Disruption of Hippocampal Neuregulin 1–ErbB4 Signaling Contributes to the Hippocampus-dependent Cognitive Impairment Induced by Isoflurane in Aged Mice

Xiao-Min Li, M.D., Fan Su, M.D., Ph.D., Mu-Huo Ji, M.D., Guang-Fen Zhang, M.D., Li-Li Qiu, M.D., Min Jia, M.D., Jun Gao, M.D., Ph.D., Zhongcong Xie, M.D., Ph.D., Jian-Jun Yang, M.D., Ph.D.

ABSTRACT

Background: A prolonged isoflurane exposure may lead to cognitive decline in rodents. Neuregulin 1 (NRG1)–ErbB4 signaling plays a key role in the modulation of hippocampal synaptic plasticity through regulating the neurotransmission. The authors hypothesized that hippocampal NRG1–ErbB4 signaling is involved in isoflurane-induced cognitive impairments in aged mice.

Methods: Fourteen-month-old C57BL/6 mice were randomized to receive 100% O_2 exposure, vehicle injection after 100% O_2 exposure, vehicle injection after exposure to isoflurane carried by 100% O_2, NRG1-β1 injection after exposure to isoflurane carried by 100% O_2, and NRG1-β1 and an ErbB4 inhibitor AG1478 injection after exposure to isoflurane carried by 100% O_2. Fear conditioning test was used to assess the cognitive function of mice 48-h postexposure. The brain tissues were harvested 48-h postexposure to determine the levels of NRG1, ErbB4, p-ErbB4, parvalbumin, and glutamic acid decarboxylase 67 in the hippocampus using Western blotting, enzyme-linked immunosorbent assay, and immunofluorescence.

Results: The percentage of freezing time to context was decreased from 50.28 ± 11.53% to 30.82 ± 10.00%, and the hippocampal levels of NRG1, p-ErbB4/ErbB4, parvalbumin, and glutamic acid decarboxylase 67 were decreased from 172.79 ± 20.85 ng/g, 69.15 ± 12.20%, 101.68 ± 11.21%, and 104.71 ± 6.85% to 112.92 ± 16.65 ng/g, 42.26 ± 9.71%, 75.89 ± 10.26%, and 73.87 ± 16.89%, respectively, after isoflurane exposure. NRG1-β1 attenuated the isoflurane-induced hippocampus-dependent cognitive impairment and the declines in the hippocampal NRG1, p-ErbB4/ErbB4, parvalbumin, and glutamic acid decarboxylase 67. AG1478 inhibited the rescuing effects of NRG1-β1.

Conclusion: Disruption of NRG1–ErbB4 signaling in the parvalbumin-positive interneurons might, at least partially, contribute to the isoflurane-induced hippocampus-dependent cognitive impairment after exposure to isoflurane carried by 100% O_2 in aged mice. **(Anesthesiology 2014; 121:79-88)**

POSTOPERATIVE cognitive decline or dysfunction occurs frequently in the elderly after surgery and anesthesia, with the impairments in recent memory, attention, language comprehension, and social integration.[1–3] It may prolong the patients' hospital stay, diminish the quality of life, and affect the recovery process.[1–4] In clinical practice, patients usually undergo surgery with general anesthesia; however, previous documents have suggested that patients receiving general anesthesia are associated with increased risk of developing postoperative cognitive dysfunction compared with the risk associated with patients receiving regional anesthesia.[5,6] Recently, accumulating evidence has shown that surgery induces cognitive impairments in rodents.[7–10] Anesthetics have also been reported to elicit cognitive impairment in rodents.[11–14] However, the effects of anesthetic isoflurane on cognitive function in aged mice and the underlying mechanisms remain largely to be determined. We therefore set out to assess the effects of isoflurane

What We Already Know about This Topic

- Postoperative cognitive decline or dysfunction occurs frequently in the elderly after surgery and anesthesia, but the role of anesthetics (*i.e.*, isoflurane) in this process remains unclear

What This Article Tells Us That Is New

- Isoflurane in 100% oxygen significantly impaired the hippocampus-dependent cognitive function assessed at 48 h by the fear conditioning test in aged mice
- This effect was mediated at least in part *via* disruption of the neurotrophic factor–dependent neuregulin 1–ErbB4 signaling in the parvalbumin-positive interneurons

The first two authors contributed equally to this work (X.-M.L. and F.S.).

Submitted for publication September 5, 2013. Accepted for publication February 3, 2014. From the Department of Anesthesiology, Jinling Hospital, School of Medicine, Nanjing University, Nanjing, China (X.-M.L., M.-H.J., G.-F.Z., L.-L.Q., M.J., J.-J.Y.); Department of Anesthesiology, Affiliate Hospital, Shandong Medical University of Traditional Chinese Medicine, Jinan, China (F.S.); Departments of Neurobiology and Pharmacology, Key Laboratory for Neurodegenerative Disease of Jiangsu Province, Nanjing Medical University, Nanjing, China (J.G.); and Geriatric Anesthesia Research Unit, Department of Anesthesia, Critical Care, and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts (Z.X.).

 Anesthesiology 2014; 121:79-88

on learning and memory function in aged mice and to study the potential mechanism.

Neuregulin 1 (NRG1), a member of the epidermal growth factor family, acts by promoting the tyrosine kinase of ErbB receptor autophosphorylation.[15] ErbB4, one of the ErbB receptors, has a high affinity to NRG1.[16] Recent data suggest a key role of the NRG1–ErbB4 signaling in neurotransmission, synaptic plasticity, and synchronization of neuronal network activity in the cortex and hippocampus through raising precisely timed γ-aminobutyric acid release, which is of great importance for cognition, learning, and memory.[15–17]

Notably, the distribution of ErbB4 is largely restricted to specific classes of interneurons, particularly in the parvalbumin-positive interneurons.[18] It has been reported that cognitive processes are regulated by the parvalbumin-positive interneurons, in which ErbB4 is required in the procedure of NRG1 modulating the pyramidal neuronal activity and the long-term potentiation in the adult mouse brain.[19] In the ErbB4$^{-/-}$ mice, parvalbumin-positive and glutamic acid decarboxylase (GAD) 67–positive interneurons are significantly decreased.[20] In the NRG1–ErbB4 signaling–mutant mice, the hippocampus-dependent memory was impaired notably.[19,21] Therefore, we hypothesized that the cognitive impairments induced by the isoflurane exposure in aged mice may be attributed to the disruption of hippocampal NRG1–ErbB4 signaling probably in the parvalbumin-positive interneurons by down-regulating the levels of parvalbumin and GAD67.

Materials and Methods

The animal care and the experiment were approved by the Ethics Committee of Jinling Hospital, Nanjing, China, and were performed according to the Guide for the Care and Use of Laboratory Animals approved by the National Institutes of Health of the United States.

