

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 Hirofata 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

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

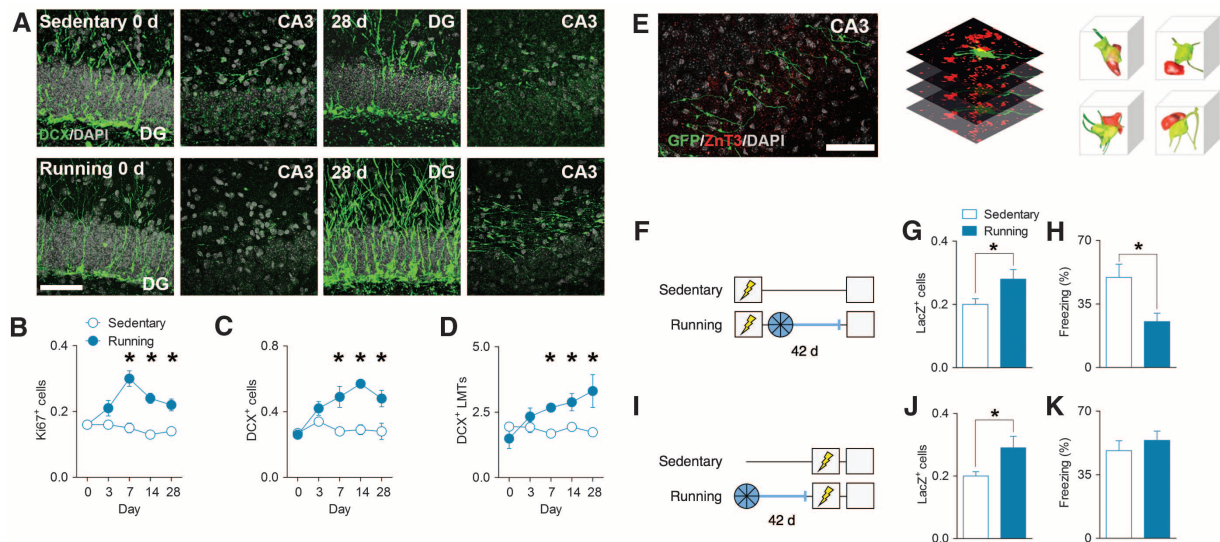
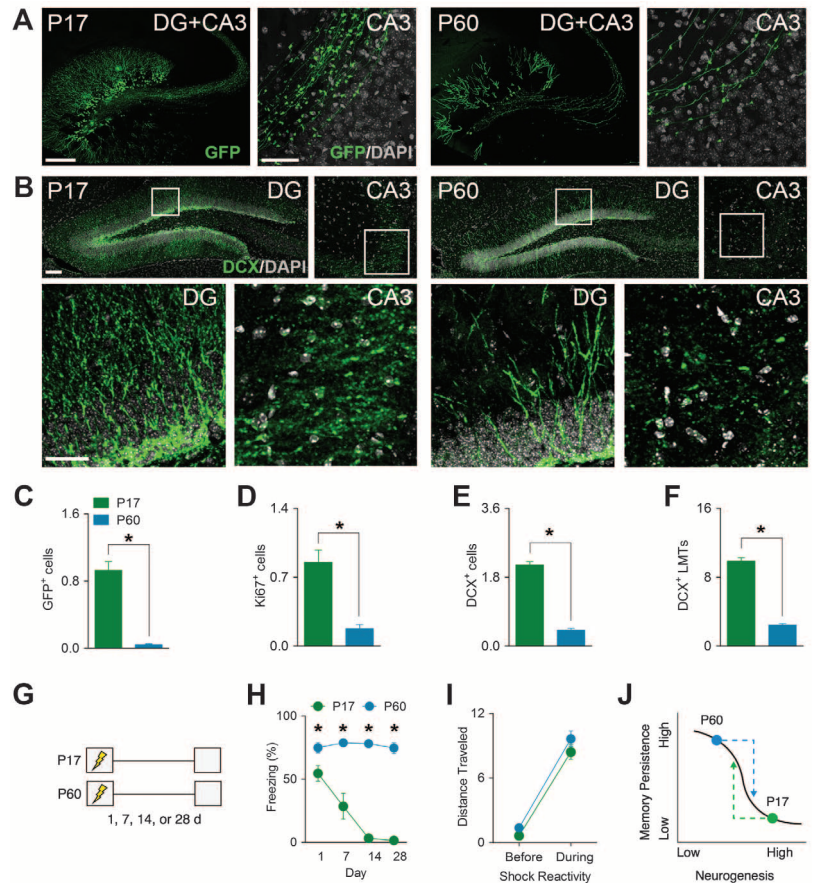


Fig. 2. Voluntary running increases neurogenesis and promotes forgetting in adult mice. (A) Adult (P60) mice given access to a running wheel for 28 days showed more immature (DCX⁺) neurons in the DG and LMTs in the CA3 than sedentary mice. Scale bar, 50 μm . (B to D) Adult mice were given access to a running wheel for 0, 3, 7, 14, or 28 days. Running increased the number of (B) proliferating (Ki67⁺) cells ($n = 8$ per group; *running* \times *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* \times *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* \times *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 . * $P < 0.05$.

