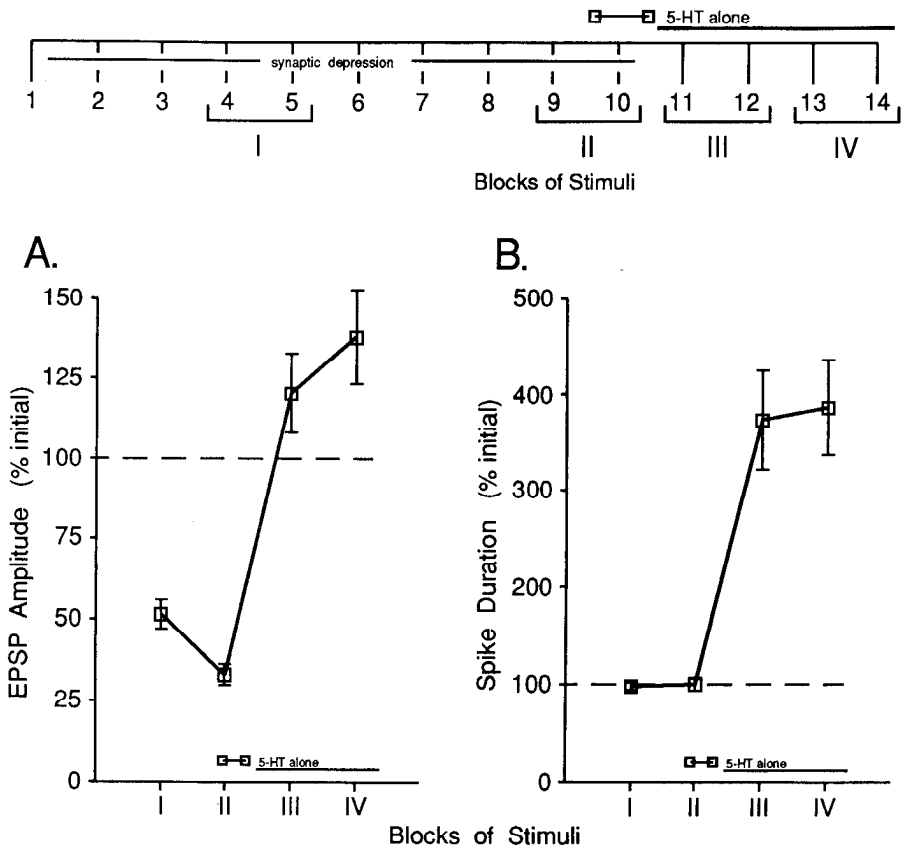

Figure 2. Effects of 5-HT on EPSP amplitude and spike duration: group data. *A*, Application of 5-HT alone (25 μM; $n = 13$) produced facilitation of the depressed EPSP to 137.8 ± 15.0% of its initial amplitude (*block IV*). *B*, Application of 5-HT alone broadened the spike to 385.9 ± 48.4% of its initial duration (*block IV*). Data were normalized to initial values (100%). Blocks of stimuli (*I–IV*) represent averaged pairs of normalized values for sequential EPSP amplitudes and spike durations as illustrated on the *time line*. The *broken lines* at 100% represent initial values for EPSP amplitude and spike duration. *Error bars* represent SEM.

2*A*, block II). Despite the dramatic synaptic depression, spike duration remained relatively constant (Figs. 1; 2*B*, blocks I, II). Thus, synaptic depression can occur independent of changes in the width of the presynaptic spike.

Application of 5-HT alone (25 μM) to depressed synapses produced a facilitation of the EPSP (Figs. 1; 2*A*, blocks III, IV). The EPSP significantly increased from 33.1 ± 3.0% (Fig. 2*A*, block II) to 119.9 ± 12.4% (block III) of its initial amplitude ($q_{102,4} = 11.89$, $p < 0.001$). The EPSP increased further during the following period (block IV) to 137.8 ± 15.0% of its initial amplitude. The enhancement of the EPSP, in depressed synapses with prebroadened spikes, is attributed to 5-HT–induced activation of the SDI process of facilitation. In addition, 5-HT produced an increase in spike duration (Figs. 1; 2*B*, blocks III, IV). The average duration of the initial spike was 15.9 ± 1.6 msec ($n = 13$). Application of 5-HT alone significantly broadened the spike from 101.2 ± 4.6% (Fig. 2*B*, block II) to 373.7 ± 51.0% (block III) of its initial duration ($q_{102,4} = 10.40$, $p < 0.001$).

SCP_b fails to activate the SDI process of facilitation

The experiment presented in Figure 3 demonstrates the effect of SCP_b. The pronounced increase in spike duration produced by SCP_b was accompanied by very modest facilitation. The application of SCP_b (4–25 μM) did not produce significant facilitation of the EPSP (Fig. 4*A*, blocks III, IV, solid circles). The average amplitude of the initial EPSP was 8.1 ± 2.0 mV ($n = 11$). Application of SCP_b to depressed synapses increased the EPSP from 31.2 ± 2.7% (Fig. 4*A*, block II) to 50.1 ± 8.2% (block III) of its initial amplitude, and this increase was not significant. The facilitatory response to SCP_b was significantly less than that produced by 5-HT alone at both time periods examined (SCP_b vs 5-HT alone at block III: $q'_{34,3} = 4.56$, $p < 0.01$; at block IV: $q'_{34,3} = 7.16$, $p < 0.01$). SCP_b did, however, produce an increase in spike duration (Figs. 3; 4*B*, blocks III, IV). The average duration of the initial spike was 16.4 ± 1.0 msec ($n = 11$). Application of SCP_b significantly broadened the spike from 97.6 ± 3.4% (Fig. 4*B*, block II) to 266.2 ± 22.4% (block III) of its initial duration ($q_{102,4} = 5.92$, $p < 0.001$). SCP_b-induced spike broadening was not significantly different from that produced by 5-HT alone at block III, but at block IV a difference was detected (SCP_b vs 5-HT alone at block IV: $q'_{34,3} = 3.03$, $p < 0.01$). In general, 5-HT–induced spike broadening persisted longer than that of SCP_b. Nevertheless, SCP_b, like 5-HT, produced significant broadening of the presynaptic spike but, unlike 5-HT, produced little facilitation of the EPSP. These data suggest that SCP_b does not significantly facilitate via the SDI process.

The ability of 5-HT to activate the SDI process subsequent to application of SCP_b was also examined. As seen in Figure 3, application of 5-HT (4 min after application of SCP_b) produced pronounced facilitation, yet (in this example) somewhat less spike broadening than that produced by SCP_b. Application of 25 μM 5-HT (4–7 min after application of SCP_b, which was still present in the bath; $n = 11$) produced a pronounced facilitation of the EPSP (Fig. 4*A*, blocks V, VI), but only increased the spike duration a small amount above the maximum broadening seen with SCP_b alone (Fig. 4*B*, compare blocks III, VI). The EPSP was increased from 28.2 ± 4.5% (Fig. 4*A*, block IV) to 138.2 ± 28.4% (block VI) of its initial amplitude, and the spike was broadened from 189.3 ± 12.2% (Fig. 4*B*, block IV) to 280.1 ± 22.2% (block VI) of its initial duration. The fact that 5-HT (after