Animal Groups

Ninety male C57BL/6 mice with the age of 14-month old were purchased from the Animal Center of Jinling Hospital, Nanjing, China. The mice were housed five per cage in a 12-h light–dark cycle in a room of 22° ± 1°C with food and water available *ad libitum*. After 7 days of accommodation, the mice were randomly divided into five groups (n = 18): control group, dimethyl sulfoxide (vehicle) group, isoflurane group, isoflurane plus NRG1-β1 (a recombinant NRG1-1β; R&D Systems, Minneapolis, MN) group, and isoflurane plus NRG1-β1 plus AG1478 group (an ErbB4 inhibitor; Tocris Bioscience, Bristol, United Kingdom).

Gas Exposure

The isoflurane exposure was performed by placing mice with spontaneous breathing in an anesthesia chamber prefilled with 1.5% isoflurane carried by 100% O_2. The anesthesia was induced and maintained with 1.5% isoflurane carried by 100% O_2 of 1.5 l/min for 2 h. Temperatures of the mice were maintained at 37° ± 0.5°C during anesthesia by heating lamps and heating pads. In our preliminary experiment, we collected more than 0.5 ml of arterial blood from both the isoflurane-exposed mice and the 100% O_2–exposed mice immediately after the gas exposure with the same protocol of the current study. The blood gas analysis has shown no hypoxia or acidosis immediately after the isoflurane exposure or the 100% O_2 exposure in the mice. After the gas exposure, the mice were placed back into the home cage for emergence with 100% O_2 of 1.5 l/min supply. The mice in the control group and the vehicle group were not anesthetized but were given 100% O_2 of 1.5 l/min for 2 h in the identical chamber.

Intracerebroventricular Cannulation and Drug Interventions

We implanted a stainless steel cannula into the left lateral ventricle (0.45 mm posterior, 1.08 mm lateral to bregma, and 2.50 mm below dura) during the anesthesia with intraperitoneal injection of 60 mg/kg sodium pentobarbital except for the mice in the control group. Then, the mice were given a period of 1 week for postoperative recovery.

NRG1-β1 (10 μM) and/or AG1478 (5 mM) were intracerebroventricularly injected 1 h before the behavior tests for 2 days. The doses of NRG1-β1 and AG1478 were selected according to the previous studies.[12,18] The time points for the drug injection were selected on the basis of the results of our preliminary experiment, showing that the hippocampal p-ErbB4 levels peaked 1 h after the intracerebroventricular injection of NRG1-β1 in mice (fig. 1). The total injection volume was 2 μl. The mice in the vehicle group and the isoflurane group received an equivalent volume of vehicle.

Open-field Test

The locomotor and exploratory activities of the mice (n = 10 for each group) were evaluated by the open-field test 24 h after the gas exposure. Each mouse was released in the center of the white plastic chamber (30 cm × 30 cm× 30 cm, XR-XZ301; Shanghai Softmaze Information Technology Co., Ltd., Shanghai, China) and left to explore it for 5 min while the activities were automatically recorded by a video-tracking system. At the end of testing, the arena was cleaned with 75% alcohol to avoid the presence of olfactory cues.

Fear Conditioning Test

The fear conditioning test is a simple and sensitive test of hippocampus-dependent and hippocampus-independent memory function. After the open-field test, the mouse was placed in the conditioning chamber (XR-XC404; Shanghai Softmaze Information Technology Co., Ltd.) for 3 min as an accommodation period and then one tone-foot-shock

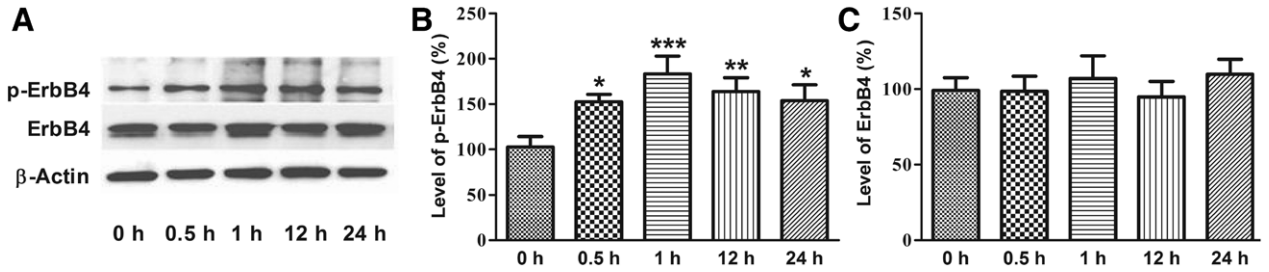

Fig. 1. The hippocampal levels of p-ErbB4 and ErbB4 after neuregulin 1 (NRG1)-β1 injection at different time points. The hippocampal p-ErbB4 levels increased significantly and peaked 1 h after the injection of NRG1-β1 (*A*, *B*) without significant change in ErbB4 levels (*A*, *C*). Data are mean ± SD. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ *vs.* the 0 h group.)

pairing (tone, 30 s, 65 dB, 1 kHz; foot-shock, 2 s, 0.75 mA) was delivered. The mouse was allowed to explore the chamber for another 30 s after the shock to study postshock freezing. The contextual fear conditioning test was assessed 24 h after the training by placing the mice back in the same test chamber for 5 min. The tone fear conditioning test was assessed 2 h after the contextual fear conditioning test in a novel chamber changed in the shape, color, and smell and the training tone was delivered for 3 min. Freezing behavior, defined as the absence of all visible movement of the body except the movement necessitated by respiration, was scored by an observer software. At the end of testing, the chamber was cleaned with 75% alcohol to avoid the presence of olfactory cues.

Brain Harvest

The brain tissues of eight mice without any behavior tests from each group were harvested 48 h after the gas exposure to determine the levels of NRG1, ErbB4, p-ErbB4, parvalbumin, and GAD67 in the hippocampus using Western blotting, enzyme-linked immunosorbent assay, and immunofluorescence.

Western Blotting

The levels of ErbB4, p-ErbB4, parvalbumin, and GAD67 in the hippocampus of mice (n = 4 for each group) were assessed by the Western blotting 48 h after the gas exposure. The normalized protein samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and then were transferred onto polyvinylidene difluoride membranes. Membranes were blocked with 5% skim milk in Tris-buffered saline tween for 1 h and then incubated with rabbit anti-ErbB4 (1:500; Santa Cruz Biotechnology, Dallas, TX), rabbit anti-p-ErbB4 (1:500; Santa Cruz Biotechnology), rabbit anti-β-actin (1:1,000; Cell Signaling Technology, Boston, MA), rabbit anti-parvalbumin (1:1,000; Abcam, Cambridge, United Kingdom), and mouse anti-GAD67 (1:500; Santa Cruz Biotechnology) overnight in a room at 4°C temperature. After thorough washing, membranes were incubated in Tris-buffered saline tween with the secondary antibody (goat anti-rabbit and goat anti-mouse; Santa Cruz Biotechnology) diluted 1:1,000 for 1 h at room temperature. Bands were visualized by the enhanced chemiluminescence and quantitated with the Image Quant Software (Syngene, Cambridge, United Kingdom).

