Sevoflurane Anesthesia Does Not Impair Acquisition Learning or Memory in the Morris Water Maze in Young Adult and Aged Rats

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ABSTRACT

Background: Sevoflurane has been found to increase apoptosis and pathologic markers associated with Alzheimer disease, provoking concern over their potential contribution to postoperative cognitive dysfunction.

Methods: The effects of anesthesia with 1 minimum alveolar concentration of sevoflurane for 4 h or sham exposure on cognition were investigated in young adult and aged (20–24 months) rats at 1, 4, and 12 weeks postexposure. Spatial reference memory acquisition and retention were tested in the Morris water maze task. Latency to locate the hidden platform and swim speed were determined and compared between treatments.

Results: Sevoflurane anesthesia significantly reduced latency to find the hidden platform in both young adult (n = 10 per treatment, $P < 0.0001$) and aged rats (n = 7 per treatment, $P < 0.0001$) when tested 1 week after exposure. In young rats only, this improved acquisition learning was maintained at 4 ($P = 0.003$) but not at 12 weeks postexposure ($P = 0.061$). There were no differences in swim speed or in open field exploration between groups (no confounding effects of stress or locomotion). Retention memory measured using probe trials was not affected by exposure to sevoflurane in young adult or aged rats.

Conclusion: Sevoflurane anesthesia did not impair acquisition learning and retention memory in young adult or aged rats.

What We Already Know about This Topic

- Studies in animals have raised concerns over the possible effects of volatile anesthetics in causing postoperative cognitive dysfunction in the elderly

What This Article Tells Us That Is New

- In rats, sevoflurane, under certain circumstances, improved rather than diminished behavioral tests of cognitive function
- Whether anesthetics cause postoperative cognitive dysfunction remains unclear

RECENT preclinical studies have raised concern over the possible toxic effects of inhalational anesthetics such as sevoflurane and their potential contribution to postoperative cognitive dysfunction (POCD).[1–6] The underlying mechanisms associating POCD with anesthesia, surgery, or both, however, remain unclear.

Experimental animal studies have demonstrated variable delayed effects of inhaled isoflurane on cognitive function in adult rats[7–11] but in agreement with clinical findings[12,13] aged animals more consistently show impairments, and these effects persist for at least 3 weeks after exposure.[7,8,14] Several mechanisms through which anesthesia could potentially contribute to cognitive dysfunction have been investigated in experimental animals. For instance, a number of studies have demonstrated anesthesia-induced acceleration of ongoing neurodegenerative processes.[1–4,6] Isoflurane,[3,4] isoflurane plus nitrous oxide,[15] sevoflurane[1] and desflurane

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combined with hypoxia[6] have all been shown to induce apoptosis and increase β-amyloid protein formation in cell culture models of neurodegeneration. Volatile anesthetics can enhance aggregation of β-amyloid protein *in vitro*, providing another potential interaction between anesthesia and Alzheimer disease pathology.[16,17] Sevoflurane, an inhalational anesthetic commonly used in clinical settings, induced apoptosis in the brain tissue of naïve mice for up to 12 h after a 2-h exposure, bringing about a cascade of increased β-site amyloid precursor protein-cleaving enzyme and β-amyloid protein levels that could in turn induce further apoptosis.[1] Despite the common use of sevoflurane in clinical settings, the evidence associating its use with increased apoptosis, and the emerging role of anesthesia in the development of POCD, no studies, either experimental or clinical, have examined the delayed effects of sevoflurane anesthesia on cognitive function occurring weeks to months after exposure that are characteristic of POCD.

A fundamental characteristic of anesthetics is to produce amnesia; however, even studies using anesthetics in the interval between learning a task and memory testing have yielded differential results. Sevoflurane has been reported to inhibit consolidation of memory in experimental animals. Mice exposed to 2.6% sevoflurane for 2 h immediately after familiarization with objects showed impaired memory retention for the familiar object in a novel object recognition task.[18] Dose-dependent inhibition of memory retention occurred in rats exposed to 0.5, 1, and 2% sevoflurane for 2 h immediately after inhibitory avoidance training.[19] In contrast, low-dose sevoflurane (0.11%) administered during single trial inhibitory avoidance training enhanced 24-h memory retention in rats.[20,21] These experiments, however, differ from the clinical picture of POCD where cognitive deficits manifest many days after anesthetic exposure when pharmacokinetics predicts the anesthetic is no longer in the body.

Therefore, in the current study we aimed to study the effects of a moderate duration of sevoflurane (1 minimum alveolar concentration, (MAC), for 4 h) in both young adult and aged (20–24 months) rats on cognition at 1, 4, and 12 weeks after anesthetic exposure. We hypothesized that sevoflurane would induce cognitive deficits and that aged rats would be more vulnerable.

Materials and Methods

This study was approved by the University of Melbourne Animal Ethics Committee on the use of animals in a research project. The 35 male Sprague-Dawley rats (8–10 weeks of age, n = 20, weighing 345.8 ± 11.0 g), and ex-breeders (n = 15, 19–21 months of age and weighing 686.5 ± 22.5 g at the beginning of the experiment) used in these experiments were obtained from the Animal Resources Centre, Canning Vale, Western Australia. Rats were housed two per cage in a climate and humidity controlled room on a 12-h light-dark cycle with free access to food and water. Some rats were housed singly over the 13-week course of the experiments if a cagemate died or was euthanized.

Rats were randomly allocated to either sham or anesthesia exposure groups. Adult and aged rats were tested in separate cohorts several months apart. Rats in the anesthesia group (n = 10 young adult, n = 8 aged) were placed in groups of three to four in an anesthetic induction chamber filled with sevoflurane (5%) for approximately 1–2 min until unconscious. Rats were then removed and attached to one of the nose cones of our custom designed anesthetizing apparatus. The apparatus allowed up to 10 rats to be anesthetized simultaneously and ensured that rats were exposed to the same amount of anesthetic because each nose cone was connected to the chamber into which the anesthetic was pumped at a flow rate of approximately 1.5 l/min, and adjusted to maintain MAC, oxygen, and carbon dioxide at constant levels within the chamber. Gases within the anesthetic chamber were continuously monitored (Ohmeda Excel 210 SE anesthetic machine, Datex Instrumentarium Corp., Helsinki, Finland). In our initial testing of the apparatus we established that the concentration of anesthetic at each nose cone was identical for any given flow rate measured between 1 and 3 l/min.

