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Pathway-Specific Synaptic Plasticity: Activity-Dependent Enhancement and Suppression of Long-Term Heterosynaptic Facilitation at Converging Inputs on a Single Target

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To explore mechanisms of long-term, pathway-specific synaptic plasticity, we examined consequences of differential stimulation of *Aplysia* sensorimotor connections in culture where two sensory neuron (SN) inputs converge on a single target motor cell L7. A single pairing of tetanus in one SN with bath application of 5-HT evoked long-term (24 hr) increase in efficacy of the SN connection given paired stimulation that was comparable in magnitude to the increase in synaptic efficacy evoked with repeated applications of 5-HT. Repeated pairing of tetanus in one SN with applications of 5-HT evoked a significant increase in efficacy of the SN connection given paired stimuli, and significant reduction in facilitation that is normally evoked by repeated applications of 5-HT in the unpaired SN connection. Hyperpolarization of L7 or incubation with APV interfered with both enhancement of facilitation with paired stimulation and suppression of facilitation with unpaired stimulation, but without interfering with long-term facilitation evoked either by repeated applications of 5-HT or by a single pairing. The results suggest that a single connection can undergo at least two forms of activity-dependent, pathway-specific facilitation lasting more than 24 hr. One form, evoked with a single pairing, is initiated and maintained primarily by activity in the presynaptic neuron. The other form, evoked with repeated paired stimuli, requires target-dependent activity that differentially modulates long-term heterosynaptic facilitation at the converging inputs.

Key words: synaptic plasticity; pathway specificity; serotonin; long-term; sensory neuron; Aplysia

Activity-dependent modulation of synapses is one cellular mechanism for the storage of information in the CNS to mediate various environment-induced changes in behavior. Modulation of synaptic transmission may accommodate many properties of behavioral plasticity because these changes can be bidirectional, can last for variable durations (seconds to weeks), and can often show pathway selectivity (Bliss and Lomo, 1973; Lynch et al., 1977; Castellucci et al., 1978; Kandel and Schwartz, 1982; Frost et al., 1985; Walters, 1987b; Bounomano and Byrne, 1990; Zalutsky and Nicholl, 1990; Bear and Malenka, 1994). Synaptic sites in the hippocampus (Bliss and Collingridge, 1993; Hawkins et al., 1993; Bear and Abraham, 1996) and the SN synapses of *Aplysia* (Castellucci et al., 1976, 1978; Hawkins et al., 1983; Walters and Byrne, 1983; Frost et al., 1985) express most if not all of these different forms of plasticity depending on stimulation conditions.

Although some of the processes that initiate and maintain either the direction or duration of changes in synaptic efficacy have been identified, the mechanisms mediating long-term changes in specific sets of synaptic inputs that converge on a common target are not known. The overall number, frequency, and spacing of stimuli affect duration and direction of the modulation of *Aplysia* SN synapses (Castellucci et al., 1978; Frost et al., 1985; Walters and Byrne, 1985) and synapses in the hippocampus (Bear and Malenka, 1994). In both preparations, and in *Drosophila*, activation of cAMP-dependent processes seem to be critical in synaptic and behavioral plasticity of long duration (Kandel and Schwartz, 1982; Greenberg et al., 1987; Dudai, 1988; Schacher et al., 1988, 1993; Scholz and Byrne, 1988; Dash et al., 1990; Drain et al., 1991; Byrne et al., 1993; Frey et al., 1993; Alberini et al., 1994; Huang and Kandel, 1994; Weisskopf et al., 1994; Yin et al., 1994, 1995; Bartsch et al., 1995; Wu et al., 1995). Other second messenger pathways may be critical for initiating or maintaining intermediate- and long-term synaptic plasticity (Akers et al., 1986; Malinow et al., 1989; Silva et al., 1992; Abeliovich et al., 1993; Arancio et al., 1995; Mayford et al., 1995). However, much less is known about the mechanisms mediating pathway-specific changes. In the CA1 region of the hippocampus, an understanding of this component is complicated by controversies regarding the site of plasticity (Davies et al., 1989; Bekers and Stevens, 1990; Malinow and Tsien, 1990; Bolshakov and Siegelbaum, 1995; Isaac et al., 1995; Liao et al., 1995) and the identity of the retrograde signals mediating pathway-specific changes (Williams et al., 1989; Schuman and Madison, 1991; Kato et al., 1994; Kang and Schuman, 1995; Cash et al., 1996b).

The sensorimotor synapse of *Aplysia* undergoes a variety of short- and long-lasting changes in efficacy that correlate with behavioral plasticity. Long-term sensitization, habituation, and classical conditioning of defensive withdrawal reflexes are accompanied by changes in the efficacy of this synapse (Castellucci et al., 1978; Hawkins et al., 1983; Walters and Byrne, 1983; Frost et al., 1985) mediated primarily by changes in transmitter release from the presynaptic SN (Castellucci et al., 1978; Byrne, 1987; Dale et al., 1988). Changes accompanying long-term sensitization and habituation and their cellular analogs include macromolecular

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synthesis-dependent alterations in the number of presynaptic branches and varicosities that contain transmitter release sites (Bailey and Chen, 1988; Glanzman et al., 1990; Bailey et al., 1992b; O'Leary et al., 1995). Classical conditioning of the reflexes (Carew et al., 1981, 1983) is accompanied by SN activity-dependent modulation of presynaptic heterosynaptic plasticity (Hawkins et al., 1983; Walters and Byrne, 1983). With repeated paired stimuli, long-term pathway-specific changes in synapse efficacy may contribute to stimulus- or site-specific behavioral plasticity (Walters, 1987a,b).

To explore mechanisms associated with pathway-specific plasticity at convergent inputs, we examined activity-dependent modulation of 5-HT facilitation of *Aplysia* sensorimotor connections reestablished in cultures that had two SNs and one target L7. We compared changes in synapse efficacy evoked with asymmetric stimulation of the two inputs. The results suggest that the same connection can undergo two forms of activity-dependent pathway-specific facilitation lasting more than 24 hr. One form, evoked with a single pairing of a brief tetanus to one SN with bath application of 5-HT, is initiated and maintained primarily by activities in the presynaptic neuron. The other form, evoked with repeated pairing of tetanus to one SN with bath applications of 5-HT, requires activity-dependent changes in the postsynaptic target that evokes bidirectional regulation of plastic capabilities in the converging inputs.