Enzyme-linked Immunosorbent Assay

The levels of NRG1 in the hippocampus of mice (n = 4 for each group) were determined by the enzyme-linked immunosorbent assay kits following the protocol provided by the manufacturer (BGI, Shenzhen, China). The readings were normalized to the amount of standard protein.

Immunofluorescence

The immunofluorescence of ErbB4, parvalbumin, and GAD67 in brain sections of mice (n = 4 for each group) was assessed 48 h after the gas exposure. The brains were cut coronally. Five sections (10 μm thickness) per mouse were chosen by randomization for the immunohistochemistry studies. There was a 30-μm interval between the two sections. The tissue sections were incubated in 1% bovine serum albumin for 1 h and followed by primary antibodies: rabbit anti-ErbB4 (1:50; Santa Cruz Biotechnology), mouse anti-parvalbumin (1:500; Abcam), and mouse anti-GAD67 (1:100; Santa Cruz Biotechnology) in 1% bovine serum albumin at 4°C overnight. After washing with phosphate buffered saline, the sections were incubated in goat anti-mouse IgG-fluorescein isothiocyanate, anti-mouse IgG-Cy3, and goat anti-rabbit IgG-Cy3 (1:200; Bioworld Technology, St. Louis Park, MN) for 1 h at room temperature. Fluorescent images were captured by a confocal microscope. Coexpression of ErbB4 with GAD67 or parvalbumin was determined by counting only the neurons that were independently, clearly, and identifiable in both the red and green color channels with 4', 6-diamidino-2-phenylindole staining, rather than solely on appearance of yellow pixels in the merged images. The z-stack high-magnification colocalization images for the ErbB4 and GAD67 or parvalbumin were obtained. The slices were imaged every 1 μm across the entire slice and then collapsed together to form one image of the resulting z-stack picture. For immunohistochemistry analysis, the mean value of eight random regions (×40) across hippocampus in each section was calculated. The numerical density (cells per square millimeter) was calculated using the confocal images with the Image J, version 1.26t (National Institutes of Health, Bethesda, MD).

Statistical Analysis

Data are presented as mean ± SD. There were four samples in each group. The sample size was guided by the experience from the pilot studies. Normality of data was analyzed by using Shapiro–Wilk test, and the data were found in Gaussian distribution. Therefore, the comparisons among the groups were performed by using one-way ANOVA followed by the Tukey test (two-tailed). The data were analyzed by using the SPSS software (version 16.0; SPSS Inc., Chicago, IL). *P* value less than 0.05 was considered statistically significant.

Results

Behavior Results of Mice Postgas Exposure

Both intracerebroventricular cannulation and drug interventions did not significantly affect the total ambulatory distance traveled and the time spent in the center during the open-field test (fig. 2, A and B). The isoflurane exposure reduced the freezing time to context from 50.28 ± 11.53% to 30.82 ± 10.00% compared with that in the control group (n = 10; *P* = 0.002; fig. 2C). NRG1-β1 increased the freezing time to context to 46.45 ± 9.88% in the isoflurane plus NRG1-β1 group compared with that in the isoflurane group (n = 10; *P* = 0.017; fig. 2C), which was abolished by AG1478 in the isoflurane plus NRG1-β1 plus AG1478 group (31.97 ± 10.94%; n = 10; *P* = 0.032; fig. 2C). No significant difference was detected in respect of the freezing time to tone among the five groups (fig. 2D).

Hippocampal Levels of NRG1 and ErbB4 Postgas Exposure

The hippocampal concentration of NRG1 decreased to 112.92 ± 16.65 ng/g in the isoflurane group, but not in the isoflurane plus NRG1-β1 and isoflurane plus NRG1-β1 plus AG1478 groups, compared with that in the control group (172.79 ± 20.85 ng/g; n = 4; *P* = 0.007; fig. 3A). The hippocampal p-ErbB4/ErbB4 decreased to 42.26 ± 9.71% in the isoflurane group compared with that in the control group (69.15 ± 12.20%; n = 4; *P* = 0.024; fig. 3, B and C). The

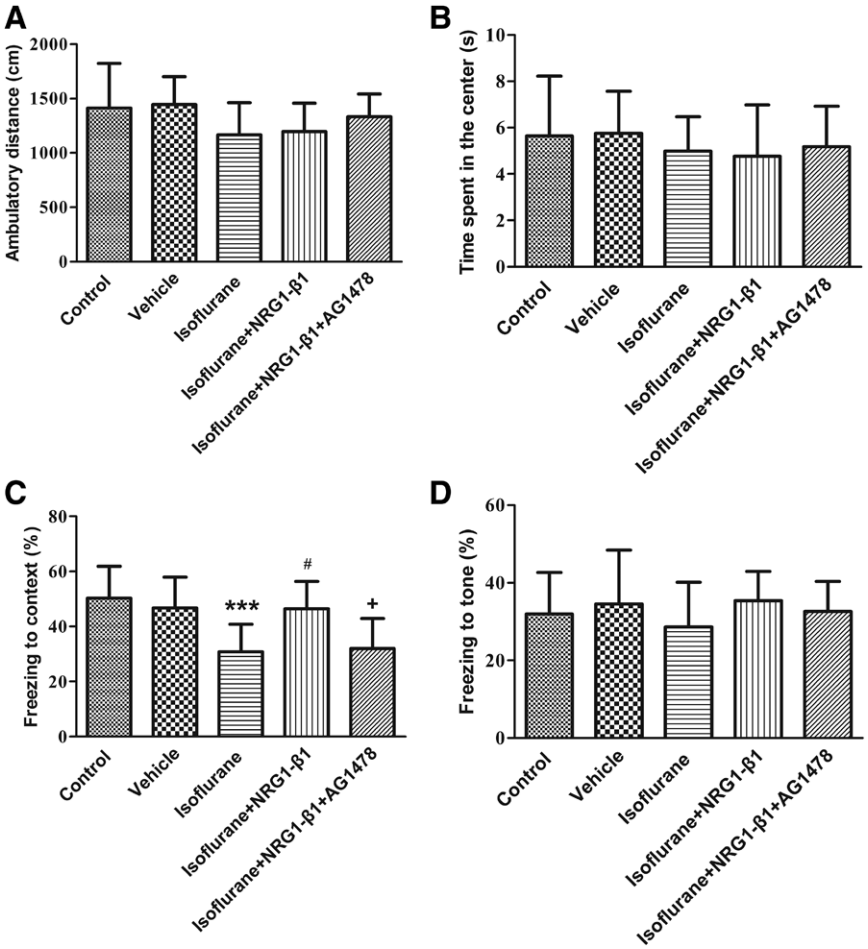

Fig. 2. The behavioral tests postisoflurane exposure. The mice had normal ambulatory distance traveled and time spent in the center in the open-field test (*A* and *B*) in the five groups 24-h postisoflurane exposure. Neuregulin 1 (NRG1)-β1 increased the freezing time to context 48-h postisoflurane exposure in the isoflurane plus NRG1-β1 group compared with that in the isoflurane group, which was abolished by AG1478 in the isoflurane plus NRG1-β1 plus AG1478 group (*C*). No significant difference was detected in the freezing time to tone among the groups (*D*) 48-h postisoflurane exposure. Data are mean ± SD (n = 10 mice for each group; ***$P < 0.005$ *vs.* the control group; #$P < 0.05$ *vs.* the isoflurane group; +$P < 0.05$ *vs.* the isoflurane plus NRG1-β1 group).