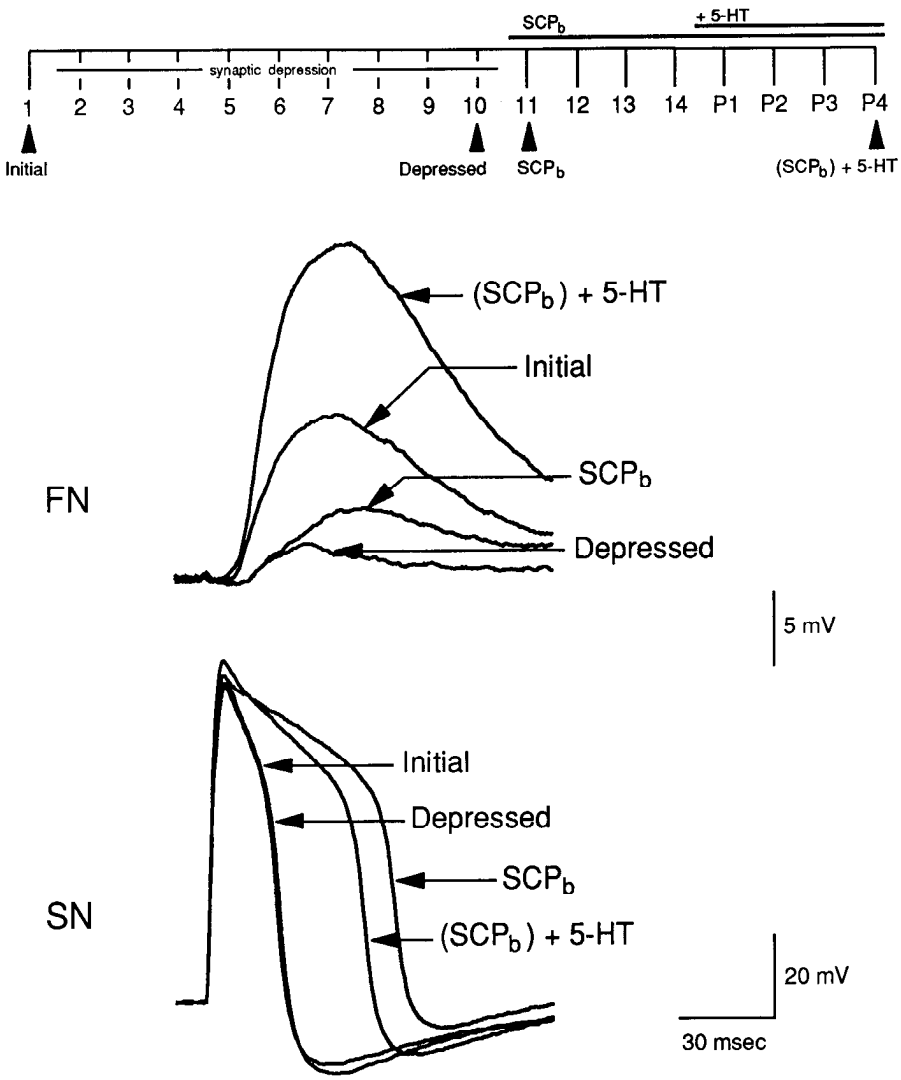

Figure 3. Changes in the durations of action potentials in a sensory neuron (*SN*) and in the amplitudes of EPSPs in a follower neuron (*FN*) during sequential applications of SCP_b and 5-HT: representative traces. Application of SCP_b (25 μM) increased the duration of the prebroadened spike but produced little facilitation of the depressed EPSP. Subsequent application of 5-HT (25 μM, in the continued presence of SCP_b) produced a pronounced facilitation of the EPSP despite the fact that, in this example, the duration of the 5-HT–broadened spike was somewhat less than that produced by SCP_b alone. Stimuli delivered in the presence of 5-HT (post-SCP_b) are denoted *P1–P4* on the *time line.*

SCP_b) produced facilitation in the same cells in which SCP_b did not (e.g., Fig. 4*A*, compare blocks III, VI) also demonstrates that these cells were capable of facilitation.

Additional insights into the differential effects of 5-HT and SCP_b were revealed when the data from experiments illustrated in Figure 4 ("blocks of stimuli") were plotted as the amplitude of the EPSP versus spike duration over the course of successive stimulations (Fig. 5). Stimuli 1–10 (Fig. 5, open squares, solid circles) produced synaptic depression but no change in spike duration. Immediately after application of 5-HT alone, there was a concomitant increase in the spike duration and the amplitude of the EPSP in the depressed synapse (Fig. 5, stimulus 11, open square). Application of SCP_b produced spike broadening and a modest enhancement of the EPSP (stimulus 11, solid circle). Thereafter, the effects of 5-HT and SCP_b diverged. Cells exposed to 5-HT alone exhibited continued increases in the amplitude of the EPSP accompanied by an increase (stimulus 12, open square), and later a decrease (stimulus 13, open square), in spike duration. In contrast, cells exposed to SCP_b exhibited a large decrease in the amplitude of the EPSP with only a small decrease in the spike duration (stimulus 12, solid circle). Application of 5-HT (4–7 min after the application of SCP_b) restored the spike duration to the maximally broadened value produced by SCP_b alone (compare stimuli P2–P4, solid circles, with stimulus 11, solid circle). The EPSP, however, was facilitated well beyond the modest enhancement produced by SCP_b alone (compare stimuli P2–P4, solid circles, with stimulus 11, solid circle). Indeed, the facilitation of the EPSP produced by 5-HT after SCP_b was comparable to that produced by 5-HT alone (compare stimuli P2–P4, solid circles, with stimuli 12–14, open squares). These data demonstrate dissociations in the relationship between the amplitude of the EPSP and the duration of the spike. Specifically, 5-HT alone produced facilitation even under circumstances in which spike duration was decreasing (e.g., stimulus 13, open square). In addition, SCP_b did not produce pronounced facilitation even though it produced pronounced spike broadening. SCP_b-induced spike broadening may have contributed to the slight facilitation produced under these conditions (e.g., stimulus 11, solid circle), but it appears that this contribution was short-lived (limited to the first minute after application of SCP_b).

FMRFamide inhibits the SDI process of facilitation

The effect of FMRFamide on the SDI process produced by 5-HT was investigated in depressed synapses with prebroadened spikes (Figs. 6, 7). The synapse was depressed by initiating five

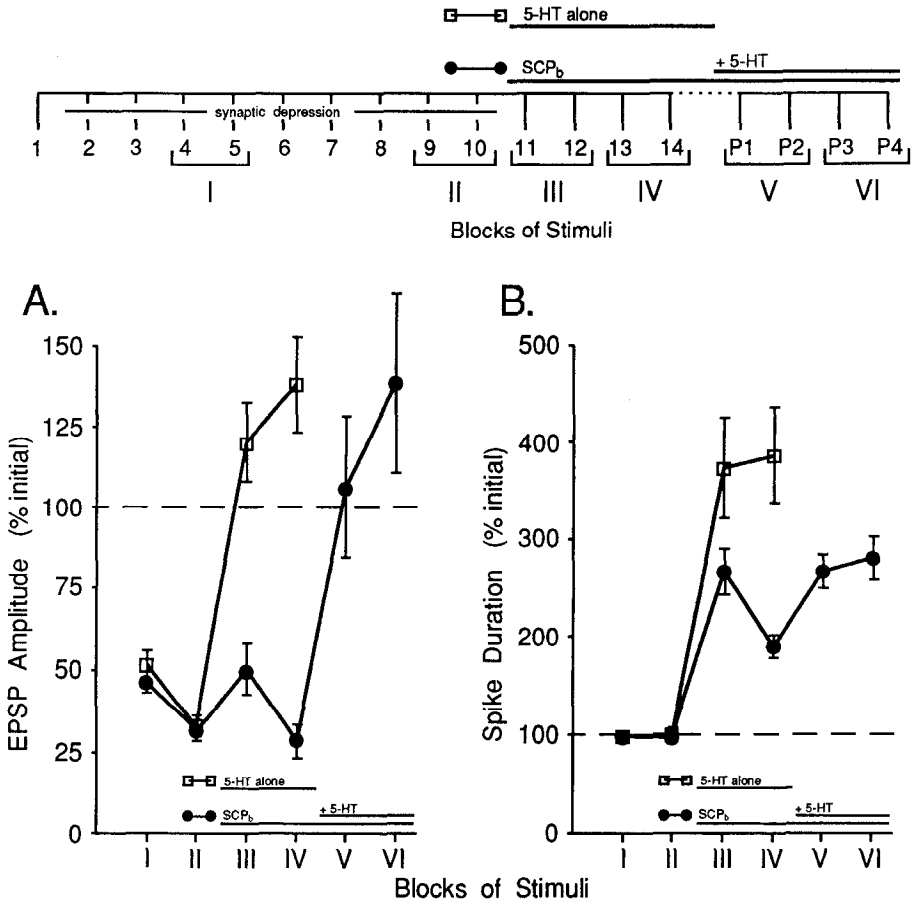

Figure 4. Effects of sequential applications of SCP_b and 5-HT on EPSP amplitude and spike duration (comparison with the effects of 5-HT alone): group data. Data from Figure 2 (5-HT alone, *open squares*) are reproduced here for comparison with SCP_b (*solid circles*). *A*, Application of SCP_b (4–25 μM; $n = 11$) increased the depressed EPSP to 50.1 ± 8.2% of its initial amplitude (*block III*), which was significantly less than that seen with 5-HT alone. Subsequent application of 5-HT (25 μM; $n = 11$) in the continued presence of SCP_b led to a facilitation of the EPSP to 138.2 ± 28.4% of its initial amplitude (*block VI*). *B*, Application of SCP_b broadened the spike to 266.2 ± 22.4% of its initial duration (*block III*). This broadening was not significantly different than 5-HT alone at *block III*, but was at *block IV*. Subsequent application of 5-HT to the experimental group broadened the spike to 280.1 ± 22.2% of its initial duration (*block VI*). Stimuli delivered after application of 5-HT in the continued presence of SCP_b (post-SCP_b; *solid circles*) are denoted *P1–P4* on the *time line*. During the period indicated by the *dotted portion* of the time line (in this and the following figure), stimulations were continued at the normal rate (60 sec intervals), but the time between stimulus *14* and the application of 5-HT (stimulus *P1*) varied between 1 and 4 min (i.e., the stimulus following the application of 5-HT, whether it was stimulus 15 or stimulus 18, is represented by stimulus *P1*).

spikes (at 60 sec intervals) in the sensory neuron, and then FMRFamide (30 μM) was added to the bath. Following initiation of five more spikes, 5-HT (25 μM) was added to the bath. Thus, the application of 5-HT alone (e.g., Figs. 1, 2) produces facilitation via the SDI process, whereas the application of 5-HT following FMRFamide represents the experimental protocol for detecting the effect of FMRFamide on this process.

