activity of sLDLR indicates that such proteins may perform independent functions that cannot be predicted from the functions of their membrane-associated analogs.

Previous studies have shown that some viruses code for soluble receptor-like molecules that block their respective cytokines, for example, IFN-γ, IL-1, and TNF. Such soluble receptors assist virus infection by suppressing host defense mechanisms (*17*). It now appears that host organisms make use of a similar type of molecule for the opposite role of controlling virus infections. Understanding the mechanism of action of sLDLR will help in determining its possible involvement in other types of viral infections.

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Long-Term Synaptic Facilitation in the Absence of Short-Term Facilitation in *Aplysia* Neurons

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Serotonin (5-HT) induces both short-term and long-term facilitation of the identified synaptic connections between sensory and motor neurons of *Aplysia*. Three independent experimental approaches showed that long-term facilitation can normally be expressed in the absence of short-term facilitation: (i) The 5-HT antagonist cyproheptadine blocked the induction of short-term but not long-term facilitation; (ii) concentrations of 5-HT below threshold for the induction of short-term facilitation nonetheless induced long-term facilitation; and (iii) localized application of 5-HT to the sensory neuron cell body and proximal synapses induced long-term facilitation in distal synapses that were not exposed to 5-HT and had not expressed short-term facilitation. These results suggest that short-term and long-term synaptic facilitation are induced in parallel in the sensory neurons and that the short-term process, because it is induced and expressed at the synapse, can occur locally, but the long-term process, because of its dependence on a nuclear signal, is expressed throughout the neuron.

It is widely accepted that there are two principal forms of memory storage: short-term memory (STM) and long-term memory (LTM). STM describes a process that retains information temporarily, after which it is thought to become incorporated or transferred into a more stable, long-term store (*1*). One way to address the interdependence between STM and LTM is to examine these processes mechanistically. We have used the identified synaptic connections between tail sensory neurons (SNs) and motor neurons (MNs) of *Aplysia*, which exhibit both short-term and long-term synaptic facilitation following learning (*2*), as a model system to explore the relation between STM and LTM. Using three independent experimental approaches, we found that the induction of the short-term synaptic process is not necessary for the induction of the long-term process.

Considerable evidence indicates that 5-HT participates in both short-term and long-term synaptic facilitation in *Aplysia* (*2*). Montarolo *et al.* (*3*) showed that in cell cultures of SNs and MNs a single application of 5-HT produces short-term facilitation, whereas repeated 5-HT applications produce long-term facilitation. We found that in the intact central nervous system (CNS) a single 5-HT application (5 μM) produced short-term facilitation and multiple applications produced long-term facilitation of the monosynaptic excitatory postsynaptic potential (EPSP) between the tail SNs and MNs (*4*) (Fig. 1A). An analysis of variance (ANOVA) revealed a significant overall effect of 5-HT ($F_{1,5} = 5.84$, $P < 0.008$) (*5*). Subsequent planned comparisons showed that a single 5-HT application produced significant short-term facilitation (mean increase = 157.1%, $t_5 = 2.14$, $P < 0.04$, one-tailed), and five applications produced significant long-term fa-

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cilitation (mean increase = 105.9%, t_5 = 3.3, $P < 0.02$) (5). In control experiments (n = 4), synapses that received no 5-HT treatment showed neither short-term nor long-term changes [mean change = −3.1% and −16.4%, respectively; t_3 = −1.53 and −2.14, not statistically significant (NS) in each case]. Thus, 5-HT applications in the intact CNS produce both short-term and long-term synaptic facilitation in the tail SNs.

