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The gender difference in effect of sevoflurane exposure on cognitive function and hippocampus neuronal apoptosis in rats

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Abstract. – OBJECTIVE: Anesthesia and surgery can induce postoperative cognitive dysfunction. Ser-133 phosphorylation sites of cAMP-response element binding protein (CREB) is a key gene that mediate a variety of downstream transcription initiation factors, regulate neuronal survival and promote the expression of a large number of genes. Thus, CREB may play a role in this impairment. We hypothesize that and sevoflurane-induced cognitive impairment possibly via inhibiting the expression of CREB downstream genes and proteins.

MATERIALS AND METHODS: To test this hypothesis, adult Sprague-Dawley rats were subjected to sevoflurane exposure and were tested with a series of behavioral experiments (open field, passive avoidance test and Morris water maze test) at different time (1 d to 95 d). Besides, blood gas changes and expiratory sevoflurane concentrations were examined at 2 h; the levels of phosphorylated CREB 1, the protein Bcl-2, Caspase-8 and Caspase-3 were assessed at 1 week and 3 months after anesthesia. We also conducted a comparison in sevoflurane-induced cognitive impairment between male and female rats.

RESULTS AND CONCLUSIONS: Here, we found that sevoflurane anesthesia can impair short-term cognitive function, which may be via down-regulating p-CREB1 and Bcl-2 expression and up-regulating Caspase-8 expression to reduce hippocampus neuronal apoptosis, and male rats suffered a more severe cognitive dysfunction than female rats. In addition, sevoflurane can produce a reversible long-term cognitive dysfunction in rats.

Key Words:
Cognitive dysfunction, Sevoflurane, cAMP-response element binding protein (CREB).

Introduction

Postoperative cognitive dysfunction (POCD) after anesthesia and surgery is the major nervous system complication that leads to impairment of learning and memory and loss of language comprehension[1-3]. Moreover, there will be personality and social capacity decline in anxiety and other serious cases. Hence, anesthesia and surgery are important factors that contribute to postoperative cognitive function[4,5]. Currently, whether inhalation anesthetics harm learning and memory remains inconclusive. Rammes et al[6] found that isoflurane can increase the expression of N-methyl-D-aspartic acid receptor subtype NR2B, enhance hippocampus long-term potentiation and improve cognitive function in adult mice[7]. However, Satomoto et al[8,9] suggested that sevoflurane anesthesia may lead to the occurrence of early cognitive dysfunction, but this result limited to one week after anesthesia. Studies assessing sevoflurane for long-term cognitive function are still rare.

N-methyl D-aspartate (NMDA) receptors play a crucial role in hippocampal synaptic function,thus modulating learning and memory[10-12]. Sevoflurane develops anesthesia and produces amnesia by inhibiting the GABA and NMDA receptors, so its effects on cognitive function may be related to inhibited NMDA receptors in hippocampus and the prefrontal cortex[13-15]. Ser-133 phosphorylation sites of cAMP-response element binding protein (CREB) is a key gene that promote the expression of a large number of genes, mediate a variety of downstream transcription initiation factors and regulate neuronal survival[16,17]. NMDA receptors play their role mainly through PKA-CREB signal pathway and present the neurotrophic effect with regulating central nervous system development, synaptic plasticity and synaptic release of neurotransmitters in physiological conditions[18-20]. Therefore, we hypothesized that CREB participated in the sevoflurane-induced cognitive

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dysfunction regardless of the length of time, and sevoflurane induces cognitive impairment possibly via inhibiting the expression of CREB downstream genes and proteins. In the present study, we subjected rats to sevoflurane exposure to identify the link between sevoflurane and cognitive dysfunction and the possible mechanisms associated with CREB.

Materials and Methods

Rat Anesthesia

Male (380-440 g) and female (180-220 g) adult Sprague-Dawley rats were used in all experiments. The rats were allowed free access to food and water before and after sevoflurane anesthesia. With approval from the Institutional Animal Care and Use Committee of the University of Soochow, Jiangsu Province, China, rats were anesthetized in groups of 14 using sevoflurane. Each group contained one cardiorespiratory control animal (total $n = 5$), which had a 24-g polyethylene catheter inserted into the tail artery after induction of general anesthesia for 2 h blood gas analysis. The cardiorespiratory control rats were not used for any other part of the study. Rats were placed in a preheated, humidified anesthetic chamber primed with 75% of oxygen containing 3.0% of sevoflurane. The chamber was part of a semi-closed anesthetic circuit incorporating a fan recirculating waste gas *via* a carbon dioxide–absorbing canister filled with soda lime and a humidifier back into the anesthetic chamber. Fresh gas flow was 4 L/min. The anesthetic concentration was titrated to 1.2 minimum alveolar concentration (MAC), and complete equilibration of inspired and brain anesthetic concentrations more than 12 min were anesthetized. The anesthetic pH, arterial hemoglobin oxygen saturation and carbon dioxide tension were analyzed by a blood gas analyzer (ABL 520, Radiometer, Copenhagen, Denmark). Blood (0.25 ml) was collected at 2 h from the tail cannula of the designated homeostatic control rat. The tail cannula was flushed with lactated Ringer's solution. After 2 h of sevoflurane anesthesia and complete recovery, rats were returned to their home cages.

Sham Anesthesia

Control-treatment rats ($n = 14$) were placed in the warmed, humidified anesthesia box insufflated with oxygen at a fraction of inspired oxygen of 75% without anesthetic gas. After 2 h, rats were returned to their home cages.

