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Original Article

Effects of propofol anesthesia on cognitive function and expression of c-fos and c-jun in rats after operation

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Abstract: Objective: This study aims to explore the effects of propofol anesthesia on cognitive function and expression of c-fos and c-jun in rats after operation. Methods: A total of 54 rats were divided into control group (N), propofol group (P) and operation + propofol anesthesia group (S) randomly. The ability of spatial learning and memory of rats were tested by Morris water maze. Hippocampal histopathological changes were determined by HE staining. The changes of c-fos and c-jun were determined by RT-PCR, western blotting and immunohistochemical methods. Results: Compared with N group, the escape latency extended, the residence time percentage of original platform and the frequency through the original platform decreased in P group and S group after operation for 1 d, 3 d and 5 d ($P<0.05$). However, there were no obvious differences among the groups after operation for 7 d ($P>0.05$). The levels of c-fos and c-jun in P group and S group increased after operation for 3 d ($P<0.05$) while there were no obvious differences among the groups after operation for 7 d ($P>0.05$). The effect of propofol anesthesia on the changes of hippocampal tissues in rats after operation gradually decreased with time-lapse. Conclusions: The effects of propofol anesthesia on the cognitive function of rats were gradually decreased with time-lapse, which maybe related with the changes of hippocampal tissues and the levels of c-jun and c-fos.

Keywords: Propofol, cognitive dysfunction, c-fos, c-jun

Introduction

Postoperative cognitive dysfunction (POCD) means the change of spirit, personality and cognitive ability after surgery. Its clinical manifestations include the declined abilities of memory, attention and language comprehension. This syndrome can last a few days, months or longer after operation. Monk found that the mortality of patients with POCD increased in the first year after surgery [1]. Steinmetz thought that POCD not only increased the mortality, but also brought the economic burden to the families and the society [2]. Hippocampus is an important part of learning, memory, and other nerve activity. The proper and complete realization of the cognitive function depends on the morphology and structure of the cells in the hippocampus. Genes c-fos and c-jun belong to the family of proto oncogene, they play important roles in cell growth, differentiation, learning and memory activities, their expression levels are low in most cells (including hippocampus). They can be activated by second messengers and express rapidly under stimulation in vitro.

Propofol is a kind of intravenous anesthetics with relatively wide clinical application and has the characters of acting fast, short action time and less adverse reactions. It was reported that the use of propofol could reduce the cognitive function of postoperative patients [3], but the impact duration remained unknown. In this study, we observed its effect time on the cognitive function of rats and the expression of c-fos and c-jun in hippocampus tissues.

Materials and methods

Experimental animals

A total of 54 SPF level male Sprague Dawley (SD) rats weighing 200±20 g were obtained

Table 1. Real-time PCR primers

Gene	Primer (5'-3')	bp
c-fos	For: GAAGGAACCAGACAGGTCCA	442
	Rev: TCACCCTGCCTCTTCTCAAT	
c-jun	For: TGGGCACATCACCACTAC AC	408
	Rev: ACTTCCTTCTCGGCGTCT	
β-actin	For: GTCGTACCACTGGCATTGTG	291
	Rev: CTCTCAGCTGTGGTGGTGAA	

from the animal experimental center of Shanghai. These SD rats were pre-feeding for 2 days with free access to food and water to adapt to the environment. They were divided into 3 groups randomly: control group (N), propofol group (P) and operation + propofol anesthesia group (S) according to the random number table. Each group has 18 rats. Cages, food and water were regularly changed. In S group, the rats were anaesthetized with intraperitoneal injection of 100 mg/kg propofol and fixed, posterior abdominal longitudinal incision was performed and peritoneum was opened, abdominal cavity was explored intermittently for 2 h, the incision was sutured after operation. Cephalosporins were injected intraperitoneally for the prevention of infection daily.

Housing and procedures involving experimental animals were in accordance with the Guide for the Care and Use of Laboratory Animals (eighth edition, published by the National Academies Press). All experimental procedures were approved by the Care of Experimental Animals Committee of our hospital.

Cognitive function measurement

The spatial learning and memory ability of rats were tested using Morris water maze. Laboratory lighting and items placed position remained unchanged during the whole cognitive function test process to eliminate the interference of environmental factors on the experimental animals.

