Hippocampal Neurogenesis Regulates Forgetting During Adulthood and Infancy

Katherine G. Akers,[1]* Alonso Martinez-Canabal,[1,2]* Leonardo Restivo,[1]* Adelaide P. Yiu,[1] Antonietta De Cristofaro,[1] Hwa-Lin (Liz) Hsiang,[1,2] Anne L. Wheeler,[1,2] Axel Guskjolen,[1,3] Yosuke Niibori,[1] Hirotaka Shoji,[4] Koji Ohira,[4] Blake A. Richards,[1] Tsuyoshi Miyakawa,[4] Sheena A. Josselyn,[1,2,3,5]† Paul W. Frankland[1,2,3,5]†

Throughout life, new neurons are continuously added to the dentate gyrus. As this continuous addition remodels hippocampal circuits, computational models predict that neurogenesis leads to degradation or forgetting of established memories. Consistent with this, increasing neurogenesis after the formation of a memory was sufficient to induce forgetting in adult mice. By contrast, during infancy, when hippocampal neurogenesis levels are high and freshly generated memories tend to be rapidly forgotten (infantile amnesia), decreasing neurogenesis after memory formation mitigated forgetting. In precocial species, including guinea pigs and degus, most granule cells are generated prenatally. Consistent with reduced levels of postnatal hippocampal neurogenesis, infant guinea pigs and degus did not exhibit forgetting. However, increasing neurogenesis after memory formation induced infantile amnesia in these species.

In both artificial systems and brain networks there is a trade-off between plasticity—the ability to incorporate new information—and stability—ensuring that the process of incorporating new information does not degrade information already stored in that network (1). In the hippocampus, new neurons continue to be generated in the subgranular zone of the dentate gyrus (DG) beyond development and into adulthood (2, 3). These new neurons synaptically integrate into hippocampal circuits (4–9) and provide potential substrates for new learning. Promoting the production of new neurons in adult mice facilitates the formation of new hippocampal memories (10, 11). However, the continuous integration of new neurons may affect memories already stored in these circuits (12). As new neurons integrate into the hippocampus, they compete with existing cells for inputs and outputs, establishing new synaptic connections that may coexist with, or even replace, older synaptic connections (5, 6, 13). As such remodeling necessarily alters the configuration of DG-CA3 circuits and likely rescales synaptic weights of preexisting connections, computational models predict that high levels of hippocampal neurogenesis will lead to forgetting of information already stored in those circuits (14–16).

Although hippocampal neurogenesis persists throughout life, rates decline dramatically with age (17, 18). Therefore, this predicted remodeling-induced forgetting should be most pronounced during infancy, when hippocampal neurogenesis is high. Consistent with this, infantile forgetting [or infantile amnesia (19)] is observed across a wide range of species (20), including humans (21). Neurobiological accounts of infantile amnesia previously emphasized that continued brain maturation might interfere with consolidation and/or storage of infant memories, rendering them inaccessible at later time points (22). Here, we test whether postnatal hippocampal neurogenesis, in particular, modulates ontogenetic changes in memory persistence.

Inverse Relationship Between Levels of Postnatal Neurogenesis and Memory Persistence

We first characterized levels of hippocampal neurogenesis in infant and adult mice using a retrovirus expressing green fluorescent protein (GFP) to label neural progenitors and their progeny (11) and immunohistochemistry. Four weeks after retroviral microinjections in infant (postnatal day 17; P17) and adult (P60) mice, we observed a pronounced age-dependent reduction in neurogenesis (i.e., reduction in the number of GFP$^+$ DG granule cells and their terminal processes in the CA3 region) (Fig. 1, A and C). From infancy to adulthood, there was also a decrease in the number of proliferating (Ki67$^+$) cells (Fig. 1D and fig. S1) and immature (doublecortin$^+$ [DCX$^+$]) neurons (Fig. 1, B and E). Postnatally generated DG granule cells project a mossy fiber that reaches the CA3 region after ~2 weeks, contacting 11 to 15 pyramidal cells (5, 7, 23). We observed a reduction in the number of immature (DCX$^+$) large mossy fiber terminals (LMTs) in

the CA3 region of adult mice (Fig. 1, B and F), consistent with a predicted reduction in synaptic rearrangements in the CA3 with age (fig. S2).

Next, we evaluated how age-dependent changes in neurogenesis influence the ability to form enduring hippocampus-dependent memories using the contextual fear conditioning paradigm (24). Infant and adult mice were placed in a novel context and presented with a series of foot shocks. One to 28 days later, separate groups of mice were returned to the context, and memory retention was evaluated by measuring freezing behavior (Fig. 1G). Mice trained as adults froze robustly regardless of retention delay. By contrast, mice trained as infants only exhibited high levels of freezing when tested 1 day after training. If infant mice were tested at longer retention delays, less freezing was observed (Fig. 1H) (25). Infant mice trained similarly but not shocked showed little freezing when tested 1 day after training (fig. S3), indicating that the freezing observed in shocked infant mice reflects conditioned fear. There were no age-related differences in reaction to the foot shock (Fig. 1I), indicating that forgetting in infant mice was not due to diminished nociception. This pattern of accelerated forgetting in infant mice was replicated using an incidental context learning paradigm (fig. S4).

Increasing Neurogenesis Promotes Forgetting in Adult Mice

To determine if levels of neurogenesis and memory persistence are causally related, we tested whether increasing hippocampal neurogenesis after learning promotes forgetting in adult mice (Fig. 1J) (26). We initially used voluntary running, a naturalistic intervention that robustly increases neurogenesis (27) (Fig. 2A). Relative to sedentary controls, running increased the number of proliferating (Ki67$^+$) cells (Fig. 2B) and immature (DCX$^+$) neurons in the DG (Fig. 2C), as well as DCX$^+$ LMTs in the CA3 of adult mice (Fig. 2D). These increases were evident within 7 days of commencing running, sustained for the duration of exercise, and not associated with increased death of developmentally generated granule cells (fig. S5). We next injected retrovirus expressing GFP into the DG of adult mice. Four weeks later, we observed many GFP$^+$ neurons in the innermost layers of the DG and GFP$^+$ LMTs in the stratum lucidum of the CA3. Counterstaining with a marker of mature presynaptic terminals, zinc transporter-3 (ZnT3) (28), revealed many new (GFP$^+$/ZnT3$^+$) LMTs in close apposition to preexisting (GFP$^-$/ZnT3$^+$) LMTs (Fig. 2E), suggesting that new contacts coexist with established connections (5). Running increased the ratio of new-to-established LMTs, indicating that running increases remodeling of DG-CA3 circuits (fig. S6).

To determine whether running-induced increases in hippocampal remodeling induce forgetting of an established hippocampus-dependent memory, we trained adult mice in contextual fear conditioning. After training, mice were given

[1]Program in Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, M5G 1X8, Canada. [2]Institute of Medical Science, University of Toronto, Toronto, M5S 1A8, Canada. [3]Department of Physiology, University of Toronto, Toronto, M5S 1A8, Canada. [4]Division of Systems Medical Science, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi, 470-1192, Japan. [5]Department of Psychology, University of Toronto, Toronto, M5S 3GM, Canada.

*These authors contributed equally to this work.
†Corresponding author. E-mail: paul.frankland@sickkids.ca (P.W.F.), sheena.josselyn@sickkids.ca (S.A.J.)

Fig. 1. Age-dependent levels of hippocampal neurogenesis and memory stability are inversely related in mice. (**A**) GFP labeling of granule cells in the DG and CA3 1 month after retroviral infection shows that infant (P17) mice had higher levels of neurogenesis than adult (P60) mice. (Left) Low magnification of DG and CA3 regions. Scale bar, 200 μm. (Right) High magnification of CA3 region showing GFP$^+$ mossy fibers and LMTs. Scale bar, 50 μm. (**B**) Top row: Infants had more DCX$^+$ cells in the DG and CA3 than adults. Scale bar, 100 μm. Bottom row: High magnification of DG and CA3. Scale bar, 50 μm. (**C** to **F**) Infants showed more (C) GFP$^+$ neurons ($t_6 = 8.57$, $P < 0.001$), (D) proliferating (Ki67$^+$) cells ($t_7 = 4.53$, $P < 0.01$), and (E) immature (DCX$^+$) neurons in the DG ($t_6 = 18.65$, $P < 0.001$), as well as more (F) DCX$^+$ LMTs in the CA3 ($t_6 = 15.03$, $P < 0.001$) than adults ($n = 4$ to 5 per group). (**G**) Separate groups of infant and adult mice were trained in contextual fear conditioning and tested 1, 7, 14, or 28 days later ($n = 8$ to 9 per group). (**H**) Context fear memory persisted for at least 28 days in adult mice, whereas infants showed intact memory shortly after training but forgetting after longer delays (age × day, $F_{3,62} = 13.07$, $P < 0.001$; post-hoc t tests, 1 day, $P < 0.05$; 7 days, $P < 0.001$; 14 days, $P < 0.001$; 28 days, $P < 0.001$). (**I**) Infants and adults showed equivalent shock reactivity during training (P17 $n = 9$, P60 $n = 8$; arbitrary units, $P > 0.05$). (**J**) Inverse relationship between neurogenesis and memory persistence predicts that increasing neurogenesis in adults will induce forgetting, whereas decreasing neurogenesis in infants will mitigate forgetting. In all panels, cells/LMTs are expressed as number of cells/LMTs per 1000 μm^2. *$P < 0.05$. For all figures, error bars represent standard error of the mean (SEM). DAPI, 4′,6-diamidino-2-phenylindole.
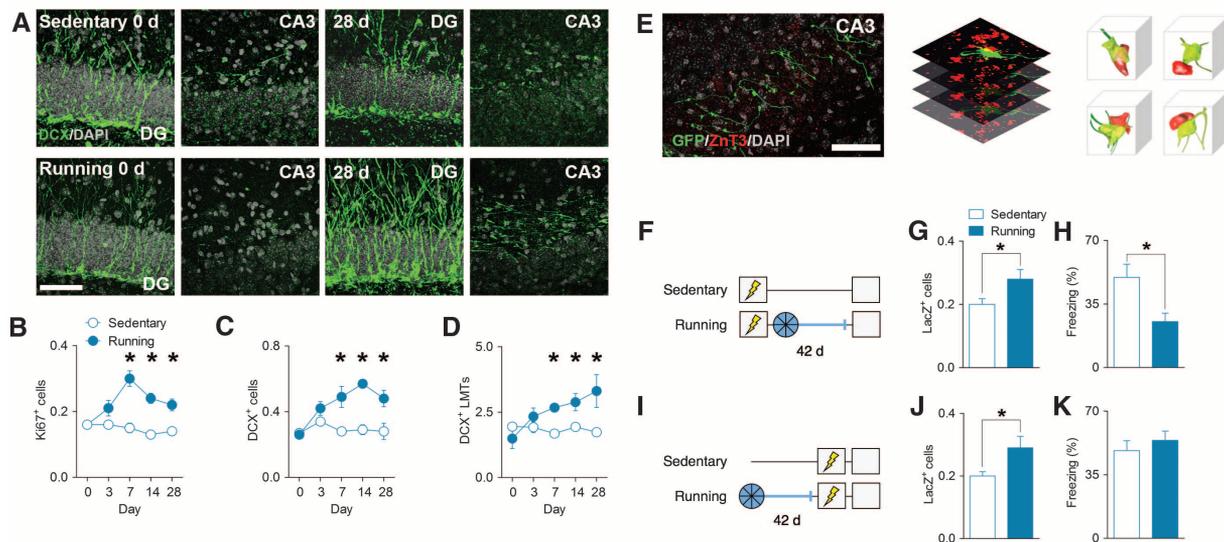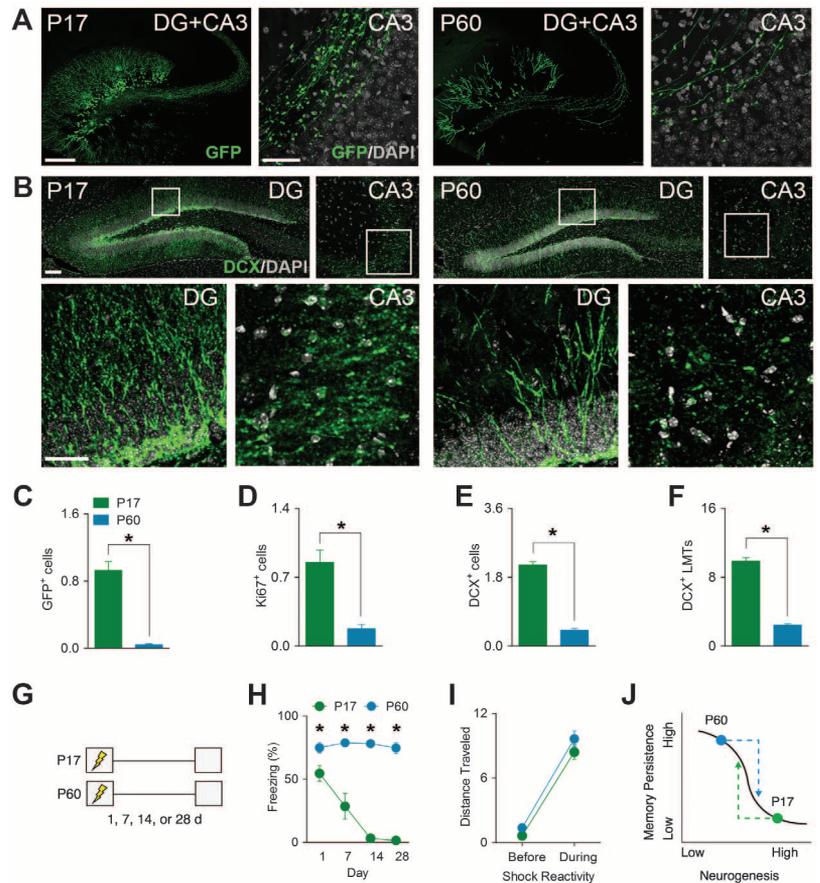

Fig. 2. Voluntary running increases neurogenesis and promotes forgetting in adult mice. (**A**) Adult (P60) mice given access to a running wheel for 28 days showed more immature (DCX$^+$) neurons in the DG and LMTs in the CA3 than sedentary mice. Scale bar, 50 μm. (**B** to **D**) Adult mice were given access to a running wheel for 0, 3, 7, 14, or 28 days. Running increased the number of (B) proliferating (Ki67$^+$) cells ($n = 8$ per group; running × day, $F_{4,70} = 7.71$, $P < 0.001$; post hoc t tests, 7 days, $P < 0.001$; 14 days, $P < 0.001$; 28 days, $P = 0.001$), and (C) immature (DCX$^+$) neurons in the DG ($n = 4$ to 8 per group; running × day, $F_{4,34} = 3.17$, $P < 0.05$; 7 days, $P < 0.05$; 14 days, $P < 0.001$; 28 days, $P < 0.05$), as well as more (D) DCX$^+$ LMTs in the CA3 ($n = 4$ per group; running × day, $F_{4,30} = 3.45$, $P < 0.05$; 7 days, $P = 0.001$; 14 days, $P < 0.05$; 28 days, $P < 0.05$) compared with sedentary controls. (**E**) Neurogenesis remodels DG-CA3 circuits.

