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Original Article

Sevoflurane exposure induces short-term impairment of hippocampal synaptic plasticity in aged rats

Jiacun Xie[1], Xin Tian[2], Aili Liu[2]

[1]*Department of Oncology Radiotherapy, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou 450003, China;* [2]*School of Biomedical Engineering, Tianjin Medical University, Tianjin 300070, China*

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Abstract: As a type of common anesthetic, sevoflurane is taken as a risk factor for postoperative cognitive dysfunction (POCD), especially in aged patients. Experimental studies in animals have been done to explore the effects of sevoflurane exposure on cognitive function. Hippocampal synaptic plasticity is well recognized to involve in learning and memory in rodents. However, sevoflurane-induced changes in hippocampal synaptic plasticity on aged rats is still unclear. This study aimed to investigate the lasting effects of sevoflurane exposure on hippocampal synaptic plasticity in aged rats. In this study, 18-month old Sprague-Dawley (SD) rats were randomly assigned to 3 groups: Con group, Sevo 24 hour group and Sevo 72 hour group. The rats in the Sevo 24 hour group and Sevo 72 hour group received 1.5 minimum alveolar concentration (MAC) sevoflurane for 2 hours. Then, long-term potentiation (LTP) and long-term depression (LTD) at hippocampal CA3-CA1 synapse was respectively evaluated at 24 and 72 hours after sevoflurane exposure via *in vivo* electrophysiological experiments. Sevoflurane anesthesia significantly suppressed the induction of hippocampal LTP and LTD at 24 hours post-exposure ($p<0.01$). In contrast, induction of hippocampal LTP and LTD was not affected 72 hours after sevoflurane exposure ($p>0.05$). These findings show that exposure to 1.5 MAC sevoflurane for 2 hours in aged rats impaired induction of hippocampal LTP and LTD at 24 hours post-exposure, but not at 72 hours after anesthesia. Sevoflurane exposure can induce the short-term impairment of hippocampal synaptic plasticity in aged rats.

Keywords: Sevoflurane, synaptic plasticity, LTP, LTD, aged rats, *in vivo*

Introduction

Postoperative cognitive dysfunction (POCD) is a common clinical phenomenon and its general characteristic is shown as cognitive decline in patients after surgery, especially in elderly surgical patients [1-3]. POCD leads to a lower quality of life, increased dependency, and mortality [4]. According to a prospective study, about 15.9% of aged patients have suffered from POCD after major non-cardiac surgery. POCD has become a significant global health issue with the increasing emphasis in aged patients [5, 6]. Evidence indicates that anesthetic agents could be a risk factors for POCD [7, 8].

As an inhalation anesthetic, sevoflurane has many advantages including low blood-gas partition coefficient, rapid induction and recovery. It has been widely used in the induction and maintenance of general anesthesia [9-11]. However, numerous animal experiments [12, 13] and clinical reports [14, 15] have also presented the neurotoxicity caused by sevoflurane. Studies have been done to uncover the effects of sevoflurane on the aged. A recent study proposed that sevoflurane reduces the frequency of electroencephalogram (EEG), alpha-band EEG power and coherence in elderly patients [16, 17]. Furthermore, several studies have reported that sevoflurane exposure induces neuronal apoptosis and memory impairment in aged rats [18-20]. Although accumulated evidence indicates that sevoflurane anesthesia induces cognitive dysfunction in aged animals [21-23], the underlying mechanisms are largely unknown.

