ber. Overall WBC counts, lymphocytes, and monocytes remained significantly associated with mating partner number (n = 8 contrasts, P = 0.01 to 0.04), whereas both neutrophils and body mass increased with transitions to greater terrestrial substrate use (n = 5 contrasts, t = 2.87, P = 0.02 and t = 2.81, P = 0.02, respectively). However, multivariate analysis of these variables using the CRUNCH algorithm (24) also revealed no significant results (n = 23 contrasts, P = 0.09 to 0.11). Finally, we tested whether the allometric relationship with neutrophils reflects an underlying life history correlate. In particular, a stronger immune system might be required in species with a longer life span. However, longevity did not account for neutrophil counts when holding body mass constant in multiple regression (b = 0.23, F(1,38) = 1.82, P = 0.19). No significant results were found in allometric analysis of other WBC types, although a negative slope for lymphocytes approached significance (b = -0.11, F(1,38) = 3.66, P = 0.06).

19. In overall, male WBC counts were highly associated with female counts in contrasts analysis (b = 0.64, F(1,38) = 24.07, P < 0.0001), as were particular WBC types (P < 0.05 in all tests). This makes sense in the case of a sexually transmitted disease: when one sex experiences increased risk, then the other sex should experience a corresponding increase (32). Male overall WBC (b = 3.14, P = 0.007), neutrophils (b = 3.15, P = 0.007), and lymphocytes (b = 1.87, P = 0.05) increased significantly over evolutionary transitions in female promiscuity (monocytes, t = 1.61, P = 0.07). Patterns of male promiscuity are difficult to analyze because there is less detailed information on male partner number, and, within species, greater variance in male mating success (i.e., sexual selection) may weaken patterns across species. Thus, analyses of discrete transitions to increased partner number in males produced significant results for neutrophils (n = 4 contrasts, t = 2.56, P = 0.04) when using the same set of species, as in analyses of females. But other analyses were not significant, including those using a wider range of species to give more contrasts. In multivariate contrasts analysis, however, residual testes mass accounted for variation in male WBC in excess of that explained by female WBC counts (b = 0.008, F(1,20) = 4.86, P = 0.04). Although other explanations are possible, this relation is consistent with male mating promiscuity affecting basal WBC counts.

20. First, we compared human standard reference WBC counts (33) to values for nonhuman primates among the discrete mating categories. For overall WBC, neutrophils, and lymphocytes, ranges for humans closely matched equivalent ranges in monogamous species, whereas human monocyte values best matched ranges for the intermediate category of “1 or mates” (see Fig. 1 caption). Second, we used the midpoint of human reference values in a hierarchical cluster analysis of the apes. We found that humans align most closely with the gorilla (Gorilla gorilla), a polygynous species with low sperm competition (17), and secondarily with a monogamous gibbon (Hylobates lar).
38. We thank S. Altizer, P. Bennett, C. Carbone, K. Jones, S. Peak, C. van Schaik, and K. Winkler for comments and discussion. C. Williams for calling our attention to the International Species Information System, and M. Carosi and M. Gerald for data on Cebus apella.
region within 3 to 5 days (Fig. 1, C and D). This duration may indicate the possible turnover rate for NMDA receptor complex in the brain. NR1-GFP started to reappear within 5 days after the withdrawal of doxy from the drinking water.

We also measured the CA1 field excitatory postsynaptic potentials (EPSPs) in hippocampal slices prepared from mice treated with doxy or vehicle (30) and observed no obvious change in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor–mediated synaptc transmission between control littersmates and iCA1-KO mice (either doxy-treated or vehicle-treated, n = 4 mice each group). Moreover, we found that NMDA receptor–mediated EPSPs were indistinguishable between those of controls or vehicle-treated iCA1-KO mice, suggesting the functional rescue of NMDA receptor activity in CA1. However, upon the treatment of doxy, CA1-NMDA currents in iCA1-KO mice were completely abolished in most slices (five out of seven slices, seven mice) (Fig. 1E, top panels), although in two slices we could still elicit NMDA currents at high intensities of stimulation. This indicates the presence of residual NMDA receptors at a few CA1 synapses despite the fact that we could no longer see NR1-GFP staining in the CA1 region.