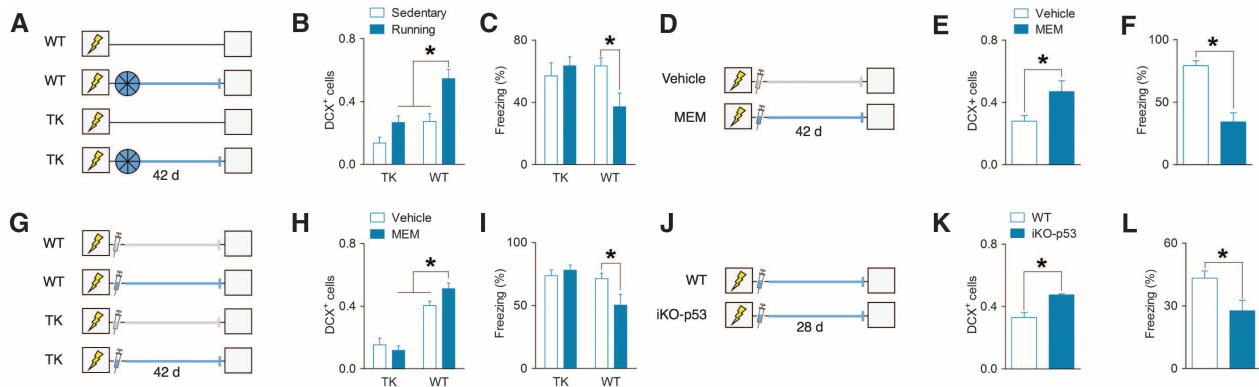


Fig. 3. Forgetting depends on increased neurogenesis. (A) Adult GAN-treated WT and TK⁺ mice remained sedentary or ran after contextual fear conditioning. Running (B) increased neurogenesis (sedentary $n = 6$, running $n = 14$; $t_{18} = 2.81$, $P < 0.05$) and (C) induced forgetting of a context fear 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 $n = 10$; $genotype \times running$ 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, $t_{14} = 8.53$, $P < 0.001$; $genotype \times drug$ interaction, $F_{1,28} = 4.69$, $P < 0.05$) and (I) forgetting (vehicle $n = 13$, MEM $n = 14$; TK⁺ vehicle versus TK⁺ MEM, $P > 0.05$; $genotype \times drug$ 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 . * $P < 0.05$.

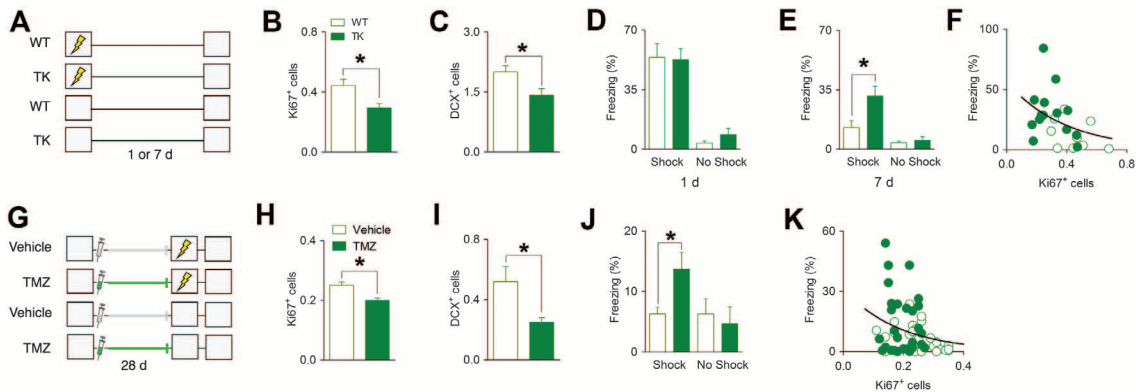


Fig. 4. Genetic and pharmacological decreases in neurogenesis mitigate forgetting in infant mice. (A) Infant GAN-treated WT or TK⁺ mice were trained in contextual fear conditioning or placed in conditioning chambers without foot shocks. TK⁺ mice had fewer (B) proliferating (Ki67⁺) cells (WT $n = 9$, 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 (H) proliferating (Ki67⁺) 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 μ m. (Right) High magnification. Scale bar, 25 μ m. (B) DCX⁺ cells in the DG of infant and adult guinea pigs. (Left) Low magnification. Scale bar, 200 μ m. (Right) High magnification. Scale bar, 25 μ m. 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* \times *delay* interaction and *age* main effect, P s > 0.05).

