Supporting Information

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SI Methods

General Procedures for Aplysia Behavior. The basic behavioral procedures followed those of previous reports (1, 2). Aplysia kurodai weighing 100~150 g were used in the behavioral experiments. To permit visualization of the entire siphon, the parapodia of animals were removed under anesthesia by cooling (4~8°C) for 10 min, and the animals were separated in individual chambers for 4 d. After separation, animals were deprived of food until the end of the behavioral procedures. We discarded any animals (~10% of all tested animals) that were already sensitized [siphon-withdrawal reflex (SWR) duration, >9 s] or that showed a reduced response (SWR duration, <2 s) without further experiments. A hand-held electrical shocker was placed onto the tail of animals for training. Training was done by applying 10 electrical shocks to the tail to sufficiently induce long-term sensitization for all animals. All training and SWR procedures were done following a blind procedure.

Drug Application for Aplysia Behavior. To examine the effect of protein synthesis inhibition on consolidation, emetine (35.586 mg/kg; Sigma-Aldrich) or vehicle (artificial sea water) was injected 30 min before the training. To examine the effect of protein synthesis inhibition or proteasome inhibition on reconsolidation, emetine, clasto-lactacystin β-lactone (βlac, 106.6 µg/kg; Calbiochem), emetine + βlac, or vehicle was injected immediately after the first long-term memory (LTM) test. Emetine and other drugs were injected into the body cavity of each animal using a syringe. Injection volume was 900 µL per 140 g of animal weight.

Cell Cultures. Sensory-to-motor cocultures and synapse recording followed previously described protocols (3–5). Sensory neurons isolated from the pleural ganglia of A. kurodai (100–150 g) were cocultured with LFS motor neurons obtained from the abdominal ganglia of adult animals (5).

Statistical Analyses. The effect of drug application across time points was examined using two-way mixed (between- and within-subjects design) ANOVA with drug groups (between-subjects) and time points (within-subjects, repeated-measures) as factors. Each drug application group was compared with other groups at specific recording time points using one-way ANOVA and the Newman–Keuls multiple-comparison post hoc test. In addition, one-sample t tests were used to compare the synaptic strength change with the basal level in each group.

**Fig. S1.** Protein synthesis-dependent consolidation and reconsolidation of LTM for behavioral sensitization. (A) (Left) Schematic of the experimental procedure used to evaluate the effect of emetine on the consolidation of LTM during *Aplysia* sensitization. (Right) Bar graph showing mean ± SEM of SWR duration in the pretest, short-term memory (STM) test, and LTM test at 24 h after training. Compared with the naïve (*n* = 5) or vehicle-injected group (veh; *n* = 6), the emetine-injected group (emetine; *n* = 7) showed impaired LTM for sensitization \[F_{(2,15)} = 34.75, P < 0.01, \text{one-way ANOVA}\]. **P < 0.01, Newman–Keuls multiple comparison test. The STM was intact in all groups. (B) (Left) Schematic of the experimental procedure used to evaluate the effect of emetine on LTM reconsolidation in *Aplysia*. (Right) Bar graph showing mean ± SEM of SWR duration on the pretest, STM test, first LTM test, and second LTM test. Compared with the group injected with vehicle after the first LTM test (test/veh; *n* = 13), the group injected with emetine after the first LTM test (test/emetine; *n* = 17) showed impaired LTM at the second LTM test. Emetine injection without the first LTM test (no/emetine; *n* = 13) or vehicle injection without the first LTM (no/veh; *n* = 12) had no effect on LTM at the second LTM test \[F_{(3,51)} = 13.64, P < 0.01, \text{one-way ANOVA}\]. **P < 0.01, Newman–Keuls multiple-comparison test; **P < 0.01, one-way ANOVA and Newman–Keuls multiple-comparison.

**Fig. S2.** Treatment with vehicle or protein synthesis inhibitor emetine without homosynaptic activation (HA) or 5 × 5-HT retreatment did not affect maintenance of facilitated synaptic strength for 48 h after the first recording. (Upper) Schematic of the experimental procedure. (Lower) Bar graphs showing mean percentage change ± SEM in excitatory postsynaptic potential (EPSP) amplitudes. On both the second and third recordings, changes in EPSP amplitudes were significantly different from the basal level in all groups (\#P < 0.05, ##P < 0.01, one-sample *t* test compared with basal level), and there was no difference among groups \[F_{(2,23)} = 3.16, P > 0.05, \text{one-way ANOVA}\]. veh, vehicle control group (*n* = 7); emetine, emetine control group (*n* = 13); only emetine, emetine-only treatment group without the second recording (*n* = 6).
Fig. S3. Proteasome inhibitor βlac does not affect the consolidation of long-term facilitation (LTF). (Upper) Schematic of the experimental procedure used to evaluate the effect of βlac treatment on LTF consolidation induced by five pulses of 5-HT. (Lower) Bar graph showing mean percentage change ± SEM in EPSP amplitude. The synaptic strength change was similar in the βlac treatment group (βlac; n = 16) and the vehicle treatment group (veh; n = 12) (P > 0.77, two-tailed unpaired Student t test).

Fig. S4. New protein synthesis is required for maintaining LTF at the sensory-to-motor synapse reactivated by 5-HT treatment at 72 h after training. (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 the 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 = 10) at 72 h after the first 5-HT treatment (training) impaired LTF at 120 h after the training, whereas treatment with vehicle alone (veh; n = 9) or emetine alone (emetine; n = 7) had no effect on LTF. *P < 0.05, **P < 0.01, one-sample t test compared with basal level [F(2,23) = 7.52, P < 0.01, one-way ANOVA]. ***P < 0.01; Newman–Keuls multiple-comparison test.
Fig. S5. Synaptic strength measured at 72 h after the first 5-HT treatment (training) with (24 h test; n = 4) or without (w/o 24 h test; n = 5) testing of synaptic strength at 24 h after the training. There was no difference between the two test groups (P > 0.89, two-tailed unpaired Student t test). Data are from the vehicle group (n = 9) shown in Fig. S4.