We aimed to administer equipotent anesthesia in 100% O_2 for 4 h to young and aged rats. Anesthetic concentration was titrated up for movement in response to toe pinch. Sham exposure rats (no anesthesia controls) were placed in the induction chamber containing 100% O_2 only for 10 min and then returned to their home cages. Because the bodies of the rats were external to the anesthetic chamber it was possible to monitor blood pressure and maintain normothermia in the anesthetized groups. Normothermia (37 ± 1°C) was maintained using warming mats and rectal temperatures were measured at 30 min intervals. Blood pressure was monitored noninvasively by tail cuff (Coda, Kent Scientific, Torrington, CT) at hourly intervals during anesthetic exposure. All rats received 0.9% saline solution (0.25 ml/100 g body weight/h) subcutaneously to maintain hydration during anesthesia.

In addition, formal MAC determinations were conducted in separate groups of rats (n = 12 young adult, 8 weeks of age, weighing 364.0 ± 7.4 g and n = 12 aged rats, 19 months, 716.3 ± 21.2 g) according to the method of Barbry *et al.*[22] Briefly, rats were commenced on approximately 80% of MAC for sevoflurane (2.9% and 95% CI: 2.79–3.0) for 1 h and then the concentration of sevoflurane was increased by 0.1–0.2% every 30 min and the percentage of rats moving in response to a forceps clamped to the first ratchet on the tail was recorded.

In separate groups of rats (young adult, 8–10 weeks and aged rats, 19 months, both n = 4) anesthetized under the same conditions as described in the previous paragraph, a catheter filled with heparinized saline (10 U/ml) was inserted into a carotid artery and connected to a pressure transducer (Cobe Argon Medical, Plano, TX). Phasic blood pressure was continuously monitored and recorded with a

data acquisition system (ADInstruments, Colorado Springs, CO). Mean arterial pressure and heart rate (beats/min) were derived from the phasic blood pressure signal (Chart 4.1.1, ADInstruments). Arterial blood samples (approximately 10 μl) were collected in capillary tubes at hourly intervals and analyzed for pH, $Paco_2$, Pao_2, and HCO^{3-} (ABL5, Radiometer Medical A/S, Bronshoj, Denmark). Normothermia was maintained using a rectal temperature probe coupled to a thermoblanket (Harvard Apparatus, Holliston, MA). These rats, referred to here as sentinel rats, were used only to determine physiologic parameters during anesthesia and were not used for any further tests.

Morris Water Maze Test

The behavioral tests were performed in a closed, quiet, light-controlled room in the Behavioral Testing Facility at the Department of Medicine, Royal Melbourne Hospital, The University of Melbourne. Rats were acclimatized to the conditions of the facility for at least 30 min before testing on each day.

Each rat was placed in a 160-cm diameter black plastic pool filled to a depth of 30 cm with clear water maintained at 23 ± 1°C and was required to locate a submerged platform using visual cues around the edges of the pool and within the room as previously described[23] with modifications.[24] On each trial a rat was gently placed into the pool at one of four different locations and allowed 90 s to locate a 10-cm diameter black platform submerged 2 cm below the surface of the water that was located in one of four randomly assigned positions. The position of the platform was kept constant for each individual rat during each acquisition test. If unsuccessful in the 90-s trial, the rat was gently guided to the platform. Once on the platform, rats were allowed to remain there for 30 s. At the end of each trial rats were removed from the pool, towel dried, and returned to their home cage. Four trials were conducted during each session with an intertrial interval of 30 min and an intersession interval of 24 h. The experiment extended for 4 consecutive days, beginning 1 week after anesthetic exposure. Each trial was videotaped and swim paths were tracked using Ethovision Video-Tracking Software (v3.1.16 Noldus Information Technology, Wageningen, Netherlands). The latency to reach the platform, distance traveled, and swim velocity were calculated for each trial and then averaged over each daily session. Four weeks after anesthetic or sham exposure a second probe trial was conducted to assess long-term memory retention. The experimental time line is shown in figure 1.

One day after the second probe trial, rats were assigned a new platform location different from the first and a reversal trial was conducted. Rats were required to learn this new platform location over four daily trials as previously described; however, criterion was reached in only 2 days in this reversal trial because rats had already learned the rules of the task, that is, when they find the platform they are taken out of the pool. Twenty-four hours after the last acquisition session, rats were tested in a third probe trial. Finally, at 3 months postexposure rats were again tested in a fourth probe trial, then subjected to another reversal trial (platform position different from week 1 and week 4) and then a final probe trial 24 h after acquisition.

Open Field Test

The open field test is widely used to assess exploratory behavior in rodents.[25,26] The open field is an arena of 1 m diameter enclosed by 20 cm high Perspex walls (P.A. Reed Pty Ltd., St. Albans, Victoria, Australia). Lighting in the center of the arena (inner circle, 66 cm diameter) is approximately 90 lux. Each rat was placed individually into the center of the open field and allowed to explore the arena for 5 min. Rats were videotaped from above and data including total distance traveled, number of entries into, and the time spent in the inner circle were quantified using Ethovision tracking software (v3.1.16 Noldus Information Technology). All rats were tested once only on day 6 postanesthetic or postsham exposure.

Statistics

Sample size estimates were based on pilot data showing a difference in means between two treatment groups of 15.1% with a pooled SD of 12.5%. A sample size of 10 should enable the detection of at least 15% difference with a power of 80% and α of 5%, with an assumed SD of 12.5%.

