A cellular model of memory reconsolidation involves reactivation-induced destabilization and restabilization at the sensorimotor synapse in *Aplysia*

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The memory reconsolidation hypothesis suggests that a memory trace becomes labile after retrieval and needs to be reconsolidated before it can be stabilized. However, it is unclear from earlier studies whether the same synapses involved in encoding the memory trace are those that are destabilized and restabilized after the synaptic reactivation that accompanies memory retrieval, or whether new and different synapses are recruited. To address this issue, we studied a simple nonassociative form of memory, long-term sensitization of the gill- and siphon-withdrawal reflex in *Aplysia*, and its cellular analog, long-term facilitation at the sensory-to-motor neuron synapse. We found that after memory retrieval, behavioral long-term sensitization in *Aplysia* becomes labile via ubiquitin/proteasome-dependent protein degradation and is reconsolidated by means of de novo protein synthesis. In parallel, we found that on the cellular level, long-term facilitation at the sensory-to-motor neuron synapse that mediates long-term sensitization is also destabilized by protein degradation and is restabilized by protein synthesis after synaptic reactivation, a procedure that parallels memory retrieval or retraining evident on the behavioral level. These results provide direct evidence that the same synapses that store the long-term memory trace encoded by changes in the strength of synaptic connections critical for sensitization are disrupted and reconstructed after signal retrieval.

memory reorganization | memory recall | 5-HT | local protein synthesis | clasto-lactacystin beta-lactone

The processes of memory reactivation (retrieval) have been the focus of several studies over the last decade. Retrieval is thought to return the memory to an unstable (labile) state, in which de novo protein synthesis-dependent reconsolidation is required to continue maintaining the memory over time (1–4). Memory reconsolidation has been reported for a variety of memory paradigms in a number of different animal models (1, 3, 5, 6); however, how memory reconsolidation works remains unclear.

At least two nonmutually exclusive hypotheses have been proposed (7). One hypothesis suggests that reconsolidation is an updating process in which the synapses that encode the preexisting memory are reorganized after memory retrieval so as to recruit new synaptic connections that allow the incorporation of new information (8–10). The second hypothesis suggests a mechanism that is a continuation of the consolidation process at the same set of synaptic connections and that serves to strengthen memory, allowing it to become longer lasting and enduring and thereby preventing forgetting (11). Both of these views of reconsolidation are consistent with retraining or retrieval. In each case, synaptic reactivation could be implicit (e.g., during sleep) or explicit, and both would presumably have the same effect of making the memory stronger, more stable, and more resistant to postretrieval interference.

Both types of reconsolidation hypotheses imply that the stored memory becomes labile after memory retrieval. To address how this occurs, we studied the retrieval of memories and found that they become labile via ubiquitin/proteasome-dependent synaptic protein degradation (9, 12). Moreover, Doyère et al. (13) found that inhibition of reconsolidation is correlated with reduced potentiation at reactivated synapses in the lateral amygdala. The foregoing studies suggest that signal retrieval activates protein degradation in the synaptic connections encoding the initial memory, and that protein synthesis is required for restoring or maintaining the memory. However, it remains unclear whether destabilization and restabilization after memory retrieval occur at the same synaptic connections where potentiation occurs for memory encoding (9), or whether different synaptic connections are involved in the retrieval process (8, 14).

To address this issue, we used the elementary neural circuit that underlies sensitization of the gill- and siphon-withdrawal reflex, a simple form of nonassociative learned fear in *Aplysia*. A critical component of this reflex that contributes significantly to this behavior is the direct monosynaptic connection from the siphon sensory neuron to gill and siphon motor neurons. The sensory-to-motor neuron synapse can be reconstituted in dissociated cell culture, where it is modulated, as in the intact animal, by serotonin (5-HT), a modulatory transmitter released during sensitization training (15). Five applications of 5-HT over a period of 1.5 h—designed to simulate five shocks to the tail that produce long-term behavioral sensitization—produce both a long-term increase in the strength of the sensory-to-motor neuron synaptic connection lasting several days [long-term facilitation (LTF)] (16) and structural remodeling and growth of new sensory-to-motor neuron synapses (17–19). The consolidation of both long-term sensitization and its cellular analog, LTF at the sensory-to-motor neuron synapse, requires de novo protein synthesis (16, 20–23).

