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Enriched Environment Attenuates Surgery-Induced Impairment of Learning, Memory, and Neurogenesis Possibly by Preserving BDNF Expression

Dan Fan · Jun Li · Bin Zheng · Lei Hua · Zhiyi Zuo

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Abstract Postoperative cognitive dysfunction (POCD) is a significant clinical syndrome. Neurogenesis contributes to cognition. It is known that enriched environment (EE) enhances neurogenesis. We determined whether EE attenuated surgery-induced cognitive impairment and whether growth factors and neurogenesis played a role in the EE effect. Eight-week-old C57BL/6J mice were subjected to carotid artery exposure. Their learning and memory were assessed by Barnes maze, and fear conditioning started 2 weeks after the surgery. Growth factor expression and cell genesis were determined at various times after the surgery. Surgery increased the time for the mice to identify the target hole in the Barnes maze and reduced context-related freezing behavior. Surgery also reduced the expression of brain-derived neurotrophic factor (BDNF) and neurogenesis in the hippocampus. These effects were attenuated by EE. EE also attenuated surgery-induced reduction of phosphorylated/activated tropomyosin-related kinase B (TrkB) and extracellular signal-regulated kinases (ERK), components of BDNF signaling pathway. ANA-12, a selective TrkB antagonist, blocked the effects of EE on cognition, phosphorylation of TrkB and ERK, and neurogenesis. These results provide initial evidence that surgery reduces BDNF expression and neurogenesis in the hippocampus. Our results suggest that EE reduces surgery-induced impairment of learning, memory, and neurogenesis by preserving BDNF expression.

Keywords Brain-derived neurotrophic factor · Enriched environment · Neurogenesis · Postoperative cognitive dysfunction

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Introduction

Postoperative cognitive dysfunction (POCD) is a clinical syndrome that affects ~30 % patients at hospital discharge after noncardiac surgery [1–3]. In addition to influencing daily activities of patients, POCD is associated with increased mortality within 1 year after the surgery [2, 4]. However, effective interventions to reduce POCD have not been developed. These interventions are urgently needed to improve patient outcome after surgery.

Current studies on POCD focus on the contribution of surgery and anesthesia to POCD and mechanisms for this effect [5, 6]. We and others have shown that neuroinflammation may be a major early pathophysiological process for the cognitive impairment after anesthesia and surgery in animals

[5, 6]. Surgery can induce neuroinflammation in humans [7]. Although various anti-inflammatory agents are used in clinical practice, they may have significant side effects. Some of these drugs have significant effects on coagulation, and their use needs to be very cautious during the perioperative period [8, 9]. Thus, using nonpharmacological methods to reduce POCD have significant advantages.

Enriched environment (EE) has been shown to improve cognitive functions and neurogenesis [10, 11]. It also can reduce neuroinflammation [12, 13]. Neuroinflammation decreases neurogenesis [14], a process that contributes to learning and memory [15]. Thus, EE may reduce POCD. Also, EE can enhance the expression of growth factors, such as brain-derived neurotrophic factor (BDNF) [16, 17], which increases neurogenesis [18]. Therefore, we hypothesize that EE attenuates POCD and increases neurogenesis via enhancing the expression of growth factors including BDNF. To test this hypothesis, we subjected C57BL/6J mice to carotid artery exposure, a surgical procedure that is a component of carotid endarterectomy. Some mice were exposed to EE after the surgery. Neurogenesis and the expression of growth factors in the hippocampus as well as the learning and memory of the mice were assessed.

Materials and Methods

The animal protocol was approved by the institutional Animal Care and Use Committee of the University of Virginia (Charlottesville, VA, USA). All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publications number 80–23) revised in 2011.

Animal Groups and Housing

Eight-week-old male C57BL/6J mice weighing 24–28 g from Charles River Laboratories International Inc. (Wilmington, MA, USA) were randomly assigned to three groups, control, surgery plus standard environment (SE), and surgery plus enriched environment (EE), in the first experiment, and three groups, control, anesthesia with 3 % sevoflurane for 2 h, and EE, in the second experiment. All mice before surgery or EE were housed under SE that was three to five mice per cage in a 27.94×15.24×11.43 cm cage on a 12-h light/dark cycle with ad libitum access to food and water. Mice were then kept in this SE or an EE starting day 0 after the surgery. For those mice that were assigned to surgery plus EE, surgery was finished before 10:00 on that day so that they could be placed in EE at 12:00. EE was performed as follows. Five mice were placed in a large cage (cage size 43.18×22.86×19.05 cm) for 6 h (12:00 to 18:00) each day. The cage contained a running wheel, tunnels, shed, and various toys. These settings were changed twice per week. The animals were housed under SE during the rest of the time each day. These housing environments were maintained until the animals have completed the behavioral tests or were sacrificed for brain tissue harvest.

Anesthesia and Surgery

The surgery was a right carotid artery exposure. Briefly, mice were anesthetized by 3 % sevoflurane. During the procedure, the mouse was kept at spontaneous respiration; rectal temperature was monitored and maintained at 37 °C with the aid of a heating blanket (TCAT-2LV, Physitemp instruments Inc., Clifton, NJ). A 1.5-cm midline neck incision was made after the mouse was exposed to sevoflurane at least for 30 min. Soft tissues over the trachea were retracted gently. One centimeter long right common carotid artery was carefully dissected free from adjacent tissues without any damage on vagus nerve. The wound was then irrigated and closed by using surgical suture. The surgical procedure was performed under sterile conditions and lasted around 10 min. After the surgery, all animals received a subcutaneous injection of 0.003 mg/kg bupivacaine. The total duration of anesthesia was 2 h. No response to toe pinching was observed during the anesthesia. Mice in the sevoflurane anesthesia group also received bupivacaine injection.

Inhibitors

As previously described [19], ANA-12 (Sigma-Aldrich, St Louis, MO), a potent tropomyosin-related kinase B (TrkB) antagonist, was dissolved in corn oil containing 1 % dimethyl sulfoxide. Mice received intraperitoneal injection of 0.5 mg/kg ANA-12 once every 12 h for 5 days with the first dose given immediately after surgery. Another group of mice received intraperitoneal injection of corn oil in the same way as for injecting ANA-12. These mice were labeled as vehicle groups.

Barnes Maze

Fourteen days after being exposed to various experimental conditions, animals were subjected to Barnes maze to test their spatial learning and memory as previously described [20]. The Barnes maze is a circular platform with 20 equally spaced holes (SD Instruments, San Diego, CA, USA). One of the holes was connected to a dark chamber that was called target box. The test was started by placing animals in the middle of the Barnes maze. Aversive noise (85 db) and bright light from a 200-W bulb shed on platform were used to provoke mice to find and enter the target box. Animals were trained in a spatial acquisition phase that took 4 days with 3 min per trial, four trials per day and 15-min interval between each trial. The

memory test was carried out on day 5 (short-term retention) and day 12 (long-term retention). No test was performed during the period from day 5 to day 12. The latency to enter the target box during each trial was recorded by an ANY-Maze video tracking system (SD Instruments).

