Social experiences switch states of memory engrams through regulating hippocampal Rac1 activity

Bo Leab,c,d, Li Leib,c,d, Shiqiang Hua,b,d, Yikai Tangab, and Yi Zhongab,d,e,f

Edited by Paul Frankland, Hospital for Sick Children, Toronto, ON, Canada; received September 23, 2021; accepted February 6, 2022 by Editorial Board Member Liqun Luo

In pathological or artificial conditions, memory can be formed as silenced engrams that are unavailable for retrieval by presenting conditioned stimuli but can be artificially switched into the latent state so that natural recall is allowed. However, it remains unclear whether such different states of engrams bear any physiological significance and can be switched through physiological mechanisms. Here, we show that an acute social reward experience switches the silent memory engram into the latent state. Conversely, an acute social stress causes transient forgetting via turning a latent memory engram into a silent state. Such emotion-driven bidirectional switching between latent and silent states of engrams is mediated through regulation of Rac1 activity–dependent reversible forgetting in the hippocampus, as stress-activated Rac1 suppresses retrieval, while reward recovers silenced memory under amnesia by inhibiting Rac1. Thus, data presented reveal hippocampal Rac1 activity as the basis for emotion-mediated switching between latent and silent engrams to achieve emotion-driven behavioral flexibility.

Animals are required to flexibly retrieve memories according to their emotional states for achieving optimal behaviors in an ever-changing environment. To understand the underlying mechanisms of such regulation, extensive effort has been devoted to studying the impact of emotion on memory processes (1–5). For instance, stress can block memory retrieval through hormones, neuroinflammation, or depression of synapses in both human and animal models (6–12), while reward and novelty can facilitate both the formation and maintenance of memories (13, 14). However, how emotion might directly impact the memory engram remains elusive. The proposed theory and experimental demonstrations have revealed the presence of multiple states of memory engrams, such as silent, latent, and active states (15–17). In the silent state, only artificial activation of engram cells is capable of inducing memory expression, whereas latent engram cells can be activated by a natural conditioned stimulus to drive the engram into the active state for memory retrieval. It is intriguing to note that training either mouse models of Alzheimer’s disease or protein synthesis-inhibited mice are reported to yield only silent engrams, and such silent engrams could be turned into a latent state (18–22) through artificial manipulations of either optical stimulation–induced long-term potentiation (LTP) or virus-driven overexpression of activated PAK1. However, the physiological significance of the different states of engrams, particularly the silent engram, remains unclear. In the course of studying the functions of reversible forgetting (23, 24), we became interested in testing the idea that the emotional impact on memory retrieval could be mediated through switching the engram between latent and silent states, while reversible forgetting may play a role in making such switching.

To investigate this idea, we tested the effects of acute social reward (SR) and social stress (SS) on memory retrieval. Since mating and fighting are widely perceived and used as behavioral stimuli for evoking feelings of reward and stress, respectively (25–27), we adopted a modified short procedure for evoking acute emotion through social interactions. For SR treatment, a single experimental male mouse is exposed to two females brought from different home cages for 10 min. Such a subtle positive experience is sufficient to enhance retrieval of contextual fear memory (28). For SS treatment, a single experimental male mouse is exposed to a group of five male littermates for 10 min. This is a hostile social environment in which the experimental mouse fights with other littermates during this time window. Such a subtle stressful experience is sufficient to significantly reduce 24-h contextual fear memory (28). Based on these two paradigms of acute social experiences, we investigated how emotion affects memory retrieval through alterations in engram states.

Significance

It is well known that silent memory engrams in pathological or artificial conditions can be artificially switched into the latent state for retrieval by natural recall cues. Thus, physiological strategies that depend on the underlying molecular mechanisms for switching between silent state and latent state are a subject for investigation. Here, we show that social experiences stimulated switching between latent and silent engrams to achieve flexible memory accessibility and also reveal the basic molecular mechanism of: 1) social reward turning silent engram to latent via suppression of Rac1 activity in CA1 neurons of the hippocampus; and 2) social stress switching latent memory engram into silent through activating Rac1. Together, this work demonstrates emotion-driven bidirectional switching between latent and silent engrams.
Results

Social Reward Could Temporarily Switch the Silent Engram into the Latent State. We first tested whether SR could switch protein synthesis inhibition–silenced engrams into the latent state so that the amnesic memory could be retrieved through natural cues. It is reported that injection of the protein synthesis inhibitor anisomycin (ANI) immediately after contextual fear conditioning (CFC) leads to retrograde amnesic memory (29) by silencing engram cells, which can be artificially activated through optogenetic stimulation to retrieve amnesic memory (19, 21). As expected, five-trial CFC-induced 24-h contextual fear memory was disrupted by posttraining administration of ANI in controls (Fig. 1 A and B). However, when the conditioned mouse was presented with SR treatment immediately before the 24-h memory test (Fig. 1A), the amnesic contextual fear memory could be retrieved by natural recall cues, which was also observed in cycloheximide (CXM, another protein synthesis inhibitor)-induced amnesic memory (SI Appendix, Fig. S1 A and B), and such SR-mediated recovery of the amnesic memory was temporary in nature, as this recovery diminished 24 h after the first recall (Fig. 1B). In addition, the presentation of SR was ineffective when given 2 h before memory recall (SI Appendix, Fig. S2 A and B).

