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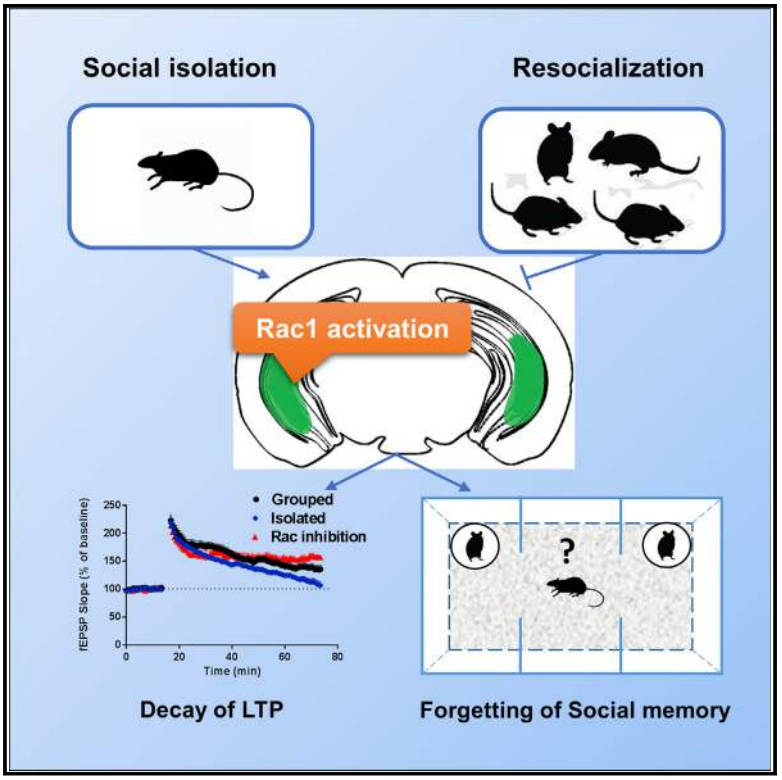
# Social Isolation Induces Rac1-Dependent Forgetting of Social Memory

## Graphical Abstract

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## In Brief

Liu et al. identify a Rac1-dependent forgetting pathway that mediates isolation-induced memory impairment. Such findings underscore the importance of maintained social interactions on cognitive function, which may have implications for autism and Alzheimer's disease.

## Highlights

- Social isolation induces forgetting of social memory and Rac1 activation
- Inhibition of Rac1 activity blocks isolation-induced forgetting of social memory
- Resocialization reverses forgetting of social memory and Rac1 activation

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# Social Isolation Induces Rac1-Dependent Forgetting of Social Memory

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## SUMMARY

**Social isolation (SI) has detrimental effects on human and animal cognitive functions. In particular, acute isolation in adult mice impairs social recognition memory (SRM). Previous accounts of this impairment have focused primarily on memory consolidation. However, the current study suggests that impaired SRM results from enhanced forgetting. SI accelerates SRM decay without affecting memory formation. The impairment is caused by elevated Rac1 activity in the hippocampus. Using adeno-associated-virus-based genetic manipulation, we found that inhibition of Rac1 activity blocked forgetting of SRM in isolated adult mice, whereas activation of Rac1 accelerated forgetting in group-housed mice. Moreover, resocialization reversed the accelerated forgetting following isolation in correlation with suppression of Rac1 activity. In addition, accelerated long-term potentiation (LTP) decay in isolated mice brain slices was rescued by inhibition of Rac1 activity. Taken together, the findings lead us to conclude that social memory deficits in isolated mice are mediated by enhanced Rac1-dependent forgetting.**

## INTRODUCTION

Social interaction is beneficial to human well-being and can reduce the risk of age-associated cognitive impairment and dementia (Crooks et al., 2008; Fratiglioni and Wang, 2007; Fratiglioni et al., 2000; Stern, 2006). Conversely, social isolation has been recognized as a major risk factor for health and normal behavior in humans and animals of all ages (Berkman et al., 2000; Cacioppo and Hawkley, 2009; Eisenberger, 2012; Friedler et al., 2015; Heinrich and Gullone, 2006). The common model used in animal research is that of prolonged post-weaning isolation, which causes a range of persistent behavioral, neuronal structural, and transcriptional changes (Fone and Porkess, 2008; Heidbreder et al., 2000; Lander et al., 2017; Lukkes et al., 2009; Makinodan et al., 2012; Wallace et al., 2009). Even short-term social isolation in adult rodents leads to impairment in social recognition memory without affecting other behaviors, such as anxiety (Gusmão et al., 2012; Kogan et al., 2000; Monteiro et al., 2014; Shahar-Gold et al., 2013). Such specific outcomes provide an opportunity to gain insights into the molecular and cellular mechanisms underlying the effect of social isolation on cognitive function during adulthood (Leser and Wagner, 2015).

Social recognition refers to the ability to identify and remember individual conspecifics, and this ability is essential to many forms of social interaction (Ferguson et al., 2002). Social recognition memory is dependent on the hippocampus (Hitti and Siegelbaum, 2014; Kogan et al., 2000; Okuyama et al., 2016). Long-term social memory is disrupted following socially isolated housing in adult mice. The impairment can be rescued by resocialization and an enriched environment (An et al., 2017; Chen et al., 2016; Gusmão et al., 2012; Monteiro et al., 2014; Shahar-Gold et al., 2013). Although cellular and molecular pathways underlying social recognition memory formation and consolidation have been extensively studied (Davis et al., 2010; Ferguson et al., 2002; Garrido Zinn et al., 2016; Hegde et al., 2016; Tanimizu et al., 2017), the mechanisms underlying isolation-induced social memory impairment remain to be elucidated.

Here, we tested the possibility that the forgetting pathway is enhanced by social isolation. Recent studies revealed that distinct molecular pathways are responsible for forgetting, in which the small GTPase Rac1 was reported to play a conserved role in active forgetting in models ranging from *Drosophila* to mice (Davis and Zhong, 2017; Jiang et al., 2016; Shuai et al., 2010; Zhang et al., 2016). Rac1 is a member of the Rho GTPase family of small G proteins and plays a critical role in regulating actin polymerization in neurons (Etienne-Manneville and Hall, 2002; Heasman and Ridley, 2008; Luo, 2000). Manipulation of Rac1 has been reported to affect synaptic plasticity and behavioral memory in animal models (Gao et al., 2015; Haditsch et al., 2009, 2013; Hayashi-Takagi et al., 2015; Oh et al., 2010; Tejada-Simon, 2015). We found that both 1-day and 7-day isolation in adult mice induced Rac1 activation in the hippocampus and caused rapid forgetting of social memory without affecting the acquisition of social recognition memory (SRM). In addition, resocialization could reverse the Rac1 activation, as well as protect memories from being forgotten.

