Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

The effect of sevoflurane on the spatial recall ability and expression of apolipoprotein E and β amyloid in the hippocampus in rats

Wanwei Jiang[1*], Qinghui Wang[1], Yajing Liao[2], Yue Sun[1], Rui Yang[1]

[1] Department of Anesthesiology, The Affiliated Zhongshan Hospital of Dalian University, Liaoning Province,116001, China
[2] Department of Surgical Operating Room, The Affiliated Zhongshan Hospital of Dalian University, Liaoning Province,116001, China

[*]**Correspondence to:** jiangwanweizgd@163.com

Received January 23, 2020; **Accepted** September 06, 2020; **Published** October 31, 2020

Doi: http://dx.doi.org/10.14715/cmb/2020.66.7.1.7

Abstract: This study aimed to observe the recent spatial recall ability and the changes of expression of hippocampal apolipoprotein E (ApoE) and amyloid β protein (Aβ) in adult rats after inhaling sevoflurane anesthetic drugs, and to analyze the mechanism of action. For this purpose, a total of 54 adult SD clean-grade rats were selected in this study and were randomly divided into the sevoflurane anesthesia group, carrier gas group and control group, 18 rats in each group. The rats in the carrier gas group were inhaled with 1 L/min of oxygen O_2+1 L/min air mixed carrier gas for 2 h, and the rats in the sevoflurane anesthesia group were given 3.2%sevoflurane for 2 hours based on the carrier gas group, the control rats were naturally reared. Before the model was copied, the Morris water maze experiment was performed before the material was taken. Some rat brain tissues were extracted on the first day (T1), the third day (T3), and the seventh day (T7) after model replication. The immunohistochemistry was used to measure the mean optical density (MOD) value of APOE and Aβ in hippocampal CA1, CA3 and DG regions. The indicators above at different time points of each group were compared and analyzed. Results showed that the number of crossing the original platform at each time point, the residence time of the original platform quadrant, the number of entering the original platform quadrant, and the percentage of the original platform quadrant residence time in the sevoflurane anesthesia group and the carrier gas group were compared, and there were no significant differences between two groups (P>0.05). Compared with the carrier gas group, the MOD values of APOE in the hippocampus at T1 and T3 time points in the sevoflurane anesthesia group were decreased (P<0.05), the MOD values of Aβ in the hippocampus at the T7 time point were increased (P<0.05). It concluded that Inhalation of 3.2%sevoflurane has no obvious damage to the recent spatial recall ability of adult rats. Within 7 days after inhalation of 3.2% sevoflurane, it can inhibit hippocampus Aβ deposition through down-regulating APOE expression level. The critical time point for hippocampal Aβ increasing was 7 days after anesthesia.

Key words: Sevoflurane; Spatial Recall Ability; Hippocampal Apolipoprotein E; Amyloid Beta Protein.

Introduction

In recent years, the incidence of postoperative cognitive dysfunction (POCD) in patients has increased year by year, and the situation is extremely severe. The research related to the specific pathological mechanism for this disease is mainly focused on Alzheimer's disease (AD) (1). The deposition of amyloid β (Aβ) is the core lesion basis of AD. The formation, deposition, degradation and initiation of Aβ are involved in the process of AD. A variety of anesthetic drugs with significant effects have been developed in clinical treatment, among them, the new inhalation anesthetic sevoflurane has many advantages such as rapid induction of anesthesia, low probability of apnea, and high hemodynamic stability (2). In addition, sevoflurane can enhance the efficacy of the inhibitory neurotransmitter GABA receptor in the central nervous system to a certain extent, in order to achieve the effect of inhibiting the activity of the key brain nuclei that maintain awakening, thus resulting in serious consequences of loss of consciousness (3). Related literature has confirmed that inhalation of anesthetic drugs, such as sevoflurane, has a serious impact on the aggravation of hippocampal Aβ deposition, which in turn impairs the learning and memory ability of rats. β and γ-secretase produce Aβ by continuous action on amyloid precursor protein (APP). In this process, various factors are involved and have different degrees of influence, among which, the effect of apolipoprotein E (ApoE) gene polymorphism on APP is mainly showed as the abnormal degradation of APP, thus leading a large amount of Aβ deposition, which seriously damages the biofilm and the stability of intracellular calcium ions.

The hippocampus in the human body plays a decisive role in the learning and memory ability and the conditioned reflex generating process. CA1, CA2, CA3 and CA4 are the four major regions of the hippocampus along with the pyramidal cell system (4). The CA1 region is connected to the subiculum, which contains small pyramidal neurons. The anterior medial region of the vertical part of the hippocampus and the dorsolateral region of the horizontal part is the CA3 region, which contains axons and moss fibers (5). The Morris water maze was first used in the study on learning and memory brain mechanisms in the 1980s. At this stage, the Morris water maze is mainly used as an experimental method for animal learning and memory ability evaluation in basic AD research. The common steps in detecting the

spatial learning and memory ability of animals through the Morris water maze test are space exploration and navigation, and these are two basic steps of evaluating the level of animal learning and memory using the Morris water maze (6-7). This study aimed to analyze the specific effects of sevoflurane on the short-term spatial recall ability and the expression of hippocampus A beta in adult rats and further explore its mechanism by observing the behavioral changes and the changes in the expression levels of hippocampal ApoE and Aβ after sevoflurane anaesthesia in rats.

Materials and Methods

Source of experimental animals

54 healthy adult male SD rats were selected for the study. All rats were weighing less than 280-320g. The rats in this study were provided by the Experimental Animal Center of the Third Military Medical University. License number: SCXK (Yu) 2012-0005.

