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Changes of CaM-CaMK-CREB signaling pathway and related neuron factors in hippocampus of rats after sevoflurane and propofol administration

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Abstract. – OBJECTIVE: To investigate the changes of CaM-CaMK-CREB signaling pathway and related neuron factors in hippocampus of rats after sevoflurane and propofol administration.

MATERIALS AND METHODS: A total of 90 SD rats, half male and half female, were selected. They were randomized into anesthesia group (AG) and control group (CG), in which 30 rats under propofol anesthesia in the AG were included in group A, 30 rats under sevoflurane anesthesia were included in group B, and 30 rats under mixed ventilation of air and oxygen were included in the CG. Before (10 rats were killed in each group) and at the end of anesthesia (2 weeks of anesthesia and 7 days of Morris water maze test), rats were respectively killed and then an appropriate amount of hippocampus was separated. Human tumor necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF), calmodulin (CaM), calmodulin-dependent kinase II a (CaMK II a), NMDA receptor 2B (NR2B), cysteine aspartate specific protease-3 mRNA (Caspase-3mRNA) and protein contents were detected.

RESULTS: The latency of rats in group A and group B, as well as their times of crossing the original platform on 1, 4, and 7d were markedly higher than those of the CG, and those in group B were markedly lower than those in group A (p<0.001). The total distance of rats in group A and group B on 1, 4, and 7d was significantly higher than that of the CG, and that in group B was dramatically lower than that in group A (p<0.001). The speed of rats in the three groups ranged from 1d to 7d, and there was no significant difference at different time points (p>0.05). At the end of anesthesia, the expression levels of CaM mRNA and protein in hippocampus of rats in group A and group B were remarkably higher than those of the CG, and those of CaMK II a mRNA and protein were remarkably lower than those of the CG; the CaM mRNA and protein expression levels in hippocampus of group A were significantly higher than those of group B, and those of CaMK IL a mRNA and protein were significantly lower than those of group B (p<0.001). Simultaneously, the expression levels of NR2BmRNA, Caspase-3mRNA and protein in hippocampus of group A and group B were substantially higher than those of the CG, and those in group B were substantially lower than those in group A (p<0.001). What's more, the TNF-a and VEGF expression levels in hippocampus of group A and B were markedly higher than those of the CG, and those in group B were dramatically lower than those of group A (p<0.001).

CONCLUSIONS: Sevoflurane anesthesia on the CaM-CaMK-CREB signaling pathway and learning and memory function of rats is less volatile, which does better in safeguarding stability.

Key Words:
Sevoflurane, Propofol, Memory function, CaM-CaMK-CREB signaling pathway, NR2B, Caspase-3.

Introduction

With the wide use of anesthetic drugs in clinical practice, relevant researchers are paying more and more attention to the relationship between them and nerve memory function[1]. General anesthetics, due to their own neurotoxicity, can affect the apoptosis of nerve cells[2]. At present, research on the animal central nervous system has become a hot topic, but the specific mechanism of nerve cell degeneration and apoptosis caused

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by anesthetics is still not very clear[3]. Different general anesthetics can delay the occurrence of central nervous system nerves and have different degrees of damage to it[4]. Sevoflurane and propofol, as common general anesthetics clinically, are currently hot topics in the study of their nerve memory function[5].

Anesthetic drugs can destroy the calcium homeostasis of brain neurons, which leads to the impairment of the learning and memory in hippocampus; in recent years, studies on the mechanism of calcium homeostasis in brain neurons have found that the CaM-CaMK-CREB signaling pathway is closely related to the learning and memory ability of the hippocampus[6,7]. Learning and memory function is the main content of cognitive function, and the balance of positive and negative regulation in hippocampus plays a crucial role in it[8]. Moreover, it has been found that the expression level of antisense oligonucleotides in hippocampus is down-regulated through negative regulation to affect the synaptic long-term potentiation; the learning and memory ability is in direct proportion to the synaptic long-term potentiation, and the former will increase with the increase of the latter[9]. At present, there is no clear research on the negative regulation of sevoflurane and propofol on hippocampal tissue, as well as the effect of the learning and memory function. In this research, we compared and investigated thoroughly the effects of CaM-CaMK-CREB signaling pathway and related neuron factors [NMDA receptor 2B (NR2B) and cysteine aspartate specific protease-3 (caspase-3)] in hippocampus of rats after receiving sevoflurane and propofol.

Materials and Methods

Data of Experimental Animals

A total of 90 SD rats, half male and half female, were selected. They were randomized into anesthesia group (AG) and control group (CG), in which 30 rats under propofol anesthesia in the AG were included in group A, 30 rats under sevoflurane anesthesia were included in group B, and 30 rats under mixed ventilation of air and oxygen were included in the CG. They were 8 weeks old, weighing (150-200) g. Both of them are in the Experimental Animal Center of Jilin University, with the certificate number of SCXK (Ji) 2008-0004. At the same time, they purchased LAD0011 feed from Nantong Trophic Animal Feed High-tech Co., Ltd, China for feeding. Indoor temperature was 21.5°C±0.5°C and humidity was 45%-65%, with free food intake and drinking water. This study was approved by the Animal Ethics Committee of The First Affiliated Hospital of Bengbu Medical College.

Methods of Grouping, Anesthesia, and Detection

(1) Methods of animal grouping and intervention. Experimental animals were enrolled in the CG. Rats in group A received intraperitoneal injection of 50 mg/kg propofol once a day, and those in group B were treated 4 h with 2% sevoflurane and 30% air-oxygen mixture, and those in the CG received mixed ventilation of air and oxygen.

(2) DetectionMethods. Materials were as below: NR2B antibody, Caspase-3 antibody (Cell Signaling Technology, China); GAPDH antibody (Huijia Biotechnology Co., Ltd., Xiamen, China; Art. No. A-AP50864); horseradish peroxidase labeled goat anti-rabbit (Bossbio, Beijing, China; Art. No. H-GR0009); horseradish peroxidase labeled goat anti-mouse (Hanlin Biotechnology Co., Ltd., China; Art. No. HS-GM-HRP-500); TNF-α ELISA kit (Guyan Biotechnology Co., Ltd., Shanghai, China); VEGF ELISA kit (Jieshikang Biotechnology Co., Ltd., Qingdao, China); automatic plate washer (Detie laboratories, Nanjing, China); Enzyme analyzer (LNB Instrument Co.,Ltd., Shanghai, China).