To gain insights into the effects of SR on the engram state that allowed the recovery of the amnesic memory, we examined engram reactivation in the CA1 (Cornu Ammonis) region of the hippocampus with regard to the retrieval of the 24-h memory (Fig. 1C) because retrievability by natural recall cues relies on the states of these engrams (17, 18, 20, 21). We injected the adeno-associated viruses (AAV)295-c-fos:tetracycline-controlled transactivator (tTA) and AAV295-tetracycline response element (TRE)-tandem dimer tomato (tdTomato) (Fig. 1D) into the CA1 region to label learning-induced engram cells (19, 24), and then detected the recall-stimulated c-Fos+ cells by c-Fos staining (Fig. 1D and SI Appendix, Fig. S3 A). We found that the number of activated latent engram cells resulting from natural recall was significantly reduced in ANI-injected mice (ANI in Fig. 1E) as compared to the saline-injected control mice (SAL in Fig. 1E). To describe this observation more rigorously, the ratio of reactivated engram cells (c-fos+ tdTomato+ double positive) to learning-activated total engram cells (tdTomato+) was decreased by ANI injection (Fig. 1F). SR treatment recovered the number of latent engram cells, which are available for natural recall (ANI+SR in Fig. 1E) and the reactivation ratio of engram cells in CA1 of the ANI-injected mice (Fig. 1F), while SR without recall has no effect on the reactivation of engram cells (SI Appendix, Fig. S4). In nonengram cells (tdTomato− cells: DAPI without tdTomato staining) was decreased by ANI (0.0001; from one-way ANOVA with Turkey test (Fig. 1G and SI Appendix, Fig. S5 A). To confirm this observation, we also examined the effect of SR on contextual fear memory induced by single-shock CFC and found that SR showed a similar rescue effect on ANI-induced amnesic memory.
Social Stress Could Temporarily Switch a Latent Engram into a Silent State. Given that acute stress was sufficient to reduce memory expression in both animal models and human (6, 7, 10), we next tested whether SS could inhibit memory retrieval by switching latent engrams to the silent state. For this purpose, a male mouse was subjected to five-trial CFC. Immediately before the 24-h memory test, the conditioned mouse was exposed to a group of five hostile male littermates (Fig. 2A). We found that 24-h memory retrieval was indeed temporarily disrupted by SS treatment, as a spontaneous recovery of memory retrieval was observed 24 h later (Fig. 2B). Consistent with the effect of SR, the presentation of SS was also ineffective when given 2 h before memory recall (SI Appendix, Fig. S2 C and D). Subsequently, we examined the effect of SS on the reactivation of engram cells during retrieval (Fig. 2C). Consistently, the ratio of reactivated engram cells (c-fos+ tdTomato+ double positive) (SI Appendix, Fig. S3B) to learning-activated total engram cells (tdTomato+ staining) was decreased by SS treatment (Fig. 2 D and E), while SS without recall has no effect on the reactivation of engram cells (SI Appendix, Fig. S4). In nonengram cells, there was no difference in the ratio of c-fos+ tdTomato+ for each group (Fig. 2E). Consistent with behavioral data, the effect of SS in the reactivation of the engram cells in CA1 was also temporary and diminished 24 h after the first recall (Fig. 2E and SI Appendix, Fig. S5B).

Hippocampal Rac1 Activity Correlates with Latent and Silent Memory Engram States. In pursuing molecular mechanisms underlying such switching between latent to silent states, we were drawn to the idea of involvement of Rac1-dependent reversible forgetting, as elevated Rac1 activity in the hippocampus leads to the forgetting of memories in multiple tasks (23, 24, 30–32), and the loss of the memory is reversed when Rac1 activity is suppressed (24). Given that multiple-trial CFC led to suppression of Rac1 activity (24), it is possible that protein synthesis inhibition disrupts the suppression of Rac1 activity induced by multiple-trial CFC so that the increase of Rac1 activity subsequently leads to reversible amnesia. To test this idea, ANI was injected (intrapertoneally [i.p.]) immediately after CFC (Fig. 3A). By immunoblotting active GTP-bound Rac1 (Rac1-GTP) in the hippocampus, we found that Rac1 activity was inhibited by five-trial CFC in controls (injected with saline), assayed 24 h after conditioning, but dramatically elevated in ANI-injected mice (Fig. 3 B–E). To confirm the causal link between elevated Rac1 activity and amnesia, we manipulated the Rac1 activity in the dorsal CA1 through injection of AAVs, which carries a mutant transgene that encodes the dominant-negative Rac1 (Rac1-DN) targeted to excitatory neurons via the CaMKIIα promotor (SI Appendix, Fig. S7A). Such expression has been shown to inhibit evoked Rac1 activity (23, 24, 31). Two weeks after the surgery, the mice were

Taken together, these data suggest a crucial role of acute social-emotional experiences in regulating states of the memory engram, as SR reverses the protein synthesis inhibition-silenced memory engram back into the latent state, while SS does not erase memory.

