Sevoflurane Anesthesia in Pregnant Mice Induces Neurotoxicity in Fetal and Offspring Mice

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ABSTRACT

Background: Each year, over 75,000 pregnant women in the United States undergo anesthesia care. The authors set out to assess the effects of the anesthetic sevoflurane on neurotoxicity in pregnant mice and on learning and memory in fetal and offspring mice.

Methods: Pregnant mice (gestational day 14) and mouse primary neurons were treated with 2.5% sevoflurane for 2 h and 4.1% sevoflurane for 6 h, respectively. Brain tissues of both fetal and offspring mice (P31) and the primary neurons were harvested and subjected to Western blot and immunohistochemistry to assess interleukin-6, the synaptic markers postsynaptic density-95 and synaptophysin, and caspase-3 levels. Separately, learning and memory function in the offspring mice was determined in the Morris water maze.

Results: Sevoflurane anesthesia in pregnant mice induced caspase-3 activation, increased interleukin-6 levels (256 ± 50.98% [mean ± SD] *vs.* 100 ± 54.12%, $P = 0.026$), and reduced postsynaptic density-95 (61 ± 13.53% *vs.* 100 ± 10.08%, $P = 0.036$) and synaptophysin levels in fetal and offspring mice. The sevoflurane anesthesia impaired learning and memory in offspring mice at P31. Moreover, interleukin-6 antibody mitigated the sevoflurane-induced reduction in postsynaptic density-95 levels in the neurons. Finally, environmental enrichment attenuated the sevoflurane-induced increases in interleukin-6 levels, reductions of synapse markers, and learning and memory impairment.

Conclusions: These results suggest that sevoflurane may induce detrimental effects in fetal and offspring mice, which can be mitigated by environmental enrichment. These findings should promote more studies to determine the neurotoxicity of anesthesia in the developing brain.

What We Already Know about This Topic

- The effects of maternal exposure to sevoflurane on fetal neurotoxicity and neurobehavioral outcome are controversial

What This Article Tells Us That Is New

- Sevoflurane may induce detrimental effects in fetal and offspring mice, which can be mitigated by environmental enrichment

ANESTHESIA neurotoxicity in the developing brain has been investigated in animals and in humans and has become a major health issue of interest to both the medical community[1] and the public.[2] Anesthesia and surgery may induce neurodevelopmental impairment and cognitive dysfunction in children (reviewed in Sun[3]). In preclinical studies, anesthesia has been shown to induce neurotoxicity and learning and memory impairment in young animals[4] (reviewed in Creeley and Olney[5]).

Each year, over 75,000 pregnant women in the United States have nonobstetric surgery and fetal intervention procedures under anesthesia.[6] Anesthesia neurotoxicity in the developing brain could occur in the fetus because (1) brain development starts as early as the second trimester of pregnancy; (2) anesthesia can induce neurotoxicity in both adult and young mice, and most general anesthetics are lipophilic

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and thus cross the placenta easily; and (3) uterine exposure to ethanol, valproic acid, and the anesthetic isoflurane have been shown to induce behavioral abnormalities in adulthood[7] (reviewed in Reitman and Flood[8]). It remains largely to be determined, however, whether anesthesia in pregnant mice can induce (1) neurotoxicity in fetal mice (the developing brain) and (2) neurotoxicity and learning and memory impairment in offspring mice after birth.

Sevoflurane is currently the most commonly used inhalation anesthetic. Previous studies have shown that anesthesia with 2.5% sevoflurane for 2 h can induce neurotoxicity in the brain tissues of adult (5-month-old) mice without statistically significant alteration in the values of blood pressure and blood gas.[9] We therefore determined whether the same sevoflurane anesthesia in pregnant mice could induce neurotoxicity and learning and memory impairment in fetal and offspring mice. Finally, we investigated whether environmental enrichment (EE), a complex living milieu that has been shown to improve learning and memory,[10–12] could ameliorate the sevoflurane-induced detrimental effects.

Materials and Methods

Mice Anesthesia

The protocol was approved by the Massachusetts General Hospital Standing Committee (Boston, Massachusetts) on the Use of Animals in Research and Teaching. Three-month-old C57BL/6J female mice (The Jackson Laboratory, Bar Harbor, ME) were mated with male mice. The pregnant mice were identified and then housed individually. The offspring mice were weaned 21 days after birth. Animals were kept in a temperature-controlled (22°–23°C) room under a 12-h light/dark period (light on at 7:00 AM); standard mouse chow and water were available *ad libitum*. At gestational day (G) 14, the pregnant mice were assigned randomly to an anesthesia group or a control group. Mice randomized to the anesthesia group received 2.5% sevoflurane in 100% oxygen for 2 h in an anesthetizing chamber. The control group received 100% oxygen at an identical flow rate for 2 h in an identical chamber as described in our previous studies.[9] The mice breathed spontaneously, and concentrations of anesthetic and oxygen were measured continuously (Datex-Ohmeda Inc., Tewksbury, MA). The temperature of the anesthetizing chamber was controlled to maintain rectal temperature of the animals at 37° ± 0.5°C. Mean arterial blood pressure was not measured in these mice because the same sevoflurane anesthesia was shown not to alter the values of blood pressure and blood gas in our previous studies.[9] Anesthesia was terminated by discontinuing sevoflurane and placing the animals in a chamber containing 100% oxygen until 20 min after return of the righting reflex. The anesthesia with 2.5% sevoflurane (approximately 1.1 minimum alveolar concentration) for 2 h in mice was used to demonstrate whether clinically relevant sevoflurane anesthesia in pregnant mice, which had been shown to induce neurotoxicity in adult mice,[9] could also induce neurotoxicity in fetal mice and then neurobehavioral deficits in offspring mice. Twenty pregnant mice were included in the experiments, which generated a sufficient number of fetal mice for the biochemistry studies (n = 6 per arm), and offspring mice for the biochemistry (n = 6 per arm) and behavioral studies (n = 15 per arm). Our pilot studies showed a mean difference of 1.5 (3 *vs.* 1.5) in platform crossing times, with an SD of 1.8 in the control group and 1.3 in the anesthesia group. From the pilot study, we also estimated a mean difference of 150% (250% *vs.* 100%) in interleukin (IL)-6 levels in brain tissues, with an SD of 51 in the control group and 54 in the anesthesia group. Assuming this study would have similar effect sizes, a sample size of 6 per arm for the biochemistry studies and a sample size of 15 per arm for the behavioral studies would lead to a 90% or larger power to detect the differences using two-sample Student *t* test with 5% type I error.

Mouse Primary Neurons

The protocol was approved by the Massachusetts General Hospital Standing Committee on the Use of Animals in Research and Teaching. The harvest of neurons was performed as described in our previous studies.[13,14] Seven to 10 days after harvesting, the neurons were treated with 4.1% sevoflurane for 6 h as described in our previous studies.[9] The treatment with 4.1% sevoflurane for 6 h was used to determine whether the sevoflurane anesthesia, which can induce cytotoxicity,[9] could also reduce levels of postsynaptic density-95 (PSD-95), the marker for synapse. The IL-6 antibody (10 μg/ml) was administrated to the neurons 1 h before the sevoflurane treatment. The neurons were harvested at the end of anesthesia and were subjected to Western blot analysis.