## RESULTS

### Social Isolation Induced a Rapid Decay of SRM through Increased Hippocampal Rac1 Activity

The social discrimination paradigm is commonly used to test SRM for its accuracy (Engelmann et al., 1995). In this study, after

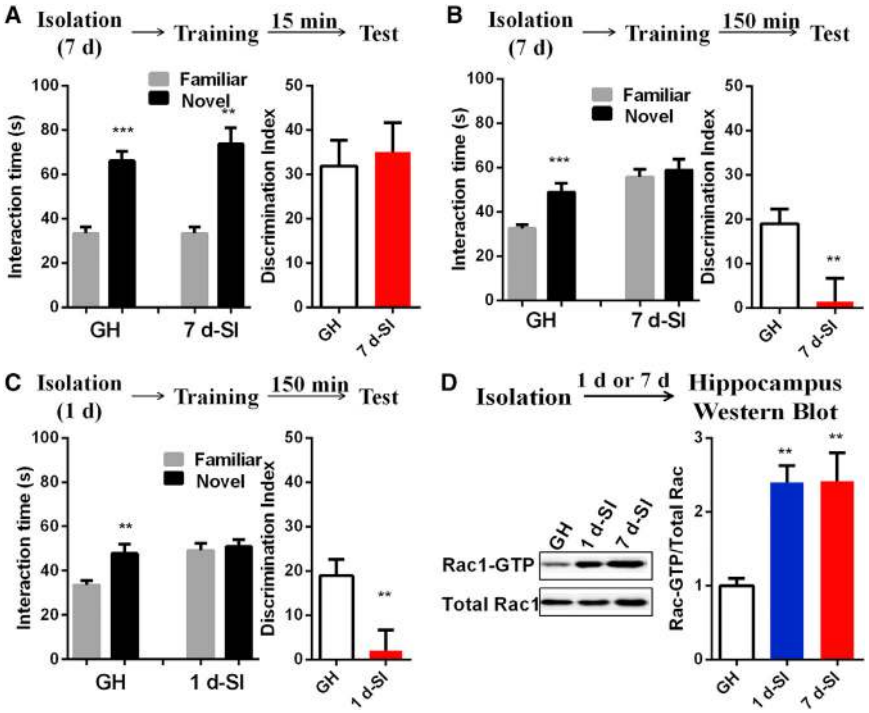


**Figure 1. Isolation-Induced Impairment of Social Recognition Memory Is Correlated with Hippocampal Rac1 Activity**

(A–C) Top: experimental procedure for social discrimination memory. Compared with group housing, 7-day isolation did not affect the formation of social memory in the 15-min test (A; $t_{(22)} = 0.3614$, $p = 0.7213$, unpaired t test), and forgetting of social memory was induced by both 7-day (B; $t_{(26)} = 2.829$, $p = 0.0089$, unpaired t test) and 1-day (C; $t_{(26)} = 2.801$, $p = 0.0095$, unpaired t test) isolation in the 150-min test. Data are shown as the interaction time (paired t tests, $^{**}p < 0.01$, $^{***}p < 0.001$, $n = 11–15$) and discrimination index (unpaired t tests, $^{**}p < 0.01$, $n = 11–15$). GH, group housing; SI, social isolation. See also Figure S1 for the schematic representation of social discrimination memory task and effects of isolation on social exploring ability during training phase.

(D) Representative blots and grouped data of Rac1 activity in isolated mice. Rac1 activity was increased in the hippocampus by both 1-day and 7-d social isolation. $F(2, 11) = 13.61$, $p = 0.0011$, one-way ANOVA ($n = 4–6$ per group, $^{**}p < 0.01$). See also Figure S1C for the effects of social isolation on RhoA activity.

All data are shown as mean ± SEM.

habituation in the social box, adult animals were allowed to explore a juvenile restricted in a cylinder for 5 min, and they were then simultaneously exposed to both the familiar juvenile and a novel juvenile for 5 min during the test (Figure S1A). The differences in the investigation times between the two juveniles were calculated to measure social memory. Previous studies have shown that SRM dissipates between 1 and 2 hr in isolated rats and mice (Kogan et al., 2000; Leser and Wagner, 2015), we therefore chose 15 min for social memory formation and 150 min for forgetting phase in our SRM paradigm. The initial memory of 7-day-isolated adult mice was comparable to that of the group-housed mice 15 min after the sample phase, indicating that memory formation was not affected by social isolation (Figure 1A). However, the 7-day-isolated adult mice showed no memory 150 min after training, as they were unable to discriminate between an already encountered familiar conspecific and a new juvenile, while the memory of the group-housed mice remained normal (Figure 1B). It should be noted that during sampling, the investigation time for the mice was approximately 80 s in total during the 5-min exploration time, and the isolated mice showed similar investigation times as the group-housed mice (Figure S1B). Impaired social memory was induced even with 1-day isolation (Figure 1C). Thus, isolation leads to a phenotype of accelerated forgetting.

Since the hippocampus is known to have a critical role in SRM (Kogan et al., 2000; Okuyama et al., 2016) and as Rac1 is reported to mediate forgetting of hippocampus-dependent memory (Jiang et al., 2016; Liu et al., 2016), we assayed Rac1 activity in the hippocampus after isolation for 1 or 7 days in the adult mice. Interestingly, both 1-day- and 7-day-isolated mice showed increased Rac1 activity (Figure 1D). We then measured activity of another small GTPase, RhoA, in a similar procedure, and we found RhoA activity in the hippocampus is not affected by both 1-day and 7-day isolation (Figure S1C), implying a specific role of Rac1 in the forgetting of social memory in isolated mice. Therefore, we examined whether the rapid decay in social memory in isolated mice is mediated by elevated Rac1 activity.

We constructed recombinant adeno-associated viruses (rAAVs) carrying a dominant-negative form of Rac1 (Rac1-DN) fused with EGFP driven by a CaMKII promoter and a constitutively active form of Rac1 (Rac1-CA) (Figure 2A). These constructs were used in our previous studies (Liu et al., 2016). Then, we injected the rAAVs into both the dorsal and ventral regions of the hippocampus (Figure 2A). Western blot data confirmed that the AAV-Rac1-DN inhibited whereas AAV-Rac1-CA increased Rac1 activity significantly in the hippocampus (Figure S2A).

We found that inhibition of Rac1 activity had no impact on the formation of social memory in isolated mice. After recovery from the adeno-associated virus-control (AAV-ctrl) or AAV-Rac1-DN injection, the mice were either individually or group housed for 7 days, and all groups had a similar 15-min social memory (Figure 2B).

Next, we tested the effects of Rac1 inhibition on 150-min social memory. The impaired 150-min social memory in both the 7-day- and 1-day-isolated mice was rescued in mice with AAV-Rac1-DN injection (Figures 2C and 2D); this rescue appeared to be mediated by suppression of Rac1-dependent forgetting, as 15-min social memory was comparable between the AAV-Rac1-DN and AAV-ctrl injected groups (Figure 2B).