We next explored the interdependence between short-term and long-term facilitation. Mercer, Emptage, and Carew (6) recently found that the 5-HT antagonist cyproheptadine (CYP) blocks 5-HT–induced short-term synaptic facilitation. This allowed us to examine the effects of blocking short-term facilitation on the subsequent expression of long-term facilitation. Confirming previous results (6), we found that CYP (200 μM) blocked short-term synaptic facilitation; however, when the same synapse was examined 24 hours later, long-term facilitation was expressed normally (Fig. 1B). The ANOVA described above (5) revealed an overall significant interaction among the 5-HT–alone, 5-HT + CYP, and control conditions ($F_{2,2}$ = 5.16, $P < 0.004$). Subsequent planned comparisons showed that, when CYP was present during 5-HT application, short-term facilitation was blocked (mean increase = 8.5%, t_4 = 0.65, NS), but significant long-term facilitation was still expressed (mean increase = 97.6%, t_4 = 3.3, $P < 0.03$). Between-group comparisons further showed that short-term facilitation was significantly blocked in the 5-HT + CYP cells compared to the 5-HT–alone cells (t_9 = 1.83, $P < 0.05$, one-tailed), but there was no statistical difference in the long-term facilitation produced by either treatment (t_9 = 0.19, NS). Finally, CYP alone had no effect on baseline synaptic transmission (Fig. 1B) or on EPSPs that had previously been facilitated by 5-HT in either short-term or long-term form. Thus, CYP blocked the induction of short-term (but not long-term) facilitation, but it did not block the expression of either short-term or long-term facilitation.

In the next experiments we asked whether we could identify conditions under which the normal agonist for the short- and long-term processes (5-HT) could selectively induce long-term facilitation in the absence of short-term facilitation. We took advantage of our recent observation that threshold 5-HT concentrations sufficient to induce increases in excitability and input resistance in the SNs do not induce short-term synaptic facilitation (7). We thus asked whether multiple applications of 5-HT that were empirically adjusted to be below threshold for short-term facilitation could induce long-term facilitation. We found that 5-HT concentrations (mean = 2.08 μM ± 0.35) sufficient to increase SN excitability but insufficient to produce short-term synaptic facilitation (mean change = −3.7%) (Fig. 2) nonetheless still induced significant long-term facilitation (mean increase = 64.8%, t_5 = 3.63, $P < 0.01$) (8). Thus, as before (Fig. 1B), long-term facilitation was induced and expressed in the absence of short-term facilitation.

The SN cell bodies are located in the pleural ganglion, whereas their synapses onto tail MNs are located in the pedal ganglion (4) [the SNs also make monosynaptic contact with interneurons in the pleural ganglion (9)]. This anatomical arrangement allowed us to use an approach introduced by Clark and Kandel (10) to deliver 5-HT exclusively to the SN cell body and local synaptic region or to a region of remote synaptic contact between SNs and MNs (11). Using this experimental strategy, we found that prolonged exposure [1.5 hours (11)] of the remote synaptic region to 5-HT produced significant short-term facilitation (mean increase = 117.4%, t_3 = 2.9, $P < 0.03$) but 24 hours later no significant long-term facilitation was expressed (mean increase = 3.2%, t_3 = 0.18, NS) (Fig. 3A) (12). In contrast, 5-HT exposure to the SN cell body and local synaptic region produced no significant short-term facilitation of the SN-MN connection in the pedal ganglion (mean increase = 2.85%, t_4 = 0.69, NS), but it still produced long-term facilitation of that synapse (mean increase = 66%, t_4 = 3.92, $P < 0.02$) (Fig. 3B). Thus, these data show that synapses that have not been exposed to 5-HT and have not expressed short-term facilitation are nonetheless fully capable of expressing long-term facilitation 24 hours later.

Short-term and long-term synaptic facilitation in *Aplysia* SNs have been distinguished on a variety of levels of analysis including (i) stimulus requirements (3, 13–17), (ii) ionic currents (2, 13, 14), (iii) second messenger systems (14–17), and (iv) mechanisms of action (2, 3, 17–19). However, despite these differences, it was not known whether the expression of the cellular events underlying short-term synaptic facilitation were required for the subsequent expression of long-term facilitation. Our evidence shows that long-term synaptic facilitation does not require the prior expression of short-term facilitation. Thus, we propose the model illustrated in Fig. 4. A primary feature of the model is that short-