NR2BmRNA, Caspase-3mRNA, CaM mRNA and CaMK II a mRNA detected by Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) Technology

qRT-PCR was used to detect the expression levels of NR2B, Caspase-3, CaM and CaMK II a in hippocampus of rats in each group. All specimens were taken out of the liquid nitrogen tank 30 minutes before use, and total RNA of tissues was extracted according to TRIzol reagent operation instructions and dissolved in 20 μL DEPC water. The total RNA was then reversely transcribed using a reverse transcription kit. The reaction system was as follows: M-MLV 1 μl, Olig (dT) 1 μl, RNA enzyme inhibitor 0.5 μl, d NTPs 1 μl, RNase freewater supplemented up to 15 μl. They were incubated 60 min at 38°C; then, 1 μl c DNA was taken, 85°C for 5 s; finally, the syn-

thesized c DNA was used as a template for qRT-PCR amplification. The PCR reaction system was prepared: 10×PCR buffer 2.5 µl, d NTPs 1 µl, upstream and downstream primers 1 µl each, Taq DNA polymerase 0.25 µl, dd H_2O supplemented to 25 µl. The reaction conditions were as below: pre-denaturation at 95°C for 15 min, denaturation at 95°C for 15 s, annealing at 60°C for 30 s, a total of 35 cycles, extending at 72°C for 15 min. Each sample was provided with 3 multiple holes for 3 repeated tests. NR2B, Caspase-3, CaM and CaMK II a took glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as internal reference. Soon afterwards, the amplification and melting curve of Real-Time PCR were confirmed, the relative amount of the target gene was counted according to the result parameters, and its relative quantification was calculated by $2^{-\Delta CT}$ (Table I).

Expression Levels of CaM, CaMK II a, NR2B and Caspase-3 Proteins Detected by Western Bot

Before (10 rats in each group were killed) and at the end of anesthesia (2 weeks), rats were killed respectively and then appropriate amount of hippocampus was separated. After protein lysate was added, the total protein in hippocampus was split. The mixture was placed into a homogenizer (Shanghai Active Motif Biotechnology Co., Ltd., item number: 40401/40415), and 300 µL of lysate was added and the tissue pieces gradually disappeared through grinding until the lysate showed no impurities; then, let it settle and crack on ice for 30 min. They were centrifuged 20 min at 14000 r/min, and finally the supernatant was taken as the total cell protein. Protein quantification was carried out by bicinchoninic acid assay (BCA) on 6%-12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane. After selecting the corresponding band according to the target protein, it was sealed 2 h with degreased milk powder with the concentration of 5%. After washing the membrane, we supplemented 2 mL of Western Anti-dilution Night (Jiangsu Beyotime Biology Co., Ltd.) with dilution ratio of 1:1,000 and stored it at 4 °C for one night. After the first antibody was re-warmed 30 min before the experiments on the second day, Western second antibody (Jiangsu Beyotime Biology Co., Ltd.) was incubated 1 h with the same procedure, and developer was added to the dark room for exposure. PVDF film was imaged by Tocan240 automatic gel imaging system (Shanghai Lingcheng Biotechnology Co., Ltd.) and the results were analyzed by Image Lab™ software.

Concentrations of Tumor Necrosis Factor-α (TNF-α) and Vascular Endothelial Growth Factor (VEGF) detected by Enzyme-Linked Immunosorbent Assay (ELISA)

Before (10 rats were killed in each group) and at the end of anesthesia (2 weeks), rats were killed respectively and then appropriate amount of hippocampus was separated. After protein lysate was added, total proteins in hippocampus were split. The detection was carried out with reference to the operating instructions of human TNF-α ELISA detection kit and VEGF ELISA detection kit. The OD value of each hole was detected promptly at 450 nm wavelength by an enzyme-labeled analyzer, and the concentrations of TNF-α and VEGF were calculated.

Morris Water Maze Test[10]

Morris water maze (produced by Shanghai Xinruan Information Technology Co., Ltd.) was used to test the spatial learning and memory ability of rats 2 weeks after anesthesia. It consists of a circular pool and an automatic image acquisition and processing system.

Table I. Primer sequences of NR2B mRNA, Caspase-3mRNA, CaM mRNA, and CaMK II a mRNA, as well as its internal reference.

Gene name	Forward (5'>3')	Reverse (5'>3')
NR2B	5'-TGCTACAACACCCACGAGAA-3'	5'-CTCCTCCAAGGTAACGATGC-3'
Caspase-3	5'-CCCGAATAAGAGAGAACCAAC-3'	5'-CATTCCTTATATGTCTCCGTAC-3'
CaM	5'-AAGCCGAGCTGCAGGATATGA-3'	5'-CAGTTCTGCCGCACTGATGTAA-3'
CaMK II a	5'-GGCCTGGACTTTCATCGATTCTA-3'	5'-CATCAGGTGGATGTGAGGGTTC-3'
GAPDH	5'-GCAAGTTCAACGGCACAG-3'	5'-GCAAGTAGACTCCACGACAT-3'

Statistical Analysis

SPSS 19.0 (Beijing Boyi Zhixun Information Technology Co., Ltd.) software system was used for statistical analysis, [n(%)] was applied to representing the counting data and (x±s) used to represent the measurement data. Comparison of data between the two groups was conducted by *T*-value test, comparison between multiple groups was conducted by analysis of variance, expressed as *F*, and the two comparisons after analysis of variance were carried out by LSD-*t* test. A *p*-value less than 0.05 was regarded as markedly statistical difference.

Results

General Data of Rats In Each Group

Differences in weight, length, and age of rats per group were not significant ($p>0.05$; Table II).

Changes of CaM-CaMK-CREB Signaling Pathway, Related Proteins and mRNA In Hippocampus of Each Group

The levels of CaM, CaMK II a mRNA and protein in hippocampus of rats in each group before and after anesthesia were detected, and the results were as follows: there was no remarkable difference in the expression of CaM, CaMK II a mRNA and protein in hippocampus of those groups before anesthesia ($p>0.05$); at the end of anesthesia, the expression levels of CaM mRNA and protein in hippocampus of group A and group B were markedly higher than those of the CG, and those of CaMK II a mRNA and protein were substantially lower than those of the CG ($p<0.001$); thereinto, the expression levels of CaM mRNA and protein in group A were significantly higher than those in group B, and those of CaMK II a mRNA and protein in group A were significantly lower than those in group B ($p<0.001$). More details were shown in Figure 1 (A, B). The results showed that group B anesthesia had a better effect on the stability of CaM-CaMK-CREB signaling pathway related proteins CaM and CaMK ll a in the hippocampus of rats.