Brain Tissue Harvest and Protein Level Quantification

Immediately after the sevoflurane anesthesia, we performed a cesarean section to extract the fetal mice and harvested their brain tissues. We also used decapitation to kill postnatal day (P) 31 offspring mice and harvested their brain tissues. Separate groups of mice were used for the Western blot analysis and the immunohistochemistry studies, respectively. For the Western blot analysis, the harvested brain tissues were homogenized on ice using immunoprecipitation buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid, and 0.5% Nonidet P-40) plus protease inhibitors (1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin A) as described in our previous studies.[15] The lysates were collected, centrifuged at 12,000 rpm for 15 min, and quantified for total proteins with bicinchoninic acid protein assay kit (Pierce Technology Co., Iselin, NJ).[15]

Western Blot Analysis

Western blot analysis was performed using the methods described in our previous studies.[15] Whole cerebral hemispheres were used for Western blot analysis because

there would be an insufficient amount of hippocampus tissues from the fetal mice for Western blot analysis. IL-6 antibody (1:1,000 dilution; Abcam, Cambridge, MA) was used to recognize IL-6 (24 kDa). PSD-95 antibody (1:1,000; Cell Signaling Technology, Danvers, MA) was used to detect PSD-95 (95 kDa). A caspase-3 antibody (1:1,000 dilution; Cell Signaling Technology) was used to recognize full-length caspase-3 (35–40 kDa) and caspase-3 fragment (17–20 kDa) resulting from cleavage at aspartate position 175. Antibody anti–β-actin (1:10,000; Sigma, St. Louis, MO) was used to detect β-actin (42 kDa). Western blot quantification was performed as described by Xie *et al.*[16] Briefly, signal intensity was analyzed using a Bio-Rad (Hercules, CA) image program (Quantity One). We quantified the Western blots in two steps. First, we used β-actin levels to normalize (*e.g.*, determining the ratio of IL-6 to β-actin amount) protein levels and control for loading differences in the total protein amount. Second, we presented changes in protein levels in mice or neurons undergoing sevoflurane anesthesia as a percentage of those in the control group. One hundred percent of protein level changes refer to control levels for the purpose of comparison with experimental conditions.

The quantification of Western blot was based not only on the images presented in figures but also on the images not presented in the figures to have adequate effect size (*e.g.*, n = 6 in biochemistry studies).[15]

Immunohistochemistry

Immunohistochemistry was performed using the methods described in our previous studies.[17] P31 offspring mice were anesthetized with sevoflurane briefly (2.5% sevoflurane for 4 min) and perfused transcardially with heparinized saline followed by 4% paraformaldehyde in 0.1M phosphate buffer at pH 7.4. The anesthesia with 2.5% sevoflurane for 4 min in mice provided adequate anesthesia for the perfusion procedure without causing statistically significant changes in blood pressure and blood gas according to our previous studies.[9] Mouse brain tissues were removed and kept at 4°C in paraformaldehyde. Five-micron frozen sections from the mouse brain hemispheres were used for the immunohistochemistry staining.[17] The sections were incubated with the primary antibody synaptophysin (1:500; Sigma) dissolved in 1% bovine serum albumin in phosphate-buffered saline at 4°C overnight. The next day, the sections were exposed to secondary antibody (Alexa Fluor 594 goat anti-rabbit IgG [H+L]; Invitrogen, Grand Island, NY). Finally, the sections were wet mounted and viewed immediately using a fluorescence microscope (60×). We used the mouse hippocampus in the studies of immunohistochemistry density quantification to determine whether sevoflurane anesthesia can induce neurotoxicity in the hippocampus. The photographs were taken and an investigator who was blind to the experimental design counted the density of synaptophysin using ImageJ version 1.38 (National Institutes of Health, Bethesda, MD).[17]

Morris Water Maze

A round steel pool, 150 cm in diameter and 60 cm in height, was filled with water to a height of 1.0 cm above the top of a 10-cm diameter platform. The pool was covered with a black curtain and was located in an isolated room with four visual cues on the wall of the pool. Water was kept at 20°C and opacified with titanium dioxide. The P31 offspring mice were tested in the Morris water maze (MWM) four times per day for 7 days. Each of the mice was put in the pool to search for the platform, and the starting points were random for each mouse. When the mouse found the platform, the mouse was allowed to stay on it for 15 s. If a mouse did not find the platform within a 90-s period, the mouse was gently guided to the platform and allowed to stay on it for 15 s. A video tracking system recorded the swimming motions of the animals, and the data were analyzed using motion-detection software for the MWM (Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, People's Republic of China). At the end of the reference training (P37), the platform was removed from the pool and the mouse was placed in the opposite quadrant. Mice were allowed to swim for 90 s and the times the mouse swam to cross the platform area was recorded (platform crossing times). Mouse body temperature was maintained by active heating as described by Bianchi *et al.*[18] Specifically, after every trial, each mouse was placed in a holding cage under a heat lamp for 1 to 2 min until dry before being returned to its regular cage.

Environmental Enrichment

The EE in the current experiment was created in a large cage (70 × 70 × 46 cm) that included five or six toys (*e.g.*, wheels, ladders, and small mazes) as described in previous studies, with modification.[10,11] The pregnant mice were put in the EE every day for 2 h before delivery. The pregnant mice delivered offspring mice at G21. Then, the mother and the babies were put in the EE again every day for 2 h from P4 to P30. The objects were changed two to three times per week to provide newness and challenge.

Statistical Analysis

The nature of the hypothesis testing was two-tailed. Data were expressed as mean ± SD. The data for platform crossing time were not distributed normally and thus were expressed as median and interquartile range (IQR). The number of samples varied from 6–15, and the samples were distributed normally, with the exception of platform crossing time (tested by normality test, data not shown). Two-way ANOVA was used to determine the interaction of IL-6 antibody and sevoflurane treatment, and the interaction of EE and sevoflurane anesthesia. Interaction between time and group factors in a two-way ANOVA with repeated measurements was used to analyze the difference of learning curves (based on escape latency) between mice in the control group and mice treated with anesthesia in the

MWM. Multiple comparisons in escape latency of MWM were adjusted using the Bonferroni method (with seven tests and a threshold of 0.05/7 = 0.0071). There were no missing data for the variables of MWM (escape latency and platform crossing time) during the data analysis. The Student two-sample *t* test was used to determine the difference between the sevoflurane and control conditions on levels of IL-6, PSD-95, and synaptophysin. Finally, the Mann–Whitney *U* test was used to determine the difference between the sevoflurane and control conditions on platform crossing times. Values of $P < 0.05$ were considered statistically significant. SAS software version 9.2 (SAS Institute Inc., Cary, NC) was used to analyze the data.

Results

Sevoflurane Anesthesia in Pregnant Mice Induced Learning and Memory Impairment in Offspring Mice

The pregnant mice were either treated with 2.5% sevoflurane anesthesia for 2 h or under the control condition at G14. The mice delivered offspring mice at G21, and the offspring mice were tested in the MWM from P31 to P37. Comparison of the time that each mouse took to reach a platform during reference training (escape latency) showed that there was a statistically significant interaction between time and group based on escape latency in the MWM between mice following the control condition and mice that were given sevoflurane anesthesia (fig. 1A) ($P = 0.012$, two-way ANOVA with repeated measurement). Comparison of the number of times that each mouse crossed the location of an absent platform at the end of reference training (platform crossing times) indicated that there was a nonsignificant difference in the platform crossing times between the control condition (median, 2; IQR, 2–4.5) and the sevoflurane anesthesia (median, 1; IQR, 1–3) ($P = 0.051$, Mann–Whitney *U* test) (fig. 1B). There was no statistically significant difference in mouse swimming speed between the sevoflurane anesthesia and the control group (data not shown). Considered together, these data suggest that sevoflurane anesthesia in pregnant mice may induce learning and memory impairment in offspring mice.