MATERIALS AND METHODS

Cell culture. Mechanosensory neurons (SNs) of *Aplysia* were isolated from pleural ganglia dissected from adult animals (70–100 gm) and cocultured with identified motor cell L7 isolated from abdominal ganglia of juvenile animals (1–3 gm; University of Miami Mariculture Facility, Miami, FL) and maintained for 6 days as described previously (Rayport and Schacher, 1986; Glanzman et al., 1991; Bank and Schacher, 1992; Sun and Schacher, 1996). Each culture contained a single L7 cocultured with two SNs. Cells were isolated with proximal segments of their original axons (100–200 μm for SNs and 400–800 μm for L7). To minimize the formation of electrotonic connections between the SNs, SNs were plated on opposite sides of the proximal portion of the motor cell axon with their stumps placed near the motor axon and about 200 μm apart (see Fig. 1 in Sun and Schacher, 1996).

Electrophysiology and treatments. Each L7 was current clamped with an intracellular microelectrode (filled with 2 M K-acetate, 0.5 M KCl, and 10 mM K-HEPES, pH 7.4) at −85 mV (~30 mV below resting potential) to permit accurate measurement of the initial amplitude of the EPSP on day 5 and the change in EPSP amplitude after treatment. EPSPs were evoked in L7 by stimulating each SN with a brief (0.3–0.5 msec) depolarizing pulse using an extracellular electrode (Montarolo et al., 1988). The designation of the SNs as either SN1 or SN2 in each culture was based on the order of testing the initial evoked EPSP. The same connections were reexamined either 30 min after treatments (see below) or 24 hr later.

To explore the long-term consequences of pairing action potential activity with bath applications of 5-HT, we treated each culture beginning 10 min after the initial test of EPSP amplitude with one of the following treatments: (1) bath application of 5-HT (final concentration of 1 μM), lasting 3 min, either one time or four times with a 22 min wash interval between each application; (2) bath application of control solution either one time or four times with perfusion medium consisting of 1 part seawater and 1 part modified L15 medium (Sigma) made isotonic with seawater; (3) tetanus (20 Hz for 2 sec) to SN2 either one time or four times at 25 min intervals; (4) tetanus plus 5-HT application beginning 0.5 sec after the onset of the 2 sec tetanus and lasting an additional 3 min either one time or four times with 22 min wash intervals between each pairing as described previously (Schacher et al., 1990; Eliot et al., 1994). An intracellular electrode in L7 was used to monitor whether each depolarizing stimulus to the SN during the tetanus evoked an EPSP in L7 (see Fig. 4 in Eliot et al., 1994). To ensure one-for-one correspondence between each extracellular stimulus to the SN and evoked EPSP in L7, the strength of the extracellular stimulus was increased by 20% above threshold (Eliot et al., 1994). 5-HT (1× or 4×) was applied using a Hamilton syringe containing 50 μl of 50 μM 5-HT that was pointed at the cultures and placed near the cells such that the 5-HT first reached the cells within 500 msec (Schacher et al., 1990). A small transient change in membrane potential in L7 indicated the presence of 5-HT. Thus, 5-HT and SN activity overlapped for about 1 sec. Bath perfusion was stopped and then resumed 3 min later. The treatments described above were given: (1) when L7 potential was maintained at resting level (about −55 mV); (2) when L7 potential was hyperpolarized to −105 mV beginning 1 min before treatment until washout of 5-HT (total of 4 min); and (3) after cells were perfused for 7 min (2.5 bath changes) with 50 μM APV for a total exposure to APV of 10 min with each treatment. When L7 was at rest, tetanic stimulation in the SN on occasion (20% to 30% of the cultures) evoked one or two action potentials in L7. With 4× pairing of tetanus and 5-HT, the frequency of action potentials evoked in L7 increased to about 50% by the last pairing. Hyperpolarization of L7 or 10 min exposures to APV did not affect the amplitude of evoked EPSPs or the ability of 5-HT to produce short-term facilitation comparable in magnitude and duration as controls; increases in EPSPs of 125–150% that lasted at least 5 min after a 3 min exposure to 5-HT ($n = 4$ cultures for each treatment).

Statistical analyses. Two-way ANOVAs were used to determine overall differences with treatment within cultures and between cultures. Multicomparison test (Scheffe *F*-test) was used to determine significant differences between control and experimental groups and between appropriate experimental groups. In all histograms, bar height of 100% represents no change in efficacy.

RESULTS

Single pairing of tetanus with 5-HT evokes pathway-specific, long-term facilitation

Synapses between SN and motor cell L7 form rapidly and reliably in cell culture (Rayport and Schacher, 1986; Glanzman et al., 1989; Zhu et al., 1994). By day 4, SN synapses with L7 are stable and show multiple forms of short-term homosynaptic and heterosynaptic plasticity (Rayport and Schacher, 1986; Schacher et al., 1990). Changes in efficacy of stable SN synapses lasting more than 24 hr (long-term) are evoked by a subset of the stimuli that also evoke synaptic plasticity lasting minutes to hours. Long-term changes are evoked with repeated (four or five) applications of specific neuromodulators such as 5-HT or FMRFamide (Montarolo et al., 1986, 1988; Schacher et al., 1990). Activity in SNs (tetanus of 20 Hz for 2 sec) that evokes potentiation of synapse efficacy, comparable in magnitude and duration to that evoked by a single application of 5-HT, failed to evoke a long-lasting (24 hr) change in efficacy of SN synapses *in vitro* (Schacher et al., 1990; but see Lin and Glanzman, 1994a,b, for LTP-like changes in SN synapses *in vitro* lasting several hours). However, temporal pairing of tetanus in SNs with application of 5-HT results in significant and cell-specific increases in both magnitude and duration (up to 30 min) of short-term plasticity evoked either by neuromodulator alone or tetanus alone (Eliot et al., 1994). We first examined in 5-d-old cultures consisting of 2 SNs cocultured with a single L7 (see Glanzman et al., 1991; Bank and Schacher, 1992; Eliot et al., 1994; Sun and Schacher, 1996, for details on these cultures) whether a single pairing of tetanus in one SN with bath application of 5-HT would evoke a long-term change of at least 24 hr in the SN connection receiving paired stimulation.