Prior to the application of FMRFamide, the average amplitude of the initial EPSP was 7.8 ± 1.9 mV ($n = 13$). FMRFamide was applied after five spikes had been evoked in the sensory neuron (Figs. 6; 7, block II). When 5-HT was added to the bath 5 min after the application of FMRFamide (Figs. 6; 7*A*, blocks III, IV), the facilitatory effect of 5-HT was inhibited as compared to 5-HT alone. Application of 5-HT (in the continued presence of FMRFamide) produced an enhancement of the EPSP from 20.0 ± 3.9% (Fig. 7*A*, block II) to 62.7 ± 12.2% (block III) of its initial amplitude, and this increase was significant ($q_{102,4} = 5.85, p < 0.001$). The 5-HT–induced facilitation in the presence of FMRFamide was significantly less than that seen with 5-HT alone, however, at both time periods examined (FMRFamide + 5-HT vs 5-HT alone at block III: $q'_{34,2} = 3.89, p < 0.01$; at block IV: $q'_{34,2} = 6.17, p < 0.01$). The facilitation produced by 5-HT alone was still increasing during the later time period (Fig. 7*A*, block IV), while that produced by 5-HT in the presence of FMRFamide was decreasing. These results indicate that FMRFamide inhibits the SDI process of facilitation produced by 5-HT.

Application of 5-HT in the continued presence of FMRFamide (Figs. 6; 7*B*, blocks III, IV) also produced an increase in spike duration. The average duration of the initial spike was 20.3 ± 2.4 msec. 5-HT (applied after a 5 min preexposure to FMRFamide) significantly broadened the spike from 74.5 ± 4.9% (Fig. 7*B*, block II) to 250.1 ± 52.6% (block III) of its initial duration ($q_{102,4} = 6.70, p < 0.001$). This broadening was not significantly different from that seen with 5-HT alone (FMRFamide + 5-HT vs 5-HT alone at block III: $q'_{34,3} = 1.99$; at block IV: $q'_{34,2} = 1.88$).

When the data from experiments illustrated in Figure 7 ("blocks of stimuli") were plotted as amplitude of the EPSP vs spike duration over the course of successive stimulations (Fig. 8), the effects of 5-HT in the presence of FMRFamide (solid triangles) resembled those of SCP_b alone (compare Fig. 5, solid circles). Immediately after the application of 5-HT (after stimulus 10), in either the absence or presence of FMRFamide, there was a concomitant increase in both spike duration and the amplitude of the EPSP, although FMRFamide attenuated both effects (Fig. 8, stimulus 11, solid triangle) as compared to 5-HT alone (stimulus 11, open square). Thereafter, the inhibitory effect of FMRFamide on 5-HT–induced facilitation became clearer, as the amplitude of the EPSP decreased even though the duration of the spike increased (stimulus 12, solid triangle). These data demonstrate a further dissociation in the relationship between the amplitude of the EPSP and spike duration. Specifically, 5-HT in the presence of FMRFamide did not produce

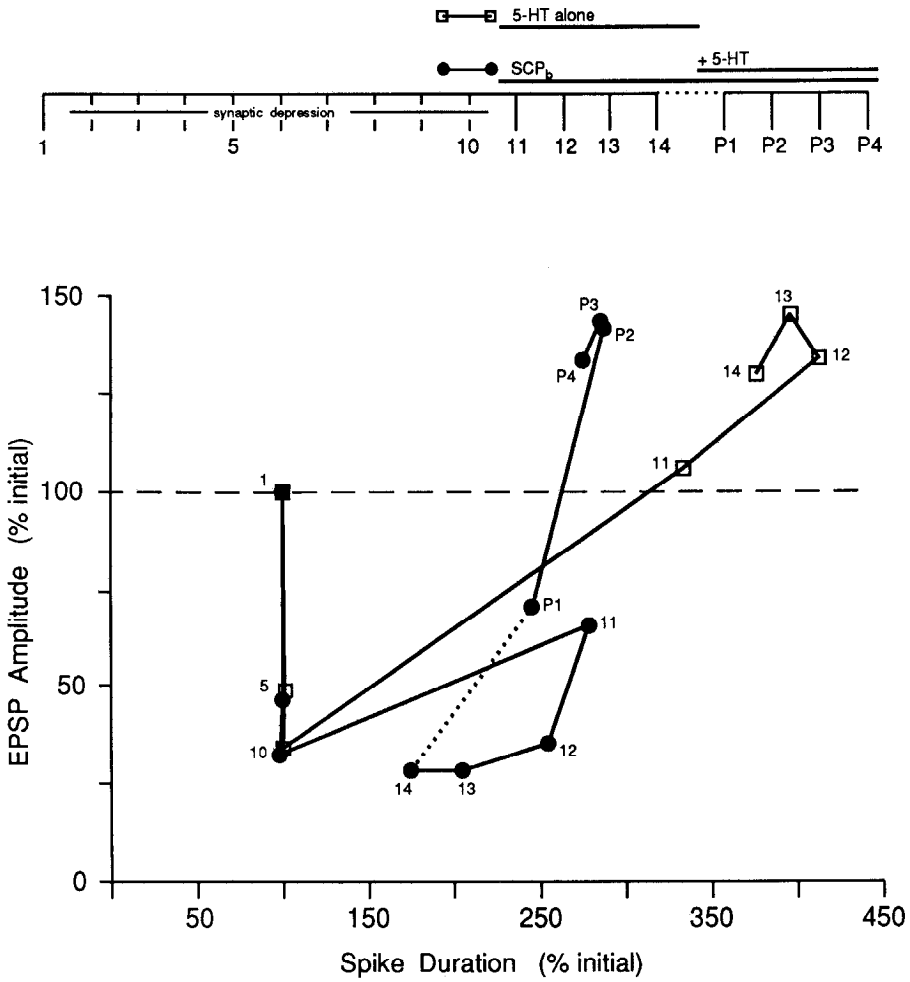

Figure 5. Differential effects of SCP$_b$ and 5-HT on the relationship between EPSP amplitude and spike duration over the course of successive stimulations. Data from experiments illustrated in Figure 4 ("blocks of stimuli") are plotted here to reveal the relationship between the amplitude of the EPSP and spike duration on a minute-by-minute basis. Both 5-HT (*open squares*; $n = 13$) and SCP$_b$ (*solid circles*; $n = 11$) increased spike duration, but only 5-HT produced facilitation of the EPSP above initial values (e.g., stimuli *11–14*). Application of 5-HT after SCP$_b$ produced facilitation (e.g., stimuli *P2–P4*) that was approximately equivalent to that seen with 5-HT alone. The spike broadening that accompanied the (post-SCP$_b$) 5-HT–induced facilitation, however, was similar to that seen with SCP$_b$ alone. The EPSPs and spikes plotted are indicated on the *time line*. Stimuli delivered in the presence of 5-HT (post-SCP$_b$; *solid circles*) are denoted *P1–P4* on the time line. The *broken line* at 100% in this figure represents the initial value for EPSP amplitude. For explanation of *dotted portion* of the time line, see Figure 4 caption.

pronounced facilitation even though it produced pronounced spike broadening. Furthermore, as with SCP$_b$, if 5-HT–induced spike broadening did contribute to the enhancement of the EPSP exhibited in the presence of FMRFamide (e.g., stimulus 11, solid triangle), it appears that this contribution was limited to the first minute after application of 5-HT.