Grouping of animals

The rats were randomly divided into three groups: carrier gas group, sevoflurane anesthesia group and blank control group, 18 rats in each group. The brain tissues of rats were extracted on day 1 (T1), day 3 (T3) and day 7 (T7) after model replication, and the mean optical density (MOD) of APOE and Aβ in hippocampal CA1, CA3 and DG regions were detected by immunohistochemistry. The average in the DG region was measured.

Experimental instruments and reagents

The instrument used in the Morris water maze test was purchased from Chengdu Taimeng Technology Co., Ltd., the version was MT-200. The instruments for anesthesia and gas monitoring were purchased from Drager, Germany. The versions of the instruments were Fabius anesthesia machine and Vamos gas monitor. The anesthetic used in this study was inhaled sevoflurane (production batch number 65150103) purchased by Lunan Pharmaceutical Group. The Aβ antibody and APOE antibody used in this study were purchased from Abcam and Beijing Boaosen, respectively. GTVisionTM III anti-mouse/rabbit universal immunohistochemistry test kit was purchased from Shanghai Gene Technology Co., Ltd.

Replication of anesthesia model

Inhalation of anesthetic drugs using a self-made 75 cm × 30 cm × 15 cm plexiglass inhalation anesthesia box, the anesthesia machine was connected to the two side holes of the anesthesia box through a threaded tube, and the position of the sevoflurane concentration in the tank was monitored by a gas monitor. Soda-lime with a thickness of 2 cm was laid on the bottom of the box (8). Rats participating in the Morris water maze test were placed in an anesthesia box at 1 day before the anesthesia was performed, and the freedom of movement of the rats was not restricted within 15 minutes, in order to enhance the familiarity of the rats to the anesthesia chamber environment, thus to avoid the restless mood in rats in an unfamiliar environment at the beginning of anesthesia which can affect the experiment (9). Rats in the sevoflurane anesthesia group were inhaled with 1L/min O_2+1L/min air carrier gas and 3.2% sevoflurane for 2 hours, the rats in the carrier gas group were continuously inhaled with carrier gas the same with the sevoflurane anesthesia group for 2 hours, and the rats in the blank control group were naturally reared (10). The skin, respiratory rate and mucosal color changes of the rats in each group during the model replication were closely monitored. The environmental conditions of the rats were 50%-65% relative temperature and 21°C±2°C ambient temperature, and natural day and night cycle illumination (11).

Morris water maze test

Experimental instruments

The main components of the Morris water maze experiment were a circular pool, a platform that can move freely while hiding under the water surface, and a system for automatically acquiring images (12). The diameter of the study pool was 180 cm (13). When the quadrant was divided, the pool was divided into four parts according to the northeast (NE), southeast (SE), southwest (SW), and northwest (NW). The animal inlet point was the midpoint of the arc of the quadrant, and the platform can be placed in any quadrant, but mostly in the SE quadrant (14). The image acquisition and analysis system were used to record the animal swimming trajectory data in detail, and the steps of extracting and analyzing the indicators were completed.

Experimental process

The main contents of the Morris water maze experiment: a one-day adaptive training, a five-day positioning navigation experiment, and a space exploration experiment lasting one day. The purpose of adaptive training was to screen out rats with swimming disorders (15). (1) Specific operation steps of positioning navigation experiment: positioning navigation experiment also named acquisition training. The rats were moved to the experimental environment one night before the experiment to improve the adaptability of the rats to the experimental environment and to reduce the possibility of restless mood in rats in an unfamiliar environment in the laboratory which can affect the experiment. On the first day of the experiment, the rats were placed in the pool to make them swim freely to achieve the purpose of adapting to the experimental environment but to avoid excessive consumption of physical strength, free-swimming was limited to 1-2 minutes (16). On the second day of the experiment, the platform was placed in any quadrant, and the rats were subjected to acquired training, 4 times/day, once in each quadrant. When the rats were placed in water, they were kept facing the wall. The experiment interval was 15 s and the training was continued for 4-5 days. Rats usually rest for 10-15 s after finding the platform during the experiment. If the rats did not find the platform within the specified time, the experimental operator should guide the rats to rest on the platform for the same time, and then proceed to the next experimental step. If the rat did not fully rest and then jumped back into the water, the rats should be returned to the platform for rest and re-timed (17). (2) Space exploration experiments specific operation steps: remove the platform and set the water inlet point to the opposite side of the platform. The time limit was wit-

hin 120 s. Record the number of crossing the platform, the time of crossing the platform quadrant, the number of entering the platform quadrant, and the percentage of time across the platform quadrant. When the above steps were completed, the experimental environment must ensure the following conditions: the water temperature was around (25±1) °C, the platform was placed 2 cm below the water surface, and the milk powder was added to the water to conceal the platform. The experiment was carried out at 10 am every day. Morris water maze experiments were performed on rats in each group before model replication and sampling.

Notes

There are many factors in the experiment that will affect the experimental data to a certain extent, resulting in data bias and reducing the accuracy of the research conclusions. The notes during the experiment are as follows:

[1] Control of time. The time of positioning navigation laboratory needs to be controlled within 4-5 days. The control of time plays a key role in the animal experiment of learning memory disorder, for example, the excessive consumption of body energy in rats will directly lead to errors in the experimental results. The training time should be controlled within 1 minute. If the experimental rats did not find a platform for rest within 1 minute, there was no need to extend the time, the rats were less likely to find the platform and would aggravate the body energy consumption of the rats, which was not conducive to reducing the incidence of experimental errors, and this time, the experimental operator should manually guide the rats to rest on the platform. Normally, the spatial search duration is 1 day, and the experimental rats are placed in the water for searching for 60 s, and the operation is repeated once completed. In addition, maintaining a consistent daily test time can reduce the chance of a reactive difference in rats due to time, and reduce the magnitude of data fluctuations in turn to ensure data accuracy.