Sevoflurane Anesthesia in Pregnant Mice Induced Neurotoxicity in Fetal Mice

Given that the sevoflurane anesthesia in pregnant mice can induce learning and memory impairment in offspring mice, we assessed the effects of sevoflurane anesthesia on the levels of proinflammatory cytokine IL-6, PSD-95, and caspase-3 activation, the neurotoxicity which may represent underlying mechanisms of learning and memory impairment.[19–28] The pregnant mice received anesthesia with 2.5% sevoflurane for 2 h or the control condition at G14. We harvested the brain tissues of the fetal mice at the end of the experiment, and these tissues were subjected to Western blot analysis. Immunoblotting of IL-6 showed that the sevoflurane

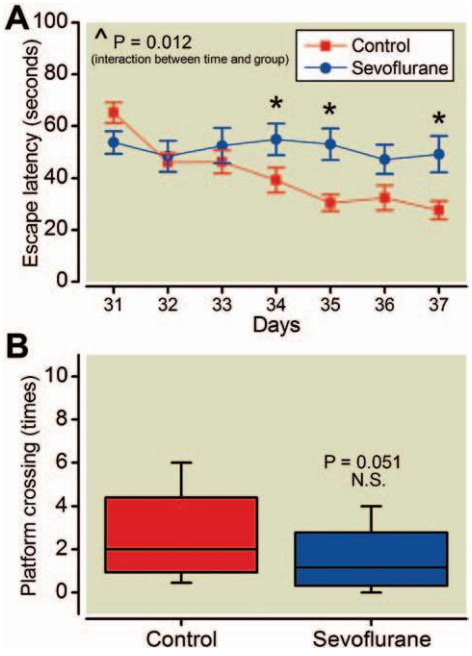

Fig. 1. Anesthesia with 2.5% sevoflurane for 2 h in pregnant mice at G14 induces learning and memory impairment in offspring mice tested at P31. (*A*) Sevoflurane anesthesia increases escape latency time of mice swimming in the Morris water maze (MWM) as compared with the control condition. Two-way ANOVA with repeated measurement analysis shows that there is a statistically significant interaction between time and group based on escape latency between mice following the control condition and mice following the sevoflurane anesthesia in the MWM (^*P* = 0.012). *Asterisk* indicates that there is a statistically significant difference in the escape latency between the control group and the sevoflurane group. (*B*) Sevoflurane anesthesia reduces the platform crossing times of mice swimming in the MWM (median, 1; IQR, 1–3) as compared with the control condition (median, 2; IQR, 2–4.5; *P* = 0.051, Mann–Whitney *U* test). IQR = interquartile range (n = 15 per arm).

anesthesia induced more visible bands representing IL-6 as compared with the control condition (fig. 2A). There was no significant difference in β-actin levels between the control condition and the sevoflurane anesthesia. Quantification of the Western blot showed that the sevoflurane anesthesia increased IL-6 levels in the brain tissues of fetal mice as compared with the control condition (256 ± 50.98% *vs.* 100 ± 54.12%, $P = 0.026$) (fig. 2B).

Next, we investigated the effects of the sevoflurane anesthesia in pregnant mice on levels of PSD-95, the marker of synapse, in the brain tissues of the fetal mice. Immunoblotting of PSD-95 showed that the sevoflurane anesthesia in pregnant mice produced less visible bands representing PSD-95 in the Western blot as compared with the control condition (fig. 2C). Quantification of the Western blot showed that the sevoflurane anesthesia in pregnant mice reduced PSD-95 levels in the brain tissues of fetal mice as compared with the control condition (61 ± 13.53% *vs.* 100 ± 10.08%, $P = 0.036$) (fig. 2D). Finally, we assessed the effects of sevoflurane anesthesia in pregnant mice

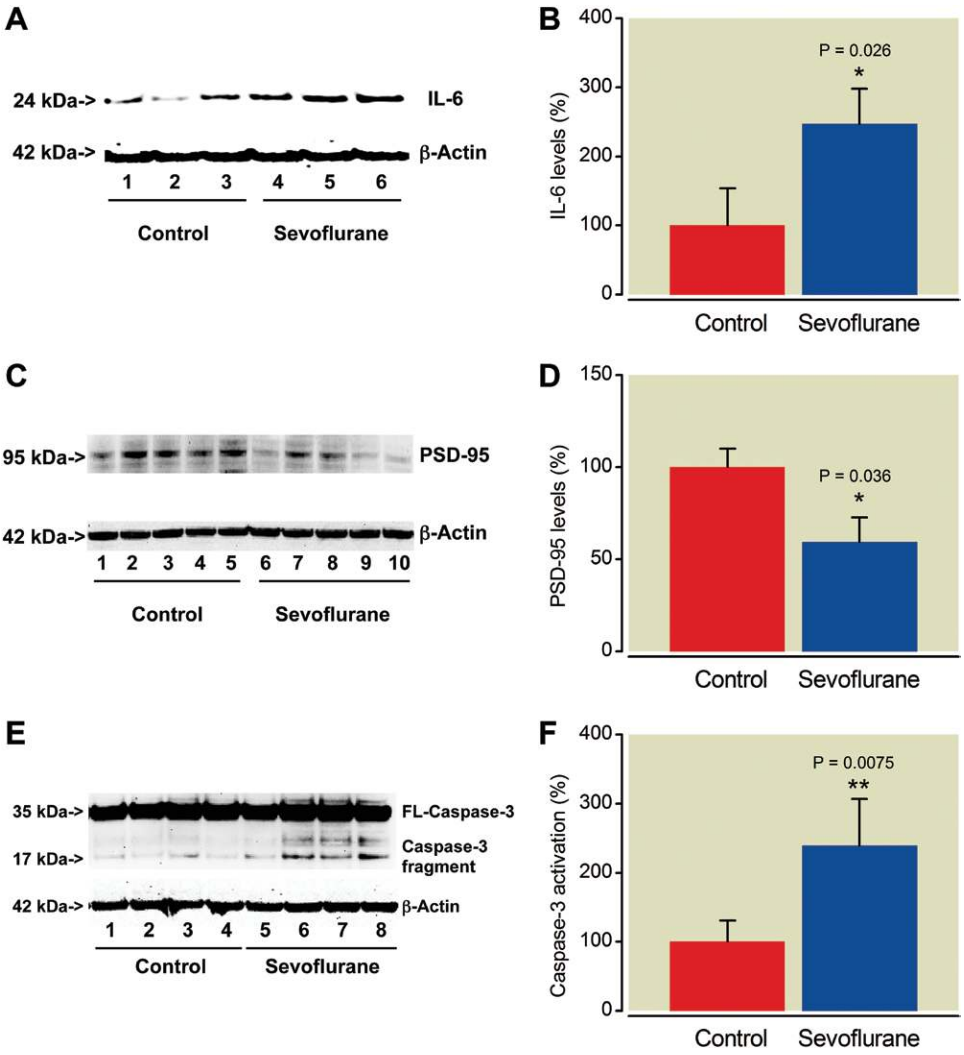

Fig. 2. Anesthesia with 2.5% sevoflurane for 2 h in pregnant mice at G14 increases IL-6 levels, decreases PSD-95 levels, and induces caspase-3 activation in the brain tissues of fetal mice. (*A*) Sevoflurane anesthesia increases IL-6 levels in the brain tissues of fetal mice as compared with the control condition on Western blot analysis. There is no statistically significant difference in the amounts of β-actin in the mouse brain tissues following the sevoflurane anesthesia or control condition. (*B*) Quantification of the Western blot shows that sevoflurane anesthesia increases IL-6 levels in the mouse brain tissues as compared with the control condition (**P* = 0.026). (*C*) The sevoflurane anesthesia reduces PSD-95 levels in the brain tissues of fetal mice as compared with the control condition on Western blot analysis. There is no statistically significant difference in the amounts of β-actin in the mouse brain tissues following the sevoflurane anesthesia or control condition. (*D*) Quantification of the Western blot shows that sevoflurane anesthesia reduces PSD-95 levels in the mouse brain tissues as compared with the control condition (**P* = 0.036). (*E*) The sevoflurane anesthesia induces caspase-3 activation in the brain tissues of fetal mice as compared with the control condition on Western blot analysis. There is no significant difference in the amounts of β-actin in the mouse brain tissues following the sevoflurane anesthesia or control condition. (*F*) Quantification of the Western blot shows that sevoflurane anesthesia induces caspase-3 activation in the mouse brain tissues as compared with the control condition (***P* = 0.0075). FL = full length (n = 6 per arm); IL-6 = interleukin-6; PSD = postsynaptic density-95.

on caspase-3 activation in the brain tissues of fetal mice. Caspase-3 immunoblotting showed that the sevoflurane anesthesia in pregnant mice increased levels of caspase-3 fragment without statistically significant changes in the levels of full-length caspase-3 in the brain tissues of fetal mice (fig. 2E). The quantification of the Western blot, based on the ratio of caspase-3 fragment to full-length caspase-3, revealed that the sevoflurane anesthesia in pregnant mice induced caspase-3 activation as compared with the control condition (198 ± 35% *vs.* 100 ± 21%, *P* = 0.0075) (fig. 2F). Considered together, these results suggest that anesthesia with 2.5% sevoflurane for 2 h in pregnant mice may induce neurotoxicity, including increases in proinflammatory cytokine levels, a reduction in synapse marker numbers, and caspase-3 activation in fetal mice, which may then lead to learning and memory impairment.