Open Field

Spontaneous locomotor activity in an open field was measured in an automated Flex-Field/Open Field Activity System (DigBehv-LR4 System, JiLiang Software Technology Co. Ltd, Shanghai, China). Mice were placed in one of four identical clear plastic chambers (40 cm × 40 cm × 65 cm) for 20 min, with four Open Field arrays detecting total locomotor activity (distance traveled in cm) and center of locomotor activity in each of the four open field zones. A video camera mounted 100 cm above the arena was used to collect the data that were analyzed by JLDigBehv-LR4 System software (Jiliang Software Technology Co. Ltd, Shanghai, China). The apparatus was thoroughly cleaned with 70% ethanol between trials.

Passive Avoidance Test

The inhibitory avoidance chamber was consisted of a rectangular-shaped perspex box, divided into a safe compartment and a shock compartment by an automatically operated sliding door (DigBehv-STR System, Jiliang software technology Co. Ltd, Shanghai, China). The safe compartment was white and illuminated, and the shock compartment was dark and made of black perspex. Foot shocks were delivered to the grid floor of the shock compartment via a constant current scrambler circuit. A single animal was placed in the light compartment, and learned to avoid the adjacent dark compartment that was previously associated with a mild foot shock. The animals randomly chosen for the training were handled daily for 3 days (5 min/day) before starting the experiments. In the training session, the animals were placed in the lighted compartment and allowed to explore for 10 s. The sliding door was then opened, and the step-through latency for animals to enter the dark compartment was measured. As soon as the animals entered the dark compartment, an inescapable foot-shock (0.5 mA for 3 s) was delivered through the grid floor with a constant current shock generator (DigBehv-STR System, Jiliang software technology Co. Ltd, Shanghai, China). Entering the dark compartment within 300 s by all of the animals was determined as cut-off latency in the training session and received a foot-shock. The

test session was performed at 24 h after the training session using the same paradigm, but without the foot-shock, and the step-through latency for animals to enter the dark compartment was measured. When an animal did not enter the dark room within 300 s, the step-through latency was recorded as 300 s.

Morris Water Maze

A *post hoc* experiment was performed to exclude motor impairment as a cause of altered post-anesthetic performance. Respectively, male and female rats were randomly assigned to a control or anesthesia group (n =14 per group) as described previously, and swim speed was tested in a Morris water maze for 4 days beginning at 48 h after anesthesia. The maze consists of a circular 180-cm tank filled with water heated to 24°C. The object is for the rat to locate a plexiglass escape platform that extends from the floor of the tank to approximately 1.5 cm below the surface of the water. Black curtains affixed with four high-contrast black and white visual cues positioned at the north, east, south, and west sides surrounded the tank. Activity is taped using a small video camera located above the center of the maze and connected to a tracking system and videocassette recorder. Each animal's swim speed was collected using DigBehv-MG Water for Windows (DigBehv-MG, Jiliang software technology Co. Ltd, Shanghai, China). For each trial, the rat was placed in the pool along the perimeter of the tank, facing the tank wall, with the four entry points (north, east, south, and west) randomized across trials, but the same sequence of start points was used for each rat. The trial ended when the rat located the escape platform or when 60 s had elapsed, at which time the rat was guided to the platform. In either case, the rat was allowed to remain on the platform for 20 s before being returned to a holding cage for a 1-min interatrial interval. The average escape latency, swimming distance and swim speed (mm/s) per day was recorded and analyzed.

Preparation of Protein Extracts

After the Morris water maze test at 7 days, 3 rats in each group were sacrificed. And the remaining rats were decapitated after completing the morris water maze test at 95 days. Brain hippocampus tissues of each mouse were dissected out, weighed, and snap-frozen in liquid nitrogen. Subsequently, the samples were minced and homogenized in cytoplasmic and nuclear protein extraction (K0311, Thermo Scientific, Rockford, IL, USA) and protease inhibitors cocktail with ultrasound cracking for 3 times and each time lasted for 10 s, the homogenate was centrifuged at 15,000 g for 30 min at 4°C. The supernatant solutions were separated and protein concentration was normalized using Coomassie brilliant blue G-250 staining. All samples were kept immediately at –80°C until they were further used. The amount of protein in each sample was measured with the assistance of a protein assay kit (BCA; Pierce, Rockford, IL, USA).

Western Blot Analysis

Western blot assay was conducted to determine expression of the caspase-3, caspase-8, bcl-2 and phosphorylation of CREB1 in the hippocampus. Equal amounts of proteins were loaded onto the gel; following electrophoresis, the proteins were transferred to polyvinylidene fluoride (PVDF) membrane. After blocking with 0.1% Tris-HCl buffer solution and Tween (TBST) containing 5% non-fat milk at room temperature (RT) for 2 h, primary antibody was added for overnight incubation. The membrane was washed with 0.1% TBST three times, 10 minutes each, and incubated with secondary antibody at RT for 1 h. The membrane was then washed as above, and color development was performed using the ECL kit. Images were acquired using the Fuji Digital Science Imager (Fuji Co., Tokyo, Japan) and analyzed with ImageJ Launcher Broken Symmetry Software to measure the integrated optimal density (IOD) values of specific bands.

Statistical Analysis

To reduce variance from different rats, we averaged the data from all rats through training before anesthesia and excluding inactive rats and rats unable to swim. Statistical analysis was performed using SAS 8.2 software (SAS Institute, Cary, NC, USA). Results of blood gas analysis of the indicators were analyzed using Student's t-test. Data from western blotting and other behavioral studies were analyzed using Student's t-test for comparison of two groups or by ANOVA followed by Fisher's post hoc multiple comparison tests for those with more than two groups. In all experiments, differences were considered statistically significant at a $p < 0.05$.