Hippocampal synaptic plasticity is well recognized as a cellular mechanism of learning and

Table 1. Arterial blood analysis (mean ± SEM)

Group	Time	PH	PaO_2 (mmHg)	$PaCO_2$ (mmHg)
Con	Before	7.39 ± 0.02	93.15 ± 3.62	32.67 ± 1.57
	After	7.37 ± 0.05	94.63 ± 4.79	31.24 ± 3.15
Sevo 24 h	Before	7.39 ± 0.01	95.03 ± 2.87	30.33 ± 4.01
	After	7.38 ± 0.03	94.25 ± 3.45	31.39 ± 2.84
Sevo 72 h	Before	7.38 ± 0.04	94.23 ± 2.27	31.13 ± 3.01
	After	7.37 ± 0.03	93.75 ± 3.05	32.39 ± 1.84

PH: potential of hydrogen, PaO_2: partial pressure oxygen, $PaCO_2$: partial pressure carbon dioxide. Data are presented as mean ± SEM.

memory [24, 25], but the effects of sevoflurane exposure on hippocampal synaptic plasticity in age is still relatively lacking. Additionally, the lasting effects of sevoflurane on synaptic plasticity remains unclear. Therefore, in the present study, the synaptic plasticity of hippocampal CA3-CA1 synapses was evaluated at 24 hours and 72 hours after administration of 1.5 minimum alveolar concentration (MAC) sevoflurane via long-term potentiation (LTP) and long-term depression (LTD) to explore the lasting effects of sevoflurane exposure on synaptic plasticity.

Materials and methods

Animals and groups

All experimental procedures were approved by the Animal Care and Use Committee of Tianjin Medical University. Male Sprague-Dawley (SD) rats (18 months, 500-550 g) were obtained from the Experimental Animal Center of Tianjin Medical University (Tianjin, China). All rats were housed in a temperature-controlled room under 12 hour light-dark cycle with *ad libitum* access to water and food.

Rats were randomly divided into Control (Con) group, Sevo 24 (24 hours after sevoflurane anesthesia) group and Sevo 72 (72 hours after sevoflurane anesthesia) group. Rats in each group were randomly assigned to measure LTP and LTD at CA3-CA1 synapses in the hippocampus.

Sevoflurane anesthesia

Rats in the sevoflurane anesthesia groups (Sevo 24 group and Sevo 72 group) were placed in an anesthetic induction box. The box bottom was covered with calcium lime to adsorb carbon dioxide and allowed three rats to be anesthetized at the same time. During the anesthesia, 1.5 MAC sevoflurane was taken into the induction box by oxygen (flow rate of approximately 4 L/min) from sevoflurane vaporizein. The whole process lasted for 2 hours and was monitored by a gas detector (Detex-Ohmeda, Louisville, KY, USA). The body temperature of rats was maintained at 36 ± 1°C with electric blanket. The rats in the Control group were placed in the same induction box and passed oxygen for 2 hours at the same flow rate. After sevoflurane exposure, rats were placed back into rearing cages after a 30-minute recovery.

Blood gas analysis

Before and after the intervention experiment (sevoflurane exposure and oxygen exposure), all groups of rats were subjected to blood gas analysis. Arterial blood samples were collected from femoral artery to measure the blood pH, $PaCO_2$ (arterial carbon dioxide tension) and PaO_2 (arterial oxygen tension) with blood gas analyzer (GEM Premier 3000, Instrumentation laboratory, Bedford, MA). The results showed no significant difference appeared in the values of blood pH, $PaCO_2$ and PaO_2 between before and after the intervention (**Table 1**).

Electrophysiological experiment

All groups of rats were performed *in vivo* electrophysiological experiments including hippocampal CA3-CA1 synapses LTP and LTD, as described previously [26].