A single pairing of tetanus with bath application of 5-HT evoked a selective and significant ($p < 0.01$; Scheffe *F*-test) long-term change in synapse efficacy in the SN (SN2) receiving the paired stimuli (Fig. 1*A*, *Tet + 5-HT* group; $n = 5$). The change in EPSP amplitude evoked in the SN connection with 1× pairing was 47.0 ± 7.0%, compared with a change of 3.2 ± 4.9% in the other SN in response to the application of 5-HT (the baseline at 100% represents 0% change in efficacy). The

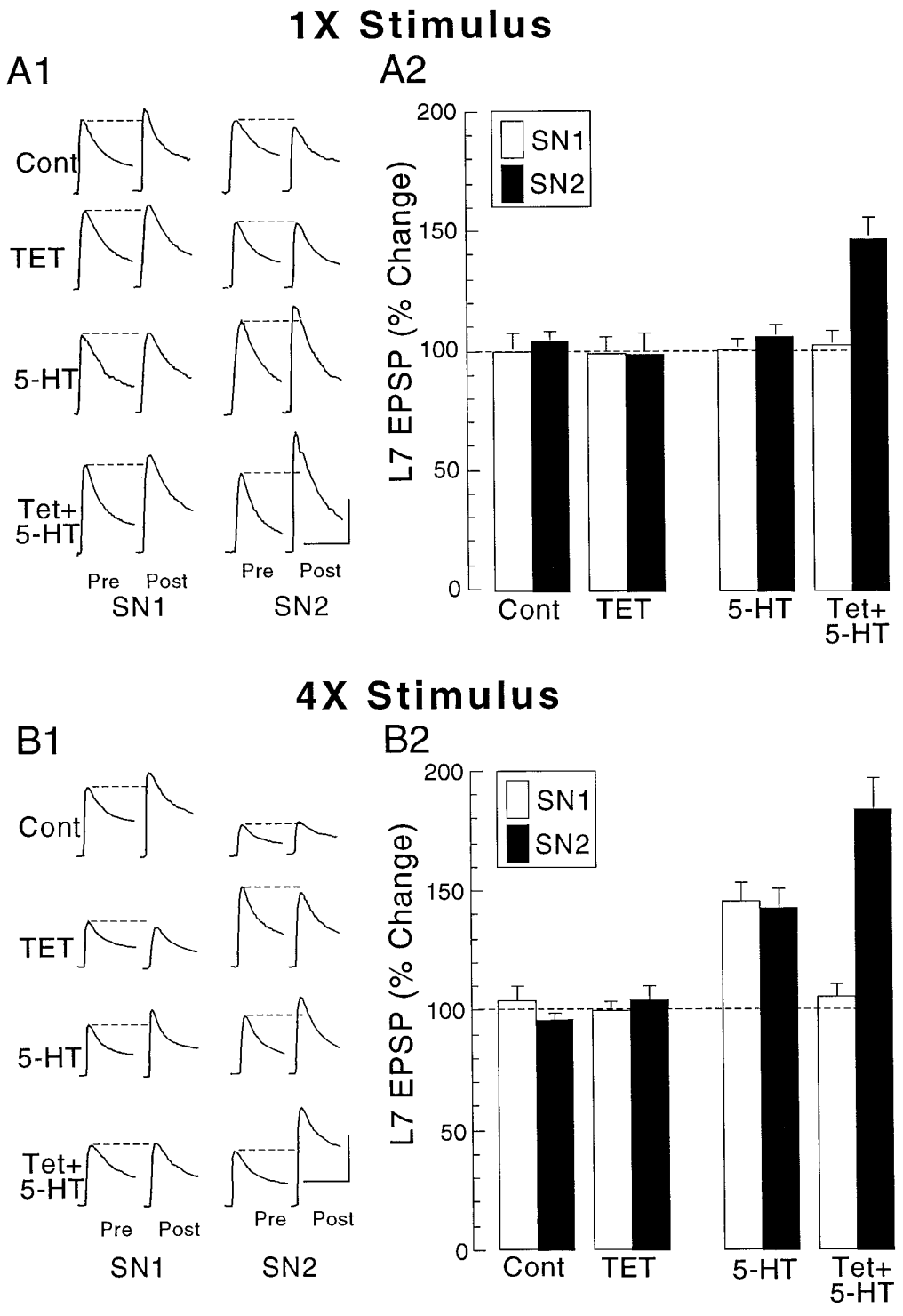

Figure 1. Pathway-specific long-term facilitation evoked with single (*1×*) or repeated (*4×*) pairing. *A*, Single (*1×*) pairing evoked long-term facilitation in SN given paired stimulation. *A1*, EPSPs evoked in SN1 and SN2 before (*Pre*) and 24 hr after (*Post*) treatment (see text for details). *Vertical bar* is 10 mV; *horizontal bar* is 25 msec. *A2*, Summary of long-term changes evoked with 1× treatment. A two-way ANOVA indicated an overall effect of treatments ($F_{(3,16)} = 8.609$; $p < 0.001$). Tet + 5-HT to SN2 evoked a significant increase in EPSP amplitude compared with the other treatments ($F = 4.556$, $p < 0.01$ vs control; $F = 5.237$, $p < 0.01$ vs tetanus; $F = 3.741$, $p < 0.04$ vs 5-HT). *B*, Repeated (*4×*) pairing evoked both an enhancement and suppression of 5-HT long-term facilitation. *B1*, EPSPs evoked in SN1 and SN2 before (*Pre*) and 22 hr after (*Post*) treatments (see text for details). *Vertical bar* is 15 mV; *horizontal bar* is 25 msec. *B2*, Summary of long-term changes evoked with 4× treatments. A two-way ANOVA indicated an overall effect of treatments ($F_{(3,24)} = 12.567$; $p < 0.001$). 5-HT (*5-HT*) significantly increased EPSP amplitude in SN1 and SN2 compared with control ($F = 6.68$, $p < 0.01$ and $F = 4.744$, $p < 0.04$). The change in EPSP amplitude evoked by SN1 (unpaired) in Tet + 5-HT group was not different from the change evoked by SN1 in controls ($F = 0.018$, $p > 0.5$). There was a significant increase in the EPSP evoked by SN2 given 4× Tet + 5-HT compared with the other groups ($F = 16.3$, $p < 0.005$ vs control; $F = 13.194$, $p < 0.005$ vs tetanus; and $F = 3.557$, $p < 0.05$ vs 5-HT). 5-HT (*5-HT*) evoked a significant change in EPSP evoked by SN1 compared with the actions of repeated 5-HT applications on the change evoked in SN1 in Tet + 5-HT group (Scheffe $F = 6.007$; $p < 0.01$).

increase in synapse efficacy in SNs given 1× pairing was comparable in magnitude to the change evoked with four repeated applications of 5-HT only (see below and Fig. 1*B*). As expected (Montarolo et al., 1986; Schacher et al., 1990), a single application of 5-HT or tetanus failed to evoke long-term changes in EPSP amplitudes (Fig. 1*A*, *Cont*, *5-HT*, and *Tet* groups; $n = 5$ for each group). Thus, a single pairing of tetanus with 5-HT produces long-term pathway-specific plasticity. We next examined whether pathway-specific changes are expressed with 4× pairings of the stimuli.