The time of onset of FMRFamide-induced inhibition of facilitation is greater than 1 min

We have begun to examine to what extent the FMRFamide-induced inhibition of the activation of the SDI process by 5-HT is dependent on the timing of the applications of these modulatory agents. As a first step, the lag time between applications of FMRFamide (30 μM) and 5-HT (25 μM) was reduced from 5 min to 1 min (Fig. 9). For this study, a new set of experiments examining the effects of 5-HT alone were performed in which 5-HT was applied after the sixth stimulation. There was no significant difference between the 5-HT–induced enhancement of the EPSP in the absence and presence of a 1 min preexposure to FMRFamide (Fig. 9*A*; two-factor ANOVA: $F_{1,10} = 0.13$ for treatment; $F_{3,30} = 0.86$ for treatment–time interaction). The average amplitude of the initial EPSP was 14.9 ± 3.8 mV ($n = 6$) in the 5-HT alone group and 11.1 ± 2.0 mV ($n = 6$) in the experimental group (prior to the application of FMRFamide). FMRFamide (1 min preexposure) did not inhibit the facilitation produced by 5-HT (Fig. 9*A*, blocks III, IV). Addition of 5-HT to the bath 1 min after the application of FMRFamide increased the EPSP to 88.7 ± 9.1% of its initial amplitude, as compared to 99.4 ± 19.0% for the 5-HT alone group (Fig. 9*A*, block III). This is in contrast to the inhibitory effect of FMRFamide on 5-HT–induced facilitation seen with a 5 min preexposure (compare Fig. 7*A*). These data imply that FMRFamide may need a period greater than 1 min (but ≤5 min) in which to initiate or express its inhibitory effect prior to the onset of 5-HT–induced activation of the SDI process.

There was also no significant difference between the absence and presence of FMRFamide pretreatment on 5-HT–induced spike broadening (Fig. 9*B*; two-factor ANOVA: $F_{1,10} = 1.09$ for treatment; $F_{3,30} = 1.30$ for treatment–time interaction). The average duration of the initial spike was 15.7 ± 1.2 msec ($n = 6$) in the 5-HT alone group and 16.5 ± 1.0 msec ($n = 6$) in the experimental group. FMRFamide (1 min preexposure) did not inhibit the spike broadening produced by 5-HT (Fig. 9*B*, blocks III, IV). Addition of 5-HT to the bath (1 min after the application of FMRFamide) broadened the spike to 304.7 ± 54.9% of its initial duration, as compared to 245.4 ± 28.3% for 5-HT alone (Fig. 9*B*, block III). Although FMRFamide (1 min preexposure) did not have an inhibitory effect on 5-HT–induced facilitation, it was nevertheless capable of affecting other processes, such as the input resistance of the follower neuron (see below).

FMRFamide decreases input resistance in postsynaptic cells

In a subset of the experiments illustrated in Figures 2, 4, 7, and 9, the input resistance of follower neurons was assessed by delivering brief hyperpolarizing current pulses at 60 sec intervals

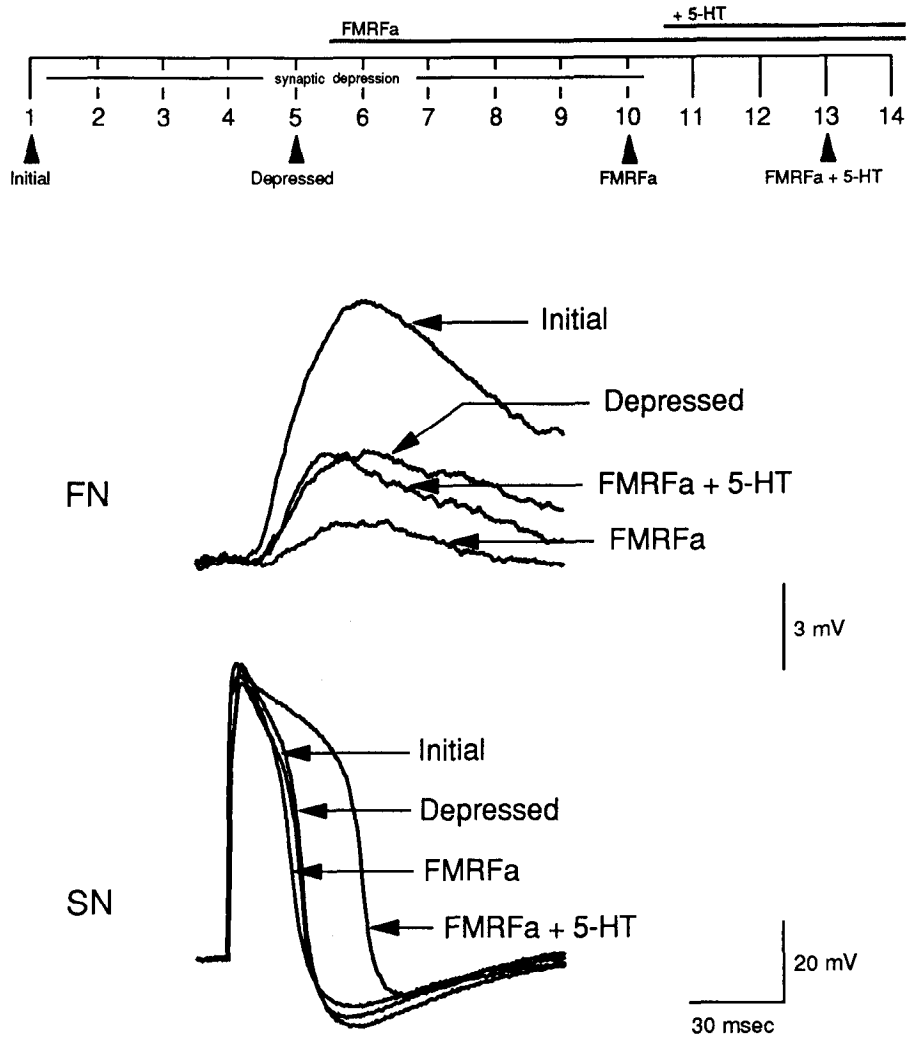

Figure 6. Changes in the durations of action potentials in a sensory neuron (*SN*) and in the amplitudes of EPSPs in a follower neuron (*FN*) during sequential applications of FMRFamide (*FMRFa*) and 5-HT: representative traces. Application of FMRFamide (30 μM) decreased the duration of the prebroadened spike and may have contributed to the further depression of the EPSP. Application of 5-HT (25 μM) in the presence of FMRFamide (5 min preexposure) increased the duration of the prebroadened spike, but produced little facilitation of the EPSP.

(30 sec before each stimulation). Of the three agents tested, only FMRFamide had a significant effect on input resistance. FMRFamide decreased the input resistance in experiments in which its application preceded that of 5-HT by both 5 min (Fig. 10*A*) and 1 min (Fig. 10*B*).

In experiments in which 5-HT alone was applied after 10 spikes (e.g., Fig. 2), the average initial value for input resistance was 25.4 ± 3.1 MΩ ($n = 3$). In experiments in which SCP_b was applied (e.g., Fig. 4) and those in which 5-HT was applied after a 5 min preexposure to FMRFamide (e.g., Fig. 7), average initial values were 17.7 ± 3.7 MΩ ($n = 4$) and 20.9 ± 4.8 MΩ ($n = 6$), respectively. A two-factor ANOVA comparing the effects of these agents gave a nonsignificant treatment effect ($F_{2,10} = 3.57$) and a significant interaction effect ($F_{6,30} = 6.36$, $p < 0.001$). In experiments in which 5-HT alone was applied after six spikes (e.g., Fig. 9) and those in which 5-HT was applied after a 1 min preexposure to FMRFamide (e.g., Fig. 9), average initial values for input resistance were 22.2 ± 3.2 MΩ ($n = 6$) and 31.5 ± 7.7 MΩ ($n = 5$), respectively. A two-factor ANOVA comparing the effects of these agents gave a nonsignificant treatment effect ($F_{1,9} = 4.86$) and a significant interaction effect ($F_{3,27} = 4.67$, $p < 0.02$).