To confirm this idea, we then tested whether Rac1 activation could lead to forgetting of social memory in group-housed

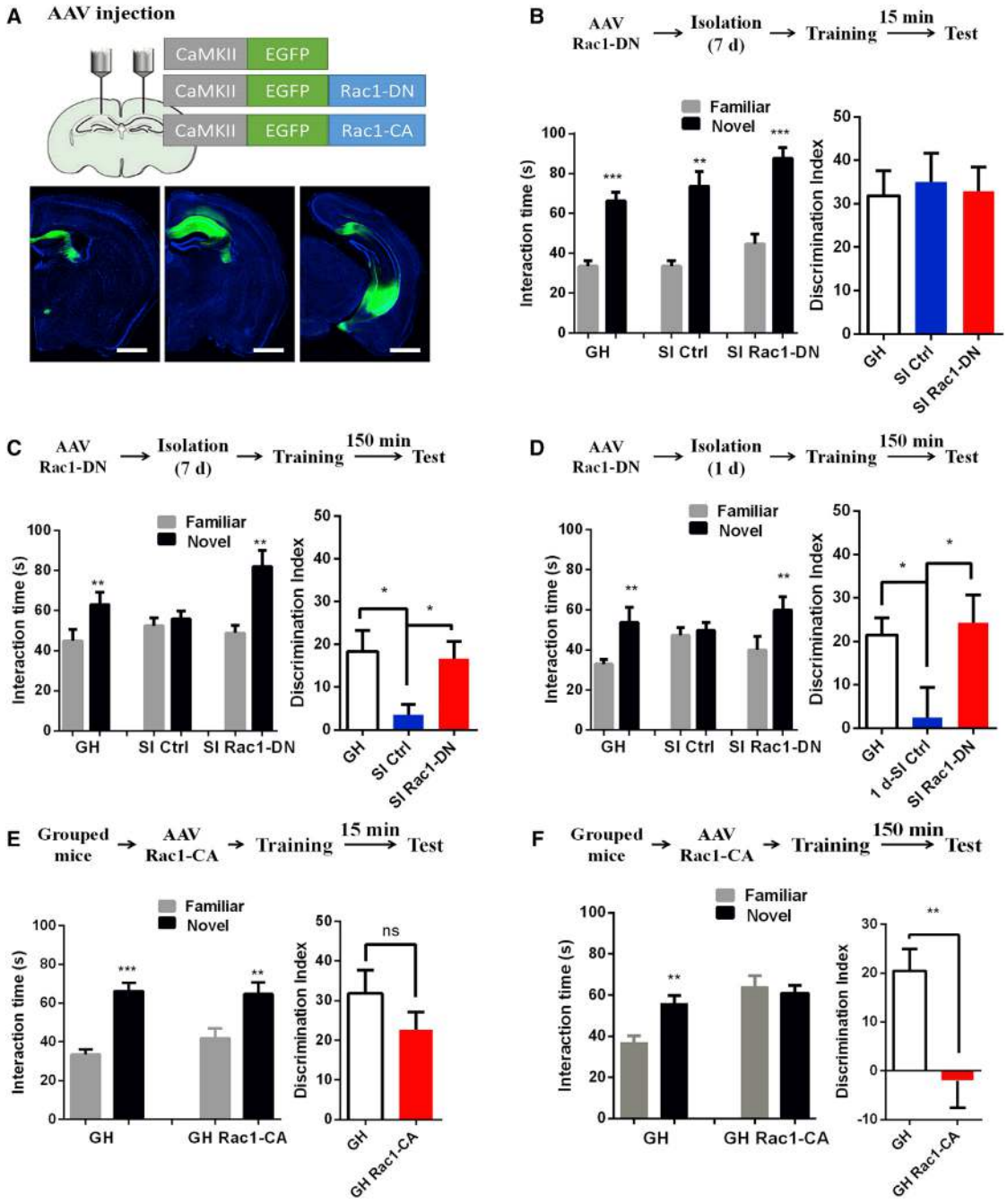


**Figure 2. Manipulation of Rac1 Activity Affects the Retention, but Not the Acquisition, of Social Memory**

(A) Top: diagram of mice that were injected in the hippocampus with AAV-expressing EGFP alone (Ctrl), the dominant-negative form of Rac1 (Rac1-DN), or the constitutively active form of Rac1 (Rac1-CA) fused with EGFP driven by the CaMKIIa promoter. Bottom: representative image of brain slices from mice injected with AAV-expressing EGFP in the dorsal and ventral hippocampus. Scale bar, 1 mm. See also Figures S2A–S2F for effects of AAV injection on Rac1 activity and effects of Rac1 inhibition on forgetting of social memory in specific hippocampal areas.

(B–D) Top: experimental procedure for social discrimination memory with AAV-Rac1-DN injection. Formation of social memory (15-min test) was not affected by AAV-Rac1-DN in the isolated mice (B; $F(2, 30) = 0.07409$, $p = 0.9288$, one-way ANOVA, n = 10–13). Additionally, 150-min social memory was impaired by both 7-day (C; $F(2, 34) = 4.819$, $p = 0.0144$, one-way ANOVA, n = 12–14) and 1-day (D; $F(2, 27) = 4.224$, $p = 0.0253$, one-way ANOVA, n = 10–12) social isolation and was rescued by AAV-Rac1-DN injection in the isolated mice (paired t tests for interaction time data, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n = 10–14). GH, group housing; SI, social isolation. See also Figures S3A and S3B for effects of Rac1 inhibition on interference-induced forgetting of social memory.

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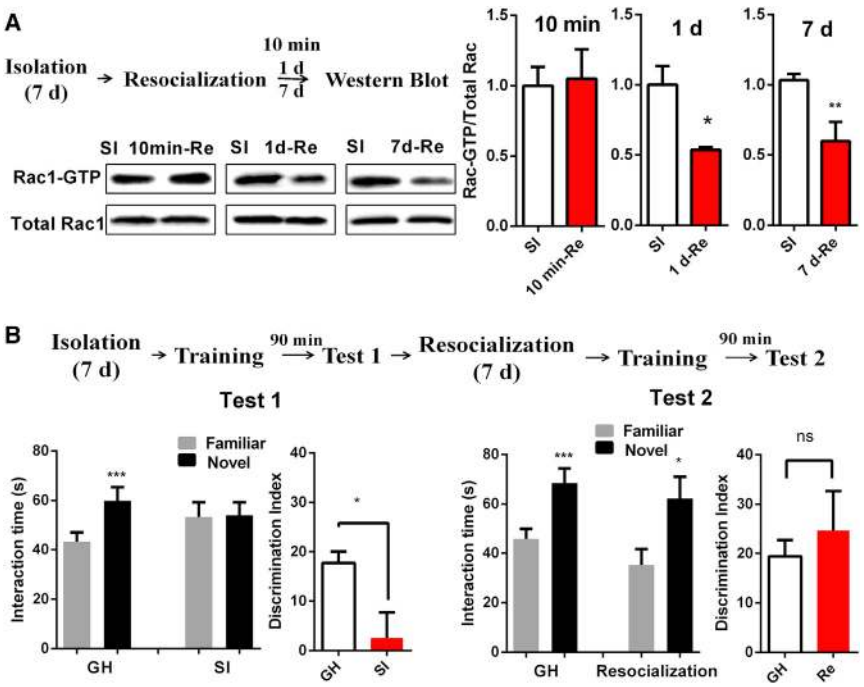


**Figure 3. Effects of Social Isolation on Rac1 Activity and Social Memory Are Reversed by Resocialization**

(A) Representative blots and grouped data of Rac1 activity in resocialized mice. After the 7-day isolation, the mice were resocialized. Hippocampal Rac1 activity was reduced by both 1-day (unpaired t test, $t_{(5)} = 2.883$, $^{*}p < 0.05$, n = 3–4) and 7-day (unpaired t test, $t_{(8)} = 3.575$, $^{**}p < 0.01$, n = 4–6), but not 10-min, resocialization (unpaired t test, $t_{(6)} = 0.1991$, p = 0.8487, n = 4). GH, group housing; Re, resocialization; SI, social isolation.

