

Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood

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Neurogenesis is known to take place in the adult brain. This work identifies T lymphocytes and microglia as being important to the maintenance of hippocampal neurogenesis and spatial learning abilities in adulthood. Hippocampal neurogenesis induced by an enriched environment was associated with the recruitment of T cells and the activation of microglia. In immune-deficient mice, hippocampal neurogenesis was markedly impaired and could not be enhanced by environmental enrichment, but was restored and boosted by T cells recognizing a specific CNS antigen. CNS-specific T cells were also found to be required for spatial learning and memory and for the expression of brain-derived neurotrophic factor in the dentate gyrus, implying that a common immune-associated mechanism underlies different aspects of hippocampal plasticity and cell renewal in the adult brain.

Neurogenesis in the hippocampus continues throughout adult life¹ and is a prerequisite for certain aspects of brain cognitive activity^{2,3}. The controlled activity of T cells directed to autoantigens in the CNS is needed for postinjury neuronal survival and functional recovery⁴⁻⁷. Such T cells, after they home to sites of damage and become locally activated, shape the behavior of microglia in a way that makes their phenotype supportive of neural cell survival⁸⁻¹⁰ and renewal¹¹. The profile of these activated microglia differs from that of the classically activated microglia known to be associated with inflammation^{8,9}.

The above findings led us to suspect that a fundamental role of autoimmune T cells, known to be present in healthy individuals, is to help maintain the integrity of the healthy CNS and that their remedial effect under neurodegenerative conditions is a manifestation of the same homeostatic role. We further postulated that if such autoimmune T cells have a role in the healthy CNS, it might well have to do with neurogenesis in adult life, possibly by contributing, through their dialogue with microglia, to the conditions needed for cell renewal.

In this study, using a rat model of environmental enrichment¹², we demonstrated an association between T cells, microglial activity and hippocampal neurogenesis. We further showed that neurogenesis is impaired in immune-deficient mice under both regular and environmental enriched conditions and can be restored by T cells directed to brain autoantigen but not by T cells directed to an irrelevant (nonself) antigen. Similar dependence on T cells was found with respect to spatial learning and memory.

RESULTS

Immune cell recruitment in environmentally enriched rats

Our working hypothesis was that both CNS resident and systemic immune cells are active participants in adult hippocampal neurogenesis. With this objective, we looked for a model in which neurogenesis is augmented physiologically without any intervention¹². Mental and physical activities promote neurogenesis in adulthood by increasing the survival and proliferation, respectively, of newly formed neurons in the dentate gyrus¹³. We anticipated that if T cells and activated resident microglia participate in neurogenesis, they would reach detectable levels under enriched conditions.

Adult Sprague-Dawley rats were housed in standard cages (control) or in an enriched environment (**Supplementary Figure 1** online). After 6 weeks, each rat received a 5-d course of daily intraperitoneal (i.p.) injections of 5-bromodeoxyuridine (BrdU) to enable the detection of newly formed cells. After 1 week, the rats were killed and their hippocampi were examined with antibodies to BrdU, the neuronal marker NeuN and the microglial marker isolectin B4 (IB-4). As expected, significantly more newly formed neurons (BrdU⁺ NeuN⁺) were found in the dentate gyri of rats housed in the enriched environment than in control rats ($P < 0.01$; **Fig. 1a**). Compared to control rats, there was a significant increase in the numbers of microglia (IB-4⁺) as well as of newly formed microglia (IB-4⁺ BrdU⁺) in the dentate gyri of rats kept in the enriched environment (**Fig. 1b-f**). To avoid the possibility that BrdU labeling in IB-4⁺ cells reflects phagocytosis of dying proliferating cells, we were careful to count only IB-4⁺ cells with the typical ramified morphology, in which the BrdU staining

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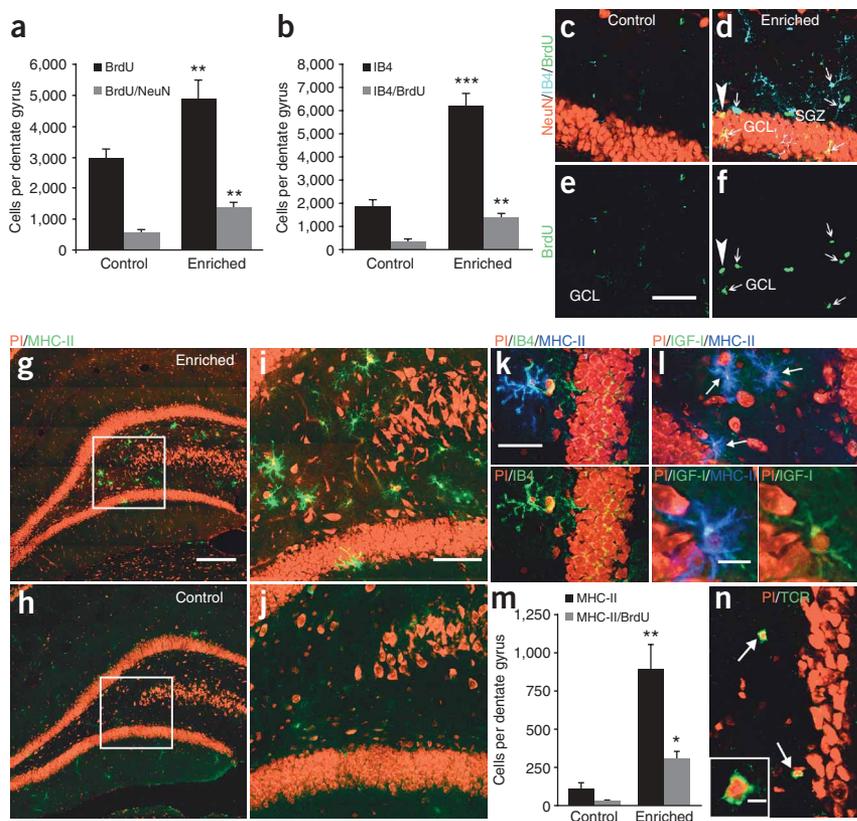