Fear Conditioning

Fear conditioning test was performed 24 h after the Barnes maze test as previously described [20]. Mice were placed in a test chamber wiped with 70 % alcohol and subjected to three tone-foot shock pairings (tone 2000 Hz, 85 db, 30 s; foot shock 0.7 mA, 2 s) with 1-min intertrial interval in a relatively dark room. Mice were removed from the test chamber 30 s after training and returned to their regular cages. Mice were placed back 24 h later to the same chamber for 8 min without tone and shock. The freezing behavior was recorded in an 8-s interval. Two hours later, mice were placed in a new test chamber that had different context and smell from the first test chamber. This chamber was wiped with 1 % acetic acid and was in a relatively light room. Freezing behavior was recorded for 3 min without any stimuli. The tone stimulus was then turned on for three cycles with each cycle for 30 s followed by 1-min inter-cycle interval (total 4.5 min). The freezing behavior in this 4.5 min was recorded too. All freezing behavior was recorded by a camera, and the video was scored by an observer who was blinded to group assignment of animals.

Brain Tissue Harvesting

Mice were deeply anesthetized with isoflurane at 1 or 5 days after the surgery. Mice in the surgery plus EE group had EE for 1 or 5 days before they were used for brain tissue harvesting. They were perfused with normal saline. The bilateral hippocampi were dissected out for ELISA assay and Western blotting. A coronal brain slice between Bregma −2 and −4 mm was harvested 2 weeks after the surgery for immunofluorescent staining. Mice in the groups that were exposed to EE only had EE for 2 weeks before the brain slices were harvested. These slices containing hippocampus were fixed with 4 % paraformaldehyde. All dissection procedures were performed on ice. Thus, three separate cohorts of mice were used for the experiments: one for the learning and memory tests that were started 2 weeks after the surgery or anesthesia, one for hippocampal harvest at 1 or 5 days after the surgery for ELISA and Western blotting analyses, and the third cohort for brain slice harvest at 2 weeks after the surgery.

ELISA Assay of Neurotrophic Factors in the Brain Tissues

The protein levels of BDNF, nerve growth factor (NGF), and glial cell-derived neurotrophic factor (GDNF) were determined by using ELISA kits (Promega, Madison, WI) according to the manufacturer's instruction and as described previously [21]. Briefly, the hippocampus was homogenized on ice in the RAPA buffer containing 25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1 % sodium deoxycholate, and 0.1 % SDS (Thermo Scientific, Rockford, IL) as well as protease inhibitor cocktail (10 mg/ml aproteinin, 5 mg/ml peptastin, 5 mg/ml leupetin, and 1 mM phenylmethanesulfonylfluoride) (Sigma-Aldrich). Homogenates were centrifuged at 13,000*g* for 20 min at 4 °C. The supernatant was collected and used for ELISA detection. The amount of BDNF, NGF, and GDNF in each sample was then normalized by its protein content.

Western Blot Analysis

Total protein lysates were prepared by lysing the hippocampus in the lysis buffer (Thermo Scientific, Rockford, IL) containing protease inhibitors cocktail (Sigma-Aldrich) and phosphatase inhibitors (phosSTOP phosphatase Inhibitor Cocktail Tablets; Roche, Nutley, NJ). About 20 μg total protein per lane was separated on a polyacrylamide gel and then transferred onto PVDF membranes. The membranes were blocked with Protein-Free T20 Blocking Buffer (Thermo Scientific, Logan, UT) for 2 h at room temperature and incubated with the following primary antibodies overnight at 4 °C: rabbit polyclonal anti-phospho-TrkB (Y705) antibody (Abcam, 1:1000); rabbit polyclonal anti-TrkB antibody (Abcam, 1:1000); rabbit monoclonal anti-phospho-extracellular signal-regulated kinases (ERK) (Thr202/Tyr204) (Cell Signaling Technology, 1:1000); rabbit polyclonal anti-ERK antibody (Cell signaling Technology, 1:1000), and rabbit polyclonal anti-β actin antibody (Cell signaling Technology, 1:1000). After being incubated with appropriate secondary antibodies, the protein complex was revealed with enhanced chemiluminescence reagents (Pierce, IL, USA) and was visualized by using Genesnap version 7.08. All protein band density was quantified by using Genetools version 4.01 and normalized to the density of β-actin in the same sample. The results from mice after various experimental conditions then were normalized by the mean values of the corresponding control animals.

BrdU Administration and Immunofluorescent Staining

Two days after surgery, mice were given seven consecutive intraperitoneal injections of 50 mg/kg 5′-bromo-2′-deoxyuridine (BrdU; Sigma-Aldrich) at 17:00 once daily as previously described [22]. Mice were sacrificed 5 days later for harvesting hippocampus. Hippocampus was fixed in 4 % paraformaldehyde in 0.1 M phosphate-buffered saline at 4 °C for 24 h and embedded in paraffin. Five microns thick coronal brain sections were cut sequentially from Bregma −2 to −4 mm. Antigen retrieval was performed by incubating the