Four pairings of tetanus with 5-HT evoke both enhancement and suppression of long-term facilitation in converging inputs

Repeated applications of 5-HT (Montarolo et al., 1986; Schacher et al., 1990) or bath application of cell-permeable analog of cAMP (Schacher et al., 1988) evoke long-term facilitation of all SN connections with a common motor cell target. Temporal pairing of tetanus with application of 5-HT evoked increases in magnitude and duration of short-term facilitation and long-term facilitation without affecting changes normally evoked in the unpaired

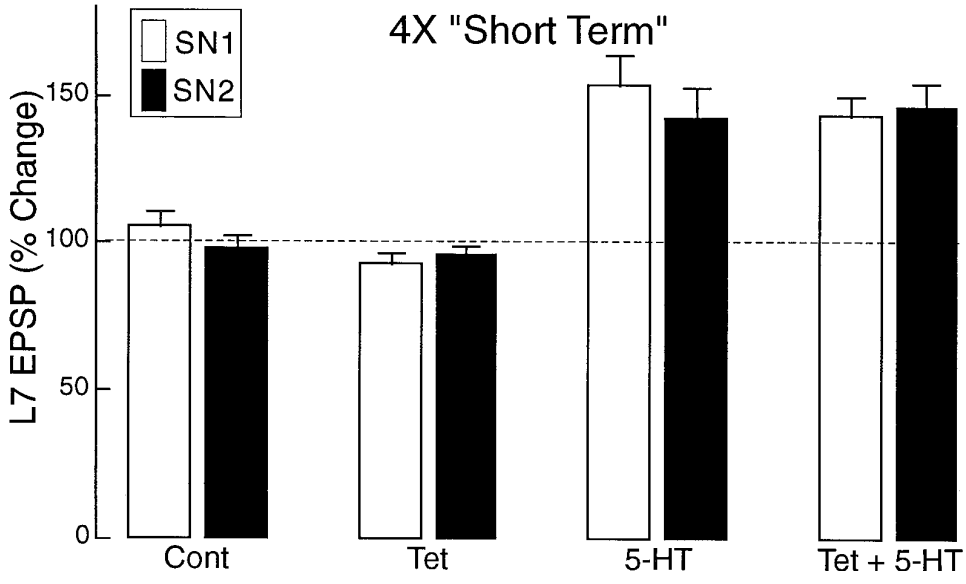

Figure 2. Repeated (4×) pairings do not evoke a significant change at an early time point after treatments (see text for details). EPSPs evoked 30 min after the last treatment were compared with those evoked before treatment. Two-way ANOVA indicated no overall effect of treatments ($F_{(3,18)} = 0.514$; $p > 0.65$). Although treatment alone evoked a significant effect ($F = 25.041$; $p < 0.001$), there was no significant difference in changes in SN1 compared with SN2 for each treatment ($F = 0.622$; $p > 0.45$). 5-HT (*5-HT*) evoked a significant change in EPSP amplitude compared with controls ($F = 7.039$, $p < 0.01$ for SN1; $F = 5.431$, $p < 0.01$ for SN2). Unlike the situation at 24 hr, 5-HT treatment given to SN1 in Tet + 5-HT group evoked a significant increase in EPSP amplitude at 30 min compared with the change evoked in SN1 connections in controls ($F = 5.245$; $p < 0.01$). Paired treatment given to SN2 also evoked a significant increase in EPSP amplitude at 30 min compared with controls ($F = 6.244$; $p < 0.01$). There were no significant differences (Scheffe F tests) in changes evoked in SNs treated with 5-HT (*5-HT*) compared with changes in SNs in the Tet + 5-HT paired group.

SN connection (Eliot et al., 1994; Fig. 1*A* above). As expected, 4× bath application of 5-HT ($n = 7$) evoked significant increases in EPSP amplitudes ($p < 0.01$; Scheffe F-test) in both SN connections (45.7 ± 7.4% and 42.9 ± 8.0% above the baseline of 100%) compared with changes of 3.3 ± 7.2% and −4.0 ± 2.6% in the control group ($n = 7$; see Fig. 1*B*, *Cont* and *5-HT* groups). As reported previously (Schacher et al., 1990), 4× tetanus failed to evoke a long-term change in the active SN (SN2: 4.7 ± 5.3%; $n = 7$) compared with control. In addition, repeated activity in one SN did not evoke a long-term change in efficacy of the nonstimulated SN (SN1) in the same cultures [−0.4 ± 4.4% (see Fig. 1B, *Tet* group)].

We expected that the consequences of repeated pairing would be changes in both SNs. The efficacy of one SN connection (SN1) undergoes a long-term change as a result of 4× bath applications of 5-HT, whereas the other SN (SN2) that receives 4× paired stimulation undergoes additional increases in facilitation via activity-dependent enhancement of 5-HT long-term facilitation. Although we found that the EPSP amplitude evoked at SN connections (SN2) given 4× paired stimulation (Fig. 1*B*, *Tet + 5-HT* group; $n = 7$) increased significantly ($p < 0.05$; Sheffe F-test) by 83.9 ± 12.5%, compared with the change evoked in SN2 connections with 4× application of 5-HT only (Fig. 1B, *5-HT* group), the efficacy of the unpaired SN connections (SN1) exposed to the repeated applications of 5-HT failed to show a significant increase ($p > 0.5$; Scheffe F-test) compared with controls (5.4 ± 5.5%). This change was significantly lower than the change evoked in cultures where both SNs are treated with repeated applications of 5-HT only ($p < 0.05$; Sheffe F-test).

Although repeated tetanus activity in one SN did not evoke long-term heterosynaptic depression in the nonstimulated SN, short-lasting heterosynaptic depression evoked by repeated activity in one pathway may contribute to the suppression of long-term facilitation by paired activity in the other SN by disrupting early or intermediate changes in EPSP amplitude evoked by 5-HT. The long-term change in EPSP amplitude for the nonstimulated SN that normally would be evoked by 5-HT may be reduced by short-lasting activity-dependent heterosynaptic depression. To test this possibility, we examined the efficacy of connections 30 min after the final (fourth) treatment (Fig. 2). Repeated tetani did not evoke a significant change in EPSP amplitude in either the stimulated (−4.2 ± 3.4% for SN2) or the nonstimulated (−7.2 ± 3.8% for SN1) SN, compared with the change evoked in SN connections in controls (Fig. 2, *Cont* and *Tet* groups; $n = 5$ each). Repeated applications of 5-HT (Fig. 2, *5-HT* group; $n = 6$) evoked an increase in EPSP amplitude at both SN connections. In addition, EPSP ampltiudes of SN connections given paired stimuli (SN2 in Tet + 5-HT group; $n = 6$) and the unpaired SN connections treated with 5-HT (SN1 in the Tet + 5-HT group) increased by similar levels at this time point (46.8 ± 7.9% for SN2 and 41.7 ± 7.1% for SN1). Unlike changes found at 24 hr (Fig. 1*B*), there was no significant difference in the enhancement evoked in both SNs with 5-HT and with pairing of tetanus plus 5-HT. Thus, it is unlikely that the failure by nonstimulated SNs in the Tet + 5-HT group to express a long-term change is a direct consequence of short-lasting heterosynaptic depression affecting the ability of 5-HT to enhance the EPSP amplitude of the nonstimulated SN either in the short-term (Eliot et al., 1994) or at an intermediate time point. The repeated paired stimulation to one SN seems to evoke both enhancement of 5-HT long-term facilitation in stimulated SNs and suppression of 5-HT long-term facilitation in nonstimulated SNs.