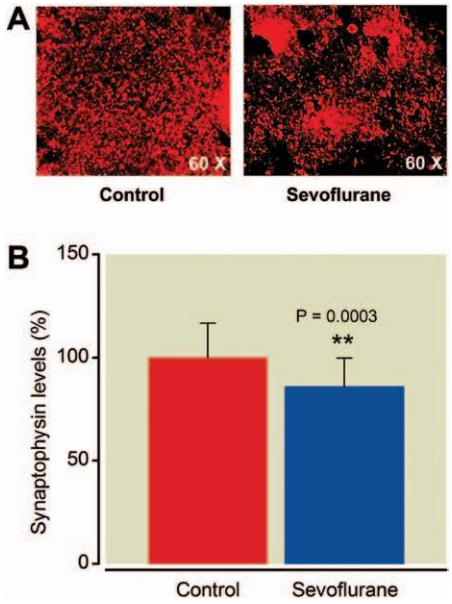

Fig. 3. Anesthesia with 2.5% sevoflurane for 2 h in pregnant mice at G14 decreases synaptophysin levels in the hippocampus of offspring mice examined at P31. (*A*) Sevoflurane anesthesia decreases synaptophysin levels in the brain tissues of offspring mice as compared with the control condition on immunohistochemistry analysis. (*B*) Quantification of the immunohistochemistry image shows that sevoflurane anesthesia decreases synaptophysin levels in the mouse brain tissues as compared with the control condition (**P = 0.0003) (n = 6 per arm).

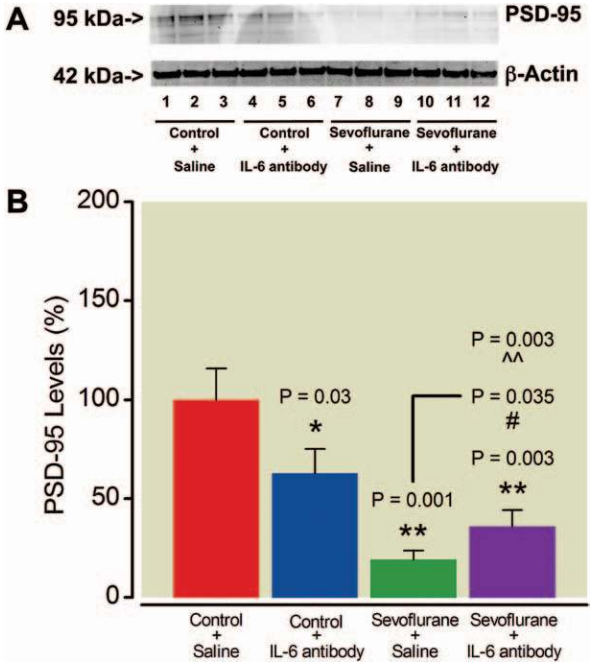

Fig. 4. IL-6 antibody mitigates the sevoflurane-induced reduction in PSD-95 levels in mouse primary neurons. (*A*) Treatment with 4.1% sevoflurane for 6 h (lanes 7–9) reduces PSD-95 levels as compared with the control condition (lanes 1–3). The treatment of IL-6 antibody (lanes 10–12) mitigates the sevoflurane-induced reduction in PSD-95 levels. There is no significant difference in the amounts of β-actin in the mouse primary neurons following the treatments of sevoflurane, IL-6 antibody, or control condition. (*B*) Quantification of the Western blot shows that sevoflurane treatment decreases PSD-95 levels as compared with the control condition (**P = 0.001). Treatment with sevoflurane plus IL-6 antibody leads to a lesser degree of reduction in PSD-95 levels as compared with treatment with sevoflurane plus saline (#P = 0.035). IL-6 antibody itself also decreases PSD-95 levels (*P = 0.03). Two-way ANOVA shows that there is an interaction of IL-6 antibody and sevoflurane, and that IL-6 antibody mitigates the sevoflurane-induced reduction in PSD-95 levels (^^P = 0.003) (n = 6 per arm). IL-6 = interleukin-6; PSD-95 = postsynaptic density-95.

Sevoflurane Anesthesia in Pregnant Mice Reduced Synaptophysin Levels in the Hippocampus of Offspring Mice

Given that sevoflurane anesthesia may cause acute neurotoxicity in fetal mice and learning and memory impairment in offspring mice at a later time (*e.g.*, P31), we assessed the effects of the sevoflurane anesthesia on levels of synapse markers in the hippocampus of P31 mice. Immunohistochemistry analysis showed that the sevoflurane anesthesia reduced levels of synaptophysin, the synapse marker,[29] in the hippocampus of P31 mice (fig. 3A). Quantification of the immunohistochemistry image showed that the sevoflurane anesthesia decreased levels of synaptophysin (77 ± 14.00% *vs.* 100 ± 16.73%, P = 0.0003) (fig. 3B). These results suggest that the sevoflurane anesthesia in pregnant mice may induce synaptic loss at a later time (*e.g.*, P31), leading to learning and memory impairment.

The Sevoflurane-induced Reduction in PSD-95 Level Was Dependent on the Sevoflurane-induced Increases in IL-6 Level

Given that the sevoflurane anesthesia increased IL-6 levels and decreased PSD-95 levels in brain tissues of fetal mice at G14, we then determined their potential association in mouse primary neurons. Treatment with 4.1% sevoflurane for 6 h reduced PSD-95 levels in mouse primary neurons as compared with the control condition (fig. 4A). The treatment with sevoflurane reduced PSD-95 levels as compared with the control condition, but IL-6 antibody mitigated the sevoflurane-induced reduction in PSD-95 levels, evidenced by more visible bands representing PSD-95 following the treatment of sevoflurane plus IL-6 antibody than following the treatment of sevoflurane plus saline (fig. 4A). Quantification of the Western blot showed that the sevoflurane treatment reduced PSD-95 levels (20 ± 4.58% *vs.* 100 ± 19%, P = 0.001) and IL-6 antibody mitigated the sevoflurane-induced reduction in PSD-95 levels (36 ± 8.33% *vs.* 20 ± 4.58%, P = 0.035) (fig. 4B). Two-way ANOVA indicated that there was an interaction between IL-6 antibody and sevoflurane, and that IL-6 antibody mitigated the sevoflurane-induced reduction in PSD-95 levels (P = 0.003) (fig. 4B). These results suggest that the sevoflurane-induced reduction in PSD-95 level may be dependent on the sevoflurane-induced increases in

IL-6 level. Interestingly, IL-6 antibody also reduced PSD-95 levels in the primary neurons (fig. 4, A and B).