[2] Platform setting and calibration. Usually, the platform is placed in the third quadrant (SW) to ensure that the platform and the edge of the pool wall are kept at a long distance, preventing the rat from swimming around the pool wall and touching the platform, thus resulting in the possibility of non-memory standing on the rat. It is necessary to fix the platform to avoid the situation where the water level fluctuates due to rat swimming and moves the platform, and to reduce the error that the data acquisition cannot be stopped automatically due to the platform shift after rats rest on the platform. It is necessary to use an object similar to the color of the rat to be scaled in the target area, and the coverage area is the entire platform, which can also reduce the incidence of data collection that cannot be stopped after the rats are on the stage, thus ensuring the accuracy of the experimental data.

[3] Control experimental stress. Controlling the consistency of experimental conditions can effectively control the experimental stress. Under the premise of ensuring the same conditions of weight, strain and age of rats, the temperature of the experimental pool should be controlled at a suitable temperature, enhance the familiarity of the experimental operators and rats, and maintaining the laboratory environment and objects in place can effectively control the stress of experimental animals (18). It is also important to keep the experimental environment quiet and the operator's body odor unchanged; administration before the experiment should be carried out by the operator who performed the water maze test, so as to achieve the effect of establishing good familiarity between the experimental operator and the rat, and reduces the stress caused by the experimental operation. The tail suspension can significantly affect the spatial learning and memory ability of rats. When performing underwater experiments, it is necessary to observe the tail state of the rats at all times. The tail suspension may also cause water to enter the respiratory tract to cause stress. Therefore, in the experiment, the tail suspension state should be avoided.

[4] Experimental control. The positioning navigation test can specify the number of daily swimming refer to the state of the animal rat, in order to avoid excessive swimming and consuming a large amount of physical energy of the rat, thus affecting the authenticity of the data.

Immunohistochemistry

The rats were intraperitoneally injected with 1% pentobarbital sodium at a dose of 50 mg/kg. The extracted brain tissue was placed on an ice tray and fixed with 4% paraformaldehyde, followed by paraffin embedding. The hippocampus coronal should be exposed and sliced according to a thickness of 3 μm. The expression level of Aβ and ApoE in the hippocampus was determined by the immunohistochemical SP method. The sections were dewaxed and dehydrated, and the exposed antigen was repaired by high pressure. The primary antibody (Aβ antibody 1:200 dilution, APOE antibody 1:100 dilution) and secondary antibody bind, DAB color development, hematoxylin counterstaining, microscopic examination, sealing and then taking images. Image-Pro Plus system was used for analysis.

Statistically analysis

The data were entered into SPSS 20.0 software for statistical analysis. The mean±standard deviation ($\bar{x}\pm s$) was used to represent the measurement data. The analysis of variance analysis Mauchly spherical test was used to test the escape latency data of each group ($P<0.10$, need to correct F value). One-way analysis of variance was used to analyze the data of spatial exploration experiment and the biochemical indicators, LSD t-test was used and the results were pairwise compared, $P<0.05$ was considered significant.

Results

Comparison of escape latency before rat model replication in each group

Before model replication, the navigational experiments were carried out on rats in each group.

With the increasing of training days, the latency of rats in each group showed a gradual shortening trend (T1: $F=9.815$, $P=0.001$; T3: $F=33.071$, $P = 0.000$; T7: $F = 14.943$, $P = 0.000$). The escape latency of rats in the control group, the carrier gas group and the sevoflurane anesthesia group were compared, and variance analysis

Table 1. Comparison of escape latency of rats in each group before model replication ($\bar{x}$±s).

Group	Day 1	Day 2	Day 3	Day 4	Day 5
T1					
Blank control	39.78±8.84	28.09±15.46	20.20±11.45	22.09±14.56	16.43±11.88
Carrier gas	54.13±27.57	21.20±18.27	18.67±15.85	14.78±13.02	14.43±6.25
Sevoflurane anesthesia	28.13±11.13	14.42±12.52	12.23±6.46	12.15±5.81	8.59±3.33
T3					
Blank control	47.00±7.96	32.92±13.18	13.52±8.47	13.87±12.10	9.52±4.74
Carrier gas	45.66±18.16	19.16±5.18	10.17±7.46	19.42±17.04	7.83±5.60
Sevoflurane anesthesia	35.20±17.98	21.91±9.00	13.19±11.78	17.85±13.30	7.65±2.75
T7					
Blank control	46.90±20.85	20.79±17.90	20.97±7.67	11.85±4.40	10.01±6.28
Carrier gas	56.14±31.50	29.57±18.48	11.60±6.06	8.70±4.66	8.50±4.73
Sevoflurane anesthesia	30.64±12.58	16.68±11.24	11.82±9.61	8.99±3.31	6.97±3.20

P<0.05, the difference was statistically significant.

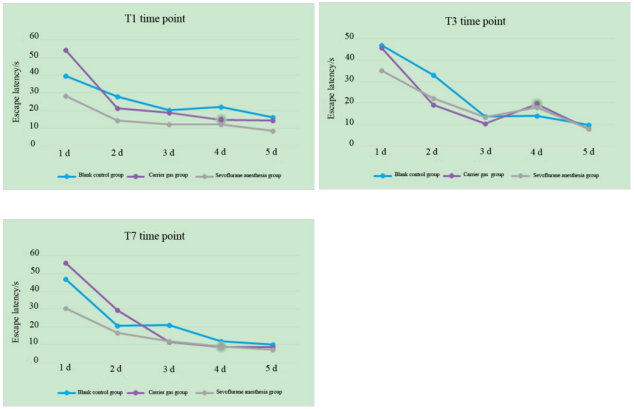

Figure 1. Comparison of escape latency of rats in each group.

of the comparison results showed that there were significant differences in the escape latency data between the three groups at three-time points, P>0.05, and there was statistical significance (T1: F=0.711, P=0.631; T3: F=0.779, P=0.624; T7: F=1.394, P=0.242) (Table 1, Figure 1).