The rats were fixed on a stereotaxie apparatus (SN-2, Narishige, Tokyo, JAPAN), and the scalp was cut using scalpel to clearly expose the skull and bregma. The positions of the stimulating electrode and recording electrode are shown in **Figure 1A**. The stimulating electrode (diameter 300 μm) was bipolar Teflon wrapped stainless steel wire and implanted into schaffer collateral (position: 3.5 mm lateral and 4.0 mm posterior to bregma; depth from brain surface: 3-3.5 mm) (**Figure 1B**). The recording electrode was made by drawing the silicate capillary glass tube through a P-97 horizontal puller (Sutter Instrument, CA, USA) and positioned in the stra-

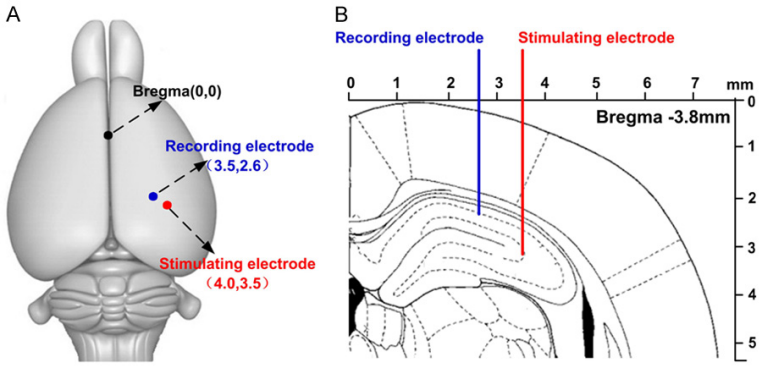

Figure 1. Positions of stimulating and recording electrodes implanted in the rat brain. A. Dorsal view of rat brain. Bregma, represented as black dot, is defined as origin of coordinate (0, 0). Blue dot showed the location of recording electrode (2.6 mm lateral and 3.5 mm posterior to bregma), while the location of stimulating electrode (3.5 mm lateral and 4.0 mm posterior to bregma) is marked as hot dot. B. Diagram of coronal sections from rat brain (3.8 mm posterior to bregma, from the *Stereotaxic Coordinates* [27]). The positions of stimulating and recording electrodes at CA3-CA1 synapses in hippocampus was showed.

tum radiatum area of hippocampal CA1 (position: 2.6 mm lateral and 3.5 mm posterior to bregma; depth from brain surface: 2-2.5 mm) (**Figure 1B**). The resistance of recording electrode filled with sodium chloride (2 mol/L) was 4-6 MΩ. Referring to the locations of stimulating and recording electrodes, two small holes were drilled using a dental drill on the skull. To facilitate implanting electrodes, the dura mater was carefully removed to expose the cortex.

After surgery, a series of basic stimulus (frequency: 0.033 Hz, intensity: 0.4-0.8 mA) was produced by Master-8 stimulator (A.M.P.I, Jerusalem, Israel) to evoke the field excitatory postsynaptic potentials (fEPSPs) at hippocampal CA3-CA1 synapses. The details were as follows: first, the stimulating electrode was gradually stepped into the sub-cortex approximately 2.5 mm and then the recording electrode was stepped into the sub-cortex about 1.5 mm. At this moment, a positive waveform would appear at the location about 10 ms behind the stimulus artifact (**Figure 2Aa**). Second, the depth of recording electrode was maintained and the stimulating electrode was slowly moved down. The wave amplitude gradually in-creased and then declined. Additionally, a negative waveform appeared prior to the positive waveform (**Figure 2Ab-d**). Third, the stimulating electrode was persistently moved down. The amplitude of negative waveform gradually increased and the positive waveform disappeared (**Figure 2Ae**). Finally, the positions of the stimulating electrode and recording electrode were adjusted slightly until the amplitude of negative waveform reached the maximum (**Figure 2Af**).