(B) Top: experimental procedure for social discrimination memory with resocialization. In test 1, the 7-day isolation caused forgetting of social memory at 90 min (unpaired t test, $t_{(26)} = 2.680$, p = 0.0126, n = 14). In test 2, the isolated mice were resocialized for 7 days, and their social memory was comparable to that of the group-housed mice (unpaired t test, $t_{(24)} = 0.6091$, p = 0.5482, n = 13). Data are shown as the interaction time and the discrimination index (paired t tests for the interaction time data, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n = 14–15).

All data are shown as mean ± SEM.

mice. Adult mice were injected with AAV-Rac1-CA to increase the Rac1 activity in the hippocampus and were then subjected to the social discrimination paradigm. We found that formation of social memory was not affected by AAV-Rac1-CA injection in group-housed mice during the 15-min test (Figure 2E). However, the AAV-Rac1-CA injection led to rapid forgetting of social memory during the 150-min test in the group-housed mice (Figure 2F). Taken together, the findings indicated that both 7-day and 1-day social isolation induced hippocampal neuronal Rac1 activation, which led to rapid forgetting of social memory.

Because it has been reported that ventral CA1 plays a necessary and sufficient role in social memory (Okuyama et al., 2016), we tested whether Rac1 activity in ventral CA1 alone is critical for forgetting of SRM. Interestingly, we found that inhibition of Rac1 activity with AAV-Rac1-DN injection in ventral CA1 blocked the 7-day social-isolation-induced forgetting of SRM at 150 min without affecting the 15-min memory formation (Figures S2B–S2D). Because CA2 has also been shown to play a critical role in social memory (Hitti and Siegelbaum, 2014; Stevenson and Caldwell, 2014), we then performed AAV-Rac1-DN injection targeting dorsal hippocampal areas, including CA2 and possibly adjacent regions; however, it has no effects on forgetting of 150-min SRM induced by 1-day isolation (Figures S2E and S2F).

To further validate the idea of Rac1-dependent forgetting of social memory, we performed an experiment using the modified interference-induced forgetting paradigm (Engelmann, 2009; Perna et al., 2015), in which the animals were exposed to another two unfamiliar conspecific juveniles 2 hr after the initial learning as interference. The animals showed disrupted social memory during the first encountered mice when tested 24 hr later. Interestingly, using a pharmacological approach, we found that interference-induced forgetting of social memory in the grouped mice was suppressed by inhibiting Rac1 activity with the Rac-specific inhibitor Ehop016 (Humphries-Bickley et al., 2015; Montalvo-Ortiz et al., 2012), further supporting a role of Rac1 activity in regulating the forgetting of social memory (Figures S3A and S3B).

## Resocialization Reversed Isolation-Induced Memory Defects and Elevated Rac1-Activity

It has been reported that resocialization can reverse isolation-induced impairment in social memory (An et al., 2017; Shahar-Gold et al., 2013). If the presented conclusion is correct, then one would expect that isolation-induced Rac1 activity could be reversed by resocialization. Mice were individually housed for 7 days and then resocialized for various time periods, and hippocampal Rac1 activity was detected by western blot. We found that 1-day and 7-day, but not 10-min, resocialization reduced Rac1 activity in isolated mice (Figure 3A), indicating that increased Rac1 activity is indeed reversible through resocialization. Next, using the social discrimination paradigm, we tested whether 7-day resocialization could reverse the forgetting of

(E and F) Top: experimental procedure for social discrimination memory with AAV-Rac1-CA injection. Formation of social memory (15-min test) was not affected by AAV-Rac1-CA in the group-housed mice (E; unpaired t test, $t_{(21)} = 1.187$, p = 0.2485, n = 10–13). Forgetting of social memory (150-min test) was induced by AAV-Rac1-CA injection (F; unpaired t test, $t_{(17)} = 3.094$, p = 0.0066, n = 9–10) in the group-housed mice (paired t tests for the interaction time data, $^{*}p < 0.05$,$^{**}p < 0.01$, $^{***}p < 0.001$, n = 9–13). Data are shown as the interaction time and the discrimination index.

All data are shown as mean ± SEM.

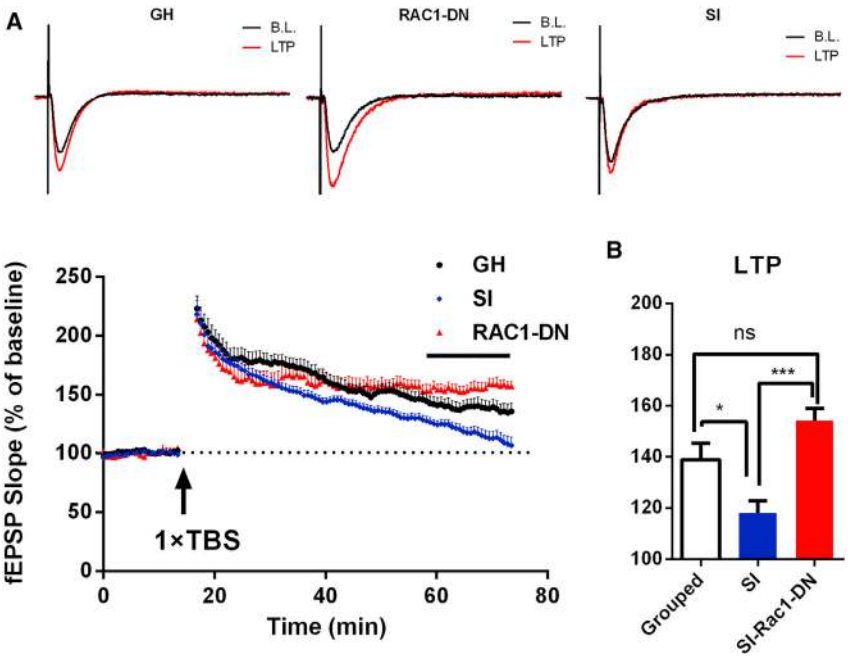


**Figure 4. LTP Decay in Isolated Mice Is Rescued by Inhibiting Rac1 Activity**

(A) Top: typical fEPSP trace at baseline and during the LTP recordings. LTP was recorded in the Schaffer collateral pathway of the hippocampal slices for 1 hr after one train of theta burst stimulation. LTP decay was accelerated in social isolation (SI) mice and slowed by AAV-Rac1-DN injected in isolated mice compared with the grouped mice, as indicated by the fEPSP slope. GH, group housing; SI, social isolation.

(B) Average fEPSP slope during the last 15 min of the LTP recording. LTP deficiency in isolated mice was rescued by AAV-Rac1-DN injection ($F(2, 36) = 11.94$, $p = 0.0001$, one-way ANOVA, $n = 11–16$). All data are shown as mean ± SEM. *$p < 0.05$; ***$p < 0.001$; ns, not significant.

SRM in isolated mice. After the 7-day isolation, mice underwent a SRM test 90 min after the initial interaction; similar to the above results, these isolated mice showed memory loss compared with that of the group-housed mice. Then, mice were resocialized for 7 days and tested using the same protocol; these resocialized animals showed memory abilities comparable to those of the group-housed mice tested at 90 min, indicating that social interacting may protect memories from being forgotten by inhibiting Rac1-dependent forgetting (Figure 3B).