Comparison of data from spatial exploration experiments performed before rat model replication in each group

Before the model replication, the space exploration experiment was carried out on rats in each group, and the experimental data of rats in each group at T1, T3 and T7 time points were integrated and compared, including the number of rats crossing the platform, the time of crossing the platform quadrant, and the number of entering the platform quadrant and the percentage of time across the platform quadrant. The variance analysis of the above indicators showed that there were no significant differences in the indicators above between groups, and the differences were not statistically significant, P>0.05, Table 2.

Comparison of data from spatial exploration experimental performed after rat model replication between groups

When the model replication was completed, the spatial exploration data of rats in each group at T1, T3 and

Table 2. Comparison of data from spatial exploration experiments performed before model replication in each group ($\bar{x}$±s).

Group	Number of rats crossing the original platform	Number of entering the platform quadrant	Residence time of original platform quadrant/s	Percentage of residence time of original platform quadrant /%
T1				
Blank control	2.6±1.8	22.6±1.5	25.47±3.10	21.86±5.98
Carrier gas	3.7±2.8	25.7±8.8	25.07±5.55	22.91±5.82
Sevoflurane anesthesia	4.9±2.3	27.6±3.1	28.41±9.53	28.50±5.63
F	1.447	1.190	0.455	2.251
P	0.266	0.311	0.645	0.141
T3				
Blank control	3.1±1.0	22.5±3.1	30.28±2.37	25.33±2.76
Carrier gas	3.2±1.1	25.1±6.1	26.34±4.13	20.37±5.47
Sevoflurane anesthesia	4.9±1.7	24.8±5.2	27.94±6.97	23.8±5.87
F	2.898	0.361	0.856	1.440
P	0.088	0.703	0.445	0.270
T7				
Blank control	2.8±1.7	23.4±5.5	27.14±2.79	21.61±3.14
Carrier gas	4.4±1.7	26.0±7.6	27.21±5.21	22.44±1.46
Sevoflurane anesthesia	4.8±1.5	28.6±4.3	26.73±2.88	22.82±2.56
F	2.759	1.133	0.027	0.370
P	0.094	0.347	0.974	0.696

P<0.05, the difference was statistically significant.

T7 time points were compared, including the number of crossing platforms, the number of entering the platform quadrant, time of crossing the platform quadrant, and the percentage of time across the platform quadrant. The variance analysis of the above indicators showed that there were no significant differences in the indicators above between groups, and the differences were not statistically significant, P>0.05, Table 3.

Comparison of the expression levels of Aβ in rat hippocampus

The variance analysis of Aβ expression levels in the hippocampus at T1 and T3 time points showed that there were no significant differences between the above indicators in each group, P>0.05, and the difference was not statistically significant. The variance analysis of Aβ expression level in the hippocampus at the T7 time point showed that there was a significant difference in data of rats between groups, P<0.05, and the difference was statistically significant. The LSD-t test was used to further analyze the data, the result showed that there was no significant difference in the MOD value of Aβ in the CA1, CA3 and DG hippocampus at the T7 time point in between the carrier gas group and blank control group (P>0.05). There was a significant difference in the MOD value of Aβ in the hippocampus between the sevoflurane anesthesia group and carrier gas group (P<0.05) Table 4, Figure 2.

Comparison of ApoE expression levels in rat hippocampus

The hippocampal ApoE expression levels in the hippocampus at T1 and T3 time points into each group were integrated, and the variance analysis was performed, and the results showed that there was a significant difference in ApoE expression level in rat hippocampus in each group at T1 and T3 time points (P<0.05). The expression levels of hippocampal ApoE in the T7 time

Table 3. Comparison of data from spatial exploration experiment performed after rat model replication between groups ($\bar{x}$±s).

Group	Number of crossing platforms	Number of entering the platform quadrant	Residence time of original platform quadrant/s	Percentage of residence time of original platform quadrant /%
T_1				
Blank control	5±2	21±6	27.45±7.38	23.33±6.34
Carrier gas	3±1	24±8	24.18±7.50	20.72±6.40
Sevoflurane anesthesia	7±4	28±7	30.99±5.54	26.31±4.63
F	3.623	1.369	1.476	1.348
P	0.051	0.285	0.261	0.290
T_3				
Blank control	6±1	24±9	24.63±7.69	20.25±5.23
Carrier gas	2±2	19±7	22.51±2.6	18.98±4.85
Sevoflurane anesthesia	6±4	26±6	30.40±8.96	26.84±7.25
F	2.851	1.436	1.727	2.990
P	0.092	0.270	0.212	0.083
T_7				
Blank control	4±2	24±8	27.22±8.19	23.18±6.97
Carrier gas	4±2	23±7	26.05±7.70	22.16±6.54
Sevoflurane anesthesia	5±1	28±4	33.68±5.76	26.89±1.69
F	0.582	1.106	1.907	1.188
P	0.572	0.356	0.184	0.333

P<0.05, the difference was statistically significant.

Table 4. Comparison of Aβ expression level in rat hippocampus ($\bar{x}$±s).

Group	CA1	CA3	DG
T_1			
Blank control	0.18±0.02	0.18±0.01	0.19±0.02
Carrier gas	0.19±0.02	0.18±0.02	0.18±0.02
Sevoflurane anesthesia	0.19±0.02	0.19±0.03	0.19±0.03
F	0.692	0.208	0.786
P	0.505	0.812	0.458
T_3			
Blank control	0.19±0.02	0.19±0.02	0.19±0.02
Carrier gas	0.18±0.02	0.19±0.02	0.18±0.02
Sevoflurane anesthesia	0.18±0.03	0.18±0.02	0.19±0.03
F	0.396	2.304	0.961
P	0.674	1.107	0.385
T_7			
Blank control	0.18±0.02	0.18±0.02	0.18±0.02
Carrier gas	0.18±0.02	0.18±0.03	0.18±0.03
Sevoflurane anesthesia	0.20±0.03	0.20±0.03	0.21±0.03
F	8.829	4.908	14.382
P	0.000	0.011	0.000

P<0.05, the difference was statistically significant.