Activity-dependent changes in postsynaptic target contribute to expression of pathway-specific, long-term facilitation evoked with repeated pairing

The fast excitatory response evoked in L7 with SN stimulation is most likely produced by glutamate (Dale and Kandel, 1993). Recent evidence suggest that SN connections express a homosynaptic LTP-like plasticity lasting several hours that is mediated by an influx of calcium through channels activated by a postsynaptic NMDA-like glutamate receptor (Lin and Glanzman, 1994a;b). Thus, hyperpolarization of the motor cell or incubation with antagonists of NMDA-like glutamate receptors reduce signifi-

cantly facilitation evoked with repetitive firing of action potentials in the SN. After determining that hyperpolarization of L7 and incubation with APV (25–50 μM) had little or no significant effect on the steady-state efficacy of connections or on the ability of 5-HT to evoke short-term facilitation (data not shown), we examined whether "activity" in L7 contributes to expression of pathway-specific long-term facilitation.

Hyperpolarization of L7 or the presence of APV (see Materials and Methods for details on treatment) did not interfere with long-term facilitation evoked by 1× pairing of tetanus + 5-HT (Figs. 3*A*, 4*A*, respectively). Paired stimulation while L7 is hyperpolarized to −105 mV still evoked a significant increase ($p < 0.005$; Scheffe *F*-test) in the EPSP amplitude (SN2) of 41.7 ± 7.1%, compared with the change in EPSP amplitude in SN2 evoked in controls when L7 is hyperpolarized (Fig. 3*A*, *Cont* and *Tet + 5-HT* groups; $n = 6$ each). As expected, a single application of 5-HT failed to evoke a significant change (Fig. 3*A*, *5-HT* group; $n = 6$). Incubation with APV did not affect efficacy of SN connections in control cultures ($n = 6$) or cultures treated with single application of 5-HT (Fig. 4*A*, *Cont* and *5-HT* groups; $n = 6$ each). Paired stimulation evoked a significant increase ($p < 0.05$; Scheffe *F*-test) in EPSP amplitude (SN2) of 27.5 ± 6.9% (Fig. 4*A*, *Tet + 5-HT* group; $n = 6$). Thus, long-term, pathway-specific synaptic plasticity after a single pairing is not affected by interfering with some activity-dependent processes in the postsynaptic target during the presentation of the stimuli.

Both hyperpolarizing L7 ($n = 7$ for each treatment) and incubation with APV ($n = 7$ for each treatment) interfered with the expression of pathway-specific plasticity evoked with repeated pairing. Neither suppression of long-term facilitation evoked by 4× application of 5-HT when the other SN receives repeated paired stimulation nor enhancement of facilitation with paired stimulation were expressed. Unlike the situation when L7 is maintained at resting potential (Fig. 1*B*), 4× paired stimulation to one SN (SN2) when L7 was hyperpolarized (Fig. 3*B*, *Tet + 5-HT* group) evoked significant increases in EPSP amplitudes for both SNs (38.4 ± 2.9% in SN1 and 44.7 ± 8.9% in SN2), compared with controls. Similarly, 4× paired stimulation to one SN (SN2) in the presence of APV (Fig. 4*B*, *Tet + 5-HT*) also evoked significant increases in EPSP amplitudes for both SNs (31.9 ± 4.9% in SN1 and 38.6 ± 6.5% in SN2). These changes were not significantly different than changes evoked in both SNs treated with 5-HT (Figs. 3*B*, 4*B*, *5-HT* groups). Thus, both manipulations applied during the presentation of the stimuli affecting activity in the postsynaptic cell interfere with pathway-specific changes evoked with repeated differential activation of inputs.

DISCUSSION

Our results are consistent with the hypothesis that some forms of long-term, pathway-specific plasticity in synaptic inputs converging on a common target require contributions from both pre- and postsynaptic neurons. Repeated activation of an APV-sensitive receptor at specific sites along the surface of the postsynaptic neuron may act as a "switch" in modulating the expression of some forms of long-term presynaptic facilitation at converging inputs that are differentially activated.

Presynaptic mechanisms mediate pathway-specific plasticity with a single pairing

Pathway-specific plasticity with a single pairing is mediated primarily by cell-specific induction of long-term changes in the presynaptic SN. Single application of 5-HT or single tetanus evoke only short-lasting changes in the efficacy of SN connections. Pairing both stimuli not only prolongs short-term facilitation (Eliot et al., 1994; Bao and Hawkins, 1995, 1996), but also evokes long-term change in synaptic efficacy of the active pathway lasting more than 24 hr (see also Walters, 1987b; Buonomano and Byrne, 1990). The coincidence of activity in the SN with 5-HT increases the levels of cAMP produced by adenylyl cyclase as a result of 5-HT binding to appropriate receptors on SNs (Occor et al., 1985; Abrams et al., 1991). The increase in cAMP levels in SNs with a single pairing may parallel changes evoked with repeated applications of 5-HT or when cAMP is injected directly into the SN cell body (Scholz and Byrne, 1988; Nazif et al., 1991; Backsai et al., 1993; Schacher et al., 1993; Sun and Schacher, 1996). The suprainduction of cAMP levels in one SN leads to increases in cAMP-dependent processes in the nucleus and regulates expression of transcription factors that influence the synthesis of effector gene products that mediate long-term changes in the connections and the structure of the activated SN (Dash et al., 1990; Backsai et al., 1993; Kaang et al., 1993; Alberini et al., 1994; Bartsch et al., 1995). Timely and appropriate local changes in the properties of the postsynaptic cell, such as changes in the distribution of cell adhesion molecules or neurotransmitter receptors (Bailey et al., 1992a; Mayford et al., 1992; Trudeau and Castellucci, 1995), are likely to accompany the presynaptic changes that include formation of new transmitter release sites (Glanzman et al., 1990; Nazif et al., 1991; Schacher et al., 1993; O'Leary et al., 1995). By contrast, the other SN receives a stimulus of 5-HT that is not sufficient to raise cAMP to appropriate levels and fails to express long-term changes (Montarolo et al., 1986; Bartsch et al., 1995). Increases in cAMP-dependent processes seem to be critical in the expression of LTP in hippocampus (Frey et al., 1993; Huang et al., 1994; Weisskopf et al., 1994) and in synaptic plasticity accompanying some forms of long-term associative learning in *Drosophila* (Dudai, 1988; Drain et al., 1991; Yin et al., 1994, 1995). These presynaptic factors seem to be sufficient for evoking long-term plasticity with a single pairing because hyperpolarizing L7 or incubating with APV do not interfere with the long-term changes evoked with a single pairing. However, recent evidence (Bao and Hawkins, 1995, 1996) suggests that in addition to the presynaptic changes described above, depolarization in the motor cell and changes in postsynaptic calcium may contribute to short-term enhancement of facilitation in *Aplysia* SN connections after a single pairing of tetanus and 5-HT. This raises a possibility, to be tested in future experiments, that under some stimulation conditions, postsynaptic changes may contribute only to some forms of short-, intermediate-, and/or long-term plasticity at a particular synapse. This would be consistent with the hypothesis that parallel processes contribute to synapse plasticity of varying duration (Emptage and Carew, 1993; Byrne and Kandel, 1996).

