



FULL LENGTH ARTICLE

The effects of repeated propofol anesthesia on spatial memory and long-term potentiation in infant rats under hypoxic conditions

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Abstract Propofol is widely used as an intravenous drug for induction and maintenance in general anesthesia. Hypoxemia is a common complication during perianesthesia. We want to know the effect of propofol on spatial memory and LTP (Long-term potentiation) under hypoxic conditions. In this study, 84 seven-day-old Sprague–Dawley rats were randomly assigned into six groups ($n = 14$)-four control groups: lipid emulsion solvent + 50% oxygen (CO), lipid emulsion solvent + room air (CA), lipid emulsion solvent + 18% oxygen (CH), and propofol + 50% oxygen (propofol–oxygen, PO); and two experiment groups: propofol + room air (propofol–air, PA), and propofol + 18% oxygen (propofol–hypoxia, PH). After receiving propofol (50 mg/kg) or the same volume of intralipid intraperitoneal (5.0 ml/kg), injected once per day for seven consecutive days, the rats were exposed to 18% oxygen, 50% oxygen and air, until recovery of the righting reflex. We found that the apoptotic index and activated caspase-3 increased in the PH group ($P < 0.05$) compared with the PA group, fEPSP (field excitatory post-synaptic) potential and success induction rate of LTP reduced in all propofol groups ($P < 0.05$). Compared with the PO group, the fEPSP and success induction rate of LTP reduced significantly

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in the PA and PH groups ($P < 0.05$). Moreover, compared with CH group, the average time of escape latency was longer, and the number of platform location crossings was significantly reduced in the PH group ($P < 0.05$). Thus, we believe that adequate oxygen is very important during propofol anesthesia.

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Introduction

Propofol is the most frequently used intravenous anesthetic for induction and maintenance of anesthesia. Hypoxemia is a common complication during anesthesia. In a previous clinical study, the incidence of mild hypoxemia (SpO_2 86–90%) was 53% and 55% in the operation room and post-anesthetic ICU (PACU), respectively, and severe hypoxemia with SpO_2 values $<81\%$ was 20% and 13%, respectively.¹ In an Australian study of 959 patients, 17% had $\text{SpO}_2 <90\%$ and 6.6% had $\text{SpO}_2 <85\%$.² Many doctors have been working on improving hypoxemia.^{3,4} The occurrence of apnea is more common when the patient is treated with a bolus dose of propofol than with a barbiturate. Apnea resulting from propofol anesthesia will lead to a gradual SaO_2 (oxygen saturation) decrease, further hypoxia, and even death without supplementary oxygen.⁵ We have previously shown that propofol per se or hypoxia per se did not directly induce significant neuronal apoptosis.⁶ However, respiratory depression induced by propofol could produce lower oxygen concentrations in the blood under air or mild hypoxia conditions and thereby result in neuronal degeneration. Meanwhile, we also found that propofol per se induced short-term cognitive impairment; however, propofol in combination with hypoxia severely impaired cognitive function in newborn rats. Additionally, propofol causes LTP impairment.^{7–11} LTP, which is believed to involve mechanisms that underlie memory formation, has been studied most extensively in the hippocampus.^{12,13} But the effect of propofol on LTP under hypoxic conditions is unknown. In the present study, we imitated clinical drug and oxygen administration to investigate the effect of propofol anesthesia on spatial memory, neuronal apoptosis, and long-term potentiation in the CA1 region of infant rat hippocampal slices.

Materials and methods

Ethics statement

The Ethics Committee of Chongqing Medical University (license number: CXK [YU] 20110016) approved all protocol of animal procedures in the experiments.

Animals

Seven-day-old male and female Sprague–Dawley rats (12–16 g) were housed individually under standardized conditions (12-h light/12-h darkness, temperature $22 \pm 2^\circ\text{C}$, and humidity $55 \pm 5\%$). They were maintained with free access to food and water. All efforts were made to minimize animal's suffering and to reduce the number of animals used.

Experimental protocol

At postnatal day 7 (P7), 84 rats were randomly divided into six groups ($n = 14$) and randomized either for propofol or control groups. Rats in the propofol group were exposed to 50% oxygen (group propofol–oxygen, PO), room air (group propofol–air, PA), or 18% oxygen (group propofol–hypoxia, PH) and were given an intraperitoneal injection of 50 mg/kg propofol. Rats in the control group were exposed to 50% oxygen (group control–oxygen, CO), room air (group control–air, CA), or 18% oxygen (group control–hypoxia, CH) and were intraperitoneally (ip) injected with an identical volume of intralipid (5.0 ml/kg). Either the anesthesia or the control group received one injection per day for seven consecutive days. SaO_2 (%) and respiratory rate (RR) were monitored throughout the procedure.