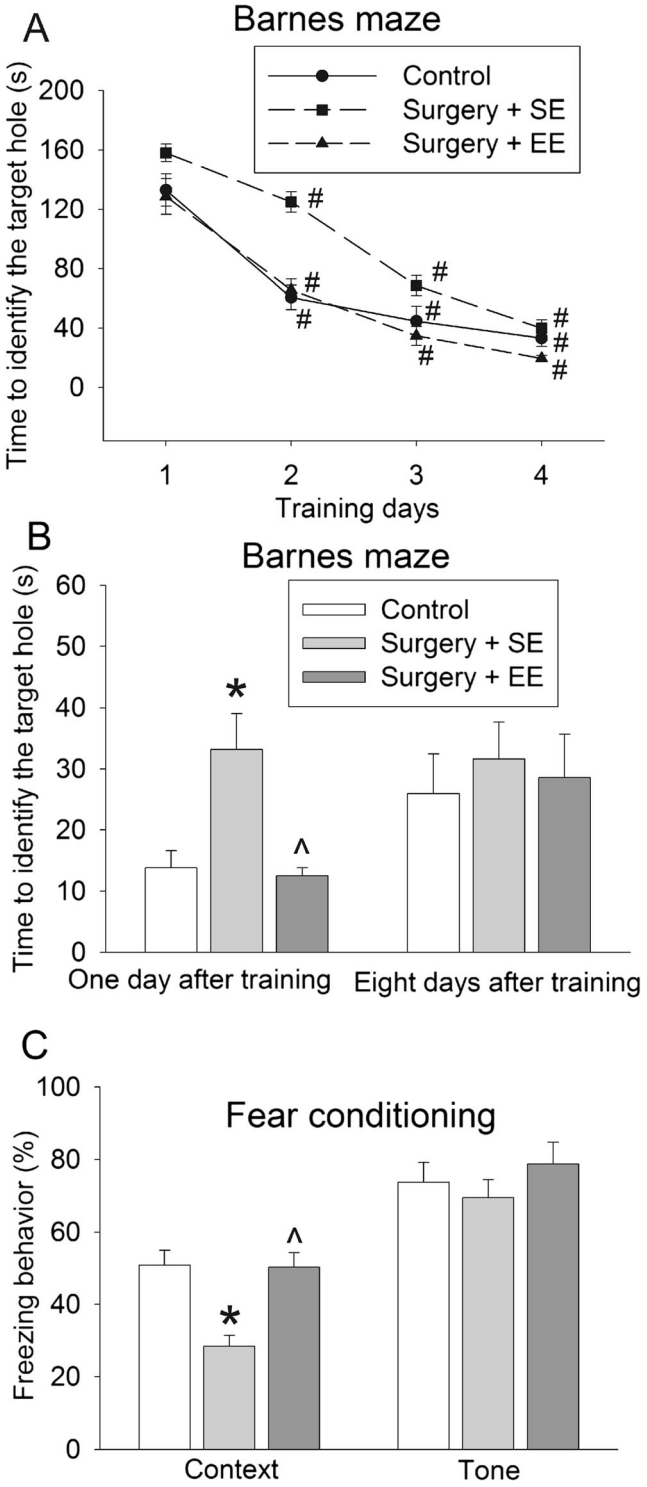


Fig. 1 Enriched environment (*EE*) attenuated surgery-induced learning and memory. Eight-week-old mice were subjected to right carotid artery exposure under sevoflurane anesthesia. They were started to be tested by Barnes maze and fear conditioning at 2 weeks after the surgery. **a** Performance during the training sessions of Barnes maze. **b** Performance during the memory phase test of Barnes maze. **c** Performance in fear conditioning test. Results are means±S.E.M. (n=12). #P<0.05 compared with the corresponding values at time 0; *P<0.05 compared with control; ^P<0.05 compared with surgery plus standard environment (SE)

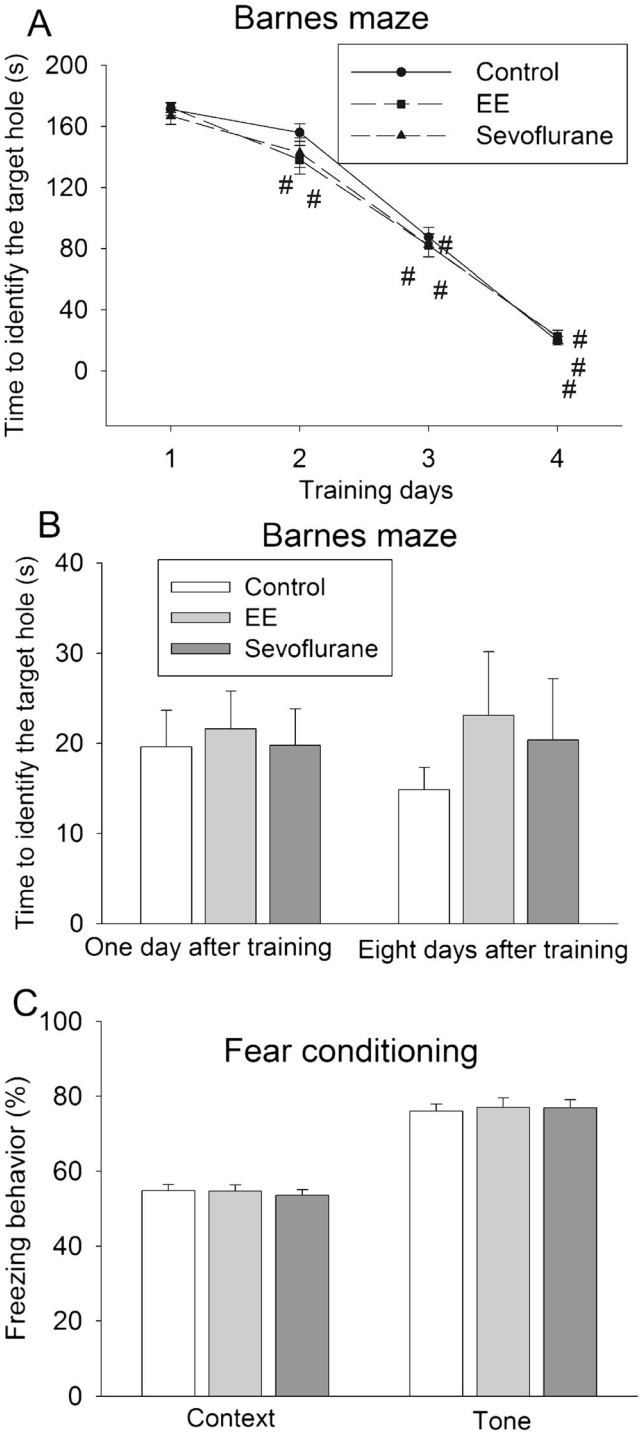


Fig. 2 No effects of sevoflurane anesthesia and enriched environment (*EE*) on learning and memory under control condition. Eight-week-old mice were subjected to sevoflurane anesthesia or EE. They were started to be tested by Barnes maze and fear conditioning at 2 weeks after the anesthesia or being on EE. EE was maintained during the period of Barnes maze and fear conditioning. **a** Performance during the training sessions of Barnes maze. **b** Performance during the memory phase test of Barnes maze. **c** Performance in fear conditioning test. Results are means± S.E.M. (n=10–12). #P<0.05 compared with the corresponding values at time 0