Place navigation test

Escape latency was the time needed for rats from diving water to climb up the platform. Escape latency was recorded as 120 s if rats could not find the platform in 120 s. The rats were tested 3 times per experiment after operation for 1 d, 3 d, 5 d and 7 d respectively and the average value of 3 times were calculated.

Spatial probe test

The platform was withdrawn after place navigation test. The frequency through the original platform and residence time in the original platform quadrant in 120 s was recorded.

Sample collection

8 rats in each group were sacrificed after operation for 3 d and 7 d respectively, brain hippocampus tissues were taken out and stored for the follow experiments.

Quantitative RT-PCR (qRT-PCR)

Total RNA was extracted using E.Z.N.A.® Total RNA Kit I (Omega Bio-Tek, Inc. Norcross, GA, USA) according to the manufacturer's instructions. cDNAs were synthesized using PrimeScript RT Master Mix (Takara Bio, Inc. Shiga, Japan). The RT-PCR reaction was performed using ABI 7500 Fast (Applied Biosystems, Foster City, CA, USA) with SYBR Premix Ex Taq II (Takara). The cycle condition was: denaturation at 95°C for 30 sec, 40 amplification cycles at 95°C for 3 sec and 60°C for 30 sec. β-actin was used as the control. Primer sequences used in this study were shown in **Table 1**.

HE staining

The hippocampus samples were fixed in 10% formalin and embedded in paraffin routinely. The paraffin blocks of specimen were cut into continuous sections with 5 μm respectively. The sections were dewaxed with xylene and washed with ethanol and water. They were stained with hematoxylin after that and then differentiated, washed and stained with eosin, then dehydrated, hyalinized and finally mounted on slides and observed under microscope, pictures were taken.

Immunofluorescent staining

Samples fixed in 10% formalin were subsequently embedded in paraffin, and sections of 4-mm thickness were cut from the formalin-fixed samples. The sectioned tissue was deparaffinized in xylene and then rehydrated in a graded ethyl alcohol series. For increased specificity and sensitivity, tissues were microwaved for 10 min for antigen retrieval. Following cooling and rinsing in distilled water, endogenous peroxide activity was blocked with 3%

Table 2. Comparison of escape latency after operation in different group (s, $\bar{x}\pm s$)

Group	Postoperative 1 d	Postoperative 3 d	Postoperative 5 d	Postoperative 7 d
N	29.51±5.28	23.51±5.28[c]	16.37±7.25[c,d]	10.28±4.03[c,d,e]
P	45.78±11.46[a]	35.72±12.64[a,c]	22.46±6.59[a,c,d]	11.37±3.51[c,d,e]
S	55.69±16.34[a,b]	40.08±7.69[a,b,c]	27.52±5.97[a,b,c,d]	11.67±4.92[c,d,e]

Compared with N group, [a]$P<0.05$; Compared with P group, [b]$P<0.05$; Compared with postoperative 1 d, [c]$P<0.05$; Compared with postoperative 3 d, [d]$P<0.05$; Compared with postoperative 5 d, [e]$P<0.05$.

Table 3. Comparison of residence time in original platform (%, $\bar{x}\pm s$)

Group	Postoperative 1 d	Postoperative 3 d	Postoperative 5 d	Postoperative 7 d
N	27.51±5.28	33.59±6.25[c]	37.52±8.26[c,d]	41.56±7.12[c,d,e]
P	24.69±9.24[a]	30.72±10.64[a,c]	34.45±6.59[a,c,d]	39.96±5.69[c,d,e]
S	20.97±7.02[a,b]	23.58±5.61[a,b,c]	29.59±5.97[a,b,c,d]	39.47±4.07[c,d,e]

Compared with N group, [a]$P<0.05$; Compared with P group, [b]$P<0.05$; Compared with postoperative 1 d, [c]$P<0.05$; Compared with postoperative 3 d, [d]$P<0.05$; Compared with postoperative 5 d, [e]$P<0.05$.

Table 4. Comparison of the frequency through the original platform (time, $\bar{x}\pm s$)

Group	Postoperative 1 d	Postoperative 3 d	Postoperative 5 d	Postoperative 7 d
N	4.51±1.20	5.89±1.23[c]	6.52±1.26[c,d]	6.56±1.12[c,d]
P	3.31±0.73[a]	4.72±0.69[a,c]	5.45±1.53[a,c,d]	6.26±1.53[c,d,e]
S	2.91±1.04[a]	3.58±0.97[a,b,c]	4.98±5.97[a,b,c,d]	6.07±2.01[c,d,e]

Compared with N group, [a]$P<0.05$; Compared with P group, [b]$P<0.05$; Compared with postoperative 1 d, [c]$P<0.05$; Compared with postoperative 3 d, [d]$P<0.05$; Compared with postoperative 5 d, [e]$P<0.05$.