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During sensitization training, stimulating the tail activates interneurons that release 5-HT onto the mechanoreceptor sensory neurons that innervate the siphon skin (24), resulting in a strengthening of the sensory-to-motor neuron synapses that control the siphon-withdrawal reflex (SWR) (25–27). Furthermore, the molecular mechanisms that govern behavioral long-term sensitization also govern the learning-related synaptic plasticity exhibited by the sensory-to-motor neuron synapses (16, 21, 28, 29). Therefore, the long-term memory (LTM) for sensitization in *Aplysia* and its sensory-to-motor neuron synapses are useful tools for studying fundamental properties of synapses, such as destabilization and restabilization after memory retrieval.

Here we first examined whether reconsolidation is required for maintaining behavioral LTM after retrieval or retraining of sensitization in *Aplysia*. We then investigated whether synaptic disruption and reconstruction are also necessary, at the same set of synaptic connections between the sensory and motor neurons that initially stored the memory, for maintaining LTF (the cellular correlate of sensitization) after synaptic reactivation protocols that mimic retrieval or retraining of the behavioral modification in the intact animal.

Results

Consistent with previous results, repetitive tail shocks induced behavioral long-term sensitization in *Aplysia* (Fig. S1*A*) (21, 25, 26, 30). The duration of the SWR was increased significantly at 24 h after training; however, in the group that was injected with the protein synthesis inhibitor emetine, LTM was specifically impaired, whereas short-term memory (STM) remained intact (Fig. S1*A*). Two-way ANOVA with time points and drug groups as factors revealed significant effects of time points [$F_{(2,30)}$ = 65.73, $P < 0.01$], drug groups [$F_{(2,15)}$ = 4.67, $P < 0.05$], and the interaction between them [$F_{(4,30)}$ = 9.64, $P < 0.01$].

A growing body of evidence supports the idea that when a memory is recalled and thereby reactivated, it undergoes a labile state, and that a new, protein synthesis-dependent reconsolidation process is required to maintain that memory (1–4). To examine whether new protein synthesis is also required to maintain the behavioral LTM for sensitization in *Aplysia* after memory retrieval, we injected emetine immediately after retrieval of the sensitized response (Fig. S1*B*). We found that emetine injection immediately after the first reactivation test (retrieval by brushing the siphon) significantly impaired LTM in the second reactivation test performed 24 h later, whereas vehicle injection had no effect on the second test (Fig. S1*B*). Moreover, vehicle or emetine injection in the absence of a first reactivation test did not affect LTM after the second test (Fig. S1*B*). Two-way ANOVA with time points (for pretest and second LTM test) and drug groups as factors revealed significant effects of time points [$F_{(1,51)}$ = 188.2, $P < 0.01$], drug groups [$F_{(3,51)}$ = 13.17, $P < 0.01$], and the interaction between them [$F_{(3,51)}$ = 12.49, $P < 0.01$]. These results suggest that storage of the behavioral LTM for sensitization in the animal becomes labile after memory retrieval, and that de novo protein synthesis-dependent reconsolidation is required for its long-term maintenance.

Our recently reported data in mice demonstrated that a reactivated fear memory becomes labile via ubiquitin/proteasome-dependent protein degradation (9). In addition, it is well known that ubiquitin-proteasome system is critical for long-term changes, such as LTF and long-term depression in *Aplysia* (31–34). We thus tested whether the behavioral LTM for sensitization in *Aplysia* is also destabilized via ubiquitin/proteasome-dependent protein degradation after memory retrieval. To do so, we first determined the concentration of the ubiquitin/proteasome inhibitor clasto-lactacystin β-lactone (βlac) needed to examine the effect of ubiquitin/proteasome inhibitor βlac on the LTM for sensitization of the SWR in *Aplysia*. We found that 0.5 μM βlac did not affect the duration of behavioral sensitization, whereas higher concentrations (≥1.0 μM) increased this duration (25). Consistent with previous results regarding fear memory (9), we found that inhibiting ubiquitin/proteasome with the injection of 0.5 μM βlac after the first reactivation test (retrieval by brushing the siphon) prevented the memory disruption induced by emetine injection (Fig. 1). βlac injection alone after the first reactivation test did not affect long-term sensitization (Fig. 1). Two-way ANOVA with time points (for pretest and second LTM test) and drug groups as factors revealed significant effects of time points [$F_{(1,40)}$ = 296.9, $P < 0.01$], drug groups [$F_{(3,40)}$ = 13.08, $P < 0.01$], and the interaction between them [$F_{(3,40)}$ = 10.68, $P < 0.01$]. In addition, two-way ANOVA with the application of emetine and βlac as factors at the second LTM test showed a significant effect of the interaction between the two factors [$F_{(1,40)}$ = 11.24, $P < 0.01$]. These results indicate that inhibition of ubiquitin/proteasome prevents LTM from becoming labile after retrieval, and that ubiquitin/proteasome-dependent protein degradation is critical for retrieval-dependent disruption of long-term sensitization in *Aplysia*.