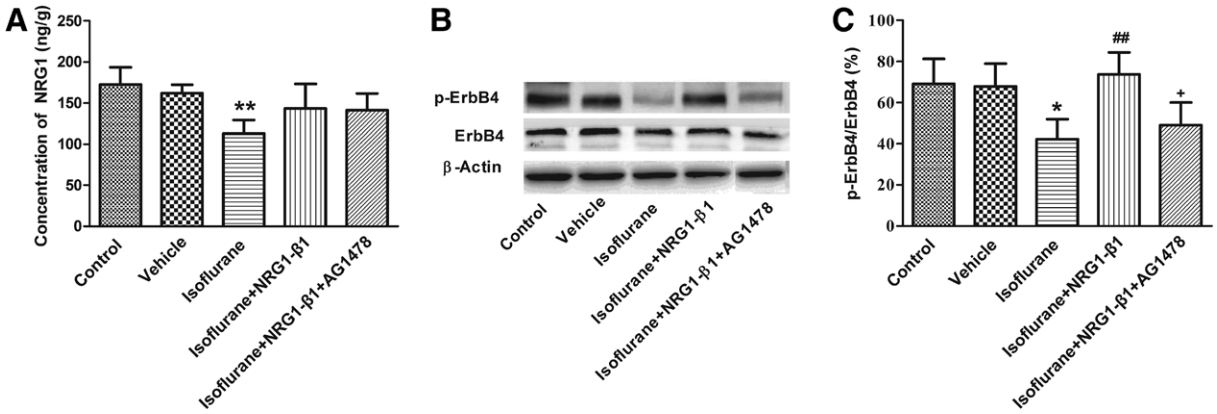

Fig. 3. The hippocampal levels of neuregulin 1 (NRG1), p-ErbB4, and ErbB4 48-h postisoflurane exposure. The isoflurane exposure reduced the hippocampal concentrations of NRG1 in the isoflurane group (*A*) (n = 4 mice for each group). The isoflurane exposure reduced the hippocampal p-ErbB4/ErbB4 significantly in the isoflurane group (*B* and *C*) (n = 4 mice for each group). NRG1-β1 reversed the decrease in the hippocampal p-ErbB4/ErbB4 induced by the isoflurane exposure in the isoflurane plus NRG1-β1 group (*B* and *C*), which was abolished by AG1478 in the isoflurane plus NRG1-β1 plus AG1478 group (*B* and *C*) (n = 4 mice for each group). Data are mean ± SD. (*$P < 0.05$, **$P < 0.01$ *vs.* the control group; ##$P < 0.01$ *vs.* the isoflurane group; +$P < 0.05$ *vs.* the isoflurane plus NRG1-β1 group.)

NRG1-β1 injection reversed the decrease in the hippocampal p-ErbB4/ErbB4 induced by the isoflurane exposure in the isoflurane plus NRG1-β1 group (up to 73.69 ± 10.77%, n = 4; $P = 0.007$; fig. 3, B and C), which was abolished by the AG1478 injection in the isoflurane plus NRG1-β1 plus AG1478 group (reduction to 49.16 ± 10.86%, n = 4; $P = 0.043$; fig. 3, B and C).

Distribution of ErbB4 in the Hippocampus

In the current study, the detected levels of NRG1, p-ErbB4, and ErbB4 were from the whole hippocampus, rather than from the GAD67- or parvalbumin-positive interneurons in the hippocampus, we therefore were unable to confirm the relation between the NRG1–ErbB4 signaling and the parvalbumin-positive interneurons. Then, we used the double-immunofluorescence to detect the colocalization of ErbB4 and γ-aminobutyric acidergic interneuron marker GAD67, and ErbB4 and parvalbumin in the hippocampus. The results indicated that ErbB4 was primarily expressed in the GAD67-immunoreactive interneurons (fig. 4A). The average coexpression of parvalbumin and ErbB4 in parvalbumin-positive interneurons was 82.73 ± 11.37% and in ErbB4-positive neurons was 63.73 ± 10.05% across the hippocampus (fig. 4, B and C; n = 4).

Hippocampal Levels of Parvalbumin and GAD67 Postgas Exposure

To investigate the possible mechanism underlying the isoflurane exposure–induced cognitive impairment mediated by NRG1–ErbB4 signaling, we detected the hippocampal levels of parvalbumin and GAD67 using the Western blotting. As shown in figure 5, the levels of parvalbumin and GAD67 decreased to 75.89 ± 10.26% and 73.87 ± 16.89%, respectively, in the isoflurane group compared with that in the control group (parvalbumin: 101.68 ± 11.21%, n = 4, $P = 0.011$; GAD67: 104.71 ± 6.85%, n = 4, $P = 0.014$). The NRG1-β1 injection reversed these reductions in the isoflurane plus NRG1-β1 group (all $P < 0.05$), which was abolished by the AG1478 injection in the isoflurane plus NRG1-β1 plus AG1478 group (all $P < 0.05$). These data were consistent with the results of the immunofluorescence (all $P < 0.05$; fig. 6).

Discussion

The current study demonstrated that the isoflurane-exposed mice presented poorer performance in contextual fear conditioning test, which was consistent with the recent findings showing isoflurane anesthesia–induced cognitive impairments in rodents.[12,22] More importantly, we found for the first time that NRG1-β1 increased the freezing time to context 48-h postisoflurane exposure, which was abolished by an ErbB4 receptor antagonist AG1478. These results indicate that dysfunction of hippocampal NRG1–ErbB4 signaling plays a key role in the isoflurane exposure–induced hippocampus-dependent cognitive impairment in aged mice.

Isoflurane, a commonly used inhalation anesthetic in clinical practice, has been shown to induce cognitive impairments after the long-time exposure in the rodent models, although it is also reported to improve cognitive impairments in the ischemia animal models.[23,24] Many neuropathology mechanisms such as the disruption in the neurotransmission, the alteration of intracellular calcium homeostasis, and the damage of synaptic plasticity have been suggested to play key roles in the isoflurane-induced cognitive impairments.[25–27] However, the underlying mechanisms of these cognitive impairments remain largely to be determined.