Application of 5-HT alone did not have a significant effect on input resistance in postsynaptic cells, whether it was added to the bath 10 min after the initial spike (Fig. 10*A*, block II vs III, open squares) or 6 min after the initial spike (Fig. 10*B*, block II vs III, open squares). Application of SCP_b also did not have a significant effect on input resistance (data not shown). FMRFamide (solid triangles), however, significantly decreased the input resistance in follower neurons when its application preceded that of 5-HT by either 5 min (Fig. 10*A*; block I vs II: $q_{30,4} = 8.88$, $p < 0.001$) or 1 min (Fig. 10*B*; block I vs II: $q_{27,4} = 6.06$, $p < 0.005$). In both cases (Fig. 10*A,B*), the FMRFamide-induced decrease remained significant after the application of 5-HT (blocks III and IV, solid triangles).

An FMRFamide-induced increase in the postsynaptic conductance could account, in part, for the attenuation of the amplitude of the EPSP seen when FMRFamide is applied alone. Even though the input resistance remained decreased in the presence of 5-HT whether cells were preexposed to FMRFamide for 5 min or 1 min (Fig. 10*A,B*), FMRFamide inhibited 5-HT–induced facilitation only after a 5 min preexposure (e.g., Fig. 7*A*). Thus, it seems unlikely that a decrease in input resistance plays a significant role in FMRFamide-induced inhibition of the facilitation produced by 5-HT.

Discussion

The present study demonstrates the differential effects of 5-HT, SCP_b, and FMRFamide on the SDI process of facilitation. The aminergic modulator 5-HT produced pronounced facilitation

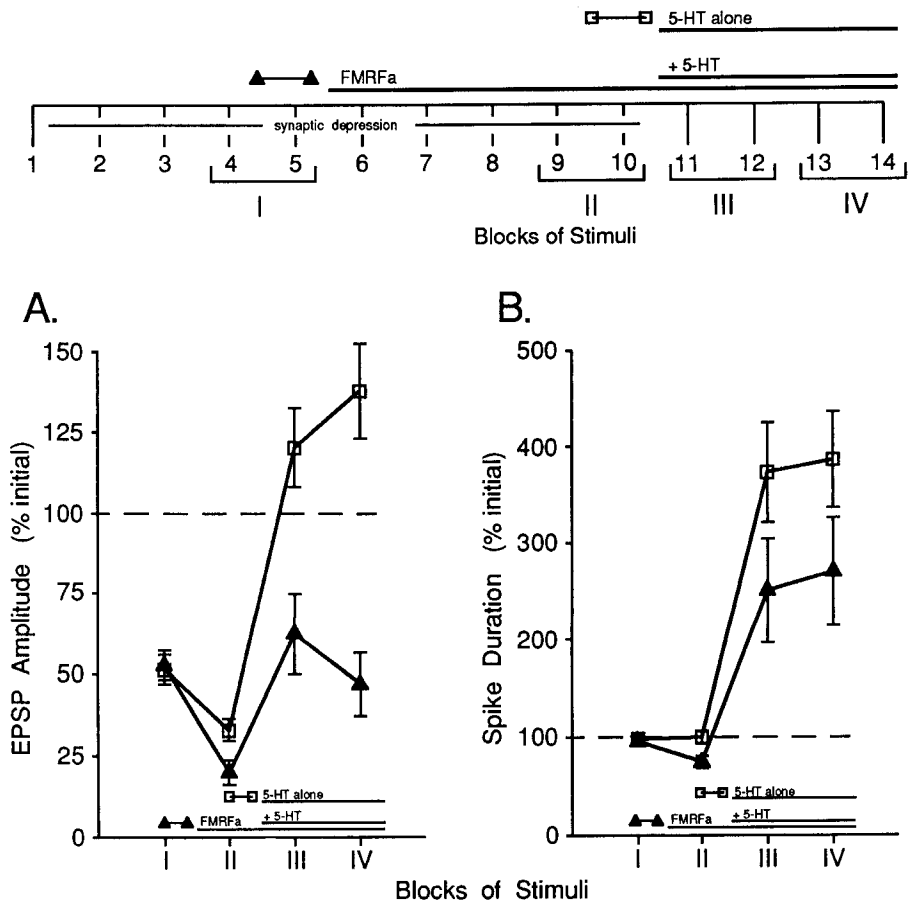

Figure 7. Effects of sequential applications of FMRFamide and 5-HT on EPSP amplitude and spike duration (comparison with 5-HT alone): group data. Data from Figure 2 (5-HT alone, *open squares*) are reproduced here for comparison with 5-HT in the presence of FMRFamide (*solid triangles*). *A*, Application of 5-HT (25 μM; $n = 13$) after a 5 min preexposure to FMRFamide (30 μM) produced an enhancement of the EPSP to 62.7 ± 12.2% of its initial amplitude (*block III*), which was significantly less than that seen with 5-HT alone. *B*, Application of 5-HT, in the continued presence of FMRFamide, broadened the spike to 269.3 ± 55.2% of its initial duration (*block IV*). This broadening was not significantly different from that seen with 5-HT alone.

in depressed synapses via activation of the SDI process. In contrast, the peptide SCP_b, which can facilitate nondepressed synapses (e.g., Schacher et al., 1990), failed to activate the SDI process in our preparation. The peptide FMRFamide inhibited the 5-HT–induced activation of the SDI process, but only after a sufficient period of preexposure. FMRFamide was unique among the three agents tested in having an effect on the input resistance of postsynaptic cells.

Multiple processes involved in presynaptic facilitation

The existence of a facilitatory SDI process (or processes) was proposed as a result of modeling studies. The model indicated that increases in spike duration could not fully account for the facilitation produced by 5-HT, both in depressed synapses and in synapses with prebroadened presynaptic spikes (Gingrich and Byrne, 1985, 1987; Gingrich et al., 1988). This facilitatory process (referred to as mobilization) was modeled as a flux of transmitter-containing vesicles (possibly controlled by one or more second messenger systems) from a storage pool to a releasable pool that is adjacent to the presynaptic active zone. In depressed synapses, for example, mobilization would be responsible for replenishing stores of vesicles in depleted active zones. This model agrees with the morphological finding that synaptic depression is associated with a decreased number of vesicles in the region of the active zone (Bailey and Chen, 1988). Alternatively, the efficiency of the release mechanism itself, rather than or in addition to the availability of transmitter, may be increased by 5-HT (see Gingrich and Byrne, 1985; Hochner et al., 1986; Dale and Kandel, 1990). Our studies verify that 5-HT significantly activates or amplifies a process(es) other than spike broadening, such as mobilization, that is responsible, to a large degree, for facilitation of the EPSP in depressed synapses.

SCP_b apparently acts through a single facilitatory process

The lack of a significant facilitatory effect of SCP_b in depressed synapses provides further evidence for the existence of a facilitatory process(es), such as mobilization, distinct from spike broadening. SCP_b and 5-HT have been shown to be relatively equipotent in their ability to elevate cAMP (Ocorr and Byrne, 1985), and both agents produced significant increases in spike duration. Therefore, the ability of 5-HT, and not SCP_b, to increase significantly the amplitude of the depressed EPSP distinguishes the SDI process of facilitation from that associated with spike duration. Our results agree with those of Schacher et al. (1990), who showed that SCP_b does not facilitate depressed synapses in cultured neurons. As noted previously (Schacher et al., 1990), one possible explanation for the different effects of these two agents is that 5-HT activates the Ca^{2+}/phospholipid-dependent protein kinase (PKC) system (Sacktor and Schwartz, 1990; Kruger et al., 1991), but SCP_b may not. There is some evidence that activation of the SDI process may involve an interaction between the cAMP-dependent protein kinase (PKA) and PKC systems (Braha et al., 1990; Goldsmith and Abrams, 1991). Although our study does not address the actions of SCP_b in nondepressed synapses, SCP_b can produce facilitation under that condition (Abrams et al., 1984; Schacher et al., 1990). Taken in combination with the lack of an effect on the input resistance of follower neurons, the evidence indicates that SCP_b