Retrovirus-labeled presynaptic LMTs from new neurons (GFP$^+$) in close apposition to existing presynaptic LMTs from mature neurons (ZnT3$^+$). Scale bar, 50 μm. Confocal stacks used to reconstruct representative three-dimensional images of new LMTs (green) in close contact with existing LMTs (red) in the CA3. (**F**) Adult mice ran or remained sedentary after contextual fear conditioning. (**G** and **H**) Running increased neurogenesis (sedentary $n = 8$, running $n = 8$; LacZ$^+$ cells; $t_{14} = 2.81$, $P < 0.05$) and induced forgetting (sedentary $n = 9$, running $n = 8$; $t_{15} = 2.75$, $P < 0.05$). (**I**) Adult mice ran or remained sedentary before contextual fear conditioning. (**J** and **K**) Running increased neurogenesis (sedentary $n = 7$, running $n = 8$; $t_{13} = 2.96$, $P < 0.05$) but did not affect formation of a context fear memory (sedentary $n = 10$, running $n = 9$; $P > 0.05$). In all panels, cells/LMTs are expressed as number of cells/LMTs per 1000 μm^2. *$P < 0.05$.
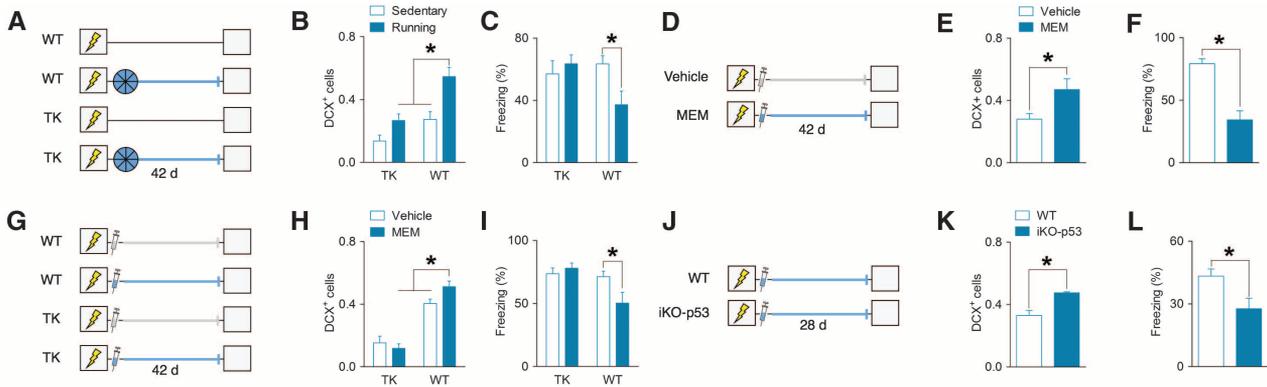
Fig. 3. Forgetting depends on increased neurogenesis. (A) Adult GAN-treated WT and TK$^+$ mice remained sedentary or ran after contextual fear conditioning. Running **(B)** increased neurogenesis (sedentary $n = 6$, running $n = 14$; $t_{18} = 2.81$, $P < 0.05$) and **(C)** induced forgetting of a context fear memory in WT mice (sedentary $n = 16$, running $n = 14$; $t_{28} = 2.63$, $P < 0.05$). By contrast, in TK$^+$ mice, both **(B)** the running-induced increase in neurogenesis (sedentary $n = 8$, running $n = 9$; TK$^+$ running versus WT sedentary, $P > 0.05$; WT running versus TK$^+$ running, $t_{21} = 3.43$, $P < 0.01$) and **(C)** forgetting (TK$^+$ sedentary $n = 12$, TK$^+$ running $n = 10$; $P > 0.05$; genotype × running interaction, $F_{1,48} = 4.88$, $P < 0.05$) were blunted. **(D)** Adult WT mice treated with MEM after contextual fear conditioning showed **(E)** increased neurogenesis (vehicle $n = 7$, MEM $n = 6$; $t_{11} = 2.55$, $P < 0.05$) and **(F)** forgetting of a context fear memory (vehicle $n = 8$, MEM $n = 8$; $t_{14} = 5.47$, $P < 0.001$) relative to vehicle-treated controls. **(G)** Adult GAN-treated

WT and TK$^+$ mice were treated with vehicle or MEM after contextual fear conditioning. MEM **(H)** increased neurogenesis (vehicle $n = 8$, MEM $n = 8$; $t_{14} = 2.25$, $P < 0.05$) and **(I)** induced forgetting of a context fear memory in WT mice (vehicle $n = 11$, MEM $n = 11$; $t_{20} = 2.23$, $P < 0.05$). By contrast, in TK$^+$ mice, both **(H)** the MEM-induced increase in neurogenesis (vehicle $n = 8$, MEM $n = 8$; TK$^+$ vehicle versus TK$^+$ MEM, $P > 0.05$; WT MEM versus TK$^+$ MEM, $t_{14} = 8.53$, $P < 0.001$; genotype × drug interaction, $F_{1,28} = 4.69$, $P < 0.05$) and **(I)** forgetting (vehicle $n = 13$, MEM $n = 14$; TK$^+$ vehicle versus TK$^+$ MEM, $P > 0.05$; genotype × drug interaction, $F_{1,45} = 5.41$, $P < 0.05$) were attenuated. **(J)** Post-training tamoxifen treatment **(K)** increased neurogenesis (WT $n = 4$, iKO-p53 $n = 4$; $t_6 = 4.39$, $P < 0.01$) and **(L)** induced forgetting of a context fear memory (WT $n = 14$, iKO-p53 $n = 9$; $t_{21} = 2.68$, $P < 0.05$) in iKO-p53 mice relative to WT controls. In all panels, cell counts are expressed as number of cells per 1000 μm^2. *$P < 0.05$.
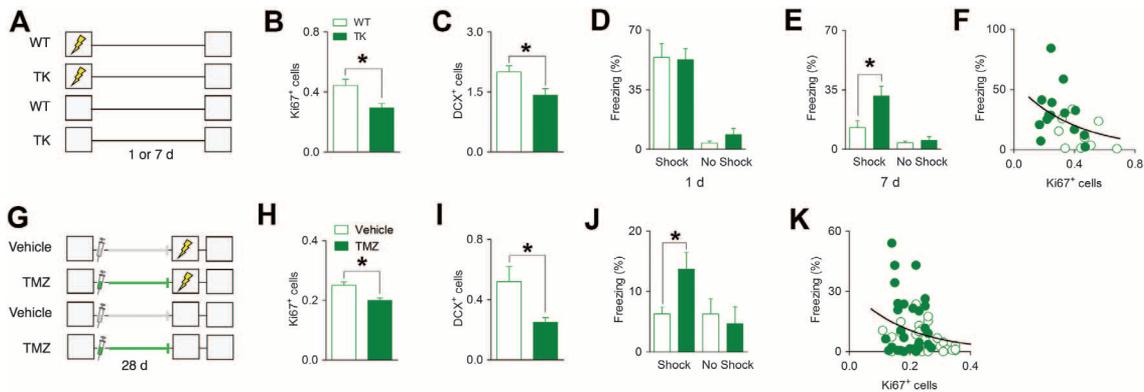

Fig. 4. Genetic and pharmacological decreases in neurogenesis mitigate forgetting in infant mice. (A) Infant GAN-treated WT or TK$^+$ mice were trained in contextual fear conditioning or placed in conditioning chambers without foot shocks. TK$^+$ mice had fewer **(B)** proliferating (Ki67$^+$) cells (WT $n = 9$, TK$^+$ $n = 14$; $t_{21} = 3.08$, $P < 0.01$) and **(C)** immature (DCX$^+$) neurons (WT $n = 6$, TK$^+$ $n = 6$; $t_{10} = 2.57$, $P < 0.05$) in the DG than WT mice. **(D)** Shocked WT and TK$^+$ mice showed equivalent context fear memory 1 day after training (WT $n = 9$, TK $n = 13$; $P > 0.05$), and nonshocked mice showed low freezing (WT $n = 7$, TK $n = 9$). **(E)** A separate group of shocked WT mice showed forgetting of a context fear memory 7 days after training, whereas this forgetting was reduced in a separate group of TK$^+$ mice (WT $n = 9$, TK$^+$ $n = 14$; $t_{21} = 2.26$, $P < 0.05$). Separate groups of nonshocked mice again showed

low freezing (WT $n = 6$, TK $n = 7$). **(F)** Inverse relationship between proliferation and memory persistence among shocked mice 7 days after training ($r = -0.46$, $P < 0.05$). **(G)** Infant mice were treated with vehicle or TMZ after training in the incidental context learning paradigm (or were similarly treated without foot shocks). TMZ-treated mice had fewer proliferating (Ki67$^+$) **(H)** cells (vehicle $n = 30$, TMZ $n = 29$; $t_{57} = 3.57$, $P = 0.001$) and **(I)** immature (DCX$^+$) neurons (vehicle $n = 5$, TMZ $n = 6$; $t_9 = 2.29$, $P < 0.05$) in the DG. **(J)** Shocked TMZ-treated mice showed less forgetting of a context-only memory (vehicle $n = 30$, TMZ $n = 29$; $t_{57} = 2.47$, $P < 0.05$). Nonshocked mice showed low freezing (vehicle $n = 9$, TMZ $n = 9$). **(K)** Inverse relationship between proliferation and memory persistence among shocked mice ($r = -0.31$, $P < 0.05$). In all panels, cells are expressed as number of cells per 1000 μm^2. *$P < 0.05$.

continuous access to a running wheel in their home cage or housed conventionally (i.e., sedentary; Fig. 2F). Running increased neurogenesis in the DG (Fig. 2G) and reduced context fear memory when mice were tested 6 weeks later (Fig. 2H and fig. S7). Access to a locked running wheel did not induce forgetting (fig. S8), indicating that forgetting was associated with running rather than exposure to a novel object in the home cage. Furthermore, running-induced forgetting of a con-

text fear memory was reduced after a stronger training protocol (i.e., additional foot shocks; fig. S9), suggesting that the degree of forgetting depends, in part, on initial memory strength.

As voluntary running induces several neural and physiological changes apart from increasing hippocampal neurogenesis (29), forgetting might alternatively be mediated by these non-neurogenic changes. Therefore, we first examined the impact of running before (rather than after) training

(Fig. 2I). Running before training similarly increased neurogenesis (Fig. 2J) but did not affect acquisition of a context fear memory (Fig. 2K), indicating that running does not nonspecifically alter freezing (e.g., by reducing anxiety).

Second, we tested whether preventing the running-induced increase in neurogenesis would prevent forgetting using transgenic mice in which a nestin promoter/enhancer drives expression of a modified herpes simplex virus (HSV) gene

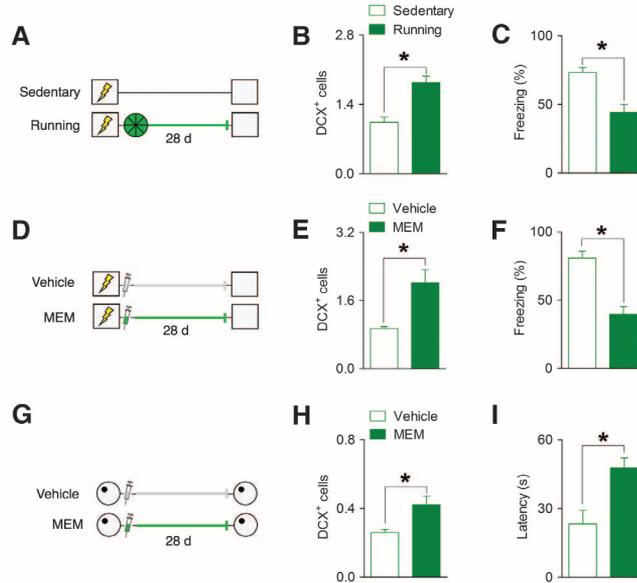**Fig. 5. Precocial degus and guinea pigs show low postnatal neurogenesis and persistent infantile memories.** (**A**) DCX$^+$ cells in the DG of infant and adult degus. (Left) Low magnification. Scale bar, 200 µm. (Right) High magnification. Scale bar, 25 µm. (**B**) DCX$^+$ cells in the DG of infant and adult guinea pigs. (Left) Low magnification. Scale bar, 200 µm. (Right) High magnification. Scale bar, 25 µm. Compared with mice (P17 n = 4 to 5, P60 n = 4), degus (P17 n = 3, P60 n = 4 to 6) and guinea pigs (GP; P17 n = 4,

P60 n = 4) showed reduced age-related declines in (**C**) proliferating (Ki67$^+$) cells and (**D**) immature (DCX$^+$) neurons in the DG. (**E**) Infant and adult degus were trained in contextual fear conditioning and tested 1 and 28 days later (P17 n = 7, P60 n = 10). (**F**) Both infant and adult degus showed persistent context fear memory (*age × delay* interaction and *age* main effect, Ps > 0.05).
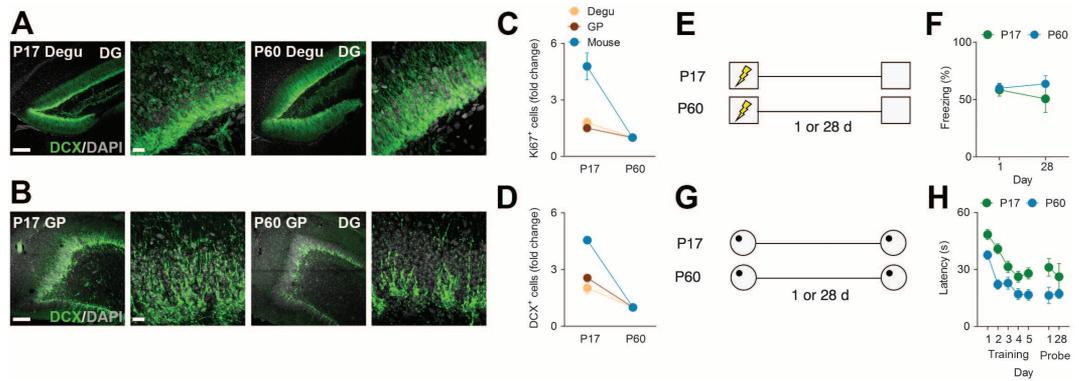
(**G**) Separate groups of infant and adult guinea pigs were trained in a water maze and tested 1 (P17 n = 8, P60 n = 8) or 28 days later (P17 n = 8, P60 n = 8). (**H**) Both infant and adult guinea pigs showed persistent spatial memory during probe tests (*age × delay* interaction, P > 0.05; *age* main effect, $F_{1,28}$ = 6.05, P < 0.05).