EE Attenuated the Sevoflurane Anesthesia–induced Learning and Memory Impairment in Offspring Mice

EE has been shown to improve learning and memory,[30,31] and we therefore assessed whether EE can ameliorate the sevoflurane-induced learning and memory impairment. Two-way ANOVA with repeated measurement analysis showed that there was a statistically significant interaction between time and group based on escape latency between mice following sevoflurane anesthesia plus standard environment (SE) and sevoflurane anesthesia plus EE, and EE mitigated the sevoflurane-induced increases in escape latency of mice swimming in the MWM ($P = 0.0004$) (fig. 5A). Sevoflurane anesthesia plus EE (median, 4; IQR, 3.75–4.25) also increased the platform crossing times of mice in the MWM as compared with sevoflurane anesthesia plus SE (median, 1; IQR, 1–3; $P = 0.003$, Mann–Whitney U test) (fig. 5B). EE alone did not alter escape latency or platform crossing times of mice swimming in the MWM (fig. 5, C and D). Two-way ANOVA with repeated measurement analysis showed that there was no statistically significant interaction between time and group based on escape latency between mice following the control condition plus SE and control condition plus EE in the MWM ($P = 0.345$) (fig. 5C), although there was a statistically significant group main effect based on escape latency between mice following the control condition plus SE and control condition plus EE ($P = 0.009$) (fig. 5C).

Finally, the swimming speeds of the mice in the MWM between all of these conditions were not different (data not shown). Considered together, these data suggest that EE may ameliorate the learning and memory impairment in the offspring mice that is caused by the sevoflurane anesthesia in the pregnant mice. These results are consistent with the findings that EE ameliorates cognitive deficits.[10,11]

EE Mitigated the Sevoflurane-induced Increase in IL-6 Levels and Reduction in Levels of PSD-95 and Synaptophysin in the Brain Tissues of Offspring Mice

Given that EE can ameliorate the sevoflurane-induced learning and memory impairment, and synapse loss is the pathologic finding closely associated with cognitive dysfunction and dementia,[24] we determined the effects of EE

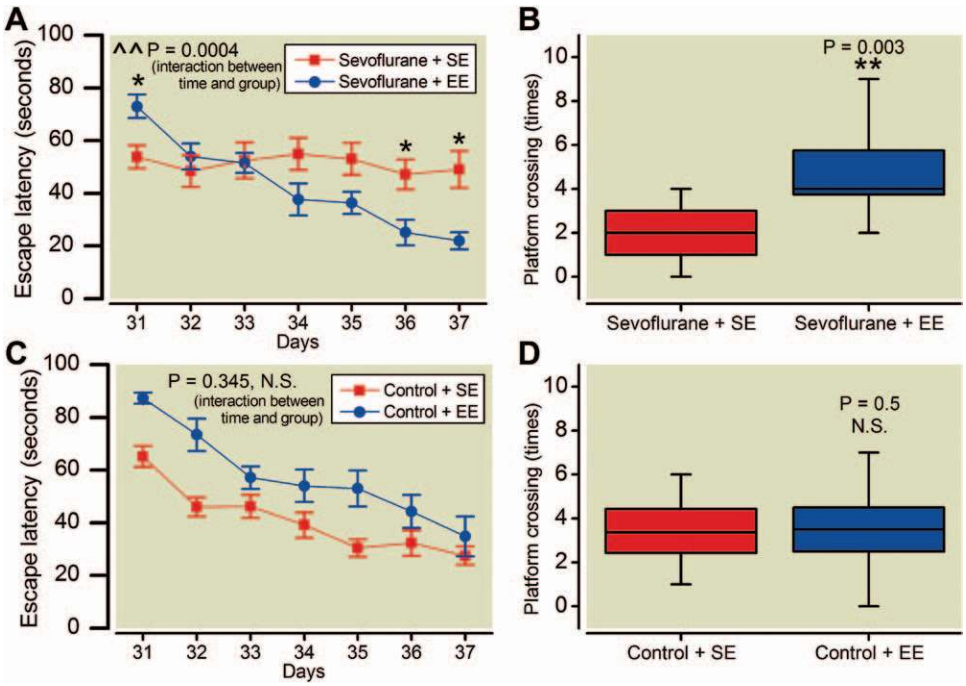

Fig. 5. Environmental enrichment (EE) attenuates the sevoflurane-induced learning and memory impairment in offspring mice. (*A*) Two-way ANOVA with repeated measurement analysis shows that there is a statistically significant interaction between time and group based on escape latency between mice following sevoflurane anesthesia plus SE and sevoflurane anesthesia plus EE (^^$P = 0.0004$). *Asterisk* indicates that there is a statistically significant difference in the escape latency between the sevoflurane plus SE group and the sevoflurane plus EE group. (*B*) Mann–Whitney *U* test shows that the platform crossing time of mice swimming in the MWM following the sevoflurane anesthesia plus EE (median, 4; IQR, 3.75–4.25) is more than that of mice following the sevoflurane anesthesia plus SE (median, 1; IQR, 1–3; **$P = 0.003$). (*C*) ANOVA shows that there is no statistically significant interaction between time and group based on escape latency of mice swimming in the MWM between the control condition plus SE and the control condition plus EE ($P = 0.345$, N.S.). (*D*) Mann–Whitney *U* test shows that there is no statistically significant difference in platform crossing time of mice swimming in the MWM between the control condition plus SE (median, 3; IQR, 2–4) and the control condition plus EE (median, 2; IQR, 2–4.25; $P = 0.499$, N.S.) (n = 15 per arm). EE = environmental enrichment; IQR = interquartile range; MWM = Morris water maze; N.S. = nonsignificant; SE = standard environment.

on the sevoflurane-induced alterations of IL-6 and synapse marker PSD-95 and synaptophysin levels in the brain tissues of offspring mice. IL-6 immunoblotting showed that sevoflurane anesthesia in pregnant mice increased IL-6 levels in the brain tissues of P31 offspring mice and that EE mitigated the effects (fig. 6A). The quantification of the Western blot illustrated that the sevoflurane anesthesia increased IL-6 levels (250 ± 77% *vs.* 100 ± 25%, $P = 0.032$). EE mitigated the sevoflurane-induced increase in IL-6 levels (89 ± 17% *vs.* 250 ± 77%, $P = 0.016$) (fig. 6B). There was no significant difference in IL-6 levels between the control condition plus SE and control condition plus EE (fig. 6C).