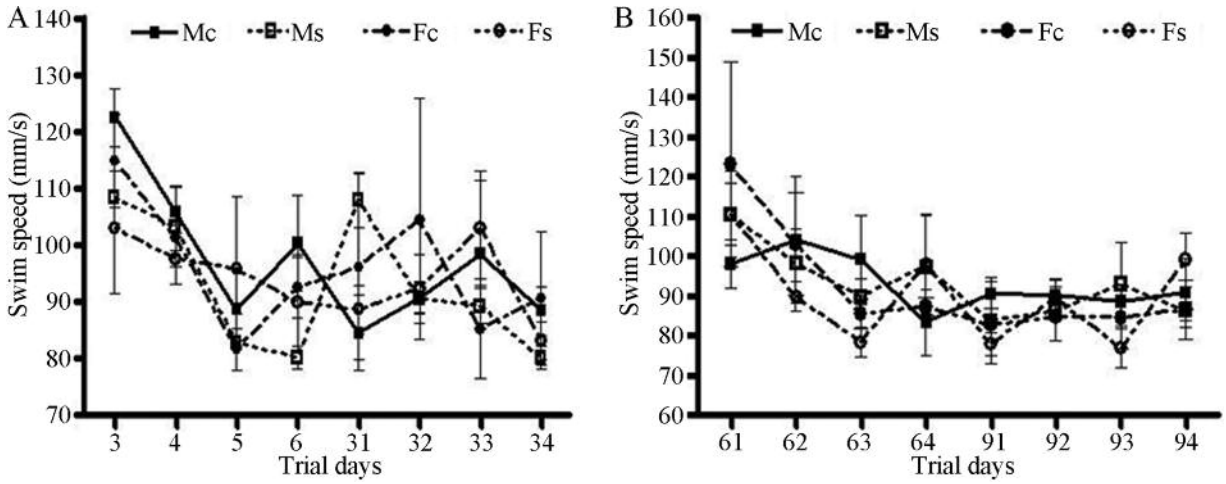

Figure 1. Swim speed. Swim speed was tested in a Morris water maze, and the average speed per trial day (8 times) was analyzed using repeated-measures ANOVA, with anesthesia group and age as between-subject factors and day of testing as the within-subject factor. However, previous anesthesia and sex factors had no effect on swim speed ($p > 0.05$), indicating that locomotion was intact in previously anesthetized rats. Data are expressed as mean SEM.

Results

Comparison of Blood Gas Changes and Expiratory Sevoflurane Concentrations Between 3% Sevoflurane Treatment and Control-Treated Groups

In the pilot study, when sevoflurane concentration was maintained at 3% for 2 h, there were no significant changes in any of the blood gas variables as compared to the controls (Table I). And rats were with red lips and toes and smooth respiratory during anesthesia. Therefore, 3% sevoflurane for 2 h was used in the subsequent formal study. There was no significant difference among the different groups in the number of trials, thus confirming the uniformity of the groups. All the animals reached the criteria during the training procedure.

Open Field Test to Estimate Effect of Sevoflurane on Locomotor Activities

We observed sevoflurane's effect on the locomotor activities through open field test, and we found that there was a significant decrease in the total distance and average speed in Fs group at 1 day after administration, compared with Fc group ($p < 0.05$) (Table II); Nonetheless, when compared with Mc group at 1 day after administration, there were no dramatic differences in Ms group at 30, 60, 90 days in the total distance and average speed. Also, we found no significant changes in the central distance in each day, which indicated that sevoflurane affected short-term locomotor activities in rats rather than long-term locomotor activities.

Passive Avoidance Test to Assess Effect of Sevoflurance on Non-Spatial Memory

Passive avoidance test was used to analyze non-spatial memory. Sevoflurane, at a concentration of 3%, decreased the non-spatial memory retrieval in passive avoidance paradigm at one week; however, this effect was not significant. Moreover, neither escape latency nor number of errors in each time duration after the anesthesia showed a statistically significant difference in rats subjected to sevoflurane exposure while compared to the control group, indicating that 3% sevoflurane anesthesia failed to affect non-spatial memory in rats.

Morris Water Maze Test to Identify the Effect of Sevoflurane on Spatial Learning and Memory

Water maze test is routinely conducted to evaluate the short-term and long-tern memory and

Table I. Effect of sevoflurane on rats blood gas analysis of the indicators at 2 h.

	pH	$PaCO_2$	PaO_2
Control-treated	7.34 ± 0.01	38 ± 3.74	96.75 ± 6.65
3% Sevo	7.35 ± 0.02	45 ± 1.30	93.00 ± 7.71

Values are mean SD. N = 4 for each group. Sevo = sevoflurane.

Table II. Effect of sevoflurane on rats locomotor function in open field test.