Fig. 6. Increasing neurogenesis promotes forgetting in infant degus and guinea pigs. (**A**) Infant degus were trained in contextual fear conditioning and then remained sedentary or given access to a running wheel for 28 days. Running (**B**) increased neurogenesis (sedentary n = 4, running n = 4; t_6 = 4.74, P < 0.01) and (**C**) induced forgetting of a context fear memory (sedentary n = 21, running n = 16; t_{35} = 4.59, P < 0.001). (**D**) Infant degus were trained in contextual fear conditioning and then treated with vehicle or MEM for 28 days. MEM treatment (**E**) increased neurogenesis (vehicle n = 8, MEM n = 5; t_{11} = 4.41, P = 0.001) and (**F**) induced forgetting of

a context fear memory (vehicle n = 12, MEM n = 11; t_{21} = 5.35, P < 0.001). (**G**) Infant guinea pigs were treated with vehicle or MEM after training in the water maze. MEM treatment (**H**) increased neurogenesis (vehicle n = 3, MEM n = 3; t_4 = 3.11, P < 0.05) and (**I**) induced forgetting of a spatial memory (vehicle n = 11, MEM n = 12; t_{21} = 3.32, P < 0.01). In all panels, cells are expressed as number of cells per 1000 µm^2. *P < 0.05.

Finally, running also induced forgetting of other hippocampus-dependent memories (incidental context learning, fig. S13; water maze, fig. S14). However, running did not induce forgetting of a conditioned taste aversion memory (fig. S15), which does not depend on the hippocampus (*35*).

Reducing Neurogenesis Increases Memory Persistence in Infant Mice

Using genetic and pharmacological strategies, we next examined whether decreasing neurogenesis mitigates forgetting normally observed in infant mice (Fig. 1J). To genetically suppress neurogenesis, we administered GAN to infant TK$^+$ and WT mice (Fig. 4A). GAN treatment reduced the number of proliferating (Ki67$^+$) cells and immature (DCX$^+$) neurons in the DG of TK$^+$ mice compared with WT littermates (Fig. 4, B and C). When tested 1 day after contextual fear conditioning, WT and TK$^+$ mice exhibited equivalent levels of freezing (Fig. 4D), indicating normal memory at this short retention delay. However, when separate groups of mice were tested 7 days after training, TK$^+$ mice froze more than WT mice (Fig. 4E), and freezing levels were inversely correlated with levels of neurogenesis (Fig. 4F). GAN-treated WT and TK$^+$ mice trained without foot shocks showed little freezing when tested 7 days after training (Fig. 4E). These low levels of freezing were similar to those observed in shocked WT mice, indicating substantial forgetting in WT but not TK$^+$ mice. Furthermore, the absence of freezing in nonshocked TK$^+$ mice suggests that suppressing neurogenesis (or GAN treatment) does not simply increase the propensity to freeze.

Using temozolomide (TMZ), a DNA alkylating agent (*36*), to pharmacologically reduce hippocampal neurogenesis in infant WT mice, we observed a similar attenuation of forgetting in an incidental context learning paradigm. Infant mice were preexposed to a context and then treated with TMZ or vehicle for 4 weeks (Fig. 4G). TMZ treatment suppressed neurogenesis (Fig. 4, H

encoding thymidine kinase (HSV-tk) (TK$^+$ mice) (*30*). In TK$^+$ mice, administration of ganciclovir (GAN) ablates only dividing cells expressing the tk transgene. TK$^+$ mice and their wild-type (WT) littermates were trained in contextual fear conditioning and then given access to a running wheel or housed conventionally for 6 weeks. During this period, mice were treated with GAN to suppress neurogenesis (Fig. 3A). GAN treatment in TK$^+$ mice limited the running-induced increase in neurogenesis to WT sedentary levels (Fig. 3B) and prevented forgetting (Fig. 3C).

Third, nonrunning interventions that increase neurogenesis might similarly induce forgetting of established memories. Administering the proneurogenic drugs memantine (MEM) (*31*) (Fig. 3,

D to F, and fig. S10) or fluoxetine (*32*) (fig. S11) after training induced forgetting [see also (*33*)]. Both the increase in neurogenesis and forgetting induced by MEM were blocked by activation of the tk transgene in TK$^+$ mice (Fig. 3, G to I). We also generated transgenic mice in which the tumor suppressor gene *p53* was inducibly deleted from neural progenitor cells and their progeny during adulthood (iKO-p53 mice) (Fig. 3J). Similar to previous studies examining global deletion of *p53* (*34*), conditional deletion of *p53* increased the number of proliferating (Ki67$^+$) cells (fig. S12) and immature (DCX$^+$) neurons (Fig. 3K) in the DG. Furthermore, post-training deletion of *p53* induced forgetting of context fear memory in adult mice (Fig. 3L).

and I) and improved retention of the context-only memory (Fig. 4J), and there was an inverse relationship between hippocampal neurogenesis and freezing (Fig. 4K). TMZ treatment did not decrease the threshold for freezing, however, as similar TMZ treatment before learning did not alter freezing levels (fig. S16). During infancy, high levels of proliferation are associated with high levels of apoptotic cell death in the subgranular zone (37). However, post-training pharmacological inhibition of cell death alone failed to attenuate forgetting (fig. S17).

Infantile Forgetting Is Absent in Precocial Guinea Pigs and Degus

As guinea pigs have a longer (~65 days) gestation than mice (~21 days), they are more neurologically mature at birth and have reduced postnatal hippocampal neurogenesis (38). This led us to predict that guinea pigs (and possibly other rodents with similarly extended gestation) will not exhibit infantile forgetting. To test this, we examined hippocampal neurogenesis and memory retention in guinea pigs [in which postnatal hippocampal neurogenesis has been described (38)] and degus, another precocial rodent (in which postnatal neurogenesis has not previously been studied). Whereas there is a considerable reduction in proliferating ($Ki67^+$) cells and immature (DCX^+) neurons from P17 to P60 in mice, this reduction was much more modest in degus and guinea pigs (Fig. 5, A to D). Unlike infant mice that showed rapid forgetting, infant degus showed normal retention of a context fear memory for up to 1 month (Fig. 5, E and F), and infant guinea pigs showed no change in spatial memory as a function of retention delay (Fig. 5, G and H), indicating that memories are persistent in infant rodent species with low postnatal hippocampal neurogenesis.

Increasing Hippocampal Neurogenesis Is Sufficient to Induce Forgetting in Infant Guinea Pigs and Degus

If levels of hippocampal neurogenesis and forgetting are causally related, then increasing hippocampal neurogenesis in degus and guinea pigs should be sufficient to induce infantile amnesia in these precocial species. To test this, infant (P17) degus were trained in contextual fear conditioning and then either housed conventionally, given access to a running wheel, or treated with MEM or vehicle for 4 weeks (Fig. 6, A and D). Both running and MEM treatment increased neurogenesis (Fig. 6, B and E) and induced forgetting (Fig. 6, C and F). Similarly, forgetting of a spatial memory was observed after MEM treatment in infant guinea pigs (Fig. 6, G to I). These results indicate that increasing hippocampal neurogenesis using mechanistically distinct interventions induces forgetting in precocial rodent species.

Discussion

The hippocampus encodes memories for places and events (39). The observation that hippocampal neurogenesis persists into adulthood led to the idea that neurogenesis modulates hippocampal memory function (40). To our knowledge, all previous studies examining the relationship between hippocampal neurogenesis and memory have used essentially the same design; they manipulated hippocampal neurogenesis before training and examined the impact of this manipulation on subsequent memory formation (i.e., they investigated the anterograde effects of manipulating neurogenesis on memory) (40). The view that emerged from these studies is that, once sufficiently mature, new neurons positively contribute to encoding of new hippocampus-dependent memories, perhaps by providing new substrates for memory storage (40). Here, we examined the retrograde impact of similar manipulations of neurogenesis on memory. Through a series of studies, we showed that high levels of neurogenesis disrupt established hippocampus-dependent memories. As such, our findings reveal a novel role for neurogenesis in forgetting or memory clearance, in line with theoretical predictions (12, 14–16).

The hippocampus is thought to rapidly and automatically encode experiences (41). Because not all experiences are ultimately remembered, it is likely that forgetting processes continuously degrade or clear stored information from the hippocampus. Our results identify neurogenesis as one such process that promotes degradation of hippocampus-dependent memories, most likely by reconfiguring DG-CA3 circuits. Successful memory retrieval may result from the reactivation of patterns of neural activity present at the time of memory encoding (i.e., pattern completion) [e.g., (42–45)]. Because neurogenesis reconfigures hippocampal circuits, this may reduce the ability of a given set of cues (or inputs) to reinvoke the same pattern of activity (i.e., pattern completion failure) (46). During infancy, when neurogenesis levels are elevated, high rates of decay render hippocampus-dependent memories [that are declarative in nature (21)] inaccessible at later time points. Reducing neurogenesis at this developmental stage can increase the persistence of hippocampus-dependent memories. During adulthood, when neurogenesis levels are lower, memories are more resistant to decay. Artificially increasing neurogenesis after learning, however, may be sufficient to induce forgetting.

References and Notes

1. W. C. Abraham, A. Robins, *Trends Neurosci.* **28**, 73–78 (2005).
2. C. Zhao, W. Deng, F. H. Gage, *Cell* **132**, 645–660 (2008).
3. G. L. Ming, H. Song, *Annu. Rev. Neurosci.* **28**, 223–250 (2005).
4. D. A. Laplagne *et al.*, *PLOS Biol.* **4**, e409 (2006).
5. N. Toni *et al.*, *Nat. Neurosci.* **11**, 901–907 (2008).
6. N. Toni *et al.*, *Nat. Neurosci.* **10**, 727–734 (2007).
7. C. Zhao, E. M. Teng, R. G. Summers Jr., G. L. Ming, F. H. Gage, *J. Neurosci.* **26**, 3–11 (2006).
8. S. Ge *et al.*, *Nature* **439**, 589–593 (2006).
9. Y. Gu *et al.*, *Nat. Neurosci.* **15**, 1700–1706 (2012).
10. A. Sahay *et al.*, *Nature* **472**, 466–470 (2011).
11. S. S. Stone *et al.*, *J. Neurosci.* **31**, 13469–13484 (2011).
12. P. W. Frankland, S. Köhler, S. A. Josselyn, *Trends Neurosci.* **36**, 497–503 (2013).
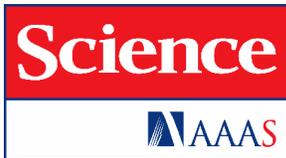13. M. Yasuda *et al.*, *Neuron* **70**, 1128–1142 (2011).
14. K. Deisseroth *et al.*, *Neuron* **42**, 535–552 (2004).
15. L. A. Meltzer, R. Yabaluri, K. Deisseroth, *Trends Neurosci.* **28**, 653–660 (2005).
16. V. I. Weisz, P. F. Argibay, *Cognition* **125**, 13–25 (2012).
17. H. G. Kuhn, H. Dickinson-Anson, F. H. Gage, *J. Neurosci.* **16**, 2027–2033 (1996).
18. T. Seki, Y. Arai, *Neuroreport* **6**, 2479–2482 (1995).
19. S. Freud, *Childhood and Concealing Memories*, in *The Basic Writings of Sigmund Freud*, A. A. Brill, Ed. (Norton, New York, 1905), pp. 30–36.
20. B. A. Campbell, in *Comparative Perspectives on the Development of Memory*, R. V. Kail, N. E. Spear, Eds. (Routledge, 1984), pp. 23-35.
21. D. C. Rubin, *Memory* **8**, 265–269 (2000).
22. B. A. Campbell, N. E. Spear, *Psychol. Rev.* **79**, 215–236 (1972).
23. L. Acsády, A. Kamondi, A. Sík, T. Freund, G. Buzsáki, *J. Neurosci.* **18**, 3386–3403 (1998).
24. J. J. Kim, M. S. Fanselow, *Science* **256**, 675–677 (1992).
25. K. G. Akers, M. Arruda-Carvalho, S. A. Josselyn, P. W. Frankland, *Learn. Mem.* **19**, 598–604 (2012).
26. S. A. Josselyn, P. W. Frankland, *Learn. Mem.* **19**, 423–433 (2012).
27. H. van Praag, G. Kempermann, F. H. Gage, *Nat. Neurosci.* **2**, 266–270 (1999).
28. R. McGonigal, N. Tabatadze, A. Routtenberg, *Hippocampus* **22**, 1242–1255 (2012).
29. H. van Praag, *Trends Neurosci.* **32**, 283–290 (2009).
30. Y. Niibori *et al.*, *Nat Commun* **3**, 1253 (2012).
31. M. Maekawa *et al.*, *Neurosci. Res.* **63**, 259–266 (2009).
32. J. E. Malberg, A. J. Eisch, E. J. Nestler, R. S. Duman, *J. Neurosci.* **20**, 9104–9110 (2000).
33. N. N. Karpova *et al.*, *Science* **334**, 1731–1734 (2011).
34. K. Meletis *et al.*, *Development* **133**, 363–369 (2006).
35. S. A. Josselyn, S. Kida, A. J. Silva, *Neurobiol. Learn. Mem.* **82**, 159–163 (2004).
36. A. Garthe, J. Behr, G. Kempermann, *PLOS ONE* **4**, e5464 (2009).
37. A. Sierra *et al.*, *Cell Stem Cell* **7**, 483–495 (2010).
38. S. Guidi, E. Ciani, S. Severi, A. Contestabile, R. Bartesaghi, *Hippocampus* **15**, 285–301 (2005).
39. H. Eichenbaum, *Neuron* **44**, 109–120 (2004).
40. W. Deng, J. B. Aimone, F. H. Gage, *Nat. Rev. Neurosci.* **11**, 339–350 (2010).
41. R. L. Redondo, R. G. Morris, *Nat. Rev. Neurosci.* **12**, 17–30 (2011).
42. X. Liu *et al.*, *Nature* **484**, 381–385 (2012).
43. L. G. Reijmers, B. L. Perkins, N. Matsuo, M. Mayford, *Science* **317**, 1230–1233 (2007).
44. B. A. Richards, P. W. Frankland, *Hippocampus* **23**, 207–212 (2013).
45. J. F. Danker, J. R. Anderson, *Psychol. Bull.* **136**, 87–102 (2010).
46. A. Treves, E. T. Rolls, *Hippocampus* **4**, 374–391 (1994).

