

AMPA receptor exchange underlies transient memory destabilization on retrieval

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Contributed by Richard W. Tsien, March 20, 2013 (sent for review February 3, 2013)

A consolidated memory can be transiently destabilized by memory retrieval, after which memories are reconsolidated within a few hours; however, the molecular substrates underlying this destabilization process remain essentially unknown. Here we show that at lateral amygdala synapses, fear memory consolidation correlates with increased surface expression of calcium-impermeable AMPA receptors (CI-AMPA receptors), which are known to be more stable at the synapse, whereas memory retrieval induces an abrupt exchange of CI-AMPA receptors to calcium-permeable AMPARs (CP-AMPA receptors), which are known to be less stable at the synapse. We found that blockade of either CI-AMPA receptor endocytosis or NMDA receptor activity during memory retrieval, both of which blocked the exchange to CP-AMPA receptors, prevented memory destabilization, indicating that this transient exchange of AMPARs may underlie the transformation of a stable memory into an unstable memory. These newly inserted CP-AMPA receptors gradually exchanged back to CI-AMPA receptors within hours, which coincided with the course of reconsolidation. Furthermore, blocking the activity of these newly inserted CP-AMPA receptors after retrieval impaired reconsolidation, suggesting that they serve as synaptic “tags” that support synapse-specific reconsolidation. Taken together, our results reveal unexpected physiological roles of CI-AMPA receptors and CP-AMPA receptors in transforming a consolidated memory into an unstable memory and subsequently guiding reconsolidation.

fear conditioning | memory reactivation |
transient memory deconsolidation | labile memory

New memories initially exist in an unstable state and are then stabilized/consolidated over a period of hours into long-term memories that can persist for even a lifetime (1). The learning and consolidation of long-term memory require stable synaptic modifications in neural circuits (2). One such modification is the postsynaptic potentiation of excitatory synaptic transmission, which has been shown to underlie associative memory in the amygdala and hippocampus (3–5). This potentiation involves an increase in postsynaptic AMPA receptors (AMPA receptors) (6), tetrameric glutamate receptors that display distinct physical characteristics according to their subunit composition (7, 8). Calcium-permeable AMPARs (CP-AMPA receptors) mostly consist of GluA2-lacking AMPA receptors and support acute synaptic potentiation, but are less stable at the synapse, whereas calcium-impermeable AMPARs (CI-AMPA receptors) make up for most of the basal synaptic transmission and are more stable at the synapse because of their interaction with synaptic molecules through the GluA2 subunit (9, 10). Accordingly, long-term potentiation (LTP), a cellular model for long-term memory consolidation, involves early increases in GluA1-containing AMPARs, which are gradually replaced by GluA2-containing AMPARs (11).

It is now known that even consolidated memories can become destabilized again with retrieval (reactivation), after which memories must undergo a de novo protein synthesis-dependent reconsolidation process to persist (12, 13). Although memory retrieval and subsequent destabilization/reconsolidation have been a major focus of memory research for the last decade, the substrate of this lability at the synaptic receptor level remains

largely unknown. Recently, two groups have demonstrated the involvement of CI-AMPA receptors or CP-AMPA receptors in “reconsolidation-update” (14), a specific variation of extinction in which conditioned fear memory is retrieved 1 h before extinction training (15, 16). Their studies focused primarily on the relationship between CI-AMPA receptors or CP-AMPA receptors and the memory-erasing effect of reconsolidation-update, and were not directed at the molecular mechanisms underlying memory destabilization on retrieval per se. We reasoned that finding manipulations that prevent the amnesic effect of combining memory retrieval with protein synthesis blockade would be vital in defining the mechanisms underlying this destabilization (12, 17). Here we show that memory retrieval induces an abrupt exchange of CI-AMPA receptors to CP-AMPA receptors that reverts back along the course of reconsolidation and is critical for memory destabilization. This molecular exchange requires both NMDA receptor (NMDAR) activation and the endocytosis of GluA2-containing CI-AMPA receptors.

Results

Memory Retrieval Causes Transient Replacement of CI-AMPA receptors by CP-AMPA receptors. Auditory-cued fear memory is believed to be stored in the form of synaptic potentiation at the thalamic input synapses to the lateral amygdala (LA), known as T-LA synapses (4, 6). We characterized the changes at these synapses induced by fear learning and subsequent memory retrieval (Fig. 1 *A* and *B*). We identified the synaptic changes in brain slices prepared at various time points after fear conditioning (5 min, 12 h, 24 h, 48 h, and 1 wk); we used two control groups for the conditioned rats: naïve controls and the tone-only group, in which a single tone was presented to naïve rats. For fear memory retrieval, conditioned rats were tested with a single conditioned stimulus (CS) in a distinct context on day 8, and slices were prepared at 5 min, 1 h, or 3 h after retrieval. As a control, conditioned rats were exposed to the memory retrieval context for the same duration as the three postretrieval groups but without tone presentation (context exposure group).

We first assessed synaptic function based on the ratio of AMPAR-mediated currents to NMDAR-mediated currents. The AMPA/NMDA ratio sharply increased after fear conditioning and remained significantly greater than the baseline value for 1 week (Fig. 1*C* and Fig. S1), consistent with a previous report (15). Intriguingly, memory retrieval had no effect on the AMPA/NMDA ratio, and neither did context exposure alone. These

Author contributions: I.H., Jeongyeon Kim, Jihye Kim, R.W.T., and S.C. designed research; I.H., Jeongyeon Kim, Jihye Kim, S.L., and H.-G.K. performed research; I.H., Jeongyeon Kim, Jihye Kim, R.W.T., and S.C. analyzed data; and I.H., Jeongyeon Kim, Jihye Kim, K.N., B.-K.K., R.W.T., and S.C. wrote the paper.