Index	Groups	1 d	30 d	60 d	90 d
Total distance (mm)	Fc	30383 ± 9098	21033 ± 5600	24909 ± 10729	20464 ± 9888
	Fs	21629 ± 9380*	22936 ± 12122	20469 ± 10209	18765 ± 12422
	Mc	20066 ± 7481	14759 ± 5073	14496 ± 5697	12441 ± 5664
	Ms	12983 ± 5993#	15079 ± 5460	16586 ± 8753	15381 ± 7208
Average speed (mm/s)	Fc	25 ± 7	18 ± 5	21 ± 9	17 ± 8
	Fs	18 ± 7*	19 ± 10	17 ± 8	16 ± 10
	Mc	16 ± 6	12± 4	12 ± 4	10 ± 5
	Ms	11 ± 5#	13 ± 5	14 ± 7	13± 6
Central distance (mm)	Fc	8287 ± 3450	4358 ± 2358	5727 ± 4348	4780 ± 3552
	Fs	4470 ± 2624	3919 ± 2376	4300 ± 2932	2603 ± 2319
	Mc	4659 ± 4480	2974 ± 3412	2494 ± 2341	2110 ± 1983
	Ms	2514 ± 2536	2527 ± 2343	2970 ± 2787	2058 ± 1548

Data were shown as mean ± SD, compared with Fc, $^{*}p < 0.05$, compared with Mc, $^{\#}p < 0.05$.

spatial learning functions of animals by measuring the escape latency duration and quadrant dwell time. We examined the effect of sevoflurane exposure on the memory and learning ability in post trained rats at different duration. After an administration of sevoflurane at concentration of 3% for 2 h post to training daily for 5 days morris water maze, SD rats were subjected to 3 monthly water maze tests after sevoflurane anesthesia, and they displayed a significant deficit in learning and memory in the first one week and one months (Figure 2A) ($p < 0.05$). However, after two months, as the escape latency period became shorter due to repeated training, the sevoflurane group started to exhibit no significantly longer latency than the control-treated group (Figure 2B). We also found that, compared with Fs group, Ms group had a longer escape latency and swimming distance in 4 to 6 days and at 34 ds after administration ($p < 0.05$, Figure 2A). Meanwhile, male rats prominently differ from female rats in swim speed (Figure 1). These results revealed a clear gender difference in the morris water maze test. Nevertheless, we failed to find any remarkable changes in the percentage of time at quadrant II and the number of times across the visible platform area which removed in Ms group when compared with Fs group ($p > 0.05$). These observations suggested a significant impairment and a gender difference in short-term learning and memory functions, as well as a reversible impairment in long-term learning and memory under the sevoflurane of 3.0% concentration condition.

Sevoflurane Exposure Decreased Phosphorylated cAMP Response Element Binding Protein 1 (P-CREB1) Activation

CREB is an important transcription factor that mediates nuclear responses underlying cognitive function and plasticity of the nervous system. We reasoned that sevoflurane anesthesia could decrease activated P-CREB1, and then performed western blot experiments to assess P-CREB1 activation after being anesthetized with sevoflurane. It was demonstrated that phospho-CREB1 expression was significantly decreased compared to the control group ($p < 0.05$), and marked increase in the same side of hippocampus female-anesthetized rats than male-anesthetized rats was shown (Figure 3A, C), suggesting that an exposure of sevoflurane with 3.0% concentration inhibited phospho-CREB1 expression at one week. Whereas, no distinct change of phospho-CREB1 expression in rats hippocampus was observed at three months after treated with anesthesia (Figure 3B, D).

Sevoflurane Exposure Decreased Bcl-2 and Increased Caspase-8 and Caspase-3 Expression

In order to explore the mechanisms involved in sevoflurane-induced hippocampus cell apoptosis, we investigated the protein Bcl-2, Caspase-8 and Caspase-3 levels using western blots. Administration of 3.0% sevoflurane for 2 h significantly increased Caspase-8 (Figure 4A, C) and Caspase-3 (Figure 5A, C) levels, decreased Bcl-2 levels (Figure 6A, C) compared to controls ($p < 0.05$). We found that equipotent dose of sevoflu-