We also observed similar results when weak behavioral training (retraining, involving two electrical shocks to the tail) was applied as a reactivation signal instead of the first reactivation test (retrieval by brushing the siphon) used in the previous experiments. Emetine injection immediately after retraining significantly impaired post-retraining (PR)-LTM 24 h later, whereas vehicle or βlac injection alone injection had no effect (Fig. 2). In addition, emetine or vehicle injection without retraining did not impair storage of the PR-LTM trace. Moreover, βlac injection prevented the impairment of PR-LTM storage induced by emetine injection (Fig. 2).

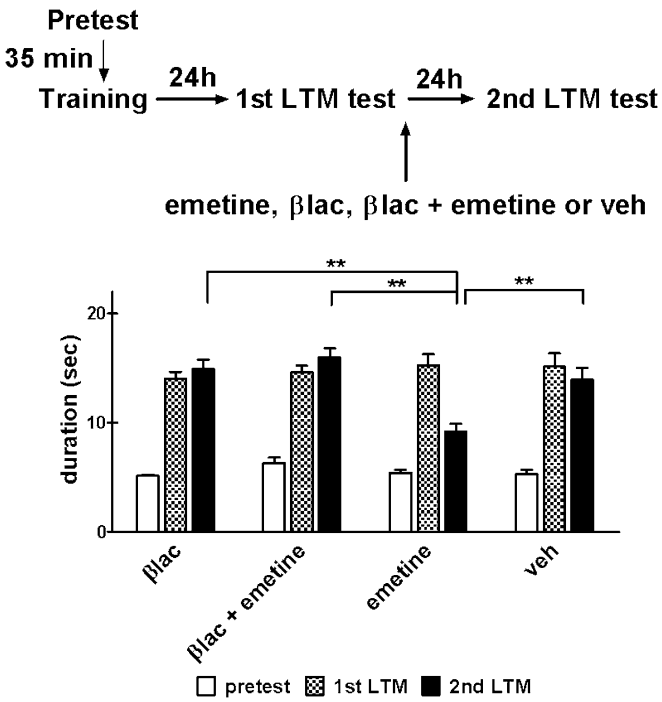

Fig. 1. Protein degradation-dependent destabilization of LTM for behavioral sensitization. (*Upper*) Schematic of the experimental procedure used to evaluate the effect of βlac on LTM after retrieval. (*Lower*) Bar graph showing mean ± SEM of SWR duration on the pretest, first LTM test, and second LTM test. Compared with the vehicle-injected group (veh; n = 10), βlac injection alone after the first LTM test (βlac; n = 11) had no effect on LTM on the second LTM test, whereas the emetine-injected group (emetine; n = 13) exhibited impaired LTM on the second LTM test. However, concurrent injection of βlac with emetine (emetine + βlac, n = 10) prevented impairment of LTM on the second LTM test induced by emetine injection [$F_{(3,40)}$ = 13.28, $P < 0.01$, one-way ANOVA]. $^{**}P < 0.01$, Newman–Keuls multiple-comparison test.