The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1305235110/-DCSupplemental.

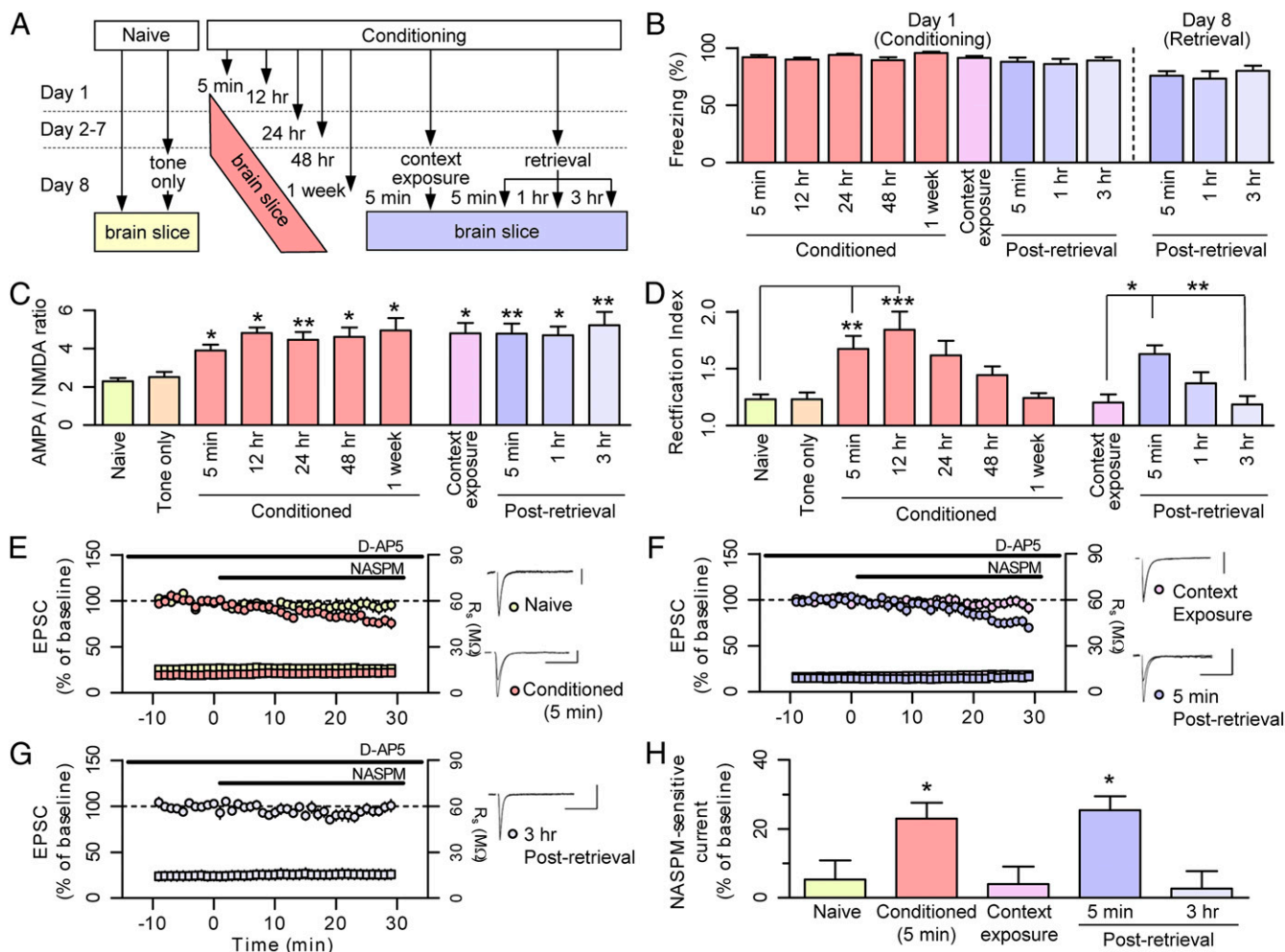


Fig. 1. Memory retrieval abruptly induces synaptic CI-AMPA to CP-AMPA exchange, without changing the synaptic strength at T-LA synapses. (A) Behavioral procedures. (B) Freezing levels in response to the last trial of fear conditioning in all conditioned groups shown in A (day 1). Memory retrieval on day 8 was tested in the three postretrieval groups (5 min postretrieval, 1 h postretrieval, and 3 h postretrieval). Naive and tone-only control groups were omitted for a better display. (C) AMPA EPSC/NMDA EPSC ratios measured after fear conditioning and subsequent retrieval. All of the conditioned groups showed significant potentiation compared with naive controls (naive, 2.3 ± 0.2 , $n = 12$; tone-only, 2.5 ± 0.3 , $n = 10$; conditioned 5 min, 3.9 ± 0.3 , $n = 14$; conditioned 12 h, 4.8 ± 0.3 , $n = 8$; conditioned 24 h, 4.5 ± 0.4 , $n = 12$; conditioned 48 h, 4.6 ± 0.5 , $n = 14$; conditioned 1 wk, 5.0 ± 0.7 , $n = 8$; context exposure, 4.8 ± 0.5 , $n = 15$; 5 min postretrieval, 4.8 ± 0.5 , $n = 15$; 1 h postretrieval, 4.7 ± 0.5 , $n = 10$; 3 h postretrieval, 5.2 ± 0.7 , $n = 10$; $F_{10,117} = 4.067$, $P < 0.0001$, one-way ANOVA; $*P < 0.05$, $**P < 0.01$, Newman-Keuls post hoc test vs. naive). (D) Rectification index of the synaptic AMPA receptor-mediated currents. Fear conditioning and retrieval induced an increase of rectification compared with naive/tone-only controls and context-exposed controls, respectively (naive, 1.23 ± 0.04 , $n = 16$; conditioned 5 min, 1.67 ± 0.11 , $n = 15$; conditioned 12 h, 1.84 ± 0.16 , $n = 9$; conditioned 24 h, 1.62 ± 0.13 , $n = 11$; conditioned 48 h, 1.44 ± 0.08 , $n = 11$; conditioned 1 wk, 1.24 ± 0.04 , $n = 16$; context exposure, 1.20 ± 0.07 , $n = 10$; 5 min postretrieval, 1.63 ± 0.08 , $n = 21$; 1 h postretrieval, 1.37 ± 0.10 , $n = 8$; 3 h postretrieval, 1.19 ± 0.06 , $n = 12$; $F_{10,127} = 6.526$, $P < 0.0001$, one-way ANOVA; $*P < 0.05$, $**P < 0.01$, Newman-Keuls post hoc test). (E–G) AMPAR-EPSC sensitivity to NASPM increased after fear conditioning (E) and memory retrieval (F), but diminished within 3 h after retrieval (G). Circles represent EPSC amplitudes, and squares indicate series resistance of whole-cell recordings. (Scale bars: 100 pA and 50 ms.) (H) Summarized results for E–G (naive, $5.3 \pm 5.6\%$, $n = 11$; conditioned 5 min, $23.0 \pm 4.6\%$, $n = 11$; context exposure, 4.0 ± 5.1 , $n = 11$; 5 min postretrieval, $15.5 \pm 4.1\%$, $n = 9$; 3 h postretrieval, $2.7 \pm 5.1\%$, $n = 8$; $F_{4,45} = 4.839$, $P < 0.005$, one-way ANOVA; $*P < 0.05$, Newman-Keuls post hoc test). All data points represent group means \pm SEM.

results, considered along with our previous observations (18), show that conditioning-induced potentiation at T-LA synapses remains largely unchanged after memory retrieval (19, 20).