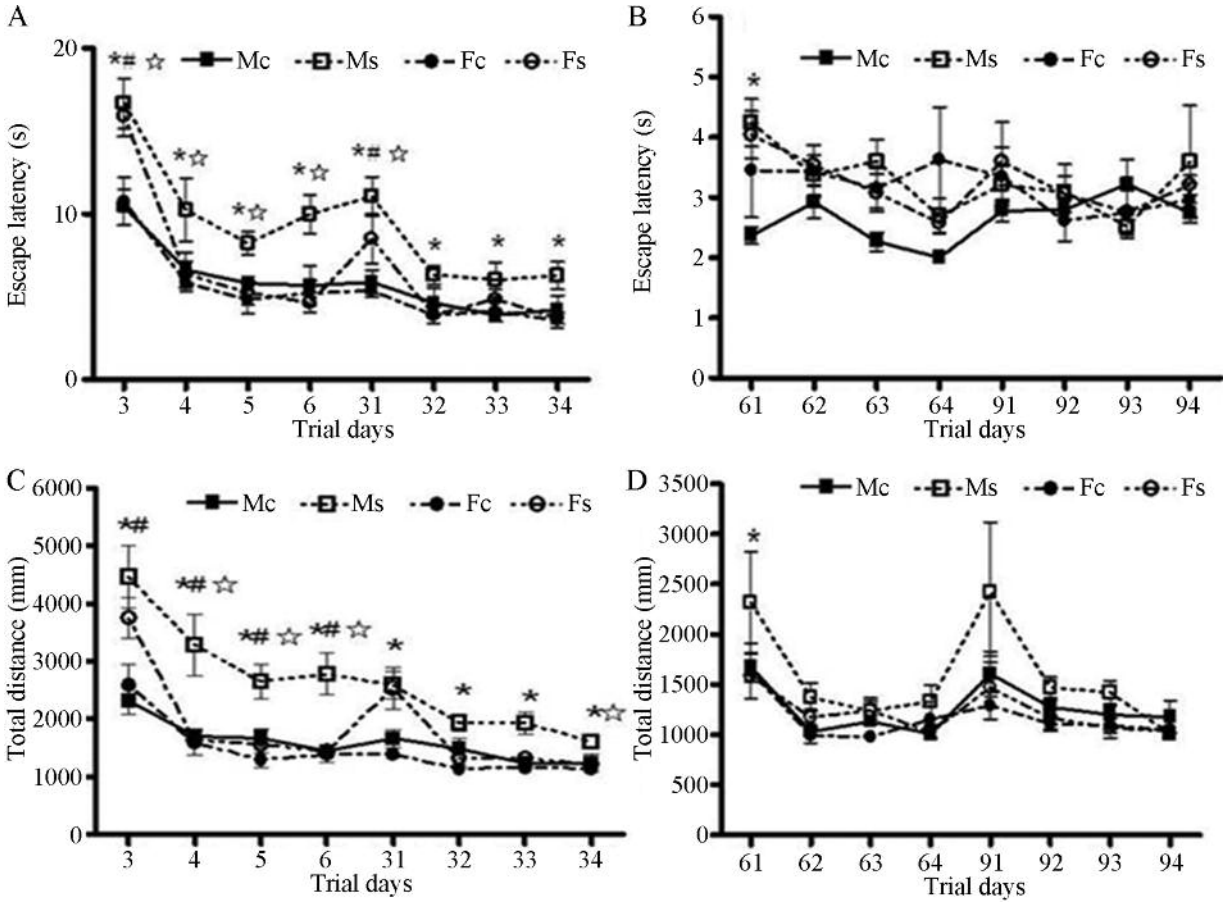

Figure 2. Effect of sevoflurane on spatial learning memory in acquisition phase in the Morris water maze. The escape latency (the time required for rats to locate the platform) and total distance for the three months are expressed as mean ± S.E.M. of 10-14 animals. ***A-B,*** $^{*}p < 0.05$, Ms group escape latency prolonged compared to Mc group, $^{\#}p < 0.05$, Fs group escape latency prolonged compared to Fc group, $^{\star}p < 0.05$, Ms group escape latency prolonged compared to male Fs group; ***C-D***, $^{*}p < 0.05$, Ms group total distance increased compared to Mc group, $^{\#}p < 0.05$, Fs group total distance increased compared to Fc group, $^{\star}p < 0.05$, Ms group total distance increased compared to male Fs group.

rane (3.0%) yield significantly sharper cuts in the Bcl-2 level in male-anesthetized rats than female-anesthetized rats (Figure 6A, C). Although there were increased Caspase-8 and Caspase-3 levels, they did not reach statistical significance. These findings illustrated that the impairment of learning and memory after anesthesia with sevoflurane was associated with apoptosis by caspases and bcl-2. Moreover, we detected that the samples from rats' hippocampus almost had equal expressions of the proteins after treated with anesthesia at three months.

Discussion

In our previous study, we found that inhaled sevoflurane could result in cognitive impairment in both old and young rats. But studies on sevoflurane's effects on adult rats are limited. In this study, we provide the molecular evidence in terms of expression and function of the phosphorylation of CREB in adult rats exposed to sevoflurane, which are likely to be responsible for the sevoflurane-induced short-term and long-term cognitive dysfunction. In this study, the pre-study results show that the arterial blood gas pH, PaO_2 and $PaCO_2$ were within the normal range after a administration of inhaled 3% sevoflurane at 2 h, thus, excluding the interference effect of hypoxia on study results during sevoflurane anesthesia. The minimum alveolar concentration (MAC) of sevoflurane in adult rats is 2.5%, and in clinical surgery a required minimum effective alveolar concentration of 1.2 MAC-1.3 MAC is commonly applied, so we choose 1.2 MAC in the present study.