Figure 1 Effects of an enriched environment on newly generated neurons and microglia in the dentate gyrus of adult rats. **(a,b)** Quantification of BrdU⁺ NeuN⁺ and BrdU⁺ IB-4⁺ cells in the dentate gyrus of rats housed in an enriched environment or under standard conditions (control). ** $P < 0.01$, *** $P < 0.001$, t -test; $n = 6$ per group. **(c-f)** Confocal micrographs of newly generated microglia (IB-4⁺ cells, blue) and neurons (NeuN⁺ cells, red) among the newly formed cells (BrdU⁺ cells, green) in standard **(c,e)** or enriched **(d,f)** environmental conditions. **(g,h)** MHC-II⁺ microglia in the dentate gyrus of rats housed in the enriched environment or under standard conditions. **(i)** Higher magnification of the boxed area in **g**. **(j)** Higher magnification of boxed area in **h**. **(k)** All MHC-II⁺ cells were colabeled with the microglial marker IB-4. **(l)** Colocalization of IGF-I⁺ cells with MHC-II⁺ cells in the dentate gyrus. **(m)** Quantification of MHC-II⁺ BrdU⁺ cells in the dentate gyrus. * $P < 0.01$, ** $P < 0.01$, t -test; $n = 6$ per group. **(n)** T cells in the dentate gyrus of rats housed in the enriched environment. Data are expressed as mean \pm s.e.m. SGZ, subgranular zone; GCL, granular cell layer. Scale bar, 100 μ m in **c-f**; 500 μ m in **g,h**; 200 μ m in **i,j**; 10 μ m in **l**; 5 μ m in **n**. PI, propidium iodide.

mice and mice that had the same genetic background but suffered from a deficiency in adaptive immunity and thus lacked both

T- and B-cell populations (severe combined immune deficiency, SCID). All mice received 4 BrdU injections (every 12 h, i.p.). The mice were killed 2 d after the first injection (that is, 12 h after the last injection) and their brains were excised and examined immunohistochemically. We observed significantly fewer BrdU⁺ cells in the SGZ of the dentate gyrus in the SCID mice than in the wild-type controls (mean \pm s.e.m. = $36.4 \pm 2.2\%$ fewer; $P < 0.05$; **Fig. 2a**). These findings suggest that when adaptive immunity is deficient, cell proliferation in the dentate gyrus is impaired.

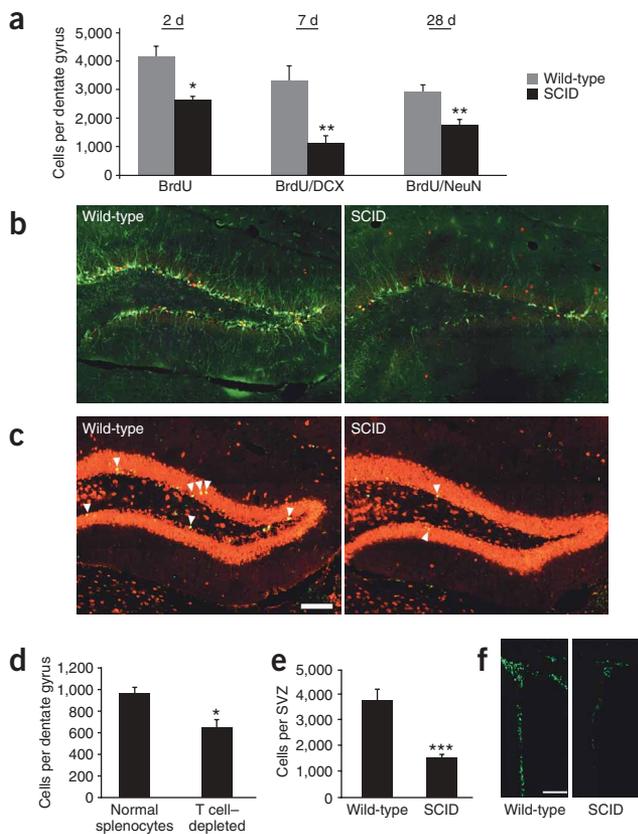
Next we examined whether a deficiency in adaptive immunity also affects the differentiation of newly proliferating cells into neurons. SCID and wild-type mice received 5 BrdU injections (every 12 h, i.p.) and were killed 7 or 28 d after the first injection. On day 7, their dentate gyri were analyzed for the presence of proliferating cells (BrdU⁺) expressing the early neuronal differentiation marker doublecortin (DCX⁺). Significantly fewer BrdU⁺ DCX⁺ cells were found in the dentate gyri of SCID mice than in those of the wild type ($65.9 \pm 8.1\%$ fewer; $P < 0.01$; **Fig. 2a**). The two groups did not differ significantly in the ratio of new neurons (BrdU⁺ DCX⁺ cells) to the total number of proliferating (BrdU⁺) cells ($60.2 \pm 4.8\%$ and $57.8 \pm 6.3\%$, respectively; $P = 0.8$, t -test).

On day 28, we saw significantly fewer cells double labeled for BrdU and NeuN in the SCID mice than in the wild type ($39.9 \pm 7.2\%$ fewer; $P < 0.01$; **Fig. 2a**). Confocal micrographs showed differences between wild-type and SCID mice in the numbers of BrdU⁺ DCX⁺ cells on day 7 (**Fig. 2b**) and of BrdU⁺ NeuN⁺ cells on day 28 (**Fig. 2c**). Taken together, these results suggest that the effect exerted by the adaptive immune system on hippocampal neurogenesis is not transient: at all time points tested, the absolute number of newly formed neurons was higher in the wild-type mice than in the SCID mice. Double staining for BrdU and the astrocytic marker glial fibrillary acidic protein (GFAP) showed no

appeared at the center of the cell. Previous studies have distinguished between liposaccharide (LPS)-activated microglia (the classically activated microglia that apparently impair neurogenesis; ref. 14) and the microglia activated by cytokines associated with adaptive immunity, which are neuroprotective⁸. Thus, for example, microglia primed with the T-helper cell type 2 (T_H2)-derived cytokine interleukin-4 (IL-4) strongly express class-II major histocompatibility complex (MHC-II) and insulin-like growth factor I (IGF-I)⁸. To determine whether the phenotype of microglia observed in the dentate gyri of rats housed in an enriched environment resembles that of microglia activated by T cell-derived cytokines, we stained adjacent sections from the same brains with antibodies to MHC-II (**Fig. 1g-j**). Analysis of the dentate gyri of the environmentally enriched rats showed that approximately 30% of the microglia (IB-4⁺) expressed MHC-II (**Fig. 1k**). These microglia were localized in the hilus, in close proximity to the subgranular zone (SGZ) of the dentate gyrus or even incorporated into the granular cell layer itself (**Fig. 1i**). Many of the MHC-II⁺ IB-4⁺ cells were also stained for IGF-I (**Fig. 1l**), a growth factor known to be associated with neurogenesis¹⁵ and neuroprotection¹⁶ induced by physical activity. Quantification of MHC-II⁺ cells in the dentate gyrus showed that their numbers were increased approximately fivefold under enriched conditions (**Fig. 1m**). Staining with antibodies to a T-cell receptor showed the presence of some T cells in the parenchyma of the hilus of environmentally enriched rats (**Fig. 1n**), suggesting that a local interaction between microglia (MHC-II⁺) and T cells was taking place.