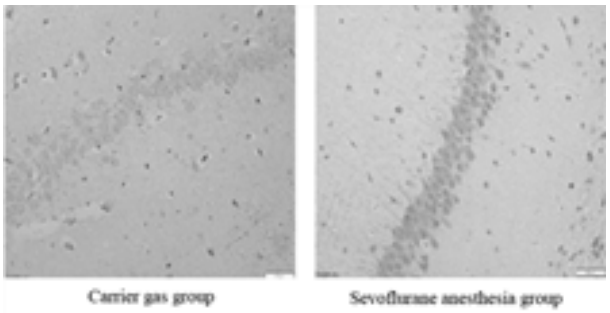

Figure 2. Aβ expressions in rat hippocampus of the sevoflurane anesthesia group and carrier gas group (×40).

point of each group were compared, and variance analysis was performed, and the result showed that there was no significant difference between the groups (P>0.05). The results of deep-level comparative analysis of the data were tested by LSD-t, and there were no significant differences in the MOD values of ApoE expression levels in CA1, CA3 and DG regions at T1 and T3 time points between the carrier gas group and blank control group (P>0.05).

The MOD value of ApoE in the hippocampus of the sevoflurane anesthesia group was significantly decreased at the T1 and T3 time points (P<0.05) Figure 3, Table 5.

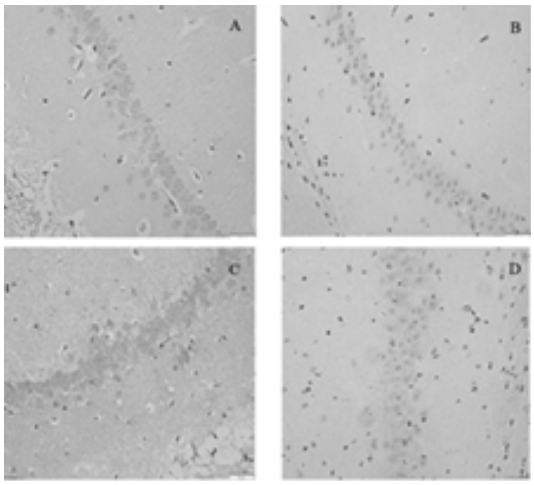

Figure 3. Expression of ApoE in the hippocampus of the sevoflurane anesthesia group and carrier gas group (×40). A: Expression of ApoE in the hippocampus of carrier gas group at T1 time point. B: Expression of ApoE in the hippocampus of the sevoflurane anesthesia group at T1 time point. C: Expression of ApoE in the hippocampus of carrier gas group at T3 time point. D: Expression of ApoE in the hippocampus of the sevoflurane anesthesia group at the T3 time point.

Discussion

The reduction of learning and memory ability and inattention is the main clinical manifestation of POCD. The probability of POCD complications is high, which causes a certain degree of damage to the anesthesiologist's clinical research and patient rehabilitation (19). The hippocampus Papez circuits and the three synaptic circuits are two important circuits that affect learning and memory (20). The CA1 and CA3 regions are located in the three synaptic circuits, and relevant research indicates that the above two regions have a significant influence on spatial discrimination learning and memory ability (21). Sevoflurane is a new inhalation anesthetic. Compared with other drugs, sevoflurane has many advantages such as rapid induction, short recovery time, and small fluctuation of anesthesia effect, which has a wide range of applications in clinical practice. Some basic researches have confirmed that inhaled anesthetics have certain effects on the learning and memory ability of rodents, but the specific mechanism has not been clarified (22). It has been confirmed by related animal experiments that inhaled sevoflurane can inhibit the memory of new things in mice, and this result is related to the inhibition of storage function and memory formation of the hippocampus. Inhalation of commonly used drugs such as enflurane, isoflurane and sevoflurane in general anesthesia is the main influencing factor of the incidence of postoperative cognitive impairment, but the unified conclusion on the specific mechanism of

Table 5. Comparison of ApoE expression levels in the hippocampus of each group ($\bar{x}$±s).

Group	CA1	CA3	DG
T_1			
Blank control	0.19±0.03	0.19±0.04	0.21±0.03
Carrier gas	0.19±0.04	0.20±0.04	0.20±0.03
Sevoflurane anesthesia	0.17±0.03	0.16±0.02	0.18±0.01
F	3.547	12.361	5.706
P	0.035	0.000	0.005
T_3			
Blank control	0.20±0.02	0.20±0.02	0.22±0.02
Carrier gas	0.19±0.03	0.19±0.03	0.21±0.03
Sevoflurane anesthesia	0.17±0.02	0.14±0.01	0.20±0.02
F	12.989	26.791	3.360
P	0.000	0.000	0.040
T_7			
Blank control	0.18±0.03	0.19±0.02	0.20±0.02
Carrier gas	0.19±0.05	0.20±0.04	0.22±0.05
Sevoflurane anesthesia	0.16±0.02	0.20±0.02	0.23±0.04
F	0.905	1.118	3.051
P	0.408	0.333	0.055

P<0.05, the difference was statistically significant.