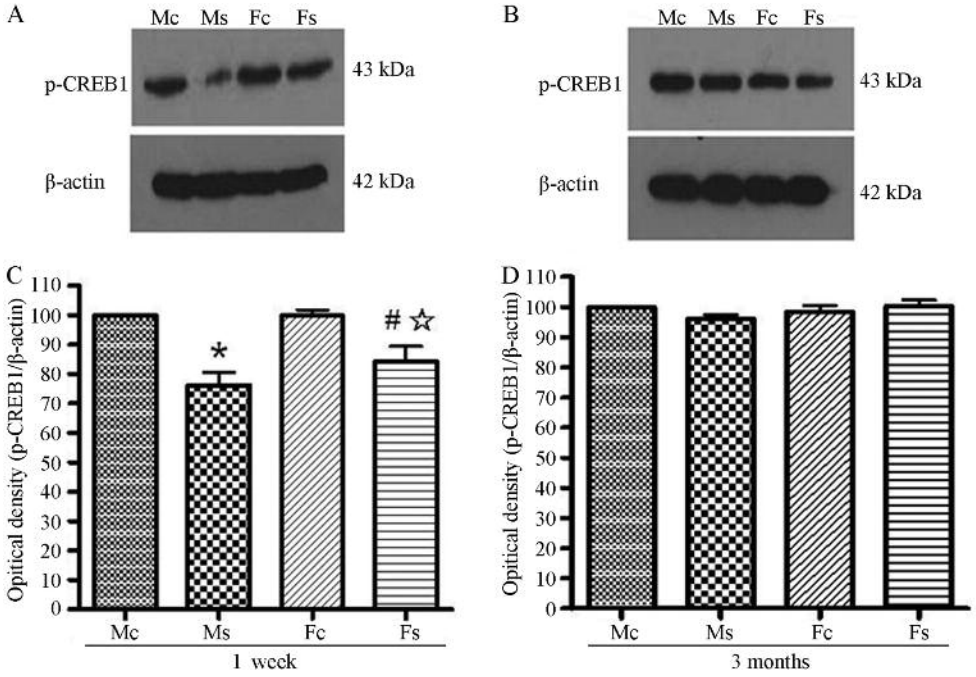

Figure 3. Expression of P-CREB1 after anesthesia exposed or control-treated at 1 week and 3 months. Data are expressed as mean SEM. Hippocampal protein at 1 week and 3 months after sevoflurane anesthesia was analyzed by western blotting for changes in hosphor-CREB1. Levels of hosphor-CREB1 were significantly decreased in male and female anesthetized rats and increased in female-anesthetized rats compared to male-anesthetized rats at 1 week ***A-C***, but no differences showed at 3 months ***(B-D)***. Values are expressed as mean SD, $^{*}p < 0.05$, male-anesthetized rats (Ms) compared to male control-treated rats (Mc), $^{\#}p < 0.05$, female-anesthetized rats (Fs) compared to female control-treated rats (Fc). ☆$p < 0.05$, female-anesthetized rats (Fs) compared to male-anesthetized rats (Ms).

Spontaneous activity is mainly observed in the natural state of animals under the new environment of their own behavior, exploratory behavior, and anxiety, it's widely used to evaluate the current drugs on the body and the spirit of the nervous system effects (such as excitatory, inhibitory)[21]. We found that both female and male rats' performance in the total distance and average speed were significantly reduced and slowed down after sevoflurane anesthesia 1 d, however, at 30 d, 60 d, and 90 d, differences were not statistically significant. The distance differences of central area time failed to show a statistical difference, which indicated that sevoflurane anesthesia in rats inhibited short-term spontaneous activity and led to depression, but didn't affect rats in their space exploration behavior.

We did not detect any changes in non-spatial memory with passive avoidance test by changing the environment to form a conditioned reflex to assess the non-spatial memory. The results suggested that sevoflurane anesthesia in all rats had no effect on non-spatial memory. In recent years, Morris water maze test is conducted as a scientific method in neuroscience research and provides important visual behavioral information, which can reflect the learning and spatial memory[22,23]. In this study, female and male rats in the anesthesia group failed to show a significant difference in swim speed and total distance to find platform compared with control group, therefore, we can exclude motor dysfunction after sevoflurane anesthesia in rats. Whereas, a single inhalation of 3.0% sevoflurane, in the Morris water maze test, can lead to learning and memory loss in rats during one week, and male rats in the test showed a greater increase in the escape latency and the total swimming distance than female rats, demonstrating that male rats suffered a more severe learning and memory impairment caused by sevoflurane than female rats. But anesthesia had no effect on both female and male rats at three months. The results suggested that sevoflurane not only can lead to short-term cognitive dysfunction in rats, but also can lead to a reversible long-term cognitive dysfunction. In addition, learning memory impairment in adult rats displayed a gender-dependent property.

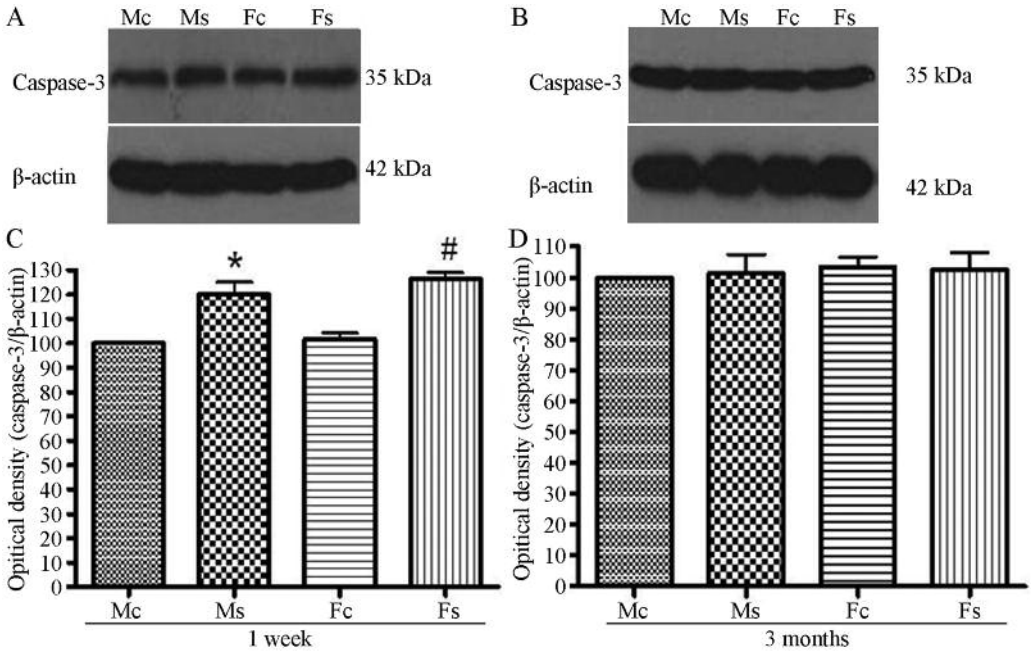

Figure 4. Expression of Caspase-8 after anesthesia exposed or control-treated at 1 week and 3 months. Data are expressed as mean SEM. The results are presented as mean SD. ***A-C***, Hippocampal protein caspase-8 at 1 week; we found that caspase-8 increased in sevoflurane exposed rats. $^{*}p < 0.05$, Ms group compared to Mc group, $^{\#}p < 0.05$, Fs group compared to Fc group. ***B-D***, Caspase-8 level showed no significant difference in hippocampus at 3 months.