Impairment of neurogenesis in immune-deficient mice

The detection of T cells in the hippocampus of adult rats under enriched conditions raised the question of whether T cells contribute to neurogenesis under normal conditions. To address this question, we compared neurogenesis in the dentate gyri of wild-type adult C57Bl/6J



differences in the ratio of BrdU⁺ GFAP⁺ cells to the total number of BrdU⁺ cells between the wild-type and SCID mice ($8.0 \pm 0.8\%$ and $7.8 \pm 1.0\%$, respectively; $P = 0.9$, t -test; $n = 5$ per group). Because the absence of adaptive immunity—as manifested in the number of newly formed neurons—seemed to be most pronounced 7 d after the first BrdU injection, this was chosen as the time point of reference for hippocampal neurogenesis throughout the rest of the study.

The *scid* mutation is also characterized by defective DNA-dependent protein kinase activity, with resulting impairment of DNA repair¹⁷. To exclude the possibility that the impairment of neurogenesis observed in the SCID mice was caused by this nonimmunological defect and to verify that the impaired neurogenesis in these mice could be attributed specifically to a deficiency of T cells, we compared neurogenesis in SCID mice replenished by the intravenous (i.v.) injection of splenocytes from wild-type matched controls ('normal splenocytes') to that in SCID mice replenished with splenocytes depleted of T cells. The mice were injected with BrdU 17 d after splenocyte replenishment and their hippocampi were analyzed 7 d later. The T-cell content of each replenished mouse on the day of hippocampal excision was verified by fluorescence-activated cell sorter (FACS) analysis (Supplementary Fig. 2 online). Significantly more BrdU⁺ DCX⁺ cells were found in the dentate gyrus of SCID mice replenished with whole splenocytes than in that of SCID mice replenished with splenocytes depleted of T cells ($P < 0.05$; Fig. 2d).

Continuous neurogenesis from neural stem cells and/or progenitor cells in the adult CNS also occurs in the subventricular zone (SVZ) of the lateral ventricles. We quantified the BrdU⁺ cells in the SVZ on day 2 after BrdU injection and found significantly fewer cells in the SCID mice than in the wild-type mice ($60.6 \pm 4.8\%$ fewer; $P < 0.001$; Fig. 2e,f), implying that immune cells contribute to progenitor cell proliferation in both germinal centers of the adult brain.

Figure 2 Neurogenesis is impaired in mice with severe combined immune deficiency. (a) C57Bl/6J mice (SCID and wild-type) were killed 2, 7 or 28 d after the first BrdU injection, and their dentate gyrus was analyzed for BrdU⁺ cells on day 2 ($*P < 0.05$, t -test; $n = 3$ per group; one of three independent experiments), BrdU⁺ DCX⁺ cells on day 7 ($**P < 0.01$, t -test; $n = 5$ and $n = 6$ for wild-type and SCID mice, respectively) and BrdU⁺ NeuN⁺ cells on day 28 ($**P < 0.01$, t -test; $n = 5$ per group). (b) Representative confocal micrographs of the dentate gyri of wild-type and SCID mice that were double stained for BrdU (red) and DCX (green) 7 d after the first BrdU injection. (c) Representative confocal micrographs of the dentate gyrus of wild-type and SCID mice that were double stained for BrdU (green) and NeuN (red) 28 d after the first BrdU injection. (d) Quantification of BrdU/DCX double-labeled cells in the dentate gyrus, 7 d after the first BrdU injection, in SCID mice replenished with syngeneic wild-type splenocytes or with splenocytes depleted of T cells. $*P < 0.05$, t -test; $n = 5$ per group. (e) Quantification of BrdU-labeled cells in the unilateral SVZ, 2 d after the first injection. $**P < 0.001$, t -test; $n = 6$ and 4 for wild-type and SCID groups, respectively. (f) Representative confocal micrographs of the SVZ of wild-type and SCID mice stained for BrdU. Data are expressed as mean \pm s.e.m. Scale bar, 100 μ m.

The above results raised a key question: can activity-induced neurogenesis occur in immune-deficient mice? We therefore examined whether environmental enrichment enhances hippocampal neurogenesis in SCID mice. The enrichment protocol and BrdU injection protocol used for these experiments in mice were the same as those used for the rat experiments (see Supplementary Methods online and Fig. 1). SCID mice housed in the enriched environment did not show an increase in neurogenesis relative to their matched controls (mice housed in standard conditions); wild-type mice showed the expected increase ($77.2 \pm 7.7\%$ increase, Fig. 3). Because we observed no overt behavioral differences between the immune-deficient and wild-type mice, these results provide evidence that enriched conditions enhance neurogenesis by a mechanism that requires peripheral immune cells.

Impairment of neurogenesis in mice devoid of T cells

To further substantiate our hypothesis that the peripheral immune cells participating in adult neurogenesis are T cells, we compared the formation of new neurons in wild-type Balb/c/OLA mice to that in strain-matched nude mice, which are deprived of their mature T-cell population but not of their B cells. As with SCID mice, 7 d after the BrdU injection we saw significantly fewer cells double labeled with BrdU and DCX in the dentate gyrus of the nude mice than in that of

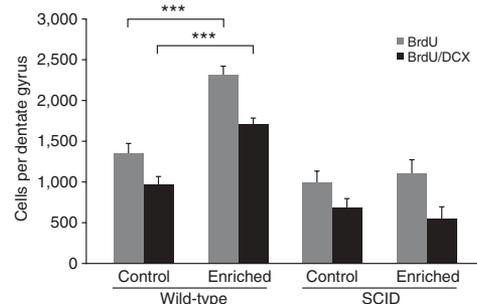


Figure 3 An enriched environment stimulates neurogenesis in Balb/c wild-type mice, but not in Balb/c SCID mice. Quantification of BrdU-labeled cells (gray bars) and BrdU/DCX double-labeled cells (black bars) in the dentate gyrus of wild-type and SCID mice housed in an enriched environment or under standard (control) conditions. $**P < 0.001$, t -test, $n = 6$ per group for the wild type; and $P = 0.3$ for BrdU-labeled cells and $P = 0.9$ for BrdU/DCX-double labeled cells, t -test, $n = 5$ per group for SCID.