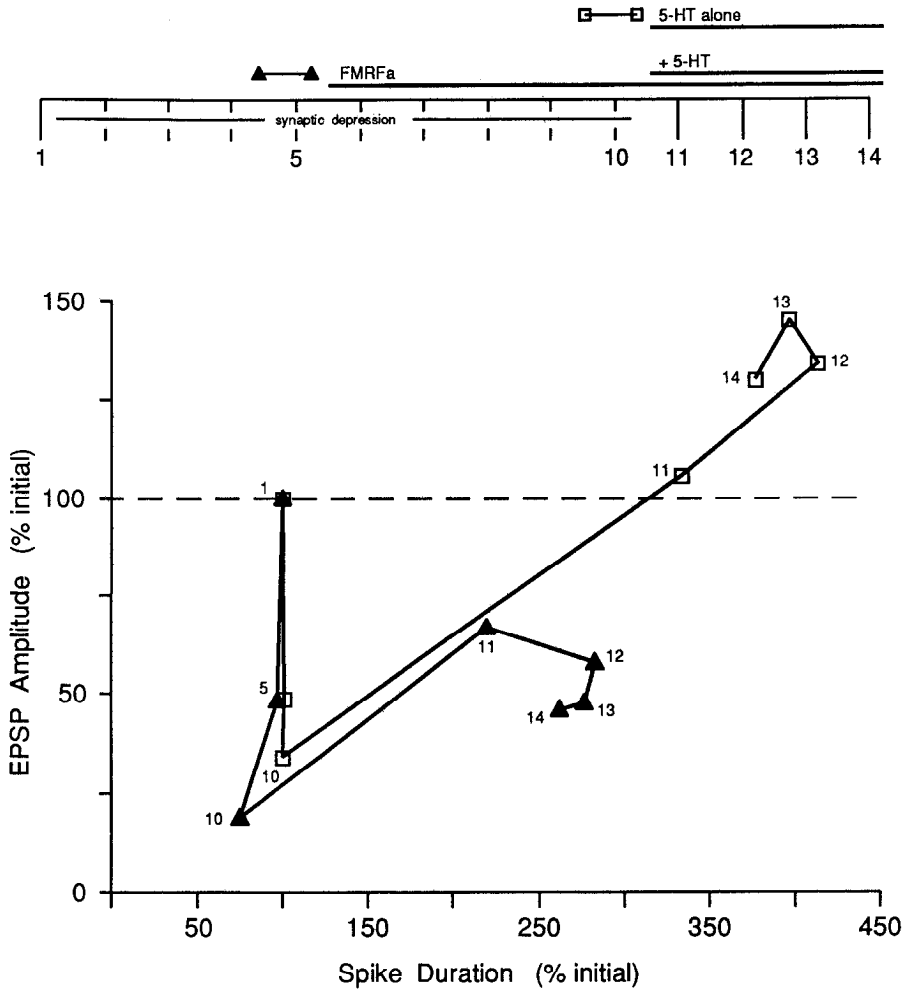

Figure 8. Differential effects of 5-HT in the absence and presence of FMRFamide on the relationship between EPSP amplitude and spike duration over the course of successive stimulations. Data from experiments illustrated in Figure 7 ("blocks of stimuli") are plotted here to reveal the relationship between EPSP amplitude and spike duration on a minute-by-minute basis. In either the absence (*open squares*; $n = 13$) or presence of FMRFamide (*solid triangles*; $n = 13$), 5-HT increased spike duration, but only 5-HT in the absence of FMRFamide (5-HT alone) produced facilitation of the EPSP above initial values (e.g., stimuli *11–14*). The *broken line* at 100% in this figure represents the initial value for EPSP amplitude.

may act through only one process (of those examined) to accomplish facilitation of the monosynaptic EPSP. SCP_b also enhances processes not examined in this study, however, such as excitability (D. A. Baxter and J. H. Byrne, unpublished observations).

The spike broadening produced by 5-HT persisted longer than that produced by SCP_b (e.g., Fig. 4*B*). The doses of SCP_b used in this study (4–25 μM) were probably sufficient to produce maximal SCP_b-induced spike broadening, as spike durations were not increased further by a higher dose (50 μM) in separate experiments (J. P. Pieroni and J. H. Byrne, unpublished observations). One possible explanation for the different activities of these two agents is that the modulation of the delayed or voltage-dependent K^+ current ($I_{K,V}$) by 5-HT, and not by SCP_b, is responsible for the difference in spike broadening (Baxter and Byrne, 1989, 1990). This possibility would seem unlikely, as $I_{K,V}$ should be blocked to a large extent by the dose of TEA (100 mM) used in these studies. In a TEA-broadened spike, however, a small, residual modulation of $I_{K,V}$ by 5-HT could have a relatively large effect on duration. The difference between 5-HT- and SCP_b-induced spike broadening may involve their differential abilities to activate the PKC system, as mentioned above. Several reports indicate that both the transient rise in intracellular levels of Ca^{2+} (in response to depolarizing voltage steps and spike activity) and Ca^{2+} currents are increased by 5-HT (Boyle et al., 1984; Blumenfeld et al., 1990; Edmonds et al., 1990) and that Ca^{2+} currents are increased by phorbol ester (Braha et al., 1988). In addition, a recent study indicates that 5-HT–induced spike broadening is accomplished, in part, through activation of the PKC system (Sugita et al., 1991).

Regardless of the mechanisms involved, the difference between the actions of 5-HT and SCP_b on spike duration probably contributes little to their differential actions on the SDI process. In the presence of TEA, the spikes were already broadened (prior to application of 5-HT or SCP_b) into a range in which additional broadening has little or no effect on facilitation of the EPSP (Gingrich and Byrne, 1985; Hochner et al., 1986). Clearly, we cannot completely rule out the possibility that the increased magnitude of broadening produced by 5-HT, and/or the persistence of that effect, may play a role (perhaps increased buildup of Ca^{2+} and a related increase in mobilization; see below) in the difference between the facilitatory effects of SCP_b and 5-HT. It is noteworthy, however, that even in instances in which SCP_b-induced spike broadening exceeds that of 5-HT, 5-HT–induced facilitation exceeds that of SCP_b (e.g., Fig. 3).

Inhibition of the SDI process by FMRFamide

FMRFamide inhibited the SDI process of facilitation produced by 5-HT. It appears, therefore, that the activation of this facilitatory process can be attenuated by at least one agent. Although we suspect that FMRFamide may have an inhibitory effect on some basal level of mobilization both *in vitro* (see also Dale and Kandel, 1990) and in the behaving animal, we did not address this possibility in the present study. There is, however, some