Curves for percentage of rats with no movement during sevoflurane exposure (MAC determination) were fitted using sigmoidal dose–response curve analysis and MAC (no movement in 50% of the animals in response to stimulus) and 95% CIs were calculated using GraphPad Prism (GraphPad Software Inc., La Jolla, CA). Percentage inhaled sevoflurane was analyzed by unpaired Student two-tailed *t* tests. Systolic and diastolic blood pressure was compared between sham and sevoflurane treated rats using a three-way ANOVA with repeated measures on time of assessment. Two-way ANOVA with factors treatment and session, the latter being repeated measures factor, was used for analysis of latency, distance traveled and velocity in the acquisition phase in the Morris water maze. Tukey honestly significant

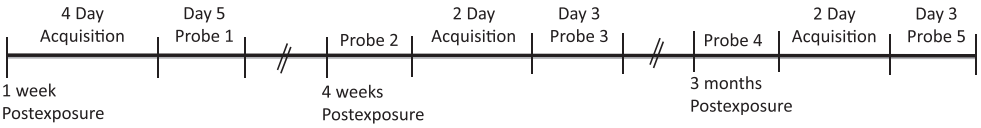

Fig. 1. Experimental timeline for Morris water maze testing showing acquisition trials at 1, 4, and 12 weeks postsevoflurane exposure and the time at which the five probe trials were conducted.

difference test (unequal n) was used for *post hoc* testing. Probe trials comparing time spent in each quadrant were also analyzed using two-way ANOVA with factors treatment, and probe number as a repeated measures factor. The open field test data were analyzed by Student unpaired two-tailed *t* test. Data were analyzed using SigmaStat (Jandel Scientific Inc., San Rafael, CA) and Statistica (Statsoft Inc., Tulsa, OK) and in all cases, statistical significance was defined as $P < 0.05$.

Results

Physiologic Variables

In the aged groups, a total of six rats died or had to be euthanized during the 13 weeks under study. One rat had a large tumor over the hindlimb that affected movement and was euthanized before group assignment. Four rats in the sevoflurane group had infected hocksore, tumor on abdomen, urinary infection, and multiple infections on the tail. One sham-exposed rat had pink eyes and significant weight loss with unknown pathology on autopsy. The aged rats were between 22 and 25 months of age by the 12-week test point and were therefore at the limit of their life expectancy for this breed of rat.

Inspired percent sevoflurane was stable over time for young adult and aged rats (fig. 2), but aged rats required significantly less sevoflurane to remain unresponsive to toe pinch than young adult rats (mean and SD of concentration measured at 30-min intervals: 2.11 ± 0.15% and 2.40 ± 0.12%, respectively, $P < 0.0001$). The MAC determined for sevoflurane in young and aged rats (MAC = 2.43% (2.17–2.69) and 2.27% (2.07–2.47) respectively, 95% CI in parentheses) see figure 2A. The concentrations used in our experiments were at the lower 95% CI of MAC for both young and aged rats (fig. 2C). This was due to the greater stimulus (tail clamp) for the formal determination compared with toe pinch for the experiment. In addition, it was expected that anesthesia concentration would be lower in the 4-h exposure as there was no surgical stimulation.

Blood pressures, measured at hourly intervals over 4 h of sevoflurane exposure, did not differ between individual rats time in the young adult group ($F_{(3,9)} = 1.16$, $P > 0.05$) or the aged group ($F_{(3,18)} = 0.35$, $P > 0.05$). Aged rats, however, had significantly higher systolic and diastolic blood pressures compared with young adult rats (Age*Blood pressure: $F_{(1,111)} = 6.01$, $P < 0.01$, ANOVA; fig. 2C). Temperatures were within normal physiologic levels throughout sevoflurane exposure in both young and aged sevoflurane exposed rats (data not shown). Physiologic parameters obtained in sentinel rats showed stable mean arterial pressure, heart rate, pH, oxygen and carbon dioxide partial pressures ($Paco_2$ and $Paco_2$) and bicarbonate ion (HCO^{3-}) over 4 h of sevoflurane exposure (table 1). The major difference between young and aged rats was greater degree of respiratory depression as evident by higher carbon dioxide partial pressure.

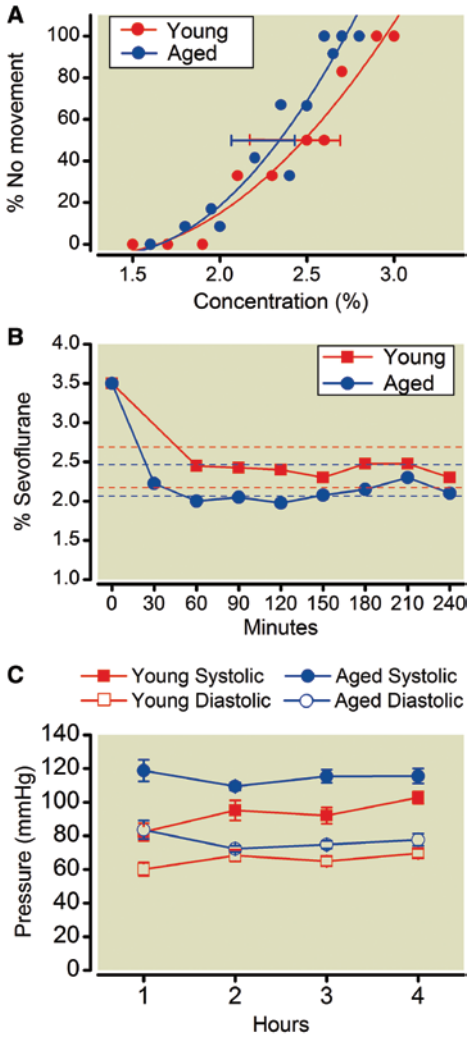

Fig. 2. (*A*) Percentage of young and aged rats with no movement for sevoflurane. Each point represents n = 12 rats. Curves were fitted using sigmoidal concentration-response analysis. The minimum alveolar concentration (MAC) for young adult and aged rats with 95% CI (*horizontal bars*) is shown. (*B*) Percentage sevoflurane required in young and aged rats to produce anesthesia over 4 h. Data are mean values of sevoflurane concentration in the anesthetic chamber in two consecutive experimental groups for each age group. n = 10 young rats and n = 7 aged rats. Superimposed on these curves are the 95% CIs from the MAC determination from *A*, *Red dashed lines* and *blue dashed lines* are the 95% CIs for young and aged rats, respectively. *Error bars* are obscured by the symbols. (*C*) Systolic (*closed symbols*) and diastolic (*open symbols*) blood pressure in young and aged sevoflurane-anesthetized rats. Data are mean ± SEM. n = 10 per treatment group in young rats and n = 7 in aged rats.