We next determined the relative contribution of CI-AMPA and CP-AMPA to AMPAR-mediated synaptic transmission at T-LA synapses (*SI Materials and Methods*). For this, we measured both AMPA excitatory postsynaptic current (EPSC) rectification and sensitivity to polyamine derivatives. CI-AMPA generally exhibit linear current-voltage (I-V) relationships, whereas CP-AMPA inwardly rectify (21) and thereby display a rectification index significantly greater than unity. High CP-AMPA content is also indicated by responsiveness of synaptic responses to 1-naphthylacetylsperimine (NASPM), a polyamine derivative that use-dependently blocks CP-AMPA activity (7).

We first confirmed Clem and Huganir's finding that CP-AMPA are transiently inserted after conditioning (15). Fear conditioning produced a transient increase in the rectification index, and the enhanced rectification indices steadily decreased after 12 h and subsided to basal levels within 1 wk (Fig. 1D and Fig. S2). Consistent with this process, AMPAR-mediated EPSCs became more responsive to NASPM inhibition after fear conditioning (residual amplitude: naive, $94.7 \pm 5.6\%$, $n = 11$; conditioned for 5 min, $77.0 \pm 4.6\%$, $n = 11$) (Fig. 1E).

We next examined whether fear memory retrieval alters surface expression of CP-AMPA. Immediately after fear memory retrieval, rectification at T-LA synapses showed a transient increase which dissipated within hours, whereas context exposure alone had no effect (Fig. 1D and Fig. S2). Consistent with the

increased rectification, fear memory retrieval rendered AMPAR-mediated EPSCs more sensitive to NASPM inhibition in slices prepared at 5 min after retrieval, whereas context exposure alone did not (5 min postretrieval, $74.5 \pm 4.1\%$, $n = 9$; context exposure, $96.0 \pm 5.1\%$, $n = 11$) (Fig. 1F). NASPM inhibition was no longer evident in slices prepared at 3 h after retrieval (3 h postretrieval, $97.3 \pm 5.1\%$, $n = 8$) (Fig. 1G). Thus, sensitivity to NASPM inhibition was transiently enhanced after fear memory retrieval ($P < 0.05$ for designated pairs, Newman–Keuls post hoc test) (Fig. 1H), but then dissipated.

These results show that fear memory retrieval leads to the rapid insertion of CP-AMPA receptors into LA synapses (*SI Materials and Methods*). Because synaptic strength is maintained despite this increase in CP-AMPA receptors on retrieval, this suggests that retrieval induces the synaptic removal of CI-AMPA receptors, which is known to occur through endocytosis involving the C-terminal tail of GluA2 (22). Collectively, these findings suggest that LA synapses undergo an abrupt exchange from CI-AMPA receptors to CP-AMPA receptors.

Blocking of CI-AMPA Receptor Endocytosis Prevents Transformation of a Consolidated Memory to an Unstable Memory. Given that GluA2 stabilizes AMPARs at synapses through interaction with synaptic proteins (11, 23–27) and also stabilizes dendritic spines (26, 28), supporting memory storage (29–31), CI-AMPA receptor endocytosis may destabilize the potentiated synaptic strength underlying consolidated memory. We tested whether the memory retrieval-induced decrease in CI-AMPA receptors is critical for rendering the memory unstable using GluA2_{3Y}. This synthetic peptide mimics the C-terminal tail of GluA2 and thus prevents GluA2-dependent endocytosis of AMPAR (32). GluA2_{3Y} was made cell membrane-permeable by fusing it to the cell membrane transduction domain of the HIV-1 transactivator of transcription (Tat) protein (32).

