Memory retrieval reduces the number of newly formed hippocampal neurons in mice with baseline and exercise-enhanced levels of neurogenesis

Karin Van der Borght1, Robbert Havekes1, Thomas Bos1, Bart J.L. Eggen2, Eddy A. Van der Zee1

1) Department of Molecular Neurobiology, Graduate school of Behavioural and Cognitive Neurosciences, and 2) Department of Developmental Genetics, Groningen Biomolecular Sciences and Biotechnology Institute; University of Groningen, P. O. Box 14, 9750 AA, Haren, The Netherlands

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Abstract

Adult hippocampal neurogenesis has been hypothesized to play a role in the formation and consolidation of memory. It has also been suggested that the integration of new neurons into the hippocampal network removes outdated information from the hippocampus. Here, we tested both theories using a 14-day exercise paradigm, which caused a significant increase in hippocampal neurogenesis. First, we investigated whether enhanced hippocampal neurogenesis improved performance in the Y-maze, a spatial hippocampus-dependent learning task. Our data show that animals with elevated levels of hippocampal neurogenesis acquired this task significantly faster than sedentary controls. Next, we tested the hypothesis that enhanced neurogenesis removes old memories from the hippocampus. Mice were trained in the Y-maze and hippocampal neurogenesis was subsequently stimulated by 14 days of wheel running. After the exercise period, we observed an improved performance in memory retention and reversal learning, indicating that retrieval of spatial information was facilitated, instead of impaired by enhanced neurogenesis. Therefore, these findings do not support the ‘memory clearance hypothesis’. Moreover, memory retrieval itself caused changes in the generation of new neurons. We observed a significant decrease in the number of newly formed doublecortin-immunoreactive neurons and pCREB-positive cells in the subgranular zone after memory retention and reversal learning. This decrease was observed in both sedentary controls and animals with enhanced neurogenesis. We hypothesize that the formation of new hippocampal neurons is transiently decreased during memory retrieval, in order to reduce interference between memory formation and the retrieval of existing memories.
Introduction

There is increasing evidence that newly formed granule neurons in the dentate gyrus play a role in learning and memory processes. Hippocampus-dependent learning can promote the survival of newly formed granule neurons (Ambrogini et al., 2000; Gould et al., 1999a), which suggests that the young neurons have a function in memory formation. Furthermore, inhibition of neurogenesis, by cytostatic drugs or brain irradiation, showed that newly formed neurons are crucial for certain types of hippocampus-dependent learning (Shors et al., 2001; Shors et al., 2002) and for memory consolidation (Bruel-Jungerman et al., 2005; Snyder et al., 2005).

There is also evidence that the incorporation of new neurons in the hippocampal circuitry results in the clearance of outdated information. Computer models of a simple network predict that turnover of cells accelerates removal of information (Chambers et al., 2004; Deisseroth et al., 2004). There is in vivo data evidence supporting this theory. Presenilin-1 (PS1) knockout mice do not show an increase in hippocampal neurogenesis upon cage enrichment. Enriched housing of PS1 knockout mice following acquisition of a contextual fear-conditioning task, resulted in a better memory retention than wildtype controls (Feng et al., 2001). These data suggest that the elevated levels of hippocampal neurogenesis in the wildtype animals promoted removal of the hippocampal memory trace.

In the present study, we further investigated the function of newly generated neurons in memory formation and memory retrieval. We hypothesized that if reduced levels of neurogenesis cause learning and memory impairments, increased numbers of newly formed hippocampal granule neurons should facilitate the acquisition of a learning task. Additionally, we predicted that if neurogenesis has a function in the deletion of old information from the hippocampus, this clearance process can be accelerated by an increased production of new neurons.

Hippocampal neurogenesis was stimulated by housing mice with a running wheel for 14 days (Aberg et al., 2003; Kronenberg et al., 2003; Van Praag et al., 1999b), which is sufficient to give the newly formed neurons the opportunity to mature and to functionally integrate (Hastings and Gould, 1999; Kempermann et al., 2004). First, we determined the effects of enhanced neurogenesis on the acquisition of the Y-maze, a hippocampus-dependent (Bannerman et al., 2003) spatial learning task. Second, mice were trained in the Y-maze and subsequently housed with a running wheel for 14 days. After this period memory retention and reversal learning performance were tested.