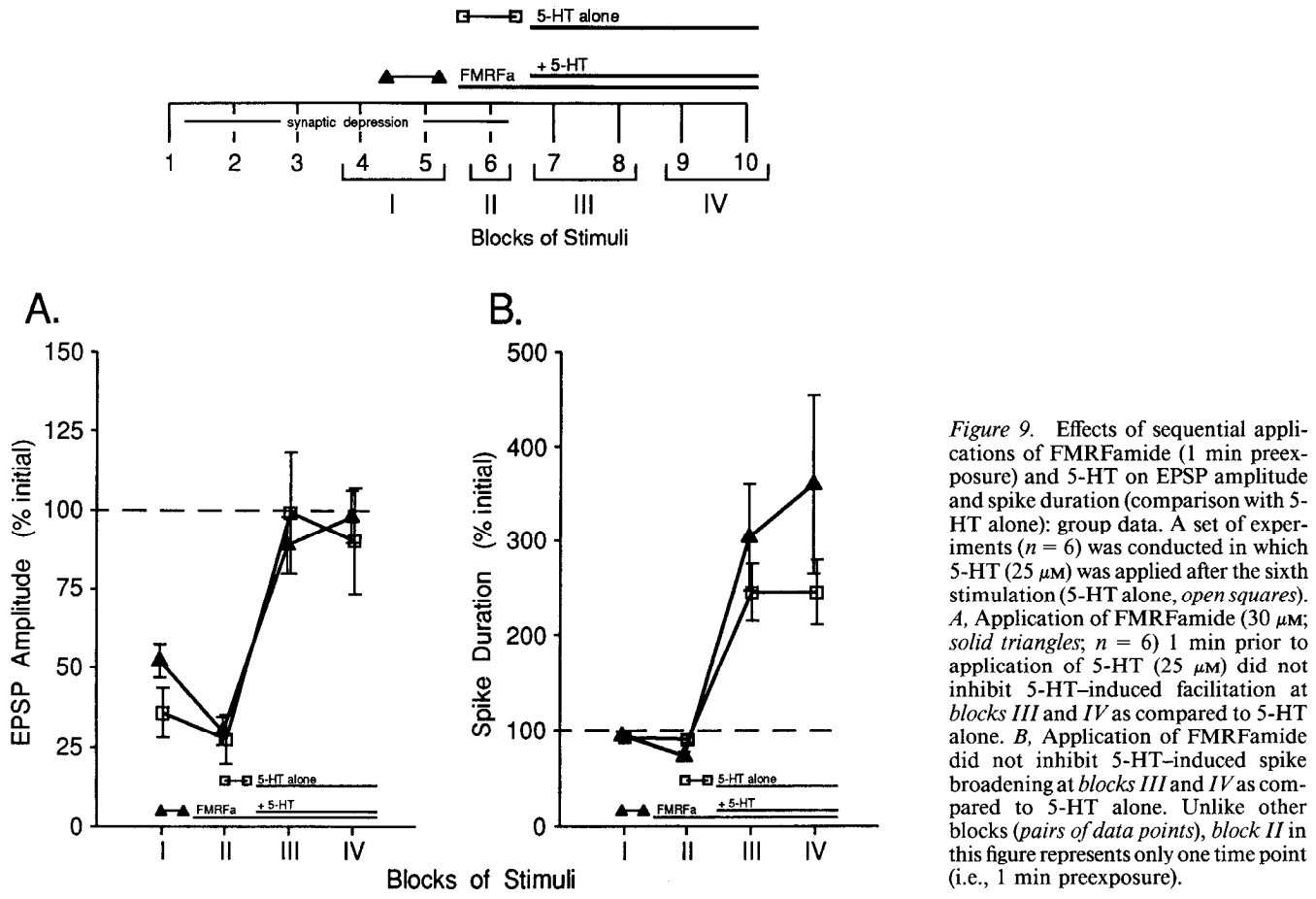

Figure 9. Effects of sequential applications of FMRFamide (1 min preexposure) and 5-HT on EPSP amplitude and spike duration (comparison with 5-HT alone): group data. A set of experiments ($n = 6$) was conducted in which 5-HT (25 μM) was applied after the sixth stimulation (5-HT alone, *open squares*). *A,* Application of FMRFamide (30 μM; *solid triangles*; $n = 6$) 1 min prior to application of 5-HT (25 μM) did not inhibit 5-HT–induced facilitation at *blocks III* and *IV* as compared to 5-HT alone. *B,* Application of FMRFamide did not inhibit 5-HT–induced spike broadening at *blocks III* and *IV* as compared to 5-HT alone. Unlike other blocks (*pairs of data points*), *block II* in this figure represents only one time point (i.e., 1 min preexposure).

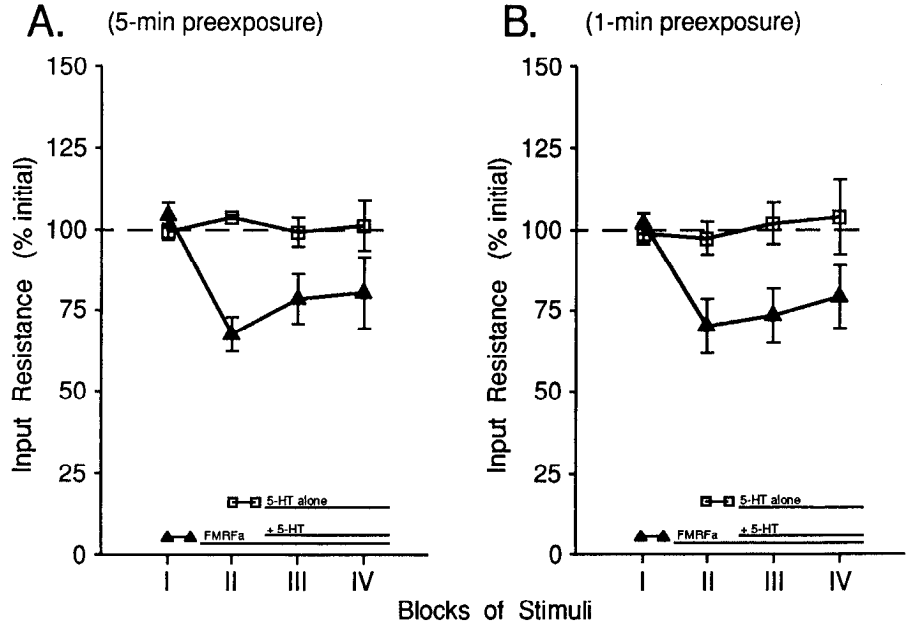

Figure 10. Effects of sequential applications of FMRFamide and 5-HT on the input resistance of follower neurons (comparison with 5-HT alone): group data. In some experiments, the input resistance of follower neurons was assessed by delivering brief, hyperpolarizing current pulses at 60 sec intervals. *A,* Application of 5-HT alone (25 μM; *open squares*; $n = 3$) 10 min after the initial spike did not have an appreciable effect on input resistance at *blocks III* and *IV*. Application of FMRFamide (30 μM; *solid triangles*; $n = 6$) significantly decreased input resistance at *block II* as compared to pre-FMRFamide values (*block I*), and this effect persisted when 5-HT was added to the bath 5 min later (*blocks III* and *IV*). *Blocks of stimuli* in *A* are the same as in Figure 7. *B,* Results are essentially the same for experiments utilizing a 1 min preexposure to FMRFamide. Application of 5-HT alone (25 μM; $n = 6$) produced no clear effect on input resistance, but FMRFamide (30 μM; $n = 5$) produced a significant decrease in input resistance (*block II*) that persisted after application of 5-HT 1 min later (*blocks III* and *IV*). *Blocks of stimuli* in *B* are the same as in Figure 9.

Table 1. Summary of effects of various modulatory agents

	Amplitude of the EPSP		Presynaptic modulatory processes	
Modulator	Short-term	Long-term	Spike duration	SDI
5-HT	Increase[1]	Increase[6,9]	Broaden[2,11,14]	Enhance or activate[4,5,12,14]
SCP_b	Increase[3]	No effect[12]	Broaden[3,14]	No effect[12,14]
FMRFamide	Decrease[3]	Decrease[10]	Narrow[3,7,8,13]	Inhibit[14]
Dopamine	Decrease[3,10]	No effect[10]	Narrow[3]	?

1, Brunelli et al., 1976; 2, Klein and Kandel, 1978; 3, Abrams et al., 1984; 4, Gingrich and Byrne, 1985; 5, Hochner et al., 1986; 6, Montarolo et al., 1986; 7, Mackey et al., 1987; 8, Piomelli et al., 1987; 9, Dale et al., 1988; 10, Montarolo et al., 1988; 11, Baxter and Byrne, 1990; 12, Schacher et al., 1990; 13, Critz et al., 1991; 14, present study.

reason to presume that the inhibition of 5-HT–induced activation of the SDI process by FMRFamide in depressed synapses has a behavioral correlate. For example, a recent behavioral study in *Aplysia* (Marcus et al., 1988) proposed an inhibitory process that attenuates dishabituation produced by strong or multiple shocks to the tail. A possible FMRFaminergic neuron in the left pleural ganglion that could be involved in such an inhibitory response in the abdominal ganglion has been identified (Mackey et al., 1987). FMRFamide-containing neurons that inhibit sensory neurons in the pleural ganglion have also been identified (Xu et al., 1991)

The observation that a 1 min preexposure to FMRFamide did not inhibit 5-HT–induced activation of the SDI process may provide some insight into the mechanisms controlling this process. The finding suggests that a slower mechanism(s) may be involved in the inhibition, as compared to the activation, of the SDI process, and that inhibition cannot be induced once the facilitatory process is engaged. Thus, the relative contributions of different modulators (especially those with opposing actions) to the ultimate response in the synapse are dependent on, and determined by, the relative timing of their release. FMRFamide may inhibit the SDI process by interacting with second messenger systems (such as PKA and PKC), perhaps via alterations in the metabolism of arachidonic acid and/or activation of a phosphatase (Piomelli et al., 1987; Sweatt et al., 1989; Ichinose and Byrne, 1991).

Other possible explanations exist, however, for the differential effects of FMRFamide (1 min vs 5 min preexposure). Perhaps the additional spikes in the presence of FMRFamide (5 min preexposure) elicit an activity-dependent enhancement of presynaptic inhibition (e.g., Small et al., 1989). It should be noted, however, that this associative cellular mechanism was produced in their study by pairing FMRFamide application with a high-frequency train of spikes. Alternatively, FMRFamide may produce its inhibitory action on facilitation by interfering with some Ca^{2+}-dependent mechanism involved in mobilization (see below). Further experiments are necessary to resolve these possibilities.