H_2O_2 for 10 min, and the samples were then rinsed in 0.01 mol/l phosphate-buffered saline (PBS, pH 7.4) for 10 min. The sections were subsequently preincubated with a protein blocking solution for 10 min, prior to incubation with the primary antibodies at 4°C overnight in a humid chamber. The slides were then washed three times in PBS and incubated with secondary biotinylated antibody for 15 min at room temperature. The streptavidin-peroxidase method was used to detect the antigen-antibody complexes, and diaminobenzidine (DAB) was used as the chromogen substrate. The sections were stained and observed under microscope.

Western blotting

Hippocampus tissues were lysed in RIPA buffer with 10% phenylmethylsulfonyl fluoride. The cell extracts were loaded on 10% SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride membranes. The membranes were blocked for 1 h at room temperature with 5% non-fat milk in TBST, and then incubated with primary antibodies at 4°C overnight. Following incubation with HRP-conjugated secondary antibody (diluted at 1:2,000, Abcam), immuno-complexes were visualized by an enhanced chemiluminescence detection under FluorChem M Systerm (ProteinSimple, San Jose, CA, USA). Endogenous β-actin was used for normalization.

Statistical analysis

Results were presented as means ± SD. Statistical analysis was performed using one-way and two-way ANOVA or the Student's t-test using SPSS 17.0 software. $P<0.05$ was considered to be statistically significant.

Results

Escape latency comparison

The comparisons were shown in **Table 2**. It showed that the escape latency in P group and S group was longer than that of N group after operation for 1 d, 3 d and 5 d ($P<0.05$) and the escape latency in S group was longer than that of P group ($P<0.05$). However, there were no obvious differences among the groups after operation for 7 d ($P>0.05$). These suggested that the effect of propofol anesthesia on the escape latency of rats after operation was short.

Comparison of residence time in original platform

As shown in **Table 3**, compared with N group, the residence time percentage of original platform in P group and S group decreased after operation for 1 d, 3 d and 5 d ($P<0.05$) and it decreased in S group compared with P group ($P<0.05$). However, there were no obvious dif-