Open Field Test

Exposure to sevoflurane had no effect on time spent in the inner arena during the open field test compared with sham-exposed rats in either young adult or aged rats (sham: 23.4 ± 5.9 s; sevoflurane: 22.5 ± 2.8 s, $P = 0.889$ and sham: 15.3 ± 9.6 s; sevoflurane: 13.4 ± 2.3 s, respectively; $F_{(1,26)} = 0.01$, $P = 0.998$). Aged rats were less active in the open field, moving only half the distance of young adult rats. Sevoflurane

Table 1. Blood Pressure, Blood Gases, and pH Measured in Young Adult and Aged Sentinel Rats Exposed to 4 h Sevoflurane

Hours of Anesthesia	MAP (mmHg)	Heart Rate (beats/min)	pH	$Paco_2$ (mmHg)	Pao_2 (mmHg)	HCO^{3-} (mm)
Young adult rats						
1	89.6 ± 3.5	316.4 ± 12.9	7.41 ± 0.05	35.0 ± 4.6	328.7 ± 4.6	21.7 ± 0.7
2	85.9 ± 3.4	308.5 ± 2.8	7.45 ± 0.03	34.7 ± 2.9	417.0 ± 9.6	23.7 ± 0.3
3	90.2 ± 10.2	327.4 ± 18.1	7.48 ± 0.03	29.3 ± 1.8	475.0 ± 65.9	21.7 ± 1.8
4	98.3 ± 7.2	333.1 ± 17.5	7.47 ± 0.00	29.0 ± 1.0	428.0 ± 50.4	20.7 ± 0.9
Aged rats						
1	92.8 ± 6.7	275.8 ± 6.3	7.23 ± 0.05	65.8 ± 2.3	315.3 ± 67.3	26.8 ± 2.8
2	95.3 ± 6.3	280.8 ± 10.9	7.33 ± 0.04	60.5 ± 4.1	361.0 ± 28.7	30.5 ± 2.1
3	90.8 ± 4.1	273.5 ± 25.7	7.30 ± 0.07	69.8 ± 9.1	337.8 ± 27.6	34.3 ± 4.8
4	88.0 ± 2.8	269.5 ± 20.4	7.33 ± 0.05	67.3 ± 10.8	259.5 ± 34.9	33.3 ± 1.3

n = 4 per age group. Data are mean ± SEM.

HCO^{3-} = bicarbonate ion; MAP = mean arterial pressure; $Paco_2$ = partial pressure carbon dioxide; Pao_2 = partial pressure oxygen.

exposure had no effect on the total distance traveled in either young adult or aged rats (young: sham: 367.0 ± 72.7 cm *vs.* sevoflurane: 336.1 ± 22.5 cm, $P = 0.822$ and aged rats: 134.2 ± 50.8 cm *vs.* sevoflurane: 117.5 ± 32.4 cm, $P = 0.99$).

Morris Water Maze Testing

Groups of young adult rats exposed to sham conditions or to sevoflurane anesthesia learned to locate the submerged platform in the water maze as evidenced by a significant decrease in latency over the four daily sessions (fig. 3A; $F_{(3,54)} = 71.94$, $P < 0.0001$). Young rats exposed to 1 MAC sevoflurane for 4 h were able to locate the hidden platform with significantly lower latencies than sham-exposed rats when tested 1 week after exposure (fig. 3A; $F_{(1,54)} = 5.74$, $P = 0.028$). This superior performance was found to persist when rats were retested with a new platform position in the reversal trial at 4 weeks postexposure (fig. 3C; $F_{(1,18)} = 11.90$, $P = 0.003$) but did not reach statistical significance at 12 weeks postexposure (fig. 3E; $F_{(1,18)} = 3.98$, $P = 0.061$).

Similar to young rats, aged rats were also able to learn the location of the platform in the water maze, exhibiting lower latencies over the four daily sessions (fig. 3B; $F_{(3,36)} = 25.40$, $P < 0.0001$). Like the young adult rats, exposure to sevoflurane in aged rats also resulted in a significantly faster latency to locate the hidden platform compared with sham controls ($F_{(3,36)} = 4.515$, $P = 0.009$). In contrast to the young adult rats, improvements observed in the sevoflurane-treated rats did not persist at 4 or 12 weeks when rats were retested with a new platform position in the reversal trials (fig. 3, D and F; $F_{(1,11)} = 0.092$, $P = 0.767$ and ($F_{(1,8)} = 3.366$, $P = 0.104$, respectively).

Improvements in latency to locate the platform in sevoflurane-treated young adult rats were accompanied by significantly shorter distances traveled on weeks 4 and 12, likely reflecting a more efficient search strategy (fig. 4A, week 1: $F_{(1,18)} = 2.62$, $P = 0.123$; fig. 4C, week 4: $F_{(1,18)} = 9.09$, $P = 0.007$; fig. 4E, week 12: $F_{(1,18)} = 4.64$, $P = 0.045$). We also assessed the distance traveled by aged rats at 1, 4, and 12 weeks during water maze acquisition, and this is shown in figure 4B, D, and F. There were no treatment effects at weeks 1, 4, or 12 on distance traveled in the aged rats (fig. 4B: $F_{(1,36)} = 1.189$, $P = 0.297$; fig. 4D: $F_{(1,11)} = 0.217$, $P = 0.650$; and fig. 4F: $F_{(1,8)} = 5.069$, $P = 0.544$).

The observed treatment effects could not be accounted for by differences in swim speed as no significant differences were found in velocity between sevoflurane or sham exposed young adult rats at any time tested (fig. 5A, week 1: $F_{(1,54)} = 0.0003$, $P = 0.986$; fig. 5C, week 4: $F_{(1,18)} = 0.238$, $P = 0.631$; figure 5E, week 12: $F_{(1,18)} = 0.331$, $P = 0.572$). Likewise in aged rats, no significant differences were found in velocity between sevoflurane and sham exposed rats at any time point (fig. 5B, week 1: $F_{(1,36)} = 0.0009$, $P = 0.977$; fig. 5D, week 4: $F_{(1,11)} = 0.53$, $P = 0.482$; fig. 5F, week 12: $F_{(1,8)} = 0.538$, $P = 0.484$), indicating that the observed differences in latency were not due to differences in swim speed.