Memory retrieval and subsequent anisomycin treatment are known to block reconsolidation, resulting in amnesia (12). Rats were conditioned on day 1 and presented with a single CS in a distinct context on day 8, followed by anisomycin microinfusion. The animals were then tested for amnesia 24 h later with a protocol for postretrieval long-term memory (PR-LTM) (Fig. 2A). Microinfusion of GluA2_{3Y} into the LA before retrieval protected the memory from the amnesic effect of anisomycin (GluA2_{3Y}/anisomycin retrieval, $68.9 \pm 5.5\%$; GluA2_{3Y}/anisomycin PR-LTM, $72.0 \pm 5.1\%$, $n = 9$; $P > 0.05$, Bonferroni post hoc test) (Fig. 2B), whereas the control peptide GluA2_{3A} spared the amnesic

effect (GluA2_{3A}/anisomycin retrieval, $69.7 \pm 6.13\%$; GluA2_{3A}/anisomycin PR-LTM, $47.2 \pm 6.5\%$, $n = 4$; $P < 0.05$, Bonferroni post hoc test). Two-way ANOVA revealed a significant session \times peptide interaction ($F_{1,11} = 10.89$, $P < 0.01$) and a significant effect of behavioral session (retrieval vs. PR-LTM, $F_{1,11} = 6.25$, $P < 0.05$), but no effect of peptide (GluA2_{3Y} vs. GluA2_{3A}, $F_{1,11} = 2.13$, $P > 0.05$). Bonferroni post hoc tests indicated significantly different freezing levels between the GluA2_{3Y}/anisomycin and GluA2_{3A}/anisomycin groups at PR-LTM ($P < 0.05$), but not at retrieval ($P > 0.05$). These results suggest that the endocytosis of GluA2-containing CI-AMPA receptors is required for the transformation of consolidated memories into unstable memories by retrieval.

This effect is not due to the effects of GluA2_{3Y} or GluA2_{3A} memory retention, given that these peptides alone, without anisomycin microinjection, did not affect either retrieval or PR-LTM (retrieval: GluA2_{3Y}/vehicle, $67.1 \pm 5.8\%$; GluA2_{3A}/vehicle, $73.0 \pm 5.7\%$, $n = 8$; $P > 0.05$, Bonferroni post hoc test; PR-LTM: GluA2_{3Y}/vehicle, $71.3 \pm 4.7\%$; GluA2_{3A}/vehicle, $66.7 \pm 5.8\%$, $n = 8$; $P > 0.05$, Bonferroni post hoc test). Two-way ANOVA revealed no significant interaction ($F_{1,14} = 1.11$, $P > 0.05$), effect of behavioral session ($F_{1,14} = 0.01$, $P > 0.05$), or effect of peptide ($F_{1,14} = 0.05$, $P > 0.05$) (Fig. 2C).

AMPA Exchange on Memory Retrieval Requires CI-AMPA Receptor Endocytosis and NMDAR Activity. To test the consequences of blocking CI-AMPA receptor endocytosis on retrieval at the synaptic level, and to further characterize the molecular mechanisms of AMPAR exchange, we combined *in vivo* behavioral manipulation and drug microinfusion with *ex vivo* whole-cell recordings (Fig. S4 and *SI Materials and Methods*). Because the microinfusion of the NMDAR antagonist D(-)-2-amino-5-phosphonopentanoic acid (D-AP5) into the amygdala is known to block retrieval-induced memory destabilization (17), we first tested whether CI-AMPA receptor-to-CP-AMPA receptor exchange would also be blocked at the same dose ($2.5 \mu\text{g}/0.5 \mu\text{L}/\text{side}$) (Fig. 3A). We found that D-AP5 microinfusion blocked the retrieval-induced increases in rectification (Fig. 3B and Fig. S5) and NASPM sensitivity (Fig. 3C and E). In contrast, robust increases in both rectification and NASPM sensitivity were detected after vehicle injection. Despite this impairment of CP-AMPA receptor insertion by D-AP5 microinjection, we detected no change in synaptic strength in either the AMPA/NMDA ratio (Fig. 3F and Fig. S6) or AMPAR miniature EPSC (mEPSC) characteristics (Fig. 3G and H and Fig. S7) compared with vehicle controls, indicating that CI-AMPA receptor removal was also blocked by inhibition of NMDARs. These results show that the CI-AMPA receptor-to-CP-AMPA receptor exchange on memory retrieval is triggered by NMDAR activation.

We then tested whether the blockade of GluA2-containing CI-AMPA receptor endocytosis inhibits AMPAR exchange. In contrast to the GluA2_{3A} control peptide, the GluA2_{3Y} peptide used earlier also blocked the rise in both EPSC rectification (Fig. 3B) and NASPM sensitivity (Fig. 3D and E) on retrieval. Moreover, the synaptic strength was unchanged by GluA2_{3Y} microinfusion and subsequent memory retrieval compared with GluA2_{3A} controls (Fig. 3F, G, and H, Fig. S6, and Fig. S7), indicating that inhibition of GluA2-containing CI-AMPA receptor endocytosis also blocks the insertion of additional CP-AMPA receptors. This finding suggests that removal of CI-AMPA receptor is a prerequisite for additional CP-AMPA receptor insertion on retrieval. Collectively, these results demonstrate that memory lability and CI-AMPA receptor-to-CP-AMPA receptor exchange share strikingly similar molecular requirements.