A large number of agents have been proposed as modulators of various facilitatory and inhibitory processes in sensorimotor synapses, but the specific arrays of the effects of each agent have not been delineated fully as yet. A possible scheme for the effects of one set of agents (see Table 1) emerges from the findings that the SDI process can be inhibited by FMRFamide but cannot be activated by SCP_b. In general, 5-HT and SCP_b have been assigned the role of facilitatory agents, whereas FMRFamide and dopamine are viewed as inhibitory agents. The possibility exists that, just as 5-HT and SCP_b share some facilitatory actions but not others, there may be an agent(s) that inhibits synaptic transmission through the spike duration–dependent process but does not inhibit the SDI process of facilitation (as FMRFamide does). Candidates for such an agent include dopamine (e.g., Abrams et al., 1984; Montarolo et al., 1988), histamine (e.g., Kretz et al., 1986; Chiel et al., 1988), and possibly myomodulin (e.g., Rosen et al., 1989; Critz et al., 1991). Dopamine is especially interesting among these, as it shares with SCP_b an inability to produce a long-term effect on the amplitude of the EPSP, as opposed to 5-HT and FMRFamide, which can produce long-term effects (Table 1). Further, the differential effects of these four agents on long-term plasticity correlate well with the cytochemical localization of the neuronal processes that contain them. Thus, as detailed by Goldstein and Schwartz (1989), SCP_b and dopamine (short-term effect only) are localized to distinct regions of the neuropil, whereas the processes containing 5-HT and FMRFamide (both long- and short-term effects) have a widespread distribution, including the area surrounding neuronal somata (see also Lo et al., 1987; Zhang et al., 1991). Further studies are necessary to determine whether dopamine has an effect on the SDI process. In addition, other agents may selectively modulate the processes that underlie presynaptic facilitation. For example, β-bag cell peptide may inhibit the SDI process without affecting spike duration (Goldsmith and Byrne, 1990, 1991), and buccalin increases excitability of sensory neurons but has variable effects on spike duration (Raymond et al., 1989; Rosen et al., 1989). If this aggregate of modulatory agents (and probably others) were involved in the neuronal circuits controlling sensitization and dishabituation, and each neuromodulator activated or inhibited a different set of processes, the animal would have an extensive repertoire of mechanisms available to fine-tune its behavioral response to a given set of stimuli.

Multiple sites for the inhibitory effects of FMRFamide

Most investigations of sensorimotor synaptic transmission in *Aplysia* have emphasized the presynaptic effects of 5-HT and FMRFamide (Klein and Kandel, 1978; Mackey et al., 1987; Piomelli et al., 1987; Baxter and Byrne, 1989, 1990; Small et al., 1989; Critz et al., 1991). Recently, however, postsynaptic effects of these agents have been detected (Walsh and Byrne, 1985; Frost et al., 1988; Belkin and Abrams, 1990, 1991). In our study, FMRFamide produced an effect on membrane conductance in follower neurons. This decrease in postsynaptic input resistance could play a general role in the inhibitory action of FMRFamide on synaptic transmission. We believe, however, that the decrease in input resistance is not responsible for the inhibition by FMRFamide of 5-HT–induced facilitation, because FMRFamide decreased input resistance even in the ex-

periments (e.g., Fig. 9) in which there was no inhibition of facilitation.

The combined actions on spike duration (e.g., Abrams et al., 1984; Mackey et al., 1987; Piomelli et al., 1987; Critz et al., 1991), the SDI process, and input resistance suggest that FMRFamide modulates multiple, distributed processes at presynaptic and postsynaptic sites. If this arrangement exists as such *in vivo*, it would provide a very potent and comprehensive means of decreasing synaptic efficacy. Our results raise related questions, such as whether there is an inhibitory agent(s) that affects the postsynaptic process exclusively, and whether there is a facilitatory agent(s) that affects all three processes.

The nature of the SDI process

Although the precise nature of the SDI process is unknown, we can speculate on the intracellular events leading to the reversal of homosynaptic depression by 5-HT. There is evidence to implicate both the PKA and PKC systems in the activation of the SDI process (Braha et al., 1990; Schacher et al., 1990; Goldsmith and Abrams, 1991; S. Sugita, J. R. Goldsmith, D. A. Baxter, J. H. Byrne, unpublished observations). Multiple second messenger systems have been associated with modulation of release mechanisms previously (e.g., Higashida, 1988). An alternative hypothesis is that the activation of the SDI process (e.g., mobilization) is directly dependent on elevated levels of Ca^{2+} in a particular presynaptic compartment. For example, Clark and Kandel (1984), Walters and Byrne (1984), and Schacher et al. (1990) have demonstrated that posttetanic potentiation of sensorimotor synapses, which presumably raises intracellular Ca^{2+} levels in the terminals, facilitates depressed synapses (see also Gingrich and Byrne, 1985). These observations raise the possibility that the effects of 5-HT and FMRFamide on the SDI process can be explained simply as a function of changes in levels of Ca^{2+}. 5-HT increases Ca^{2+} transients and Ca^{2+} current, whereas FMRFamide decreases both (Boyle et al., 1984; Blumenfeld et al., 1990; Edmonds et al., 1990).

A number of recent studies indicate, however, that 5-HT and FMRFamide may affect presynaptic release through processes other than modulation of Ca^{2+} channels. For example, although 5-HT modulates a Ca^{2+} current, it does not directly modulate the rapidly inactivating, dihydropyridine-insensitive Ca^{2+} current that plays a major role in transmitter release (Edmonds et al., 1990; Blumenfeld et al., 1991; Eliot et al., 1991). Moreover, in cultured neurons 5-HT increases and FMRFamide decreases spontaneous release of transmitter in the absence of Ca^{2+} influx (Dale and Kandel, 1990). The lack of a critical role for the modulation of Ca^{2+} channels by FMRFamide and 5-HT is also seen in other preparations. For example, in *Helisoma* neurons, FMRFamide reduces the rate of evoked transmitter release by a presynaptic mechanism that involves a pertussis toxin–sensitive G-protein and apparently functions independently of changes in the levels of intracellular Ca^{2+} (Man-Son-Hing et al., 1989; Haydon et al., 1991). Delaney et al. (1991) have shown in excitor motor nerve terminals of the crayfish that 5-HT–induced enhancement of evoked transmitter release is not due to an increase in the presynaptic concentration of Ca^{2+}. They suggest instead that 5-HT acts on the neurosecretory apparatus (perhaps through some second messenger) to enhance release.

How might 5-HT and FMRFamide modulate (via a second messenger system) the presynaptic neurosecretory apparatus in the sensory neurons to affect release? An intriguing, possible mechanism (which parallels the mobilization-type model of the SDI process) involves the synaptic regulatory proteins, the synapsins. Phosphorylation of synapsin I, and its subsequent dissociation from exocytotic vesicles, apparently allows the vesicles to detach from a cytoskeletal anchoring system and fuse with the presynaptic membrane (Llinas et al., 1985, 1991; Goldenring et al., 1986; McGuinness et al., 1989; Hackett et al., 1990; for reviews, see DeCamilli and Greengard, 1986; Südhof and Jahn, 1991). A synapsin-like protein has been identified in nervous tissue of *Aplysia* (Goelz et al., 1985). FMRFamide and 5-HT might affect the function of such a synapsin-like molecule, perhaps through a second messenger system or systems (e.g., PKA, PKC, Ca^{2+}/calmodulin-dependent protein kinase II, metabolites of arachidonic acid, etc.), and thus alter the fusion of vesicles with the membrane.

Thus, a number of second messenger systems may be involved in the activation and inhibition of the spike duration–dependent process. The same may be said for the activation and inhibition of the SDI process of facilitation, and perhaps for mechanisms involved in regulating the membrane conductance of the postsynaptic cells as well. Regardless of the mechanisms involved, however, the present study depicts a distribution of modulatory processes across presynaptic and postsynaptic sites. In principle, the differential actions of the facilitatory and inhibitory agents can have very powerful yet flexible effects on the modulation of synaptic transmission.

In conclusion, 5-HT, SCP_b, and FMRFamide have differential effects on the various processes that modulate sensorimotor synapses in *Aplysia*. Of those processes examined, SCP_b apparently acts through only one process (spike broadening) to produce a facilitatory effect, whereas 5-HT and FMRFamide engage multiple processes leading to facilitatory and inhibitory effects, respectively. The processes modulated by FMRFamide are distributed across the presynaptic and postsynaptic elements of the sensorimotor connection. These results suggest that the behavioral state of the animal may determine which processes are available and/or which modulators are relevant for the modulation of synaptic transmission. For example, modulation of the SDI process (e.g., mobilization) may be most important after the animal has been habituated. Further, SCP_b-induced facilitation (via spike broadening) would be expected to contribute to sensitization, but not to dishabituation.

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