Exposure to sevoflurane had no significant effect on any of the five probe trials conducted over the 12 weeks of testing in either young adult (fig. 6A, $F_{(1,90)} = 0.472$, $P = 0.494$) or aged rats (fig. 6, B and $F_{(1,50)} = 0.864$, $P = 0.357$; see fig. 1 for probe schedule). Each probe trial was also analyzed to determine whether rats within a treatment group showed a significant preference for the target quadrant relative to the other three quadrants, therefore indicating that they had remembered in which quadrant the learned target platform had previously been located. In all probe trials conducted 24 h after the acquisition sessions (probes 1, 3, and 5) all groups of rats showed a significant preference for the target quadrant. This was true for both young and aged rats, treated or untreated (data not shown).

Neither young or aged rats were able to recall the target quadrant when tested in probe 4, which was conducted at 12 weeks (that is, 8 weeks after the second acquisition session: $F_{(3,79)} = 0.030$, $P = 0.993$ and $F_{(3,39)} = 0.972$, $P = 0.418$, respectively).

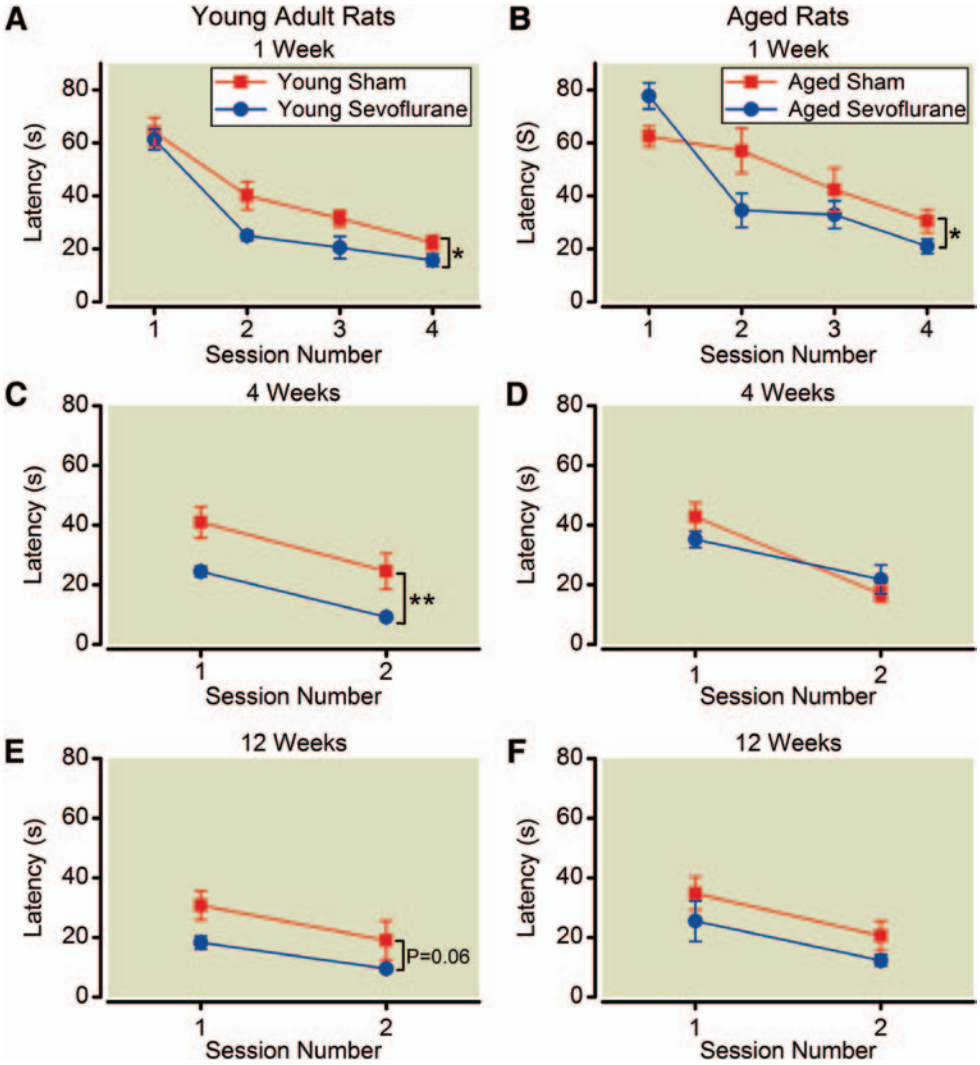

Fig. 3. Effects of 4-h exposure to sevoflurane in young adult and aged rats on latency to locate the hidden platform in the Morris water maze at (*A* and *B*) 1 week, (*C* and *D*) 4 weeks, and (*E* and *F*) 12 weeks postexposure. Sevoflurane-exposed rats performed significantly faster than sham-exposed controls at 1 week in both young (*A*) and aged (*B*) groups (*$P < 0.05$, ANOVAs and Tukey honestly significant difference test). At 4 weeks this effect was only statistically significant in young sevoflurane-treated rats compared with their sham controls (*C*). (**P less than 0.01, ANOVA). The superior cognitive performance in sevoflurane-exposed rats was not maintained at 12 weeks in either young (*E*) or aged rats (*F*) compared with their respective shams. Data are mean ± SEM. Weeks 1, 4, and 12: n = 10 rats per treatment group for young adult rats. Aged rats: week 1, n = 7; week 4, n = 7 sham, n = 6 sevoflurane; week 12, n = 6 sham and n = 4 sevoflurane-treated.

Discussion

This study is the first to test the long-term effects of sevoflurane on cognitive performance in experimental animals. The main finding is that sevoflurane does not cause detrimental effects in acquisition learning, and may even improve learning in both young adult and aged rats. Recent studies have shown that exposure of rodents to common inhalational anesthetics such as sevoflurane induces apoptosis and the formation of Alzheimer disease pathology,[1,4,6,16,27,28] leading to speculation that these effects may exacerbate Alzheimer disease and contribute to or cause POCD[1,6,29] in humans. The current findings do not support translation of any early occurrence of apoptosis or Alzheimer disease pathology into a cognitive deficit in the water maze after exposure to sevoflurane anesthesia in young adult or in aged rats.