inhalation of the above anesthetic drugs on memory ability has not been obtained (23). The positioning navigation and space exploration tests were carried out on the animals resting for a certain period with the same steps, in order to determine the preservation and reproducibility of the animal's spatial memory ability. In addition, additional experiments can be performed, removed the platform to the para-quadrant at 24h after the space exploration test completion, and re-carry out the alignment exploration test and counterpoint training, which has a certain effect on the study of animal working memory ability (24). Zhang (25) found that after performing isoflurane anesthesia, the rats were subjected to 12-arm star maze training for 24 h, and the data showed that the memory ability of the rats decreased significantly. Wang (26) used enflurane for related research, and the data obtained were quite different from the data of the scholar, and the study showed that the memory ability of mice increased significantly. The comparison of the data of the two studies confirmed that inhalation of different anesthetic drugs has a different effect on the learning and memory ability. There is no unified view on the specific mechanism of the effect of sevoflurane on memory in rats in clinical studies. Balakrishnan Shanmuganathan (27) showed that a 2.6% concentration of sevoflurane has a certain inhibitory effect on the ability of mice to remember new things. A scholar used 2.5% of anesthesia in the experiment of rat memory ability, but the conclusions obtained in this study are quite different from those in the previous literature. The possible reasons for the different results are: (1) There are differences in the dose of sevoflurane used in the study; (2) The types of memory ability studied are different, and different types of memory ability are related to the function of different parts of the brain (28). The existing literature at this stage is still controversial on the medium- and long-term effects of inhaled anesthetics on memory ability. Wang (29) found the changes in memory ability after isoflurane anesthesia, and the phenomenon lasted about 60 days. Liu (30) showed that after sevoflurane anesthesia, there was no significant difference in spatial memory ability between the 7th and 28th day Morris water maze experiments compared with the normal control group. The reason for the large difference in the conclusions of the above studies may be due to the different types of inhaled anesthetics.

Zhu (31) confirmed that 2.4% is the minimum alveolar concentration (MAC) in adult rats, and the 99% effective dose of sevoflurane is 1.3 MAC. This study simulated the clinical treatment, 3.2% sevoflurane was used to perform the replication of the inhalation anesthesia model. In order to reduce the interference of pure oxygen on the research data, 1 L/min O_2+1 L/min air was used as the carrier gas, and the blank control group was set at the same time. After model replication, the number of crossing original platform, the residence time of the original platform quadrant, number of entering the original platform quadrant, and the percentage of the residence time of the original platform quadrant, as well as the MOD values of hippocampus Aβ and ApoE were compared between the blank control group and the carrier gas group, and there were no significant differences in the indicators above between groups (P>0.05). Eliminate the interference of carrier gas, the experiment was confirmed to have higher authenticity and accuracy.

The data of the study showed that with the increasing of the training days before model replication, the escape latency of rats in each group was increased gradually. The comparison of data showed that there was no significant difference in data between groups (P>0.05). The analysis of the space exploration test showed that there were no significant differences in the number of crossing the original platform, time of entering the original platform quadrant, the residence time of the original platform quadrant, and the percentage of the residence time of the original platform quadrant of rats between groups, indicated that the rats have successfully established the stable learning and memory ability before model replication, and there are no differences in spatial exploration and recall ability, and the baseline was consistent.

Wang (32) indicated that not all rodents meet the requirements of water maze experiments, among them, KM and SAM mice are not suitable for water maze experiments because of their large gene pool, high hybridization rate, obvious individual differences, and the high incidence of not being on stage or re-enter the water after guiding on the stage. The vast majority of inbred lines BALB/C could not be tested for water maze because they could not learn to swim. The water maze test mostly used ICR mice, SD rats and Wistar rats as the main experimental animals, and most of them were male. In addition, the gender, weight, strain, and age of the animals in one experiment should be consistent to reduce the bias of the data.

After the model replication, there were no significant differences in the number of crossing the original platform, number of entering the original platform quadrant, the residence time of the original platform quadrant, and the percentage of the residence time of the original platform quadrant between the sevoflurane anesthesia group and the carrier gas group. It is confirmed that the concentration of sevoflurane commonly used in the clinic has no obvious adverse effects on the recent spatial association and recall ability of adult rats. It is speculated that factors, such as age, disease, and surgery, are related to the recent incidence of POCD in clinical work, and the inhaled anesthetic sevoflurane is not a major risk factor for recent POCD.

The data of this study showed that the expression level of ApoE in the hippocampus was decreased at 1 and 3 days after inhalation of sevoflurane, but there was no significant change in Aβ expression level; The expression level of ApoE in the hippocampus was normal on the 7th day after model replication, and the Aβ expression level was increased to a certain extent. It can be seen that ApoE and Aβ have the same change trend, remaining ApoE at a low level can keep the Aβ expression level at a normal level, the expression level of ApoE at T7 time point returns to normal, but Aβ deposition occurred, which confirmed that ApoE affects Aβ production, but not the unique relevant factor. Analysis of a large number of existing literature confirmed that presenilin and acetylcholine were involved in the process of Aβ changes. It is speculated that sevoflurane may induce the increase of Aβ level in the hippocampus by other means, and down-regulate the expression level of ApoE, inhibit the deposition of Aβ in the hippocam-

pus, so as to maintain the overall Aβ level in the normal range. However, there is a time limit for this effect, and the 7th day after surgery is a key time point for the increase of Aβ. It should be noted that sevoflurane impairs the long-term spatial recall ability of adult rats.