In the rest of the experiment, the depth of the stimulating and recording electrodes remained unchanged. After 20 minutes when the fEPSPs stabilized, the stimulus intensity was increased step by step (step=0.1 mA). The amplitude of fEPSPs increased along with stimulus intensity and tended to be stable. The stimulus intensity which activated the maximal amplitude of fEPSPs was defined as the maximum stimulus intensity (MSI) (**Figure 2B**, **2D**). Before inducing the LTP or LTD, the fEPSPs under 50% MSI was recorded for 30 minutes and calculated as baseline. High-frequency stimulation (HFS) (10 bursts of 20 pulses at 200 Hz, each burst separated by 2 seconds, 75% MSI) was used to induce LTP (**Figure 2C**) and low-frequency stimulation (LFS) (900 bursts of 4 pulses at 250 Hz, each burst separated by 1 seconds, 50% MSI) was applied to induce LTD (**Figure 2E**). After HFS or LFS, the stimulus intensity was reset to 50% MSI. The fEPSPs was continually recorded for 60 minutes. The data were obtained using Axoclamp 2B amplifier (Molecular Devices, Foster City, CA, USA), Digidata 1322A (Molecular Devices, Foster City, CA, USA) and pCLAMP 10.0 (Molecular Devices, Foster City, CA, USA).

Statistical analysis

The experiment data were analyzed offline using the pCLAMP Clampfit 10.0 (Molecular Devices, Foster City, CA, USA) and Origin 8.0 (OriginLab Corp, Northampton, MA, USA). All data are presented as mean ± standard error (S.E.M.) in the figures and tables. Statistical analysis was performed by using t-test and one-way ANOVA (IBM SPSS 19, Chicago, IL). $p<0.05$ was considered as statistical significance.