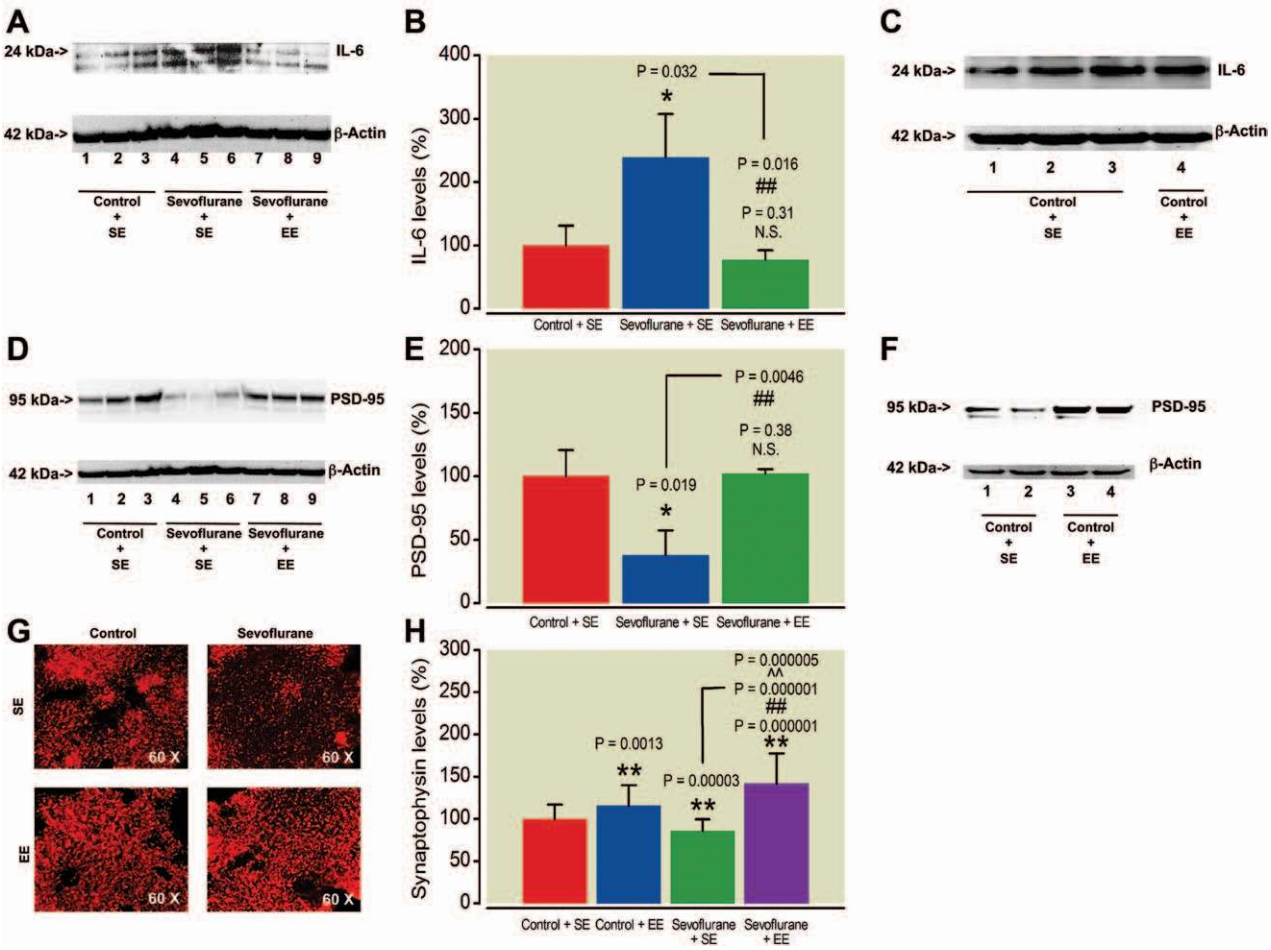

Fig. 6. EE mitigates the sevoflurane-induced increase in IL-6 levels, and reduction in levels of PSD-95 and synaptophysin in mouse brain tissues. (*A*) Sevoflurane anesthesia plus SE increases IL-6 levels as compared with the control condition plus SE. Sevoflurane anesthesia plus EE leads to lower levels of IL-6 as compared with the sevoflurane anesthesia plus SE. There is no significant difference in β-actin levels between the above treatments. (*B*) Quantification of the Western blot shows that the sevoflurane anesthesia plus SE increases IL-6 levels as compared with the control condition (*$P = 0.032$), and EE mitigates the sevoflurane-induced increase in IL-6 levels (##$P = 0.016$). (*C*) There is no significant difference in IL-6 levels between control plus EE and control plus SE. (*D*) Sevoflurane anesthesia plus SE reduces PSD-95 levels as compared with the control condition plus SE. Sevoflurane anesthesia plus EE leads to higher levels of PSD-95 as compared with the sevoflurane anesthesia plus SE. There is no significant difference in β-actin levels between the above treatments. (*E*) Quantification of the Western blot shows that the sevoflurane anesthesia plus SE reduces PSD-95 levels as compared with the control condition (*$P = 0.019$), and EE mitigates the sevoflurane anesthesia-induced reduction in PSD-95 levels (##$P = 0.0046$). (*F*) The PSD-95 level increases following control plus EE as compared with control plus SE. (*G*) Sevoflurane anesthesia plus SE leads to a reduction in synaptophysin levels in the brain tissues of offspring mice as compared with the control condition on the immunohistochemistry analysis. EE mitigates the sevoflurane-induced reduction in synaptophysin levels. The synaptophysin level increases following control plus EE as compared with control plus SE. (*H*) Quantification of the immunohistochemistry image shows that sevoflurane anesthesia plus SE (*green bar*) leads to a reduction in synaptophysin levels in the brain tissues of offspring mice as compared with the control condition plus SE (*red bar*, **$P = 0.00003$). Both EE plus control condition (*blue bar*, **$P = 0.0013$) and sevoflurane (*purple bar*, **$P = 0.000001$) cause higher synaptophysin levels in the brain tissues of offspring mice as compared with the control condition (*red bar*). Finally, there is an interaction between EE and sevoflurane anesthesia that EE mitigates the sevoflurane-induced reduction in synaptophysin levels in the hippocampus of offspring mice (^^$P = 0.00005$) (n = 6 per arm). EE = environmental enrichment; IL-6 = interleukin-6; PSD-95 = postsynaptic density-95; SE = standard environment.

Immunoblotting of PSD-95 showed that sevoflurane anesthesia in pregnant mice decreased PSD-95 levels in the brain tissues of P31 offspring mice, and EE mitigated the sevoflurane-induced reduction in PSD-95 levels in the brain tissues of offspring mice examined at P31 (fig. 6, D and E) (102 ± 3.23% for sevoflurane plus EE *vs.* 38 ± 19.39% for sevoflurane plus SE *vs.* 100 ± 20.6% for control plus SE, P = 0.0046). There was a higher level of PSD-95 in the control plus EE as compared with the control plus SE (fig. 6F). Immunohistochemistry staining showed that sevoflurane anesthesia in pregnant mice decreased synaptophysin levels in the brain tissues of P31 offspring mice as compared with the control condition (fig. 6, G and H) (77 ± 17% *vs.* 100 ± 21%, P = 0.00003), and EE mitigated the sevoflurane-induced reduction in synaptophysin levels in the brain tissues of offspring mice at P31 (fig. 6, G and H) (77 ± 17% for sevoflurane plus SE *vs.* 141 ± 36.44% for sevoflurane plus EE, P = 0.000001). Collectively, these results suggest that EE may rescue the sevoflurane-induced neuroinflammation and synaptic loss, leading to amelioration of the sevoflurane-induced learning and memory impairment.

Discussion

The widespread and growing use of anesthesia in the developing brain makes its safety a major health issue of interest[1] (reviewed in Sun[3]). This has become a matter of even greater concern with the evidence that anesthesia and surgery may induce neurodevelopmental impairment in children and that anesthetics are neurotoxic in young animals (reviewed in Sun[3]). Many pregnant women in the United States have nonobstetric surgery and fetal intervention procedures under anesthesia each year.[6,32] We therefore determined whether anesthesia with sevoflurane in pregnant mice could induce detrimental effects in fetal mice and offspring mice. We chose sevoflurane in the studies because sevoflurane is currently the most commonly used inhalation anesthetic, although sevoflurane might be less toxic than isoflurane.[33] Moreover, the effects of isoflurane in pregnant mice on behavioral changes in offspring mice have been determined.[7]

Sevoflurane anesthesia in pregnant mice induced learning and memory impairment in offspring mice at P31 (fig. 1). The same sevoflurane anesthesia induced acute neurotoxicity as evidenced by the increased levels of proinflammatory cytokine IL-6, reduced levels of synapse marker PSD-95, and caspase-3 activation in the brain tissues of fetal mice (fig. 2). The sevoflurane anesthesia in pregnant mice also increased IL-6 levels and decreased levels of PSD-95 and synaptophysin in the brain tissues of P31 offspring mice (fig. 6). Proinflammatory cytokine IL-6 can be released by the microglia cells during their activation, fueling neuroinflammation and leading to cognitive dysfunction[34–36] and mild cognitive impairment[37] in medical and surgical patients.[38] PSD-95 is a postsynaptic marker.[39,40] The reduction of PSD-95 has been shown to be associated with decreases in synapse number or synaptic loss, a part of the mechanisms underlying Alzheimer's disease–associated dementia and impairment of learning and memory[23,24] (reviewed in Querfurth and LaFerla[25]). In the *in vitro* studies, IL-6 antibody attenuated the sevoflurane-induced reduction in PSD-95 levels, which suggests that the sevoflurane-induced increase in IL-6 levels may lead to reduction in PSD-95 levels. Considered together, these data suggest that sevoflurane may increase neuroinflammation (*e.g.*, increase in IL-6 levels), which causes a reduction in synapse number, leading to learning and memory impairment. Future studies, including determination of whether anti-inflammatory medicine(s) can rescue the sevoflurane-induced synaptic loss and impairment of learning and memory, are warranted to further test this hypothesis.