Supplementary Materials for

Hippocampal Neurogenesis Regulates Forgetting During Adulthood and Infancy

Katherine G. Akers, Alonso Martinez-Canabal, Leonardo Restivo, Adelaide P. Yiu, Antonietta De Cristofaro, Hwa-Lin (Liz) Hsiang, Anne L. Wheeler, Axel Guskjolen, Yosuke Niibori, Hirotaka Shoji, Koji Ohira, Blake A. Richards, Tsuyoshi Miyakawa, Sheena A. Josselyn,* Paul W. Frankland*

*Corresponding author. E-mail: paul.frankland@sickkids.ca (P.W.F.), sheena.josselyn@sickkids.ca (S.A.J.)

This PDF file includes:

Materials and Methods
Figs. S1 to S17
References

Akers et al.

MATERIALS AND METHODS

Animals

All procedures were approved by the Animal Care and Use Committees at the Hospital for Sick Children or Fujita Health University and conducted in accordance with the policies of the Canadian Council on Animal Care (CCAC) and National Institutes of Health (NIH) Guidelines on the Care and Use of Laboratory Animals

Mice. Mice were bred in the animal facilities at the Hospital for Sick Children or Fujita Health University and maintained on a 12 hr light/dark cycle (lights on at 0700 hrs). Day of birth was designated P0. After weaning (P21), mice were group-housed (2-5 per cage) in transparent plastic cages ($31 \times 17 \times 14$ cm) with free access to food and water unless otherwise specified. For most experiments, mice were wild-type (WT) male and female F1 hybrids (C57Bl/6NTac $\times$ 129S6/SvEvTac).

For a subset of contextual fear conditioning experiments, three different lines of genetically-modified male and female mice were used. (i) LacZ reporter mice were a cross between a nestin-Cre^{ERT2} line and Rosa-LacZ or Rosa-49YFP lines maintained in a C57Bl/6 background. In these mice, injection of tamoxifen (TAM) leads to expression of the LacZ or YFP transgene only in nestin$^+$ cells and their progeny (*47*). (ii) TK$^+$ mice were maintained in a 129Svev background. In these mice, a modified HSV-1 thymidine kinase (TK) gene is expressed under the control of a nestin promoter (*48*). Administration of ganciclovir (GAN) causes premature DNA chain termination and apoptosis of only nestin$^+$ cells. (iii) iKO-p53 mice were a cross between nestin-Cre^{ERT2} and floxed-p53 lines (*49*) maintained in a C57Bl/6 background. In these mice, injection with TAM leads to the deletion of p53 only in nestin$^+$ cells. Genotypes were

2

determined using PCR analysis of tail DNA samples. For the Barnes maze experiment, WT

C57Bl/6 mice were implanted with fluoxetine (FLX) or placebo pellets.

Guinea pigs. Hartley guinea pigs were maintained on a 12 hr light/dark cycle (lights on at

0630 hrs) and group-housed (2 per cage) in transparent plastic cages ($47 \times 35.5 \times 21.5$ cm) with

free access to food and water.

Degus. Octodon degus were bred in-house. They were maintained on a 12 hr light/dark

cycle (lights on at 0630 hrs) and group-housed (5-7 per cage) in transparent plastic cages ($47 \times$

35.5×21.5 cm) with free access to food and water.

Treatments

Retrovirus. New neurons were labeled using a replication-deficient retroviral vector

(based on the Moloney murine leukemia virus) in which a CAG promoter drives green

fluorescent protein (GFP) expression (*11*, *50*). This retroviral vector was prepared by transfecting

Plat-gp cells with two plasmids containing an amphotropic envelope (vsvg) and the transgene

(pCAG-GFP), followed by collection through ultra-speed centrifugation. Plat-E cells were then

infected to generate a stable virus-producing cell line, and concentrated virus solution was

obtained by ultra-speed centrifugation (average 3.5×10^9 iu/ml). Mice were treated with atropine

(0.1 mg/kg) and anesthetized with chloral hydrate (400 mg/kg). Using stereotaxic procedures, 0.5

µl retrovirus was infused into the dentate gyrus (DG) bilaterally (-2.2 mm AP, ±1.8 mm ML, and

2.2 mm DV relative to bregma) with a glass micropipette. A pump maintained the infusion rate

at 0.15 µl/min, and the pipette was left in place for 5 min after each infusion. Mice were

postoperatively treated with ketoprofen (5 mg/kg) and perfused 4 weeks later.

Running. Mice in running groups were given voluntary access to a single running wheel placed in their home cages. Mice ran an average of 4.7 km ± 0.53 per day, similar to previous experiments (e.g., (*27*)). In one experiment, the wheel was locked to prevent running. Degus in running groups were given voluntary access to 1-2 custom-made running wheels placed in their home cages. Mice and degus in sedentary groups were similarly housed but not given running wheels.

BrdU. BrdU was dissolved in phosphate-buffered saline (PBS) and injected (100 mg/kg, i.p.) into mice twice per day (12 h apart) for 5 consecutive days.

Ganciclovir. GAN was mixed into powdered food (0.08%) and given to mice *ad libitum*, resulting in an average dose of ~70/mg/kg/day (*30*).

Tamoxifen. TAM was dissolved in sunflower seed oil containing 10% ethanol and injected (180 mg/kg, i.p.) into mice once per day for 5 consecutive days.

Memantine. Memantine (MEM) was dissolved in 0.9% saline and injected (25 mg/kg, i.p.) into mice, guinea pigs, or degus once per week (*31*).

Fluoxetine. FLX (15 mg/kg/day, 21-day release) or placebo pellets were implanted subcutaneously in the dorsal interscapular region of mice under chloral hydrate anesthesia (400 mg/kg) (*51*).

Temozolomide. Temozolomide (TMZ) was dissolved in 0.9% saline containing 10% DMSO. Four rounds of treatment at 1 week intervals were given to mice, with each round consisting of one injection (25 mg/kg, i.p.) per day for 4 consecutive days (*30*).

P7C3. P7C3 was dissolved in deionized water containing 2% DMSO, 8% Cremaphor, and 5% dextrose (pH 7.4) and injected once per day (25 mg/kg, i.p.) for 28 days (*52*).

Behavioral Testing

Contextual Fear Conditioning. For mice, contextual fear conditioning occurred in test chambers (31 cm × 24 cm × 21 cm) with shock-grid floors (bars 3.2 mm in diameter spaced 7.9 mm apart). The front, top and back of the chamber were clear acrylic and the sides were modular aluminum. During training, mice were placed in the chambers, and three foot shocks (0.5 mA, 2 s duration, 1 min apart) were delivered after 2 min. For mice in the strong contextual fear conditioning experiment, five foot shocks (0.5 mA, 2 s duration, 1 min apart) were delivered after 2 min. Mice were removed from the chambers 1 min after the last shock. During the test, mice were placed in the chambers for 5 min. For degus, contextual fear conditioning occurred in test chambers (30.5 × 25.5 × 33 cm) with shock-grid floors (bars 5.0 mm diameter spaced 20.0 mm apart). The front, top and back of the chamber were clear acrylic and the sides were modular aluminum. Degus underwent identical training and testing procedures as mice except that foot shocks were delivered at a higher intensity (2 mA). Behavior was recorded by overhead cameras. Freezing (i.e. absence of movement except for breathing) was measured using automated scoring systems for mice and degus.

Incidental Context Learning Paradigm. Incidental context learning in mice occurred in the same chambers as described above (Med Associates). During the context pre-exposure session, mice were placed in the chambers for 10 min. During the immediate shock session, mice were returned to the chambers, a single foot shock was immediately delivered (≤ 1 s after entry, 1 mA, 2 s duration), and mice were removed from the chambers after 1 min. During the test session, mice were placed in the chambers for 3 min.

Water Maze. For mice, a circular plastic pool (120 cm diameter, 50 cm height) was filled to a depth of 40 cm with water (~26º C) made opaque by the addition of nontoxic paint. A

circular escape platform (10 cm diameter) was submerged 0.5 cm below the surface of the water in the center of one of the pool quadrants. The pool was surrounded by curtains that were located at least 1 m from the pool wall and painted with distinct geometric cues. For guinea pigs, a circular plastic pool (185 cm diameter, 60 cm height) was filled to a depth of 45 cm with water (room temperature) made opaque by the addition of nontoxic paint. A circular escape platform (14 cm diameter) was submerged 2 cm below of the surface of the water. Geometric cues were located on the walls of the room. During training, mice were given 3 trials per day for 4 days, and guinea pigs were given 8 trials per day for 5 days. Trials started when animals were released into the pool, facing the wall, from one of 4 possible points. A different release point was used for each trial on each day, and the order of release points varied pseudorandomly across days. Trials ended when animals reached the hidden platform or 60 s elapsed. If an animal failed to find the platform, it was guided by the experimenter. During the probe tests, the platform was removed from the pool, and animals were allowed to swim for 60 s. Swim paths were recorded by an overhead video camera and tracked using automated software. For mice, latency to reach the platform was recorded during training, and time spent in a circular zone (15 cm radius) centered on the platform location was measured during probe tests. Density plots depicting areas of the pool visited more frequently during the probe tests were generated using a custom program developed in our laboratory. For guinea pigs, latency to reach the platform was recorded during training and probe tests.

Barnes Maze. A white circular platform (100 cm diameter, 70 cm above the floor) contained 12 holes equally spaced around its perimeter. Under one of the holes, there was a black Plexiglas escape box (17 × 13 × 7 cm) filled with paper bedding. The location of this escape hole was consistent for a given mouse but randomized across mice. To prevent navigation based on

olfactory or proximal cues within the maze, the platform was rotated before each trial, and the spatial location of the escape hole remained in a fixed location with respect to the distal room cues. During habituation, mice were allowed 5 min to freely explore the maze, with no escape box present. During training, mice were given 3 trials per day for 6 days. On each trial, mice were released in the center of the maze and allowed 5 min to enter the escape box, where it remained for 30 s. If a mouse failed to find the escape box, it was guided by the experimenter. During probe tests, the escape box was removed from the maze, and mice were allowed to search for 3 min. Time spent around each hole was recorded. Search paths were recorded by an overhead video camera and tracked using automated software based on the public domain NIH ImageJ program and custom modifications of ImageJ software

Conditioned Taste Aversion. During habituation, water-restricted mice were placed in individual cages and given access to two bottles containing tap water for gradually decreasing amounts of time across days: 4 hours on day 1, 2 hours on day 2, 1 hour on day 3, and 30 min on days 4 and 5. During conditioning, the water bottles were replaced by a single bottle containing a saccharin solution (0.1% saccharin in tap water). Mice were allowed to drink for 30 min and then injected with LiCl (0.30M in PBS, 2% body weight, i.p.). During testing, mice were given access to one bottle containing tap water and one bottle containing saccharin solution. During the delay between conditioning and testing, mice were kept on water restriction for 4 days each week, during which they were placed in individual cages and given access to two water bottles for 1 hour a day. Throughout the experiment, a limited amount of wet food was provided daily in the home cage to maintain ~90% of baseline body weight.

Histology

Tissue Preparation. Mice, guinea pigs, and degus were perfused transcardially with PBS followed by 4% paraformaldehyde (PFA). Brains were post-fixed in PFA and transferred to 30% sucrose. Coronal sections (50 μm) were cut along the entire anterior-posterior extent of the DG using a cryostat. Sections were kept in sequential order and stored free-floating in 50% glycerol and 10% ethylene glycol in PBS. A 1/4 section sampling fraction was used to create four sets (each containing sections at 200 μm intervals) for immunohistochemistry.

Immunohistochemistry. Unless otherwise stated, all incubations occurred at room temperature.

For Ki67 labeling, sections were pre-treated with 0.01M citrate buffer (pH 6.0) in a 97° C steamer for 20 min, allowed to cool to room temperature for 20 min, and then treated with 3% H_2O_2 in PBS for 10 min. Sections were then incubated with primary (rabbit anti-Ki67, 1:1000) and secondary (biotinylated goat anti-rabbit, 1:1000) antibodies. Ki67$^+$ cells were visualized using avidin-biotin-peroxidase complex (ABC) followed by diaminobenzidine (DAB).

For DCX labeling, sections were pre-treated with 0.01M citrate buffer (pH 6.0) in a 97° C steamer for 20 min and then allowed to cool to room temperature for 20 min. Sections were then incubated with primary (rabbit anti-DCX, 1:1000) and secondary (biotinylated goat anti-rabbit, 1:2000) antibodies. DCX$^+$ cells were visualized using ABC followed by signal amplification using a TSA system and SAV Alexa-488. DAPI (1:10000) was used as a counterstain.

For GFP labeling and GFP/ZnT3 double-labeling, sections were incubated with primary (rabbit anti-GFP, 1:1000; goat anti-ZnT3, 1:1000) and secondary (donkey anti-rabbit Alexa-488, 1:1000; donkey anti-goat Alexa-568, 1:1000) antibodies. DAPI was used as a counterstain.

Akers et al.