Fig. 2. Acute social stress converts latent engrams to silent state. (A) Experimental schedule. (B) The effects of SS on contextual fear memory (24 h; nCFC = 10, nSS = 15; 48 h; nCFC = 9, nSS = 9). (C) Strategy of hippocampus engram overlap and CFC. (D) Coronal section of CA1 engram cells (TRE-tdTomato) labeling with anti-c-fos (green). (Scale bar, 50 μm.) Arrowheads indicate double positive. (E and F) Percentages of c-fos+ cells in tdTomato+ and tdTomato– cells in CA1 at 24 h (E) or 48 h (I) after training. (F) 18 to 20 slices from five mice for each group. ns P > 0.05, ****P < 0.0001; from unpaired t test (E and F) and two-way ANOVA with Bonferroni test (B). Data are presented as means ± SEM.

(SI Appendix, Fig. S6). Combining the behavioral observations with the detection of reactivated engram cells, our data support the notion that SR treatment switches the protein-synthesis inhibition-silenced engram into the latent state.
bilateral injection with ANI through the cannula ([SI Appendix](https://doi.org/10.1073/pnas.2116844119), Fig. S7B) immediately after the five-trial CFC (Fig. 3G). We found that ANI injection increased Rac1 activity 24 h after CFC in the control, but failed to increase Rac1 activity in Rac1-DN mice (Fig. 3H and [SI Appendix](https://doi.org/10.1073/pnas.2116844119), Fig. S8A). Accordingly, protein synthesis inhibition–induced amnesia was also prevented in Rac1-DN mice (Fig. 3I). Thus, as soon as Rac1 activity is unable to be evoked by CFC, protein synthesis inhibition fails to cause retrograde amnesia for contextual fear memory.

We also showed that ANI injection led to a further increase in Rac1 activity in response to single-shock CFC ([SI Appendix](https://doi.org/10.1073/pnas.2116844119), Fig. S9A–C). To exclude the possibility of direct ANI stimulation of Rac1 activity, we assayed the effects of ANI on Rac1 activity without coupling to conditioning. Rac1 activity remained unaffected in mice subjected to either context-only or shock-only experience with injection of ANI ([SI Appendix](https://doi.org/10.1073/pnas.2116844119), Fig. S10).

Thus, the data presented support a role for Rac1-dependent forgetting in mediating ANI-induced reversible amnesia. Such observations led us to pursue a hypothesis that SS-dependent activation of Rac1 activity drives latent engrams to silence, while SR-dependent suppression of Rac1 activity switches silent engrams to latent engrams.

**Acute Social Experiences Switch States of Memory Engrams by Regulating Hippocampal Rac1 Activity.** The hypothesis elaborated above leads to three testable predictions. First, SR experience leads to suppression of Rac1 activity. Indeed, we found that a 10-min SR treatment was sufficient to reduce protein synthesis inhibitor–induced hyperactive Rac1 assayed 24 h after CFC (Fig. 4H and [SI Appendix](https://doi.org/10.1073/pnas.2116844119), Fig. S11A).