Immunohistochemistry

Twenty-four hours after the last injection, six pups from each group were randomly selected for immunohistochemistry of activated caspase-3. Before the brains were removed, anesthetized animals were perfused with 10 U/mL heparin saline and subsequently with 4% formaldehyde in PBS, these procedures can protect the antigenicity of neuron and prevent the tissue liquification. After the rats were decapitated, hippocampal CA1 was separated and dissected into small blocks. Blocks were fixed in paraformaldehyde, embedded in paraffin, and then cut into coronal sections 5 μm thick. These paraffin sections were used, in turn, for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and immunohistochemistry. Tissue sections were deparaffinized in xylene and rehydrated in descending concentrations of ethanol, followed by antigen retrieval in a sodium citrate buffer for 10 min in a microwave oven at 100°C and cooled to room temperature. The experiments were carried out using Histostain-Plus Kits (ZSGB-Bio, Beijing, China), and a chromogenic reaction was carried out with 3,30-diaminobenzidine (ZSGB-Bio, Beijing, China) according to the manufacturer's protocol. The primary antibodies used were for activated caspase-3 (Santa-cruz, USA) at a 1:200 dilution. The sections were counterstained with hematoxylin and mounted with resinene. The images were collected on an Olympus microscope (BX40, Tokyo, Japan).

TUNEL

TUNEL staining was performed based on the instructions of the TUNEL Kit (Roche, Germany) based on terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate

in situ nick end labeling (TUNEL). In brief, the slides were placed in a citrate buffer (0.1 M, pH 6.0) after deparaffinization and rehydration. We applied microwave irradiation for 1 min, double-distilled water was added to cool them down. 0.1 M Tris-HCl (pH 7.5, 3% BSA, 20% normal bovine serum) immersed for 30 min in. The slides were rinsed twice with PBS, TUNEL reaction mixture was added on the sections, then incubated darkly for 60 min at 37 °C, followed by converter-POD (peroxidase) incubation at 37 °C for 30 min; DAB staining was developed and redyed with methyl green. The next step involved dehydrating the slides in 50% butanol, cleared in xylene and mounted with Entellan (Electron Microscopy Sciences). Apoptotic neurons were calculated under five randomly selected high-power fields, at least 200 cells per field. The ratio of TUNEL positive cells to total cell number was defined as apoptotic index.

Field excitability postsynaptic potential recording and LTP induction success-rate detection

Twenty-four hours after the last injection, Animals were decapitated under anesthesia with isoflurane (2%) and laughing gas (N₂O, 70%), Rats were perfused with 20 ml artificial cerebrospinal fluid into the heart. Brains were rapidly placed in slice liquid of 0–4 °C oxygen saturation for 1–2 min. We cut 400 µm thick bilateral hippocampus brain slices using a vibratome (WPI, Sarasota, NVSLM-1) and placed the slices in a mixture of record fluid containing the following (in mM): 124 NaCl, 26NaHCO₃, 1.25 NaH₂PO₄, 2.8 KCl, 2 CaCl₂, 2 MgSO₄, and 10 glucose, oxygenated with 95% O₂, and 5% CO₂. Gas was continually bubbled into the mixture, which was incubated at 35 °C for 30–45 min. Following the incubation period, the brain slices were kept at room temperature for 1 h.

Field excitability postsynaptic potential recording

The hippocampal slices were continuously perfused with oxygen saturation record fluid at 35 °C, and at a rate of 1.5 ml/min. A bipolar tungsten stimulation electrode (diameter of 0.01 mm, spacing 10 µm) was put into the hippocampal CA3 area. The stimulation intensity was 0.1–0.25 mA and the pulse width was 0.5 ms stimulate. We put a glass record electrode into the CA1 area radiation layer and recorded the electrical stimulation induced field excitability postsynaptic potential (field excitatory postsynaptic potential or fEPSP). The samples were processed and preserved using pCLAMP 9.2 software.

LTP induction success-rate detection

The stimulation intensity at a maximum reaction value of 50%, induced by fEPSP, was selected as the basic stimulus, giving high-frequency LTP-induced electrical stimulation after base stability of 30 min. The fEPSP slope increased by more than 20% after high-frequency electrical stimulation and lasted for 30 min or more. We regarded this as LTP induction success. There were two groups of high-frequency electrical stimulation parameters. Their frequency was 100 Hz, their pulse width 0.5 ms 100 pulse, and each group interval was 30 s.

Morris water maze (MWM)

The Morris water maze test consists of a place navigation test (PNT) and a spatial probe test (SPT). We used the place navigation test and the spatial probe test to test each rat's ability to obtain space learning and space memory ($n = 8$). The water maze experimental design is a stainless steel circular tank (diameter 100 cm, 50 cm deep) filled with water (24 ± 0.5 °C). We used a video-tracking system to record the swimming motions of each rat and analyzed the data obtained using MWM motion-detection software (SLY-WMS, Beijing, China).