To study LTP in the hippocampal slices, we applied two 1-s trains (100 Hz, test strength, spaced 10 s apart) in Schaffer-collateral CA1 pathway. LTP was readily produced in both vehicle-treated iCA1-KO and control littersmates (210.0 ± 19.2%, n = 5 control mice; 227.6 ± 16.3%, n = 5 iCA1-KO mice). However, no LTP was observed in doxy-treated iCA1-KO mice (n = 5; 105.1 ± 6.9%) (Fig. 1, E, bottom panels, and F), suggesting the loss of NMDA-dependent plasticity after doxy treatment.

To examine the acute effect of switching-off CA1 NMDA receptors on learning, we first used a hidden-platform water maze (31). We tested three groups of young adult animals: iCA1-KO mice treated with vehicle, iCA1-KO mice treated with doxy, and control mice treated with doxy (1 mg/ml in the drinking water, starting 5 days before the training). We found that doxy-treated iCA1-KO mice exhibited longer escape latency than doxy-treated control or vehicle-treated iCA1-KO mice [F(2, 38) = 17.88, P < 0.001] (Fig. 2A). The deficits in doxy-treated iCA1-KO mice were further confirmed in the transfer test (Fig. 2B), indicating the requirement of CA1-NMDA plasticity for spatial learning and memory.

We then used a fear-conditioning task (32) with the same doxy treatment paradigm (1 mg/ml, starting 5 days before the training began), and a retention test was carried out 24 hours after training (Fig. 2, C and D). In contextual conditioning, a hippocampal-dependent task (33, 34), vehicle-treated iCA1-KO mice exhibited similar freezing responses to those of control mice, indicating that the expression of NR1-GFP has also rescued the contextual memory deficits in CA1-KO mice. However, iCA1-KO mice treated with doxy showed significantly fewer freezing responses compared with those in either control or vehicle-treated iCA1-KO mice (Fig. 2C). To further assess the behavioral specificity in iCA1-KO mice, we conducted the hippocampal-independent, cued-fear conditioning. As expected from the CA1-specific genetic manipulation, we did not find any significant difference in freezing responses among these three groups (Fig. 2D). Our additional observations further suggested the behavioral specificity of iCA1-KO mice (35).

Memory formation consists of at least three major distinct stages: acquisition, consolidation and storage, and retrieval (3–6, 36, 37). To examine whether reactivation of CA1-NMDA receptors is required for long-term memory consolidation or retrieval, we first examined the consolidation of long-term spatial memory by using the hidden-platform water-maze task. We trained both control and vehicle-treated iCA1-KO mice with a seven-session training protocol (31). Both control and iCA1-KO mice exhibited similar learning curves (Fig. 3A). After the seven-session training, both groups of mice were treated with doxy for the first week (1 mg/ml, posttraining days 1 to 7) and then with water from the second week (days 8 to 14) (Fig. 3A). Retention tests were carried out on the 15th day after training, and transfer tests were performed 24 hours later. A significant difference in the latency between doxy-treated control
The hippocampus is critical for the consolidation of short-term memories into long-term spatial memory. Lesions of the hippocampus impair the expression of emotional memories, suggesting that the hippocampus is involved in the consolidation of memory. However, the exact role of hippocampal NMDA receptors in memory consolidation is not fully understood.

We hypothesized that hippocampal NMDA receptors are involved in the consolidation of emotional memory using a fear-conditioning paradigm. The CA1 NMDA receptor is a key player in the regulation of synaptic plasticity, which is essential for memory consolidation.

To test this hypothesis, we trained two groups of mice using a 7-day training protocol. One group was treated with doxy, a selective inhibitor of the NMDA receptor, while the other group received vehicle treatment. We found that doxy treatment impaired the mice’s ability to recall the context of the training, as evidenced by a significant reduction in freezing response in the retention test.

We then performed a transfer test at the end of the 7 days of training. The mice that received doxy treatment showed a decrease in freezing response when presented with the context of the training, indicating a failure to recall the memory. The control group showed a significant increase in freezing response, indicating robust recall of the memory.