IL-6 antibody itself reduced PSD-95 levels in the primary neurons (fig. 4, A and B). This could be attributable to IL-6 antibody only mitigating the effects associated with IL-6 accumulation (*e.g.*, mitigating a reduction in PSD-95 levels). In the absence of IL-6 accumulation, however, the IL-6 antibody may have nonspecific effects. The exact mechanisms of these effects remain to be determined.

The sevoflurane anesthesia induced caspase-3 activation, increases in IL-6 levels, and a reduction in PSD-95 levels 2 h after the anesthesia in the brain tissues of fetal mice, which occurred more rapidly than in the brain tissues of adult mice (6 h).[9] These data suggest that fetal mice might be more vulnerable to neurotoxicity than adult mice.

The mechanisms by which anesthetics induce neuroinflammation remain to be determined. Anesthetics have been shown to increase cytosolic calcium levels.[41–44] The elevation of cytosolic calcium is associated with increased levels of proinflammatory cytokines,[45] potentially through activation of the nuclear factor-κB signaling pathway.[46–49] Activated nuclear factor-κB translocates to the nucleus, where it binds to the promoter region of multiple genes, including cytokine genes.[46–50] Thus, future studies will include determining whether anesthetics can increase calcium levels in neurons and microglia cells to trigger generation of proinflammatory cytokine (*e.g.*, IL-6) through the nuclear factor-κB signaling pathway.

EE, consisting of social interaction and novel stimulation, may result in various neuroplastic changes, including increased hippocampal neurons,[51] improved spatial abilities and enhanced dendritic growth,[52] increased neurogenesis,[53] and increased nerve growth factor[54] after brain injury. EE has also been shown to improve learning and memory function.[10–12] We found that EE ameliorated the sevoflurane-induced learning and memory impairment, and mitigated the sevoflurane-induced increase in IL-6 levels and reduction in synaptic markers (figs. 5 and 6). These results suggest that EE may rescue the sevoflurane-induced neuroinflammation and synaptic loss, leading to improvement of the sevoflurane-induced impairment of learning and memory.

This study has several limitations. First, we did not determine the long-term (*e.g.*, 3–6 months) effects of sevoflurane

anesthesia on learning and memory function; however, the current findings were able to illustrate the effects of sevoflurane anesthesia on behavioral changes (*e.g.*, spatial learning and memory impairment) and the potential underlying cellular mechanisms (*e.g.*, caspase activation, increases in IL-6 levels, and synaptic loss). Second, we focused on only one proinflammatory cytokine, IL-6, in the experiments because IL-6 has been shown to contribute to learning and memory impairment. Sevoflurane anesthesia in pregnant mice may also induce other changes (*e.g.*, microglia activation) in the brain tissues of fetal mice consistent with neuroinflammation and need to be investigated in future studies.

It is unknown whether the anesthesia itself contributes to the clinically observed cognitive impairment, or whether the need for anesthesia/surgery is a marker for other unidentified factors that contribute. To either rule in or rule out the contribution of anesthesia, we will determine whether anesthesia alone can induce neuroinflammation and learning and memory in young mice. Our established preclinical mouse model will be used to determine whether anesthesia alone can induce detrimental effects (*e.g.*, learning and memory impairment, and neuroinflammation) in young animals (developing brain), to reveal the underlying mechanisms and to explore targeted interventions. Moreover, nociceptive stimuli such as surgical incision and pain with formalin have been shown to potentiate the anesthesia-induced neurotoxicity and neurobehavioral deficits.[55] Future studies may also include assessing whether other perioperative factors (*e.g.*, hypothermia and hypotension) can potentiate anesthesia-induced neurotoxicity and neurobehavioral deficits.

In conclusion, clinically relevant sevoflurane anesthesia in pregnant mice can induce acute neurotoxicity, including increases in IL-6 levels and reductions in synapse marker PSD-95 and caspase-3 activation, in the brain tissues of fetal mice. The same sevoflurane anesthesia in pregnant mice also induced long-term detrimental effects, including reductions in synapse marker PSD-95 and synaptophysin, and impairment of learning and memory in offspring mice at 31 days after birth. These results suggest that sevoflurane anesthesia in pregnant mice may induce neuroinflammation, caspase activation, and synaptic loss, leading to learning and memory impairment. Finally, EE may be able to rescue the sevoflurane-induced learning and memory impairment by mitigating the sevoflurane-induced synaptic loss and neuroinflammation. These findings will promote more research in anesthesia neurotoxicity in the developing brain, especially mechanistic studies.

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References

1. Rappaport B, Mellon RD, Simone A, Woodcock J: Defining safe use of anesthesia in children. N Engl J Med 2011; 364:1387–90
2. Belluck P: F.D.A. to study whether anesthesia poses cognitive risks in young children. The New York Times. March9, 2011: Science
3. Sun L: Early childhood general anaesthesia exposure and neurocognitive development. Br J Anaesth 2010; 105(Suppl 1):i61–8
4. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, Olney JW, Wozniak DF: Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci 2003; 23:876–82
5. Creeley CE, Olney JW: The young: Neuroapoptosis induced by anesthetics and what to do about it. Anesth Analg 2010; 110:442–8
6. Kuczkowski KM: Nonobstetric surgery during pregnancy: What are the risks of anesthesia? Obstet Gynecol Surv 2004; 59:52–6
7. Palanisamy A, Baxter MG, Keel PK, Xie Z, Crosby G, Culley DJ: Rats exposed to isoflurane in utero during early gestation are behaviorally abnormal as adults. Anesthesiology 2011; 114:521–8
8. Reitman E, Flood P: Anaesthetic considerations for non-obstetric surgery during pregnancy. Br J Anaesth 2011; 107(Suppl 1):i72–8
9. Dong Y, Zhang G, Zhang B, Moir RD, Xia W, Marcantonio ER, Culley DJ, Crosby G, Tanzi RE, Xie Z: The common inhalational anesthetic sevoflurane induces apoptosis and increases beta-amyloid protein levels. Arch Neurol 2009; 66:620–31
10. Hoffman AN, Malena RR, Westergom BP, Luthra P, Cheng JP, Aslam HA, Zafonte RD, Kline AE: Environmental enrichment-mediated functional improvement after experimental traumatic brain injury is contingent on task-specific neurobehavioral experience. Neurosci Lett 2008; 431:226–30
11. Kline AE, Wagner AK, Westergom BP, Malena RR, Zafonte RD, Olsen AS, Sozda CN, Luthra P, Panda M, Cheng JP, Aslam HA: Acute treatment with the 5-HT(1A) receptor agonist 8-OH-DPAT and chronic environmental enrichment confer neurobehavioral benefit after experimental brain trauma. Behav Brain Res 2007; 177:186–94
12. Sozda CN, Hoffman AN, Olsen AS, Cheng JP, Zafonte RD, Kline AE: Empirical comparison of typical and atypical environmental enrichment paradigms on functional and histological outcome after experimental traumatic brain injury. J Neurotrauma 2010; 27:1047–57
13. Zhen Y, Dong Y, Wu X, Xu Z, Lu Y, Zhang Y, Norton D, Tian M, Li S, Xie Z: Nitrous oxide plus isoflurane induces apoptosis and increases beta-amyloid protein levels. Anesthesiology 2009; 111:741–52
14. Zhang Y, Dong Y, Wu X, Lu Y, Xu Z, Knapp A, Yue Y, Xu T, Xie Z: The mitochondrial pathway of anesthetic isoflurane-induced apoptosis. J Biol Chem 2010; 285:4025–37
15. 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
16. 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
17. 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
18. 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
19. Wan Y, Xu J, Ma D, Zeng Y, Cibelli M, Maze M: Postoperative impairment of cognitive function in rats: A possible role for cytokine-mediated inflammation in the hippocampus. Anesthesiology 2007; 106:436–43
20. Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, Pollmächer T: Cytokine-associated emotional and