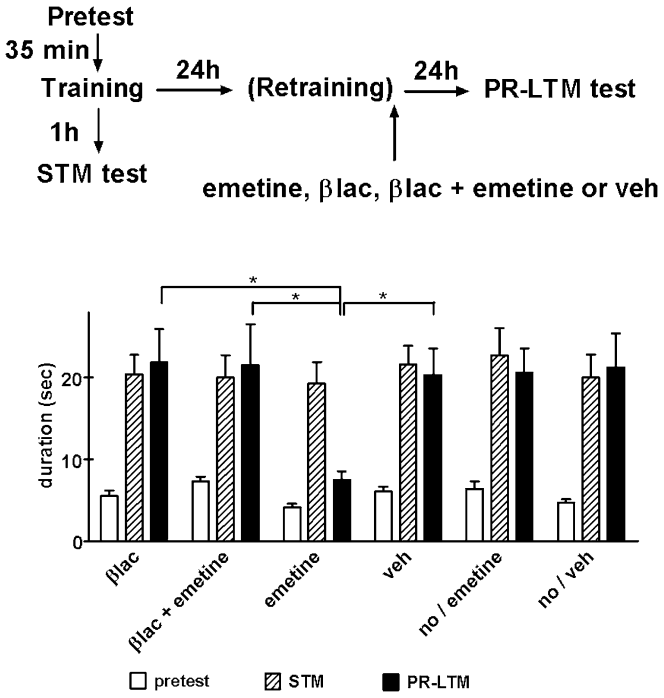

Fig. 2. Destabilization and restabilization of LTM for behavioral sensitization after retraining. (*Upper*) Schematic of the experimental procedure used to evaluate the effect of emetine or βlac on LTM after retraining. (*Lower*) Bar graph showing mean ± SEM of SWR duration on the pretest, STM test, and post-retraining (PR)-LTM test. Emetine injection just after retraining (emetine; $n = 14$) impaired PR-LTM, whereas emetine injection without retraining (no/emetine; $n = 15$), or vehicle injection with retraining (veh; $n = 16$) and without retraining (no/veh; $n = 13$) had no effect on PR-LTM test. However, concurrent injection of βlac with emetine (βlac + emetine; $n = 11$) prevented impairment of LTM on PR-LTM test induced by emetine injection. βlac injection alone (βlac; $n = 15$) had no effect on the PR-LTM test [$F_{(3,52)} = 3.88$, $P < 0.05$; one-way ANOVA]. *$P < 0.05$, Newman–Keuls multiple-comparison test.

We next asked whether the same sensory-to-motor neuron synapse that was facilitated by five pulses of 5-HT also undergoes destabilization and restabilization after synaptic reactivation. To address this question, we first measured the basal strength of the sensory-to-motor neuron synapse (first recording), and then induced LTF with five pulses of 5-HT. At 24 h after the 5-HT treatment, we retested synaptic strength (second recording) and found that it was significantly greater than the basal level (all groups, $P < 0.01$, one-sample t test compared with basal level of 0). After the second recording, we applied homosynaptic activation (HA) (21) as a reactivation (retrieval) signal by generating four action potentials (at 1-min intervals) in the sensory neuron of the sensory-to-motor neuron synapse. For the third recording (at 24 h after the second recording), the facilitated synaptic strength of the reactivated group (HA + vehicle group) was maintained and was significantly greater than the basal level ($P < 0.01$, one-sample t test compared with basal level), although the synaptic strength at the third recording showed a tendency toward a decrease compared with the strength at the second recording (Fig. 3*A*). However, combining HA with emetine after the second recording (HA + emetine group) disrupted the strength of the facilitated synapse, causing a reversion to its basal strength at the third recording ($P > 0.43$, one-sample t test compared with basal level) (Fig. 3*A*).

Vehicle application without HA (vehicle group) or emetine treatment without HA (emetine group) at the second recording did not disrupt the facilitated synaptic strength, and so the synaptic strength was significantly different from the basal level ($P < 0.05$, one-sample t test compared with basal level) (Fig. S2). The emetine-only group, in which only emetine was applied without the second recording at 24 h after the first recording, also maintained synaptic strength at the third recording (Fig. S2). Although the group to which emetine was applied 24 h after the first recording exhibited some reduction in synaptic strength, this reduction was not statistically significantly different from that in either the vehicle or emetine-only group. These results suggest that LTF at the sensory-to-motor neuron synapse reactivated by HA undergoes a reconsolidation phase after synaptic reactivation.

To determine whether ubiquitin/proteasome-dependent synaptic destabilization and protein synthesis-dependent synaptic restabilization occur at the same sensory-to-motor neuron synapse, we applied 0.1 μM βlac, which has no effect on basal synaptic strength or on consolidation of LTF, to the reactivated synapse using HA (Fig. 3 and Fig. S3). Consistent with the behavioral results, βlac treatment prevented the disruption of LTF induced by emetine (HA + βlac + emetine), whereas βlac treatment alone (HA + βlac) had no effect (Fig. 3*A*). One-way ANOVA with HA groups at the third recording revealed a significant effect of drug treatment, and application of the Newman–Keuls multiple-comparison post hoc test showed significantly lower synaptic strength in the HA + emetine group at the third recording compared with the HA + vehicle, HA + βlac + emetine, and HA + βlac groups (Fig. 3*A*). Synaptic strength in the HA + βlac + emetine and HA + βlac groups was not significantly different from that in the HA + vehicle group at the third recording. Two-way ANOVA with time points (for the first and the third recordings) and drug groups as factors revealed significant effects of time points [$F_{(1,31)} = 40.96$, $P < 0.01$], drug groups [$F_{(3,31)} = 4.38$, $P < 0.05$], and the interaction between them [$F_{(3,31)} = 4.38$, $P < 0.05$].