A role for anesthesia in exacerbating Alzheimer disease pathology and leading to POCD has previously been questioned by the findings of Bianchi *et al.*[30] In a transgenic mouse model of Alzheimer disease, increased production of amyloid plaque was found after daily exposure to halothane or isoflurane without enhanced cognitive decline compared with preanesthetic performance. Interestingly, wild-type mice—which do not develop amyloid plaques—exposed to the same anesthetic regimen did develop cognitive impairments, suggesting an alternative pathway for anesthetic-induced neurodegeneration, although a ceiling effect in the transgenic mice cannot be ruled out. In Alzheimer

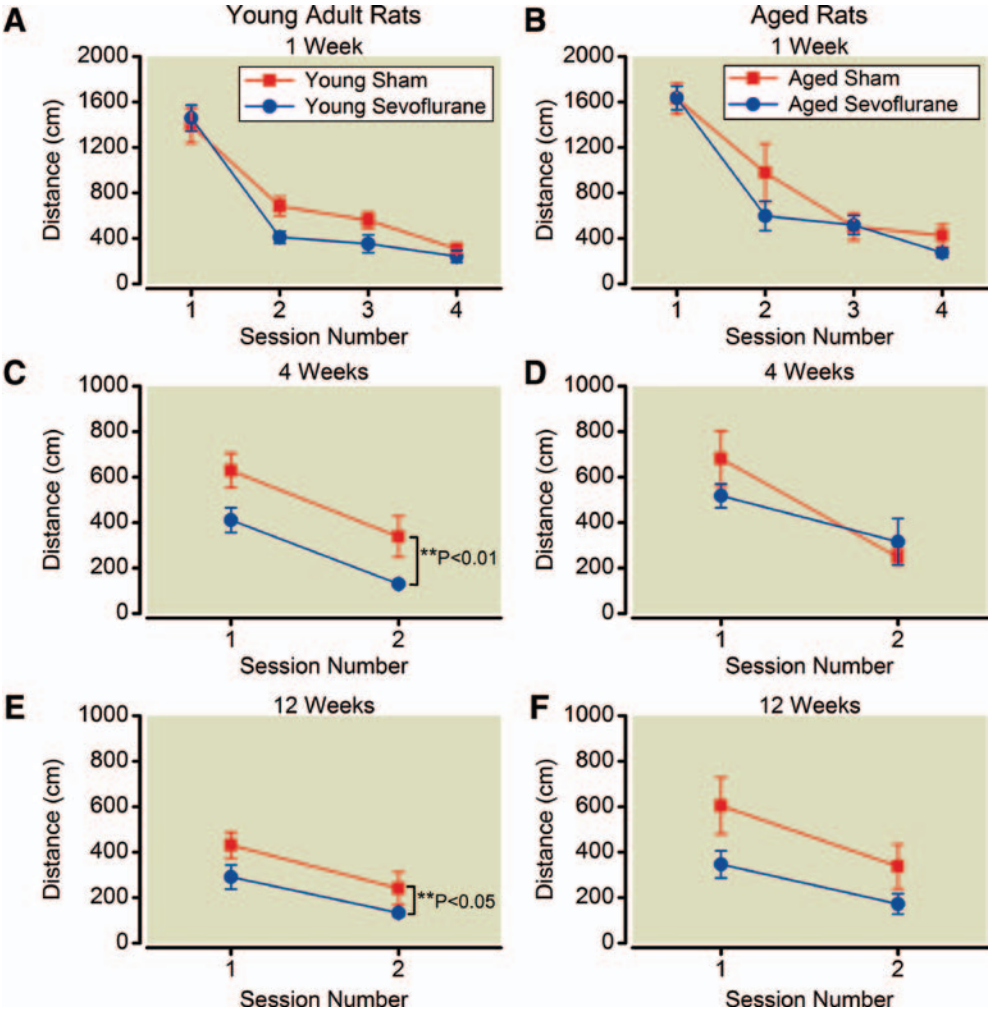

Fig. 4. Effects of 4-h exposure to sevoflurane in young adult and aged rats on distance traveled during acquisition in the Morris water maze at (*A* and *B*) 1 week, (*C* and *D*) 4 weeks, and (*E* and *F*) 12 weeks postexposure. At week 1 the effect of sevoflurane treatment on distance traveled in the water maze did not reach statistical significance ($P > 0.05$, ANOVA). At weeks 4 and 12, sevoflurane-treated rats traveled less distance than their corresponding shams (*C*, 4 weeks: $^{**}P < 0.01$, and (*E*) 12 weeks ($^{*}P < 0.01$, ANOVA and Tukey honestly significant difference posttest ANOVA). There were no significant differences between sham or sevoflurane-treated aged rats (*B*, *D*, and *F*) on distance traveled. Data are mean ± SEM. Weeks 1, 4, and 12: n = 10 rats per treatment group for young adult rats. Aged rats: week 1, n = 7; week 4, n = 7 sham, n = 6 sevoflurane; week 12, n = 6 sham and n = 4 sevoflurane-treated.

disease itself a direct connection between various pathologic markers and cognitive symptoms remains controversial[31,32] and recent studies suggest amyloid plaques are more likely to be a response to the disease, rather than its initiator.[33] Clinical studies have also yielded contradictory data with respect to anesthesia and risk of Alzheimer disease. The age of onset of Alzheimer disease has been reported to be inversely correlated with cumulative exposure to general anesthesia before 50 yr of age[34,35] but in contrast Gasparini *et al.*[36] found no association between the number of surgical operations or exposures to anesthesia and the risk of Alzheimer disease.