Comparison of the Memory Function of Rats In Each Group

The spatial learning and memory ability of rats after anesthesia for 2 weeks was tested by Morris water maze.

(1) Comparison of the latency of rats per group. The latency of rats in group A at 1, 4, and 7d was (87.20±6.89) s, (56.10±6.28) s, (28.10±6.36) s, respectively, and that in group B was (72.20±7.04) s, (38.20±6.44) s, (14.29±1.38) s, respectively, and that in CG was (53.12±7.21) s, (23.08±5.18) s, (9.16±1.05) s, respectively. The latency of rats in the three groups showed a downward trend from 1d to 7d, and the differences at different time points in group A and group B were markedly significant ($p<0.001$). The latency of rats in group A and group B on 1, 4, and 7d was significantly higher than that in CG ($p<0.001$), and that in group B was significantly lower than that in group A ($p<0.001$). The results showed that the latency of group B was shorter 2 weeks after anesthesia.

(2) Comparison of times of rats crossing the original platform per group. The number of times of rats in group A crossing the original platform on 1, 4, and 7d was (3.20±0.67) s, (4.62±0.63) s, (5.18±0.67) s, respectively, and that in group B was (5.29±0.64) s, (6.21±0.66) s, (7.20±0.64) s, respectively, and that in the CG was (7.12±0.68) s, (8.20±0.62) s, (8.46±1.69) s, respectively. The number of times of rats crossing the original platform in the three groups showed an upward trend from 1 d to 7. d, and the differences at different time points in group A and group B were statistically significant ($p<0.001$). The number of times of rats in group A and group B crossing the original platform on 1, 4, and 7d was markedly lower than that in the CG ($p<0.001$), and that in group B was markedly higher than that in group A ($p<0.05$). The results showed that the rats in group B had more times of crossing the original platform 2 weeks after anesthesia.

(3) Comparison of the total distance of rats per group. The total distance of rats in group A on

Table II. General data of rats in each group.

Group	Group A (n = 30)	Group B (n = 30)	Control group (CG) (n = 30)	*F*	*p*
Weight (g)	201.25 ± 30.20	201.79 ± 32.41	202.67 ± 33.20	0.015	0.985
Length (cm)	19.20 ± 3.00	19.30 ± 2.50	20.00 ± 2.00	0.888	0.415
Age (weeks)	8.10 ± 0.12	8.10 ± 0.14	8.10 ± 0.20	1.000	0.372