These results suggest that the CA1 NMDA receptor is involved in the consolidation of emotional memory, and its role is critical for the formation of long-term contextual fear memory. We further found that treatment of doxy in the last 8 days (days 22 to 29) has no effects on either the contextual or cued retention tests (Fig. 4, C and D), suggesting that the CA1 NMDA receptors have a time-dependent role in memory consolidation but are not required for the retrieval of fear memory.

Because the CA1 NMDA receptor is not involved in basal synaptic transmission (11–13), the reactivation of the NMDA receptor in CA1 is likely to act as a triggering mechanism for modifying synaptic efficacy. Therefore, our above results indicate that memory consolidation may require multiple rounds of site-specific synaptic modifications, possibly to reinforce plastic changes initiated during learning, thereby making memory traces stronger and more stable. Recent studies report that the learning-induced correlation states among CA1 neurons are reactivation spontaneously in the postlearning period (37, 39, 40). Such a coactivation of these neurons might suggest the existence of the natural condition within the hippocampus by which the recurrent synaptic strengthening can occur during memory consolidation.

We hypothesize that such a synaptic reen...
try reinforcement (SRR) process can also be applied to explain how the hippocampus transfers newly created memories to the cortex for permanent storage. As the hippocampus undergoes reactivation during consolidation, it may also act as a coincidence regenerator for activating neurons in the cortical areas such as the association cortex. This would allow cortical neurons previously corresponding to the different sensory modules to be reactivated together, leading to the strengthening of the connections between them through SRR. Indeed, such a coordinated reactivation of hippocampal-cortical neurons after learning has been observed recently (41). Once these cortical connections are fully consolidated and stabilized, the hippocampus itself becomes dispensable for the retrieval of the “old memory” (3–6, 42). Therefore, we postulate that the hippocampus, by serving as a coincidence regenerator, may induce the reactivation of synaptic connection within the cortex during memory consolidation as the cellular means to convert short-term memories into long-term memories.

References and Notes
21. Web fig. 1 is available at www.sciencemag.org/feature/data/1054469.shl.
22. Our design for the generation of inducible CA1 NR1 knockout mice was composed of three steps [see Web fig. 1 (21)]. First, we generated tTA transgenic lines in which the tTA transgene was driven by the β-actin promoter. The translation of tTA was prevented by an inserted translational “stop” sequence that is flanked by loxP sites (27). Second, we generated tetO-NR1 transgenic mouse lines in which the NR1-GFP transgene was under the control of the tetO minimal promoter. Third, we crossed each line of tTA and tetO-NR1 transgenic lines with the CA1-KO mice, thus switching off the NR1 expression and returning the mice to the NR1 knockout state in the CA1 region. Furthermore, removal of tetracycline from the drinking water restored NR1 expression in the CA1 region. To detect NR1 expression fusion transgene that retains both normal NMDA channel properties and GFP activity (28). Thus, the patterns and kinetics of NR1 transgene expression in the transgenic mice can be assessed through the detection of GFP.
23. The transgenic founder mice were produced by pronuclear injection of the linearized DNA into B6/Cbf1 inbred zygotes and then intercrossed with B6/Cbf1 for various analyses. The tetracycline-controlled transactivator (tTA) construct was generated with the Eco RI–Bam HI I fragment from pUD15151 and the fragment after removing LacZ from pcat2X-YStopX-LacZ plasmid. The tTA target construct containing NR1-GFP was obtained by cloning a 4-kilobase pair promoterless fragment downstream from the tTA responsive promoter (tetO) at the Xba I site of the pUHD101 vector. The NR1-GFP chimeric protein coding cDNA in pCND3 vector was generously provided by J. Marshall (28).
24. We produced a total of seven independent tTA transgenic mouse lines and five NR1-GFP
transgenic lines. After crossing each tTA line with an individual tetO-NR1/GFP line in a systematic and combinatorial manner [as illustrated in Web fig. 1 (21)], we obtained double-transgenic mice. These double-transgenic mice were then mated with our previously engineered CA1-KO mice that contained a heterozygous Cre transgene and the homozygous floxed NR1 genes (14, 16). After three generations of carefully planned crossings and subsequent histological analysis of each respective inducible knockout line, we obtained an iCA1-KO line (carrying tTA/++, NR1-GFP/++, Cre/++, INR1/INR1) with the expected expression pattern and properties. Littermates carrying INR1/INR1, tTA/+, NR1-GFP/+ were used as controls. For suppression of transgene expression, doxycycline (1 mg/ml) was added to the drinking water with 5% sucrose to mask the bitter taste.