### Inhibiting Rac1 Activity Suppressed Accelerated Decay of LTP in Isolated Mice

Long-term potentiation (LTP) is believed to be the cellular analog of learning and memory. As forgetting of social memory induced by social isolation is critically influenced by Rac1 activity in hippocampal excitatory neurons, we next examined whether the decay in LTP in isolated mice is regulated by Rac1 activity. To this end, we prepared brain slices from the group-housed mice and isolated mice injected with ctrl virus or AAV-Rac1-DN and recorded the field potentials for 1 hr from the CA1 region of these brain slices using the MED64 system. Compared with the decay in ctrl slices from the group-housed mice, the decay in LTP stimulated by one theta burst stimulation (TBS) was accelerated in 7-day-isolated mice, whereas inhibition of Rac1 activity in slices via expression of Rac1-DN suppressed the decay in LTP in isolated mice (Figure 4A). Statistical analysis of the last 15 min of the LTP confirmed these results (Figure 4B). Note that the induction of LTP was comparable among all groups. The electrophysiological data were consistent with the findings of previous studies that showed that isolation led to LTP deficiency (Kamal et al., 2014; Tada et al., 2017; Talani et al., 2011) and that Rac1 activity was involved in LTP stability, but not initiation (Liu et al., 2016; Rex et al., 2009). Such results were in accordance with behavioral data in support of the role of Rac1 activity in isolation-induced memory deficits.

## DISCUSSION

The aim of this study was to determine whether forgetting pathways contribute to social-isolation-induced deficits in SRM. Our behavioral analysis yielded observations consistent with previous reports and showed that acute social isolation did not affect short-term memory but impaired long-term social memory (Gusmão et al., 2012; Kogan et al., 2000; Monteiro et al., 2014). In the current study, short-term memory or learning referred to memory that was tested 15 min after the initial social interaction (Figures 1, 2, and 3), whereas to study isolation effects, we focused on 150-min memory at this time point for mice in 7-day or 1-day isolation; these mice showed almost no recognition of social encounters, while the memory of the group-housed mice remained significant (Figures 1 and 2). For mice in 7-day isolation, recognition memory disappeared within 90 min (Figure 3). Within the time windows tested, our study, which was based on an approach of a combination of behavioral and biochemical assays, genetic manipulation, pharmacological treatment, and electrophysiological recordings, yielded highly consistent data in support of the involvement of enhanced Rac1-dependent forgetting in social-isolation-induced social memory problems, as further described below.

We showed that Rac1 activity was significantly elevated in hippocampal tissues from adult mice in response to either 7-day or 1-day isolation (Figure 1D). Consistently, Rac1 activity is also upregulated by neonatal social isolation in the developing rat barrel cortex (Tada et al., 2017). The increase of Rac1 activity in adult mice was important to SRM for the following reasons. First, the level of increased Rac1 activity correlated well with the degree of memory deficits, regardless of the difference in isolation duration for 7 days and 1 day (Figures 1B and 1C). Second, manipulation of Rac1 activity in hippocampal excitatory neurons through virus-mediated expression of mutant Rac1 genes was capable of regulating the retention of social memory. Inhibiting Rac1 activity in isolated mice restored the 150-min memory to a level similar to that of the group-housed mice (Figures 2C and 2D). Conversely, activating Rac1 in the group-housed mice led to rapid forgetting of social memory (Figure 2F). Such

Rac1 activity manipulation had no impact on short-term (15-min) memory in either isolation duration (Figure 2B). Third, resocialization reversed not only 150-min memory but also the isolation-induced increase in Rac1 activity (Figure 3). This reverse in Rac1 activity was physiologically significant for elevated Rac1 activity and remained when resocialization was introduced for only 10 min (Figure 3A). Fourth, the LTP induced in brain slices from isolated mice decayed faster than that in group-housed mice, while this phenotype was also rescued by inhibition of Rac1 activity (Figure 4). Taking these findings together, we conclude that social isolation induces accelerated forgetting of SRM by elevating hippocampal Rac1 activity.

The Rac1 activation induced by social isolation appears to be specific, since activity of another small GTPase, RhoA, is not affected by isolation, even though RhoA is reported to antagonize Rac1 (Burridge and Wennerberg, 2004; Byrne et al., 2016; Guilluy et al., 2011). In fact, using dominant mutant-expressing approaches, it was shown that Rac1, but not RhoA, mediates forgetting in *Drosophila* (Shuai et al., 2010). In addition, pharmacological studies showed that memory formation and LTP initiation are regulated by RhoA, but not Rac1, activity in mammalians (Rex et al., 2009; Wang et al., 2013), whereas Rac1 was reported to regulate LTP stabilization and memory maintenance in previous (Liu et al., 2016; Rex et al., 2009) and current studies, suggesting Rac1 and RhoA may play distinct roles in regulating memory processes.

SRM can last for several days in group-housed mice, and its consolidation has been shown to involve the cyclic AMP (cAMP) pathway, the transcription factor CREB, and neurotransmitter systems (Garrido Zinn et al., 2016; Hegde et al., 2016; Shahar-Gold et al., 2013; Tanimizu et al., 2017). In addition, some neuropeptides, such as arginine-vasopressin (AVP) and the similar peptide oxytocin (OXT), have been found to be crucial to SRM in rats and mice (Leser and Wagner, 2015). It would be of interest to see whether Rac1 activity is related to such mechanisms.

Social isolation has been shown to be associated with the progression of cognitive deficits, such as in Alzheimer's disease (Donovan et al., 2016), in human and animal models (Hsiao et al., 2018). Thus, the current study may shed light on treatments targeting Rac1 for Alzheimer's disease.

In summary, the current study revealed a mechanism by which social isolation initiates Rac1-dependent forgetting of social memory, indicating that social interaction may protect memories from being forgotten.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
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  - Immunohistochemistry
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- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Statistics

## SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.09.033.

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## AUTHOR CONTRIBUTIONS

Y.L. conceived and designed this project and performed all behavioral experiments. L.L. performed all electrophysiological experiments. Western blot data were collected and analyzed by L.L. and L.W. The molecular cloning and rAAV packaging were conducted by Y.L. The rAAV and drug injections were performed by Y.L. and L.L. The manuscript was written by Y.L. and Y.Z.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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# STAR★METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Antibodies | | |
| Anti-Rac1 antibody | BD Transduction Laboratories | Cat. No. 610650; RRID:AB_397977 |
| HRP-conjugated goat anti-mouse IgG | Cell Signaling Technology | Cat. No. #7072; RRID:AB_331144 |
| Anti-RhoA-GTP monoclonal antibody | NewEast company | Cat. No. 26904; RRID:AB_1961799 |
| Anti-RhoA Mouse Monoclonal Antibody | NewEast company | Cat. No. 26007; RRID:AB_1961795 |
| Bacterial and Virus Strains | | |
| pAAV-CaMKIIα-EGFP-Rac1-DN | Liu et al., 2016 | N/A |
| pAAV-CaMKIIα-EGFP-Rac1-CA | Liu et al., 2016 | N/A |
| Chemicals, Peptides, and Recombinant Proteins | | |
| EHop-016 | Shanghai Sun-shine Chemical Technology Co., Ltd | Catalog No#: 102615 |
| Protease Inhibitor Cocktail | Selleck | K4000 |
| Phosphatase Inhibitor Cocktail | Selleck | K5000 |
| Critical Commercial Assays | | |
| PAK-PBD beads | Cytoskeleton | Cat. # PAK02-A |
| Experimental Models: Organisms/Strains | | |
| Mouse: C57BL/6J | Beijing Vital River Laboratory Animal Technology Co., Ltd | N/A |
| Recombinant DNA | | |
| pCyPet-Rac1 (T17N) | Machacek et al., 2009 | Addgene plasmid # 22784 |
| pCyPet-Rac1 (Q61L) | Machacek et al., 2009 | Addgene plasmid # 22783 |
| pAAV-CaMKIIα-EGFP | Edward Boyden | Addgene plasmid # 64545 |
| Software and Algorithms | | |
| Graphpad Prism 6 | Graphpad software (https://www.graphpad.com/) | RRID: SCR_002798 |
| ANY-maze Behavioral tracking software | Stoelting Co. | RRID; SCR_014289 |
| ZEN | ZEISS | RRID:SCR_013672 |

## CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and request for resources and reagents should be directed to the Lead Contact, Yi Zhong (zhongyi@tsinghua.edu.cn).