Second, direct inhibition of Rac1 activity through pharmacological treatment should mimic SR-mediated rescue effects on amnesic memory at the behavioral and engram-cell levels. We suppressed Rac1 activity by injecting the Rac1 inhibitor Ehop016 (i.p.) 30 min before testing the 24-h memory (Fig. 4B). Such treatment suppressed ANI-injection–elevated Rac1 activity ([SI Appendix](https://doi.org/10.1073/pnas.2116844119), Fig. S12) and rescued contextual fear memory from protein synthesis inhibition–induced amnesia (Fig. 4C). Consistent with the effect of SR (Fig. 1B), this rescue effect was also temporary, as memory dropped back 24 h later.
Fig. 4. Social reward switches silent memory engram to a latent state via inhibiting hippocampal Rac1 activity. (A) Immunoblotting and data showing Rac1 activity (Rac1-GTP) and total Rac1 levels in hippocampus (normalization of the value of Rac1-GFP/total Rac1: (A) Normalized to the SAL group at various retention intervals (nNaive = 7, nSAL = 7, nANI = 4, nANI+SR = 4). (B) Experimental schedule. (C) The effect of Ehop016 on contextual fear memory (nSAL+vehicle(24 h) = 8, nSAL+vehicle(48 h) = 5, nANI+vehicle(24 h) = 8, nANI+vehicle(48 h) = 9, nANI+Ehop016(24 h) = 11, nANI+Ehop016(48 h) = 10). (D) Coronal section of CA1 engram cells (TRE-tdTomato) labeling with anti-c-fos (green). (Scale bar, 50 μm.) Arrowheads indicate double positive. (E) Percentages of c-Fos+ cells in tdTomato+ and tdTomato−/C0 cells in CA1 (12 to 13 slices from five mice for each group). (F) Strategy of chemogenetic inhibition of engram cells; experimental schedule; the effect of chemogenetic inhibition of engram cells on the rescue of amnesia by Ehop016. (G) Strategy of Rac1-CA expression and experimental schedule. (H) The effect of social reward on silenced memory is abolished in mice with the expression of Rac1-CA. ns P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001; from one-way ANOVA with Tukey’s test (A, E, F, and H) and two-way ANOVA with Bonferroni test (C). Data are presented as means ± SEM.
after the Ehop016 injection (Fig. 4C, see histograms over 48 h). We next examined the engram reactivation ratio in the dorsal CA1. We found that such pharmacological inhibition of Rac1 activity reversed the decreased reactivation ratio of engram cells resulting from protein synthesis inhibition (Fig. 4D and E and SI Appendix, Fig. S3C), in a pattern consistent with the effects of SR (Fig. 1E and F). In addition, we found that Ehop016 treatment also rescued CMX-induced silenced memory (SI Appendix, Fig. S13) and ANI-induced silenced memory with single-shock CFC (SI Appendix, Fig. S11B and C). To test whether this Rac1 activity-dependent effect relied on the function of engram cells, we did a loss-of-function experiment in engram cells by expressing the chemogenetic inhibition tool hM4DG (human M4 DREADD Gi) or tetanus toxin light chain (TeTX). We found that the inhibition of Rac1 had no effect on silenced memory with inhibiting engram-cell activity (Fig. 4F) or block the synaptic output of engram cells (SI Appendix, Fig. S14). Thus, direct inhibition of Rac1 activity in the hippocampus mimics the effects of SR treatment in converting silenced engrams to the latent state. To further validate that the inhibition of hippocampal activity underlies the effect of SR, we next tested whether SR still can rescue anisomycin-induced silenced memory when hippocampal Rac1 activity is elevated. By expressing constitutively active Rac1 (Rac1-CA) (33) in CA1, we found that the effect of SR on silenced memory was abolished in mice with expressing Rac1-CA. Together, our results indicate that SR switches silent memory engrams to the latent state via inhibiting hippocampal Rac1 activity.

The third prediction derived from the hypothesis of Rac1 activity–mediated switching of engram states would suggest that SS treatment would increase Rac1 activity to silent engrams. We assayed hippocampal Rac1 activity in mice subjected to 10 min of SS treatment 24 h after a five-trial CFC. Immunoblotting showed that SS treatment indeed induced an increase in hippocampal Rac1 activity (Fig. 5A). To validate the functional significance of this SS-induced increase, we showed that pharmacological inhibition of Rac1 activity through injection of Ehop016 (i.p.) (Fig. 5B) prevented SS-induced memory disruption (Fig. 5C).

**Fig. 5.** Social stress silences memory engram via activating hippocampal Rac1 activity. (A) Immunoblotting and data showing Rac1 activity (Rac1-GTP) and total Rac1 levels in hippocampus (normalization of the value of Rac1-GTP/total Rac1) (nNaive = 7, nCFC = 7, nCFC+SS = 7). (B) Experimental schedule. (C) Effects of Ehop016 on contextual fear memory (nCFC = 10, nCFC+SS+vehicle = 10, nCFC+SS+Ehop016 = 10). (D) Coronal section of CA1 engram cells (TRE-tdTomato) labeling with anti-c-fos (green). (E) Percentages of c-Fos+ cells in tdTomato+ and tdTomato− cells in CA1 (10 to 12 slices from five mice for each group). (F) Strategy of ChR2 expression in engram cells; experimental schedule; optogenetic activation of engram cells induces memory retrieval in mice with or without immediate SS in a novel context. ns P > 0.05, *P < 0.05, **P < 0.01, and ***P < 0.0001; from paired t test (F) and one-way ANOVA with Tukey’s test (A, C, and E). Data are presented as means ± SEM.
We confirmed the role of SS-activated Rac1 activity in switching the latent memory engram to a silent engram by showing that an SS-triggered decrease in the engram-cell reactivation ratio could be rescued by inhibiting Rac1 activity (Fig. 5D and E). Furthermore, by optogenetically activating engram cells in CA1 after SS, we found that this activation could both induce memory retrieval in mice with immediate SS or not in a novel context (34), which further supports that these engram cells are silent engram cells. Thus, the data presented above confirmed all three predictions. Such strong evidence leads us to suggest that for contextual fear memory, SR treatment switches silent engrams to latent engrams through the suppression of Rac1 activity, while SS treatment switches latent engrams to silent engrams through elevations in Rac1 activity.