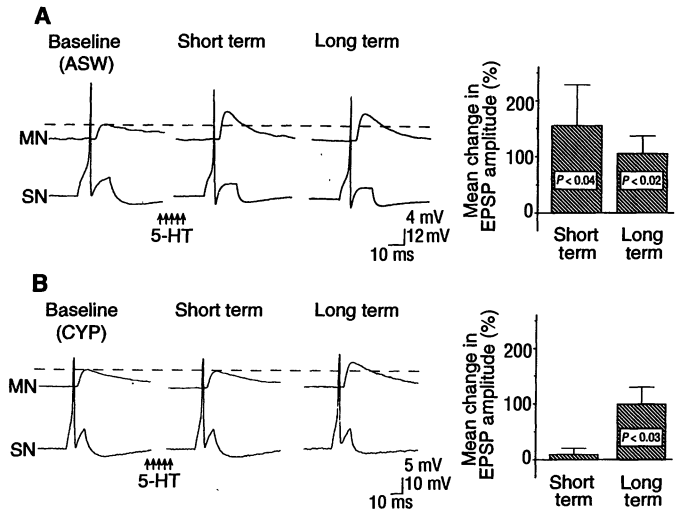

Fig. 1. Effects of cyproheptadine on short-term and long-term synaptic facilitation. (**A**) The monosynaptic EPSP from a tail SN onto a tail MN is shown. Repeated 5-HT (5 μM) applications are indicated by arrows. EPSPs are from tests, immediately after the first 5-HT application (short-term) and 24 hours later (long-term). Dashed line indicates the amplitude of the baseline EPSP. Summary of experiments (n = 6); in this and other summary graphs, data are expressed as means ± SEM. (**B**) Same experiment as (A) except CYP is present throughout baseline, during 5-HT (5 μM) application, and during the short-term test. Summary of experiments (n = 5).

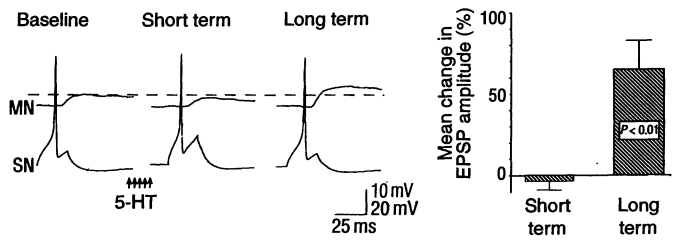

Fig. 2. Effects of 5-HT concentration on short-term and long-term facilitation. Multiple applications of 5-HT, empirically determined to be subthreshold for short-term facilitation (7), are shown (in this example, 5-HT = 2 μM). Summary of experiments (n = 6).

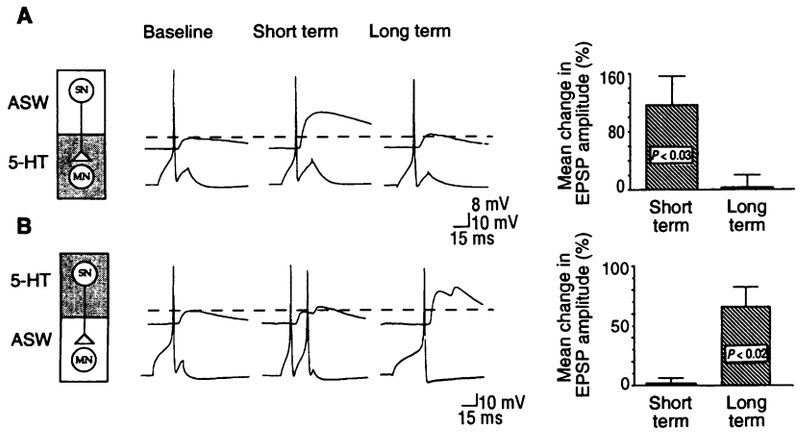

Fig. 3. Effects of local 5-HT application to either remote SN-MN synapses or to soma and proximal synapses. (**A**) Prolonged 5-HT exposure exclusively to the remote SN-MN synaptic region. Summary of experiments (n = 4). (**B**) Prolonged 5-HT exposure exclusively to the somatic and local synaptic region. Summary of experiments (n = 5).

term and long-term synaptic facilitation can be produced in parallel: the short-term process is rapidly induced and expressed locally at the synapse by 5-HT–induced spike broadening (*2*, *6*, *13*), whereas the long-term process is delayed in onset, ultimately requiring a somatic signal [for example, adenosine 3′5′-monophosphate (cAMP)] [induced by either somatic or synaptic (*12*) 5-HT input], which in turn initiates gene activation and protein synthesis (*3*, *17*–*19*). Consistent with this view, we find that, with whole CNS 5-HT application, short-term facilitation at the synapse can completely decay before the subsequent expression of long-term facilitation of that same synapse several hours later (*20*).