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

### Animal

All experiments were performed using the principles outlined in the Guide for the Care and Use of Laboratory Animals. C57BL/6J mice (age 3-5 months, male) were purchased from Vital River Laboratory (Animal Technology Co. Ltd., Beijing, China) and were maintained under standard conditions of the Tsinghua University animal facility. All experiments involving animals were approved by Tsinghua University committees on animal care and use.

## METHOD DETAILS

### Stereotactic surgery procedure

The methods used for the stereotaxic surgery have been described previously (Liu et al., 2016). The mice were anesthetized with 0.2% sodium pentobarbital (5 ml/kg). Bilateral craniotomies were performed using a 0.5-mm diameter drill. The virus was injected using a 10 μL nanofil syringe controlled by UMP3 and a Micro4 system (WPI), with a speed of 50 nl/min. The dorsal CA1 injections were bilaterally targeted to −2.0 mm AP, +/− 1.5 mm ML, and −1.5 mm DV. The ventral CA1 injections were bilaterally targeted to −3.16 mm AP, +/− 3.10 mm ML, and –3.70 mm DV. The CA2 injections were bilaterally targeted to −2.18 mm AP, +/− 2.70 mm ML,

and –2.0 mm DV. The viral volumes were 300 nL for CA1 and 150nl for CA2. After the injection, the needle remained in place for 10 minutes to ensure that the virus spread to the targeted area before it was slowly withdrawn. The mice remained on an electric blanket until they fully recovered from the anesthesia. After surgery, the mice were allowed to recover for at least 2 weeks before all subsequent experiments were performed.

### Social discrimination paradigm

Prior to the experiment, adult male mice were group housed or put into solitary cages for 1-d or 7-d isolations. During the habituation phase, the adult male mice were introduced to a clean standard cage containing two empty cylinders for a period of 15 min. In the training session, one juvenile mouse (3-4 weeks of age) was introduced into one of the clean cylinders in the cage. Then, individual adult male mice were placed into the same cage containing one empty cylinder and one juvenile mouse and were allowed to explore the cage for 5 min. The experimental animals were returned to their home cages immediately after training. For the testing phase, a second novel juvenile mouse was introduced to one of the clean cylinders in the cage. The time spent exploring the two juvenile mice was separately recorded using ANY-MAZE software. The discrimination index (DI) was calculated using the following formula: (time exploring the novel mouse – time exploring the familiar mouse)/(time exploring the novel mouse + time exploring the familiar mouse) * 100.

### Slice preparation and electrophysiology

Transverse hippocampal slices (300 μm) were prepared from 4-month-old mice using a VF-300 microtome (Precisionary Instruments Inc.) and were maintained in an interface chamber between humidified carbogen gas (95% O2/5% CO2) and artificial cerebrospinal fluid (ACSF) containing 124 mM NaCl, 3 mM KCl, 1.25 mM KH2PO4, 1 mM MgSO4, 2 mM CaCl2, 26 mM NaHCO3, and 10 mM glucose (pH 7.2–7.4). After remaining at room temperature for at least 1 hr, the slices were kept in the MED probe for 1 hr to attach the 16-channel array (MED-PG515A, Alpha MED Sciences). Extracellular field excitatory post-synaptic potentials (fEPSPs) in the Schaffer Collateral pathway were synaptically evoked at 0.025 HZ and recorded in the CA1 region. The fEPSPs were evoked using a stimulation intensity that elicited a 30% maximal response. Data acquisition and analysis were performed using the Multielectrode MED64 hardware and software packages (Panasonic). Additional analyses were performed using Microsoft Excel and GraphPad Prism. LTP was induced by 1-train theta burst stimulation (TBS), consisting of 10*100-Hz bursts (4 pulses per burst) with a 200-ms interval between bursts.

### DNA constructs and AAV production

The methods used for the AAV constructs and production were described previously (Liu et al., 2016). All plasmids were constructed using standard molecular biology procedures and were subsequently verified by double-strand DNA sequencing. The pCyPet-Rac1 (T17N), pCyPet-Rac1 (Q61L), pAAV-CaMKIIα-EGFP plasmids were formed from the Addgene. The pAAV- CaMKIIα-EGFP-Rac1-DN and pAAV- CaMKIIα-EGFP-Rac1-CA were subcloned from pCyPet-Rac1 (T17N) and pCyPet-Rac1 (Q61L) into pAAV-CaMKIIα-EGFP using BsrGI/EcoRI restriction enzymes.

The rAAV2/1 was prepared by cotransfection of the core plasmids (as described above) into HEK293 human embryonic kidney cells with the adenoviral helper plasmids (gifts from Dr. Song Sen). Sixty hours later, the transfected cells were collected for rAAV2/1 purification. After titration using RT-PCR, the virus was then diluted using PBS solution (pH 7.4) to 5.0 × 1012 genome copies (GC) per ml before injection.

### Western Blot Analysis

The relative levels of Rac1-GTP were measured using a PBD pull-down assay (Cytoskeleton). The hippocampi were isolated and homogenized in cell lysis buffer. Large cuticular debris was removed by centrifugation at 12,000 × g (10 min, 4°C). GTP-Rac1 was precipitated from the cell lysates with a GST-tagged PAK-PBD protein on colored agarose beads. The protein extracts were subjected to SDS-PAGE at 12% and transferred to nitrocellulose membranes (Millipore). The blots were incubated with antibodies against Rac1 (1:2,000) (BD Transduction Laboratories) overnight at 4°C and with HRP-conjugated goat antimouse IgG (Cell Signaling Technology) for 2 hr at room temperature.

For detection of the RhoA activity, 40 μg of hippocampal homogenates were separated by 15% SDS-PAGE and transferred to nitrocellulose membranes (Pall Corporation, Cat. No. 66485). Membranes were blocked in milk solution (5% milk in TBS and 0.1% tween 20) for one hour at room temperature. Membranes were individually incubated with primary anti-active RhoA mouse monoclonal antibody (26904, NewEast Biosciences, 1:1000) and primary Anti-RhoA (26007, NewEast Biosciences, 1:2000) overnight at 4°C. All the HRP-conjugated secondary antibodies were used at 1:2000 dilutions for membranes incubation at room temperature for one hour. The intensities of the detected bands in the western blots were analyzed with ImageJ software (National Institutes of Health).