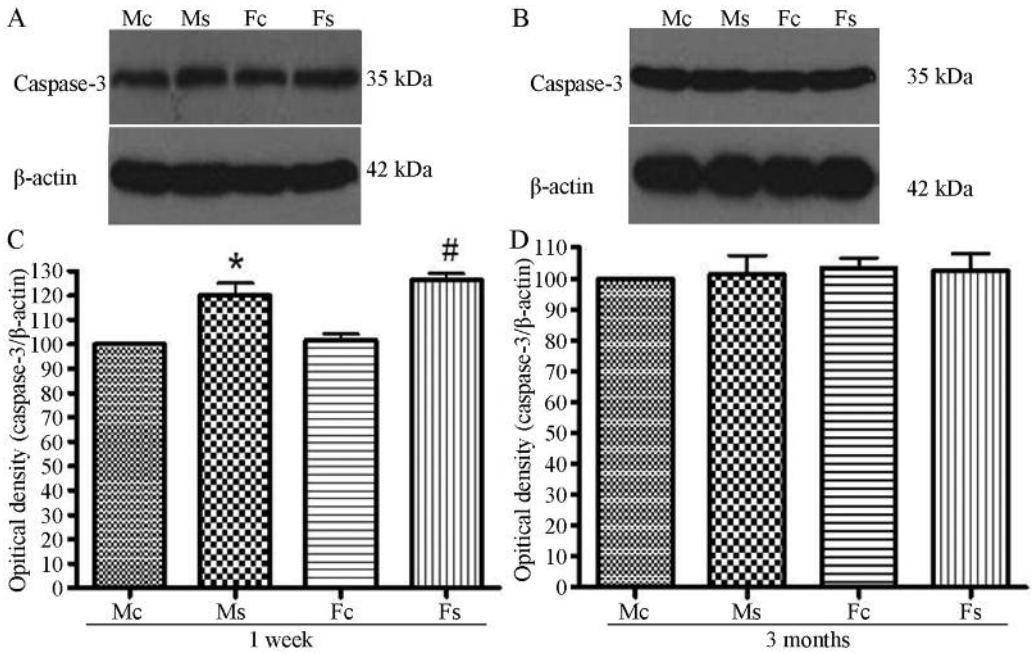

Figure 5. Expression of Caspase-3 after anesthesia exposed or control-treated at 1 week and 3 months. Data are expressed as mean SEM. Caspase-3, which is the caspase family member and the downstream effector of caspase-8 leading to apoptosis. ***A-C,*** caspase-3 increased in sevoflurane exposed rats in male and female. $^{*}p < 0.05$, Ms group compared to Mc group, $^{\#p} < 0.05$, Fs group compared to Fc group. ***B-D***, Caspase-3 level showed no significant difference in hippocampus at 3 months.

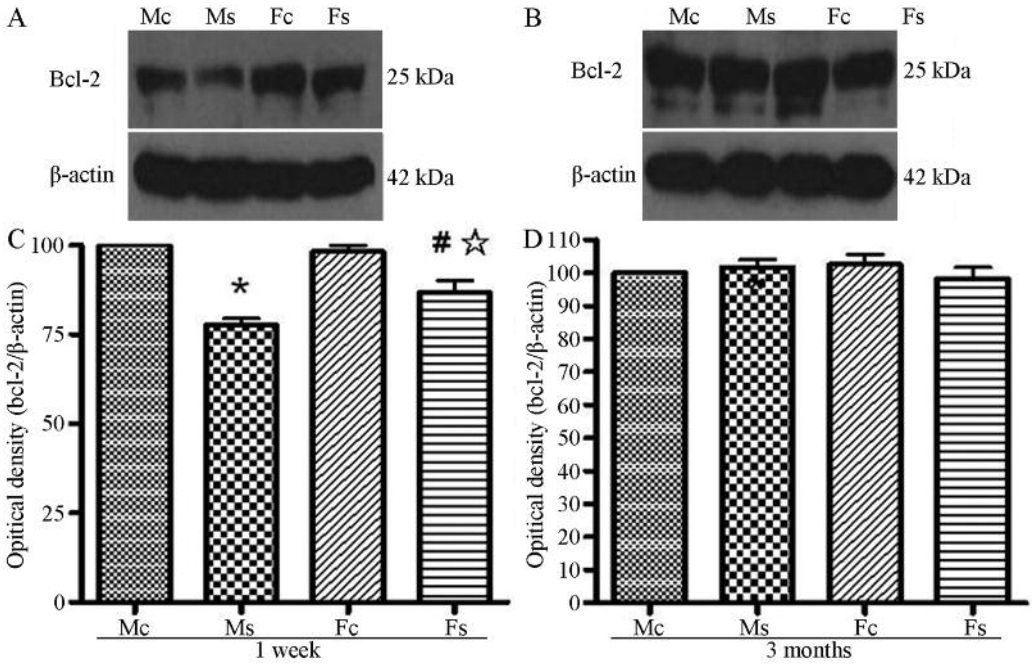

Figure 6. Expression of Bcl-2 after anesthesia exposed or control-treated at 1 week and 3 months. Data are expressed as mean SEM. ***A,*** at 1 week after sevoflurane exposure, Bcl-2 protein expression increased in rats hippocampus in both male and female rats, and Bcl-2 expression increased more in femalethan male anesthesia rats. ***C,*** $^{*}p < 0.05$, Ms group compared to Mc group, $^{\#}p < 0.05$, Fs group compared to Fc group, $^{\star}p < 0.05$, Fs group compared to male Ms group. ***B-D,*** Bcl-2 level showed no significant differences in hippocampus at 3 months.