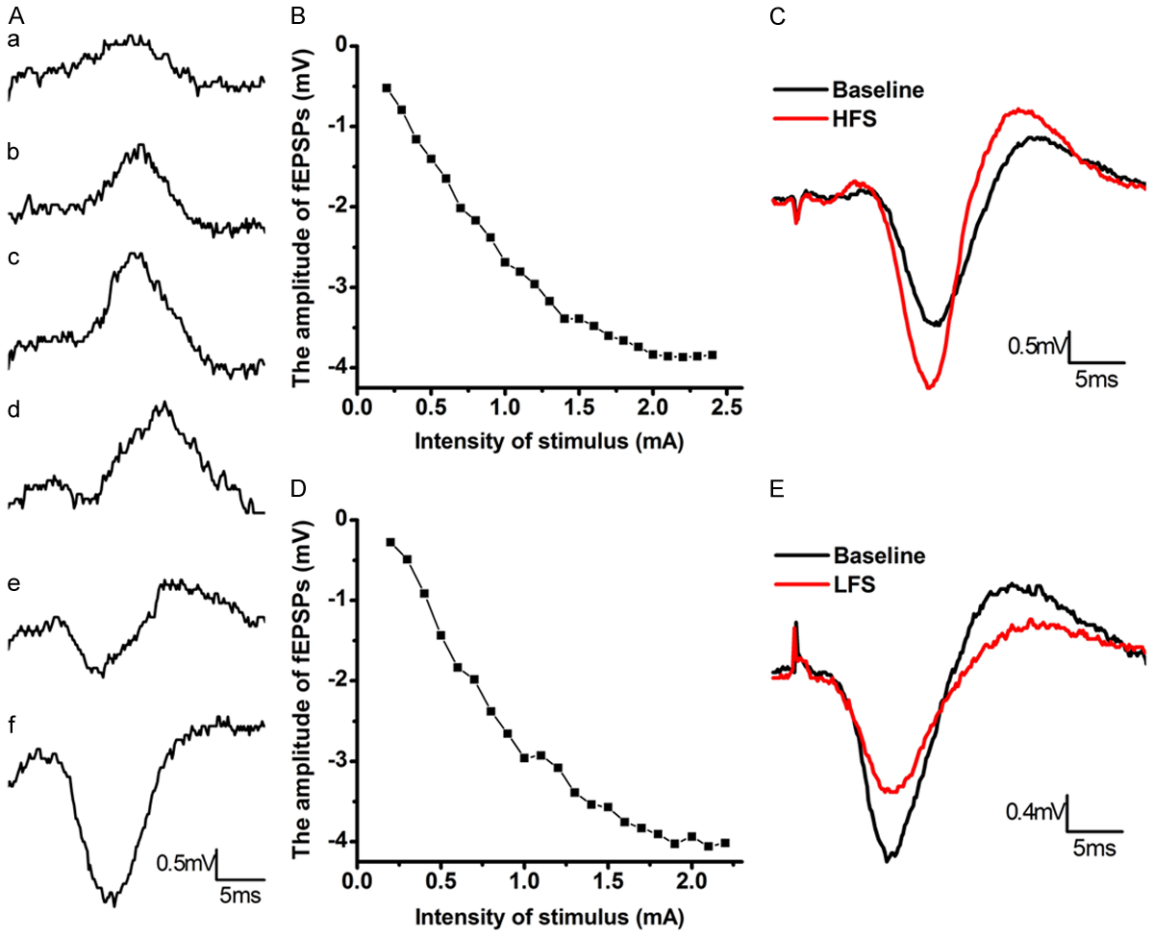

Figure 2. Induction of LTP and LTD at hippocampal synaptic plasticity in aged rats. A. Waveform changed with the positions of stimulating and recording electrodes. f showed the fEPSPs under a given stimulus. B, D. The amplitude of fEPSPs changed with increasement of stimulus intensity. Maximum stimulus intensity (MSI) said the stimulus intensity that evoked the maximal amplitude of fEPSPs. C. Comparison of fEPSPs amplitude before and after HFS. E. Comparison of fEPSPs amplitude before and after LFS.

Results

Sevoflurane exposure inhibited the induction of hippocampal LTP

After HFS, the fEPSPs were recorded for 60 minutes under basic stimulus in the Sevo 24 group and Sevo 72 group. The fEPSPs amplitude of LTP in the three groups was normalized to baseline (**Figure 3A**). The fEPSPs amplitude of LTP in Sevo 24 group was 116.27 ± 2.79%, which was significantly lower than that in the Con group (138.75 ± 3.75%) ($p<0.001$) (**Figure 3B**). Meanwhile, the fEPSPs amplitude of LTP in Sevo 72 group was 134.02 ± 2.29%, showing no statistical difference between the Sevo 72 group and Con group ($p>0.05$) (**Figure 3B**). These results showed that 1.5 MAC sevoflurane exposure induced a significant LTP suppression in the Sevo 24 group but not in the Sevo 72 group, suggesting that 1.5 MAC sevoflurane exposure induces short-term LTP inhibition in aged rats.

Sevoflurane exposure inhibited the induction of hippocampal LTD

The LTD at hippocampal CA3-CA1 synapses in aged rats was also evaluated in the Sevo 24 group and Sevo 72 group. The fEPSPs amplitude of LTD in the three groups were normalized to baseline and showed in **Figure 4A**. The fEPSPs amplitude of LTD was 68.57 ± 3.38% in the Con group and 89.99 ± 4.41% in the Sevo 24 group. The results presented significant statistical difference on the induction of LTD