### Immunohistochemistry

The mice were anesthetized with 0.2% sodium pentobarbital (5 ml/kg) before perfusion. The brains were fixed in 4% paraformaldehyde for 3 hr and dehydrated in a 30% sucrose solution overnight. Serial coronal sections (45-μm) throughout the hippocampus were cut on a vibratome and stored in a 0.1 M PBS solution (pH 7.4). Images of the immunohistochemistry were captured on a Zeiss Axio Scan.Z1.

### Drug

The Ehop016 (Shanghai Sun-shine Chemical Technology Co., Ltd.) was dissolved in a solution containing 1% DMSO/30% PEG/1% Tween-80. Mice were intraperitoneally injected (20 mg/kg) with the Ehop016 solution 2 hr ago before the retroactive interference experiment. It has been shown I.P. injection of Ehop016 (20 mg/kg) reduced hippocampal Rac1 activity within 2 hr and had no observable effects on the basic parameter of mouse behaviors, including locomotor activity and exploratory activity(Liu et al., 2016).

## QUANTIFICATION AND STATISTICAL ANALYSIS

### Statistics

Statistical analyses were performed using Student's t tests in GraphPad Prism 6.0 for normally distributed variables to determine the significance of the differences between the controls and treatments. Specifically, paired t test was used for interaction time. Experiments involving a comparison between two groups were analyzed by two-tailed unpaired t test. To compare three different groups, one-way ANOVA was used. A p value of less than 0.05 was considered statistically significant. The data are shown as the mean ± standard error of the mean (SEM). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; n.s., nonsignificant ($p > 0.05$).

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## Supplemental Information

# Social Isolation Induces Rac1-Dependent Forgetting of Social Memory

**Yunlong Liu, Li Lv, Lianzhang Wang, and Yi Zhong**

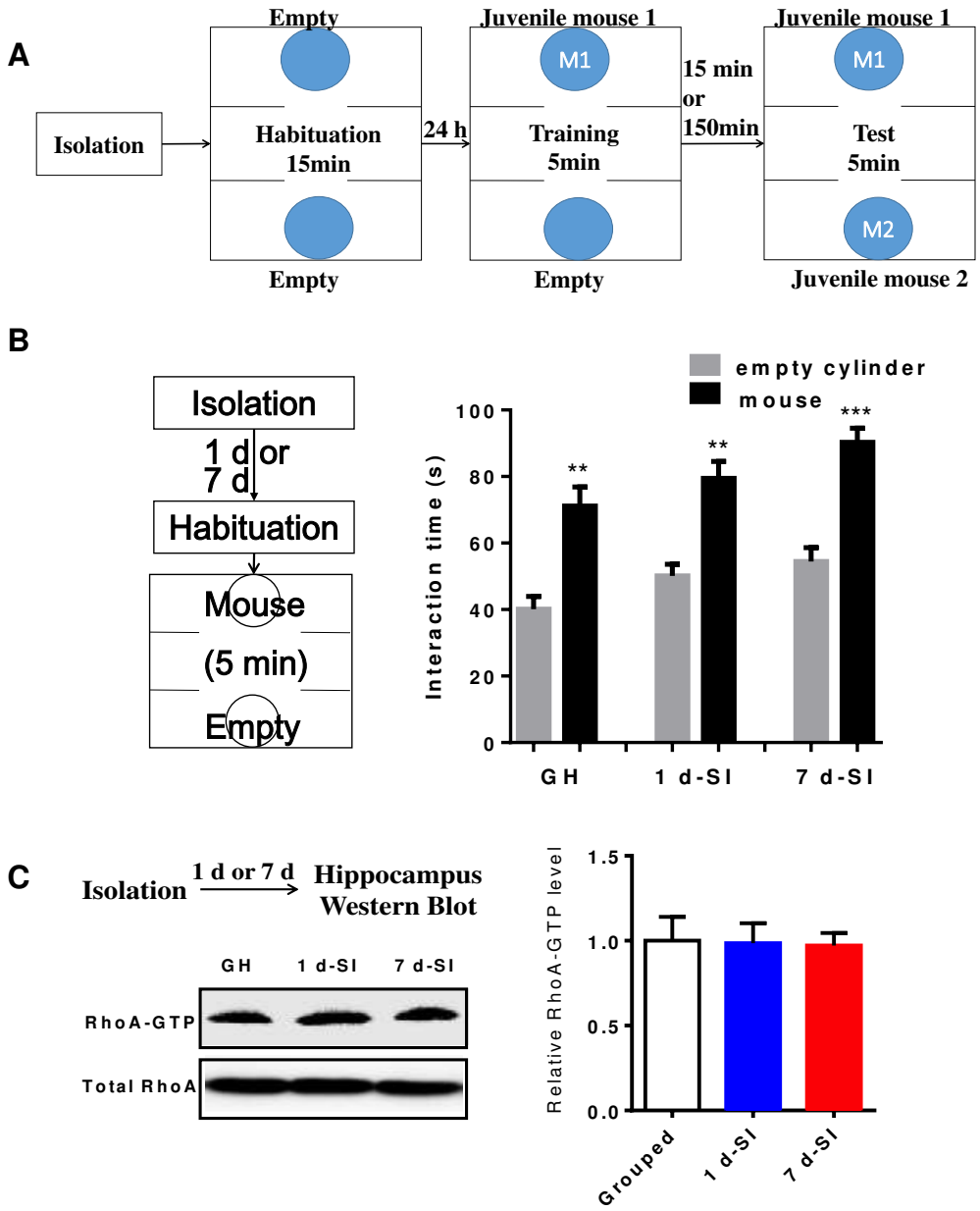


**Figure S1. Effects of social isolation on social exploring ability and RhoA activity.** Related to Figure 1. (A) Diagram of social recognition memory task. (B) Left, diagram of SRM task during training session. Right, similar to group-housed mice, the isolated mice tended to explore mice significantly and they have comparable interaction time among the groups (Paired t-test, **p < 0.01, ***p < 0.001, n=14-15). (C) Representative blots and grouped data of RhoA activity in isolated mice. RhoA activity was not affected in the hippocampus by both 1-d and 7-d social isolation. F (2, 15) = 0.09370, p = 0.9111, one-way ANOVA (n=6 per group).Group housing (GH), Social isolation (SI).Data are shown as the mean ±SEM.