29. Animals were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Coronal brain sections were successively incubated in solutions of (i) rabbit antibody to GFP (1/2500, Clontech, and Chemicon) overnight, (ii) biotinylated goat antibody to rabbit (1/2000, Jackson ImmunoRe.
30. The recording procedures are the same as previously described (18), with the exception for the doxy-treatment schedule. Briefly, transverse slices of the hippocampus from CA1-KO and control mice (3 to 4 months old) that were treated with doxy (1 mg/ml) or vehicle were rapidly prepared and maintained in an interface chamber. The slices prepared from doxy-treated mice were exposed to doxy (1 ng/ml) in the perfusate. A bipolar tungsten stimulating electrode was placed in the stratum radiatum in the CA1 region, and extracellular field potentials were recorded with a glass microelec-
31. All the behavioral data presented in this report were collected in adult animals and collected at the ages of 7.5 to 14.5 weeks old in blind fashion. Analysis of variance (ANOVA) and Student’s t test were used to determine genotype effects on the behavioral responses. The experimental protocol for the water maze test was the same as described previously (18). The training procedures consisted of a total of seven sessions in 7 days; each session contained four trials per day.
32. The experimental protocol for fear conditioning was the same as that described previously (16, 18). The unconditional stimulus was a single foot shock 0.8 mA for 2 s, paired with an 86-8B sound at 2800 Hz in the shock chamber (context).
35. We excluded the possibility that doxy has any side effect on these learning behaviors by measuring long-term treatment of doxy (1 mg/ml). After 1-month treatment, no obvious side effect on contextual or cued freezing responses in wild-type mice was found. Furthermore, we excluded the possibility that any changes in nociceptive responses might contribute to the difference observed in freezing behaviors because the amount of current to elicit flinching/running, jumping, and vocalizing was indistinguishable among all groups.
42. We thank J. Marshall and T. Hughes for generously providing the NR1-GFP fusion plasmid; J. Hopfield, S. Wang, G. Wittenberg, and O. Downs for stimulating discussion; and M. Zhuo for valuable technical assistance and advice on hippocampal slice physiology. This research was supported by funding from NIMH (J.Z.T.) and J.Z.T. is a Beckman, Burroughs Welcome Fund, and W. M. Keck Foun-
43. R. M. Schwartz and T. Hughes for generously providing the NR1-GFP fusion plasmid; J. Hopfield, S. Wang, G. Wittenberg, and O. Downs for stimulating discussion; and M. Zhuo for valuable technical assistance and advice on hippocampal slice physiology. This research was supported by funding from NIMH (J.Z.T.) and J.Z.T. is a Beckman, Burroughs Welcome Fund, and W. M. Keck Foundation Young Investigator.

Fig. 4. Memory consolidation and retrieval in the fear-conditioning task. (A) Impaired long-term contextual fear memory in iCA1-KO mice if the CA1-NMDA receptor was suppressed during early consolidation. Regular water was adminis-
terated to both control (n = 13) and iCA1-KO (n = 11) mice before training. Immediately after training, both groups received doxy treatment that continued for 2 weeks. On day 15, the doxy was stopped and switched to water for another 2 weeks. A retention test was carried out on day 29. A significant difference was observed between control and doxy-treated iCA1-KO mice (**P < 0.01, Student’s t test). (B) Normal cued conditioning in the same group of mice as described in (A). No significant difference was found in the long-term cued-fear memory between these two groups. (C) Retrieval and the late-stage consolidation of contextual fear memory are independent of CA1 NMDA receptor. Both control (n = 12) and iCA1-KO (n = 13) mice received regular water before training and after training until posttraining day 21; both groups were then treated with doxy for 8 days. A retention test was carried out on day 29. No significant difference was found in either immediately freezing (imm freezing) or contextual conditioning. (D) Normal cued freezing response in the same group of mice as described in (C). No significant difference was found in cued-fear memory between these two groups.