Tetanus activity alone seemed to have little effect on either the active or inactive pathway. Direct heterosynaptic actions of activity in one input on inactive converging inputs may require significant overlap of synaptic contacts from each input on the common postsynaptic target (White et al., 1990; Lo and Poo, 1991; Cash et al., 1996b). For example, heterosynaptic depression at *Xenopus* nerve-muscle contacts with activity in one input is expressed only when competing neural contacts are within 20 μm of each other on the muscle target (Lo and Poo, 1991; Cash et al., 1996a). Synaptic contacts by competing presynaptic SNs on a single motor cell L7 segregate from each other over time in culture. By day 4, varicosities of each SN become restricted to specific 50–100 μm segments along the main axon of L7 (Glanzman et al., 1991; Bank

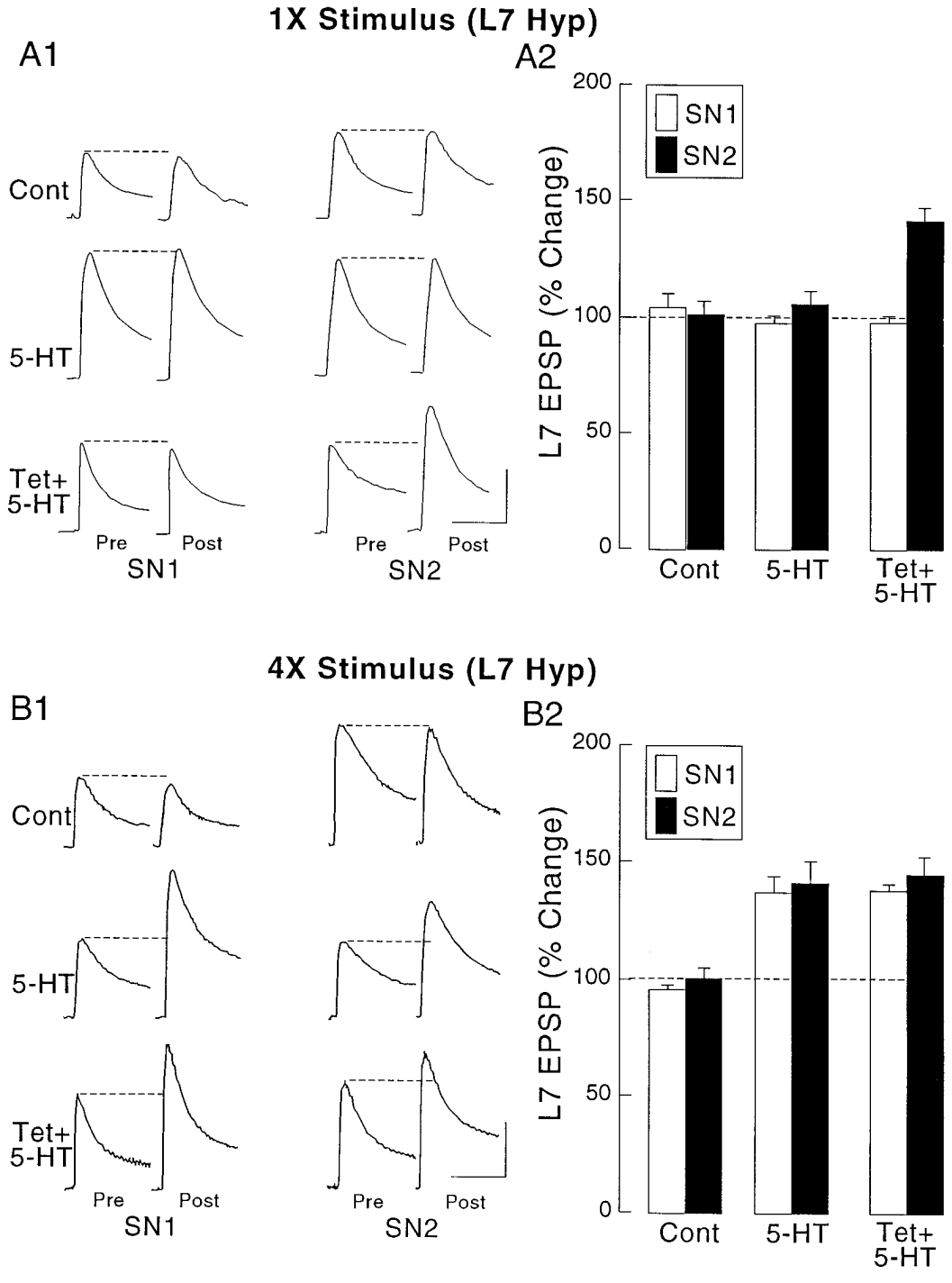

Figure 3. Hyperpolarization of L7 interferes with long-term pathway-specific facilitation evoked with 4× pairing only. *A*, Hyperpolarizing L7 does not interfere with pathway-specific plasticity evoked with 1× pairing. *A1*, EPSPs in SN1 and SN2 before (*Pre*) and 24 hr after (*Post*) treatments while L7 was hyperpolarized (see text for details). *Vertical bar* is 10 mV (same as *B1*); *horizontal bar* is 25 msec (same as *B1*). *A2*, Summary of long-term changes evoked with treatments. A two-way ANOVA indicated an overall effect of treatment ($F_{(2,15)} = 17.795$; $p < 0.001$). Only pairing Tet + 5-HT to SN2 evoked a significant change in EPSP amplitude ($F = 11.474$, $p < 0.005$ vs control; $F = 7.996$, $p < 0.01$ vs 5-HT). *B*, Hyperpolarizing L7 blocks pathway-specific plasticity with 4× pairing. *B1*, EPSPs in SN1 and SN2 before (*Pre*) and 22 hr after (*Post*) treatments (see text for details) while L7 is hyperpolarized. *B2*, Summary of the long-term changes evoked with treatments. A two-way ANOVA indicated no overall effect of treatment ($F_{(2,18)} = 0.670$; $p > 0.5$), because there was no difference in response by each SN in a given culture to treatment. Hyperpolarization did not interfere with 5-HT long-term facilitation. Compared with controls, there were significant increases in EPSP amplitudes for both SN1 and SN2 ($F = 20.457$, $p < 0.005$; $F = 4.983$, $p < 0.05$). Treatment of SN2 with 4× Tet + 5-HT did not interfere with 5-HT evoking a significant change in EPSP amplitude of SN1 compared with controls ($F = 12.968$; $p < 0.005$). Tet + 5-HT also evoked a significant long-term change in the EPSP amplitude evoked by SN2 compared with controls ($F = 5.775$; $p < 0.01$). The changed evoked in SN2 by paired stimulation was not significantly different ($F = 0.032$; $p > 0.5$) from the change evoked in SN2 in the 5-HT group.

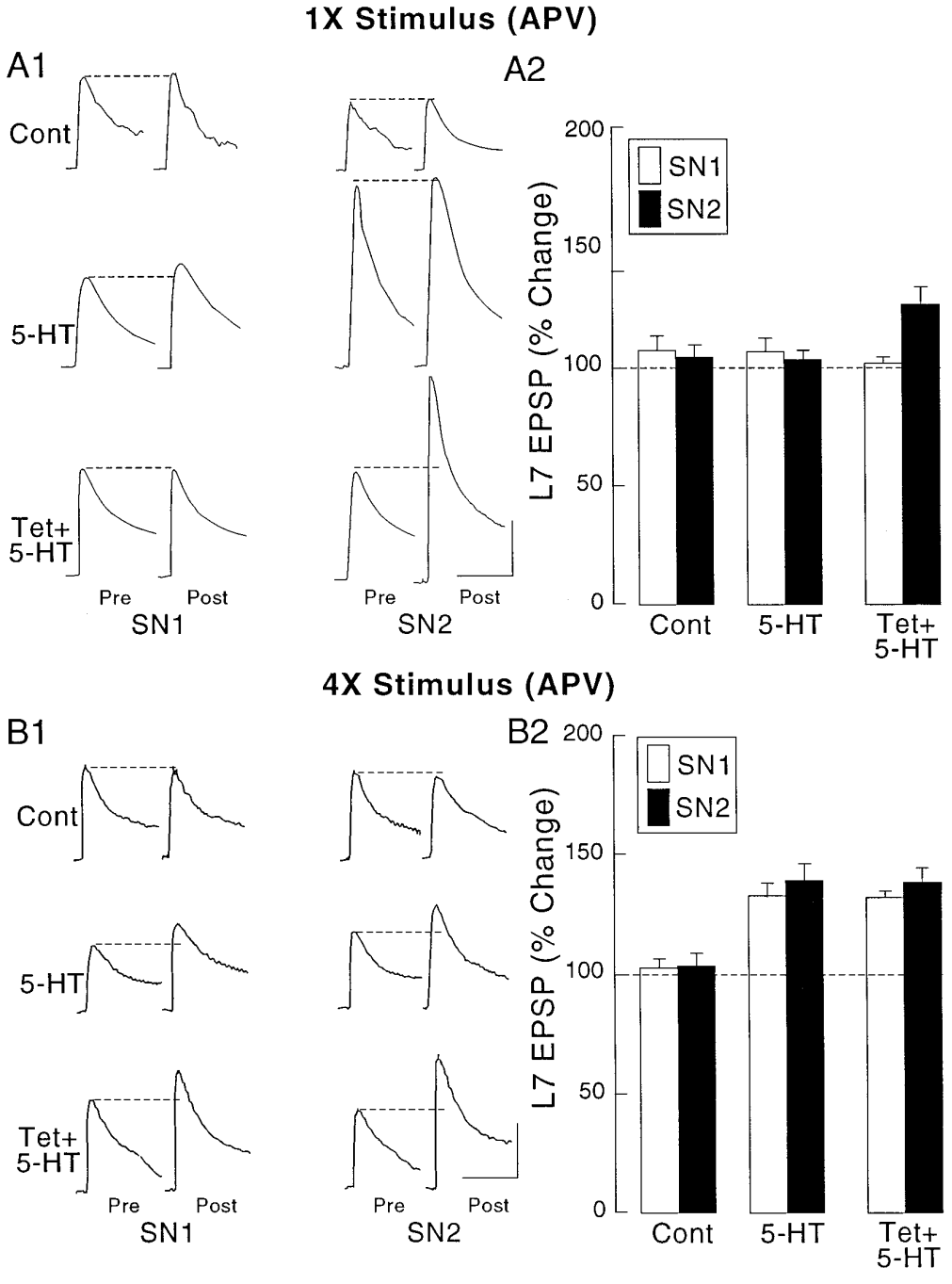

Figure 4. Incubation with APV interferes with long-term pathway-specific facilitation evoked with 4× pairing only. *A*, APV does not interfere with pathway-specific plasticity evoked with 1× pairing. *A1*, EPSPs in SN1 and SN2 before (*Pre*) and 24 hr after (*Post*) treatments in the presence of APV. *Vertical bar* is 10 mV (same as *B1*); *horizontal bar* is 25 msec (same as *B1*). *B2*, Summary of long-term changes evoked with treatments. A two-way ANOVA indicated an overall effect of treatment ($F_{(2,15)} = 7.295$; $p < 0.007$). Only Tet + 5-HT to SN2 evoked a significant change ($F = 3.265, p < 0.05$ vs controls; $F = 3.285$, $p < 0.05$ vs 5-HT). *B*, APV blocks pathway-specific plasticity with 4× pairing. *B1*, EPSPs in SN1 and SN2 before (*Pre*) and 22 hr after (*Post*) treatments in the presence of APV. *B2*, Summary of long-term changes evoked with treatments. A two-way ANOVA indicated no overall effect of treatment ($F_{(2,18)} = 0.407$; $p > 0.65$) because there was no difference in the response by each SN in a given culture to treatment. APV did not interfere with 5-HT evoking long-term facilitation in the EPSP amplitudes of SN1 and SN2 compared with the change in controls ($F = 9.87, p < 0.01$; $F = 7.51$, $p < 0.01$). Treatment of SN2 with 4× Tet + 5-HT did not interfere with 5-HT evoking a significant change in the EPSP amplitude evoked by SN1 compared with that evoked in SN1 with controls ($F = 9.229$; $p < 0.01$). Tet + 5-HT evoked a significant long-term change in the EPSP amplitude evoked by SN2 compared with that evoked after controls ($F = 7.452$; $p < 0.01$), but it was not different from the change evoked in SN2 in the 5-HT group. The changed evoked in SN2 by paired stimulation was not significantly different ($F = 0.015$; $p > 0.5$) from the change evoked in SN2 in the 5-HT group.