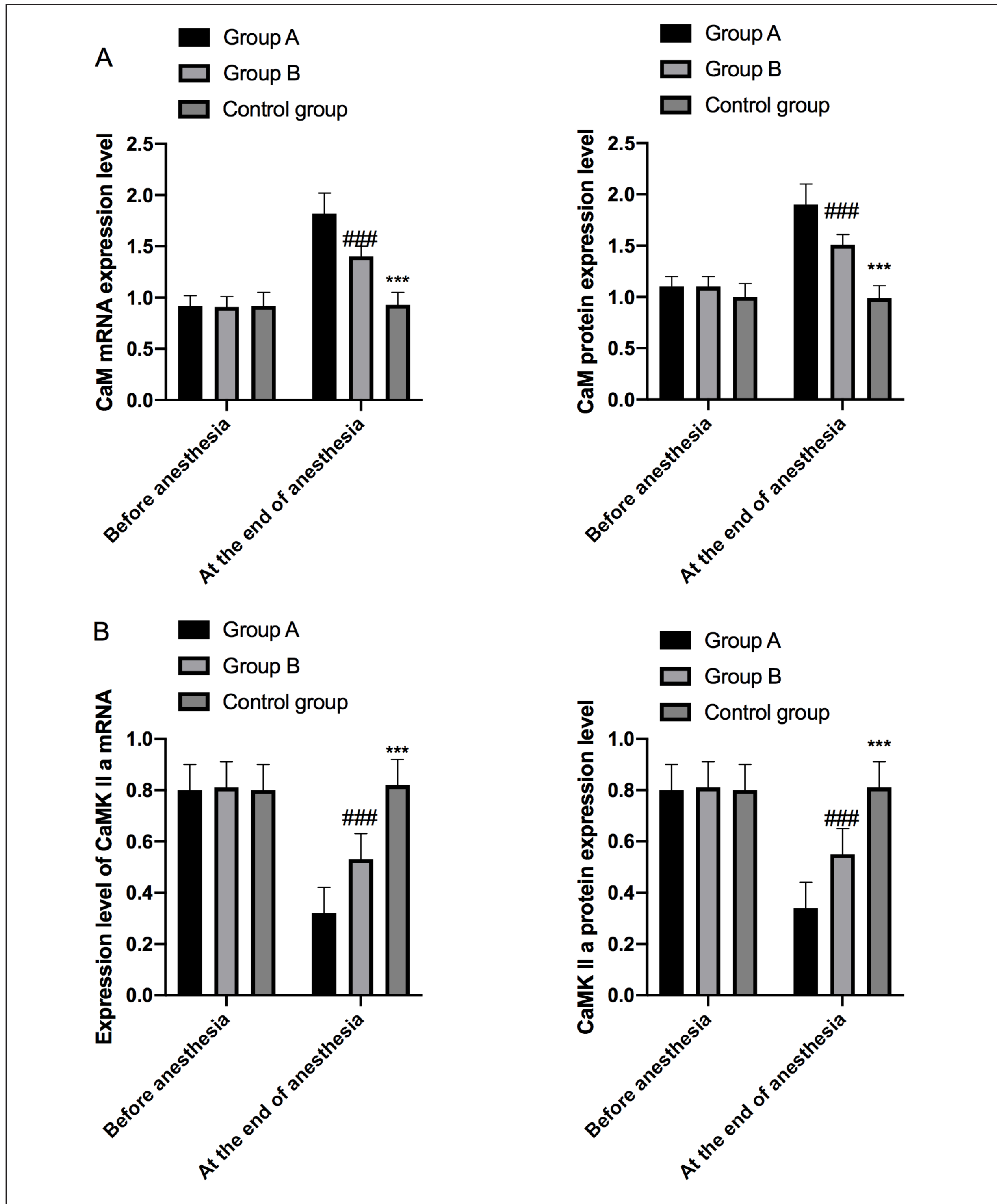

Figure 1. Changes of CaM-CaMK-CREB signaling pathway, related proteins and mRNA in hippocampus of each group. **A,** Expression of CaM mRNA and protein in hippocampus of rats in each group. *** indicates that at the end of anesthesia, the expression levels of CaM mRNA and protein in hippocampus of group A and group B were significantly higher than those of the control group (p<0.001); ### indicates that the expression levels of CaM mRNA and protein in group A were significantly higher than those in group B (p<0.001). **B,** Expression of CaMK II a mRNA and protein in hippocampus of rats in each group. *** indicates that at the end of anesthesia, the expression levels of CaMK II a mRNA and protein in hippocampus of group A and group B are significantly lower than those of the control group (p<0.001); ### indicates that the expression levels of CaMK II a mRNA and protein in hippocampus of group A are significantly lower than those of group B (p< 0.001).

1, 4, and 7d was (28.21±3.29) cm, (18.16±3.47) cm, (13.29±2.98) cm, respectively, and that in group B was (17.20±3.89) cm, (12.26±3.25) cm, (6.33±1.56) cm, respectively, and that in the CG was (13.84±1.79) cm, (7.20±1.03) cm, (3.12±0.79) cm, respectively. The total distance of rats in the three groups showed a downward trend from 1d to 7d, and the differences at different time points in group A and group B were statistically significant ($p<0.001$). The total distance of rats in group A and group B on 1, 4, and 7d was significantly higher than that in CG ($p<0.001$), and that in group B was remarkably lower than that in group A ($p<0.001$). The results showed that the total distance through maze of rats in group B was shorter 2 weeks after anesthesia.

(4) Comparison of the speed of rats per group. The speed of rats in group A on 1, 4, and 7d was (0.18±0.06) cm/s, (0.17±0.07) cm/s, (0.17±0.08) cm/s, respectively, and that in group B was (0.17±0.08) cm/s, (0.17±0.09) cm/s, (0.17±0.09) cm/s, respectively, and that in the CG was (0.17±0.09) cm/s, (0.18±0.07) cm/s, (0.17±0.09) cm/s, respectively. The speed of rats in the three groups was varied from 1d to 7d, and the differences at different time points were not statistically significant ($p>0.05$; Figure 2 (A, B). The results showed that there was no significant difference in maze crossing speed of rats in each group 2 weeks after anesthesia.

Expression Levels of NR2B, Caspase-3mRNA, and Protein In Hippocampus of Rats Per Group

The levels of NR2B, Caspase-3mRNA, and protein in hippocampus of rats per group were detected before and at the end of anesthesia respectively. The results showed as follows:

(1) Expression levels of NR2B and Caspase-3mRNA in hippocampus of rats per group. The levels of NR2BmRNA before and after anesthesia were (1.01±0.14) and (2.74±0.35) respec-

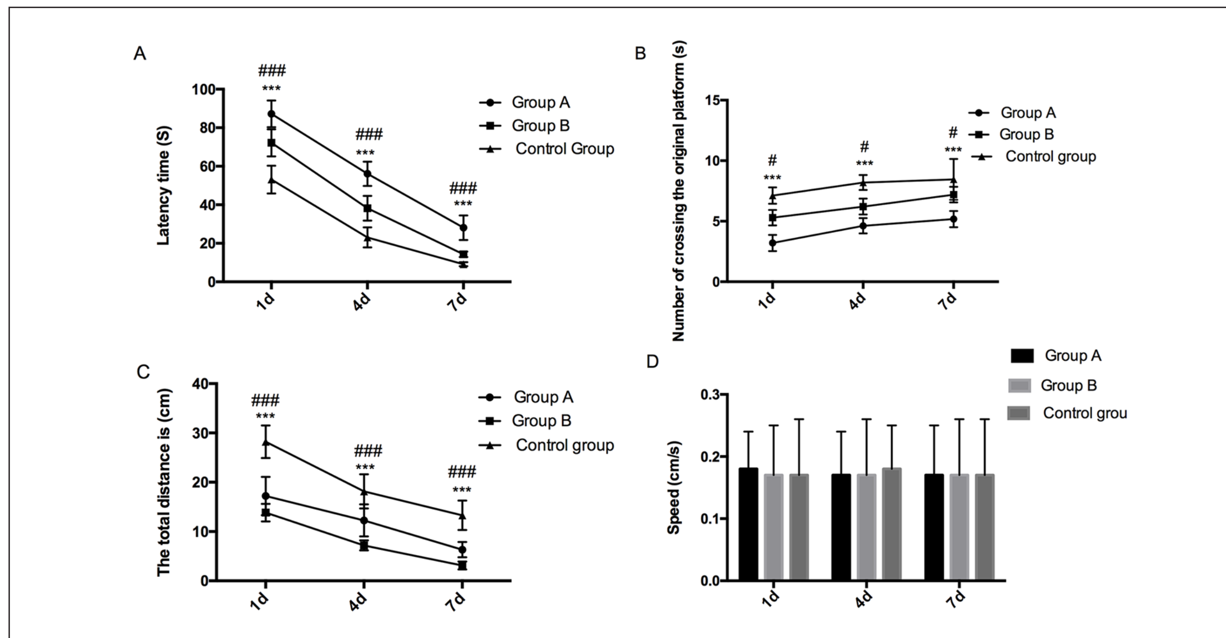

Figure 2. Comparison of the memory function of rats in each group. **A,** Comparison of the latency of rats in each group. *** indicates that the latency of rats in group A and group B on 1d, 4d, and 7d is significantly higher than that in control group ($p<0.001$); ### indicates that the latency of rats in group B on 1d, 4d, and 7d is significantly lower than that in group A ($p<0.001$). **B,** Comparison of the number of times of rats crossing the original platform in each group. *** indicates that the number of times of rats in group A and group B crossing the original platform on 1d, 4d, and 7d is significantly less than that in control group ($p<0.001$); # indicates that rats in group B crossing the original platform on 1d, 4d, and 7d significantly is significantly more than that in group A ($p<0.05$). **C,** Comparison of the total distance of rats in each group. *** indicates that the total distance of rats in group A and group B on 1d, 4d, and 7d is significantly higher than that in the control group ($p<0.001$); ### indicates that the total distance of rats in group B on 1d, 4d, and 7d is significantly lower than that in group A ($p<0.001$). **D,** Comparison of the speed of rats in each group. The speed of rats in the three groups was varied from 1d to 7d, and there was no significant difference at different time points ($p>0.05$).