A major implication of the model is that long-term facilitation of the SN synapses, because of its dependence on a somatic (nuclear) signal, should always occur cell-wide. Thus, synapses that have not been exposed to 5-HT input and have not expressed short-term facilitation can still be the recipients of long-term related gene products induced by protein synthesis in the soma (Fig. 3B). The model also allows for the possibility of the physiological induction of long-term processes in the absence of any short-term modification. A correlative aspect of this feature of the model is that it emphasizes a potentially general role in long-term modulation for synaptic input to the soma of neurons, because somatic input would be optimally placed to induce genomic activation [see also (*21*)].

In conclusion, although we have presented evidence for parallel processing of short-term and long-term synaptic facilitation in SNs, our results do not imply that behaviorally expressed STM and LTM subserved by these and other synaptic modifications in *Aplysia* are necessarily processed in parallel. Even within the SNs themselves other modulatory actions of 5-HT (for example, increased excitability) are induced in both a short-term and a long-term form (*22*) and thus could, in principle, provide some serial processing in STM and LTM (*23*).

Interestingly, there are precedents for long-term processing in the apparent absence of short-term processing in other systems. In the marine mollusk *Hermissenda* 5-HT–induced short-term enhancement of the generator potential in identified photoreceptors can be blocked without affecting long-term enhancement in the same cells (*24*). Moreover, in humans, some clinical conditions can result in impaired STM but normal LTM (*25*). Therefore, the possibility exists that at least some aspects of STM and LTM are processed in parallel in a variety of systems.

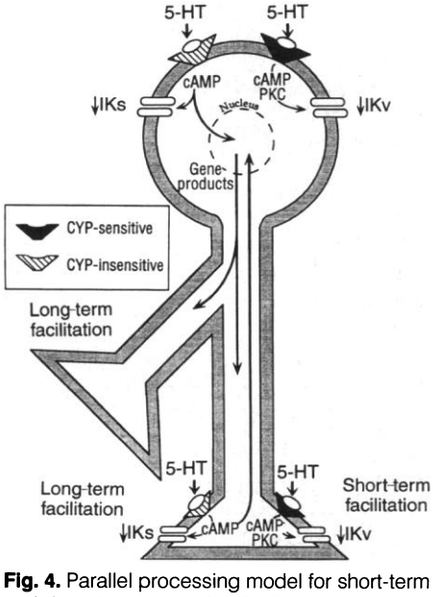

Fig. 4. Parallel processing model for short-term and long-term synaptic facilitation in which short-term facilitation can occur locally but long-term facilitation occurs cellwide; PKC, protein kinase C; IKs, 5-HT–sensitive potassium current; and IKv, delayed rectifier potassium current.

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5. We normalized the data by expressing all test EPSPs as a percentage of average baseline EPSPs. Overall significance was first determined with an ANOVA to establish a significant treatment × trials interaction. Subsequent planned within-group and between-group comparisons were made by means of t tests for correlated or independent means, respectively. All P values are two-tailed except where indicated.
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11. Pleural and pedal ganglia were placed in two chambers separated by a Plexiglas wall, which was sealed with petroleum jelly. Each chamber was independently perfused. A single 1.5-hour exposure of 5-HT was used to induce long-term facilitation because the barrier separating the chambers was easily disrupted by repeated bath exchanges required for multiple 5-HT applications.
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Mutation of Glycine Receptor Subunit Creates β-Alanine Receptor Responsive to GABA

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The amino acid at position 160 of the ligand-binding subunit, α1, is an important determinant of agonist and antagonist binding to the glycine receptor. Exchange of the neighboring residues, phenylalanine at position 159 and tyrosine at position 161, increased the efficacy of amino acid agonists. Whereas wild-type α1 channels expressed in *Xenopus* oocytes required 0.7 millimolar β-alanine for a half-maximal response, the doubly mutated (F159Y,Y161F) α1 subunit had an affinity for β-alanine (which was more potent than glycine) that was 110-fold that of the wild type. Also, γ-aminobutyric acid and D-serine, amino acids that do not activate wild-type α1 receptors, efficiently gated the mutant channel. Thus, aromatic hydroxyl groups are crucial for ligand discrimination at inhibitory amino acid receptors.