For BrdU labeling, sections were pre-treated with 1N HCl at 45°C for 30 min. Sections were then incubated with primary (rat anti-BrdU monoclonal, 1:1000) and secondary (Alexa-488 goat anti-rat, 1:1000) antibodies.

For TUNEL labeling, sections were pre-treated with 0.01M citrate buffer (pH 6.0) in a 97º C steamer for 20 min and allowed to cool to room temperature for 20 min. Sections were then processed using the DeadEnd Colorimetric TUNEL System. Methyl green was used as a counterstain.

For LacZ labeling, sections were incubated with primary (rabbit anti-Laz, 1:1000) and secondary (donkey anti-rabbit Alexa-488, 1:1000) antibodies.

Imaging and Quantification. Ki67^{+}, DCX[+] and LacZ[+] cell quantification was performed using an Olympus BX61 microscope and StereoInvestigator 9.0 software. The total number of labeled cells in the DG was estimated using the optical fractionator technique. With a 60× oil-immersion objective (N.A. 1.43), labeled cells were counted inside 90×90 µm counting frames equally spaced across a 250×250 µm grid with a 20 µm optical dissector height. The counting parameters were chosen to achieve a Gundersen coefficient of error less than 0.1 (*53*). GFP^{+} cells in WT mice and DCX^{+} cells in FLX-treated mice were counted manually. Data are reported as number of cells per 1000 µm^2.

For quantification of YFP[+] large mossy terminals (LMTs), DCX[+] LMTs, and ZnT3^{+} puncta, a LSM-710 confocal microscope with a 20× objective (N.A. 0.8) was used to acquire serial Z-stack images (step: 0.8 µm) of the CA3 region at a resolution of 1024×1024 pixels (212 $\times$ 212 µm, digital zoom: 1×). Using ImageJ software, labeled structures were counted in a 70×70 µm square within the stratum lucidum of the CA3b region. Data are reported as number of LMTs per 1000 µm^2.

9

Three-dimensional reconstructions of GFP$^+$ LMTs were created using an LSM-710 confocal microscope with Zen 2009 software. With a 63× oil-immersion objective (N.A. 1.40), serial Z-stack images were acquired (step: 0.25 µm). The contours of the GFP$^+$ LMT and ZnT3$^+$ zone were traced in 3 dimensions using Neurolucida 9.1 software, and surface reconstruction was performed using the 3D visualization module.

BrdU$^+$ and TUNEL$^+$ cell quantification was performed manually using a Nikon microscope. With a 40× objective, BrdU$^+$ cells were counted in the granule cell layer, and TUNEL$^+$ cells were counted from the entire DG. Data are reported as number of cells per section.

Specific experimental procedures

Age differences in hippocampal neurogenesis in mice. To track age-dependent changes in neurogenesis, GFP-expressing retrovirus was infused into the DG of mice at P17 or P60. Mice were perfused 28 days later, and sections were stained for GFP. In a second experiment, mice were perfused at P17, P28, P60, P120, or P180, and sections were stained for Ki67 and DCX.

Age differences in synaptic remodeling in mice. We developed a computational model to estimate differences between P17 and P60 mice in the amount of DG-CA3 synaptic remodeling occurring across time. First, the number of Ki67$^+$ cells in the DG was estimated by fitting a double exponential curve (Y = (a × exp(-b × X)) + (c × exp(-d × X)), $r^2 = 0.91$) to the data shown in Fig. 1C. Thirty percent of Ki67$^+$ cells were assumed to survive and mature into granule cells that project mossy fibers to the CA3 and grow LMTs onto CA3 pyramidal cells within 14 days (*54, 55*). Next, the number of LMTs making contact with CA3 cells was calculated by multiplying the number of LMTs per DG cell (*n* = ~14 (*56, 57*), generated by a Gaussian

function) by the number of DG cells added each day. Finally, the number of new LMTs making contacts with CA3 cells was divided by the total number of LMTs per CA3 cell ($n = 50$ (*56, 57*)). A CA3 cell was defined as being remodeled if 2-4% of its synaptic inputs were altered by new neuron addition, and cumulative predicted synaptic remodeling was expressed as the proportion of CA3 cells (from a total of 250,000 (*58)*) with altered synaptic inputs as a consequence of new neuron addition.

Age differences in memory persistence in mice. To assess age-dependent changes in memory persistence, separate groups of P17 or P60 mice were trained in contextual fear conditioning and tested 1, 7, 14, or 28 days later. Other groups of control P17 or P60 mice were treated similarly but did not receive foot shocks. These no-shock control mice were similarly tested 1 or 28 days later. Shock reactivity was assessed in a subset of shocked P17 and P60 mice by measuring the distance mice traveled immediately before and during the foot shocks. As a second way to assess age-dependent changes in memory persistence, separate groups of P17 or P60 mice were trained in the incidental context learning paradigm, and immediate shock and test sessions occurred 1, 7, 14, or 28 days later. Age-matched groups of control mice were not exposed to the context prior to immediate shock and test sessions.

Effect of TAM on acquisition of context fear memory. Because we used TAM-inducible transgenic lines (i.e., nestin-Cre[ERT2] × Rosa-LacZ, nestin-Cre[ERT2] × floxed-p53) in some experiments, we evaluated whether TAM treatment has acute effects on behavior and neurogenesis. C57Bl/6 mice (the same genetic background as TAM-inducible lines) were treated with TAM or vehicle starting at P60 and then trained in contextual fear conditioning and tested 24 hours later. Mice were then perfused, and sections were stained for DCX.

Akers et al.

Effect of running on adult hippocampal neurogenesis in adult mice. To assess whether voluntary running increases neurogenesis, separate groups of mice remained sedentary or were allowed access to a running wheel for 0, 3, 7, 14, or 28 days starting at P60. Mice were then perfused, and sections were stained for Ki67 and DCX. To control for the presence of a novel object in the home cage, a control experiment compared mice that were given a locked running wheel, mice that were allowed to run on an unlocked wheel, and mice that were not given a running wheel (i.e., sedentary) for 28 days. In a second experiment, mice were injected with BrdU starting on P30. Starting on P60, mice remained sedentary or ran for 7 days. Mice were then perfused, and sections were stained for BrdU and TUNEL. In a third experiment, GFP-expressing retrovirus was infused in the DG of mice on P60. Mice then ran for 28 days before being perfused, and sections were stained for GFP and ZnT3 and used for 3-dimensional reconstruction of pre-synaptic terminals in CA3. In a fourth experiment, LacZ/YFP reporter mice (nestin-cre^{ERT2} × YFP reporter mice) were injected with TAM starting at P55, and then remained sedentary or ran for 28 days starting at P60. Mice were then perfused, and sections were stained for GFP and ZnT3.

Effect of running on context fear memory and blockade of the running effect by genetic suppression of neurogenesis in adult mice. To test whether the running-induced increase in neurogenesis leads to forgetting, LacZ reporter mice were injected with TAM starting on P55. Half of the mice underwent contextual fear conditioning on P60, remained sedentary or ran for 42 days, and then were tested. The other half remained sedentary or ran for 42 days before conditioning and testing. Mice were perfused after testing, and sections were stained for LacZ. To test whether running induces forgetting of a stronger context fear memory, mice were trained with five (rather than three) foot shocks on P60, remained sedentary or ran for 28 days, and then

were tested. To test whether preventing the running-induced increase in neurogenesis blocks

forgetting, TK$^+$ mice and their WT littermates were trained in contextual fear conditioning on

P60, remained sedentary or ran for 42 days, and then were tested. Both TK$^+$ and WT mice were

placed on GAN-containing food starting 7 days before training and continuing until testing. Mice

were perfused after testing, and sections were stained for DCX.

Effect of pharmacological increases in neurogenesis on context fear memory and

blockade by genetic suppression in adult mice. Mice underwent contextual fear conditioning on

P60. Separate groups were then either given (1) a single injection of MEM or vehicle 24 hours

after conditioning with a test session 48 hours after conditioning, (2) weekly injections of MEM

or vehicle for 6 weeks with a test session 1 week after the final injection, or (3) weekly injections

of MEM or vehicle for 6 weeks with a test session 2 weeks after the final injection. Additional

groups of mice were trained in the incidental context learning paradigm on P60, received weekly

injections of MEM or vehicle for 4 weeks, and then given shock and test sessions. To test

whether preventing the MEM-induced increase in neurogenesis blocks forgetting, TK$^+$ mice and

their WT littermates were trained in contextual fear conditioning on P60, received weekly

injections of MEM or vehicle for 42 days, and then were tested. Mice were perfused after testing,

and sections were stained for DCX. In a second experiment, mice were subcutaneously

implanted with FLX or placebo pellets on P70 and then trained to locate an escape box in the

Barnes maze. Probe tests occurred 2 and 30 days after training. Mice were perfused after testing,

and sections were stained for DCX.

Effect of genetic increase in neurogenesis on context fear memory in adult mice. iKO-p53

mice and their WT littermates underwent contextual fear conditioning on P60, were treated with

TAM starting the next day, and were tested 28 days later. Mice were perfused after testing, and sections were stained for DCX and Ki67.

Effect of running on other hippocampus-dependent memories in adult mice. To test whether running induces forgetting of a context-only memory, mice were trained in the incidental context learning paradigm on P60, then remained sedentary or ran for 28 days, and then given immediate shock and test sessions. Separate groups of mice remained sedentary or ran for 28 days before training, shock, and test sessions. Mice were perfused after testing, and sections were stained for Ki67. To test whether running induces forgetting of a spatial memory, mice were trained to locate a hidden platform in the water maze starting on P60. An initial probe test occurred immediately after training. Mice then remained sedentary or ran for 28 days, followed by a second probe test.

Effect of running on a hippocampus-independent memory in adult mice. Mice were trained in taste aversion conditioning on P60 and then remained sedentary or ran for 28 days. A test session occurred 28 days after conditioning.

Effect of genetic reduction in neurogenesis on memory in infant mice. TK$^+$ mice and their WT littermates were administered GAN in their food (which was also available to the dam) starting on P10. On P17, TK$^+$ and WT mice were trained in contextual fear conditioning and then were tested 1 day or 7 days later. Separate groups of mice were similarly treated (placed in the chamber on P17) but did not receive foot shocks. Mice were perfused after testing, and sections were stained for Ki67 and DCX.

Effect of pharmacological reduction in neurogenesis on memory in infant mice. To test whether reducing neurogenesis mitigates infantile forgetting, P17 mice were trained in the incidental context learning paradigm, then given 4 rounds of injection with vehicle or TMZ, and

14

then given immediate shock and test sessions. Separate groups of P17 mice were given 4 rounds of injection with vehicle or TMZ before training, shock, and test sessions. Mice were perfused after testing, and sections were stained for Ki67 and DCX. To determine whether reducing cell death alleviates infantile forgetting, mice were trained in the incidental context learning paradigm on P17, then given injections of vehicle or P7C3 for 28 days, and then given immediate shock and test sessions.

Lack of age differences in hippocampal neurogenesis and memory persistence in precocial guinea pigs and degus. To assess hippocampal neurogenesis, degus and guinea pigs were perfused at P17 or P60, and sections were stained for Ki67 and DCX. Levels of neurogenesis were compared to those observed in mice at P17 and P60. To assess memory persistence, degus underwent contextual fear conditioning on P17 or P60 and were tested 1 and 28 days later. Guinea pigs were trained to locate a hidden platform in the water maze starting on P17 or P60 and were tested 1 day or 28 days later.

Effect of running and pharmacological increases in neurogenesis on memory persistence in infant degus and guinea pigs. Degus were trained in contextual fear conditioning on P17 and then either remained sedentary, ran, or received weekly vehicle or MEM injections for 28 days. After testing, degus were perfused, and sections were stained for DCX. Guinea pigs were trained to locate a hidden platform in the water maze, received weekly injections of vehicle or MEM over 4 weeks, and then were tested. Guinea pigs were perfused after testing, and sections were stained for DCX.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) and two-tailed t-tests. Statistical significance was set at $P < 0.05$. To examine individual-level relationships between measures of neurogenesis and memory, Pearson's r was reported after using nonlinear regression to calculate exponential rate of decay ($y = A \times e^{(-k \times x)}$).
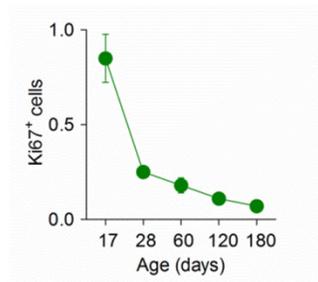
Akers et al.