brain-derived neurotrophic factor (BDNF) plays a key role in the formation and storage of memory. Gu (33) found that after knocking out the BDNF gene in mice, the formation of long-term action potentials in hippocampal neurons was inhibited. After the administration of exogenous BDNF, long-term action potentials were restored. The receptor for BDNF is a receptor tyrosine kinase B (TrkB), and the combination of the two can significantly enhance the synaptic transmission process of the excitatory neurotransmitter glutamate, and significantly increase the receptor of N-methyl-D-aspartate (NMDA) at the same time, thus achieving the purpose of regulating the formation and storage of memory. Maintaining long-term memory and forming memory are two distinct stages. The existing literature indicates that the hippocampus does not participate in the long-term maintenance of memory, and memory will be transferred to the cerebral cortex after formation in the hippocampus, and leave a stable memory trace to achieve long-term memory preservation. However, Nie (34) found that escape inhibition experiment, rats need to participate in the process of long-term maintenance of fear memory through the involvement of BDNF in the hippocampus. The expression level of BDNF mRNA in the hippocampus of rats at this stage is significantly increased, if the synthesis process of BDNF is inhibited, it can adversely affect the maintenance of fear memory. Yang (35) found that escape inhibition experiments after inhaling 0.11% sevoflurane can significantly improve the fear memory ability of rats. Chen (36) found that at the aspect of inducing neuronal apoptosis, isoflurane, midazolam and other general anesthetics have a certain effect, so as to achieve the effect of inhibiting the learning and memory ability of experimental rats, BDNF and its receptor TrkB are significantly associated with this occurrence. The decisive factor in memory ability is BDNF. Studies on gene knockout mice showed that mice with lower expression of BDNF in the hippocampus have weaker memory ability on the platform position in the Morris water maze test.

The design of the water maze experiment in this study still has some limitations. In clinical treatment, the combined anesthesia with multiple drugs in general anesthesia is the main method, and usually, sevoflurane will not be used alone. Therefore, this experiment cannot fully reflect the actual situation of clinical anesthesia. Under the training with the same intensity, the size of the pool is an important factor affecting the learning and memory ability of rats. It is necessary to expand the study sample size and expand the study on the effects of inhaled sevoflurane on the spatial recall ability of rats and the expression levels of hippocampal ApoE, Aβ and other related proteins from more research perspectives, in order to provide new ideas for the treatment and prevention of POCD.

In conclusion, inhalation of 3.2% sevoflurane had no significant effect on Aβ deposition in the hippocampus of early adult rats after surgery, and the Aβ deposition achieved mainly by down-regulating ApoE expression levels; the key time points of Aβ increasing in the hippocampus was the 7th day after inhalation of sevoflurane, but there was no significant damage to the spatial recall ability of rats.

References

1. Zhang XT, Zhang L, Zhou YY, Pei YN, Xue WG. Effect of Electro-acupuncture stimulation of GV20 and K11on expressions of hippocampal Aβ and ApoE in APP/PS1 transgenic mice. J Clinic Acupuncture Moxibustion. 2017; 33(12):73-77.
2. Zhou JJ, Que JY, Yu WW, Chen ML, Wu ZQ, Qiu LP, Zhu KJ. Methodology for detecting learning and memory levels in animals by Morris water maze. Chin J Gerontol 2017; 37(24):6274-6277.
3. Xu S, Zhu ZQ, Wang Y, Tang CC, Zhang C. Impact of sevoflurane on short-term spatial memory capacity and expressions of ApoE and amyloid beta in hippocampus of rats. Chin J Modern Med 2018; 28(5):1-6.
4. Wang HP, Wu HY, Li HL, Ma CL, Zeng QT, Cui SM, Zhu KM. Effect of angelica polysaccharide on learning and memory abilities and Aβ metabolism in model rats with Alzheimer disease. Chin J Info TCM 2018; 25(4):51-55.
5. Zheng WW, Wang XL, Meng WJ, Cui YJ, Jin YS, Lu L, Wei Y. Effect if intraventricular injected hpmocysteine on amyloid-beta protein expression and cognitive function in rats with cerebrak ischemia. Chin J Anat 2017; 40(1):26-30.
6. Zhang JC, Xu YJ, Kong QB, Ren R. Effect of β amyloid protein on endoplasmic reticulum stress in rat hippocampal neurons and its mechanism. J Toxicol 2018; 32(1):75-78.
7. HongY, Du SJ, Liu JY, Peng SJ, Liu T, Wang SP. Effect of ulinastatin pretreatment on cognitive function after ketamine anesthesia in juvenile mice. Lingnan Modern Clinic Clinic 2018;18(2):226-229.
8. Li YY, Lu HG. The Physiological and Pathological Functions of Amyloid Precursor Proteins in the Skin Tissue. Chin J Dermatovenereol 2018; 32(5):571-574.
9. Zeng HM. Analysis of the value of serum soluble CD40 ligand (sCD40L) and β-amyloid 1-42 (Aβ 1-42) results in the diagnosis of post-stroke cognitive impairment (PSCI). World Latest Med Info 2018;18(58):193-194.
10. Li MX, Hao Q, Liu Y, An S, Guo XX, Xu TR, Yang Y. The Relationships between Amyloid Precursor Protein Gene Polymorphisms and Alzheimer's Disease. Chin J Cell Biol 2018; 40(9):1599-1605.
11. Dai JW, Duan ZW. Effect of sevoflurane on cognitive function and the expression of β-amyloid protein in the hippocampus of geriatric rats. Chin J Clinic Pharmacol 2018; 34(18):2183-2186.
12. Zhang XP, Ma DL, Liu F, Wang G, Tian JZ, Cui QH. Study on learning and memory impairment of Alzheimer's Disease model rats of Xingnao Yizhi Multi-component Chinese Medicine Group. Pharmacol Clinic Chin Materia Medica 2018; 34(4):144-148.
13. Zhang LG, Zhu CM, Wu Q, Zhang JL, Wang FM. Correlation Study of Amyloid β-Protein 1-42 with Cervical Atherosclerosis and Mild Cognitive Impairment. World J Complex Med 2019; 5(5):19-21.
14. Yang Q, Zhang Lp, Song CM, Zhang YS, Dong RR, Zhang HF. Effects of Resvertrol on Spatial Memory and Synaptic Plasticity in Ovariectomized Rats. Acta Nutrimenta Sinica. 2019; 41(3):276-280.
15. Zhou QY, Huang JR, Chen X, Wang CX. Development of chemiluminescent immunoassay for human serum amyloid A. J Biol 2019; 36(4):85-88.
16. Zhang K, Zhang YT, Fu Y, Wang JW, Fan J, Wei CL, Yuang QJ. Effects of aerobic exercise intervention on spatial learning and memory ability and expression of frontal brain-derived neurotrophic factor in aging rats. Chin J Pub Health 2019; 36(4):1-5.
17. Chen JL, Yu ZH. Changes of Learning - Memory and Amyloid-