Activity of CP-AMPA Receptors Newly Inserted by Retrieval Is Required for Reconsolidation. What is the role of these newly inserted CP-AMPA receptors? Because these receptors are inserted into synapses in an activity-dependent manner, they presumably accumulate at synapses that are strongly involved in the retrieved memory. The receptors and the local calcium influx that they allow at resting potentials (33, 34) would be poised to guide the synapse-specific

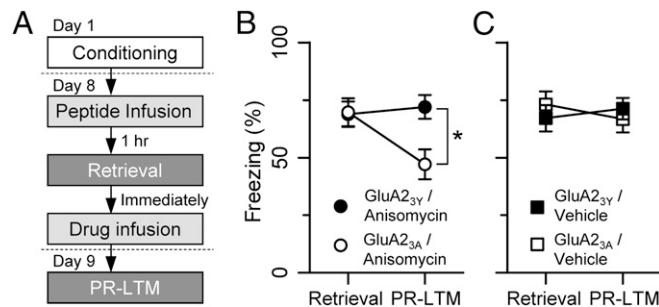


Fig. 2. Blockade of GluA2-containing AMPAR endocytosis prevents memory destabilization on retrieval. (A) Behavioral procedure. (B) Microinfusion of GluA2_{3Y} at 1 h before retrieval into the LA rendered the memory insensitive to the amnesic effect of anisomycin, whereas the control peptide GluA2_{3A} did not block this amnesic effect. (C) When vehicle was microinfused immediately after retrieval instead of anisomycin, GluA2_{3Y} (filled square) had no effect on either retrieval or PR-LTM compared with GluA2_{3A} (open square). Diffusion of the GluA2_{3Y} peptide (1.5 nmol) was confined within the LA when measured at 1 h after the microinjection (Fig. S3A), and cannula tip placement was confirmed for the experiments shown in B and C (Fig. S3B).