Recovery of Silent Memory Engrams in Multiple Hippocampus-Dependent Tasks through the Inhibition of Rac1 Activity. To gain a further understanding of the physiological significance of the identified novel Rac1-dependent mechanism that mediates switching between engram states, we expanded experiments into other hippocampus-dependent tasks, including trace fear conditioning and novel object location (NOL) recognition. In the trace fear conditioning task, a mouse was subject to a five-trial conditioning in which a tone (1,200 Hz) is paired with an electric shock, and a memory test is performed in a novel context (Fig. 6A and Materials and Methods). We found that ANI injections immediately after conditioning disrupted 24-h trace memory, while a single injection of Ehop016 before the memory test recovered the ANI-induced amnesic memory (Fig. 6B, with conditioned stimulation [CS]). Without the CS, there were no significant differences in freezing levels among the three groups of mice (Fig. 6B, without CS or pre-CS). Thus, inhibition of Rac1 activity was sufficient to reverse protein synthesis inhibition–induced amnesia in trace memory. In the novel object location task (Fig. 6C), the ANI-injected mice showed 24-h spatial memory defects in the novel object location test. Similarly, the rescue effect mediated by Ehop016 injection was observed in amnesic mice (Fig. 6D and E). Thus, the presented data support the idea that inhibition of Rac1 activity leads to switching of silent engrams to latent engrams in multiple hippocampus-dependent tasks.

Discussion
The current work investigates whether and how emotional states evoked by acute social interactions regulate memory engrams. This study leads to two major findings: First, social interaction–evoked emotions can bidirectionally switch the...
storage states of memory engrams, as SR turns a silent engram into the latent state, while SS converts a latent engram to silent in CA1 neurons of the hippocampus. Second, SR suppresses, and SS evokes the hippocampal Rac1 activity, while the effects of such Rac1 regulation are consistent with respective impacts of SR and SS on engrams. Taken together, the current work provides a neurobiological mechanism for how acute emotional changes can transiently affect cognitive processing by switching states of memory engrams stored in the hippocampus.

A major feature of the memory engram in the hippocampus is revealed as that memory can be stored not only as active or latent states of memory engrams stored in the hippocampus. Changes can transiently affect cognitive processing by switching into the latent state, while SS converts a latent engram to silent storage states of memory engrams, as SR turns a silent engram, while via some artificial approaches (22, 35), such silenced memory engrams can be converted to the latent state. However, whether and how multiple engram states, and silent engrams, in particular, are present and switchable between engram states in normal physiological or psychological conditions remain elusive. We demonstrated that acute SR treatment recovers protein synthesis inhibition–induced silenced memory, while SS temporarily disrupts memory retrieval. These transient alterations in memory retrievability are associated with increases or decreases, respectively, in the ratio of reactivated engram cells to learning-activated total engram cells after ANI injection (Fig. 1).

Our data suggest that such diverse states of memory engrams confer the dynamics and flexibility to memory, allowing animals to retrieve prior knowledge according to the current conditions.

For molecular mechanisms underlying the switching of engram states, we presented multiple lines of evidence supporting the idea that such switching is mediated through emotion-dependent regulation of Rac1 activity. First, hippocampal Rac1 activity correlates well with memory engram states, as Rac1 activity is inhibited after CFC and dramatically elevated with protein synthesis inhibition. Furthermore, the inhibition of Rac1 activity can antagonize the effect of protein synthesis inhibition (Fig. 2). In addition, silenced memory engrams and elevated hippocampal Rac1 activity are both observed in Alzheimer’s disease model mice (32, 35), supporting our conclusion. Second, SR suppresses and SS evokes the regulation of hippocampal Rac1 activity to achieve their respective impact (Figs. 4 and 5), and the overexpression of constitutively active Rac1 blocks the effect of SR while the pharmacological inhibition of Rac1 (Fig. 4) prevented SS switching of a latent engram into the silent state (Fig. 5). Supportively, previous studies have reported that Rac1 activity responses to social stress and mediates the regulation of related behaviors (36, 37). Third, chemogenetically inhibiting activity (Fig. 4B) or blocking synaptic outputs (SI Appendix: Fig. S14) of engram cells made pharmacological inhibition of Rac1 activity fail to recover protein synthesis inhibition–induced silenced memory, suggesting observed Rac1 effects act through engram cells. Fourth, such Rac1-dependent regulation of engram states is not confined to CFC memory but is also observed in other hippocampus-dependent memories (Fig. 6).

In light of the anisomycin-induced impairment of synaptic connection underlying the silence of memory engrams (19, 21, 22), and given that Rac1 is crucial for the dynamics of synaptic connectivity (38–40), this Rac1-dependent switching between latent and silent engram states may recruit the downstream of Rac1 to regulate synaptic structure in engram cells. A recent study reported the possible downstream of Rac1 for regulating memory: Rac1 activates a WASP family protein SCAR/WAVE to cause active forgetting of memory, and Rac1/SCAR may function with formin diaphanous, a nucleator that facilitates linear actin polymerization, to regulate memory forgetting by modifying synaptic connectivity (41). It provides a possible explanation for how Rac-1 mediates such emotion-mediated switching of engram states.

Although the involvement of hippocampal Rac1 activity in emotion-mediated regulation of memory is strongly supported via the data presented, we could not rule out an involvement of other signaling pathways in switching memory engram states, considering that learning-evoked protein synthesis induces expression of diverse molecules (42). Specifically, brain-derived neurotrophic factor (BDNF) is capable of restoring amnesic memory similar to Rac1 effects (43), and the activation of PAK-1 also can restore the silenced memory engram (21). However, it remains unclear whether these signaling molecules act through pathways independent of Rac1.