Here, we show that enhancement of neurogenesis by wheel running improved learning in the Y-maze. In contrast to what would be expected on the basis of the ‘memory clearance hypothesis’, memory retrieval was also facilitated by exercise. These data suggest that newly generated neurons do not remove information from the hippocampus, but seem to play an important role in retrieval. Therefore, we further studied the effects of memory
retention and reversal learning on hippocampal neurogenesis by staining the brains for doublecortin and pCREB, both of which are markers that are expressed by newly formed neurons. Here, we show that both memory retention and reversal learning reduced hippocampal neurogenesis.
Materials and Methods

Animals and housing conditions
Eighty male C57Bl/6 mice (Harlan, Horst, The Netherlands, 25.8 ± 0.2 g at the beginning of the experiment) were individually housed, had free access to drinking water and were kept under a 12/12h light/dark cycle (lights on at 8.00 a.m.). Throughout the entire experiment, all animals were food restricted to about 85% of their original bodyweight, which means that they received on average 3 to 4 g of food per day. Animals were fed daily between 3:00 p.m. and 5:00 p.m., after Y-maze training. Food restriction started 1 day prior to the start of the experiment. All procedures concerning animal care and treatment were approved by the ethical committee for the use of experimental animals of the University of Groningen (DEC 4089A and 4089B).
**Y-maze training procedure**

Behavioral testing was conducted in an enclosed plexiglass Y-maze. The home cage of each animal was provided with a small sliding door that could be connected to the maze. Both the stem arm (27.5 cm long) and the two arms forming the Y (both 27.5 cm long and diverging at a 60° angle from the stem arm) were 5 cm in diameter. Perforations at the endings of the two arms forming the Y allowed odors from food (standard lab chow, Hope Farms, Woerden, The Netherlands) placed under the perforations to enter both arms. Small plastic blocks (1 cm high) were placed 4 cm from the endings of the arms to prevent visual inspection for food presence from a distance. Each arm was equipped with a trapdoor halfway the arm which could be operated manually from the experimenter’s position.

The day prior to the start of the training, animals were allowed to freely explore the maze for five minutes. Next, they received two trials, one in which the food was located in the left arm and one in which the food was positioned in the right arm. This procedure prevented the development of a preference for one of the arms. During the training procedure, the animal voluntarily entered the maze and whenever it visited one of the two arms, the trapdoor of the non-visited arm was

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**Figure 3: Effect of exercise on memory retention and reversal learning.**

A) Schematic overview of the experimental procedure. The upper line represents the experimental protocol for animals with enhanced neurogenesis, the lower line show the procedure for sedentary mice; hab = habituation to the Y-maze, sc = standard cage, p = perfusion; numbers indicate days. B) All animals were fully trained in the Y-maze (grey squares). Directly after the last training session, mice were either housed with a running wheel or kept in a standard cage for 14 days. After this 14-day interval, which is shown by a break in the x-axis, animals with enhanced neurogenesis performed significantly better in the retention test compared to sedentary controls. C) Also in the reversal task, in which the food was located in the arm opposite to the one during training, mice with enhanced neurogenesis showed an improved performance compared to sedentary mice. The learning curves show the average percentage of correct arm choices per session of six trials ± S.E.M.
closed. The mouse was allowed to eat the food crumble and after it had re-entered its home cage, the arm connected to the home cage was closed. After thorough cleaning of the arms, the animal was allowed to enter the maze again for the next trial.

**Effect of 14 days of exercise on hippocampal neurogenesis**

Eight mice were housed with a running wheel (diameter 13 cm) for 14 days. Sedentary mice (n=6) were housed under standard conditions. Running wheel activity was recorded and analyzed with specialized software (ERS system, Haren, The Netherlands). After the exercise period, mice were sacrificed and brains were processed for immunocytochemistry (see below).

**Effect of enhanced neurogenesis on memory formation**

Mice (n=16) were housed with a running wheel for 14 days. Sedentary mice (n=16) were kept in a standard cage during this period. After the exercise period, all mice were placed in a clean, standard cage and one day later eight exercise animals and eight sedentary animals were habituated to the Y-maze. Training took place on the following four days and consisted of two sessions per day, each session containing six trials. The other half of the exercise and the sedentary animals were kept in the home cage during the training period and served as naive controls for the Y-maze. Animals were sacrificed one day after the last training session.