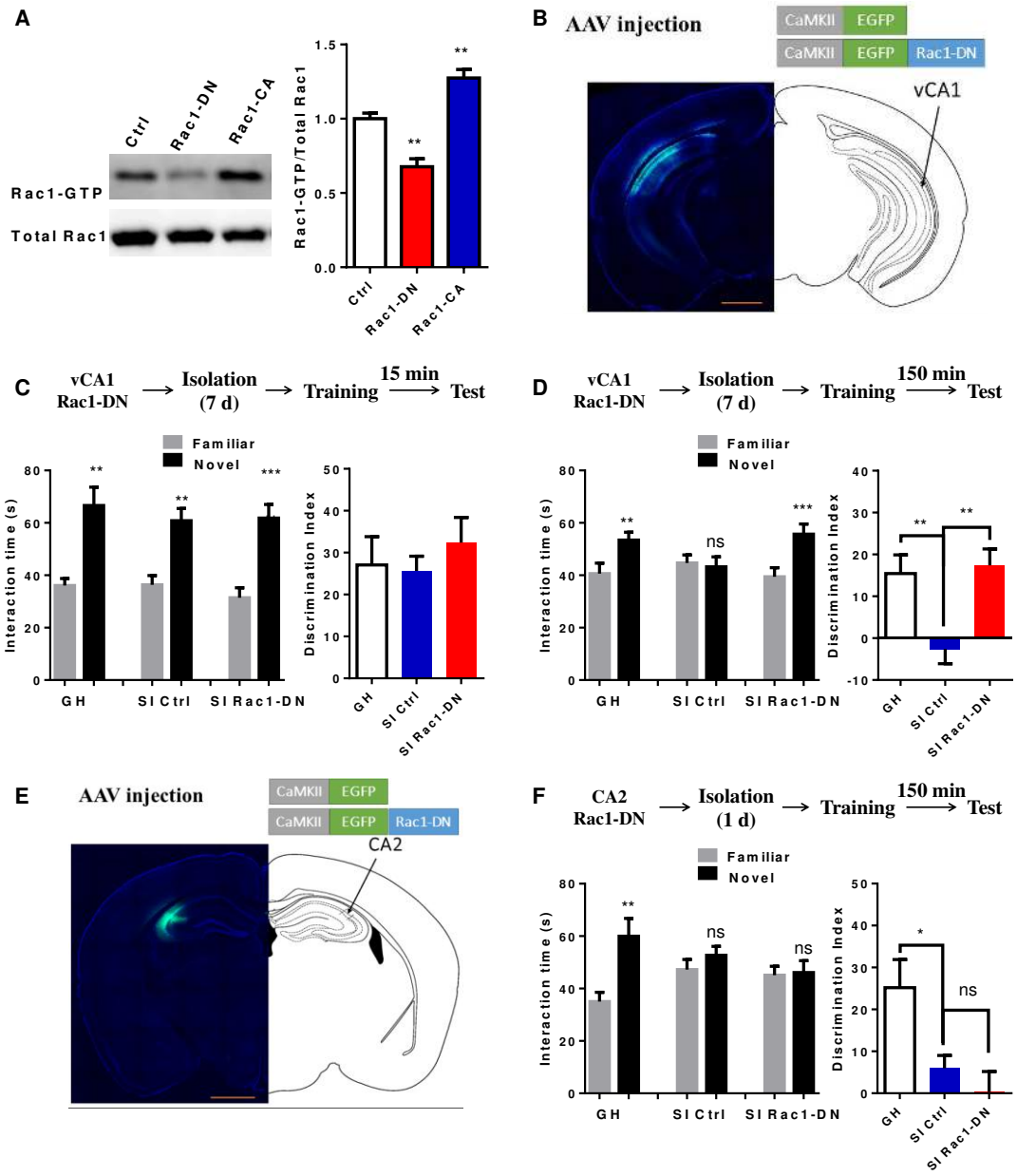


**Figure S2. Inhibition of Rac1 activity in ventral CA1 suppressed isolation induced forgetting of social memory.** Related to Figure 2. (A) Representative blots and group data of the AAV-Rac1 mutants in the hippocampus. Rac1 activity was decreased by injection with Rac1-DN and increased by Rac1-CA ($F(2, 9) = 35.91$, $P < 0.0001$, one-way ANOVA, n = 3–4). (B) Representative image of brain slices from mice injected with AAV-expressing EGFP in the ventral CA1. Scale bar, 1 mm. (C) Top, experimental procedure for social discrimination memory with AAV-Rac1-DN injection in the ventral CA1. Formation of social memory (15-min test) was not affected by AAV-Rac1-DN in the isolated mice ($F(2, 30) = 0.07409$, $p= 0.9288$, one-way ANOVA, n=11-12). (D) 150-min social memory was impaired by 7-d social isolation and was rescued by AAV-Rac1-DN injection in the isolated mice ($F(2, 32) = 7.309$, $p= 0.0024$, one-way ANOVA, n=11-12). (E) Representative image of brain slices from mice injected with AAV-expressing

EGFP in the CA2. Scale bar, 1 mm (F) Experimental procedure for social discrimination memory with AAV-Rac1-DN injection in the CA2 and adjacent areas. Forgetting of social memory (150-min test) was not affected by AAV-Rac1-DN in the CA2 ($F(2, 32) = 6.500$, $p = 0.0043$, one-way ANOVA, n=11-12). Group housing (GH), Social isolation (SI). Data are shown as the interaction time and the discrimination index (Paired t-tests for the interaction time data, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, n=11-12).
All the above data are shown as the mean ±SEM.

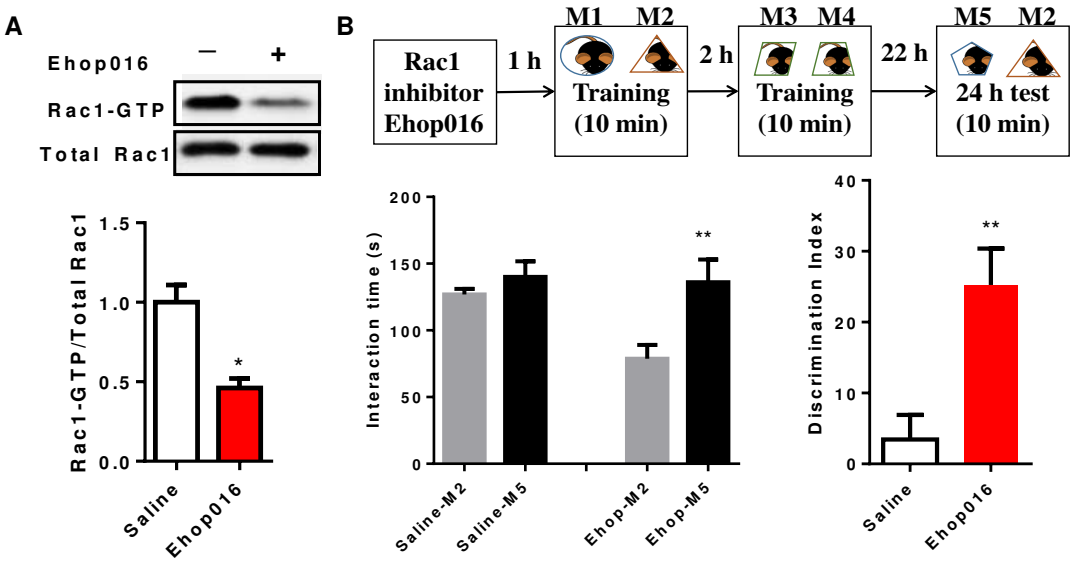


**Figure S3. Inhibition of Rac1 activity by Ehop016 suppressed interference-induced forgetting of social memory.** Related to Figure 2. (A) The mice were I.P. injected with 20 mg/kg Ehop016 and the hippocampus were isolated 24 hr later for western blotting; Rac1 activity was significantly reduced by Ehop016 (unpaired t-test, $t_{(4)}=4.310$, $p= 0.0125$, n=3). (B) Top, retroactive interference (RI) paradigm in social recognition memory. A second 10-min exposure to two mice was introduced as RI 2 hr after the first training. Bottom, forgetting of the original memory was induced by 2-hr RI in saline injection mice but not the Rac inhibitor (Ehop016) injected mice. Data are shown as the interaction time (paired t-tests, $**p < 0.01$, n=9-11) and the discrimination index (unpaired t-test, $t(18)=3.621$, $**p = 0.0020$, n=9-11). Mean ±SEM.