tively in group A, (1.01±0.13) and (1.82±0.26) respectively in group B, and (1.01±0.15) and (0.60±0.15) respectively in the CG. Caspase-3mRNA levels before and after anesthesia were (1.03±0.11) and (3.25±0.36) respectively in group A, (1.03±0.10) and (1.79±0.39) respectively in group B, and (1.02±0.10) and (1.03±0.10) respectively in the CG. The NR2B and Caspase-3mRNA expression levels in hippocampus of rats in the three groups before anesthesia showed no significant difference (p>0.05). At the end of anesthesia, the expression levels of NR2B and Caspase-3mRNA in hippocampus of rats in group A and group B were significantly higher than those in the CG (p<0.001). Among them, the NR2B and Caspase-3mRNA expression levels in hippocampus of rats in group B were markedly lower than those in group A (p<0.001; Figure 3 (A, B). The results showed that the effect of NR2B mRNA and Caspase-3 mRNA in hippocampus of group B was better than that of group A.

(2) Expression levels of NR2B and Caspase-3 proteins in hippocampus of rats per group. The levels of NR2B protein before and after anesthesia in group A were (0.58±0.15) and (1.73±0.24) respectively, and those in group B were (0.58±0.14) and (1.18±0.25) respectively, and those in the CG were (0.59±0.15) and (0.60±0.15) respectively. The levels of Caspase-3 protein before and after anesthesia in group A were (0.12±0.04) and (0.52±0.10) respectively, and those in group B were (0.12±0.03) and (0.28±0.07) respectively, and those in the CG were respectively (0.12±0.05) and (0.13±0.05). The expression levels of NR2B and Caspase-3 proteins in hippocampus of rats in the three groups before anesthesia showed no significant difference (p>0.05). At the end of anesthesia, the expression levels of NR2B and Caspase-3 proteins in hippocampus of rats in group A and group B were markedly higher than those in the CG (p<0.001). Among them, the expression levels of NR2B and Caspase-3 proteins in hippocampus of rats in group B were significantly lower than those in group A (p<0.001; Figure 4 A, B). The results showed that the down-regulation effect of NR2B and Caspase-3 protein in hippocampus of group B was better than that of group A.

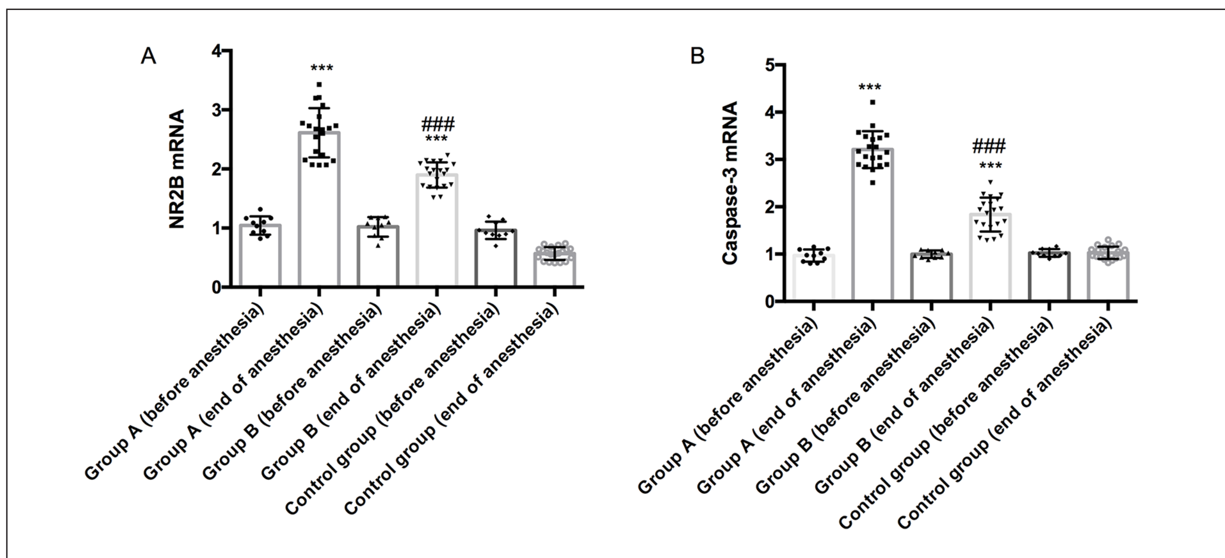

Figure 3. Expression levels of NR2B and Caspase-3mRNA in hippocampus of rats in each group. **A,** Expression levels of NR2BmRNA in hippocampus of rats in each group. *** indicates that at the end of anesthesia, the expression level of NR2BmRNA in hippocampus of rats in group A and group B is significantly higher than that in control group (p<0.001); ### indicates that the expression level of NR2BmRNA in hippocampus of rats in group B is significantly lower than that in group A (p<0.001). **B,** Expression levels of Caspase-3mRNA in hippocampus of rats in each group *** indicates that at the end of anesthesia, the expression level of Caspase-3mRNA in hippocampus of rats in group A and group B is significantly higher than that in control group (p<0.001); ### indicates that the expression level of Caspase-3mRNA in hippocampus of rats in group B is significantly lower than that of group A (p<0.001).

Expression Levels of Related Inflammatory Factors In Hippocampus of Rats Per Group after Anesthesia

At the 8th week after the intervention, the contents of cytokines TNF-a (ng/mL) and VEGF (Pg/mL) in the brain tissues of rats in the three groups were analyzed as follows: the content of TNF-a in the brain tissues of rats in group A and group B was higher than that in the CG, and that of VEGF was lower than that in the CG (p<0.05); the content of TNF-a in group B was lower than that in group A, and that of VEGF was higher than that in group A. The results showed that the effect of group B anesthesia on TNF-a and VEGF in hippocampus was more evident. (p<0.05; Table III and Figure 5 A, B).