beta Protein in Rats With Heart-qi Deficiency. Chin J of Alzheimer's Dis Related Disord 2018; 1(2):143-147.
18. Tang MW, Mei X, Wang FB. Effect of electroacupuncture combined with rich environment on spatial learning and memory ability of diabetic rats. Mil Med J Southeast China 2019; 21(1):17-20.
19. Wang Y, Tao YM, Sun GJ, Xiao JH, Du YJ. Effect of electroacupuncture on inflammatory factors in serum and brain of AD rats induced by Aβ1 ~ 42. Chin J Gerontol 2019; 39(8):1921-1927.
20. Kong XR, Xiu Y. Morris Water Maze Test for the Study of Cognitive Impairment in a Mouse Model of Glutamate Dysfunction. World J Complex Med 2019; 5(1):25-27.
21. Tang L, Wang R, Zong C, Shi GF. Effect of Danggui Buxue Decoction on spatial memory ability of D-galactose-induced aging rats. J Ethnomed Ethnopharm 2019; 28(4):15-17.
22. Zheng NZ, Wang M, Zhao J, Zhang YM. A study of the correlation between amyloid beta protein and the neural network and pathology in the rat model of epilepsy complicated with alzheimer's disease. Chin J Clin Neurosci 2019; 27(3):252-258.
23. Cao GF, Zhang YA, Zhang SM, Zhang HM, Zhu L, Zhu J, Zhou XL, Chen T. Effects of different doses of methamphetamine on spatial learning and memory in mice and the mechanism. J Xi'an Jiaotong Univers (Med Sci). 2019; 40(4):559-563.
24. Xia TG, Diao YF, Bi Y, Zhang J, Chen JL, Dong HJ, Dong YQ. Effect of glycated β-amyloid protein on cognitive function of Alzheimer's disease-like rats. J Xinxiang Med Univers 2019; 36(6):511-516.
25. Zhang XT. Effect of electroacupuncture "Yongquan, Baihui" on amyloid beta protein and apolipoprotein E in hippocampus of APP/PS1 double transgenic mice. Beijing Univers Chin Med 2018.
26. Wang MC, Liu TL, Jiang YH, Han L, Wang WW, Miao YW. SWI in evaluation of abnormal β-amyloid protein deposition in hippocampus of rabbits with type 2 diabetes mellitus. Chin J Med Imag Technol 2019;17(8):1159-1163.
27. Shanmuganathan B, Sathya S, Balasubramaniam B, Balamurugan K, Devi KP. Amyloid-β induced neuropathological actions are suppressed by Padina gymnospora (Phaeophyceae) and its active constituent α-bisabolol in Neuro2a cells and transgenic Caenorhabditis elegans Alzheimer's model. Nitric Oxide. 2019; 91:52-66.
28. Gerhard Leinenga, Wee Kiat Koh, Jürgen Götz. Scanning ultrasound in the absence of blood-brain barrier opening is not sufficient to clearβ-amyloid plaques in the APP23 mouse model of Alzheimer's disease. Brain Res Bullet 2019; 16(8):1158-1161.
29. Wang X. 1. Chitinase-1 enhances TGFβ-Smad signaling pathway and phagocytosis of β-amyloid in microglia; 2. Analysis of incidence of mental behavioral symptoms in patients with memory impairment in memory disorders outpatients. Chongqing Med Univers 2018.
30. Liu N, Liao YH, Lai S, Huang J, Li Y, Huang ZS. Effect of TSG on the Expressions of Caspase-3 and APP in Brain Tissues of APP/PS1 Double Transgenic Mouse Model of Alzheimer's Disease. Chin General Pract 2017; 20(15):1854-1859.
31. Zhu HM, Zhang FL, Xiao ZC, Chen F. The Application and Research Progress of the Role of APP in Neurogenesis and Alzheimer`s Disease. Chin J Ethnomed Ethnopharm 2017; 26(12):74-79.
32. Wang ZG, Zhan Y, Wu XD, Guo J, Lu GX, Zhang T, Zhang GX. Clinical Value of Plasma β-amyloid in the Early Diagnosis of Alzheimer's Disease. Labeled Immunoassays Clinic Med 2017; 24(9):976-978.
33. Gu Y, Wang H, Li JM, Liu J. Diagnostic efficacy of serum soluble CD40 ligand and β-amyloid 1-42 levels on post-stroke cognitive dysfunction. Shandong Med J 2017; 57(43):49-51.
34. Nie JL, Tan SM, Huang QF, Lin X. The protective mechanism of pratensein on Beta-amyloid-induced cognitive deficits in Wistar rats. Pharmacol Clinic Chin Materia Med 2017; 32(3):20-24.
35. Yang WD. Effects of early electroacupuncture on hippocampal ultrastructure, expression of Aβ and Tau in SAMP8 mice. Fujian Univers Trad Chin Med 2019.
36. Chen JL. 1. Ang-(1-7) attenuates AD-like neuropathy in diabetic rats by activating Mas receptor. 2, metformin relieves diabetic encephalopathy Tau protein lesions by promoting autophagy. Chongqing Med Univers 2017.