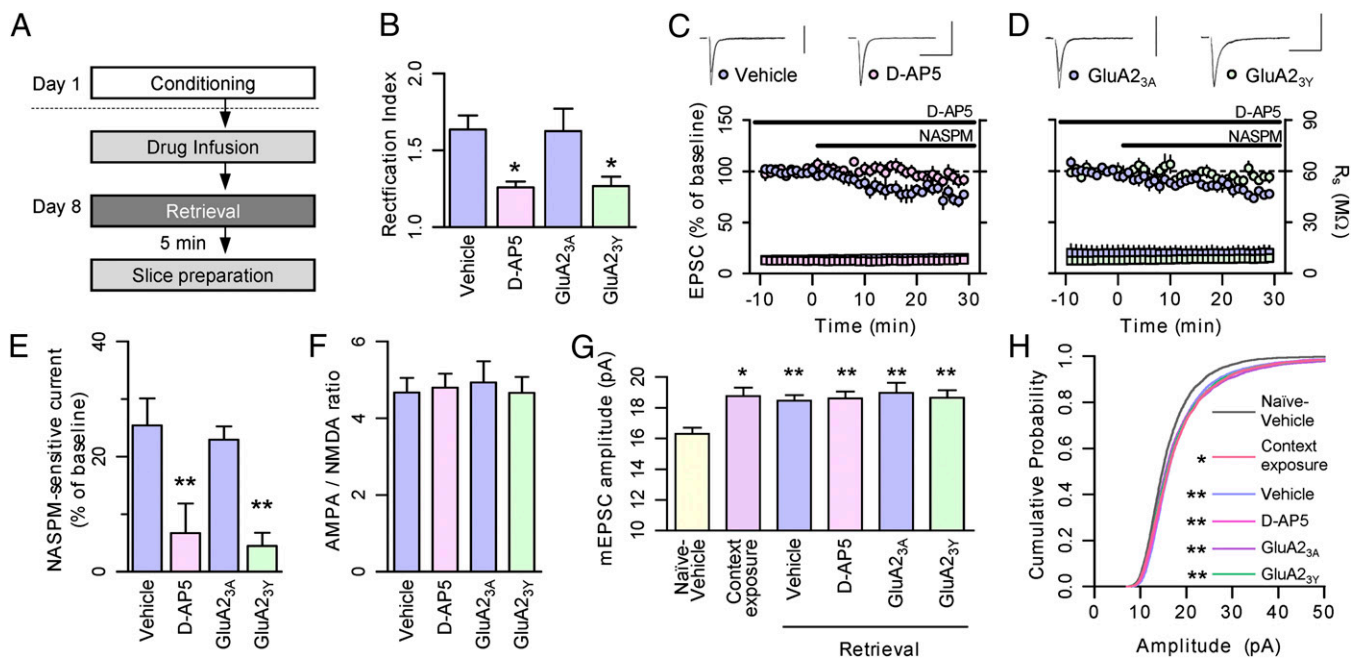


Fig. 3. GluA2-AMPA receptor endocytosis and NMDAR activity are required for AMPAR exchange on retrieval. (A) Behavioral procedure. AMPAR-mediated transmission changes on retrieval were monitored ex vivo after drug (or peptide) microinjection and subsequent retrieval. (B) Rectification index of the synaptic AMPA receptor-mediated currents measured after drug (or peptide) infusion and subsequent retrieval. D-AP5 and GluA2_{3Y} microinjection attenuated the increase of rectification on retrieval compared with vehicle and GluA2_{3A} controls, respectively (vehicle, 1.64 ± 0.09 , $n = 15$; D-AP5, 1.26 ± 0.03 , $n = 10$; GluA2_{3A}, 1.63 ± 0.14 , $n = 10$; GluA2_{3Y}, 1.27 ± 0.06 , $n = 15$; $F_{3,46} = 5.537$, $P < 0.005$, one-way ANOVA; $*P < 0.05$, Newman-Keuls post hoc test). (C and D) Enhanced sensitivity to NASPM after memory retrieval was blocked in slices prepared from D-AP5- (C) or GluA2_{3Y}-injected animals (D) compared with vehicle or GluA2_{3A}-injected animals, respectively. Circles represent EPSC amplitudes, and squares indicate series resistance of whole-cell recordings. (Scale bars: 100 pA and 50 ms.) (E) Summarized results for C and D (vehicle, $25.5 \pm 4.6\%$, $n = 6$; D-AP5, $6.7 \pm 5.2\%$, $n = 6$; GluA2_{3A}, $23.0 \pm 2.3\%$, $n = 5$; GluA2_{3Y}, $4.5 \pm 2.3\%$, $n = 7$; $F_{3,20} = 8.039$, $P < 0.005$, one-way ANOVA; $**P < 0.01$, Newman-Keuls post hoc test vs. designated control). (F) AMPA EPSC/NMDA ratios measured after drug (or peptide) infusion and subsequent retrieval. All four groups exhibited similarly potentiated ratios (vehicle, 4.7 ± 0.4 , $n = 16$; D-AP5, 4.8 ± 0.4 , $n = 14$; GluA2_{3A}, 4.9 ± 0.5 , $n = 12$; GluA2_{3Y}, 4.9 ± 0.5 , $n = 12$; $F_{3,50} = 0.085$, $P > 0.05$, one-way ANOVA; $P > 0.05$ for all pairs, Newman-Keuls post hoc test). (G and H) AMPAR-mediated mEPSCs measured after drug (or peptide) infusion and subsequent retrieval. All five of the conditioned groups (context exposure-vehicle, vehicle, D-AP5, GluA2_{3A}, and GluA2_{3Y}) displayed enhanced mEPSC amplitude compared with the naive vehicle group irrespective of drug infusion or memory retrieval, but mEPSC amplitude was not significantly different among the five conditioned groups (naive-vehicle, 16.3 ± 0.4 pA, $n = 14$; context exposure-vehicle, 18.8 ± 0.5 pA, $n = 12$; vehicle, 18.4 ± 0.4 pA, $n = 15$; D-AP5, 18.6 ± 0.4 pA, $n = 14$; GluA2_{3A}, 18.9 ± 0.7 pA, $n = 17$; GluA2_{3Y}, 18.7 ± 0.5 pA, $n = 18$; $F_{5,84} = 3.301$, $P < 0.001$, one-way ANOVA; $*P < 0.05$; $**P < 0.01$, Newman-Keuls post hoc test vs. naive-vehicle). Thus, AMPAR mEPSC amplitude was potentiated by fear conditioning, but neither amplitude nor frequency changed on retrieval compared with context exposure-vehicle controls irrespective of drug infusion (Fig. S7), consistent with the results of the AMPA/NMDA ratio measurements (Fig. 1C), as well as with our previous findings (18). Cannula tip placement was confirmed for the experiments shown in B–H (Fig. S4E).

molecular events required for reconsolidation (18, 19). Thus, we tested whether blocking CP-AMPA receptors immediately after retrieval would impair the reconsolidation of destabilized memory. Rats were conditioned on day 1 and presented with a single CS in a distinct context on day 8 (Fig. 4A). Immediately after retrieval, NASPM was microinfused into LA, and memory retention was tested for PR-LTM 24 h later. Two-way ANOVA revealed a significant session \times drug interaction ($F_{2,30} = 4.38$, $P < 0.05$) and a significant effect of behavioral session (retrieval vs. PR-LTM, $F_{1,30} = 7.83$, $P < 0.01$), but no effect of drugs (high-dose NASPM vs. low-dose NASPM vs. vehicle, $F_{2,30} = 1.37$, $P > 0.05$). PR-LTM was selectively impaired in the NASPM-treated group (4 $\mu\text{g}/0.5 \mu\text{L}/\text{side}$) compared with the vehicle-treated group (high-dose NASPM: retrieval, $75.4 \pm 3.7\%$; PR-LTM, $59.5 \pm 7.8\%$, $n = 11$; vehicle: retrieval, $73.5 \pm 4.2\%$; PR-LTM, $77.0 \pm 2.3\%$, $n = 13$; high-dose NASPM vs. vehicle: retrieval, $P > 0.05$; PR-LTM, $P < 0.05$, Bonferroni post hoc test) (Fig. 4B and C). This amnesic effect of NASPM was dose-dependent, as demonstrated by the lesser effect of the low-dose NASPM (0.4 $\mu\text{g}/0.5 \mu\text{L}/\text{side}$: retrieval, $80.9 \pm 2.5\%$, PR-LTM, $67.2 \pm 5.4\%$, $n = 9$; NASPM low-dose vs. vehicle: both retrieval and PR-LTM, $P > 0.05$, Bonferroni post hoc tests) (Fig. 4C). A subset of these rats were reconditioned, when both the vehicle-treated and high-dose NASPM-treated groups showed robust memory retention

(NASPM, $70.2 \pm 6.1\%$, $n = 6$; vehicle, $70.3 \pm 4.8\%$, $n = 6$; $P > 0.05$, unpaired t test), suggesting that the amnesic effect was not due to permanent damage to the LA neurons.

This amnesic effect of NASPM required memory retrieval. When NASPM was microinfused into the LA of rats that received only context exposure without tone (CS) presentation, memory retention was similar in both groups with testing 24 h after the context exposure (NASPM, $71.5 \pm 6.8\%$, $n = 10$; vehicle, $73.5 \pm 4.6\%$, $n = 14$; $P > 0.05$, unpaired t test) (Fig. 4D).