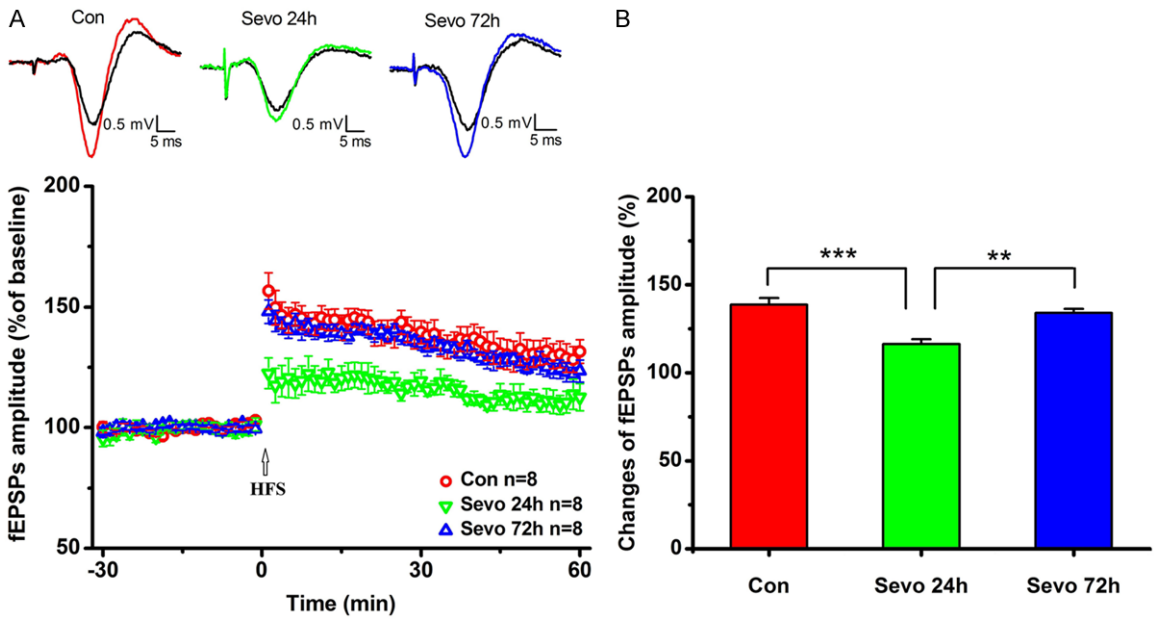

Figure 3. Effects of sevoflurane exposure on the induction of LTP in aged rats. A. The amplitude of fEPSPs before and after HFS. All data were normalized to baseline and plotted against time. The arrow indicates the application of HFS (10 bursts of 20 pulses at 200 Hz, each burst separated by 2 sec.). Insets showed the waveform of fEPSPs before and after HFS in the three groups. B. Changes of fEPSPs amplitude in the three groups. All data were represented by mean ± SEM. **p<0.01, ***p<0.001, n=8.