Discussion

At the present stage, studies on the changes of the nervous system after the central nervous system is given general anesthesia drugs show that repeated intraperitoneal injections of general anesthesia drugs can damage the spatial learning and memory function of rats[11]. When they are exposed to general anesthesia drugs for too long, the degeneration and death rate of nerve cells is accelerating. Moreover, the damage to the central nervous system and acceleration of apoptosis of nerve cells caused by anesthesia drugs are irreversible[12].

Sevoflurane and propofol are both common clinical intravenous general anesthesia drugs[13]. Relevant reports suggest that patients who have been anesthetized with sevoflurane or propofol during surgery will continue to suffer temporary learning, language, and cognitive disorders for a period of time after drug withdrawal[14]. However, in animal studies, it is also found that sevoflurane or propofol anesthesia can affect short-term memory dysfunction in rats[15]. Sevoflurane is usually used in the surgery of children, and has certain side effects on their nervous and cardiac systems[16]. Propofol is a short-acting analgesic of alkyl acids. Anesthesia is rapid and stable, but the analgesic effect is weak[17]. Animal experiments have found that propofol abuse can significantly damage the spatial learning and memory function of rats and cause degeneration of brain neurons in animals[18]. This study aims to observe the memory ability of rats and changes of hippocampal related factors after intraperitoneal injection of propofol or sevoflurane and 30% air-oxygen mixture. Results of Morris water maze test verified that there was no significant difference in the speed of rats

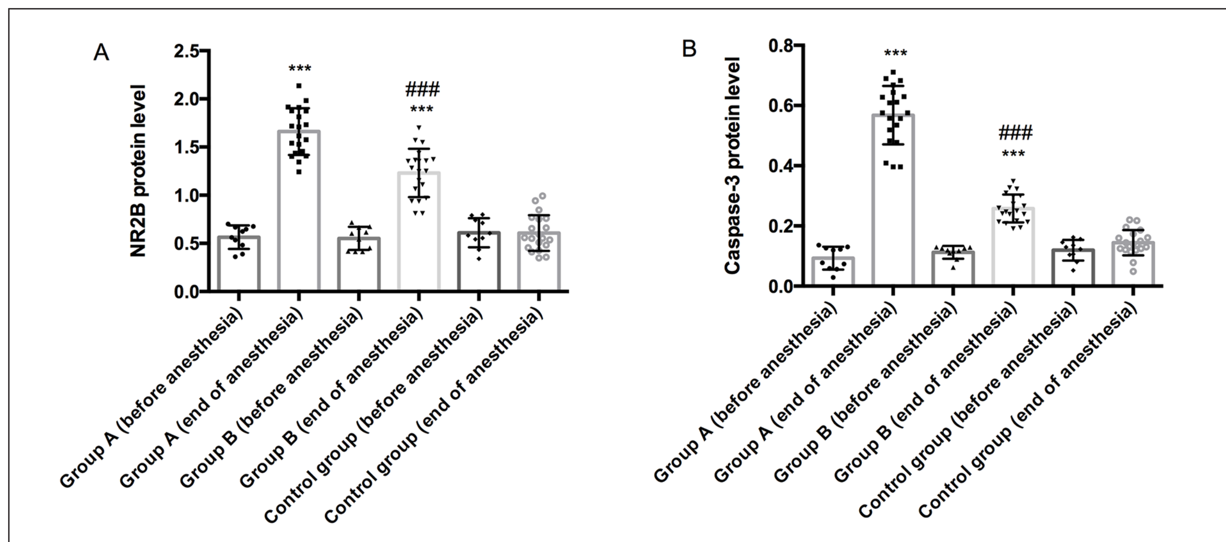

Figure 4. Expression levels of NR2B and Caspase-3 proteins in hippocampus of rats in each group. **A,** Expression levels of NR2B protein in hippocampus of rats in each group. *** indicates that at the end of anesthesia, the expression level of NR2B protein in hippocampus of rats in group A and group B is significantly higher than that in control group (p<0.001); ### indicates that the expression level of NR2B protein in hippocampus of rats in group B is significantly lower than that of group A (p<0.001). **B,** Expression levels of Caspase-3 protein in hippocampus of rats in each group. *** indicates that at the end of anesthesia, the expression level of Caspase-3 protein in hippocampus of rats in group A and group B is significantly higher than that in control group (p<0.001); ### indicates that the expression level of Caspase-3 protein in hippocampus of rats in group B is significantly lower than that in group A (p<0.001).

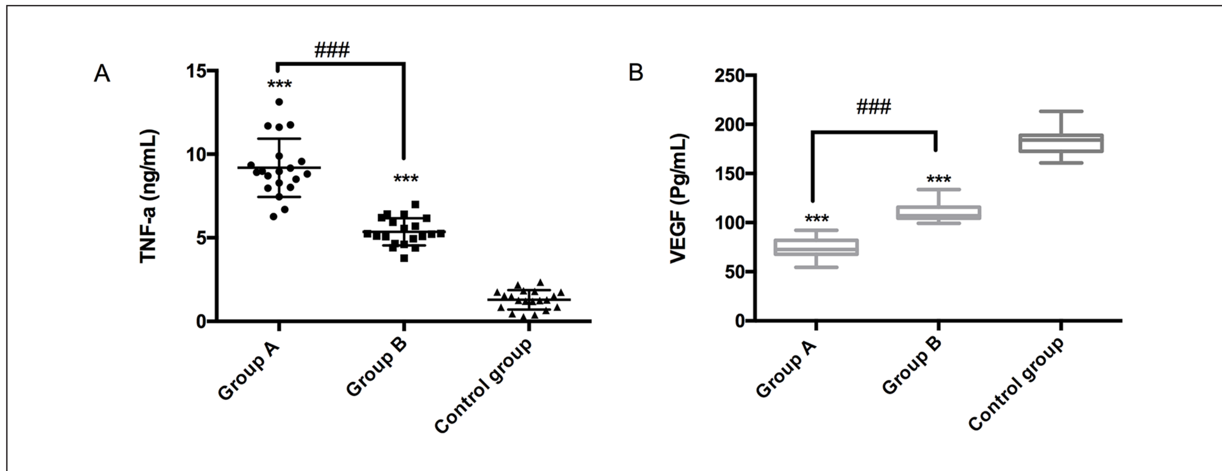

Figure 5. Expression levels of related inflammatory factors in rats of each group. **A,** Contents of TNF-a in brain tissue of rats in the three groups. *** indicates that at the end of anesthesia, the expression level of TNF-a in hippocampus of rats in group A and group B is significantly higher than that in the control group (p<0.001); ### indicates that the expression level of TNF-a in hippocampus of rats in group B is significantly lower than that in group A (p<0.001). **B,** Contents of VEGF in brain tissue of rats in the three groups. *** indicates that at the end of anesthesia, the expression level of VEGF in hippocampus of rats in group A and group B is significantly lower than that in control group (p<0.001); ### indicates that the expression level of VEGF in hippocampus of rats in group B is significantly higher than that in group A (p<0.001).