**Effect of enhanced neurogenesis on memory retention and reversal learning**

Mice (n=34) were trained in the Y-maze for three days, using two sessions per day. Directly after the last training session, 16 animals were housed with a running wheel for 14 days and the rest of the mice remained in a standard cage during this period. One day after the end of the exercise period, all mice were placed in a clean, standard cage for one day. The next day, memory retention was tested in half of the exercise animals and half of sedentary mice. These animals were placed again in the Y-maze, with the food in the same arm as they had learned during training. The other half of the animals was tested in a reversal learning task, in which the food was located in the arm that was not baited during the training sessions. Animals were exposed to the retention or reversal learning paradigm for two sessions per day for four days and were sacrificed one day later.

**Brain processing and Immunocytochemistry**

Animals were transcardially perfused with heparinized saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed, kept in 0.01 M PBS overnight and subsequently cryoprotected in 30% sucrose for 48h. Next, 30 μm sections, spanning the dorsal hippocampus (Bregma -1.46 to -2.80), were cut on a cryostat microtome. Brains were stained for doublecortin (DCX) and Ser133-phosphorylated CREB (pCREB). Sections were treated with 0.3% H2O2, blocked with 3% normal serum and the cell membrane was permeabilized with 0.1% Triton-X100. Goat-anti-DCX (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and rabbit-anti-pCREB (1:300, Cell Signaling Technology,
Beverly, MA, USA) were applied for 72h at 4°C. Sections were incubated with a biotinylated secondary antibody (rabbit-anti-goat or goat-anti-rabbit, 1:400, Jackson Immunolabs, West Grove, PA, USA) for 2h at room temperature, followed by incubation with Avidin-Biotin-Complex (1:400, ABC Elite kit, Vector Laboratories, Burlingame, CA, USA) for 2h. Staining was visualized with 20 mg/100 ml DAB and 0.03% H₂O₂.

For the DCX/pCREB double labeling procedure, a pCREB staining was performed as described above, but without the use of normal serum. The staining was visualized with DAB (20 mg/100 ml), nickelammoniumsulfate (200 mg/100 ml) and 0.03% H₂O₂. Subsequently, sections were treated with a high dose of H₂O₂ (1%), in order to remove all HRP reactivity. Then, sections were stained for DCX, as described above. The DCX-positive cells were visualized with DAB (15 mg/100 ml) and 0.01% H₂O₂.

Quantification of the immunostainings
DCX and pCREB-positive cell numbers were determined in 3-4 sections per animal, randomly chosen between -1.46 to -2.80 mm from Bregma. Cells were counted throughout the entire thickness of the sections, using a 40x magnification. In order to prevent the inclusion of cell profiles in the DCX analysis, only cells were included with a cell soma that was larger than 8 μm in diameter. The inner and outer blades of the granule cell layer were counted separately. For every animal, the average number of immunopositive cells/section was calculated.

A second measure of the DCX-immunostaining was taken in order to verify the DCX cell count analysis. Since most DCX-immunopositive cells possess only one primary dendrite that projects through the granule cell layer, we determined the density of DCX-immunoreactive dendrites in the granule cell layer as a measure for the number of DCX-positive cells. For the density measurements the same sections were used as for the cell counts. With a computerized system (Leica Qwin, Rijswijk, The Netherlands), two equally sized areas of the inner blade of the granular cell layer and two areas of the outer blade of each hippocampus were delineated. Within the demarcated areas, the percentage of the total surface that was covered with immunopositive dendrites was calculated. For every animal, an average area percentage was calculated from the different measurements.

Statistics
The learning curves of the Y-maze were analyzed using a repeated measures ANOVA. Potential differences in DCX and pCREB immunoreactivity after 14 days of exercise were statistically tested with an independent-samples t-test. For the statistical analysis of the DCX-measurements and the pCREB data of the different Y-maze groups, a two-way ANOVA was used, with exercise and Y-maze training as between-subjects variables. Whenever this revealed a significant difference, pairwise comparisons were made with a post hoc LSD test. Data are expressed as averages ± S.E.M.
Results

Running wheel activity in combination with dietary restriction promotes hippocampal neurogenesis

This study aimed to look at the effects of enhanced neurogenesis on various aspects of Y-maze learning. Running wheel activity has repeatedly been shown to promote hippocampal neurogenesis. In the experiments reported here, exercise was combined with a food restriction paradigm. We examined whether neurogenesis is still enhanced by exercise under conditions of reduced food intake.