Thus, the current study develops two emotional paradigms to switch engram reversibly from a silent state to a latent state and provides a potential molecular mechanism for understanding how the current emotional state affects memory accessibility. Multiple lines of evidence are presented to support the idea that hippocampal Rac1 activity plays a crucial role in mediating the emotion-dependent switching of engram states.

**Materials and Methods**

**Animals.** C57BL/6J (age 3 to 4 mo, male) were purchased from the Vital River Laboratory (Animal Technology) and were housed in groups under standard conditions according to the Tsinghua University animal facility. Mice were maintained on a 12-h light/dark cycle and tested during the light phase of the cycle. All animal work complied with ethical regulations for animal testing and research, and was done in accordance with Institutional Animal Care and Use Committee approval by Tsinghua University and followed all Association for Assessment and Accreditation of Laboratory Animal Care guidelines.

**Drugs.** Ehop016 (Shanghai Sun-shine Chemical Technology Co., Ltd.) was dissolved in a solution containing 1% dimethylsulfoxide (DMSO)/30% polyethylene glycol 300/1% Tween-80. Mice were intraperitoneally injected with 20 mg/kg of Ehop016 solution or an equivalent volume of saline 30 min before the test. At the dose used, Ehop016 has high efficiency to block Rac1 activity. Anisomycin (Selleck, Cat. No. S7409) was dissolved in 0.9% saline and the pH was adjusted to 1 N HCl to 7.0 to 7.4. Mice received a single 150 mg/kg of anisomycin injection immediately or multiple 50 mg/kg of anisomycin injections immediately, 2, 4, and 6 h after acquisition. A total of 150 mg/kg anisomycin was shown to effectively inhibit cerebral protein synthesis in mice (~96%). The multiple injections of anisomycin should sustain protein synthesis inhibition at levels >90% until 2 h after the final injection, resulting in strong protein synthesis inhibition for 8 h in mice that received multiple injections. For the CXM experiment, 9 mg/kg CXM, or equivalent SAL, was delivered subcutaneously immediately after training.

**Surgery and Viral Injections.** Mice were anesthetized with 0.2% sodium pentobarbital (5 mL/kg) and fixed to a stereotaxic frame. Their body temperature was kept at 36 °C by a heating pad and their skull was exposed. Bilateral craniotomies were performed using a 0.5-mm diameter micromotor drill. The injection of the virus was performed using a 10-μL NanoFil syringe under the control of the UMP3 and Micro4 system (WPI), with a speed of 50 nL/min. The dorsal CA1 injections were bilaterally targeted to ~2.0 mm anterior-posterior (AP), 1.5 mm medial-lateral (ML), and ~1.5 mm dorsal-ventral (DV). The viral volumes of the AAV-CaMKII-rac1-DN-EGFP, and AAV-CaMKII-EF (as the control group) were 400 nL for the CA1 excitatory neurons. For labeling of the CA1 engram cells, a virus mixture AAV2/9-c-fos:tfA (300 nL of tFA) and 300 nL of TRE-tTomato into the CA1 (−2.2 mm AP, ±1.7 mm ML, and −1.6 mm DV). For the gain- and loss-of-function experiments in engram cells, AAV2/9-TRE-hChR2-eYFP, AAV2/9-TRE-TeTX-P2A-eGFP, and AAV2/9-TRE-hM4DGi-mCherry were used. All viral vectors were aliquoted and stored at −80 °C until use. After the injections, the needle remained in place for 10 min before slowly being withdrawn and the
wound was sutured. After surgery, animals were allowed to recover for 2 wk prior to the performance of all subsequent experiments. All the AAVs were purchased from BrainVIA and Vigene Biosciences.

**Cannula Placements and Drug Infusion.** Mice were implanted under 0.2% sodium pentobarbital (5 mL/kg) with guide cannula (RWD Life Science, Cat. No. 62522) in the dorsal CA1 region of the hippocampus in accordance with coordinates 2.0 mm AP, ±1.5 mm ML, and −2.0 mm DV. The cannulae were fixed to the skull with dental acrylic. Immediately after the training, WPI injectors were lowered through the guide cannula and mice received 0.25 μL of anisomycin (100 μg/μL) or saline per side at the rate of 0.1 μL/min.