These results suggest that consolidated LTF at the sensory-to-motor neuron synapse is destabilized via ubiquitin/proteasome-dependent protein degradation after synaptic reactivation (retrieval), and that synaptic destabilization and restabilization after synaptic reactivation occur at the same synaptic connections. Possible cellular signaling cascades, such as alterations in intracellular Ca^{2+} levels, that may be recruited by HA of sensory neurons also might be involved in synapse destabilization.

Because reactivation of the behavioral LTM for sensitization by retraining (two electrical shocks to the tail) also demonstrated labile and reconsolidation phases similar to reactivation of LTM by retrieval (brushing the siphon), we investigated another reactivation method in sensory-to-motor neuron cocultures. We retreated the synaptic connection with five pulses of 5-HT to mimic the retraining protocol of electrical shocks used in the behavioral experiments. We found that five pulses of 5-HT increased the synaptic strength of sensory-to-motor neuron synapses at the second recording (Fig. 4). After the second recording 24 h later, we again applied five pulses of 5-HT as a reactivation (retraining) signal, then retested synaptic strength. Emetine treatment during and after 5-HT retreatment (5 × 5-HT + emetine) significantly disrupted the facilitation of the synapse, whereas retreatment with vehicle and 5-HT (5 × 5-HT + vehicle) did not affect maintenance of synaptic strength (Fig. 4). Moreover, βlac treatment combined with emetine (5 × 5-HT + βlac + emetine group) prevented emetine-induced disruption of LTF, whereas βlac treatment (5 × 5-HT + βlac) alone had no effect (Fig. 4). Results for the 5 × 5-HT + βlac + emetine group and the 5 × 5-HT + βlac group were not significantly different from those for the 5 × 5-HT + vehicle group at the third recording. Two-way ANOVA with time points (for the first and the third recordings) and drug groups as factors revealed significant effects of time points [$F_{(1,47)} = 21.98$, $P < 0.01$], drug groups [$F_{(3,47)} = 4.85$, $P < 0.01$], and the interaction between them [$F_{(3,47)} = 4.85$, $P < 0.01$]. Although we cannot exclude the possibility that a single stimulation of the sensory neuron used to evoke