Place navigation test (PNT)

At postnatal day 28, the day before formal MWM testing, we placed the rats into the swimming pool for 2 min for a single adaptation trial. The rats received four acquisition trials per day for six consecutive days (T1–T6). During the place trials, the rats were placed in the middle of one of the four virtual quadrants in the swimming pool, facing the wall, and randomly positioned in the pool. An inter-trial interval was used during which the rats were allowed to stand on the platform for 60 s before the next trial. For each trial, we used a computerized tracking system to measure the swim speed, the time spent (escape latency), and the distance traveled (escape path length) to reach the platform. Escape latency was recorded as 120 s. We placed animals that did not find the platform within 120 s onto the escape platform.

Spatial probe test (SPT)

To examine spatial reference memory, we administered a probe test 24 h after the last training session. During the probe test, we removed the platform from the pool to measure spatial bias for the previous platform location. We placed each rat in the pool in the quadrant opposite to the trained platform location and tracked the rat for 120 s, measuring the percentage of time spent in the previous target quadrant as well as the number of crossings over the previous platform location.

Statistical analysis

We used SPSS software, version 13.0 (SPSS Incorporated, Chicago USA) for the statistical analysis. Comparisons between groups were analyzed using one-way analysis of variance, and differences were considered significant when $P < 0.05$. The data were expressed as means $\pm$ SD.

Results

Respiratory depression was induced by propofol

SaO₂ (oxygen saturation) and respiratory rate (RR) were monitored during propofol anesthesia. Compared with control groups, all propofol-treated animals had signs of respiratory distress with a significantly reduced respiratory rate. The levels of SaO₂ in propofol-treated animals decreased significantly when exposed to air and mild

Table 1 RR, SaO₂, fEPSP and the success induction rate of LTP (n = 6).

Group	RR (bpm)	SaO ₂ (%)	fEPSP(mv)	Success rate of LTP (%)
PH	36.00 ± 5.40 ^a	68.83 ± 3.86 ^a	0.13 ± 0.03 ^a	22.91 ± 12.28 ^a
PA	37.33 ± 6.21 ^b	82.50 ± 4.18 ^b	0.21 ± 0.08 ^b	31.25 ± 10.45 ^b
PO	39.16 ± 6.55 ^c	96.83 ± 1.72 ^{cde}	0.38 ± 0.06 ^{cde}	43.75 ± 10.45 ^{cde}
CH	133.50 ± 12.66	97.50 ± 1.04	0.51 ± 0.08	66.66 ± 10.20
CA	136.66 ± 7.50	98.00 ± 1.41	0.56 ± 0.09	60.41 ± 9.40
CO	129.16 ± 9.06	98.83 ± 0.75	0.50 ± 0.11	58.33 ± 10.20

Results are mean ± SD. Differences among groups were tested by one-way ANOVA. CO: group control–oxygen; CA: group control–air; CH: group control–hypoxia; PO: group propofol–oxygen; PA: group propofol–air; PH: group propofol–hypoxia; RR: respiratory rate.

^ap < 0.05, compared with CH group.

^bp < 0.05, compared with CA group.

^cp < 0.05 compared with CO group.

^dp < 0.05, compared with PA group.

^ep < 0.05, compared with PH group.

hypoxia. Interestingly, when propofol-treated animals were given supplemental oxygen, there was no significant change compared with the control animals (Table 1).

The result suggests propofol may induce respiratory depression and subsequent hypoxia under air circumstances.

Propofol induced neuron apoptosis under air and mild hypoxia conditions

In our experiment, propofol resulted in significantly increased apoptosis in brains under air and mild hypoxia conditions. As shown in Table 2 and Fig. 1, there were only a few TUNEL-positive cells in the CO, CA, CH, and PO groups, and the apoptotic index of the PA and PH groups increased significantly. The apoptotic indexes of the PA and PH groups were significantly higher than the CA and CH groups (P < 0.05), and the mean TUNEL index value in the PA and PH groups was higher than in the PO group (P < 0.05). The results showed that apoptosis in the rat brains was significantly increased by propofol under air conditions, and this phenomenon was more serious in mild hypoxia environments.

Caspase-3 expression

Caspase-3 expression was dramatically upregulated in the hippocampus in the PA and PH groups. Immunohistochemistry, as shown in Fig. 2, was obviously enhanced in the PA and PH groups when compared with the control group and the PO group — expression of caspase-3.