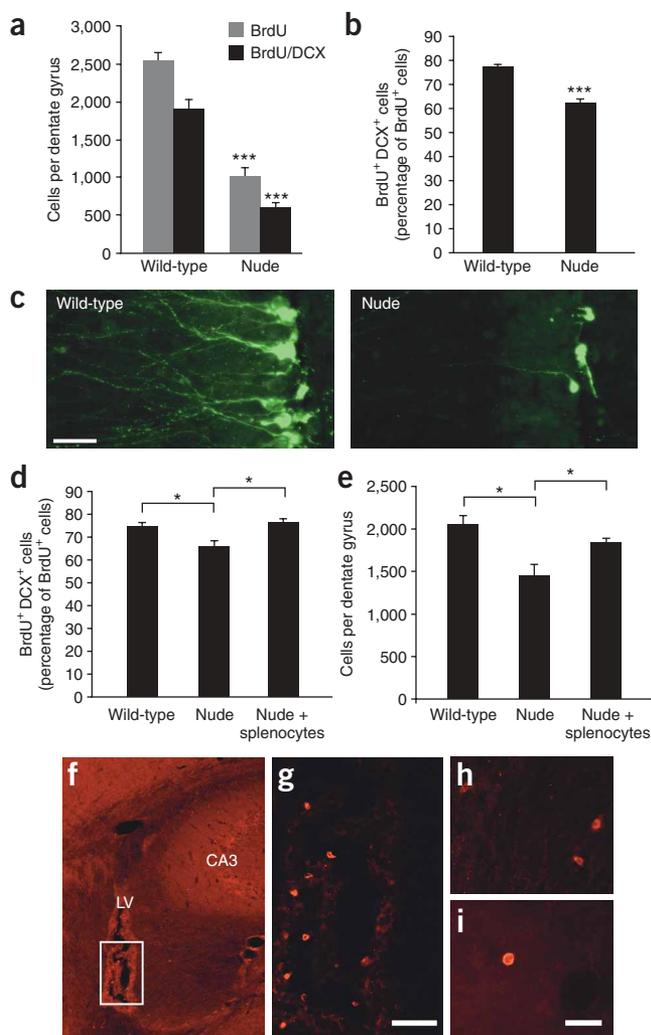


Figure 4 Impaired neurogenesis in T cell-deficient mice. **(a)** Quantification of BrdU⁺ cells and BrdU⁺ DCX⁺ cells in the dentate gyrus of nude and wild-type mice 7 d after the first BrdU injection. *** $P < 0.001$, t -test; $n = 4$ per group. **(b)** Number of BrdU⁺ DCX⁺ cells as a percentage of the total number of BrdU⁺ cells in the dentate gyrus. *** $P < 0.001$, t -test; $n = 4$ per group. **(c)** Dendritic arborization examined by DCX staining in wild-type and nude mice. **(d)** Number of BrdU⁺ DCX⁺ cells as a percentage of the total number of BrdU⁺ cells in the dentate gyrus of wild-type, nude and splenocyte-replenished nude mice, 7 d after the first BrdU injection. * $P < 0.05$, ANOVA; $n = 5$ per group. **(e)** Total numbers of PCNA⁺ cells in the dentate gyrus of wild-type, nude and splenocyte-replenished nude mice. * $P < 0.05$, ANOVA; $n = 4$ per group. **(f)** T cells (CD3⁺) in the brain of a replenished nude mouse. **(g)** Higher magnification of the marked area in **f**. **(h)** CD3⁺ cells lining the wall of the third ventricle in the brain of a wild-type mouse. **(i)** CD3⁺ cells in the parenchyma adjacent to a blood vessel in the brain of a replenished nude mouse. Data are expressed as mean \pm s.e.m. LV, lateral ventricle; CA3, CA3 of the hippocampus. Scale bar, 20 μ m in **c**, 50 μ m in **g** and 25 μ m in **h** and **i**.

numbers were similar to those in the wild type ($P = 0.2$, ANOVA) (**Fig. 4e**). Thus, T cell-induced enhancement of proliferation in the nude mice was observed 17 d after replenishment (as in the case of SCID mice; **Fig. 2d**), but not 10 d after replenishment. These findings supported our suggestion that the impaired neurogenesis observed in nude mice was T cell-dependent and could be partially restored in the adult animal.

Using antibodies to CD3 (anti-CD3), we were able to detect T cells in the brain parenchyma of both wild-type and splenocyte-replenished nude mice, mainly around the walls of the ventricles adjacent to the hippocampus (**Fig. 4f–i**). The location of the T cells was not restricted, however, to areas of active adult neurogenesis.

CNS-specific T cells contribute to adult neurogenesis

Antigenic specificity to CNS autoantigens, such as certain peptides of myelin basic protein (MBP), is required for the manifestation of the beneficial effect of T cells under traumatic or degenerative conditions^{4,5,18}. To determine whether the same specificity rule is also applicable to the T-cell effect on adult neurogenesis, we used transgenic mice possessing a normal B-cell population and a genetically engineered excess of monospecific T cells directed either to MBP (T_{MBP}) or to an irrelevant (nonself) antigen. The ‘ T_{MBP} -transgenic’ mice express a transgene encoding a T-cell receptor that recognizes an epitope (Ac1–11) of MBP, and therefore approximately 98% of the T-cell pool in these mice consists of Ac1–11 T_{MBP} cells. The remaining 2% are endogenous T cells whose regulatory activity is apparently sufficient to prevent the T_{MBP} -transgenic mice from spontaneously developing autoimmune encephalomyelitis¹⁹. As a control for the antigenic specificity, we used

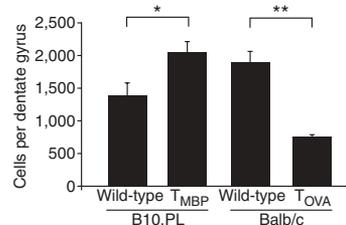


Figure 5 Neurogenesis is maintained by T cells specific to MBP but not by those specific to ovalbumin. BrdU/DCX double-labeled cells in the dentate gyrus of B10.PL wild-type and T_{MBP} -transgenic mice and in that of Balb/c wild-type mice and T_{OVA} -transgenic mice, 7 d after the first BrdU injection. * $P < 0.05$, t -test; $n = 5$ and $n = 8$, respectively (one of two independent experiments). ** $P < 0.01$, t -test; $n = 5$ per group. Data are expressed as mean \pm s.e.m.

the wild type ($68 \pm 8\%$ fewer; $P < 0.001$; **Fig. 4a**). Notably, unlike in the SCID mice, the ratio of new neurons (BrdU⁺ DCX⁺ cells) to the total number of proliferating (BrdU⁺) cells was significantly smaller in the nude mice than in the wild type ($P < 0.001$; **Fig. 4b**), suggesting that T cells can affect not only proliferation but also neuronal differentiation. Moreover, the dendritic arborization of DCX⁺ cells in the two groups differed markedly: dendrites in the nude mice were less abundant and shorter than those in the wild type (**Fig. 4c**).