The hippocampus is important to learning and memory, in which anesthetic-induced acute memory impairment

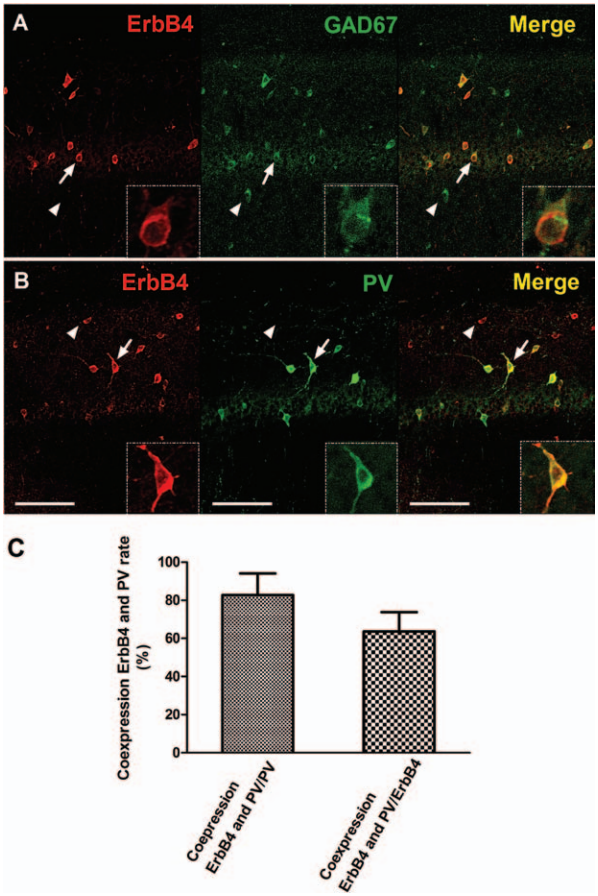

Fig. 4. The colocalization of ErbB4 and GAD67 and ErbB4 and parvalbumin in the hippocampus in the control mice. Double-immunofluorescence indicates that ErbB4 (*red*) is primarily expressed in the GAD67 (*green*)-immunoreactive interneurons in the CA1 (*A*; *arrow*: coexpress ErbB4 and GAD67; *arrowhead*: express GAD67 without ErbB4; the neuron indicated by *arrow* was magnified in the *dashed rectangle*). A high number (more than half) of the parvalbumin (*green*)-immunoreactive interneurons coexpress ErbB4 (*red*) as indicated by *yellow pixels* (*B*; *arrow*: coexpress ErbB4 and parvalbumin; *arrowhead*: express ErbB4 without parvalbumin; the neuron indicated by *arrow* was magnified in the *dashed rectangle*) in the CA1. Quantification of coexpression of ErbB4 and parvalbumin in parvalbumin interneurons or in ErbB4 neurons across the hippocampus (*C*; n = 4 mice for each group). Original magnification = ×40; *scale bar* = 100 μm. GAD67 = glutamic acid decarboxylase 67; PV = parvalbumin.

parallels the inhibition of synaptic plasticity.[28,29] In the current study, isoflurane exposure and the drug interventions did not affect the locomotor activity and the exploratory behavior. Moreover, the isoflurane-exposed mice had decreased freezing time to context, but no difference in the freezing time to tone compared with that in the control mice, suggesting that the isoflurane exposure impairs the early hippocampus-dependent learning and memory in aged mice. These behavior results were still somewhat different from the results of the studies by Zhang *et al.*[13] and Lin *et al.*,[30] which may be attributed to the differences in the animal species, the methods of the anesthetic exposure, the time points of the performance of the memory tests, and especially the animal age which has been increasingly reported to contribute to postoperative brain dysfunction in the previous works.[1,13]

NRG1–ErbB4 signaling is critical in various neurophysiology processes including neuron development and migration, synapse plasticity, and synthesis and secretion of neurotransmitter.[15] In the hippocampus, NRG1–ErbB4 signaling has been implicated in the modulation of cognitive processes through regulating the activity of neural networks and synchronizing the pyramidal cells,[31] whereas the disturbance of NRG1–ErbB4 signaling is associated with specific impairments in the hippocampus-dependent memory.[32] In the current study, the isoflurane exposure elicited the hippocampus-dependent cognitive impairment and reduced the hippocampal NRG1 level significantly in aged mice, whereas NRG1-β1 administration reversed this cognitive deficit. Furthermore, an ErbB4 receptor antagonist AG1478 was shown to abolish the beneficial effects of NRG1-β1, implying the involvement of ErbB4 in this procedure. These results suggest that NRG1–ErbB4 signaling contributes to the hippocampus-dependent cognitive impairment induced by isoflurane exposure in aged mice.

Parvalbumin-positive interneurons, the major subpopulation of γ-aminobutyric acidergic interneurons, express the calcium-binding parvalbumin and are characterized by the fast-spiking discharge pattern.[33] They are the main recipient of recurrent glutamatergic innervations and phase the output of pyramidal innervations in the hippocampal circuitry.[34,35] Accumulating evidence suggests that parvalbumin-positive interneurons are the major cellular target of NRG1–ErbB4 signaling in regulating the neuron network activity,[36,37] which can be confirmed by the findings that parvalbumin-Cre; ErbB4$^{-/-}$mice have contextual fear conditioning impairment, in which ErbB4 is knocked out specifically in parvalbumin-positive interneurons, whereas the CaMKII-Cre; ErbB4$^{-/-}$ mice display normal freezing response in which ErbB4 is knocked out in excitatory neurons.[19,38] To determine whether the disruption of NRG1–ErbB4 signaling in the parvalbumin interneurons contributes to the cognitive impairments induced by the isoflurane exposure, we investigated the distribution of ErbB4 in the hippocampus. The results indicated the colocalization of ErbB4 and γ-aminobutyric acidergic interneuron marker GAD67 and parvalbumin, two markers of parvalbumin interneurons, supporting the view that ErbB4 is largely expressed in the parvalbumin-positive interneurons in the hippocampus.[18,39] In this study, both Western blotting and immunofluorescence results suggest that NRG1-β1 improves the cognitive impairment induced by the isoflurane exposure probably by the up-regulation of parvalbumin and GAD67 in the hippocampus.