**Fear Conditioning.** The fear conditioning test was conducted using the HABIT-EST Modular Behavioral Test System. A Coulbourn Habitest chamber (27 cm × 28 cm × 30.5 cm) had a stainless-steel rod floor, which was connected to a shock generator in a sound-attenuating box. In single-shock CFC sessions, mice were individually placed in the conditioning and they freely explored the area for 3 min. Then, mice were exposed to one footshock (2 s, 0.8 mA) and returned to their home cage 30 s after. In the five-shock contextual fear conditioning task, the footshock (2 s, 0.8 mA) was delivered at 180 s, 240 s, 300 s, 360 s, and 420 s. Mice remained in the conditioning chamber for a total of 450 s. During testing, mice were placed back in the conditioning chamber for 4 min. For trace fear conditioning, the mice were placed in the chamber and allowed to explore for 3 min. Then, a 20-s tone (80 dB, 1,200 Hz) was administered five times, followed by a 2 s ± 0.6 mA shock, with a 4-min interval between repetitions. The mice were removed 30 s after the last shock, and the tone and shock were separated by a 20-s interval. For the trace fear memory test, the mice were placed in a new environment with a 3-min period followed by three 20-s tones (CS), with a 220-s interval between repetitions. For the context-only group, mice were placed into the fear conditioning chamber for 3 min with no shock being delivered and then put back into their homecages. For the shock-only group, mice were exposed to an immediate electric shock (2 s, 0.8 mA) and then put back into their homecages. For all experiments, freezing levels of mice were accessed by an automated motion detection software (Freezeframe software; Actimetrics).

**Activity-Dependent Cell Labeling.** Mice were bilaterally injected with a virus mixture (300 nL of AAV2-tdTomato:1A) and 300 nL of the AAV2-c-fos:tdTomato into the dorsal CA1. Mice were allowed to recover for 2 wk and raised on food containing 40 mg/kg doxycycline (Dox). In order to open a window of activity-dependent labeling for CFC, the mice were then taken off Dox for 24 to 30 h. After CFC, Dox diets were resumed immediately. For detection of the c-fos immunoreactivities in the CA1, animals were perfused and their brains were fixed with 4% paraformaldehyde (PFA) after the exposure to recall cues (the conditioned context). Coronal slices were incubated with anti-c-fos antibody (Cell Signaling Technology, Cat. No. 2250). The images of the immunohistochemistry were captured on a Zeiss LSM 710 confocal microscope.

**Statistical.** Statistical analyses were performed in GraphPad Prism. All data were analyzed with an unpaired t test, one-way ANOVA, or two-way ANOVA where appropriate. The data are shown as the mean ± SEM and ns indicates nonsignificance (P > 0.05). The significant levels were set as P = 0.05. Significant for comparison as follows: *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. The sample sizes (n), specific statistical tests used, main effects, and the P values for each experiment can be found in SI Appendix, Table S1.