We also transferred splenocytes (containing a normal population of mature T cells) from wild-type mice to syngeneic nude mice and, as in the SCID mice, analyzed the hippocampi 17 d later. To analyze the results in the same splenocyte-recipient mice at an additional time point, we used two markers of cell proliferation: BrdU (injected 10 d after splenocyte transfer) and an endogenous marker of proliferating cells, proliferating cell nuclear antigen (PCNA). The ratio of BrdU⁺ DCX⁺ cells to the total number of BrdU⁺ cells was significantly higher in the dentate gyri of nude mice replenished with splenocytes than in those of nude mice without such replenishment ($P < 0.05$; **Fig. 4d**). Whereas the total numbers of BrdU-labeled cells in the dentate gyrus did not differ significantly between the replenished nude mice and the nonreplenished nude controls ($P = 0.9$, analysis of variance (ANOVA); data not shown), PCNA-labeled cells (which detected the proliferating cells at the day of excision, day 17) in the replenished nude mice were significantly more numerous than in the controls, and their mean

transgenic mice that express a T-cell receptor that recognizes ovalbumin (' T_{OVA} -transgenic' mice) and thus bears mainly T_{OVA} cells⁵.

Because the two types of transgenic mice had different genetic backgrounds, we compared each of them to their matched wild-type controls. Significantly more BrdU⁺ DCX⁺ cells were found in the dentate gyrus of T_{MBP} -transgenic mice than in that of their wild-type counterparts ($P < 0.05$; Fig. 5). Under the same experimental conditions, significantly fewer BrdU⁺ DCX⁺ cells were seen in the T_{OVA} -transgenic mice than in their controls ($P < 0.01$; Fig. 5). In addition, the enriched environment did not induce enhanced neurogenesis in T_{OVA} -transgenic mice: the number of BrdU⁺ DCX⁺ cells in the dentate gyri of T_{OVA} -transgenic mice that were housed in an enriched environment did not differ from that in T_{OVA} -transgenic mice housed under standard conditions (962 ± 45 and $1,054 \pm 77$, respectively; $P = 0.5$, t -test; $n = 3$ and $n = 4$, respectively). These findings imply that the T cells that contribute to activity-induced neurogenesis in adult mice are CNS specific. Comparison of glial differentiation in the T_{MBP} - and T_{OVA} -transgenic mice and their relevant wild-type controls showed no significant differences between them ($P = 0.9$ and $P = 0.3$, respectively; t -test; data not shown).

To verify that the effect of CNS-specific T cells on neurogenesis is exerted by means of their activation of microglia, we used the antibiotic drug minocycline, which blocks microglial activity in the CNS under pathological inflammatory conditions²⁰. The experiment was carried out with T_{MBP} -transgenic mice because of the likelihood that their higher incidence of CNS-specific T cells and enhanced neurogenesis relative to the wild type would make it easier to detect any potential effect of the drug. Treatment of the T_{MBP} -transgenic mice with a daily dose of minocycline resulted in a significant decrease in the number of BrdU⁺ DCX⁺ cells in the dentate gyrus relative to controls injected with phosphate-buffered saline (PBS) (769 ± 55 and 990 ± 54 , respectively, $P < 0.05$, t -test; $n = 3$ per group). This finding supported our hypothesis that the effect of CNS-specific T cells on neurogenesis is mediated, at least in part, by their interaction with microglia.

CNS-specific T cells contribute to spatial learning

Recent findings point to an association between adult neurogenesis and certain hippocampal activities^{3,21}, although an association between adult neurogenesis and performance on the hippocampus-dependent, spatial learning and memory task in the Morris water maze (MWM) is still a matter

of debate^{3,22}. Spatial learning and memory, as assessed by MWM performance²³, is impaired in immune-deficient mice²⁴. This, together with our finding that hippocampal neurogenesis is significantly impaired in mice deficient in CNS-specific T cells, prompted us to examine whether spatial learning and memory are dependent on the presence of autoimmune T cells. We compared the MWM performance of T_{MBP} mice and T_{OVA} mice relative to their respective matched wild-type controls. To ensure that any observed differences in performance in the MWM could be attributed to cognitive activity rather than to motor ability, we first subjected all the mice to a rotarod test. We recorded the time that each mouse spent balancing on the rotating rod before falling and found no significant differences in fall latencies among all tested groups (T_{MBP} , 39 ± 2.3 s and B10.PL wild-type, 37.6 ± 1.1 s, $F_{(1,6)} = 0.0635$, $P = 0.04$, ANOVA; T_{OVA} , 40.7 ± 1.9 s and Balb/c wild-type, 40.7 ± 4.2 s, $F_{(1,5)} = 0.004$, $P = 0.96$, ANOVA).

When tested in the MWM, the T_{MBP} -transgenic mice performed better than their controls in all three phases of the task (Fig. 6a–b). In the acquisition phase, the T_{MBP} mice took significantly less time than the controls to find the hidden platform (three-way repeated-measures analysis of variance (ANOVA); groups: $F_{1,9} = 6.9$, $P < 0.03$; trials: $F_{3,27} = 21.3$, $P < 0.0001$; days: $F_{3,27} = 6.8$, $P < 0.002$; Fig. 6a). In the extinction phase, in which the platform was removed from the water maze, T_{MBP} mice spent a significantly larger proportion of time than their controls in the quadrant that formerly contained the platform (one-way ANOVA; $F_{1,9} = 35.1$, $P < 0.0003$). In the reversal phase of the task, when the platform was placed in a position opposite to its former location, the T_{MBP} mice again took significantly less time than the controls to find it (three-way repeated-measures ANOVA; groups: $F_{1,9} = 5.3$, $P < 0.05$; trials: $F_{3,27} = 4.5$, $P < 0.01$; days: $F_{1,9} = 18.1$, $P < 0.003$; Fig. 6b). In contrast to the T_{MBP} mice, the performance of T_{OVA} -transgenic mice was significantly worse than that of their