cognitive disturbances in humans. Arch Gen Psychiatry 2001; 58:445–52

21. Sparkman NL, Buchanan JB, Heyen JR, Chen J, Beverly JL, Johnson RW: Interleukin-6 facilitates lipopolysaccharide-induced disruption in working memory and expression of other proinflammatory cytokines in hippocampal neuronal cell layers. J Neurosci 2006; 26:10709–16
22. Weaver JD, Huang MH, Albert M, Harris T, Rowe JW, Seeman TE: Interleukin-6 and risk of cognitive decline: MacArthur studies of successful aging. Neurology 2002; 59:371–8
23. Hongpaisan J, Sun MK, Alkon DL: PKC ε activation prevents synaptic loss, Aβ elevation, and cognitive deficits in Alzheimer's disease transgenic mice. J Neurosci 2011; 31:630–43
24. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R: Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. Ann Neurol 1991; 30:572–80
25. Querfurth HW, LaFerla FM: Alzheimer's disease. N Engl J Med 2010; 362:329–44
26. Burguillos MA, Deierborg T, Kavanagh E, Persson A, Hajji N, Garcia-Quintanilla A, Cano J, Brundin P, Englund E, Venero JL, Joseph B: Caspase signalling controls microglia activation and neurotoxicity. Nature 2011; 472:319–24
27. 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
28. 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 USA 2010; 107:20518–22
29. Calhoun ME, Jucker M, Martin LJ, Thinakaran G, Price DL, Mouton PR: Comparative evaluation of synaptophysin-based methods for quantification of synapses. J Neurocytol 1996; 25:821–8
30. Bouet V, Freret T, Dutar P, Billard JM, Boulouard M: Continuous enriched environment improves learning and memory in adult NMRI mice through theta burst-related-LTP independent mechanisms but is not efficient in advanced aged animals. Mech Ageing Dev 2011; 132:240–8
31. Veena J, Srikumar BN, Mahati K, Bhagya V, Raju TR, Shankaranarayana Rao BS: Enriched environment restores hippocampal cell proliferation and ameliorates cognitive deficits in chronically stressed rats. J Neurosci Res 2009; 87:831–43
32. Van De Velde M, De Buck F: Anesthesia for non-obstetric surgery in the pregnant patient. Minerva Anestesiol 2007; 73:235–40
33. Liang G, Ward C, Peng J, Zhao Y, Huang B, Wei H: Isoflurane causes greater neurodegeneration than an equivalent exposure of sevoflurane in the developing brain of neonatal mice. Anesthesiology 2010; 112:1325–34
34. Teeling JL, Perry VH: Systemic infection and inflammation in acute CNS injury and chronic neurodegeneration: Underlying mechanisms. Neuroscience 2009; 158:1062–73
35. van Gool WA, van de Beek D, Eikelenboom P: Systemic infection and delirium: When cytokines and acetylcholine collide. Lancet 2010; 375:773–5
36. Willard LB, Hauss-Wegrzyniak B, Wenk GL: Pathological and biochemical consequences of acute and chronic neuroinflammation within the basal forebrain cholinergic system of rats. Neuroscience 1999; 88:193–200
37. Schuitemaker A, Dik MG, Veerhuis R, Scheltens P, Schoonenboom NS, Hack CE, Blankenstein MA, Jonker C: Inflammatory markers in AD and MCI patients with different biomarker profiles. Neurobiol Aging 2009; 30:1885–9
38. Hudetz JA, Gandhi SD, Iqbal Z, Patterson KM, Pagel PS: Elevated postoperative inflammatory biomarkers are associated with short- and medium-term cognitive dysfunction after coronary artery surgery. J Anesth 2011; 25:1–9
39. Takeuchi M, Hata Y, Hirao K, Toyoda A, Irie M, Takai Y: SAPAPs: A family of PSD-95/SAP90-associated proteins localized at postsynaptic density. J Biol Chem 1997; 272:11943–51
40. Liu Q, Trotter J, Zhang J, Peters MM, Cheng H, Bao J, Han X, Weeber EJ, Bu G: Neuronal LRP1 knockout in adult mice leads to impaired brain lipid metabolism and progressive, age-dependent synapse loss and neurodegeneration. J Neurosci 2010; 30:17068–78
41. Zhang G, Dong Y, Zhang B, Ichinose F, Wu X, Culley DJ, Crosby G, Tanzi RE, Xie Z: Isoflurane-induced caspase-3 activation is dependent on cytosolic calcium and can be attenuated by memantine. J Neurosci 2008; 28:4551–60
42. Zhang J, Dong Y, Xu Z, Zhang Y, Pan C, McAuliffe S, Ichinose F, Yue Y, Liang W, Xie Z: 2-Deoxy-D-glucose attenuates isoflurane-induced cytotoxicity in an in vitro cell culture model of H4 human neuroglioma cells. Anesth Analg 2011; 113:1468–75
43. 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
44. Yang H, Liang G, Hawkins BJ, Madesh M, Pierwola A, Wei H: Inhalational anesthetics induce cell damage by disruption of intracellular calcium homeostasis with different potencies. Anesthesiology 2008; 109:243–50
45. Kim D, Cho SH, Kim JS, Jo SH, Lee SJ, Kim KT, Choi SY: Human astrocytic bradykinin B(2) receptor modulates zymosan-induced cytokine expression in 1321N1 cells. Peptides 2010; 31:101–7
46. Meffert MK, Chang JM, Wiltgen BJ, Fanselow MS, Baltimore D: NF-kappa B functions in synaptic signaling and behavior. Nat Neurosci 2003; 6:1072–8
47. Vexler ZS, Yenari MA: Does inflammation after stroke affect the developing brain differently than adult brain? Dev Neurosci 2009; 31:378–93
48. Baeuerle PA, Henkel T: Function and activation of NF-kappa B in the immune system. Annu Rev Immunol 1994; 12:141–79
49. Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, Schwaninger M: NF-kappaB is activated and promotes cell death in focal cerebral ischemia. Nat Med 1999; 5:554–9
50. Neumann M, Naumann M: Beyond IkappaBs: Alternative regulation of NF-kappaB activity. FASEB J 2007; 21:2642–54
51. Kempermann G, Kuhn HG, Gage FH: More hippocampal neurons in adult mice living in an enriched environment. Nature 1997; 386:493–5
52. Leggio MG, Mandolesi L, Federico F, Spirito F, Ricci B, Gelfo F, Petrosini L: Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. Behav Brain Res 2005; 163:78–90
53. Olson AK, Eadie BD, Ernst C, Christie BR: Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. Hippocampus 2006; 16:250–60
54. Torasdotter M, Metsis M, Henriksson BG, Winblad B, Mohammed AH: Environmental enrichment results in higher levels of nerve growth factor mRNA in the rat visual cortex and hippocampus. Behav Brain Res 1998; 93:83–90
55. Shu Y, Zhou Z, Wan Y, Sanders RD, Li M, Pac-Soo CK, Maze M, Ma D: Nociceptive stimuli enhance anesthetic-induced neuroapoptosis in the rat developing brain. Neurobiol Dis 2012; 45:743–50