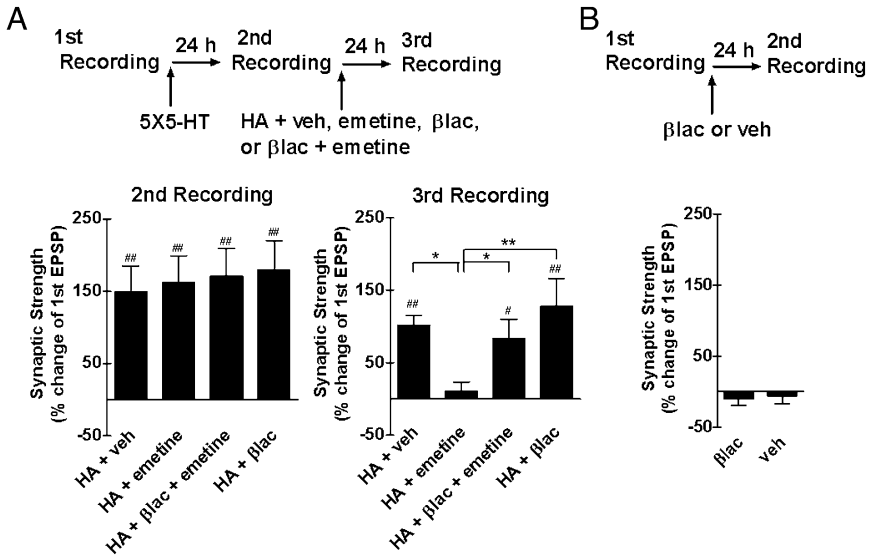

Fig. 3. Synaptic destabilization and restabilization of LTF at the sensory-to-motor neuron synapse after reactivation by HA. (*A*) (*Upper*) Schematic of the experimental procedure used for evaluating the effect of synaptic reactivation by HA on LTF. (*Lower*) Bar graph showing mean percentage change ± SEM in EPSP amplitudes. (*Lower*, *Left*) On the second recording, the changes of EPSP amplitudes were not significantly different among groups. (*Lower*, *Right*) Compared with the vehicle application after HA (HA + veh; $n = 8$), emetine treatment after HA (HA + emetine; $n = 10$) impaired LTF on the third recording, whereas βlac treatment alone after HA (HA + βlac; $n = 9$) had no effect. However, βlac treatment immediately after the second recording prevented impairment of LTM induced by emetine treatment (HA + βlac + emetine; $n = 8$). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, one-sample *t* test compared with basal level. [$F_{(3,31)} = 4.38$, $P < 0.05$, one-way ANOVA], $^{*}P < 0.05$, $^{**}P < 0.01$, Newman–Keuls multiple-comparison test. There was no significant difference among groups in the EPSP amplitudes of the first recording [$F_{(3,31)} = 1.35$, $P > 0.27$; one-way ANOVA], and no significant correlation between the EPSP amplitudes of the first recording and the facilitated level of the second recording ($P > 0.11$, Pearson correlation). (*B*) Proteasome inhibitor βlac did not affect basal synaptic transmission. (*Upper*) Schematic of the experimental procedure used to evaluate the effect of βlac treatment on basal synaptic transmission. (*Lower*) Bar graphs showing mean percentage change ± SEM in EPSP amplitudes. Neither βlac ($n = 5$) nor vehicle (veh; $n = 6$) treatment affected basal synaptic transmission.

the excitatory postsynaptic potential (EPSP) during the second recording provides sufficient activity to account for a partial effect of emetine (Fig. S2), our results do suggest that emetine has a stronger effect when applied in association with a multiple stimulation paradigm (HA) or 5-HT retraining that likely mimics stronger retrieval stimuli. Thus, once reactivated, LTF at the sensory-to-motor neuron synapse undergoes labile and reconsolidation phases.

Taken together, our data suggest that at both the cellular and behavioral levels, LTM becomes labile after retrieval or retraining, and that de novo protein synthesis-dependent reconsolidation is required to maintain memory storage both in the reflex of the animal and in the underling synaptic mechanism in the neural circuit of the reflex.

A previous study found that the persistence of LTF in culture still requires local protein synthesis at the sensory-to-motor neuron synapse at 24 h and 48 h after treatment with five pulses of 5-HT, suggesting that the time window for consolidation is a natural consequence of long-term training (35). Consequently, we tested whether de novo protein synthesis-dependent reconsolidation is also required for maintaining LTF at 72 h after the synaptic training—a time point at which inhibitors of local protein synthesis no longer disrupt the 5-HT–induced newly formed sensory neuron varicosities or LTF. The facilitated synapse was reactivated by five pulses of 5-HT at 72 h (retraining) after the first 5-HT treatment (training), and the strength was retested 48 h later (120 h after the training; Fig. S4), because the effect of emetine on both LTF and growth is more pronounced at this time point than at 24 h after its application (35). Consistent with the results shown in Fig. 4, the inhibition of protein synthesis combined with synaptic reactivation impaired the facilitated synaptic strength, which returned to the basal level, whereas the facilitated synaptic strength induced by 5 × 5-HT treatment after the first recording was maintained in the vehicle and emetine groups (Fig. S4).

These data suggest that even at 72 h after the initial 5-HT training, when the facilitated synapse is quite stable (perhaps now more "fully consolidated") and not disrupted by local application of inhibitors of protein synthesis, simple reactivation of the sensory-to-motor neuron synapse can still induce the protein synthesis-dependent reconsolidation required to maintain the increase in synaptic strength. Although there is a possibility that our synaptic recording from 0 h to 48 h was obtained in a time window when the consolidation process was still in process, the data clearly show that memory storage, even in a partially consolidated state, can be further destabilized by a reactivating stimulus. Thus, even during earlier stages in the process of consolidation (at 24 h and 48 h after initial training), it is possible that reactivated synapses also may undergo a phase that requires new protein synthesis compared with nonreactivated synapses.