and Schacher, 1992; Sun and Schacher, 1996). However, on day 2, there is significant overlap in the position of SN varicosities regenerated by each SN on the L7 axon. This absence of segregation at the earlier time point may explain why differential activation of inputs with a tetanus on day 2 resulted in significant changes in the development of synaptic interactions by both SNs. A single tetanus evoked long-term increases in efficacy of the active SN connections and suppression of changes in synaptic efficacy that normally developed in the nonstimulated SN connections (Sun and Schacher, 1996). The postsynaptic target contributed to activity-induced changes because hyperpolarization of L7 blocked the changes in both SNs. Analysis of structural changes accompanying changes in synaptic efficacy indicated that activity increased stability of existing varicosities (synaptic contacts) in the activated SN and decreased stability of existing varicosities in the nonstimulated SN. Such pathway-specific changes associated with activity-dependent modulation of competitive interactions during an early stage in establishing synapses may be recapitulated when stable synaptic interactions are differentially and repeatedly activated with paired stimulation.

Postsynaptic target regulates pathway-specific plasticity with repeated pairing

Although long-term facilitation of SN-L7 synapses by 5-HT is maintained primarily by an increase in transmitter release (Dale et al., 1988) and includes formation and long-term maintenance of new SN branches and varicosities (Glanzman et al., 1990; Schacher et al., 1993; Wu et al., 1995), expression of structural

changes requires the presence of the postsynaptic motor cell. Long-term structural plasticity is not expressed when isolated SNs are treated with repeated applications of 5-HT on day 5 (Glanzman et al., 1990). Moreover, both long-term functional and structural changes in SN-L7 connections are blocked when cells are exposed after treatment with 5-HT to monoclonal antibodies that bind a family of cell adhesion molecules (Zhu et al., 1995). The expression of these cell adhesion molecules on the surface of L7 contributes significantly to the formation of new synapses by SNs (Zhu et al., 1994, 1995). Thus, changes in the properties of the postsynaptic target may alter the cascade of events triggered primarily in the presynaptic neuron that initiate or maintain long-term synaptic plasticity.

Our results suggest that the same synapse in *Aplysia* may be capable of expressing at least two different forms of long-term facilitation (potentiation) lasting more than 24 hr. One form is initiated via a mechanism that is independent of activity in the postsynaptic cell and mediates long-term changes with repeated application of 5-HT or pathway-specific facilitation evoked with a single pairing. Postsynaptic activity-independent forms of long-term facilitation at SN-L7 synapses is initiated primarily by changes in the presynaptic SNs via heterosynaptic presynaptic facilitation through the actions of neuromodulators on second messenger cascades in the SNs (Byrne et al., 1993; Hawkins et al., 1993). Such cellular changes may play a significant role in producing long-term site-specific behavioral changes after a single or brief series of paired stimulation or training trials (Carew et al., 1983; Walters, 1987b). A second form is dependent on activity in the postsynaptic target and mediates pathway-specific long-term facilitation with repeated differential activity in converging inputs. With repeated paired stimuli spaced over time, activation of an APV-sensitive receptor on L7 and depolarization are required for both enhancement and suppression of long-term 5-HT facilitation initiated in presynaptic SNs. As at some synaptic sites in hippocampus, 4× pairing of stimuli at SN-L7 connections evokes pathway-specific changes in synaptic efficacy via local presynaptic activation of a NMDA-like glutamate receptor on the postsynaptic cell. This postsynaptic activity influences long-term changes at both active and inactive SN synapses and is consistent with the hypothesis that one role of the NMDA-type glutamate receptor is to serve as a "binary switch" to initiate long-term pathway-specific synaptic plasticity (Bear and Malenka, 1994). A suppression of long-term changes in the nonstimulated but sensitized pathway in the same preparation after associative/nonassociative training protocols has not been reported or examined directly in previous cellular and behavioral studies in *Aplysia*. Buonomano and Byrne (1990) compared the long-term effects of temporally paired versus temporally unpaired stimuli on the efficacy of converging sensory neuron inputs and found that the stimulation conditions used (different than those used here) evoked changes in both connections, including an enhancement of synaptic facilitation in the SN given temporally paired stimuli. One possibility, to be tested in future experiments, is that unpaired stimulation in the other SN interferes with site-specific changes in the motor cell (see below) required to suppress long-term changes in the converging SN input.

How does activity in L7 with repeated pairing of activity in one SN with applications of 5-HT enhance 5-HT long-term facilitation in the stimulated SN and suppress 5-HT long-term facilitation in the nonstimulated SN? One possibility is that local changes in the "substrate" properties of L7 gate the initiation and/or stabilization of structural and other changes that accompany long-term facilitation evoked by 5-HT in the active SN but not the inactive SN. Local pre- and postsynaptic activity may modulate 5-HT induced changes in expression and distribution of cell adhesion molecules on the surface of the interacting cells that seem to regulate growth and formation of new presynaptic structures (Bailey et al., 1992a; Mayford et al., 1992; Zhu et al., 1995) and/or of glutamate receptors on L7 (Trudeau and Castellucci, 1995) at sites of SN contacts that may accompany structural changes in the presynaptic SN. The critical local activity in the postsynaptic cell may be calcium influx through channels opened by NMDA-like glutamate receptors (Glanzman, 1994). A local increase in postsynaptic calcium may trigger a second messenger cascade that influences targeting of the postsynaptic changes. Persistent local activation of kinases (Malinow et al., 1988; Lisman, 1989) may affect local organization of the cytoskeleton, which in turn influences organelle transport and the anchoring, distribution, and mobilization of molecules (cell adhesion molecules and neurotransmitter receptors) within the postsynaptic membrane that are critical for the expression of long-term facilitation with 5-HT. The failure of L7 to target critical postsynaptic substrate changes to sites of interaction with the nonstimulated SN that only received repeated applications of 5-HT, while increasing those changes at sites of interaction with the SN given paired stimulation, may involve some of the same mechanisms associated with activity-dependent modulation of competitive interactions during early stages of synapse formation in which synaptic sites of the activated SN are stabilized selectively (Bank and Schacher, 1992; Sun and Schacher, 1996). Future experiments will be directed at identifying the local activity-dependent changes in the postsynaptic target that are critical for long-term maintenance and stabilization of specific presynaptic SN contacts.

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