To our knowledge there are no previous studies showing long-term improvement in learning after sevoflurane anesthesia. Enhanced memory, however, has been reported after exposure to other volatile anesthetics. Facilitation of memory in an avoidance task was reported 22 h after exposure of mice to 2 h of halothane, enflurane, or isoflurane.[37] Rammes *et al.*[38] reported isoflurane-induced enhancement of long-term potentiation (thought to be the molecular/cellular correlate of memory) in CA1 hippocampal neurons *in vitro* as well as improved hippocampal-dependent cognitive performance in the modified hole board test in mice 24 h after anesthesia with 1 MAC (1.3 vol%) isoflurane for 2 h. This improvement was accompanied by a hippocampus-selective elevation of *N*-methyl-D-aspartate receptor 2B subunit expression providing a mechanism through which these effects might be mediated. Sevoflurane could potentially act through a similar mechanism to enhance cognitive performance as all volatile anesthetics stimulate *N*-methyl–D-aspartate receptors.[39] However, in contrast to the current findings of a persistent improvement in memory performance in the young adult rats at 1, 4, and 12 weeks and in aged rats at 1 week after

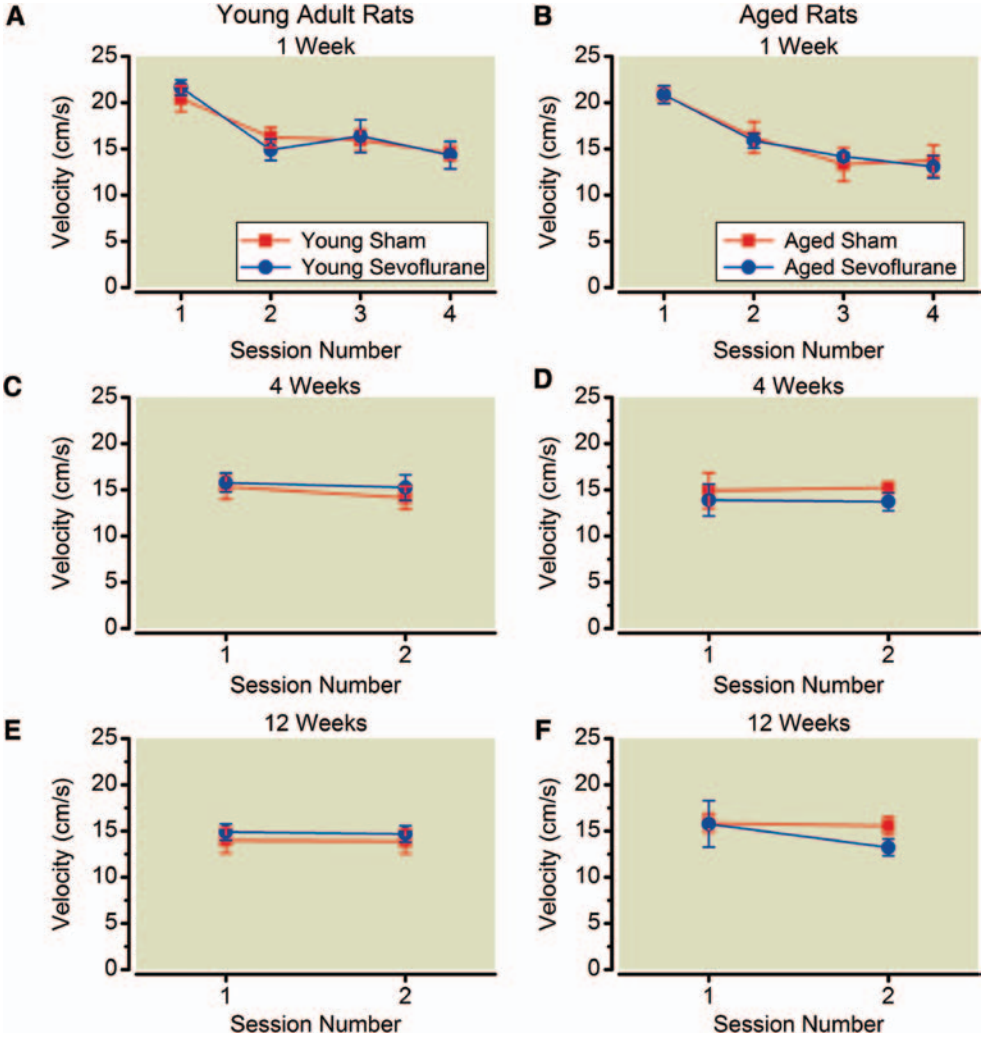

Fig. 5. Effects of 4-h exposure to sevoflurane in young adult and aged rats on velocity of swimming during acquisition in the Morris water maze at (*A* and *B*) 1 week, (*C* and *D*) 4 weeks, and (*E* and *F*) 12 weeks postexposure. There were no significant differences between sevoflurane or sham treated rats or between age groups on swim velocity in the maze ($P < 0.05$ at all time points, ANOVA). Data are mean ± SEM. Weeks 1, 4, and 12: n = 10 rats per treatment group for young adult rats. Aged rats: week 1, n = 7; week 4, n = 7 sham, n = 6 sevoflurane; week 12, n = 6 sham and n = 4 sevoflurane-treated.

sevoflurane exposure, the isoflurane-mediated improvements in cognitive performance as well as the *N*-methyl–D-aspartate receptor 2B subunit elevation reported by Rammes *et al.*[38] had returned to control levels 7 days after anesthesia.

Improved memory after sevoflurane exposure in the current study could not be accounted for by differences in swimming speed between treatment groups. Aged rats as a whole tended to swim slower and this has previously been reported by our group.[11] In young adult rats the distance traveled when searching for the platform was unrelated to treatment at 1 week but at 4 and 12 weeks young adult sevoflurane-treated rats traveled a shorter distance when searching for the platform. Because velocity was not different between young sham and sevoflurane rats, this difference in distance traveled likely reflects a difference in search strategy, which may improve over the course of the study. Sevoflurane-treated aged rats did not differ in distance traveled at 1 and 4 weeks compared with their corresponding sham rats but a significant difference was found at 12 weeks postsevoflurane. Some rats in this study were observed to float before swimming toward the platform location, although formal quantification of this was not performed.

In the current study, it could be argued that superior performance in the 1-week acquisition trials gives sevoflurane-anesthetized rats an advantage in the following 4- and 12-week acquisition sessions, and the improvement observed at these times may not represent a permanent neurologic change. Whether the memory-enhancing effects of sevoflurane truly represent permanent neurologic and cognitive change requires further study.