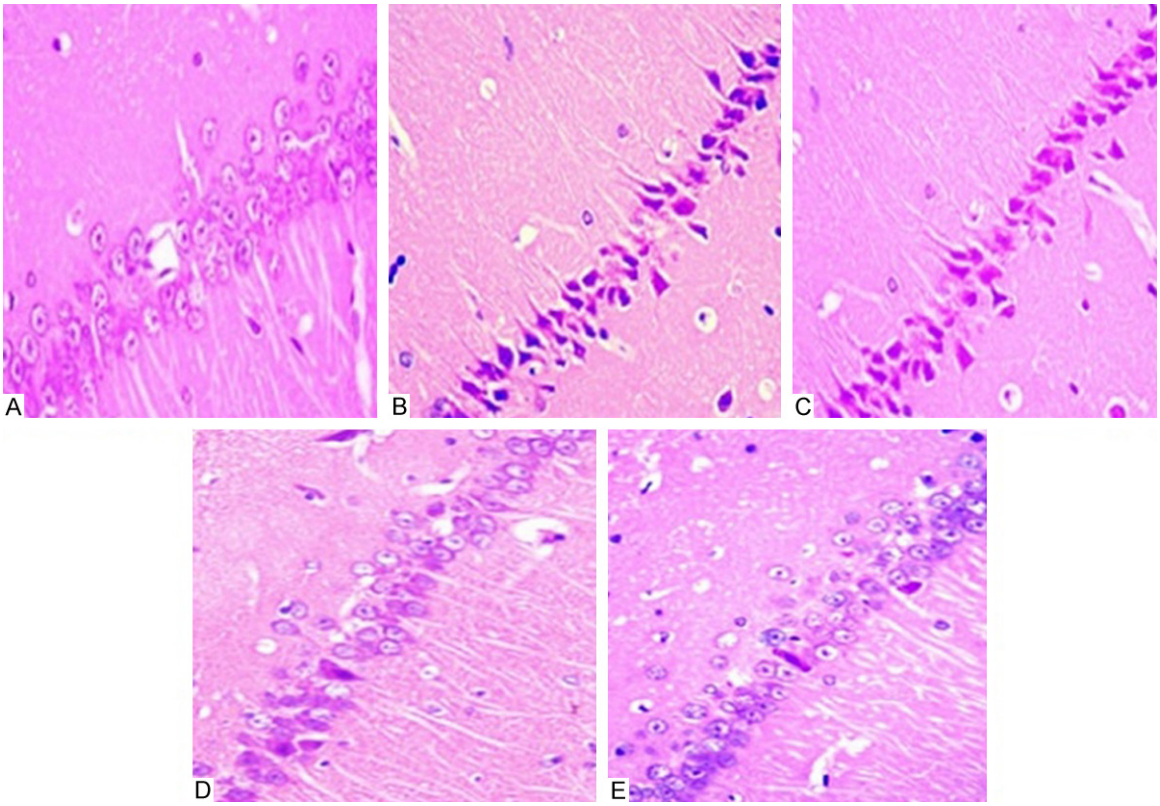

Figure 1. Pathological changes of hippocampus tissues in rats. A: Postoperative 3 d in N group; B: Postoperative 3 d in P group; C: Postoperative 3 d in S group; D: Postoperative 7 d in P group; E: Postoperative 7 d in S group.

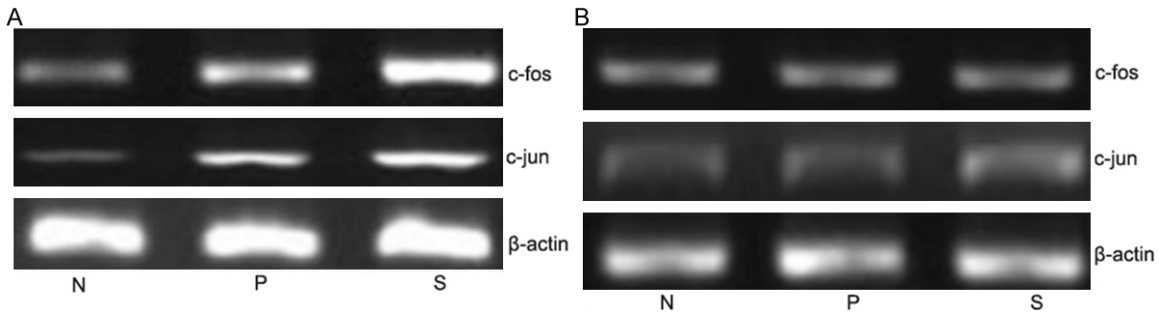

Figure 2. RT-PCR results of c-fos and c-jun in different groups. A: Postoperative 3 d; B: Postoperative 7 d.

ferences among the groups after operation for 7 d (P>0.05). These suggested that the effect of propofol anesthesia on the residence time percentage of original platform of rats after operation was short.

Comparison of the frequency through the original platform

The frequency through the original platform in different groups was shown in **Table 4**. It showed that the frequency through the original platform in P group and S group decreased compared with N group after operation for 1 d, 3 d and 5 d (P<0.05) and it decreased in S group compared with P group (P<0.05). However, there were no obvious differences among the groups after operation for 7 d (P>0.05). These suggested that the effect of propofol anesthesia on the frequency through the original platform of rats after operation was short.

Pathological changes of hippocampus tissues in rats

The HE staining results were shown in **Figure 1**. It showed that neuronal cells were conical or elliptical and arranged neatly in N group after

Table 5. Normalized expression of c-fos and c-jun mRNA in different groups ($\bar{x}\pm s$)

Group	c-fos		c-jun	
	Postoperative 3 d	Ppostoperative 7 d	Postoperative 3 d	Postoperative 7 d
N	0.33±0.06	0.25±0.03[c]	0.52±0.06	0.36±0.02[c]
P	0.52±0.13[a]	0.26±0.09[c]	0.75±0.03[a]	0.36±0.09[c]
S	0.78±0.14[a,b]	0.27±0.07[c]	0.98±0.27[a,b]	0.37±0.10[c]

Compared with N group, [a]$P<0.05$; Compared with P group, [b]$P<0.05$; Compared with postoperative 3 d, [c]$P<0.05$.