SUPPLEMENTAL FIGURES

Fig. S1. Age-dependent decrease in proliferation in the DG**.** The number of proliferating

(Ki67$^+$) cells in the mouse DG decreased with age (P17 $n = 5$, P28 $n = 5$, P60 $n = 4$, P120 $n = 4$,

P180 $n = 4$; *age* main effect, $F_{4,17} = 23.62$, $P < 0.001$).
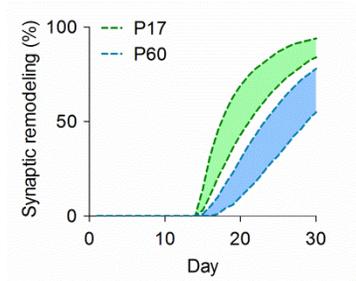
Fig. S2. Computational model of how the addition of new neurons might impact DG-CA3

circuits. During infancy, the addition of many new neurons altered contacts on virtually all CA3

pyramidal cells after one month. By contrast, during adulthood, the addition of fewer new

neurons led to more modest synaptic rearrangements.
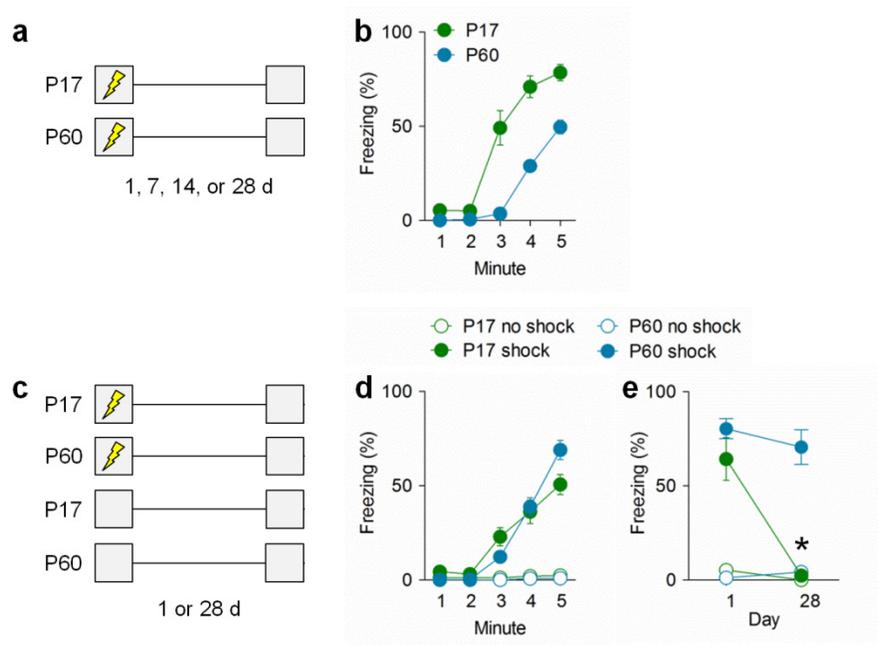
Fig. S3. Forgetting of context fear memory in infant mice. (**a**) Infant (P17) and adult (P60) mice were trained in contextual fear conditioning and then tested 1, 7, 14, or 28 days later. (**b**) During training, infant ($n = 36$) and adult ($n = 34$) mice displayed increasing levels of freezing after each foot shock (3 shocks delivered before minutes 3, 4, and 5). (**c**) Infant and adult mice were trained in contextual fear conditioning or placed in the conditioning chamber with no foot shock and then tested 1 or 28 days later. (**d**) During training, shocked mice (P17 $n = 12$, P60: $n = 17$) displayed increasing levels of freezing after each foot shock, whereas non-shocked (P17 $n = 10$, P60: $n = 16$) mice did not exhibit freezing. (**e**) During testing, shocked adult mice showed stable context fear memory for 28 days (1 day $n = 8$, 28 days $n = 9$; 1 vs. 28 days, $P > 0.05$), whereas shocked infant mice showed forgetting (1 day $n = 5$, 28 days $n = 7$; 1 vs. 28 days, $t_{10} = 6.52$, $P < 0.001$). Non-shocked infant (1 day $n = 4$, 28 days $n = 6$) and adult (1 day $n = 8$, 28 days $n = 8$) mice did not exhibit freezing during testing.
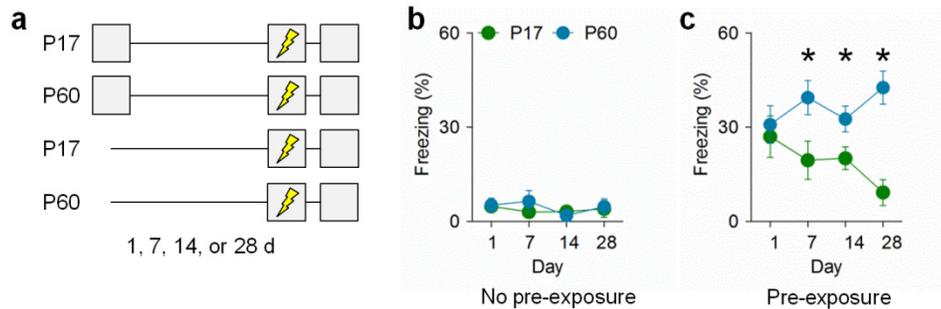
Fig. S4. Forgetting of context-only memory in infant mice. (**a**) Infant (P17) and adult (P60) mice were pre-exposed or not pre-exposed to a context. One, 7, 14, or 28 days later, separate groups of mice received an immediate shock in that context and then were tested the following day. (**b**) Infant and adult mice that were not pre-exposed to the context displayed an immediate shock deficit during testing (n = 8-10 per group). (**c**) Context pre-exposure rescued the immediate shock deficit in adult mice regardless of the delay between context pre-exposure and immediate shock training (n = 13-15 per group). By contrast, context pre-exposure was ineffective in rescuing the immediate shock deficit in infant mice after longer delays (n = 13-15 per group), indicating forgetting of the context-only memory (*age* × *delay* interaction, $F_{3,103}$ = 2.94, $P < 0.05$; post hoc t-test, 7 d, $P < 0.05$; 14 d, $P < 0.05$; 28 d, $P < 0.001$).
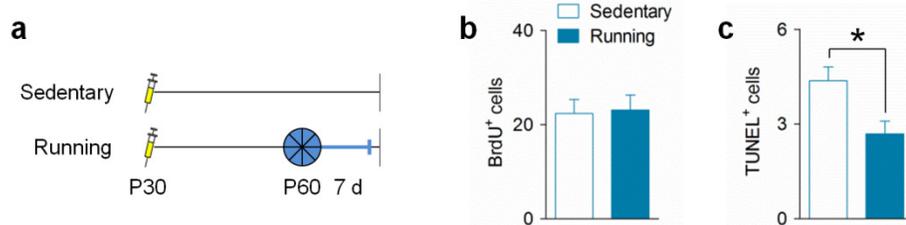
Fig. S5. Running during adulthood does not induce death of developmentally-generated granule cells. (**a**) Adult (P60) mice remained sedentary ($n = 8$) or ran ($n = 8$) after BrdU injection on P30. (**b**) Compared to sedentary mice, running mice showed equivalent numbers of surviving BrdU$^+$ cells and fewer numbers of cells undergoing apoptosis (i.e., TUNEL$^+$; **c**, $t_{14} = 2.82$, $P < 0.05$). These results indicate that running-induced increases in neurogenesis are not associated with increased death of dentate granule cells generated earlier during development.
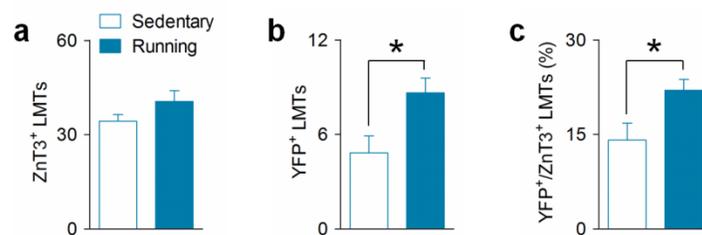
Fig. S6. Running increases the ratio of new-to-established LMTs in the CA3. GFP-expressing retrovirus was injected into the DG of adult (P60) mice to label newborn dentate granule cells and their processes. Mice ran ($n = 6$) or remained sedentary ($n = 6$) for 4 weeks, and tissue was counterstained for ZnT3, a marker of mature presynaptic terminals. (**a**) Numbers of LMTs in the CA3 were equivalent in running and sedentary mice. However, running was associated with an increase in (**b**) number ($t_{10} = 2.67$, $P < 0.05$) and (**c**) proportion ($t_{10} = 2.47$, $P < 0.05$) of newly-generated (GFP$^+$/ZnT3$^+$) LMTs.
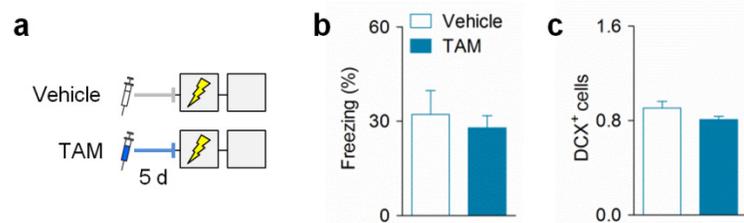
Fig. S7. TAM treatment does not impact context fear memory formation or neurogenesis. (**a**) Adult mice were treated with TAM ($n = 8$) or vehicle ($n = 9$) and then trained in contextual fear conditioning. TAM- and vehicle-treated mice showed no differences in (**b**) acquisition of a context fear memory ($P > 0.05$) or (**c**) number of immature (DCX+) neurons in the DG ($P > 0.05$).
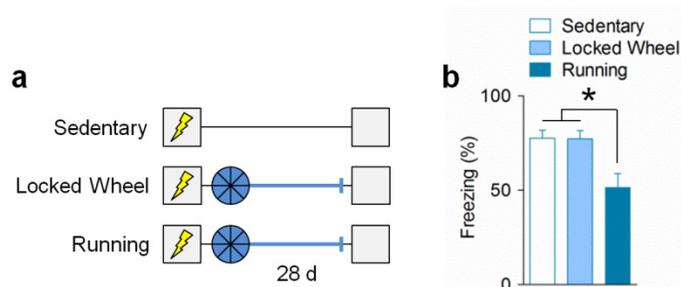

Fig. S8. The presence of a locked running wheel does not lead to forgetting of a context fear memory. (**a**) Adult (P60) mice were trained in contextual fear conditioning then remained sedentary ($n = 12$), given a locked wheel (i.e., no running; $n = 8$), or allowed to run ($n = 12$) for 28 days, after which they were tested. (**b**) Running mice showed less freezing in the trained context compared with sedentary ($t_{22} = 3.08$, $P < 0.01$) or locked wheel ($t_{18} = 2.65$, $P < 0.05$) mice (*group* main effect, $F_{2,29} = 6.95$, $P < 0.01$).
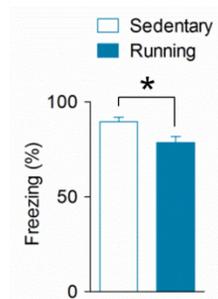
Fig. S9. Running-induced forgetting of a strong context fear memory. Adult (P60) mice were trained with five (rather than three) foot shocks and then ran ($n = 10$) or remained sedentary ($n = 10$) for 28 days. Post-learning running induced less (although still significant) forgetting of a strong context fear memory ($t_{18} = 2.71$, $P < 0.05$).
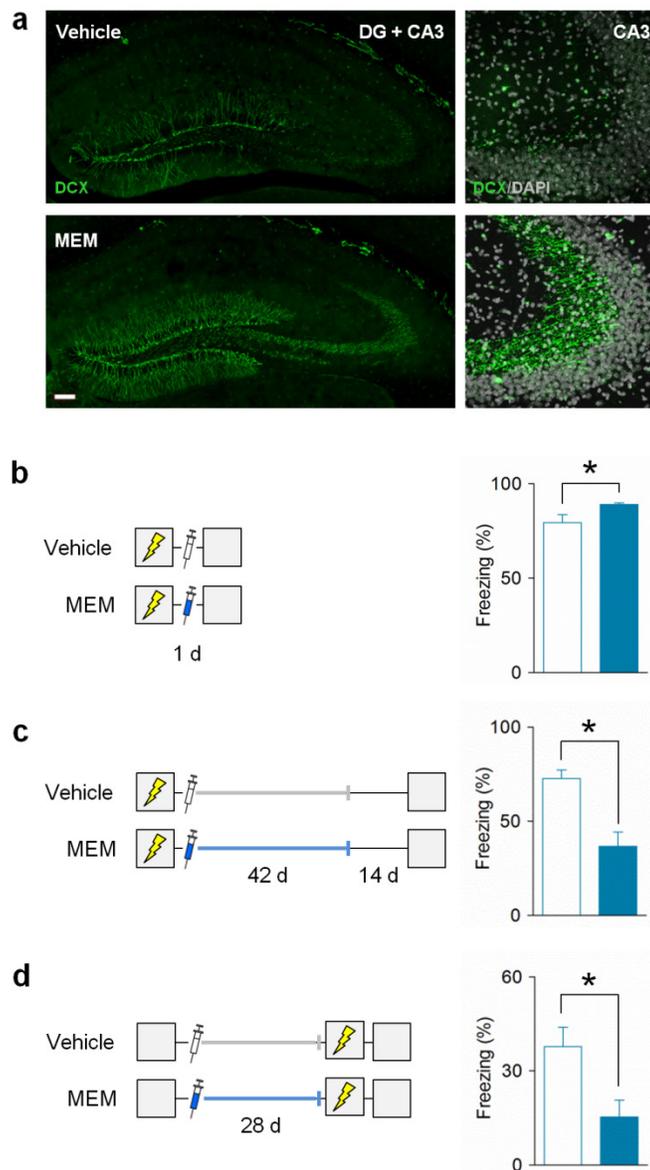
Fig. S10. MEM increases neurogenesis and induces forgetting of context memories in adult (P60) mice. (**a**) MEM treatment (6 injections, spaced 1 week apart) increased the number of DCX$^+$ cells in the DG (left, scale bar = 100 μm) and DCX$^+$ processes in the CA3 (right, scale bar = 50 μm) of adult mice. (**b**) A single post-training MEM injection did not disrupt initial consolidation of a context fear memory (MEM $n = 8$, vehicle $n = 8$; $t_{14} = 2.26$, $P < 0.05$). (**c**) Prolonged post-training MEM treatment (6 injections, spaced 1 week apart) resulted in forgetting

Akers et al.

of a context fear memory 8 weeks later (MEM $n = 7$, vehicle $n = 7$; $t_{12} = 4.11$, $P = 0.001$) later.