Animals were housed with a running wheel for 14 days and perfused one day later. An overview of the experimental procedure that was used is presented in Fig. 1A. DCX-immunocytochemistry showed that the number of immature neurons was significantly increased by the exercise procedure (Fig. 1B, P≤0.01). This increase was visible both in the inner and the outer blade of the granule cell layer (separate data sets not shown). The difference between runners and control mice was confirmed by the DCX-positive dendrite measurements (Fig. 1C, P≤0.001). In addition, brains were stained for pCREB. Figure 1D shows that 14 days of exercise significantly increased the number of pCREB-positive cells in the DG (P≤0.01), and this was observed in both blades of the GCL (separate data sets not shown).

Running wheel activity facilitates Y-maze acquisition, retention and reversal learning

We investigated the consequences of enhanced neurogenesis caused by 14 days of voluntary exercise on various aspects of Y-maze learning. First, the effects of wheel running on acquisition of the Y-maze were studied (Fig. 2A). Animals were exposed to two training sessions per day and each session consisted of six trials. Overall, the runners performed significantly better than sedentary mice (Fig. 2B, repeated measures ANOVA: F(1,14)=30.1, P≤0.001; session*group interaction: F(7,98)=3.88, P≤0.001). A more detailed examination of the first session (Fig. 2C) shows that the mice with enhanced neurogenesis did not have an initial bias for the correct arm, but readily learned the position of the food within the first training session (repeated measures ANOVA: F(1,14)=13.5, P≤0.01).

Next, we investigated whether 14 days of exercise, starting directly after Y-maze training was completed, had an effect on memory retention. This was tested by re-exposing the animals to the maze after the 14-day exercise period and to test their memory for the position of the food (Fig. 3A). Animals quickly relearned the position of the food (repeated measures ANOVA, session effect: P≤0.05). Furthermore, there was a significant session*group interaction (F(7,105)=2.4, P≤0.05), indicating that running wheel activity facilitated Y-maze retention (Fig. 3B).

Third, the effects of running wheel activity on reversal learning were tested. A similar protocol was used as for the retention test (Fig. 3A), except for the fact that during reversal learning, the food was placed in the arm that was opposite to the one that was rewarded during training. In the first reversal session, both sedentary and exercise animals...
showed an impaired performance, which was significantly improved during the following sessions (Fig. 3C, P≤0.001). The significant session*group interaction (F(7,105)=2.1, P≤0.05) showed that runners learned to find the new location of the food faster than sedentary mice.

**Y-maze retention and reversal learning reduce hippocampal neurogenesis**

Since we observed that enhanced neurogenesis facilitated acquisition and retrieval of spatial information, we further studied the effects of these aspects of Y-maze learning on hippocampal neurogenesis. The brain material was stained for the immature neuron marker DCX and for ser133-phosphorylated CREB (Fig. 4, page 142). DCX can be used as a suitable marker for investigating the absolute number of newly formed neurons (Rao and

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Figure 5: Effect of acquisition on hippocampal neurogenesis. A) Y-maze acquisition caused a moderate reduction in immature neuron number in sedentary mice, but not in animals with enhanced neurogenesis. B) The density of DCX-positive dendrites in the granule cell layer was reduced in sedentary mice after acquisition of the Y-maze, but remained unchanged in mice with enhanced neurogenesis. C) Acquisition of the Y-maze did not cause changes in the number of pCREB-positive cells, neither in sedentary animals, nor in mice with enhanced neurogenesis. *: P≤0.05. Cell numbers are shown as the average number of cells/section ± S.E.M.
Shetty, 2004). CREB phosphorylation has also been reported to occur in newly formed, PSA-NCAM positive granule neurons during maturation (Nakagawa et al., 2002; Zhu et al., 2004b). Since there is an almost complete overlap between DCX-expression and NCAM polysialylation (Nacher et al., 2001a), we supposed that the pCREB-positive cells in the granule cell layer were in fact newly formed neurons. This assumption was confirmed by the significant correlation that we found between the number of DCX-expressing neurons and the number of pCREB-positive cells ($R^2=0.62, P \leq 0.001$). In addition, a double labeling for DCX and pCREB was performed (Fig. 4, page 142). Random investigation of sections from the different experimental groups showed that the majority of the DCX-positive cells colocalized with pCREB. Occasionally, a DCX single labeled cell could be found. However, not all pCREB positive cells also expressed DCX, suggesting that some existing mature granule neurons contain pCREB.