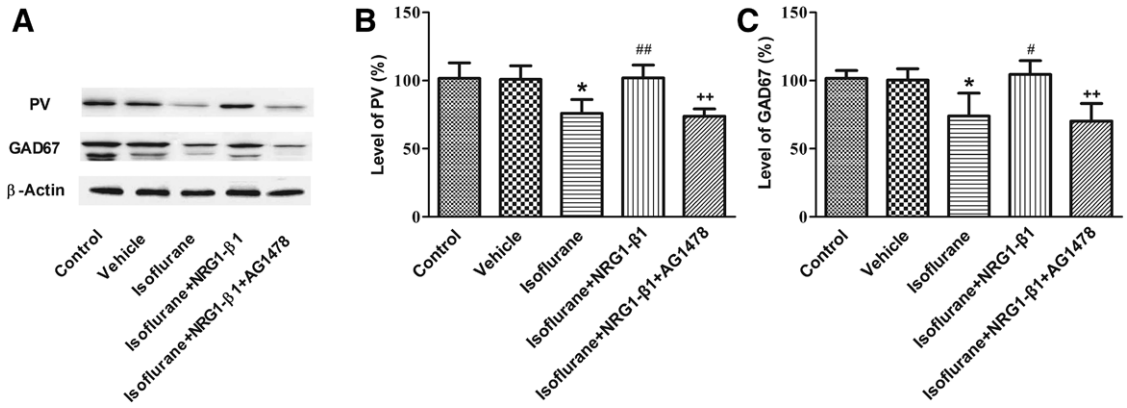

Fig. 5. The hippocampal levels of parvalbumin and GAD67 determined by the Western blotting 48-h postisoflurane exposure. The isoflurane exposure reduced the levels of parvalbumin (*A* and *B*) and GAD67 (*A* and *C*) in the isoflurane group. Neuregulin 1 (NRG1)-β1 reversed these reductions in the isoflurane plus NRG1-β1 group (*A–C*), which was abolished by AG1478 in the isoflurane plus NRG1-β1 plus AG1478 group (*A–C*). Data are mean ± SD (n = 4 mice for each group; **P* < 0.05 *vs.* the control group; #*P* < 0.05, ##*P* < 0.01 *vs.* the isoflurane group; ++*P* < 0.01 *vs.* the isoflurane plus NRG1-β1 group). GAD67 = glutamic acid decarboxylase 67; PV = parvalbumin.

The studies by Terrando *et al.*[7] and Cibelli *et al.*[8] have suggested that anesthesia may not strongly influence memory dysfunction in rodents, rather the surgery-induced neuroinflammation leads to the cognitive impairment. However, other studies have shown that anesthetic isoflurane and sevoflurane induce neuroinflammation, leading to cognitive impairment.[13,14] The data from the current studies also suggest that anesthesia with 1.5% isoflurane for 2 h induces cognitive impairment in aged mice. Note that the anesthetics in the studies by Terrando *et al.* were 2.1% isoflurane and buprenorphine (0.1 mg/kg).[7] The anesthetics in the other studies were 1.4 or 1.5% isoflurane for 2 h once or 3.0% sevoflurane for 2 h daily for 3 days.[14,40,41] It is conceivable that different anesthesia regimens could have different effects on learning and memory function in rodents. The future studies may include determining whether the surgery and anesthesia can potentiate each other, leading to a greater cognitive impairment. These studies would lead to a better understanding of the underlying mechanism of postoperative cognitive dysfunction observed in patients.

There are limitations in the current study. First, although most of the ErbB4 are expressed in the parvalbumin-positive interneurons in the current study, we still cannot verify whether isoflurane exposure affects the NRG1–ErbB4 signaling especially in the parvalbumin-positive interneurons. Second, for the technical reasons, we cannot identify the disturbance in NRG1–ErbB4 signaling in the parvalbumin interneurons as a mechanism underlying isoflurane exposure–induced cognitive decline. Therefore, further studies using parvalbumin-Cre; ErbB4$^{-/-}$mice and CaMKII-Cre; ErbB4$^{-/-}$ mice or electrophysiological techniques are needed to demonstrate whether isoflurane exposure affects the NRG1–ErbB4 signaling activity selectively in the parvalbumin-positive interneurons. In addition, the data in the current experiment were obtained after exposure to isoflurane carried by 100% O_2 in the aged mice. The future studies to investigate the effects of isoflurane or other inhalational anesthetics carried by different concentrations of oxygen on the NRG1–ErbB4 signaling in parvalbumin-positive interneurons are warranted.

In conclusion, exposure to isoflurane carried by 100% O_2 impairs the early hippocampus-dependent cognitive function and decreases the hippocampal levels of NRG1, p-ErbB4, parvalbumin, and GAD67 in aged mice, which is reversed by the NRG1-β1. All the effects of NRG1-β1 are blocked by the ErbB4 inhibitor AG1478. We provided additional evidence that the disruption of NRG1–ErbB4 signaling in parvalbumin-positive interneurons contributes, at least partially, to the isoflurane-induced hippocampus-dependent cognitive impairment after exposure to isoflurane carried by 100% O_2 in aged mice, pending further studies.

Acknowledgments

This work was supported by the grants from the National Natural Science Foundation of China, Beijing, China (Numbers 81271216 and 81222013) and the Natural Science Foundation of Jiangsu Province, Nanjing, China (Number BK2012778) and R01 GM088801 and R01 AG041274 from National Institutes of Health, Bethesda, Maryland, and Cure Alzheimer's Fund, Wellesley, Massachusetts.

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Yang: Department of Anesthesiology, Jinling Hospital, 305 East Zhongshan Road, Nanjing 210002, China. yjyangjj@126.com. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this