(**d**) Prolonged post-training MEM treatment (4 injections, spaced 1 week apart) also resulted in

forgetting of a content-only memory (MEM $n = 10$, vehicle $n = 10$; $t_{18} = 2.73$, $P < 0.05$).
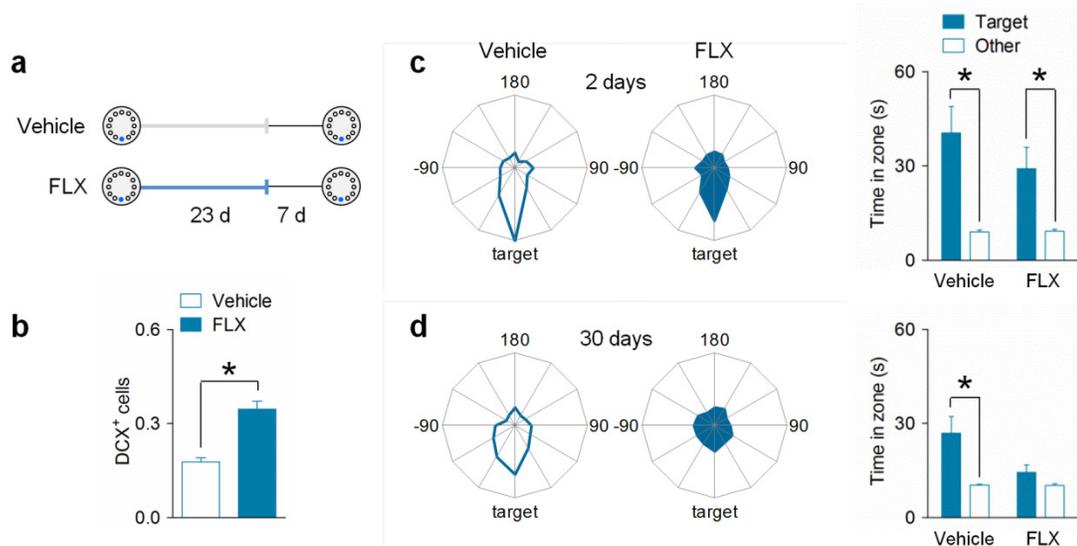

Fig. S11. Fluoxetine (FLX) increases neurogenesis and induces forgetting of a spatial memory

in adult (P70) mice. (**a**) Chronic FLX treatment increased the number of DCX$^+$ cells in the DG

(vehicle $n = 5$, FLX $n = 5$; $t_8 = 5.97$, $P < 0.001$). (**b**) Adult mice were treated with vehicle or FLX

after learning an escape location in the Barnes maze. (**c**) One day after training, vehicle-treated

($n = 10$) and FLX-treated ($n = 8$) mice showed a spatial bias for the escape location (target)

compared to other locations (vehicle, $t_9 = 3.53$, $P < 0.01$; FLX, $t_7 = 2.71$, $P < 0.05$). (**d**) Twenty-

eight days after training, vehicle-treated but not FLX-treated mice showed a spatial bias for the

escape location (target) compared to other locations (vehicle, $t_9 = 2.96$, $P < 0.05$; FLX, $P > 0.05$).
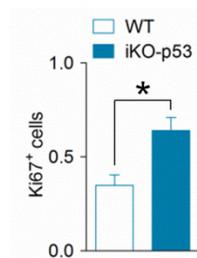
Fig. S12. Inducible deletion of p53 from neural progenitor cells increases proliferation in the DG. Adult (P60) iKO-p53 mice had more proliferating (Ki67^{+}) cells in the DG than WT mice (iKO-p53: $n = 3$, WT: $n = 4$; $t_5 = 3.29$, $P < 0.05$).
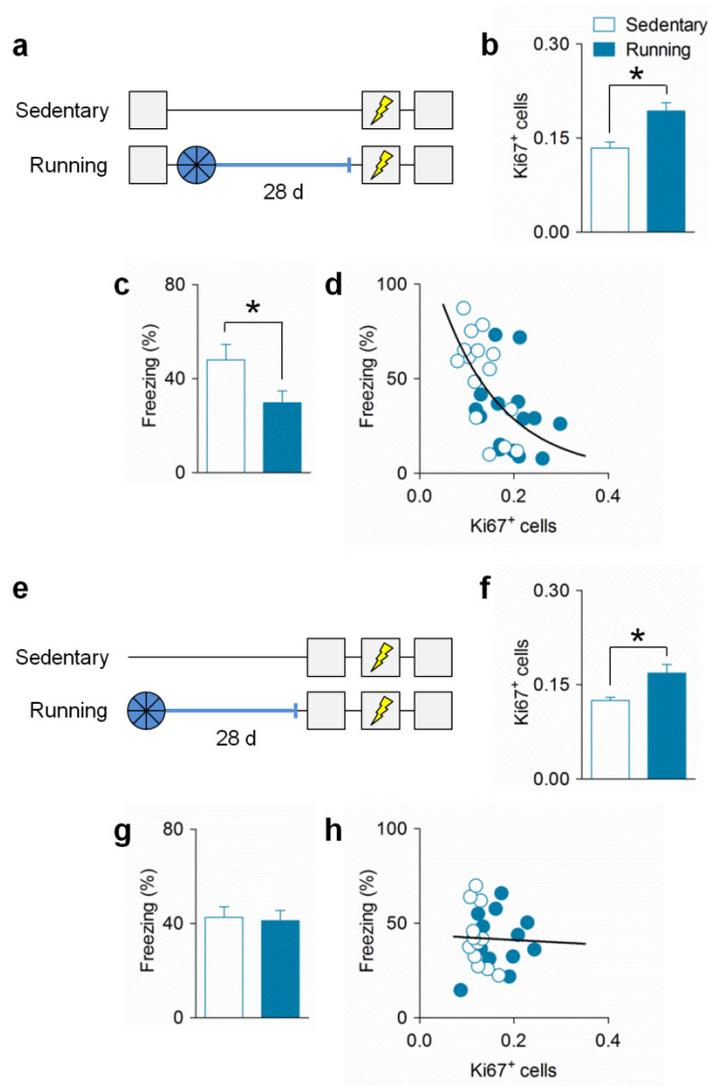
Fig. S13. Post-learning running induces forgetting of a context-only memory in adult (P60) mice. Running after context pre-exposure (**a**) increased the number of proliferating (Ki67^{+}) cells in the DG (**b**; sedentary $n = 15$, running $n = 15$; $t_{28} = 3.62$, $P = 0.001$) and reduced the ability of context pre-exposure to rescue the immediate shock deficit during the test (**c**; sedentary $n = 16$, running $n = 16$; $t_{30} = 2.20$, $P < 0.05$). (**d**) There was an inverse relationship between proliferation (Ki67^{+} cells) and memory ($r = -0.56$, $P = 0.001$). Running before context pre-exposure (**e**) increased the number of proliferating (Ki67^{+}) cells in the DG (**f**; sedentary $n = 12$, running $n =$

12; $t_{22} = 3.06$, $P < 0.01$), but did not alter the ability of context pre-exposure to rescue the immediate shock deficit during the test (**g**; sedentary $n = 12$, running $n = 12$, $P > 0.05$). (**h**) There was no relationship between proliferation (Ki67$^+$ cells) and memory (freezing score during test) ($r = -0.04$, $P > 0.05$).
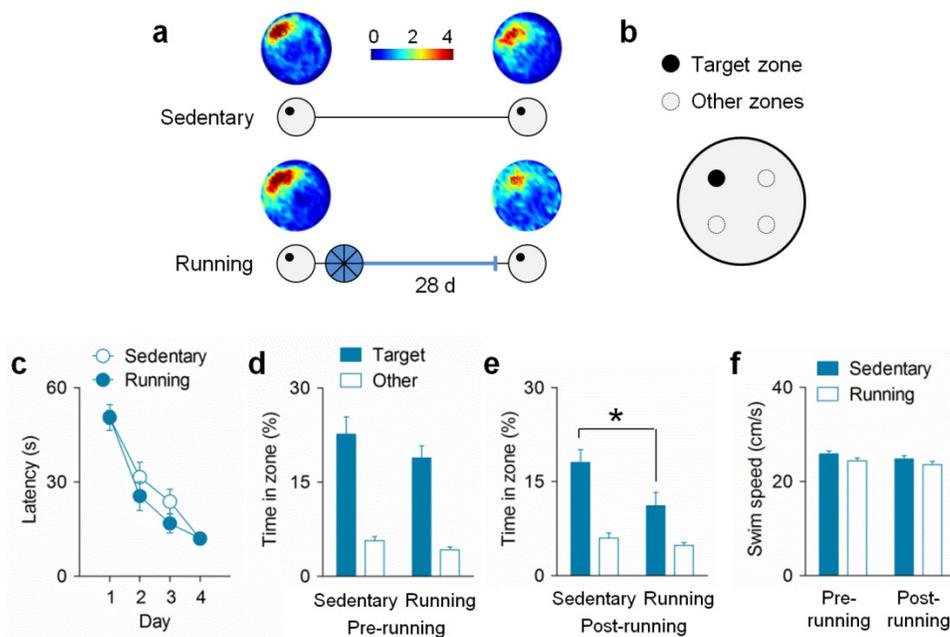
Fig. S14. Post-learning running induces forgetting of a spatial memory. (**a**) Adult (P60) mice were trained to locate a hidden platform in the water maze (density plots show where mice concentrated their search for the platform during probe tests; color legend = average number of visits per mouse per 5 × 5 cm area). (**b**) During probe tests, time spent in a zone (15 cm diameter) that formerly contained the platform (target) was compared with time spent in three other zones. Both groups of mice (**c**) learned to locate the platform during training ($P > 0.05$) and (**d**) exhibited equivalent spatial bias toward the target zone during a pre-running probe test ($P > 0.05$). (**e**) However, mice that ran after water maze training ($n = 16$) showed weaker memory for the platform location during a post-running probe test ($t_{29} = 2.38$, $P < 0.05$) compared with mice that remained sedentary ($n = 15$). (**f**) Sedentary and running mice showed equivalent swim speed during the probe tests ($P > 0.05$).
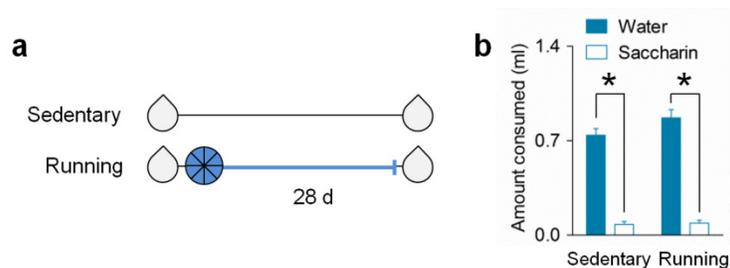
Fig. S15. Running did not lead to forgetting of a non-hippocampal conditioned taste aversion memory. (**a**) Adult (P60) mice were trained to avoid a LiCl-paired saccharin solution, then ran (n = 12) or remained sedentary (n = 12) for 28 days, and then were tested. (**b**) During the test, running and sedentary mice expressed equivalent aversion of the saccharin solution (*fluid* main effect, $F_{1,22}$ = 309.84, P < 0.001; sedentary, t_{11} = 13.92, P < 0.001; running, t_{11} = 11.71, P < .001).
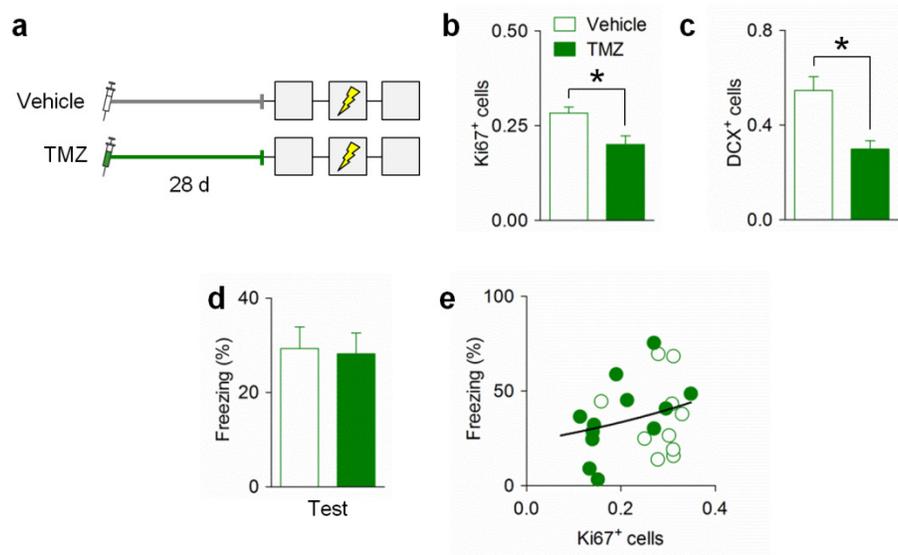
Akers et al.

Fig. S16. Disrupting neurogenesis before training did not impact context-only memory in infant (P17) mice. (**a**) Infant mice were treated with vehicle or TMZ before training in the incidental context learning paradigm. TMZ-treated mice had fewer (**b**) proliferating (Ki67^{+}) cells (vehicle n = 10, TMZ n = 12; t_{20} = 2.92, $P < 0.01$) and (**c**) immature (DCX^{+}) neurons (vehicle n = 7, TMZ n = 7; t_{12} = 3.66, $P < 0.01$) in the DG compared to vehicle-treated mice. However, infant mice treated with TMZ before training showed no change in freezing during the test (**d**; vehicle n = 22, TMZ n = 22; $P > 0.05$), and there was no relationship between proliferation and freezing (**e**; r = 0.27, $P > 0.05$).
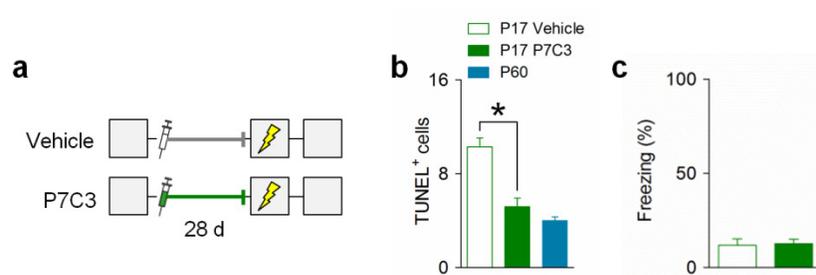
Fig. S17. Reducing cell death in infant mice does not prevent forgetting. (**a**) Infant (P17) mice were pre-exposed to a context and then treated with vehicle ($n = 13$) or the anti-apoptotic drug P7C3 ($n = 13$) for 28 days. They were then given immediate shock training and tested 24 hours later. (**b**) P7C3 treatment reduced apoptosis (TUNEL$^+$ cells; vehicle $n = 7$, P7C3 $n = 8$; $t_{14} = 4.88$, $P < .001$) to a level comparable to adults ($n = 8$) but (**c**) did not alleviate infantile amnesia.