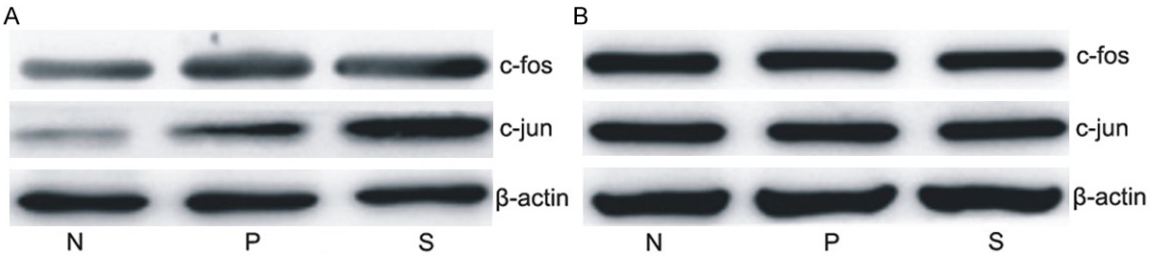

Figure 3. Western blotting results of c-fos and c-jun in different groups. A: Postoperative 3 d; B: Postoperative 7 d.

Table 6. Protein levels of c-fos and c-jun in different groups ($\bar{x}\pm s$)

Group	c-fos		c-jun	
	Postoperative 3 d	Postoperative 7 d	Postoperative 3 d	Postoperative 7 d
N	0.43±0.05	0.95±0.14[c]	0.22±0.03	0.96±0.21[c]
P	0.72±0.23[a]	0.96±0.18[c]	0.45±0.03[a]	0.94±0.19[c]
S	0.98±0.14[a,b]	0.96±0.15[c]	1.08±0.27[a,b]	0.92±0.16[c]

Compared with N group, [a]$P<0.05$; Compared with P group, [b]$P<0.05$; Compared with postoperative 3 d, [c]$P<0.05$.

operation for 3 d (**Figure 1A**). Cell layer was significantly reduced with deformed cells, cytosolic concentration and nuclear condensation in P group and S group after operation for 3 d (**Figure 1B**, **1C**). However, the cells almost recovered in P group and S group after operation for 7 d (**Figure 1D**, **1E**). These suggested that the effect of propofol anesthesia on the changes of hippocampal tissues in rats after operation gradually decreased with time-lapse.

Expression level changes of c-fos and c-jun mRNA

RT-PCR results were shown in **Figure 2** and **Table 5**. The expression levels of c-fos and c-jun mRNA in P group and S group were significant higher than that of N group after operation for 3 d ($P<0.05$) and the expression levels of c-fos and c-jun mRNA in S group were significant higher than that of P group after operation for 3 d ($P<0.05$). However, there were no obvious differences among the groups after operation for 7 d ($P>0.05$).

Western blotting results

Western blotting results were shown in **Figure 3** and **Table 6**. They showed that the protein levels of c-fos and c-jun in P group and S group were significant higher than that of N group after operation for 3 d ($P<0.05$) and the levels of c-fos and c-jun in S group were significant higher than that of P group after operation for 3 d ($P<0.05$). However, there were no obvious differences among the groups after operation for 7 d ($P>0.05$).

Immunohistochemical results

The immunohistochemical results of c-fos protein after operation for 3 d and 7 d were shown in **Figure 4** and the immunohistochemical result of c-jun protein after operation for 3 d and 7 d were shown in **Figure 5**. They showed that the protein levels of c-fos and c-jun in P group and S group were higher than that of N group after operation for 3 d. However, there were no obvious differences among the groups after operation for 7 d.