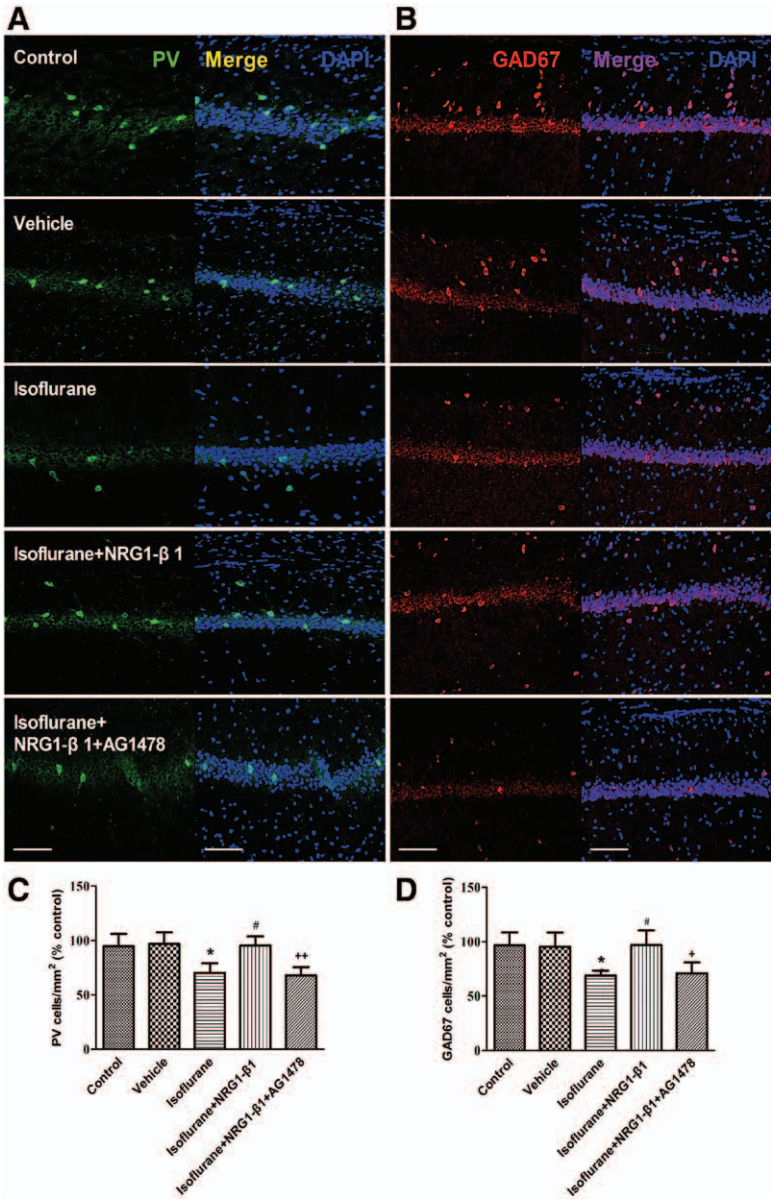

Fig. 6. Parvalbumin- and GAD67-positive interneurons in the hippocampus 48-h postisoflurane exposure. Representative immunoreactivity of parvalbumin (*green*) or GAD67 (*red*) in the CA1 (*A* and *B*). Quantification of parvalbumin- and GAD67-positive interneurons across the hippocampus (*C* and *D*; n = 4 mice for each group). The isoflurane exposure reduced the numbers of the parvalbumin- (*C*) and GAD67-positive (*D*) interneurons in the isoflurane group. Neuregulin 1 (NRG1)-β1 reversed these reductions in the isoflurane plus NRG1-β1 group, which was abolished by AG1478 in the isoflurane plus NRG1-β1 plus AG1478 group (*C* and *D*). Data are mean ± SD. (*$P < 0.05$ *vs.* the control group; #$P < 0.05$ *vs.* the isoflurane group; +$P < 0.05$, ++$P < 0.01$ *vs.* the isoflurane plus NRG1-β1 group.) Original magnification = ×40; *scale bar* = 100 μm. DAPI = 4', 6-diamidino-2-phenylindole; GAD67 = glutamic acid decarboxylase 67; PV = parvalbumin.

References

1. Moller JT, Cluitmans P, Rasmussen LS, Houx P, Rasmussen H, Canet J, Rabbitt P, Jolles J, Larsen K, Hanning CD, Langeron O, Johnson T, Lauven PM, Kristensen PA, Biedler A, van Beem H, Fraidakis O, Silverstein JH, Beneken JE, Gravenstein JS: Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD investigators. International Study of Post-Operative Cognitive Dysfunction. Lancet 1998; 351:857–61
2. Bryson GL, Wyand A: Evidence-based clinical update: General anesthesia and the risk of delirium and postoperative cognitive dysfunction. Can J Anaesth 2006; 53:669–77
3. Hovens IB, Schoemaker RG, van der Zee EA, Heineman E, Izaks GJ, van Leeuwen BL: Thinking through postoperative cognitive dysfunction: How to bridge the gap between clinical and pre-clinical perspectives. Brain Behav Immun 2012; 26:1169–79