Figure 6: Effect of memory retention on hippocampal neurogenesis. A) The retention test caused a significant reduction in DCX-positive cell number, both in sedentary mice and animals with enhanced neurogenesis. B) The reduction in DCX-positive cell number was reflected in the decline in DCX-positive dendrite density. C) Quantification of pCREB-positive cells showed that memory retention also resulted in a decline in CREB phosphorylated cell number. *: $P \leq 0.05$; ***: $P \leq 0.001$. Cell numbers are shown as the average number of cells/section ± S.E.M. Note that the data presented for the sedentary naive animals and the naive mice with enhanced neurogenesis are similar to Fig. 5.
In order to statistically analyze potential effects of exercise or any of the Y-maze conditions on the number of newly formed hippocampal neurons, a two-way ANOVA was performed with the 4 different Y-maze groups (naive, acquisition, retention and reversal) and the housing condition (sedentary or exercise) as between-subjects variables. Both for DCX (F(1,66)=36.8, P≤0.001) and pCREB (F(1,66)=36.4, P≤0.001), a significant effect of exercise was observed, with runners having more DCX and pCREB-positive cells. The Y-maze groups also differed significantly from each other with respect to DCX-positive cell number (F(3,66)=20.7, P≤0.001), DCX-positive dendrite density (F(3,66)=20.8, P≤0.001) and pCREB-positive cell number (F(3,66)=12.1, P≤0.001). For pCREB, there was also a near-significant interaction between exercise and learning (P≤0.10). A post hoc LSD test was performed to test which Y-maze groups differed from naive controls.

Y-maze acquisition did not cause major changes in newly formed cell numbers. A post hoc LSD-test showed that in the sedentary animals, a mild reduction was observed in DCX-positive cell number (Fig. 5A, P≤0.05) and in the density of DCX-positive dendrites (Fig. 5B, P≤0.05). However, the number of pCREB-positive neurons did not differ between Y-maze naive and trained mice (Fig. 5C). In mice with enhanced neurogenesis, Y-maze acquisition did not cause any changes in DCX-positive cell number (Fig. 5A), DCX-positive dendrites (Fig. 5B) or CREB phosphorylation in the granule cell layer (Fig. 5C).

Next, we studied whether re-exposure to the Y-maze task 14 days after mice had acquired the task caused changes in the number of newly formed cells. Figure 6A shows a significant reduction in DCX-positive cell number after memory retention, both in sedentary mice (P≤0.001) and animals with enhanced neurogenesis (P≤0.001). The DCX-positive dendrite measurements also revealed a decline in animals that were exposed to the retention test (Fig. 6B, sedentary: P≤0.001, enhanced neurogenesis: P≤0.001). In addition, the number of pCREB-positive cells was significantly reduced after memory retention (Fig. 6C, sedentary: P≤0.05, enhanced neurogenesis: P≤0.001).

Furthermore, we investigated hippocampal neurogenesis after relocation of the food reward, 14 days after mice had acquired the Y-maze task. Reversal learning resulted in changes in the number of newly generated neurons that were comparable to those observed after memory retention. Both DCX-positive cell number (Fig. 7A, sedentary: P≤0.001, enhanced neurogenesis: P≤0.001) and dendrite density (Fig. 7B, sedentary: P≤0.001, enhanced neurogenesis: P≤0.001) were significantly reduced after reversal learning. In addition, we found a significant decrease in the number of pCREB-positive cells after reversal learning (Fig. 7C, sedentary: P≤0.05, enhanced neurogenesis: P≤0.001). All changes reported for DCX-immunoreactivity or pCREB-positive cell number were visible in both blades (separate data set not shown).
Figure 7: Effect of reversal learning on hippocampal neurogenesis. A) Both in sedentary animals and in mice with enhanced neurogenesis, reversal learning caused a decrease in the number of immature neurons. B) Also the coverage of the granule cell layer with DCX-positive dendrites was reduced upon reversal learning. C) The number of pCREB-positive cells was significantly lower in animals that were exposed to the reversal learning paradigm. *: P≤0.05; ***: P≤0.001. Cell numbers are shown as the average number of cells/section ± S.E.M. Note that the data presented for the sedentary naive animals and the naive mice with enhanced neurogenesis are similar to Fig. 5.
Discussion