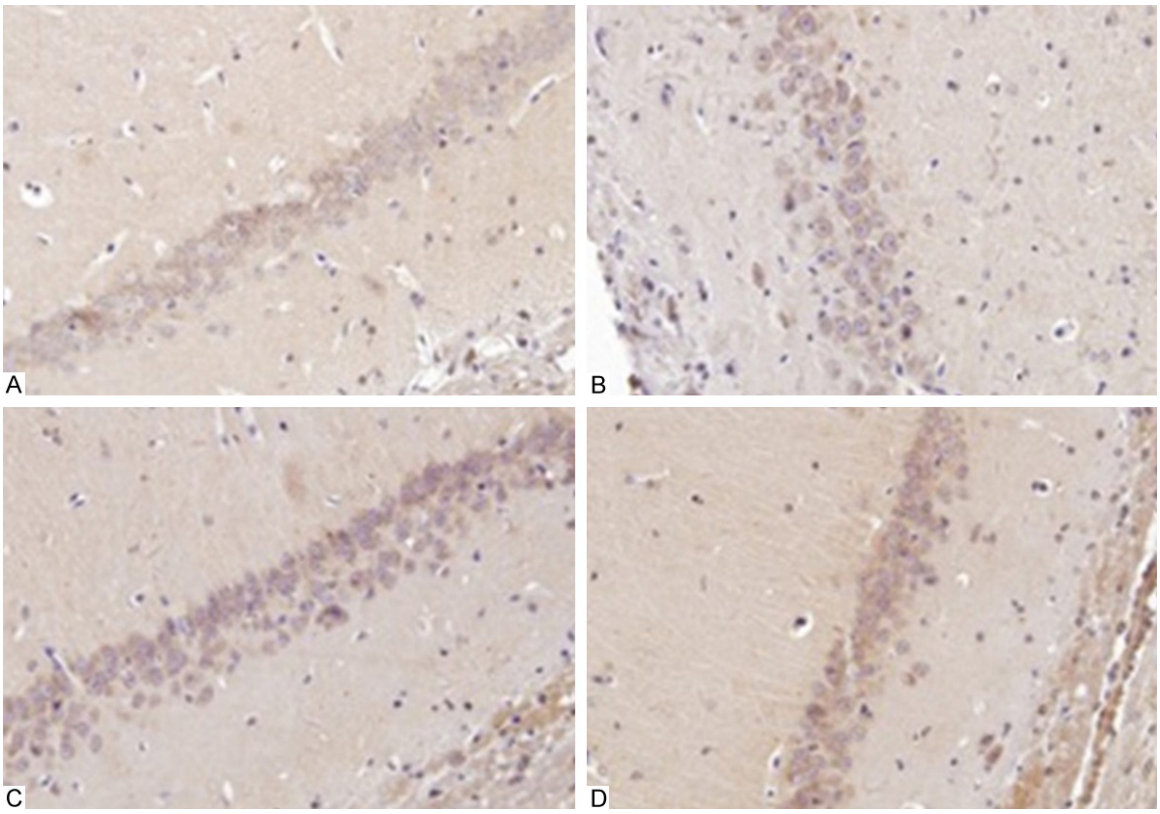

Figure 4. The immunohistochemical results of c-fos protein after operation for 3 d and 7 d. A: Postoperative 3 d in N group; B: Postoperative 3 d in P group; C: Postoperative 3 d in S group; D: Postoperative 7 d in S group.