We further reasoned that if CP-AMPA receptor insertion were prevented during retrieval, then blockade by NASPM would lose its effect on memory. We tested this idea by microinfusing either GluA2_{3Y} peptide or control peptide before memory retrieval, followed by NASPM immediately after retrieval (Fig. 4E). Consistent with our hypothesis, infusion of the GluA2_{3Y} peptide prevented the memory impairment induced by the NASPM treatment (GluA2_{3Y}/NASPM retrieval, $75.3 \pm 2.6\%$; GluA2_{3Y}/NASPM PR-LTM, $73.9 \pm 4.3\%$, $n = 13$; $P > 0.05$, Bonferroni post hoc test) (Fig. 4F), unlike the sparing of the NASPM effect seen with the control peptide (GluA2_{3A}/NASPM retrieval, $77.3 \pm 2.5\%$; GluA2_{3A}/NASPM PR-LTM, $59.6 \pm 6.2\%$, $n = 10$; $P < 0.05$, Bonferroni post hoc test). Infusion of peptide alone before retrieval demonstrated no effect on memory retrieval or retention (Fig. 2C). Two-way ANOVA revealed a significant session \times

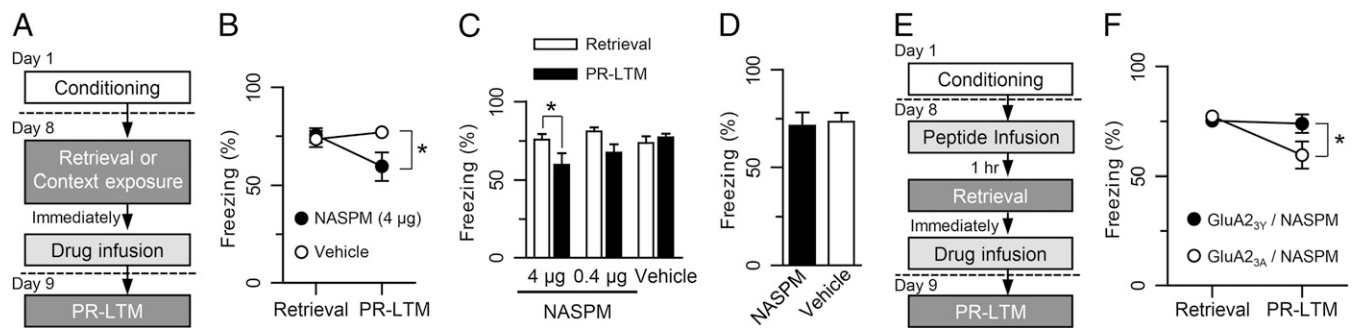


Fig. 4. Blockade of newly inserted CP-AMPA receptors on retrieval impairs memory reconsolidation. (A) Behavioral procedure for the experiments shown in B–D. (B and C) NASPM infusion immediately after retrieval dose-dependently impaired PR-LTM compared with vehicle controls. $*P < 0.05$. (D) Memory retention at 24 h after context exposure was similar in the NASPM- and vehicle-treated groups. (E) Behavioral procedure for the experiments shown in F. (F) GluA2_{3Y} peptide infusion prevented the memory impairment induced by NASPM compared with the GluA2_{3A} controls. Cannula tip placement was confirmed for the experiments shown in B, C, D, and F (Fig. S3 C–E).

drug interaction ($F_{1,21} = 4.47$, $P < 0.05$) and a significant effect of peptide (GluA2_{3Y} vs. GluA2_{3A}, $F_{1,21} = 6.17$, $P < 0.05$), but no effect of behavioral session (retrieval vs. PR-LTM, $F_{1,21} = 6.17$, $P > 0.05$). Bonferroni post hoc tests indicated significantly different freezing levels between the GluA2_{3Y}/NASPM and GluA2_{3A}/NASPM groups when tested for PR-LTM ($P < 0.05$), but not at retrieval ($P > 0.05$). These findings suggest that CP-AMPA receptors that are inserted into synapses during retrieval have a critical role in memory reconsolidation.

Discussion

Here we show that the exchange from CI-AMPA receptors to CP-AMPA receptors is the major synaptic change on memory retrieval. Importantly, blocking the synaptic removal of GluA2-containing CI-AMPA receptors during retrieval prevented this AMPAR composition exchange, and also protected the consolidated memory against becoming labile. Because GluA2 stabilizes dendritic spines (26, 28) and retains AMPARs at synapses through direct interaction with synaptic proteins (11, 23–27), the loss of CI-AMPA receptors may destabilize potentiated synaptic strength, and hence memory. Our results suggest that other manipulations leading to synaptic removal of CI-AMPA receptors may destabilize consolidated memory as well.

The CP-AMPA receptors inserted on memory retrieval appear to be labile at the synapse and to undergo gradual replacement by CI-AMPA receptors over the course of memory reconsolidation. This conversion from CP-AMPA receptors to CI-AMPA receptors also has been observed during the stabilization of LTP (11, 33, 35), in association with preservation of enlarged synapse and spine structures (7). Intriguingly, a recent study in cultured hippocampal neurons showed that the replacement of CP-AMPA receptors by CI-AMPA receptors after synaptic potentiation requires the ongoing activity of newly inserted CP-AMPA receptors (33), very much like the reconsolidation described here.

NASPM has multiple molecular targets, but is known to be more selective for CP-AMPA receptors (7). Accordingly, researchers have used NASPM *in vivo* as a specific CP-AMPA receptor antagonist (36). In our study, NASPM had no effects on learned fear when injected without retrieval (Fig. 4D), indicating that the *in vivo* NASPM effect requires retrieval-induced changes (e.g., CP-AMPA receptor insertion). Furthermore, microinjection of the GluA2_{3Y} peptide into the LA and subsequent retrieval blocked both CP-AMPA receptor insertion (Fig. 3) and the *in vivo* NASPM effect (Fig. 4F). Taken together, these findings indicate that NASPM may exert its amnesic effect by acting on the CP-AMPA receptors inserted after retrieval; however, we cannot rule out the possibility that other calcium-permeating ion channels recruited after retrieval are involved as well.