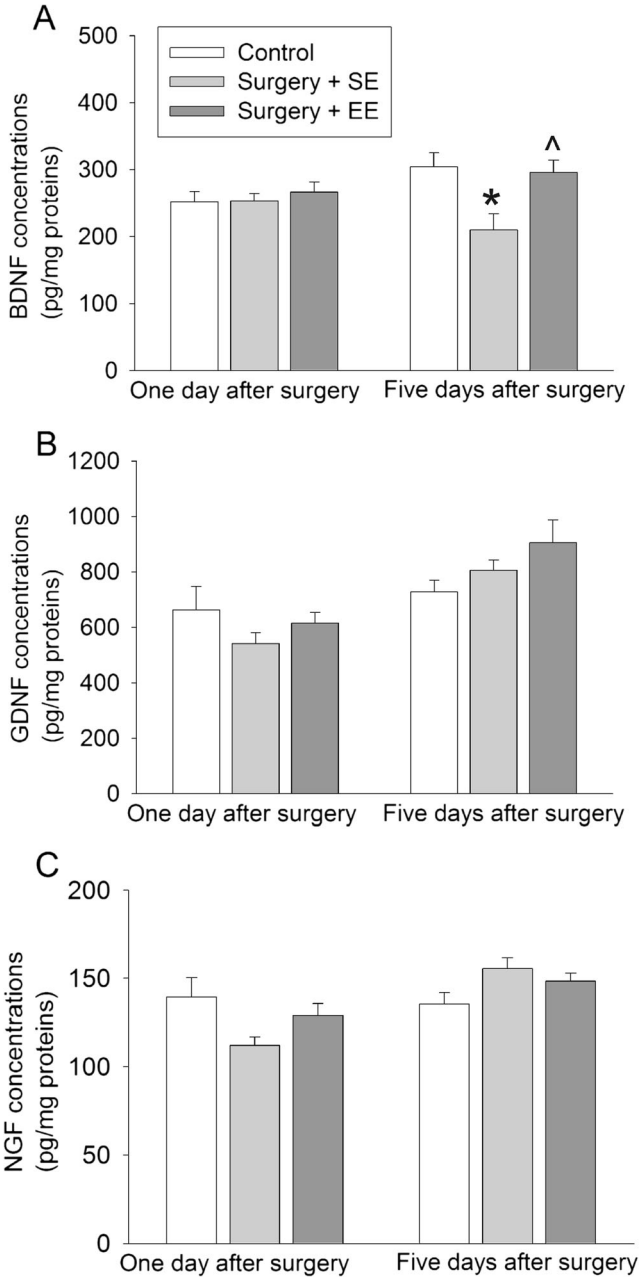


Fig. 3 Enriched environment (*EE*) attenuated surgery-induced decrease of BDNF expression. Eight-week-old mice were subjected to right carotid artery exposure under sevoflurane anesthesia. Hippocampus was harvested at 1 or 5 days after the surgery. **a** BDNF expression. **b** GDNF expression. **c** NGF expression. Results are means±S.E.M. (n=8). *P<0.05 compared with control; ^P<0.05 compared with surgery plus standard environment (*SE*)

sections with sodium citrate buffer containing 10 mM sodium citrate, 0.05 % Tween 20 (pH 6.0) at 95–100 °C for 20 min. DNA denaturation was done by incubating with 1 N HCl (Sigma-Aldrich) on ice for 3 min, 2 N HCL at room temperature for 3 min, and at 37 °C for 6 min. Sections were blocked with 5 % donkey serum in phosphate-buffered saline containing 0.5 % triton-X 100 for 2 h at room temperature. The sections were then incubated overnight at 4 °C with the following primary antibodies: rat monoclonal anti-BrdU antibody (Abcam, 1:100), rabbit monoclonal anti-Ki67 antibody (Abcam, 1:50), rabbit polyclonal anti-doublecortin antibody (Abcam, 1:100), or mouse monoclonal anti-glial fibrillary acidic protein (GFAP) antibody (Millipore, 1:200). The sections were incubated with donkey anti-rat IgG antibody conjugated with Alexa Fluor 594 (Invitrogen, 1:500), donkey anti-rabbit IgG antibody conjugated with Alexa Fluor 488 (Invitrogen, 1:500), or donkey anti-mouse IgG antibody conjugated with Alexa Fluor 488 (Invitrogen, 1:500) for 1 h at room temperature in a dark room. After washed in phosphate-buffered saline, sections were counterstained with Hoechst 33342 (Thermo Scientific), rinsed and mounted with Vectashield mounting medium (Vector Labs). Images were acquired with a LSM700 confocal microscopy system (ZEISS). For each mouse brain, six sequential hippocampal sections were used for cell counting. The number of all cells positively stained for an interesting marker or the combination of two markers in the subgranular zone of the dentate gyrus of each section was counted.

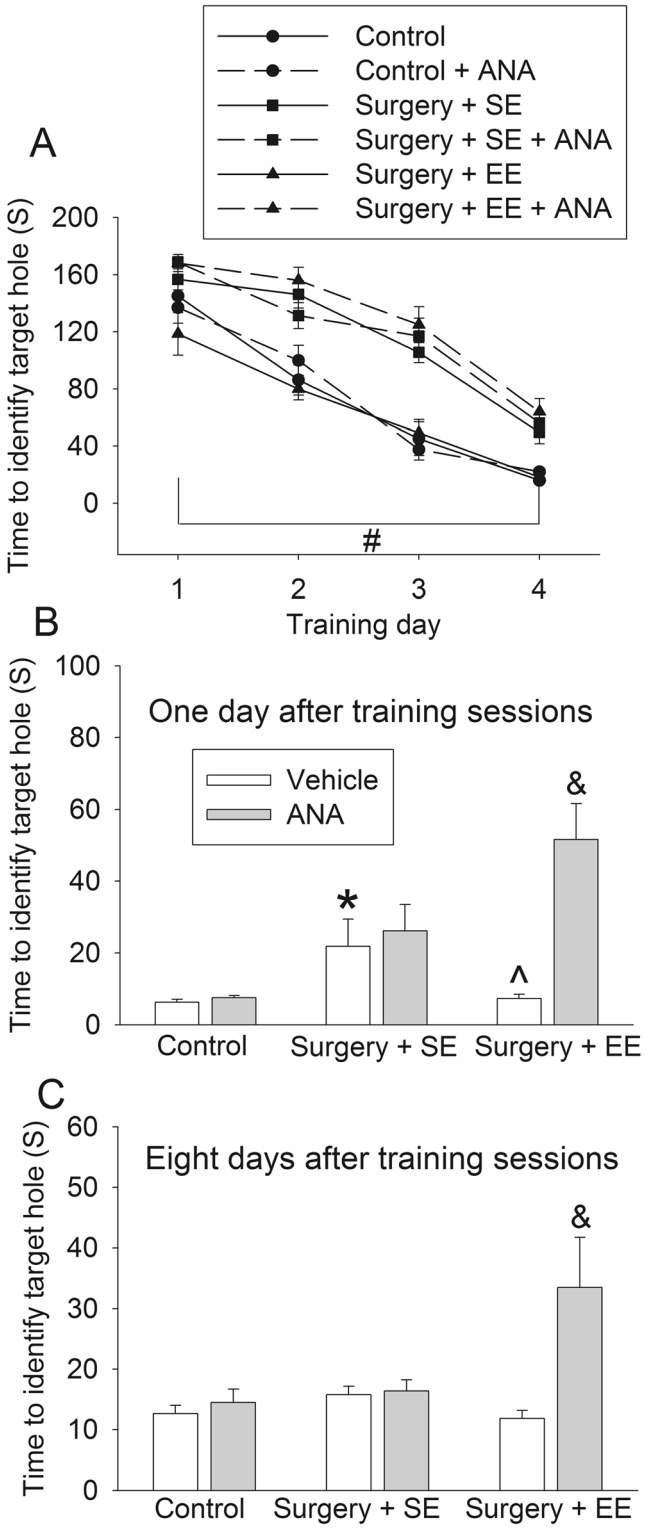
Statistical Analysis

Results are presented as means±S.E.M. (n≥7). Data from the training sessions of Barnes maze were analyzed by two-way repeated measures analysis of variance followed by Tukey's test. The other data were analyzed by one-way analysis of variance followed by Tukey's test or by Student's t test as appropriate. Differences were considered significant at a P<0.05. All statistical analyses were performed with SigmaStat (Systat Software, Inc., Point Richmond, CA, USA).