Discussion

Our results suggest that sensory-to-motor neuron synapses, the primary components of the neural circuit underlying behavioral sensitization, are destabilized by means of ubiquitin/proteasome-dependent protein degradation after memory retrieval or retraining and are restabilized by a protein synthesis-dependent reconsolidation process. These results demonstrate that, at least in this model learning system, reconsolidation after memory retrieval or retraining involves transient and regulated changes of the stored memory trace at the same synaptic connections that were initially modified for storage of that trace. Furthermore, these synaptic mechanisms in *Aplysia* are likely to share important similarities with those that underlie reconsolidation in the mammalian brain, because many molecular mechanisms of learning and memory storage are shared by invertebrates and vertebrates.

The ubiquitin/proteasome system is known to have a critical role in the consolidation of long-term sensitization in *Aplysia* (32, 36) and FMRFamide-induced depression (34). The ubiquitin/proteasome

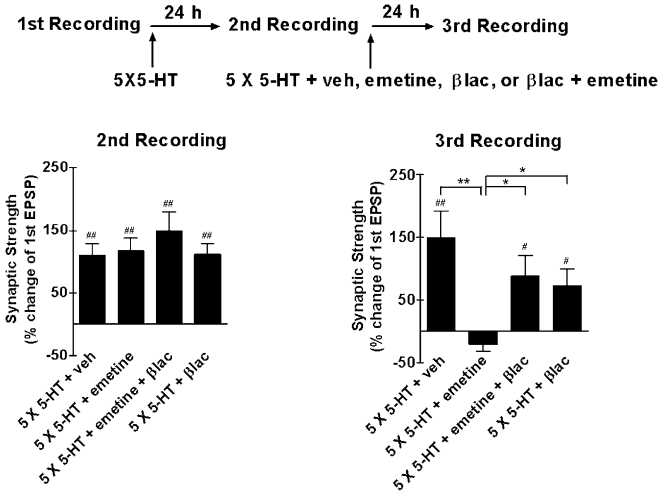

Fig. 4. Synaptic destabilization and restabilization of LTF at the sensory-to-motor neuron synapse after reactivation by 5-HT treatment. (*Upper*) Schematic of the experimental procedure used to evaluate the effect of emetine on LTF after synaptic reactivation with 5-HT treatment. (*Lower*) Bar graphs showing mean percentage change ± SEM in EPSP amplitudes. (*Lower*, *Left*) On the second recording, the changes in EPSP amplitudes were significantly different from the basal level recorded at the first recording, and were similar across groups. (*Lower*, *Right*) Concurrent application of emetine with five pulses of 5-HT (5 × 5-HT + emetine; $n = 12$) after the second recording impaired LTF at the third recording, whereas 5-HT (5 × 5-HT + veh; $n = 12$) or βlac treatment (5 × 5-HT + βlac; $n = 14$) alone after the second recording had no effect on LTF. βlac treatment combined with emetine (5 × 5-HT + βlac + emetine; $n = 13$) prevented LTF impairment on the third recording. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, one-sample t test compared with basal level [$F_{(3,47)} = 4.85$, $P < 0.01$; one-way ANOVA]. $^{*}P < 0.05$, $^{**}P < 0.01$, Newman–Keuls multiple-comparison test).

system also functions as an inhibitory constraint on synaptic strength and growth in a translation-dependent, but not a transcription-dependent, manner (37). Indeed, consistent with results of Zhao et al. (37), we also observed an increase of both basal synaptic transmission at sensory-to-motor neuron synapses in culture and reflex behavior in the presence of higher concentrations (1 μM) of βlac. However, the treatment of βlac at lower concentrations (0.1 μM for synapse recording and 0.5 μM for reflex behavior) affected only the reconsolidation and had no affect on the basal level or consolidation process in cultured synapses (Fig. 3*B* and Fig. S3). These results suggest that compared with the consolidation process, the reconsolidation process may be more sensitive to inhibition of the ubiquitin/proteasome system.

Taken together, our results show that long-term behavioral sensitization in *Aplysia*, as well as in its cellular representation, LTF at the sensory-to-motor neuron synapse, is destabilized and restabilized in response to memory reactivation signals. Kim et al. (38) reported that synapses in the amygdala also undergo a labile phase initiated by reactivation; however, because multiple synaptic connections were examined together in their experimental system, whether synaptic destabilization and restabilization occurred at the same synapses that initially encoded the memory was not clear.