References and Notes

1. W. C. Abraham, A. Robins, Memory retention—the synaptic stability versus plasticity dilemma. *Trends Neurosci.* **28**, 73–78 (2005). Medline doi:10.1016/j.tins.2004.12.003

2. C. Zhao, W. Deng, F. H. Gage, Mechanisms and functional implications of adult neurogenesis. *Cell* **132**, 645–660 (2008). Medline doi:10.1016/j.cell.2008.01.033

3. G. L. Ming, H. Song, Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* **28**, 223–250 (2005). Medline doi:10.1146/annurev.neuro.28.051804.101459

4. D. A. Laplagne, M. S. Espósito, V. C. Piatti, N. A. Morgenstern, C. Zhao, H. van Praag, F. H. Gage, A. F. Schinder, Functional convergence of neurons generated in the developing and adult hippocampus. *PLOS Biol.* **4**, e409 (2006). Medline doi:10.1371/journal.pbio.0040409

5. N. Toni, D. A. Laplagne, C. Zhao, G. Lombardi, C. E. Ribak, F. H. Gage, A. F. Schinder, Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nat. Neurosci.* **11**, 901–907 (2008). Medline doi:10.1038/nn.2156

6. N. Toni, E. M. Teng, E. A. Bushong, J. B. Aimone, C. Zhao, A. Consiglio, H. van Praag, M. E. Martone, M. H. Ellisman, F. H. Gage, Synapse formation on neurons born in the adult hippocampus. *Nat. Neurosci.* **10**, 727–734 (2007). Medline doi:10.1038/nn1908

7. C. Zhao, E. M. Teng, R. G. Summers Jr., G. L. Ming, F. H. Gage, Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J. Neurosci.* **26**, 3–11 (2006). Medline doi:10.1523/JNEUROSCI.3648-05.2006

8. S. Ge, E. L. Goh, K. A. Sailor, Y. Kitabatake, G. L. Ming, H. Song, GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* **439**, 589–593 (2006). Medline doi:10.1038/nature04404

9. Y. Gu, M. Arruda-Carvalho, J. Wang, S. R. Janoschka, S. A. Josselyn, P. W. Frankland, S. Ge, Optical controlling reveals time-dependent roles for adult-born dentate granule cells. *Nat. Neurosci.* **15**, 1700–1706 (2012). Medline doi:10.1038/nn.3260

10. A. Sahay, K. N. Scobie, A. S. Hill, C. M. O'Carroll, M. A. Kheirbek, N. S. Burghardt, A. A. Fenton, A. Dranovsky, R. Hen, Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* **472**, 466–470 (2011). Medline doi:10.1038/nature09817

11. S. S. Stone, C. M. Teixeira, L. M. Devito, K. Zaslavsky, S. A. Josselyn, A. M. Lozano, P. W. Frankland, Stimulation of entorhinal cortex promotes adult neurogenesis and facilitates spatial memory. *J. Neurosci.* **31**, 13469–13484 (2011). Medline doi:10.1523/JNEUROSCI.3100-11.2011

12. P. W. Frankland, S. Köhler, S. A. Josselyn, Hippocampal neurogenesis and forgetting. *Trends Neurosci.* **36**, 497–503 (2013). Medline doi:10.1016/j.tins.2013.05.002

13. M. Yasuda, E. M. Johnson-Venkatesh, H. Zhang, J. M. Parent, M. A. Sutton, H. Umemori, Multiple forms of activity-dependent competition refine hippocampal circuits in vivo. *Neuron* **70**, 1128–1142 (2011). Medline doi:10.1016/j.neuron.2011.04.027

14. K. Deisseroth, S. Singla, H. Toda, M. Monje, T. D. Palmer, R. C. Malenka, Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron* **42**, 535–552 (2004). Medline doi:10.1016/S0896-6273(04)00266-1

15. L. A. Meltzer, R. Yabaluri, K. Deisseroth, A role for circuit homeostasis in adult neurogenesis. *Trends Neurosci.* **28**, 653–660 (2005). Medline doi:10.1016/j.tins.2005.09.007

16. V. I. Weisz, P. F. Argibay, Neurogenesis interferes with the retrieval of remote memories: Forgetting in neurocomputational terms. *Cognition* **125**, 13–25 (2012). Medline doi:10.1016/j.cognition.2012.07.002

17. H. G. Kuhn, H. Dickinson-Anson, F. H. Gage, Neurogenesis in the dentate gyrus of the adult rat: Age-related decrease of neuronal progenitor proliferation. *J. Neurosci.* **16**, 2027–2033 (1996). Medline

18. T. Seki, Y. Arai, Age-related production of new granule cells in the adult dentate gyrus. *Neuroreport* **6**, 2479–2482 (1995). Medline doi:10.1097/00001756-199512150-00010

19. S. Freud, *Childhood and Concealing Memories*, in *The Basic Writings of Sigmund Freud*, A. A. Brill, Ed. (Norton, New York, 1905), pp. 30–36.

20. B. A. Campbell, in *Comparative Perspectives on the Development of Memory,* R. V. Kail, N. E. Spear, Eds. (Routledge, 1984), pp. 23–35.

21. D. C. Rubin, The distribution of early childhood memories. *Memory* **8**, 265–269 (2000). Medline doi:10.1080/096582100406810

22. B. A. Campbell, N. E. Spear, Ontogeny of memory. *Psychol. Rev.* **79**, 215–236 (1972). Medline doi:10.1037/h0032690

23. L. Acsády, A. Kamondi, A. Sík, T. Freund, G. Buzsáki, GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *J. Neurosci.* **18**, 3386–3403 (1998). Medline

24. J. J. Kim, M. S. Fanselow, Modality-specific retrograde amnesia of fear. *Science* **256**, 675–677 (1992). Medline doi:10.1126/science.1585183

25. K. G. Akers, M. Arruda-Carvalho, S. A. Josselyn, P. W. Frankland, Ontogeny of contextual fear memory formation, specificity, and persistence in mice. *Learn. Mem.* **19**, 598–604 (2012). Medline doi:10.1101/lm.027581.112

26. S. A. Josselyn, P. W. Frankland, Infantile amnesia: A neurogenic hypothesis. *Learn. Mem.* **19**, 423–433 (2012). Medline doi:10.1101/lm.021311.110

27. H. van Praag, G. Kempermann, F. H. Gage, Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci.* **2**, 266–270 (1999). Medline doi:10.1038/6368

28. R. McGonigal, N. Tabatadze, A. Routtenberg, Selective presynaptic terminal remodeling induced by spatial, but not cued, learning: A quantitative confocal study. *Hippocampus* **22**, 1242–1255 (2012). Medline doi:10.1002/hipo.20998

29. H. van Praag, Exercise and the brain: Something to chew on. *Trends Neurosci.* **32**, 283–290 (2009). Medline doi:10.1016/j.tins.2008.12.007

30. Y. Niibori, T. S. Yu, J. R. Epp, K. G. Akers, S. A. Josselyn, P. W. Frankland, Suppression of adult neurogenesis impairs population coding of similar contexts in hippocampal CA3 region. *Nat Commun* **3**, 1253 (2012). [Medline](#) [doi:10.1038/ncomms2261](#)

31. M. Maekawa, T. Namba, E. Suzuki, S. Yuasa, S. Kohsaka, S. Uchino, NMDA receptor antagonist memantine promotes cell proliferation and production of mature granule neurons in the adult hippocampus. *Neurosci. Res.* **63**, 259–266 (2009). [Medline](#) [doi:10.1016/j.neures.2008.12.006](#)

32. J. E. Malberg, A. J. Eisch, E. J. Nestler, R. S. Duman, Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J. Neurosci.* **20**, 9104–9110 (2000). [Medline](#)

33. N. N. Karpova, A. Pickenhagen, J. Lindholm, E. Tiraboschi, N. Kulesskaya, A. Ágústsdóttir, H. Antila, D. Popova, Y. Akamine, A. Bahi, R. Sullivan, R. Hen, L. J. Drew, E. Castrén, Fear erasure in mice requires synergy between antidepressant drugs and extinction training. *Science* **334**, 1731–1734 (2011). [Medline](#) [doi:10.1126/science.1214592](#)

34. K. Meletis, V. Wirta, S. M. Hede, M. Nistér, J. Lundeberg, J. Frisén, p53 suppresses the self-renewal of adult neural stem cells. *Development* **133**, 363–369 (2006). [Medline](#) [doi:10.1242/dev.02208](#)

35. S. A. Josselyn, S. Kida, A. J. Silva, Inducible repression of CREB function disrupts amygdala-dependent memory. *Neurobiol. Learn. Mem.* **82**, 159–163 (2004). [Medline](#) [doi:10.1016/j.nlm.2004.05.008](#)

36. A. Garthe, J. Behr, G. Kempermann, Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies. *PLOS ONE* **4**, e5464 (2009). [Medline](#) [doi:10.1371/journal.pone.0005464](#)

37. A. Sierra, J. M. Encinas, J. J. Deudero, J. H. Chancey, G. Enikolopov, L. S. Overstreet-Wadiche, S. E. Tsirka, M. Maletic-Savatic, Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* **7**, 483–495 (2010). [Medline](#) [doi:10.1016/j.stem.2010.08.014](#)

38. S. Guidi, E. Ciani, S. Severi, A. Contestabile, R. Bartesaghi, Postnatal neurogenesis in the dentate gyrus of the guinea pig. *Hippocampus* **15**, 285–301 (2005). [Medline](#) [doi:10.1002/hipo.20050](#)

39. H. Eichenbaum, Hippocampus: Cognitive processes and neural representations that underlie declarative memory. *Neuron* **44**, 109–120 (2004). [Medline](#) [doi:10.1016/j.neuron.2004.08.028](#)

40. W. Deng, J. B. Aimone, F. H. Gage, New neurons and new memories: How does adult hippocampal neurogenesis affect learning and memory? *Nat. Rev. Neurosci.* **11**, 339–350 (2010). [Medline](#) [doi:10.1038/nrn2822](#)

41. R. L. Redondo, R. G. Morris, Making memories last: The synaptic tagging and capture hypothesis. *Nat. Rev. Neurosci.* **12**, 17–30 (2011). [Medline](#) [doi:10.1038/nrn2963](#)

42. X. Liu, S. Ramirez, P. T. Pang, C. B. Puryear, A. Govindarajan, K. Deisseroth, S. Tonegawa, Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature* **484**, 381–385 (2012). [Medline](#)

43. L. G. Reijmers, B. L. Perkins, N. Matsuo, M. Mayford, Localization of a stable neural correlate of associative memory. *Science* **317**, 1230–1233 (2007). Medline doi:10.1126/science.1143839

44. B. A. Richards, P. W. Frankland, The conjunctive trace. *Hippocampus* **23**, 207–212 (2013). Medline doi:10.1002/hipo.22089

45. J. F. Danker, J. R. Anderson, The ghosts of brain states past: Remembering reactivates the brain regions engaged during encoding. *Psychol. Bull.* **136**, 87–102 (2010). Medline doi:10.1037/a0017937

46. A. Treves, E. T. Rolls, Computational analysis of the role of the hippocampus in memory. *Hippocampus* **4**, 374–391 (1994). Medline doi:10.1002/hipo.450040319

47. B. P. Zambrowicz, A. Imamoto, S. Fiering, L. A. Herzenberg, W. G. Kerr, P. Soriano, Disruption of overlapping transcripts in the ROSA βgeo 26 gene trap strain leads to widespread expression of β-galactosidase in mouse embryos and hematopoietic cells. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 3789–3794 (1997). Medline doi:10.1073/pnas.94.8.3789

48. T. S. Yu, G. Zhang, D. J. Liebl, S. G. Kernie, Traumatic brain injury-induced hippocampal neurogenesis requires activation of early nestin-expressing progenitors. *J. Neurosci.* **28**, 12901–12912 (2008). Medline doi:10.1523/JNEUROSCI.4629-08.2008

49. S. Marino, M. Vooijs, H. van Der Gulden, J. Jonkers, A. Berns, Induction of medulloblastomas in p53-null mutant mice by somatic inactivation of Rb in the external granular layer cells of the cerebellum. *Genes Dev.* **14**, 994–1004 (2000). Medline

50. A. T. Leslie, K. G. Akers, A. D. Krakowski, S. S. Stone, M. Sakaguchi, M. Arruda-Carvalho, P. W. Frankland, Impact of early adverse experience on complexity of adult-generated neurons. *Transl. Psychiatry* **1**, e35 (2011). 10.1038/tp.2011.38 Medline doi:10.1038/tp.2011.38

51. K. Ohira, T. Miyakawa, Chronic treatment with fluoxetine for more than 6 weeks decreases neurogenesis in the subventricular zone of adult mice. *Mol. Brain* **4**, 10 (2011). Medline doi:10.1186/1756-6606-4-10

52. A. A. Pieper, S. Xie, E. Capota, S. J. Estill, J. Zhong, J. M. Long, G. L. Becker, P. Huntington, S. E. Goldman, C. H. Shen, M. Capota, J. K. Britt, T. Kotti, K. Ure, D. J. Brat, N. S. Williams, K. S. MacMillan, J. Naidoo, L. Melito, J. Hsieh, J. De Brabander, J. M. Ready, S. L. McKnight, Discovery of a proneurogenic, neuroprotective chemical. *Cell* **142**, 39–51 (2010). Medline doi:10.1016/j.cell.2010.06.018

53. H. J. G. Gundersen, E. B. V. Jensen, K. Kiêu, Nielsen J, The efficiency of systematic sampling in stereology—reconsidered. *J. Microsc.* **193**, 199–211 (1999). Medline doi:10.1046/j.1365-2818.1999.00457.x

54. G. Kempermann, F. H. Gage, Genetic influence on phenotypic differentiation in adult hippocampal neurogenesis. *Brain Res. Dev. Brain Res.* **134**, 1–12 (2002). Medline doi:10.1016/S0165-3806(01)00224-3

55. A. G. Dayer, A. A. Ford, K. M. Cleaver, M. Yassaee, H. A. Cameron, Short-term and long-term survival of new neurons in the rat dentate gyrus. *J. Comp. Neurol.* **460**, 563–572 (2003). Medline doi:10.1002/cne.10675

56. D. A. Henze, N. N. Urban, G. Barrionuevo, The multifarious hippocampal mossy fiber pathway: A review. *Neuroscience* **98**, 407–427 (2000). [Medline](#) [doi:10.1016/S0306-4522(00)00146-9](#)

57. D. G. Amaral, J. A. Dent, Development of the mossy fibers of the dentate gyrus: I. A light and electron microscopic study of the mossy fibers and their expansions. *J. Comp. Neurol.* **195**, 51–86 (1981). [Medline](#) [doi:10.1002/cne.901950106](#)

58. M. Hosseini-Sharifabad, J. R. Nyengaard, Design-based estimation of neuronal number and individual neuronal volume in the rat hippocampus. *J. Neurosci. Methods* **162**, 206–214 (2007). [Medline](#) [doi:10.1016/j.jneumeth.2007.01.009](#)