**Data Availability.** All study data are included in the article and SI Appendix.

**ACKNOWLEDGMENTS.** We greatly thank Wantong Hu for help with imaging analysis, Lianzhang Wang for help with the experiments, and all the members of the Y.Z. laboratory for their support. This work was supported by grants from the National Natural Science Foundation of China (32021002), the Peking University–Tsinghua University–National Institute of Biological Science Joint Graduate Program, and the Tsinghua-Peking Center for Life Sciences.
1. J. J. Kim, D. M. Diamond, The stressed hippocampus, synaptic plasticity and lost memories. Nat. Rev. Neurosci. 3, 453–462 (2002).
2. G. Fernández, R. G. M. Morris, Memory, novelty and prior knowledge. Trends Neurosci. 41, 654–659 (2018).
3. M. Joëls, G. Fernandez, B. Rozenendaal, Stress and emotional memory: A matter of timing. Trends Cogn. Sci. 15, 280–288 (2011).
4. S. H. Wang, R. G. M. Morris, Hippocampal-neocortical interactions in memory formation, consolidation, and reconsolidation. Annu. Rev. Psychol. 61, 49–79, C1–C4 (2010).
5. A. J. Duszewska, C. G. McKinna, S. Takeuchi, L. Genzel, Novelty and dopaminergic modulation of memory persistence: A tale of two systems. Trends Neurosci. 42, 102–114 (2019).
6. D. J. F. de Quervain, B. Roozendaal, J. L. McGaugh, Stress and glucocorticoids impair retrieval of long-term spatial memory. Nature 394, 787–790 (1998).
7. T. P. Wong et al., Hippocampal long-term depression mediates acute stress-induced spatial memory retrieval impairment. Proc. Natl. Acad. Sci. U.S.A. 104, 11471–11476 (2007).
8. T. Takahashi et al., Social stress-induced cortisol elevation acutely impairs social memory in humans. Neurosci. Lett. 363, 125–130 (2004).
9. X. D. B. McKim et al., Neuroinflammatory dynamics underlie memory impairments after repeated social defeat. J. Neurosci. 36, 2599–2604 (2016).
10. A. M. Smith, V. A. Fiovec, A. K. Thomas, Retrieval practice protects memory against acute stress. Science 354, 1046–1048 (2016).
11. H. Hu et al., Emotion enhances learning via noradrenergic regulation of AMPA-receptor trafficking. Cell 131, 160–173 (2007).
12. S. A. Gannon, A. D. Wagner, Acute stress and episodic memory retrieval: Neurobiological mechanisms and behavioral consequences. Ann. N. Y. Acad. Sci. 1369, 55–75 (2016).
13. T. Takeuchi et al., Locus coeruleus and dopaminergic consolidation of everyday memory. Nature 537, 357–362 (2016).
14. A. C. Singer, L. M. Frank, Rewarded outcomes enhance reactivation of experience in the hippocampus. Neuron 64, 910–921 (2009).
15. D. L. Schacter, J. E. Eich, L. Tulving, Richard Semon’s theory of memory. J. Verbal Learning Verbal Behav. 17, 721–743 (1978).
16. S. Tonegawa, X. Liu, S. Ramirez, R. Redondo, Memory engraving cells have come of age. Neuron 87, 918–931 (2015).
17. S. A. Josselyn, S. Tonegawa, Memory engrams: Recalling the past and imagining the future. Science 355, eaaw4325 (2020).
18. T. Kitamura et al., Engrams and circuits crucial for systems consolidation of a memory. Science 356, 73–78 (2017).
19. T. J. Ryan, D. S. Roy, M. Pignatelli, A. Bors, S. Tonegawa, Memory. Engram cells retain memory under retrograde amnesia. Science 348, 1007–1013 (2015).
20. S. Tonegawa, M. D. Morrissey, T. Kitamura, The role of engraving cells in the systems consolidation of memory. Nat. Rev. Neurosci. 19, 485–498 (2018).
21. D. S. Roy, S. Muralidhar, L. M. Smith, S. Tonegawa, Silent memory engrams as the basis for retrograde amnesia. Proc. Natl. Acad. Sci. U.S.A. 114, E9972–E9979 (2017).
22. J. Yokose et al., Overlapping memory trace indispensable for linking, but not recalling, individual memories. Science 355, 398–403 (2017).
23. Y. Liu et al., Hippocampal activation of Rac1 regulates the forgetting of object recognition memory. Curr. Biol. 26, 2351–2357 (2016).
24. L. Liu et al., Interplay between nNOS and Rac1 activity determines dynamic maintenance of long-term memory. Nat. Commun. 10, 5313 (2019).
25. M. B. Baker, H. Nicole Sloan, A. D. Hall, J. Leo, J. K. Maner, Matting and memory. Can mating cues enhance cognitive performance? Evolut. Psychol. 13, 1474/09415623280 (2015).
26. N. D. Powell et al., Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via β-adrenergic induction of myeloidopoiesis. Proc. Natl. Acad. Sci. U.S.A. 110, 16574–16579 (2013).
27. R. Nardos et al., Oxytocin-dependent recovery of a social reward learning critical period with MDMA. Nature 569, 116–120 (2019).
28. L. Lu et al., Adult newborn granule cells confer emotional-state-dependent plasticity in memory retrieval. bioRxiv [Preprint] (2020). https://doi.org/10.1101/2020.07.14.2024481. Accessed 14 July 2020.
29. T. Abel et al., Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. Cell 88, 615–626 (1997).
30. R. L. Davis, Y. Zhang, The biology of forgetting—A perspective. Neuron 95, 490–503 (2017).
31. Y. Liu, L. Lu, L. Wang, Y. Zhong, Social isolation induces Rac1-dependent forgetting of social memory. Cell Rep. 25, 288–295.e3 (2018).
32. W. Wu et al., Inhibition of Rac1-dependent forgetting alleviates memory deficits in animal models of Alzheimer’s disease. Protein Cell 10, 745–757 (2019).
33. M. C. Subauste et al., Rho family proteins modulate rapid apoptosis induced by cytotoxic T lymphocytes and Fas. J. Biol. Chem. 288, 9725–9733 (2000).
34. X. Liu et al., Optogenetic stimulation of a hippocampal engraving activator decreases fear memory. Nature 484, 381–385 (2012).
35. D. S. Roy et al., Memory retrieval by activating engraving cells in mouse models of early Alzheimer’s disease. Nature 531, 508–512 (2016).
36. J. Wang et al., Epigenetic modulation of inflammation and synaptic plasticity promotes resilience against stress in mice. Nat. Commun. 9, 477 (2018).
37. S. A. Golden et al., Epigenetic regulation of Rac1 induces synaptic remodeling in stress disorders and depression. Nat. Med. 19, 337–344 (2013).
38. A. Hayashi-Takagi et al., Labelling and optical erasure of synaptic memory traces in the motor cortex. Nature 525, 333–338 (2015).
39. Y. Luo, Rho GTPases in neuronal morphogenesis. Nat. Rev. Neurosci. 1, 173–180 (2000).
40. W. J. Wright et al., Silent synapses dictate cocaine memory destabilization and reconsolidation. Nat. Neurosci. 23, 32–46 (2020).
41. Y. Guo et al., Genetic dissection of active forgetting in labile and consolidated memories in animal models of Alzheimer’s disease. Proc. Natl. Acad. Sci. U.S.A. 116, 21191–21197 (2019).
42. E. R. Kandel, Y. Dudai, M. R. Mayford, The molecular and systems biology of memory. Cell 157, 163–186 (2014).
43. P. Bekinschtein et al., Persistence of long-term memory storage requires a late protein synthesis and BDNF-dependent phase in the hippocampus. Neuron 53, 261–277 (2007).