Results

Enriched Environment Attenuated Surgery-Induced Learning and Memory Impairment

The time for mice in the control, surgery plus SE, and surgery plus EE groups to identify the target hole was decreased with increased training sessions. This time was significantly shorter on day 2, day 3, and day 4 than that on day 1 for all three groups of mice (Fig. 1a). Surgery had a significant effect on the time to identify the target hole during training sessions [F(1,22)=32.871, P<0.001]. EE attenuated this surgical effect [F(1,22)=43.16, P<0.001]. Similarly, surgery increased the time needed for mice to identify the target hole on the first day after the training sessions. This effect was blocked by EE. However, the time to identify the target hole at 8 days after the training sessions was not different among the three groups of



◀ **Fig. 4** TrkB inhibition blocked enriched environment (*EE*)-induced improvement of learning and memory after surgery. Eight-week-old mice were subjected to right carotid artery exposure under sevoflurane anesthesia. They were started to be tested by Barnes maze at 2 weeks after the surgery. **a** Performance during the training sessions. **b** Performance at 1 day after the training sessions. **c** Performance at 8 days after the training sessions. Results are means±S.E.M. (n=8). #P<0.05 compared with the corresponding values at time 0 for all six groups; *P<0.05 compared with control; ^P<0.05 compared with surgery plus standard environment (*SE*); &P<0.05 compared with surgery plus EE. *ANA* ANA-12

mice (Fig. 1b). These results suggest that surgery impairs the spatial learning and memory and that this impairment was blocked by EE.

Similar to Barnes maze results, surgery reduced the context-related freezing behavior, and this reduction was attenuated by EE. However, the tone-related freezing behavior was not affected by surgery or EE (Fig. 1c). These results suggest that EE reverses surgery-induced impairment of hippocampus-dependent learning and memory. Thus, evaluation of neurogenesis and biochemical changes was performed in the hippocampus.

Mice in the sevoflurane anesthesia and EE only groups took less time with more training sessions to identify the target hole. They did not behave differently when compared with mice in the control group in the Barnes maze and fear conditioning tests (Fig. 2).

Enriched Environment Blocked Surgery-Induced Reduction of BDNF Expression in the Hippocampus

To determine whether surgery and EE affect growth factor expression, hippocampus was harvested 1 or 5 days after the surgery. Surgery significantly reduced BDNF expression 5 days after the surgery. This surgery-induced reduction was blocked by EE. There was no significant difference in the BDNF levels among control mice, mice in the surgery plus SE, and mice in the surgery plus EE at 1 day after the surgery (Fig. 3a). There was also no difference in the expression of nerve growth factor (NGF) and glial cell-derived neurotrophic factor (GDNF) among the three groups of mice at 1 and 5 days after the surgery (Fig. 3b, c). Since BDNF is known to affect learning, memory, and neurogenesis [18], these results suggest a possible role of BDNF in the effects of surgery and EE on cognition and neurogenesis.

TrkB Inhibition Blocked Enriched Environment-Induced Improvement of Learning and Memory After Surgery

To imply the role of BDNF signaling pathway in the EE effect, we used ANA-12, a selective TrkB antagonist [19]. TrkB is a BDNF receptor. BDNF can bind and activate TrkB, which then activates the downstream signaling proteins, such as extracellular signal-regulated kinases (ERK) [23]. Consistent with the first set of experiment on learning and memory, surgery was a significant factor to affect the time for mice to identify the target hole, and this effect was attenuated by EE during the training sessions of the Barnes maze test. However, ANA-12 reversed the EE effect [F(1,14)=57.153, P<0.001]

(Fig. 4a). Similarly, ANA-12 also reversed the effects of EE on the time to identify the target hole at 1 day after the training sessions (Fig. 4b). Although surgery and EE did not affect the time for the mice to identify the target hole 8 days after the training sessions, ANA-12 significantly increased the time for the mice subjected to surgery plus EE to identify the target hole (Fig. 4c). These results suggest that TrkB signaling may be involved in the EE effects on cognition. Of note, the time to identify the target hole at 1 or 8 days after the training sessions in the Barnes maze test was not different between mice subjected to surgery plus SE plus ANA-12 and surgery plus EE plus ANA-12 (Fig. 4b, c). These results suggest that ANA-12 impairs the learning and memory of mice treated with EE to the same level as the mice without EE treatment after the surgery.

TrkB Inhibition Blocked Enriched Environment-Induced TrkB and ERK Activation in the Hippocampus

Similar to the change pattern of BDNF, surgery reduced the phosphorylation/activation of TrkB and ERK. This reduction was attenuated by EE. ANA-12 blocked the effects of EE on the phosphorylation of TrkB and ERK. On the other hand, surgery, EE, and ANA-12 did not significantly affect the expression of total TrkB and ERK (Fig. 5). These results suggest that ANA-12 inhibited the TrkB signaling activation in the mouse hippocampus after surgery plus EE.

TrkB Inhibition Blocked Enriched Environment-Induced Neurogenesis in the Hippocampus

Surgery significantly reduced the number of cells positively stained for BrdU in the dentate gyrus. This reduction was reversed by EE. The effect of EE was attenuated by ANA-12. A similar change pattern was observed for the number of cells positively stained for doublecortin, doublecortin and BrdU, BrdU and GFAP, and Ki67 in the dentate gyrus (Figs. 6 and 7). Sevoflurane anesthesia and EE alone did not change the number of cells stained positively for BrdU, doublecortin, doublecortin and BrdU, BrdU and GFAP, and Ki67 in the dentate gyrus (Figs. 8 and 9). These results suggest that surgery but not anesthesia alone inhibits neurogenesis and astrogenesis. This inhibition was attenuated by EE. ANA-12 blocks the effects of EE on neurogenesis and astrogenesis.