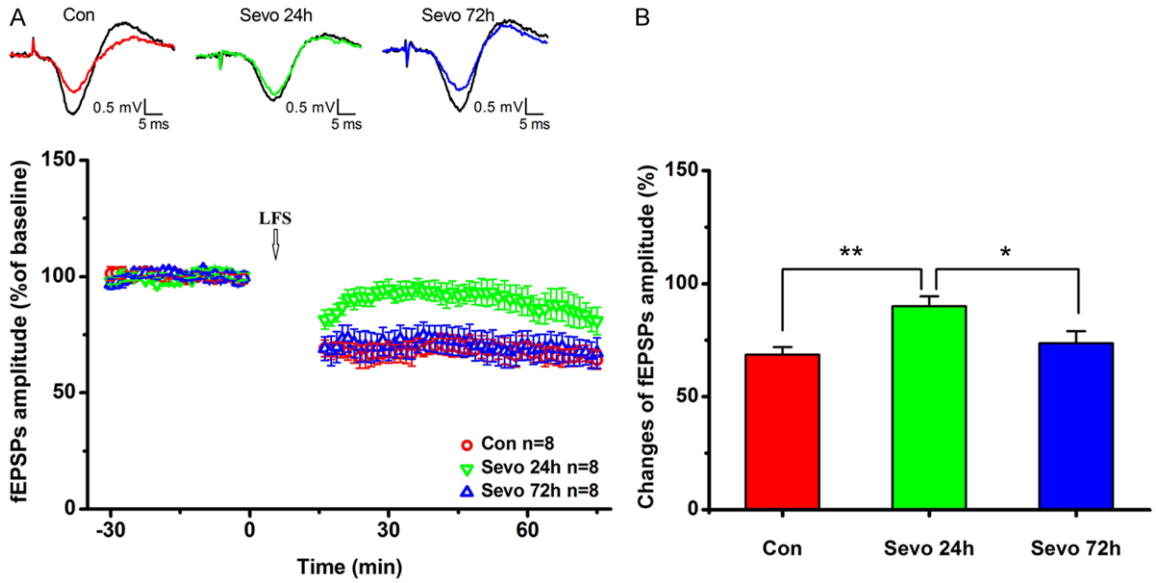

Figure 4. Effects of sevoflurane exposure on the induction of LTD in aged rats. A. The amplitude of fEPSPs before and after LFS. All data were normalized to baseline and plotted against time. The arrow indicates the application of LFS (900 bursts of 4 pulses at 250 Hz, each burst separated by 1 sec.). Insets showed the waveform of fEPSPs before and after LFS in the three groups. B. Changes of fEPSPs amplitude in the three groups. All data were represented by mean ± SEM. **p<0.01, *p<0.05, n=8.

between the Con group and Sevo 24 group (p<0.01) (**Figure 4B**). The fEPSPs amplitude of LTD (73.63 ± 5.33%) in the Sevo 72 group showed no statistical difference compared with Control (p>0.05, **Figure 4B**). These results indicate that induction of LTD was inhibited in the

Sevo 24 group but not in the Sevo 72 group. The sevoflurane-induced hippocampal LTD inhibition in aged rats was also short-term.

Discussion

With age, the brain and cognition are more likely to be affected. It has been confirmed that the alterations of synaptic connectivity in hippocampus were closely related to age-related impairment of cognitive function [28-32]. In this study, the effect of sevoflurane exposure on synaptic plasticity of CA3-CA1 in aged rats was detected and it was explored how long the sevoflurane-induced influence would last.

First, *in vivo* electrophysiological experiments were respectively performed on aged rats at 24 and 72 hours after exposure to 1.5 MAC sevoflurane to evaluate the LTP of hippocampal CA3-CA1 synapse. The results show that the fEPSPs amplitude of LTP in the Sevo 24 group was significantly lower than that in the Con group. However, there was no significant difference in LTP induction between the Sevo 72 group and Con group. The results suggest that the blocked of 1.5 MAC sevoflurane for the induction of hippocampal LTP was short-term. Furthermore, induction of hippocampal LTD at CA3-CA1 synapses of the aged rats at 24 and 72 hours after 1.5 MAC sevoflurane exposure. Results showed that the fEPSPs amplitude of LTD in Sevo 24 group was significantly greater than that in Con group. While the fEPSPs amplitude of LTD in the Sevo 72 group has no statistical difference comparing with that in Con group. These results indicate that sevoflurane-induced inhibition in the induction of hippocampal LTD in aged rats was also short-term.

Although sevoflurane suppressed the induction of hippocampal LTP and LTD in aged rats at 24 hours after anesthesia, the changes were not found at 72 hours. Our findings were consistent with a previous study about effects of sevoflurane on aged rats, which found that 1.5 MAC sevoflurane exposure for 2 hour induced cognitive impairment by conducting the Y-maze test in aged rats [33]. In addition, a similar study also revealed that sevoflurane impaired the spatial learning and memory at 1 day after anesthesia exposure in aged mice with 1 hour daily 3% sevoflurane anesthesia for three consecutive days, but not at 21 days following sevoflurane exposure [21]. Further studies are expected to investigate the mechanism underlying sevoflurane-induced short-term impairment on hippocampal synaptic plasticity.

In conclusion, these results indicate that sevoflurane exposure (1.5 MAC for 2 hours) to aged rats impaired the hippocampal synaptic plasticity, but the impairment was short-term. It is expected to provide insights to the mechanism underlying the POCD induced by sevoflurane on aging brain.

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Address correspondence to: Aili Liu, School of Biomedical Engineering, Tianjin Medical University, 22 Qixiangtai Road, Tianjin 300070, China. Tel: 18630883216; E-mail: liuzitong.aili@163.com

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