Memory retrieval is known to trigger the protein degradation that is crucial for destabilization (37). Because GluA2 is known to undergo activity-dependent ubiquitination (38), it is conceivable that memory retrieval may evoke the degradation of CI-AMPA receptors along with other synaptic proteins, thereby contributing to the lability of memory. Accordingly, GluA2 is a likely candidate for resynthesis and incorporation during reconsolidation, insofar as signaling pathways known to promote GluA2 expression are required for reconsolidation (39, 40). Together with previous studies, our results suggest that the abrupt CI-AMPA receptor-to-CP-AMPA receptor exchange and subsequent gradual reversion represent a coordinated molecular scheme underlying memory destabilization and reconsolidation.

How do CP-AMPA receptors contribute to reconsolidation after retrieval? CP-AMPA receptors are already thought to be inserted after LTP and support the consolidation of LTP (33, 35), a leading cellular model of long-term memory. Because they allow calcium permeation at resting membrane potentials, these channels may serve as synaptic “tags” (41) that support the consolidation of synapse-specific potentiation (19), and thus memory, during spontaneous activity or off-line neuronal “replay” (42). These receptors themselves and/or the Ca²⁺ microdomains that they induce (34) may guide the incorporation of newly synthesized synaptic molecules (33) in the synapse-specific manner required for proper memory update on reconsolidation. Intriguingly, CP-AMPA receptors accumulate at synapses during the daytime and decrease during sleep (43, 44). In light of the deep involvement of sleep in

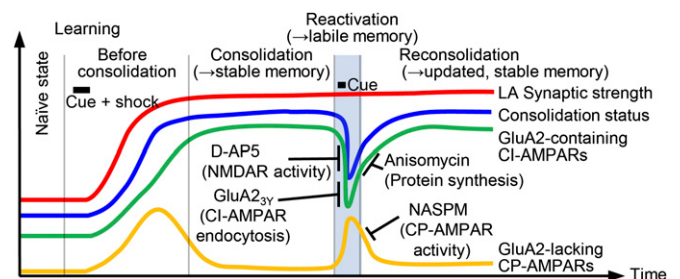


Fig. 5. Hypothetical model of synaptic mechanisms involved in memory consolidation, retrieval, and reconsolidation. After learning, LA synaptic strength is persistently potentiated, first mostly by CP-AMPA receptors but later almost solely by CI-AMPA receptors. On memory retrieval, NMDAR activity induces CI-AMPA receptor endocytosis and subsequent CP-AMPA receptor insertion, causing an unstable state of synaptic potentiation. Blocking this NMDAR activity or CI-AMPA receptor endocytosis prevents memory lability, occluding subsequent effects of anisomycin. The newly inserted CP-AMPA receptors contribute to memory reconsolidation and update, but are in turn removed from synapses over the course of reconsolidation.

consolidation and reconsolidation (45), it is tempting to hypothesize that daytime learning and retrieval induces CP-AMPA, which in turn guide consolidation during subsequent sleep.

Is the AMPAR exchange characterized here a unique phenomenon occurring only on memory retrieval and reconsolidation or a general mechanism of synaptic destabilization and stabilization occurring also with memory acquisition and consolidation? Clem and Hugarir (15) have shown that fear conditioning induces the synaptic insertion of CP-AMPA, which are gradually replaced by CI-AMPA. It would be interesting to pin down the precise role of CP-AMPA and CI-AMPA in memory consolidation as tested here with NASPM and reconsolidation after memory retrieval. Nonetheless, the findings of Clem and Hugarir and our results dovetail well in yielding a global picture of the synaptic changes occurring on memory acquisition and retrieval (Fig. 5).

Our experiments indicate that as much as ~30% of the potentiated LA synaptic transmission encoding fear memory undergoes an abrupt change from CI-AMPA to CP-AMPA. One might question how a minority fraction of receptors could play such a critical role in the stabilization of consolidated synapses and memories. One possibility is that a small but key fraction of CI-AMPA at potentiated synapses are involved in stabilizing memory-storing dendritic spines, and that selective removal of these receptors thus exerts a strong effect on the stability of consolidated memory. Alternatively, or additionally, the temporary insertion of CP-AMPA may allow a sufficient calcium influx to

contribute to the gradual induction of synaptic depression or depotentiation (15), thereby causing a large decrement in synaptic strength. Whatever the downstream mechanism, our experiments identify CI-AMPA to CP-AMPA exchange as a key molecular event supporting transient memory destabilization. This reinforces the idea that the presence of CI-AMPA at memory-encoding synapses is critical for the persistence of consolidated memory, while also providing fresh targets for possible clinical disruption of aberrant memory.

Materials and Methods

Male Sprague–Dawley rats were fear-conditioned to an auditory cue (3 pairings of cue and footshock; cf. ref. 46), and a single nonreinforced tone was used for subsequent memory retrieval. Brain slices were prepared for electrophysiological recordings as described previously (47). Rats were cannulated to enable microinfusion of drugs or peptides into the LA (18, 47). Details of this and all other experimental procedures are provided in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We are grateful to Beomjong Song for his assistance in electrophysiology and helpful discussions. We thank J. E. Le Doux and three expert reviewers for helpful comments. This research was supported by Grant 2012K001111 from the Brain Research Center of the 21st Century Frontier Research Program, funded by the Korean Ministry of Education, Science and Technology, and by National Institutes of Health Grant MH071739 (to R.W.T.). I.H. was supported by National Research Foundation of Korea (NRF) Fellowship 2012R1A6A3A01019438, and J.K. and J.K. were supported by Brain Korea 21 (BK21) Research Fellowships.

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