Discussion

Our results showed that surgery but not sevoflurane anesthesia alone reduced the number of cells positively stained for BrdU

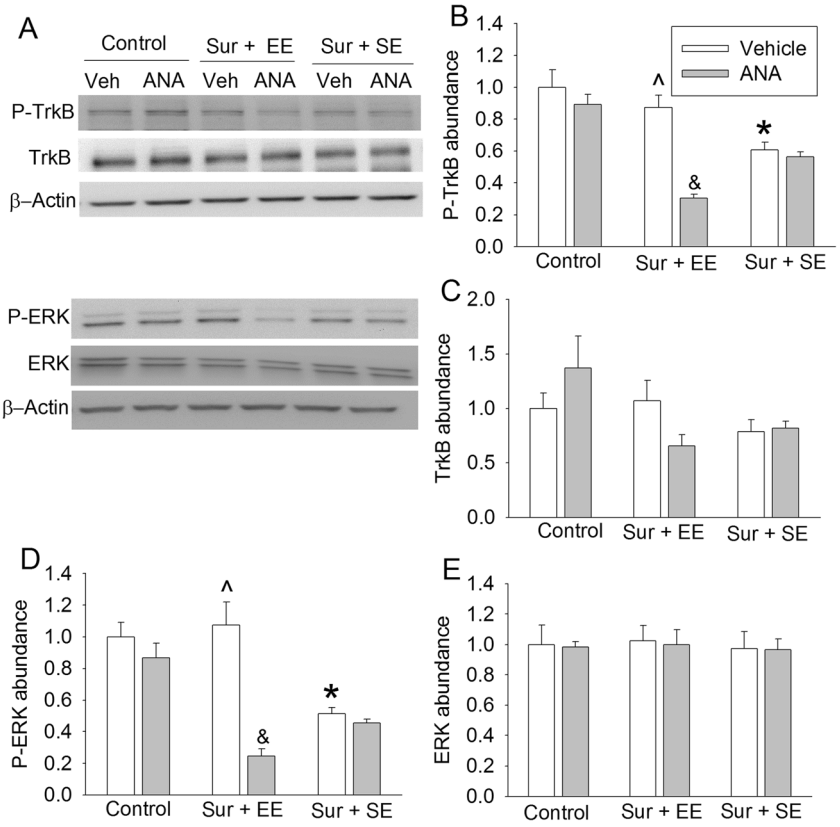


Fig. 5 TrkB inhibition blocked enriched environment (*EE*)-induced recovery of BDNF signaling after surgery. Eight-week-old mice were subjected to right carotid artery exposure under sevoflurane anesthesia. Hippocampus was harvested at 5 days after the surgery. **a** Representative Western blot images. **b** Quantitative results of phospho-TrkB (P-TrkB) abundance. **c** Quantitative results of TrkB abundance. **d** Quantitative results of phospho-ERK (P-ERK) abundance. **e** Quantitative results of ERK abundance. Results in the bar graph are means±S.E.M. (n=8). *P<0.05 compared with control; ^P<0.05 compared with surgery plus standard environment (*SE*), &P<0.05 compared with surgery plus EE. *Veh* vehicle, *Sur* surgery

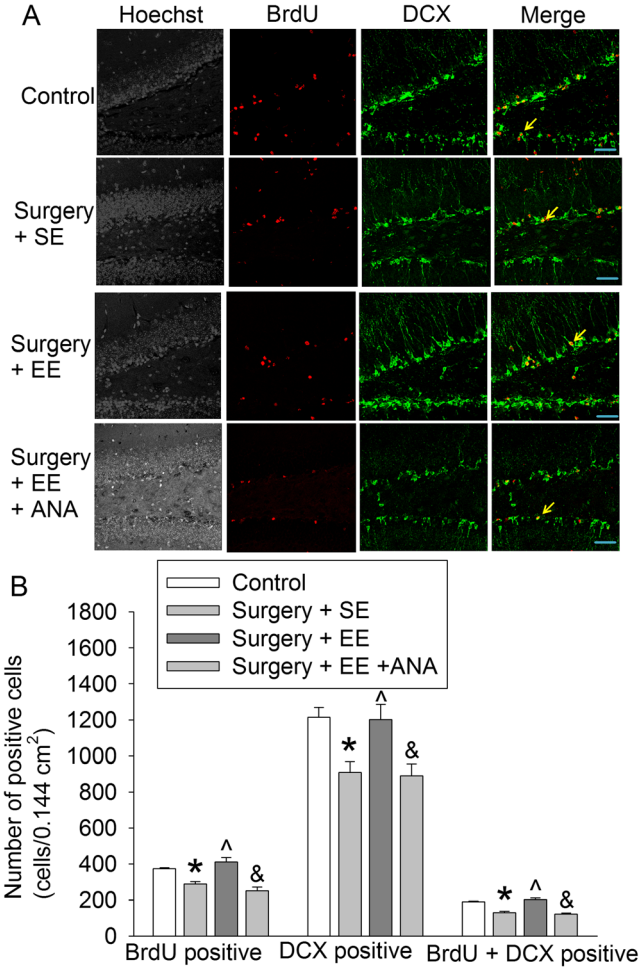


Fig. 6 TrkB inhibition blocked enriched environment (*EE*)-induced neurogenesis after surgery. Eight-week-old mice were subjected to right carotid artery exposure under sevoflurane anesthesia. Hippocampus was harvested at 2 weeks after the surgery. **a** Representative fluorescent staining images. BrdU staining is in *red* and doublecortin (DCX) is in *green*. *Scale bar*=50 μm. *Arrows* indicate cells that are positively stained for both BrdU and DCX. **b** Quantitative results. Results in the *bar graph* are means±S.E.M. (n=8). *P<0.05 compared with control; ^P<0.05 compared with surgery plus standard environment (*SE*); &P<0.05 compared with surgery plus EE. *ANA* ANA-12 (color figure online)

plus doublecortin and BrdU plus GFAP. Doublecortin is a marker for neuronal precursor cells and immature neurons [24]. GFAP is an astrocytic marker [25]. Surgery also reduced the number of cells positively stained for Ki67, a marker for cell proliferation [26]. These results provide initial evidence that surgery inhibits neurogenesis and astrogenesis. These effects may be due to the inhibition of BDNF signaling pathway because surgery reduced BDNF expression and the activation of BDNF signaling pathway. Also, EE attenuated the surgery-induced inhibition of BDNF expression, BDNF signaling, neurogenesis, and astrogenesis in the hippocampus but did not affect cell genesis under control condition. Finally, ANA-12 inhibited the effects of EE on BDNF signaling, neurogenesis, and astrogenesis. Consistent with the possible role of BDNF in surgery-induced neurogenesis and astrogenesis, BDNF has been shown to enhance neurogenesis [18].