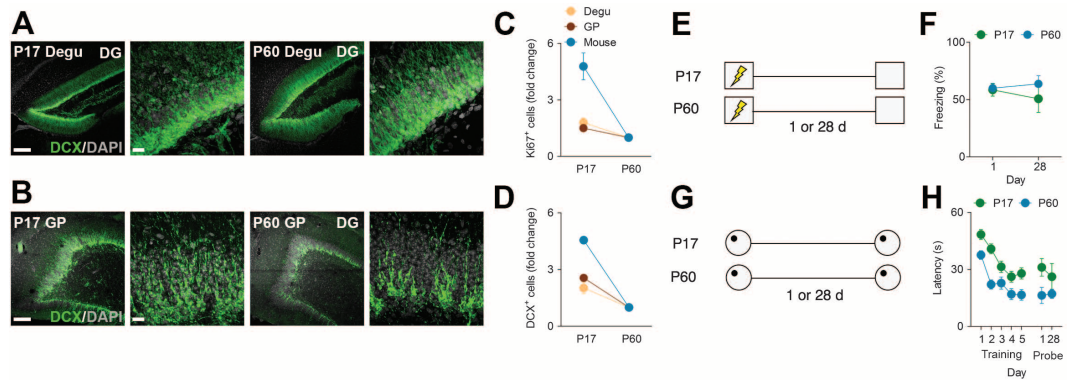
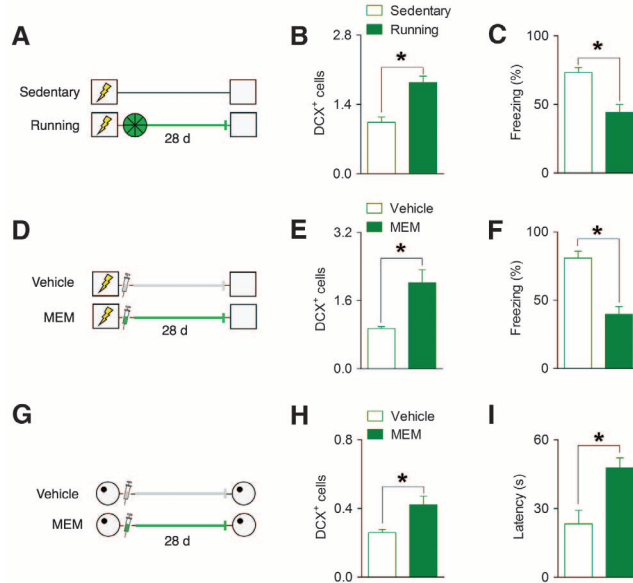


Fig. 6. Increasing neurogenesis promotes forgetting in infant degus and guinea pigs.

(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 μ m². * P < 0.05.



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 pro-neurogenic 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).

(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* \times *delay* interaction, P > 0.05; *age* main effect, $F_{1,28}$ = 6.05, 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

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 hippo-

campal 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 reinvolve 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 Freud 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. Sik, 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).

Acknowledgments: This work was supported by grants from Brain Canada to P.W.F., the Canadian Institutes of Health Research (CIHR) to P.W.F. (MOP86762) and S.A.J. (MOP74650), the Centre for Brain and Behavior at the Hospital for Sick Children to P.W.F. and S.A.J., and the Core Research for Evolutional Science and Technology of Japan Science and Technology Agency (JST-CREST) and Ministry of Education, Culture, Sports, Science and Technology Grants-in-Aid for Scientific Research (KAKENHI) to T.M. K.G.A. and H.-L.H. (CIHR), A.M.-C. (El Consejo Nacional de Ciencia y Tecnología), A.P.Y. (Donald and Doris Milne Alzheimer's Disease Fellowship), A.L.W. (Ontario Mental Health Foundation), and A.G. and B.A.R. (Natural Sciences and Engineering Research Council of Canada) were supported by fellowships. Data are archived in the Dryad repository (doi:10.5061/dryad.s7q3c).

Supplementary Materials

www.sciencemag.org/content/344/6184/598/suppl/DC1
Materials and Methods
Figs. S1 to S17
References (47–58)

25 November 2013; accepted 1 April 2014
10.1126/science.1248903

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of June 30, 2015):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/344/6184/598.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2014/05/07/344.6184.598.DC1.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/344/6184/598.full.html#related>

This article **cites 56 articles**, 14 of which can be accessed free:

<http://www.sciencemag.org/content/344/6184/598.full.html#ref-list-1>

This article has been **cited by** 11 articles hosted by HighWire Press; see:

<http://www.sciencemag.org/content/344/6184/598.full.html#related-urls>

This article appears in the following **subject collections**:

Neuroscience

<http://www.sciencemag.org/cgi/collection/neuroscience>