Neurotransmitter receptors mediate synaptic transmission in the nervous system and constitute important target sites of many therapeutic drugs. The ligand-gated ion channel subclass of neurotransmitter receptors consists of families of oligomeric proteins, which are composed of homologous ligand-binding and structural subunits (*1*). The mechanism of agonist discrimination at these receptors is not understood. Covalent labeling and site-directed mutagenesis experiments indicate that several discontinuous domains in the NH_2-terminal extracellular region of the ligand-binding subunits contribute to the formation of the binding pocket (*2–5*).

In the central nervous system, amino acids are the major excitatory and inhibitory neurotransmitters. The glycine receptor (GlyR) is the most abundant inhibitory neurotransmitter receptor in the spinal cord (*6*), whereas subtype A of the γ-aminobutyric acid receptor ($GABA_AR$) mediates inhibition in the mammalian brain (*7*). The GlyR, isolated from spinal cord (*8*), contains five membrane-spanning subunits (*9*) of apparent molecular masses of 48 kD (α) and 58 kD (β); both subunits are homologous to $GABA_AR$ and, to a much lesser extent, nicotinic acetylcholine receptor (nAChR) proteins (*1*, *10*). Several GlyR α subunits (α1, α2, α2*, and α3) have been identified by complementary DNA (cDNA) cloning (*11–13*). On heterologous expression these ligand-binding subunits generate agonist-gated Cl^- channels whose pharmacology mimics that of the postsynaptic GlyR (*11–15*).

Studies on mutated GlyR α1 and α2 subunits have identified a conserved glycine residue (corresponding to position 160 of the α1 subunit; Fig. 1) as an important determinant of ligand binding (*12*, *16*, *17*). Interestingly, two aromatic amino acids flanking this residue are conserved at the corresponding positions of the agonist-binding subunits of the $GABA_AR$ (*18*). However, residue 159, where a phenylalanine is found in all GlyR subunits, is replaced by a tyrosine in the $GABA_AR$ polypeptides (Fig. 1). We therefore introduced by site-directed mutagenesis (*19*) a tyrosine at position 159 (F159Y) and a phenylalanine at position 161 (Y161F) of the GlyR α1 subunit and exchanged both aromatic residues in the double mutant $\alpha1^{F159Y,Y161F}$. Voltage-clamp analysis of the mutated proteins expressed in *Xenopus* oocytes (*20*) revealed an unfamiliar agonist pharmacology. First, the glycine concentrations required to gate the $\alpha1^{F159Y,Y161F}$ channels were about 1/10 [concentration for eliciting a half-maximal response (EC_{50}) of 22 μM] of those required for the wild-type α1 GlyR (EC_{50} = 260 μM) (Fig. 2 and Table 1). Second, the glycinergic agonist β-alanine (*7*) became more potent than glycine (Fig. 2). Its EC_{50} value decreased about 110-fold from 720 μM for the wild-type α1 GlyR to 6.5 μM for the double mutant (Table 1). Moreover, the Hill coefficient (h) for glycine changed from 2.4 ± 0.2 (mean ± SEM) for the α1 GlyR to 1.8 ± 0.2 for the $\alpha1^{F159Y,Y161F}$ receptor. The response to β-alanine showed an inverse behavior, with $h = 1.6 \pm 0.1$ for wild-type α1, and $h = 2.5 \pm 0.2$ for the mutant $\alpha1^{F159Y,Y161F}$. Also, the mean maximal currents (I_{max}) obtained for the double mutant with β-alanine and glycine were

GlyR	α1	154	M	Q	L	E	S	F	G	Y	T	M	N	D	L
$GABA_AR$	α1	155	L	K	F	G	S	Y	A	Y	T	R	A	E	V
$GABA_AR$	β1	152	L	E	I	E	S	Y	G	Y	T	T	D	D	I
nAChR	β1	144	M	K	L	G	T	W	T	Y	D	G	S	V	V

Fig. 1. Alignment of partial GlyR, $GABA_AR$, and nAChR subunit sequences. The regions homologous to amino acids 154 to 166 of the GlyR α1 subunit are shown; this segment is conserved in the mammalian GlyR α1, α2, and α3 subunits (*13*). Amino acids homologous to residues 159 to 161 of the GlyR α1 subunit are boxed. Sequences are from the following references: human GlyR α1 subunit (*11*); bovine $GABA_AR$ α and β subunits (*18*); and bovine nAChR α1 subunit (*28*).

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