Our results provide first evidence that EE can attenuate surgery-induced learning and memory impairment. Our study showed that surgery impaired spatial and context-related learning and memory. These effects were attenuated by EE. The surgery-induced learning and memory impairment may be due to the reduced BDNF signaling and neurogenesis because EE reversed surgery-induced cognitive impairment and the inhibition of BDNF signaling and neurogenesis in the hippocampus. In addition, ANA-12 inhibited the effects of EE. These results suggest a critical role of BDNF signaling and neurogenesis in the EE effects on surgery-induced learning and memory impairment. In supporting this possibility, BDNF has been shown to improve cognitive functions [18], and neurogenesis is a process for learning and memory [15, 27]. We showed here that EE-induced increase of BDNF concentrations and neurogenesis in the hippocampus was detected at 5 days and 2 weeks, respectively, after the surgery. This time sequence supports the idea that EE leads to BDNF increase, which then results in a long-lasting increase of neurogenesis to improve the learning and memory of mice after surgery.

Studies have indicated the role of neuroinflammation in the learning and memory impairment caused by anesthesia and surgery [5, 6, 20]. However, neuroinflammation lasts for only a few days after surgery. Learning and memory impairment exists when neuroinflammation is no longer apparent [20]. It has been difficult to interpret these findings. Long-lasting effects after neuroinflammation to lead to the learning and memory impairment caused by anesthesia and surgery have been proposed [20]. Inhibition of BDNF signaling and neurogenesis may be those effects because we clearly observed in this study that neurogenesis was deceased when the learning and memory impairment was present. In addition, neuroinflammation has been shown to inhibit neurogenesis [14], and proinflammatory cytokines reduce BDNF expression [28]. EE that attenuated surgery-induced learning and memory impairment in this study has been shown to reduce neuroinflammation induced by mild brain trauma and influenza infection [12, 13]. However, further studies are needed to determine the role of neuroinflammation in the surgery-induced decrease of BDNF signaling and neurogenesis.

Our data indicate the impairment of hippocampus-dependent learning and memory after surgery because context-related fear conditioning was impaired. Tone-related fear conditioning was not affected by the surgery, suggesting components other than hippocampus in the fear circuit, such as amygdala, may not be the source of learning and memory dysfunction. This pattern is consistent with the finding of a previous study in which mice had a tibal fracture and fixation surgery [5]. However, our study cannot exclude the possibility

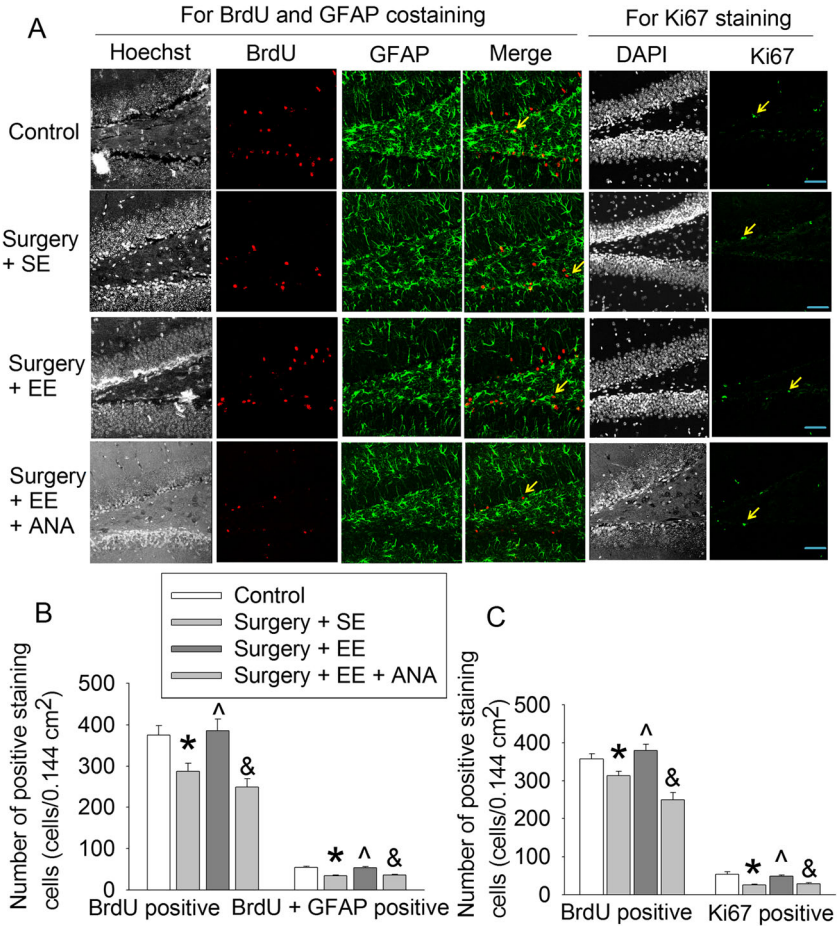


Fig. 7 TrkB inhibition blocked enriched environment (*EE*)-induced astrogenesis after surgery. Eight-week-old mice were subjected to right carotid artery exposure under sevoflurane anesthesia. Hippocampus was harvested at 2 weeks after the surgery. **a** Representative fluorescent staining images. BrdU staining is in *red* and GFAP and Ki67 are in *green*. *Scale bar*= 50 μm. *Arrows* indicate cells that are positively stained for both BrdU and GFAP or Ki67. **b**, **c** Quantitative results. Results in the *bar graph* are means±S.E.M. (n= 8). *P<0.05 compared with control; ^P<0.05 compared with surgery plus standard environment (SE); &P<0.05 compared with surgery plus EE. *ANA* ANA-12 (color figure online)

that the function of brain regions other than hippocampus is affected by the surgery because spatial learning and memory assessed by Barnes maze were affected by the surgery. Nevertheless, our results and the previous study [5] suggest that hippocampus-dependent learning and memory are very sensitive to surgery. Interestingly, there was a significant difference between control mice and mice after surgery in the time to identify the target hole at 1 day but not at 8 days after the training sessions in the Barnes maze. The reasons for the disappearance of the difference at 8 days after the training sessions are not known. One possibility is that the memory after the training sessions was not permanent so that the difference between control mice and mice after surgery in the time to identify the target hole becomes smaller with increased time.

We showed that neurogenesis in the DG is reduced by surgery. This effect should reduce the total number of neurons in the hippocampus. However, the difference in the total number of neurons between control mice and mice after surgery may be very small, considering that the reduction in the neurogenesis after surgery was small and the total number of neurons is huge in the hippocampus.