NMDA receptors and GABA receptors are both important targets of sevoflurane anesthesia to produce amnesia. When the NMDA receptors are excitatory, the Na^+, K^+ permeability of cell membrane increase, then fast excitatory postsynaptic potentials are generated, causing a postsynaptic membrane epolarization and opening of Ca^{2+} channels. The influx of Ca^{2+} can combine with calcium/calmodulin-dependent protein kinase (CaMK), activate the CaMK, CaMK activation of adenylyl cyclase and result in the ATP decomposition to cyclic adenosine monophosphate (cAMP), thus causing increased cAMP level in hippocampal neuronswhich leads to C subunit of PKA (catalytic subunit) and R subunit (regulatory subunit) dissociation. The following phosphorylation of PKA and entrance of C-subunit into the nucleus can activate CREB phosphorylation. When the CREB is phosphorylated, it forms the dimer binding to the CRE of the target gene sequence to promote transcription of CRE sequence and regulates downstream gene transcription to promote the production of neurotrophic factor (BDNF) and the expression of growth factors such as tPA, as well as other functional proteins, to participate in long-term formation of synaptic plasticity and modulate the intrinsic excitability of neurons. By the aforementioned pathway, the NMDA receptors can promote and sustain learning and memory formation[24].

As we know, the CREB gene is with a variety of splicing patterns and it can be affected by the stability of mRNA. Moreover, post-translational modification differences and other impacting factors could lead to the production of a number of different spliceosome in cell, such as CREB1, CREB2[16,17]. And CREB1 is positively correlation with learning memory function. Especially, Ser-133 phosphorylation of CREB1 sites is of vital importance[25].

The cAMP/PK-CREB signaling pathway plays a crucial role in short-term and long-term synaptic plasticity and learning memory formation[17]. Studies have shown that sevoflurane may inhibit the activity of PKA, and suppress NMDA receptors or GABA receptors, thereby, affecting nuclear CREB phosphorylation and binding to CRE, and regulating its downstream gene products as pro-apoptotic protein Caspase-8 and anti-apoptotic protein Bcl-2, etc. In this way, sevoflurane can inhibite hippocampal

neurogenesis and promote apoptosis of hippocampal neurons, hence cause cognitive dysfunction[16,25]. The results of behavior tests showed that sevoflurane anesthesia impaired learning and memory, and western blot found a marked reduction of phosphorylated CREB1 protein expression and anti-apoptotic protein Bcl-2 expression, but an increase expression of pro-apoptotic protein Caspase-8 and Caspase-3after being administered with sevoflurane. However, we did not find any change in learning memory and phosphorylated CREB1, Caspase-8, caspase-3 and Bcl-2 expression at 3 months after being anesthetized. These results suggested that sevoflurane anesthesia might play its role via inhibiting CREB1 phosphorylation, enhancing expression of pro-apoptotic protein Caspase-8 and Caspase-3 and reducing anti-apoptotic protein Bcl-2 expression which promotes apoptosis in hippocampal neurons and cause cognitive dysfunction in rats.

The present study shows that male rats had a comparatively serious cognitive impairment than female rats at one week after sevoflurane anesthesia, but no discrepancy was detected between the two groups at three months. According to the recent work, we know that estrogen can enhance learning and memory. Besides, studies[26] have shown that estradiol has a significantly protective effect on oxidative stress-induced neuronal apoptosis, and it can reduce excessive oxygen radicals caused by sevoflurane anesthesia in neuronal injury, while increase cerebral blood flow in rats which plays a neuroprotective action in isoflurane anesthesia[27,28]. So, we assume that the negative damage effects of sevoflurane on cognition are related to their ability to affect oxygen radicals and cerebral blood flow. Estrogen acting as a selective modulator may have a direct role in nuclear transcription sites (CREB and AP1) to promote transcription and combination with cell membrane receptors to regulate transcription promote the expression of downstream genes and enhance cognitive function. And we found that there was an increase of bcl-2 and a smaller inhibition of phosphorylated CREB1 in female anesthetized compared with male anesthetized. These results are similar to Luine, which support the conclusion that gender difference in sevoflurane anesthesia on cognitive function may be due to estrogen which enhance CREB1 protein phosphorylation and activate Bcl-2 protein expression, finally lessen hippocampal neuronal apoptosis.

Conclusions

Sevoflurane anesthesia can impair short-term cognitive function, which may be via down-regulating p-CREB1 and Bcl-2 expression and up-regulating Caspase-8 expression to reduce hippocampus neuronal apoptosis. This pernicious role played by sevoflurane is much bigger in male than in female rats. In addition, sevoflurane can produce a reversible long-term cognitive dysfunction, which may because sevoflurane anesthesia interfered with the excitement of hippocampal NMDA receptor activity and thus affected expression of phosphorylated CREB in hippocampus and contributed to memory loss in rats. But the recovery of learning and memory ability of rats at 95d is accomplished whether through restored excitement of NMDA receptor activity or compensatory increased NMDA receptor still needs further studies to demonstrate.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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