in each group. However, the latency and times of crossing the original platform of sevoflurane anesthetized rats in one week were significantly higher than those of propofol anesthetized rats. Nevertheless, the total distance of sevoflurane anesthetized rats was markedly lower than that of propofol anesthetized rats. Morris water maze test is widely used in neurobiology research and is a common method to detect the function of animal hippocampus in animal experiments; it is a standard experiment to study spatial learning and memory and it can objectively evaluate the memory function of animals[19]. Based on the results of Morris water maze test, we believe that sevoflurane and propofol have different effects on the learning and memory function of rats, and the effect of sevoflurane on their learning and memory function fluctuates slightly.

Hippocampus is the central nervous system part in human and mammalian cerebral cortex responsible for long-term memory storage, conversion, and orientation[20]. At the end of anesthesia, the expression levels of CaM mRNA and protein in hippocampus of group A and group B were remarkably higher than those of the CG; thereinto, the expression levels of CaM mRNA and protein in hippocampus of group A were markedly higher than those of group B, and those of CaMK II a mRNA and protein were opposite. CaM and CaMK II a are important regulators of CaM-CaMK-CREB signaling pathway, in which CaM is a vital signaling molecule in hippocampal neurons, and its complex participates in learning and memory effect; CaMK II a is a mechanism involved in gene transcription regulation in hippocampal neurons, which has been proved to be abnormally reduced in the mouse model of cognitive impairment[21]. Therefore, sevoflurane and propofol have different effects on the learning and memory function of rats, and the effect of sevoflurane on the CaM-CaMK-CREB signaling pathway fluctuates little. Studies have shown that the hippocampus has the ability to produce new neurons, and

Table III. Expression levels of inflammatory factors related to rats in each group.

Group	Group A (n=20)	Group B (n = 20)	Control group (CG) (n = 20)	*F*	*p*
TNF-a (ng/mL)	9.20 ± 1.77	5.10 ± 0.85	1.29 ± 0.76	211.800	< 0.001
VEGF (Pg/mL)	75.19 ± 9.23	110.32 ± 10.91	189.56± 15.20	473.100	< 0.001

their survival is related to the normal function of the hippocampus[22]. NR2B is one of the subtypes of NMDA receptor, which is relevant to the brain's learning, cognition, judgment, and memory. The expression changes of NR2B mediate the apoptosis and anti-apoptosis process of neuron cells and indirectly affect the function of the hippocampus[23]. Caspase-3 is a cysteine protease of aspartic acid, and its change is the central link in neuronal apoptosis pathway. Caspase-3 induced cascade reaction is the key to the process of apoptosis[24]. Hence, Caspase-3 and NR2B were selected as monitoring indicators in this study. According to the test results, at the end of anesthesia, the expression levels of Caspase-3, NR2BmRNA, and protein in hippocampus of rats in group A and group B were markedly higher than those with mixed inhalation of air and oxygen; however, the expression levels of Caspase-3, NR2BmRNA, and protein in hippocampus of rats in group B were significantly lower than those in group A. Hence, compared with normal rats, we indicate that sevoflurane and propofol anesthesia have a greater impact on the mRNA and protein expression of NR2B and Caspase-3 in hippocampus of rats; it is speculated that the effects of sevoflurane and propofol anesthesia on the mRNA and protein expression of NR2B and Caspase-3 are tied to the changes of cognitive function in rats, but the long-term effects of specific impact mechanisms need further study. At last, we analyzed the expression level of relevant cytokines in hippocampus of rats after anesthesia, and found that the content of TNF-a in brain tissue of rats in the two groups after anesthesia intervention was higher than that in the CG, and that of VEGF was lower than that in the CG. Among them, the level of TNF-a in brain tissue of sevoflurane anesthetized rats was lower than that of propofol anesthetized rats, and that of VEGF was higher than that of propofol anesthetized rats. Studies have shown that nerve function damage is caused by the increase of secretion of various injured cytokines, decrease of secretion of protective cytokines during anesthesia, and excessive apoptosis of neurons[25]. TNF-α and VEGF are common related cytokines in neuronal injury and apoptosis; the difference is that the former is an inflammatory factor that damages cells, while the latter is a protective cytokine[26,27]. In similar studies, it was also found that sevoflurane anesthesia had little effect on the changes of TNF-α and VEGF in brain tissue and did better in safeguarding stability.

Conclusions

To sum up, sevoflurane anesthesia on the CaM-CaMK-CREB signaling pathway and learning and memory function of rats is less volatile, which does better in safeguarding stability.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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References

1) Zhu C, Gao J, Karlsson N, Li Q, Zhang Y, Huang Z, Li H, Kuhn HG, Blomgren K. Isoflurane anesthesia induced persistent, progressive memory impairment, caused a loss of neural stem cells, and reduced neurogenesis in young, but not adult, rodents. J Cereb Blood Flow Metab 2010; 30: 1017-1030.
2) Kuo MC, Leung LS. Disruption of hippocampal multisynaptic networks by general anesthetics. Anesthesiology 2017; 127: 838-851.
3) Xu L, Shen J, McQuillan PM, Hu Z. Anesthetic agents and neuronal autophagy. What we know and what we don't. Curr Med Chem 2018; 25: 908-916.
4) Kurumaji A, Dewar D, McCulloch J. Metabolic mapping with deoxyglucose autoradiography as an approach for assessing drug action in the central nervous system. Imaging drug action in the brain. Routledge, 2017.
5) Purdon PL, Pavone KJ, Akeju O, Smith AC, Sampson AL, Lee J, Zhou DW, Solt K, Brown EN. The ageing brain: age-dependent changes in the electroencephalogram during propofol and sevoflurane general anaesthesia. Br J Anaesth 2015; 115: i46-i57.
6) Zuo D, Liu Y, Liu Z, Cui J, Zhou X, Liu Y, Li Z, Wu Y. Alcohol aggravates ketamine-induced behavioral, morphological and neurochemical alterations in adolescent rats: the involvement of

CREB-related pathways. Behav Brain Res 2018; 349: 80-90.