**Beneficial effects of exercise on Y-maze acquisition, retention and reversal learning**

Our data show that 14 days of exercise increased the rate of acquisition in the Y-maze, improved retention of previously acquired information and facilitated reversal learning. The fact that exercise had a positive effect on Y-maze acquisition is in line with other studies (Fordyce and Farrar, 1991; Fordyce and Wehner, 1993; Shaw et al., 2003; Van Praag et al., 1999a) and supports the idea that newly formed neurons participate in memory formation (Gould et al., 1999a; Snyder et al., 2005). Newborn neurons form a specific population of cells that may serve as a substrate for the formation of new memories. They are not yet involved in other memory traces and they show extraordinary morphological (Hastings and Gould, 1999; Seki and Arai, 1991; Seki and Arai, 1993) and synaptic (Schmidt-Hieber et al., 2004; Snyder et al., 2001) plasticity. They may therefore play a specific role in the formation of memories (Gould et al., 1999c).

Here we present that physical exercise not only promotes the acquisition of a spatial learning task, but that 14 days of running wheel activity, starting after mice have mastered a task, also has beneficial effects on retention of information and on the reversal learning ability. These data contradict with our initial hypothesis, based on the report by Feng and colleagues (2003), stating that hippocampal neurogenesis serves to remove old data from the hippocampus. However, there are major differences in the experimental setup between the present study and the experiments by Feng et al. We used another learning task (Y-maze versus contextual fear conditioning), a different method to increase neurogenesis (wheel running instead of environmental enrichment) and wildtype mice instead of knockout animals. Nevertheless, with our experimental paradigm we cannot confirm that hippocampal neurogenesis erases existing memory traces from the hippocampus.

The question remains whether and how the elevated levels of hippocampal neurogenesis may have contributed to the improved memory retrieval. The excess in newly generated cell number was created after the animals had mastered the task. This implies that the extra neurons that were produced during exercise were not involved in memory formation. However, the enhanced hippocampal neurogenesis following the acquisition may have caused a more efficient memory consolidation. An increased capacity of the granule cell layer reduces the chance that the cells that were involved in Y-maze acquisition are subsequently involved in other memory traces. The incorporation of a cell into multiple memory traces increases the likelihood for mistakes during retrieval (Deisseroth et al., 2004). Increasing the capacity of the granule cell layer may minimize this problem.

The positive effects of physical exercise on various aspects of Y-maze learning can probably not all be attributed to the increase in hippocampal neurogenesis. Directly after 14 days of exercise, the rise in pCREB-positive cell number exceeded the increase in the number of DCX-positive cells, suggesting that the improved learning performance could have been caused by increased transcription of genes that promote long-term memory formation (Bourtchuladze et al., 1994; Guzowski and McGaugh, 1997; Kida et al., 2002;
Kogan et al., 1997; Silva et al., 1998). In addition, running has been shown to increase levels of various growth factors and neurotrophic factors (Fabel et al., 2003; Farmer et al., 2004; Gomez-Pinilla et al., 1997; Oliff et al., 1998; Trejo et al., 2001) and to promote, for instance, cerebral blood flow (Endres et al., 2003), angiogenesis (Swain et al., 2003) and cholinergic synaptic communication (Floyd and Farrar, 1991), all of which may positively influence learning and memory. Finally, it is important to realize that exercise-induced improvements in learning, retention and reversal learning are not necessarily mediated by the same mechanism.

Memory retention and reversal learning reduce hippocampal neurogenesis both in sedentary mice and animals with enhanced neurogenesis
We further determined the effects of training, memory retention and reversal learning in the Y-maze on the number of newly formed neurons. Hippocampal neurogenesis was determined by staining for DCX and pCREB. DCX is expressed by immature neurons (Brown et al., 2003b; Couillard-Despres et al., 2005) and quantification of DCX expression therefore provides direct information on the number of newly formed neurons (Rao and Shetty, 2004). CREB phosphorylation has also been shown to occur in maturing neurons (Fujioka et al., 2004; Nakagawa et al., 2002). We found a highly significant correlation between pCREB-positive cell number and DCX-expression. Moreover, double labelings confirmed that DCX and pCREB were strongly colocalized in the granule cell layer and therefore both predominantly represent immature neurons.