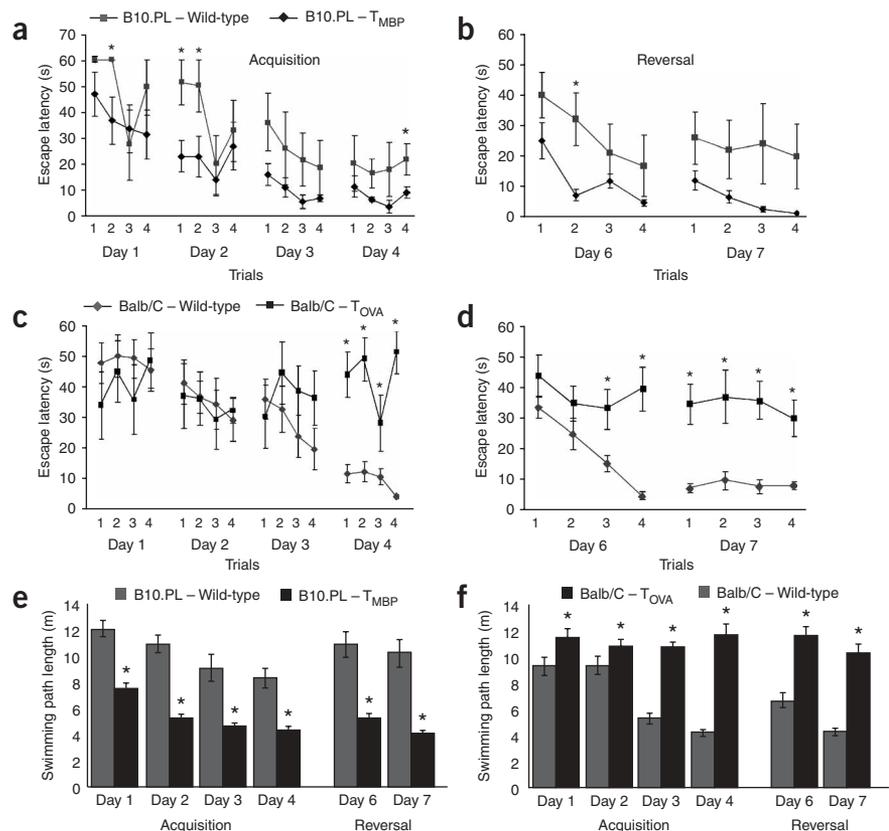


Figure 6 Spatial learning and memory are maintained by T cells specific to MBP but not by those specific to ovalbumin. (a–d) Monitoring T_{MBP} (a,b) and T_{OVA} (c,d) transgenic mice and their wild-type controls in acquisition (a,c) and reversal phases (b,d) of a spatial learning and memory task in the Morris water maze. (e,f) Average swimming path lengths during training sessions of T_{MBP} (e) and T_{OVA} (f) mice and their respective wild-type controls. No significant intergroup differences were found in swimming speed. * $P < 0.05$ (Scheffé test), indicating significant *post-hoc* differences between individual groups. Data are expressed as mean \pm s.e.m.

matched controls in all phases of the MWM: acquisition (three-way repeated-measures ANOVA; groups: $F_{1,18} = 24.4$, $P < 0.0003$; trials: $F_{3,54} = 5.8$, $P < 0.002$; groups \times trials: $F_{3,54} = 6.3$, $P < 0.0002$; groups \times days: $F_{3,54} = 3.2$, $P < 0.035$), extinction (one-way ANOVA; $F_{1,19} = 5.3$, $P < 0.035$) and reversal (three-way repeated-measures ANOVA; groups: $F_{1,18} = 63.1$, $P < 0.0001$; trials: $F_{3,54} = 3.3$, $P < 0.03$) (Fig. 6c–d). We measured the length of the path taken by each mouse to reach the hidden platform (Fig. 6e,f) and found that, relative to their respective wild-type controls, the path taken by the T_{MBP} mice was shorter (two-way repeated-measures ANOVA; acquisition phase—groups: $F_{1,9} = 73.5$, $P < 0.0001$; trials: $F_{3,27} = 14.9$, $P < 0.0001$; and reversal phase—groups: $F_{1,9} = 45.07$, $P < 0.0001$) but the path taken by the T_{OVA} mice was longer (two-way repeated-measures ANOVA; acquisition phase—groups: $F_{1,18} = 105.6$, $P < 0.0001$; trials: $F_{3,54} = 19.2$, $P < 0.0001$; groups \times trials: $F_{3,54} = 11.4$, $P < 0.0001$; and reversal phase—groups: $F_{1,18} = 60.49$, $P < 0.0001$; trials: $F_{3,54} = 29.59$, $P < 0.0001$). The performance of T_{OVA} mice in this test was similar to that of the SCID mice in a previous study²⁴.

T cells can provide neurotrophic factors²⁵, such as brain-derived neurotrophic factor (BDNF), and can regulate, by means of their secreted cytokines, the production of growth factors (for example, IGF-I) by other CNS-resident cells such as the microglia⁸. In the absence of external stimuli (for example, an enriched environment), microglia expressing MHC-II or IGF-I are hardly detectable in the dentate gyrus. We postulated that additional factors might have a role in the constitutive T cell–dependent neurogenesis. BDNF is an essential component of many hippocampal activities, including both spatial learning and memory²⁶ and adult neurogenesis²⁷. We compared the expression of BDNF in the hippocampal dentate gyri of SCID, T_{MBP} -transgenic and T_{OVA} -transgenic mice to that in their wild-type counterparts. Relative to their respective wild-type controls, BDNF immunoreactivity was significantly lower in both SCID mice and T_{OVA} -transgenic mice, but significantly higher in T_{MBP} -transgenic mice ($P < 0.05$; Fig. 7a,b). Staining for BDNF and NeuN showed that the cellular source of BDNF in the hippocampus was neurons (Fig. 7c). It should be noted, however, that BDNF expression in the nude mice did not differ significantly from that of the wild type ($P = 0.3$; t -test; $n = 5$ per group; data not shown), suggesting the existence of a compensatory mechanism in nude mice.

DISCUSSION

The present work identifies CNS-specific autoimmune T cells as being important to adult brain plasticity and shows that their involvement occurs, at least in part, by means of their cross-talk with resident microglia. This new finding is in line with earlier demonstrations that CNS-specific T cells, provided that the onset, duration and intensity of their activity are well controlled, exert a beneficial effect on neuronal survival after CNS injury^{4,28,29}.