7) Zhang Y, Lin R, Tao J, Wu Y, Chen B, Yu K, Chen J, Li X, Chen LD. Electroacupuncture improves cognitive ability following cerebral ischemia reperfusion injury via CaM-CaMKIV-CREB signaling in the rat hippocampus. Exp Ther Med 2016; 12: 777-782.
8) Frick KM, Kim J, Tuscher JJ, Fortress AM. Sex steroid hormones matter for learning and memory: estrogenic regulation of hippocampal function in male and female rodents. Learn Mem 2015; 22: 472-493.
9) Duarte-Guterman P, Yagi S, Chow C, Galea LA. Hippocampal learning, memory, and neurogenesis: effects of sex and estrogens across the lifespan in adults. Horm Behav 2015; 74: 37-52.
10) Daniels BP, Sestito SR, Rouse ST. An expanded task battery in the Morris water maze reveals effects of Toxoplasma gondii infection on learning and memory in rats. Parasitol Int 2015; 64: 5-12.
11) Cortese GP, Olin A, O'Riordan K, Hullinger R, Burger C. Environmental enrichment improves hippocampal function in aged rats by enhancing learning and memory, LTP, and mGluR5-Homer1c activity. Neurobiol Aging 2018; 63: 1-11.
12) Soler JE, Robison AJ, Núñez AA, Yan L. Light modulates hippocampal function and spatial learning in a diurnal rodent species: a study using male Nile grass rat (Arvicanthis niloticus). Hippocampus 2018; 28: 189-200.
13) Cho YJ, Nam K, Kim TK, Choi SW, Kim SJ, Hausenloy DJ, Jeon Y. Sevoflurane, propofol and carvedilol block myocardial protection by limb remote ischemic preconditioning. Int J Mol Sci 2019; 20: 269.
14) Kumari M, Sood S, Dhir G. Comparison of sevoflurane and propofol for insertion of laryngeal mask airway in adults. J Evol Med Dent Sci 2018; 7: 5224-5228.
15) Shiraishi S, Cho S, Akiyama D, Ichinomiya T, Shibata I, Yoshitomi O, Maekawa T, Ozawa E, Miyaaki H, Hara T. Sevoflurane has postconditioning as well as preconditioning properties against hepatic warm ischemia–reperfusion injury in rats. J Anesth 2019; 33: 390-398.
16) Ydemann M, Nielsen BN, Henneberg S, Jakobsen JC, Wetterslev J, Lauritsen T, Steen N, Edstrøm B, Afshari A. Intraoperative clonidine for prevention of postoperative agitation in children anaesthetised with sevoflurane (PREVENT AGITATION): a randomised, placebo-controlled, double-blind trial. Lancet Child Adolesc Health 2018; 2: 15-24.
17) Li H, Yang H, Wu Y, Pan Z, Liu W. Comparison of effects of sevoflurane and propofol on cerebral oxygen metabolism in pediatric anesthesia. Neuro Quantology 2018; 16: 858-862.
18) O'Donohoe PB, Turner PJ, Huskens N, Buckler KJ, Pandit JJ. Influence of propofol on isolated neonatal rat carotid body glomus cell response to hypoxia and hypercapnia. Respir Physiol Neurobiol 2019; 260: 17-27.
19) Bye CM, Hong NS, Moore K, Deibel SH, McDonald RJ. The effects of pool shape manipulations on rat spatial memory acquired in the Morris water maze. Learn Behav 2019; 47: 29-37.
20) Tao R, Davis KN, Li C, Shin JH, Gao Y, Jaffe AE, Gondré-Lewis MC, Weinberger DR, Kleinman JE, Hyde TM. GAD1 alternative transcripts and DNA methylation in human prefrontal cortex and hippocampus in brain development, schizophrenia. Mol Psychiatry 2018; 23: 1496-1505.
21) Sun H, Wu H, Liu J, Wen J, Zhu Z, Li H. Prenatal stress impairs spatial learning and memory associated with lower mRNA level of the CAMKII and CREB in the adult female rat Hippocampus. Neurochem Res 2017; 42: 1496-1503.
22) Arellano JI, Harding B, Thomas JL. Adult human hippocampus: no new neurons in sight. Cereb Cortex 2018; 28: 2479-2481.
23) Sullivan JA, Zhang XL, Sullivan AP, Vose LR, Moghadam AA, Fried VA, Stanton PK. Zinc enhances hippocampal long-term potentiation at CA1 synapses through NR2B containing NMDA receptors. PLoS One 2018; 13: e0205907.
24) Rami A, Benz A. Exclusive activation of caspase-3 in mossy fibers and altered dynamics of autophagy markers in the mice hippocampus upon status epilepticus induced by kainic acid. Mol Neurobiol 2018; 55: 4492-4503.
25) Othman FN, Muthuraju S, Noor SSM, Abdullah S, Mohd Yusoff AA, Tharakan J, Bhaskar S, Mahmood MS, Kassim F, Rafia H, Mohd Haspani MS, Alias A, Pando RH, Abdullah JM, Jaafar H. Human tuberculosis brain promotes neuronal apoptosis but not in astrocytes with high expression of vascular endothelial growth factor. Tuberculosis (Edinb) 2018; 112: 45-51.
26) Sun JJ, Zhang XY, Qin XD, Zhang J, Wang MX, Yang JB. MiRNA-210 induces the apoptosis of neuronal cells of rats with cerebral ischemia through activating HIF-1α-VEGF pathway. Eur Rev Med Pharmacol Sci 2019; 23: 2548-2554.
27) Fadda LM, Ali HM, Mohamed AM, Hagar H. Prophylactic administration of carnosine and melatonin abates the incidence of apoptosis, inflammation, and DNA damage induced by titanium dioxide nanoparticles in rat livers. Environ Sci Pollut Res Int 2019 May 4. doi: 10.1007/s11356-019-05059-4. [Epub ahead of print].