Although volatile anesthetics have been reported to improve cognition, there are as many reports of impaired cognition. Experimental studies where sevoflurane at doses from 0.5 to 2.6% has been administered either during or immediately after a learning task have shown inhibition of memory retention in experimental animals.[18–21,40] Low-dose sevoflurane (0.11%)

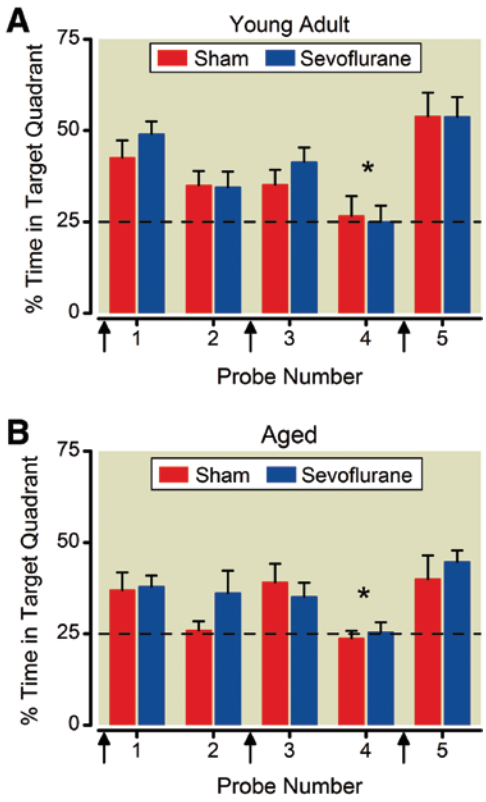

Fig. 6. Probe trials over 3 months showing % time spent in target quadrant by rats exposed to sevoflurane or sham exposure in (*A*) young rats and (*B*) aged rats. *Arrows* on x-axis indicate time of acquisition testing relative to probe trials. Both young and aged rats were unable to remember the target quadrant on probe trial number 4 performing at chance level only (*$P < 0.05$ compared with performance on other probe trials, ANOVA). Data are mean and SEM. The *dotted line* represents 25% or chance performance. Weeks 1, 4, and 12: n = 10 rats per treatment group for young adult rats. Aged rats: week 1, n = 7; week 4, n = 7 sham, n = 6 sevoflurane; week 12, n = 6 sham and n = 4 sevoflurane-treated.

administered during single-trial inhibitory avoidance training enhanced 24-h memory retention in rats. Taken together, these data suggest a dose-dependent effect of anesthesia on memory with low, subanesthetic doses enhancing and higher doses inhibiting memory. *In vitro* studies support a dose-response relationship showing subanesthetic concentrations of sevoflurane enhance postsynaptic excitatory transmission and long-term potentiation in the hippocampal CA1 region, whereas at anesthetic concentrations presynaptic inhibition occurs.[38,41] Results of studies in mouse brain slices suggest that the margin between enhancement and inhibition may be quite narrow.[38] Although these studies offer some insight into the possible mechanisms of action of anesthetics during and shortly after exposure, POCD arise many days after anesthetic exposure when pharmacokinetics predict the anesthetic is no longer in the body. It is unknown how in the current study sevoflurane exposure 7 days before a new learning paradigm could enhance subsequent memory performance.

Some limitations of this study warrant discussion. First, the young and aged rat studies were conducted at different times due to the lag time in obtaining aged rats and therefore cannot be statistically compared. Second, there were a large number of deaths of aged rats over the 13-week period of testing resulting in small group size by the 12-week water maze test. There were more spontaneous deaths and euthanized rats in the sevoflurane group (n = 4) than the sham group (n = 1) however, the variety of causes and time of death after treatment makes attributing this to a treatment effect unlikely. Sprague-Dawley rats are reaching the end of their expected life span by 24 months and hence deaths are not unusual. Increased mortality has been linked to POCD[42] but not to a specific anesthetic agent. There is no evidence to suggest that sevoflurane may cause increased mortality. However, in view of the small sample size in aged rats at 12 weeks and the loss of statistical power, we urge caution in interpretation of our data at this time point in aged rats.

The concentrations of sevoflurane used in our experiments were at the lower 95% CIs for both young and aged rats. This was due to the greater stimulus (tail clamp) for the published MAC determination method compared with toe pinch used in our experiments. Furthermore, it is to be expected that the concentration required in our experiments would be at the lower range of MAC, as there was no surgical stimulus. Our data confirmed that MAC is lower in aged rats compared with young rats. Importantly, both young and aged rats were at the lower level of MAC determined in our study, and we are confident that they received an equivalent dose exposure. Our MAC values for sevoflurane were comparable with previously published values of 2.90% (2.79–3.00)[22] and 2.68 ± 0.19% for young and 2.29 ± 0.19 for older rats.[43]

Some of the rats in this study were singly housed during the course of testing due to death or euthanasia of a cagemate. Rats are social animals and single housing (isolation) can affect behavioral outcome and indeed has been reported to enhance spatial memory in the water maze.[44] However, isolation studies have examined isolation from weaning, not brief isolation periods in aged rats. As single-housed rats accounted for only three rats (n = 1 sham and n = 2 sevoflurane-treated), it is unlikely that single housing affected the outcome of these experiments.

Sham rats were exposed to only 10 min of 100% O_2 in contrast with the treated rats receiving sevoflurane in 100% O_2 for 4 h. Hyperoxia has been reported both to trigger cognitive impairment[45] and to improve cognitive performance in Alzheimer transgenic mice.[46] However, in both studies hyperoxia had no effect in nontransgenic or wild-type mice. We are not aware of any evidence to suggest that the different oxygen exposure between sham and sevoflurane-treated rats could account for the current findings.

In conclusion, the current study has demonstrated that 4 h of sevoflurane exposure does not impair acquisition learning or retention memory in young adult or aged rats

in the Morris water maze test. We have previously found under identical conditions that 1 MAC isoflurane exposure for 4 h results in a retention memory deficit in young adult rats but not in middle-aged rats.[11] Taken together with the current results, our findings may suggest a differential effect of volatile anesthetics on cognitive outcome. This hypothesis requires further investigation.

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