The findings of this study suggest that T cells affect adult neurogenesis, both in the dentate gyrus and in the SVZ, primarily by means of their effect on progenitor-cell proliferation. We cannot rule out the possibility that T cells have a role not only in the proliferation of progenitor cells but also in their neuronal differentiation, as indicated by the results obtained with nude mice.

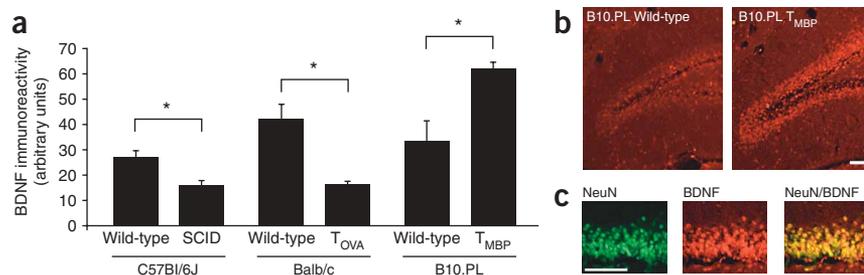


Figure 7 Expression of BDNF in the hippocampus correlates with hippocampal neurogenesis and spatial learning and memory. Coronal sections of the hippocampus were stained for BDNF. (a) Quantification of BDNF immunoreactivity (in arbitrary units) in the dentate gyrus of C57Bl/6J wild-type and SCID mice ($n = 3$ per group), Balb/c wild-type and T_{OVA} -transgenic mice ($n = 4$ and $n = 3$, respectively), and B10.PL wild-type and T_{MBP} -transgenic mice ($n = 3$ per group). (b) BDNF staining in the dentate gyrus of B10.PL wild-type and T_{MBP} -transgenic mice. (c) Confocal images showing colocalization of BDNF and NeuN in the dentate gyrus. * $P < 0.05$, t -test. Data are expressed as mean \pm s.e.m. Scale bar, 100 μ m.

The fact that hippocampal neurogenesis was impaired in mice with T-cell deficiencies of different causations argues against the possibility of a nonimmunological effect resulting, for example, from gene mutation. Moreover, the fact that neurogenesis was partially restored by replenishment of the T-cell population in immune-compromised adult animals further argues in favor of T-cell participation in adult neurogenesis. In this study, we did not use mice whose immune deficiency results from the knockout of the recombination-activating gene *Rag1*, because *Rag1* transcript is present in the mouse brain and specifically in the hippocampus³⁰ and its function there is currently unknown.

Differences in the numbers of BrdU⁺ cells between wild-type and SCID mice were found at all time points tested after BrdU injection but were greatest after 7 d. These results suggest a possible effect of T cells on the survival of newly formed neurons, in addition to their effect on cell proliferation. This could not be accurately evaluated, however, because cell proliferation rates were slower in immune-deficient mice than in the corresponding wild-type mice. Thus, the increased difference between wild-type and SCID mice observed on day 7 (relative to day 2) after BrdU injection might be attributable both to their continued proliferation at different rates and to an earlier loss of newly formed cells in the absence of T cells. Notably, comparison of the cell numbers on day 7 and day 28 showed that the rate of cell loss during the period between these two time points seemed to be higher in the wild-type mice than in the SCID mice. The apparently smaller loss of neurons in the SCID mice might be attributable to their relatively lower rate of proliferation; this may, in turn, have contributed to the relatively late increase in the number of detectable BrdU⁺ cells in SCID mice, unlike in the wild type in which the high proliferation rate could result in the dilution of BrdU beyond detectable levels.

T_{MBP} -transgenic mice showed increased neurogenesis and improved spatial-learning abilities relative to the wild type, whereas both neurogenesis and spatial learning were impaired in T_{OVA} mice; this finding suggests that the T cells, if they are to function properly in brain plasticity, should become activated by their cognate brain antigens. Thus, the mere presence of T cells is not enough to maintain neurogenesis and learning abilities; these T cells need, in addition, to be directed to CNS antigens. The relevant CNS-specific antigen is probably not restricted to MBP; previous studies using various models of CNS injuries^{4–7} have shown that other CNS antigens might be similarly effective. Specificity to autoantigens is a requirement for the homing of T cells to sites in which their relevant antigens reside and in

which they are needed; it does not (as in the traditional perception of autoimmunity) imply an attack on the self, as long as the activity of the T cells is rigorously regulated²⁸. It is possible that the relevant T cells, which continuously survey the healthy CNS (refs. 31 and 32), serve as mobile mini-factories for growth factors^{25,33,34}.

In our view, T cells interact locally with microglia¹⁰ and possibly also with other cellular components (such as endothelial cells and astrocytes) of the special microenvironment known as the 'stem-cell niche' (ref. 35). Local immune activity, supporting progenitor-cell proliferation outside the CNS, occurs in the stem-cell niche of the mouse colon³⁶. The finding that microglia expressing MHC-II proteins can be seen adjacent to a site of increased neurogenesis (induced by an enriched environment) further supports the notion that T cells can interact locally with microglia through these proteins. The reduction in neurogenesis observed in T_{MBP} -transgenic mice after treatment with minocycline supports our suggestion that T cells can benefit neurogenesis by activating microglia (or other CNS-resident antigen-presenting cells such as, for instance, perivascular dendritic cells). This does not argue against reported observations that severe inflammation, whether induced experimentally (by LPS) or as a result of neurodegenerative conditions (such as Alzheimer disease or severe chronic multiple sclerosis), impairs neurogenesis²⁰. In contrast, our results might explain why pathological conditions such as acute CNS insult and experimental autoimmune encephalomyelitis might be accompanied by an increase in neurogenesis as well as by the induction of neurogenesis in non-neurogenic areas^{37,38}. Indeed, specifically activated microglia can induce neurogenesis from adult neural progenitor cells *in vitro*^{11,39}. Such microglia locally produce growth factors such as IGF-I. Accordingly, it is feasible that 'resting' microglia, recently described as highly dynamic surveillants of the brain⁴⁰, function in the dentate gyrus as standby cells not only for housekeeping and repair but also to support neurogenesis. The finding that microglia proliferate in the cortex of mice after physical activity and environmental enrichment⁴¹ might be in line with our data.

The reduction in BDNF in the absence of functional CNS-specific T cells points to the participation of the immune system in additional molecular pathways related to neurogenesis and hippocampal plasticity, and suggests that immune-deficient mice might show impairment in other aspects of brain plasticity as well. It is likely, moreover, that besides IGF-I and BDNF, other factors affected directly or indirectly by T cells are also important in the functioning of the stem-cell niche.