Sevoflurane anesthesia alone did not affect mouse learning and memory in this study. Anesthesia with volatile anesthetics, such as isoflurane, for 2 h has been shown to impair cognition in adult rats [29]. Exposure to sevoflurane for 1 h per month for five consecutive months also induces learning and memory impairment in adult mice [30]. Different anesthetics and exposure methods may explain the difference in learning and memory functions after anesthetic exposure in this study and previous studies [29, 30]. Consistent with the failure of inducing learning and memory impairment, sevoflurane exposure did not affect the neurogenesis under control condition in our study. Similar to our study, a previous study showed that isoflurane anesthesia did not affect neurogenesis in the hippocampus of rats [31]. Our results that surgery under sevoflurane anesthesia impairs learning, memory, and neurogenesis but sevoflurane anesthesia alone did not affect learning, memory, and neurogenesis suggest that surgery may be the major factor to induce the impairment of learning, memory, and neurogenesis in our mice.

Our study showed that EE did not affect learning, memory, and neurogenesis under control condition. A previous study showed that EE for at least 3 weeks improved learning and

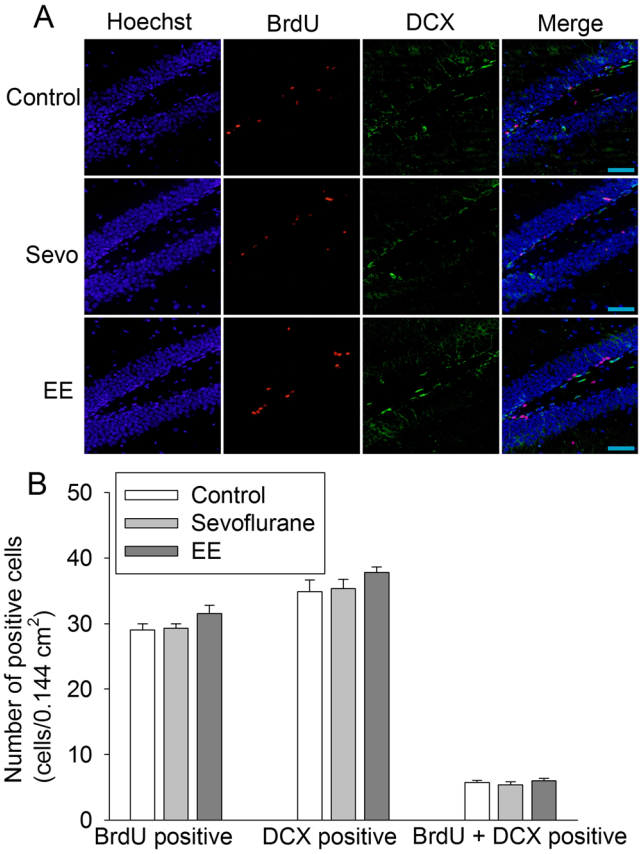


Fig. 8 No effect of sevoflurane and enriched environment (*EE*) on neurogenesis under control condition. Eight-week-old mice were subjected to sevoflurane anesthesia or EE. Hippocampus was harvested at 2 weeks after the anesthesia or EE. **a** Representative fluorescent staining images. BrdU staining is in *red* and doublecortin (DCX) is in *green*. *Scale bar*=50 μm. **b** Quantitative results. Results in the *bar graph* are means±S.E.M. (n=7) (color figure online)

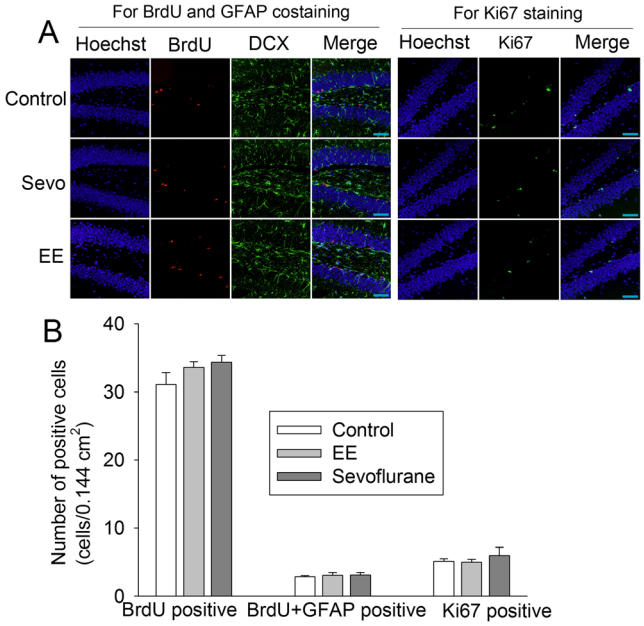


Fig. 9 No effect of sevoflurane and enriched environment (*EE*) on astrogenesis under control condition. Eight-week-old mice were subjected to sevoflurane anesthesia or EE. Hippocampus was harvested at 2 weeks after the anesthesia or EE. **a** Representative fluorescent staining images. BrdU staining is in *red* and GFAP and Ki67 are in *green*. *Scale bar*=50 μm. **b**, **c** Quantitative results. Results in the *bar graph* are means±S.E.M. (n=8) (color figure online)

memory and that 6-week EE was needed to increase neurogenesis in the hippocampus under control condition in young adult rats [10]. Thus, it is possible that the failure to improve learning, memory, and neurogenesis of mice by EE under control condition is due to a relatively short observation time in our study. Nevertheless, our results as a whole indicate that EE reverses surgery-induced impairment in learning, memory, and neurogenesis and that this effect of EE on cognition and neurogenesis of mice after surgery is most likely not due to the enhanced neurogenesis and cognition under control condition.

Our studies have significant implications. Surgery is performed in millions of patients each year in the USA. We showed here that a small surgical procedure in mice induced a significant decrease of neurogenesis. If this finding is confirmed in human, our result would suggest that a peripheral surgery can induce a long-term brain structural change. Fortunately, this surgery-induced change can be easily reversed by EE, a condition that often exists or can be easily provided in human after surgery.

Our studies have limitations. We subjected mice to a relatively mild surgical procedure. It is not clear whether EE can block learning and memory impairment after a major surgery. Also, we subjected mice to EE for 6 h each day. Our study has not provided information to differentiate whether the EE-induced improvement in learning, memory, and neurogenesis is due to EE-induced increase of exercises or exploratory brain activity. Finally, we showed that surgery inhibited BDNF expression but did not affect the expression of NGF and GDNF in the hippocampus. Future studies are needed to determine how this selectivity of the effects on growth factor expression occurs.

In summary, we showed that carotid artery exposure impaired mouse learning and memory. This surgery also reduces BDNF signaling, neurogenesis, and astrogenesis in the hippocampus. EE attenuated these surgical effects via maintaining BDNF signaling.

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Author Contributions Z.Z. conceived the concept of the project. D.F. and Z.Z. designed research; D.F., J.L., B.Z., and L.H. performed research; D.F., J.L., B.Z., and Z.Z. analyzed data; and D.F. and Z.Z. wrote the paper.

Conflicts of Interest None.

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