We show that training in the Y-maze did not cause changes in immature neuron number in mice with enhanced neurogenesis. These data confirm the results of other studies, also from our own group, in which spatial learning had no effect on hippocampal neurogenesis (Snyder et al., 2005; Van der Borght et al., 2005c; Van Praag et al., 1999b). However, in sedentary mice, Y-maze acquisition resulted in a reduction in DCX-expressing cells, but not in pCREB-immunoreactive cells. Hippocampal neurogenesis has been shown to be differentially regulated throughout the different phases of learning (Dobrossy et al., 2003). The fact that we found changes in DCX-expression in sedentary animals, but not in mice with enhanced neurogenesis may be due to the fact that the two groups were in a different phase of the learning curve at the moment of termination. Sedentary mice had just reached the level of 80% correct trials, whereas the mice with enhanced neurogenesis already performed at maximal levels for 3 days.

We further explored the effects of memory retention and reversal learning on hippocampal neurogenesis. Both paradigms caused a dramatic reduction in the number of newly formed cells, both in control animals and in mice with enhanced neurogenesis. The common feature of memory retention and reversal learning is that both processes require retrieval of the stored spatial information, so it can be suggested that retrieval of spatial information reduced hippocampal neurogenesis.

The decrease in the number of newly formed neurons can be caused by increased apoptosis of newly formed cells. Alternatively, the generation of new cells may have been inhibited during retention testing and reversal learning. A third option is that memory
retrieval caused an accelerated maturation of neural precursors. However, since the length of DCX-expression has been shown to remain stable, even in conditions such as aging or hippocampal injury (Rao and Shetty, 2004), the latter explanation is not very likely.

The data we present here suggest that neurogenesis in the dentate gyrus is actively suppressed during memory retrieval. Other studies have shown a reduction in hippocampal activity after memory retrieval. Retention testing of mice in a radial maze, 25 days after acquisition, decreased hippocampal metabolic activity, measured by (14C)-deoxyglucose uptake, below baseline levels (Bontempi et al., 1999). In addition, repeated exposure of rats to a familiar environment significantly reduced hippocampal CREB phosphorylation compared to naive controls (Winograd and Viola, 2004). However, in those studies no differentiation was made between the different hippocampal subregions.

The changes we observed occurred in the dentate gyrus, a part of the hippocampus which is known to be crucially involved in the formation of memories, but much less in the retrieval of information (Eldridge et al., 2005; Lee and Kesner, 2004). The fact that neurogenesis in the dentate gyrus is inhibited during retrieval, suggests that the presence of highly plastic newly formed neurons in the ‘gateway’ to the hippocampus may negatively affect the retrieved memory. Fourteen days after the animals have learned the Y-maze, the acquired information is most likely stored in the cortex and retrieval is independent of the hippocampus (Beylin et al., 2001; Kim and Fanselow, 1992; Takehara et al., 2003). However, re-exposure to the same context is thought to re-activate the hippocampal memory trace and to return it to a more labile state which is sensitive to disruption (Debiec et al., 2002; Nader, 2003). We hypothesize that during this labile state of the retrieved memory, neurogenesis is inhibited in order to prevent the formation of a new, but redundant, memory trace of a familiar context. We propose that the simultaneous retrieval and formation of a memory of the same contextual situation may cause interference and hinder the consolidation process.

In summary, we show here that 14 days of physical exercise facilitates acquisition, memory retention and reversal learning in the Y-maze. Possibly, these beneficial effects on various aspects of spatial learning are mediated by the increase in hippocampal neurogenesis after exercise, which persisted for at least six days after the running wheel had been removed from the cage. Secondly, we show a significant reduction in hippocampal neurogenesis following retrieval of spatial information in the Y-maze. Since newborn neurons may form an important substrate for the formation of new memory traces, we propose that active suppression of neurogenesis during re-exposure to a familiar environment may prevent the formation of redundant memories and reduce possible interference between the existing and the newly formed memory trace.
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