Additional factors that are indirectly affected by T-cell deficiency and that might also contribute to the observed impaired neurogenesis in immune-deficient mice could be related to the hypothalamic-pituitary-adrenal (HPA) axis. Stress-related malfunction of the HPA axis, with no differences in basal levels of corticosterone, impairs adult neurogenesis⁴². This might be relevant to nude mice, which have a T cell-related impairment of HPA axis function with normal basal corticosterone⁴³, and would suggest that one mechanism whereby T cells affect neurogenesis involves the regulation of the HPA axis function.

The active participation of CNS-specific T cells in brain-cell renewal does not exclude the possibility that T cells are needed for neurogenesis during development as well. A link between immune-related molecules (such as MHC-I or CD3- ζ) and neuronal development has been proposed in the context of neuron-neuron synapses^{44,45}, but not in the context of synapses between neurons and immune cells.

The results of this study might partially explain the age-related loss of certain cognitive activities by viewing the decline in terms of an aging immune system⁴⁶. They might also explain why conditions of immune compromise (such as AIDS) result in cognitive impairment (HIV-associated dementia; ref. 47).

Systemic manipulations of the immune system, based on increasing the numbers of T cells directed to weak agonists of autoantigens, beneficially affect neurodegenerative conditions by promoting neuronal survival^{4,28,29}. It is likely that the same manipulations (for example, T cell-based vaccination) might lead to increased neurogenesis. Such an approach would therefore yield new ways to maintain the integrity of the aging brain and the diseased mind.

METHODS

Animals. Male Sprague-Dawley rats (12 weeks old) were supplied by the Animal Breeding Center of the Weizmann Institute of Science. C57Bl/6J and Balb/c/OLA wild-type and SCID mice, B10.PL T_{MBP} -transgenic mice and Balb/c/OLA nude and T_{OVA} -transgenic mice, all aged 8–16 weeks, were supplied by the Animal Breeding Center of the Weizmann Institute of Science. The B10.PL wild-type mice were purchased from the Jackson Laboratory. Male mice were used in all experiments except for the enriched-environment experiments, in which female mice were used because male mice can show territorial behavior and aggression leading to stressful consequences that can affect neurogenesis.

Administration of BrdU and tissue preparation. Rats and mice housed under either standard or environmentally enriched conditions were injected i.p. with BrdU (Sigma-Aldrich; 50 mg per kg body weight), once daily for 5 d, after being kept for 6 weeks in their respective environments. They continued to live in those environments for 1 week after the last injection. They were then killed and perfused transcardially, first with PBS and then with 4% paraformaldehyde. Their brains were removed, postfixed overnight and equilibrated in phosphate-buffered 30% sucrose. Free-floating, 40- μ m-thick coronal hippocampal sections were collected on a freezing microtome (Leica SM2000R) and stored at 4 °C before immunohistochemistry. Under standard conditions, BrdU was injected (i.p., 50 mg per kg body weight) at 12-h intervals for 2 d unless differently indicated. Free-floating, 16- μ m or 30- μ m sections were collected and stored at 4 °C before immunohistochemistry.

Antibodies and reagents for immunohistochemistry. Primary antibodies: *Bandeiraea simplicifolia* IB-4 (1:50; Sigma-Aldrich), mouse antibody to MHC-II (1:50; IQ Products), mouse antibody to T-cell receptor ($\alpha\beta$, 1:20; Acris Antibodies), goat antibody to IGF-I (1:20; R&D Systems), rat antibody to BrdU (1:200; Oxford Biotechnology), mouse antibody to NeuN (1:200; Chemicon), goat antibody to DCX (1:400; Santa Cruz Biotechnology), rabbit antibody to GFAP (1:200; Dako), chicken antibody to BDNF (1:100; Promega) and hamster antibody to CD3 (1:200; Southern Biotech). Secondary antibodies: fluorescein avidin D-conjugated donkey anti-goat antibody, Cy-3-conjugated donkey anti-mouse antibody, Cy-3- or Cy-5-conjugated donkey anti-rat antibody, Cy-3-conjugated donkey anti-chicken antibody, biotin-conjugated anti-hamster antibody and Cy-3-conjugated streptavidin antibody (all from Jackson ImmunoResearch). For detailed immunohistochemistry protocols, see **Supplementary Methods** online.

Morris water maze behavioral test. Spatial learning and memory were assessed by performance on a hippocampus-dependent, visuospatial learning task in the MWM. For a detailed protocol, see **Supplementary Methods** online.

Quantification. For microscopic analysis, we used a Zeiss LSM 510 confocal laser scanning microscope (40 \times magnification) or a Nikon E800. Proliferation was assessed by bilateral counting of BrdU⁺ cells in the SGZ of the dentate gyrus (defined as a zone of the hilus, the width of two cell bodies, along the base of the granular layer). Neurogenesis in the dentate gyrus was evaluated by counting the cells that were double labeled with BrdU and DCX or with BrdU and NeuN. Microglial numbers were obtained by counting cells that were double labeled with BrdU and markers of microglia (IB-4) or antigen-presenting cells (MHC-II). We counted the number of labeled cells in nine coronal sections (330 μ m apart) per rat brain and six coronal sections (370 μ m apart) per mouse brain that were stained and mounted on coded slides. To obtain an estimate of the total number of labeled cells per dentate gyrus, the total number of cells counted in the selected coronal sections from each brain was multiplied by the volume index (the ratio

between the volume of the dentate gyrus and the total combined volume of the selected sections).

For quantification of proliferation in the SVZ, we used the protocol previously described⁴⁸; four coronal sections per brain (145 μ m apart), spanning from 0.38 mm anterior to bregma to 0.34 mm posterior to bregma, were stained with BrdU and mounted on coded slides. BrdU⁺ cells were counted manually in the lateral walls of both lateral ventricles. Values obtained from this count were multiplied by the volume index to obtain an estimate of the total number of proliferating cells per lateral ventricle wall. BDNF immunoreactivity was quantified with Image Pro Plus 4.5 software (Media Cybernetics) by measuring the intensity per unit surface area at the granule cell layer of the dentate gyrus. At least three hippocampal sections per mouse were used for this analysis.

Statistical analysis. A two-tailed unpaired Student's *t*-test was used for analyses of the experiments presented in **Figures 1, 2, 5 and 7**. The data from the experiments presented in **Figures 4 and 6** were analyzed with ANOVA.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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