4. Price CC, Garvan CW, Monk TG: Type and severity of cognitive decline in older adults after noncardiac surgery. Anesthesiology 2008; 108:8–17
5. Luger TJ, Kammerlander C, Gosch M, Luger MF, Kammerlander-Knauer U, Roth T, Kreutziger J: Neuroaxial *versus* general anaesthesia in geriatric patients for hip fracture surgery: Does it matter? Osteoporos Int 2010; 21(suppl 4):S555–72
6. Mason SE, Noel-Storr A, Ritchie CW: The impact of general and regional anesthesia on the incidence of post-operative cognitive dysfunction and post-operative delirium: A systematic review with meta-analysis. J Alzheimers Dis 2010; 22(suppl 3):67–79
7. Terrando N, Monaco C, Ma D, Foxwell BM, Feldmann M, Maze M: Tumor necrosis factor-alpha triggers a cytokine cascade yielding postoperative cognitive decline. Proc Natl Acad Sci U S A 2010; 107:20518–22
8. Cibelli M, Fidalgo AR, Terrando N, Ma D, Monaco C, Feldmann M, Takata M, Lever IJ, Nanchahal J, Fanselow MS, Maze M: Role of interleukin-1beta in postoperative cognitive dysfunction. Ann Neurol 2010; 68:360–8
9. Wan Y, Xu J, Meng F, Bao Y, Ge Y, Lobo N, Vizcaychipi MP, Zhang D, Gentleman SM, Maze M, Ma D: Cognitive decline following major surgery is associated with gliosis, β-amyloid accumulation, and τ phosphorylation in old mice. Crit Care Med 2010; 38:2190–8
10. Terrando N, Brzezinski M, Degos V, Eriksson LI, Kramer JH, Leung JM, Miller BL, Seeley WW, Vacas S, Weiner MW, Yaffe K, Young WL, Xie Z, Maze M: Perioperative cognitive decline in the aging population. Mayo Clin Proc 2011; 86: 885–93
11. Saab BJ, Maclean AJ, Kanisek M, Zurek AA, Martin LJ, Roder JC, Orser BA: Short-term memory impairment after isoflurane in mice is prevented by the α5 γ-aminobutyric acid type A receptor inverse agonist L-655,708. Anesthesiology 2010; 113:1061–71
12. Bianchi SL, Tran T, Liu C, Lin S, Li Y, Keller JM, Eckenhoff RG, Eckenhoff MF: Brain and behavior changes in 12-month-old Tg2576 and nontransgenic mice exposed to anesthetics. Neurobiol Aging 2008; 29:1002–10
13. Zhang Y, Xu Z, Wang H, Dong Y, Shi HN, Culley DJ, Crosby G, Marcantonio ER, Tanzi RE, Xie Z: Anesthetics isoflurane and desflurane differently affect mitochondrial function, learning, and memory. Ann Neurol 2012; 71:687–98
14. Shen X, Dong Y, Xu Z, Wang H, Miao C, Soriano SG, Sun D, Baxter MG, Zhang Y, Xie Z: Selective anesthesia-induced neuroinflammation in developing mouse brain and cognitive impairment. Anesthesiology 2013; 118:502–15
15. Mei L, Xiong WC: Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. Nat Rev Neurosci 2008; 9:437–52
16. Eto K, Hommyo A, Yonemitsu R, Abe S: ErbB4 signals Neuregulin1-stimulated cell proliferation and c-fos gene expression through phosphorylation of serum response factor by mitogen-activated protein kinase cascade. Mol Cell Biochem 2010; 339:119–25
17. Fisahn A, Neddens J, Yan L, Buonanno A: Neuregulin-1 modulates hippocampal gamma oscillations: Implications for schizophrenia. Cereb Cortex 2009; 19:612–8
18. Fazzari P, Paternain AV, Valiente M, Pla R, Luján R, Lloyd K, Lerma J, Marín O, Rico B: Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. Nature 2010; 464:1376–80
19. Wen L, Lu YS, Zhu XH, Li XM, Woo RS, Chen YJ, Yin DM, Lai C, Terry AV Jr, Vazdarjanova A, Xiong WC, Mei L: Neuregulin 1 regulates pyramidal neuron activity *via* ErbB4 in parvalbumin-positive interneurons. Proc Natl Acad Sci U S A 2010; 107:1211–6
20. Neddens J, Buonanno A: Selective populations of hippocampal interneurons express ErbB4 and their number and distribution is altered in ErbB4 knockout mice. Hippocampus 2010; 20:724–44
21. Yin DM, Chen YJ, Lu YS, Bean JC, Sathyamurthy A, Shen C, Liu X, Lin TW, Smith CA, Xiong WC, Mei L: Reversal of behavioral deficits and synaptic dysfunction in mice overexpressing neuregulin 1. Neuron 2013; 78:644–57
22. Culley DJ, Baxter MG, Yukhananov R, Crosby G: Long-term impairment of acquisition of a spatial memory task following isoflurane-nitrous oxide anesthesia in rats. Anesthesiology 2004; 100:309–14
23. Rammes G, Starker LK, Haseneder R, Berkmann J, Plack A, Zieglgänsberger W, Ohl F, Kochs EF, Blobner M: Isoflurane anaesthesia reversibly improves cognitive function and long-term potentiation (LTP) *via* an up-regulation in NMDA receptor 2B subunit expression. Neuropharmacology 2009; 56:626–36
24. Li H, Yin J, Li L, Deng J, Feng C, Zuo Z: Isoflurane postconditioning reduces ischemia-induced nuclear factor-κB activation and interleukin 1β production to provide neuroprotection in rats and mice. Neurobiol Dis 2013; 54: 216–24
25. Wei H, Liang G, Yang H, Wang Q, Hawkins B, Madesh M, Wang S, Eckenhoff RG: The common inhalational anesthetic isoflurane induces apoptosis *via* activation of inositol 1,4,5-trisphosphate receptors. Anesthesiology 2008; 108:251–60
26. Stratmann G, Sall JW, May LD, Bell JS, Magnusson KR, Rau V, Visrodia KH, Alvi RS, Ku B, Lee MT, Dai R: Isoflurane differentially affects neurogenesis and long-term neurocognitive function in 60-day-old and 7-day-old rats. Anesthesiology 2009; 110:834–48
27. Xie Z, Culley DJ, Dong Y, Zhang G, Zhang B, Moir RD, Frosch MP, Crosby G, Tanzi RE: The common inhalation anesthetic isoflurane induces caspase activation and increases amyloid beta-protein level *in vivo*. Ann Neurol 2008; 64: 618–27
28. Martin LJ, Zurek AA, MacDonald JF, Roder JC, Jackson MF, Orser BA: Alpha5GABAA receptor activity sets the threshold for long-term potentiation and constrains hippocampus-dependent memory. J Neurosci 2010; 30:5269–82
29. Jutras MJ, Fries P, Buffalo EA: Oscillatory activity in the monkey hippocampus during visual exploration and memory formation. Proc Natl Acad Sci U S A 2013; 110:13144–9
30. Lin D, Zuo Z: Isoflurane induces hippocampal cell injury and cognitive impairments in adult rats. Neuropharmacology 2011; 61:1354–9
31. Min SS, An J, Lee JH, Seol GH, Im JH, Kim HS, Baik TK, Woo RS: Neuregulin-1 prevents amyloid β-induced impairment of long-term potentiation in hippocampal slices *via* ErbB4. Neurosci Lett 2011; 505:6–9
32. Fuchs EC, Zivkovic AR, Cunningham MO, Middleton S, Lebeau FE, Bannerman DM, Rozov A, Whittington MA, Traub RD, Rawlins JN, Monyer H: Recruitment of parvalbumin-positive interneurons determines hippocampal function and associated behavior. Neuron 2007; 53: 591–604
33. Morris NP, Harris SJ, Henderson Z: Parvalbumin-immunoreactive, fast-spiking neurons in the medial septum/diagonal band complex of the rat: Intracellular recordings *in vitro*. Neuroscience 1999; 92:589–600
34. Sohal VS, Zhang F, Yizhar O, Deisseroth K: Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 2009; 459:698–702
35. Gulyás AI, Megías M, Emri Z, Freund TF: Total number and ratio of excitatory and inhibitory synapses converging onto single interneurons of different types in the CA1 area of the rat hippocampus. J Neurosci 1999; 19:10082–97
36. Kwon OB, Longart M, Vullhorst D, Hoffman DA, Buonanno A: Neuregulin-1 reverses long-term potentiation at CA1 hippocampal synapses. J Neurosci 2005; 25:9378–83

37. Pitcher GM, Kalia LV, Ng D, Goodfellow NM, Yee KT, Lambe EK, Salter MW: Schizophrenia susceptibility pathway neuregulin 1-ErbB4 suppresses Src upregulation of NMDA receptors. Nat Med 2011; 17:470–8

38. Chen YJ, Zhang M, Yin DM, Wen L, Ting A, Wang P, Lu YS, Zhu XH, Li SJ, Wu CY, Wang XM, Lai C, Xiong WC, Mei L, Gao TM: ErbB4 in parvalbumin-positive interneurons is critical for neuregulin 1 regulation of long-term potentiation. Proc Natl Acad Sci U S A 2010; 107:21818–23

39. Vullhorst D, Neddens J, Karavanova I, Tricoire L, Petralia RS, McBain CJ, Buonanno A: Selective expression of ErbB4 in interneurons, but not pyramidal cells, of the rodent hippocampus. J Neurosci 2009; 29:12255–64

40. Wu X, Lu Y, Dong Y, Zhang G, Zhang Y, Xu Z, Culley DJ, Crosby G, Marcantonio ER, Tanzi RE, Xie Z: The inhalation anesthetic isoflurane increases levels of proinflammatory TNF-α, IL-6, and IL-1β. Neurobiol Aging 2012; 33: 1364–78

41. Tan W, Cao X, Wang J, Lv H, Wu B, Ma H: Tau hyperphosphorylation is associated with memory impairment after exposure to 1.5% isoflurane without temperature maintenance in rats. Eur J Anaesthesiol 2010; 27:835–41.