Our findings provide the first direct evidence that the same specific synaptic connections that initially encode the stored memories are selectively destabilized and restabilized after memory retrieval. This is consistent with the idea that reconsolidation represents a continuation of the consolidation process at the same set of synaptic connections and may serve to strengthen memory storage by allowing it to become longer-lasting and more stable. Whether or not reconsolidation in *Aplysia* also may represent an updating process in which the synapses that encode the preexisting memory are further reorganized after memory retrieval so as to recruit new synaptic connections that allow the incorporation of new information was not addressed in this study. Future imaging studies in *Aplysia* cultures where the "circuit" of a sensory neuron is expanded by the addition of more than one type of target cell may help answer this question.

The simplicity of our model synaptic system should facilitate addressing a set of questions that are fundamental to a further understanding of the function of reconsolidation. For example, does the process of reconsolidation recruit some of the same cellular and molecular mechanisms that underlie consolidation? Do CPEB and synaptic growth, which are essential for the stable maintenance of LTM in *Aplysia*, also play a role in the reconsolidation-dependent strengthening of memory storage? What is the nature of the reorganization induced by memory retrieval at the presynaptic and postsynaptic sites? Are these changes coordinated? Which synaptic proteins are degraded and which proteins are resynthesized during the reorganization process? Answers to these questions will provide a molecular foundation that should lead to a better understanding not only of the mechanisms that underlie the processes of retrieval and reconsolidation, but also of the contribution of these processes to the storage of LTM.

Methods

***Aplysia* Behavior Task.** In all experiments, behavioral results were videotaped, and the duration of the SWR was measured by a blinded observer. Before the sensitization training, SWR duration was measured by briefly (~1 s) brushing the siphon (pretest). At 35 min after the pretest, 10 electrical tail shocks (100 mA for the training and retraining used in Fig. 2, and 50 mA for other experiments, 60 Hz AC, for 1.5 s, with a 10-min interval between shocks) were given to the freely moving *Aplysia* (training). For retraining, two electrical shocks (100 mA for 1.5 s, with 10-min interval between shocks) were used. SWR duration was measured again at 1 h (STM test), 24 h (LTM or first LTM test), or 48 h [second LTM test or post-retraining (PR)-LTM] after the training.

Electrophysiology. At 4 d after sensory-to-motor coculture, the first EPSP was evoked in an LFS motor cell by stimulating the sensory neurons with a brief depolarizing stimulus using an extracellular electrode (first recording). During EPSP measurement, the motor cell was impaled intracellularly with a glass microelectrode filled with 2 M K-acetate, 0.5 M KCl, and 10 mM K-Hepes (10–15 MΩ), and the membrane potential was held at –40 mV below its resting value. Then the sensory-to-motor neuron synapses received five pulses of 5-HT (10 μM, 5 min/pulse, with a 15-min interval between pulses) to induce LTF. To reactivate the facilitated synapse, HA or five pulses of 5-HT treatment was performed. HA represents activation induced by four action potentials in the sensory neuron of the sensory-to-motor synapse with a 1-min interval. We used this multiple recording paradigm to mimic stronger retrieval stimuli. To avoid providing long-term potentiation (39) or long-term depression (40) stimuli to sensory cells, a 1-min interval was applied. When the synaptic facilitation was examined at 120 h after the training (Fig. S3), half of the synapses were also tested at 24 h after the training to confirm the successful initiation of LTF; these synapses were no different from the other synapses at later testing time points (Fig. S5). To examine the effect of protein synthesis inhibition or proteasome inhibition after the reactivation, we applied emetine (100 μM; Sigma-Aldrich) or βlac (0.1 μM; Calbiochem) in the bath for 2.5 h immediately after the second recording. Bath application affects both the cell body and synapses simultaneously, which can serve to mimic our behavioral experimental conditions (i.p. injection). To examine the effect of proteasome inhibition on basal synaptic transmission, we applied βlac (0.1 μM) or vehicle (0.1% DMSO) for 2.5 h immediately after the first recording, and performed the second recording 24 h later. When emetine or βlac was applied with 5-HT treatment, the inhibitor (emetine or βlac) was bath-applied at 30 min before the first application of 5-HT and remained in the bath throughout the 5-HT treatment and for 30 min after the 5-HT treatment. For the reactivation experiments, we excluded sensory-to-motor neuron synapses showing less than a 20% increase in synaptic strength on the second recording compared with the first recording.

Information on general procedures and drug application for the behavioral experiments, cell culture, and statistical analysis is provided in *SI Methods*.

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