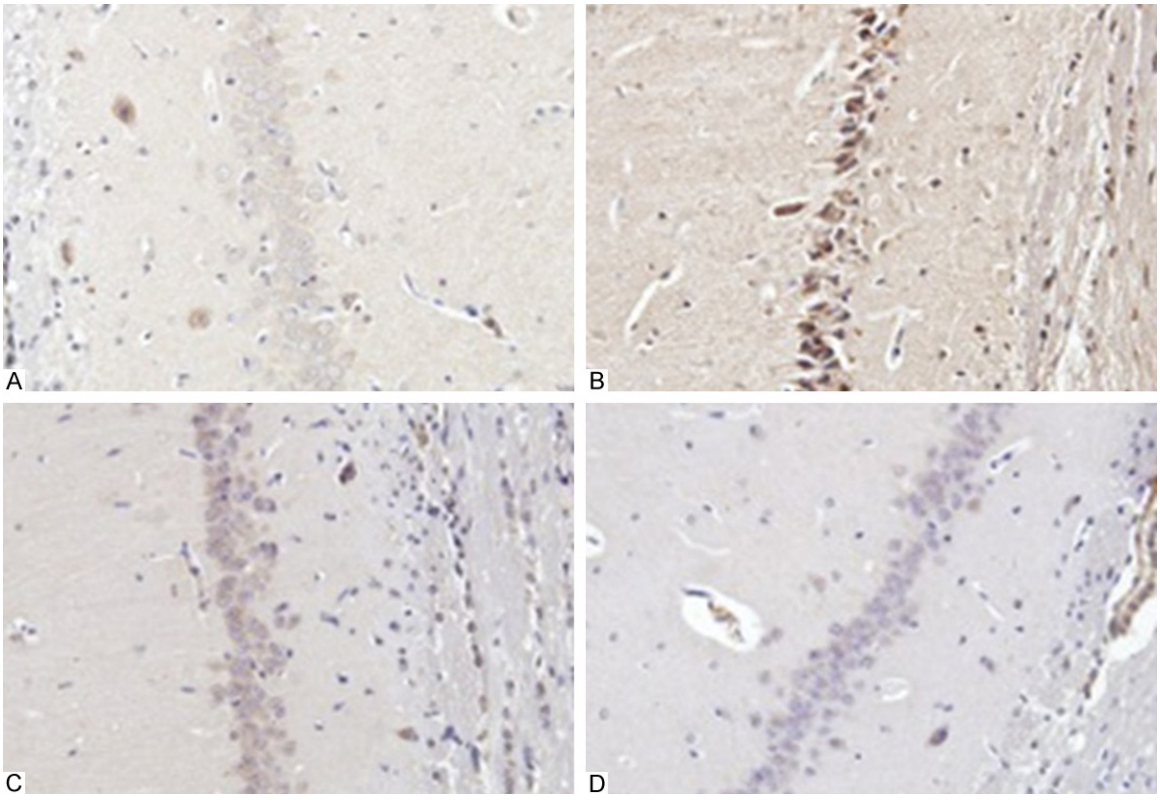

Figure 5. The immunohistochemical results of c-jun protein after operation for 3 d and 7 d. A: Postoperative 3 d in N group; B: Postoperative 3 d in P group; C: Postoperative 3 d in S group; D: Postoperative 7 d in S group.

Discussion

It was reported that small dose of propofol could cause compliance amnesia and large dose of propofol could cause retrograde amnesia in patients [4]. Pratico also found that large dose of propofol could cause retrograde amnesia in rats [5]. Amnesia induced by propofol is the intervention of the learning and memory process. Ramsay confirmed that sedation dose of propofol did not affect the spatial memory of rats while anesthetic dose of propofol could affect the spatial memory of rats in a short time [6]. Sarasin found that continuous intravenous infusion of propofol could cause POCD in patients with oral operation for a short time [7]. It was also confirmed that propofol could damage the spatial memory of rats and inhibit the long-term potentiation [8].

Morris water maze is a classical model for studying the function of spatial learning and memory in animal. It can respond the changes of the qualitative and positioning capability accurately. In this study we found that the effect of propofol on spatial learning and memory in rats gradually decreased with time-lapse after operation and almost had no effect after operation for 7 days. These were consistent with previous studies [9, 10]. However, Shang found that anesthetic dose of propofol almost had no effect after operation for 4 days in rats [11].

The destruction of hippocampus could lead to the decline of learning and memory ability. Lynch found that it could result in a decrease in memory and spatial learning ability of rats when the hippocampus was damaged bilaterally [12]. Hippocampus was related with the short-term memory [13]. In this study we found that propofol could induce cytosolic concentration and nuclear condensation after operation for 3 days in rats, and they recovered after operation for 7 days. The main target of propofol in brain tissue is N-methyl-D-aspartate (GABA) receptor and N-(NMDA) receptor [14]. There are a large number of NMDA and GABA neurons in the hippocampus. So the spatial learning and memory based on the hippocampus can be damaged by propofol.

When the hippocampus is stimulated by external stimuli, the presynaptic membrane releases the glutamate transmitter and activates AMPA and NMDA receptors in the postsynaptic membrane, which leading to the activation and expression of c-fos gene. The c-fos protein and c-Jun nuclear protein can form the heterologous dimmer and combine with specific DNA sequence, the synaptic plasticity was produced finally. The expression of c-fos and c-jun gene in hippocampus of rats could be induced by various learning and training methods, the expression of c-jun and c-fos in hippocampus was related to memory function [15, 16]. Hamaya confirmed that cognitive dysfunction induced by propofol anesthesia in rats was related with the increase expression of c-fos in CA1 region of the hippocampus in rats [17]. Kidambi also found that effect of propofol anesthesia on the memory function of rats was related with the expression of c-fos [18]. We found that propofol significantly affected the expression of c-jun and c-fos genes after operation for 3 days in rats, while there was no effect after operation for 7 days.

In a word, the effects of propofol anesthesia on the cognitive function of rats were gradually decreased with time-lapse, and there was no effect after operation for 7 days, which maybe related with the changes of hippocampal tissues and the levels of c-jun and c-fos.

Disclosure of conflict of interest

None.

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