The fEPSP and reduced success induction rate of LTP under hypoxic conditions

Field excitability postsynaptic potential

With the same intensity of electrical stimulation, fEPSP amplitudes had no significant differences among the CO,

CA, and CH groups (P > 0.05). Compared with the control rats, fEPSP amplitudes were reduced in propofol-treated rats (P < 0.05), and fEPSP reduced more significantly in PA and PH groups than in the PO group (P < 0.05, Table 1 and Fig. 3).

The fEPSP was reduced in the PO, PA, and PH groups. There were no significant differences among the three control groups.

The success induction rate of LTP

The fEPSP slope before high-frequency stimulation (HFS) was defined as base value. In control rats, the fEPSP was obviously enhanced after HFS, and the success induction rate of LTP was up to 60% (Fig. 3A–C, Table 1). The fEPSP of the PO group was enhanced dramatically and the success induction rate of LTP was about 43% after HFS (Fig. 3D, Table 1). In the PA and PH groups, the EPSP did not change significantly and the success induction rate of LTP was about 31% and 22%, respectively (Fig. 3E, F, Table 1). Compared with the control groups, the success induction rate of LTP reduced in propofol-treated rats (P < 0.05, Table 1). The success induction rate of LTP in the PA and PH groups were lower than in the PO group (P < 0.05, Table 1).

Propofol exposure impaired long-term spatial learning and memory under hypoxic conditions

Compared with the CO group, RR decreased and the average time of escape latency was significantly longer on the first and second day in the PO group (P < 0.05). Compared with the CA group, the average time of escape latency was longer and the number of platform location crossings was reduced in the PA group (P < 0.05). Compared with the CH group, the average time of escape latency was longer and the number of platform location crossings was significantly reduced in the PH group (P < 0.05). Compared with the PO group, time of escape latency was longer and the number of platform location crossings was reduced in

Table 2 Apoptotic index in each group (%), n = 6).

CO	CA	CH	PO	PA	PH
0.25 ± 0.15	1.42 ± 0.28	0.50 ± 0.06	0.30 ± 0.22	18.22 ± 4.35 ^{a,c}	23.97 ± 1.82 ^{b,c}

^aP < 0.05, compared with CA; ^bP < 0.05 compared with CH; ^cP < 0.05, compared with PO.

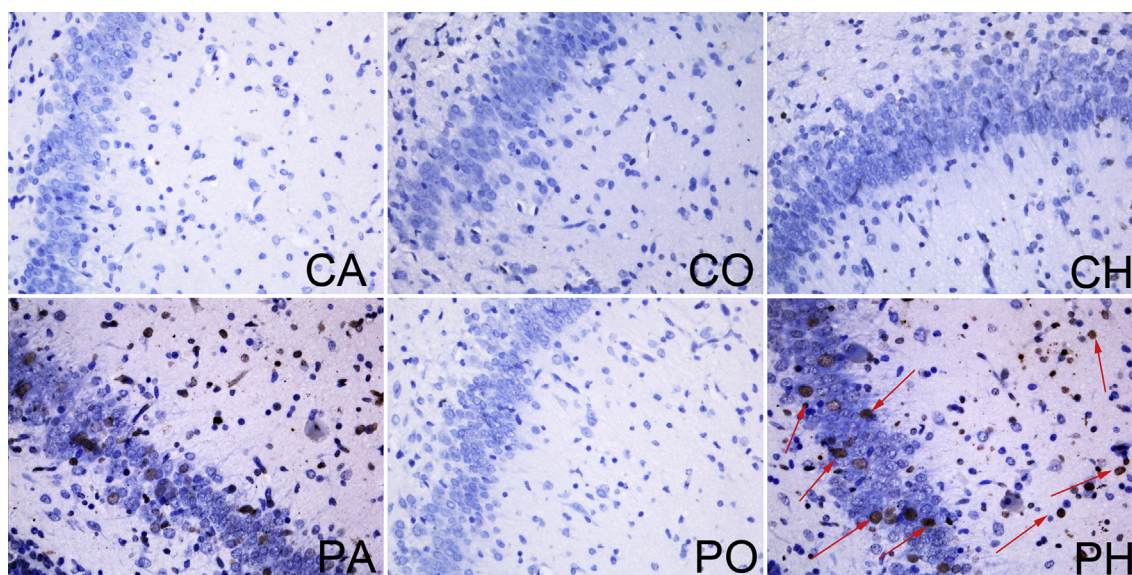


Figure 1 Apoptotic index in each group — red arrows indicate TUNEL-positive neurons.

the PA and PH groups ($P < 0.05$). There were no significant differences in the CO, CA, and CH groups ($P > 0.05$). Data are shown in [Tables 3 and 4](#), and [Figs. 4 and 5](#).

Discussion

General anesthetics can induce neuronal cell death and persistent behavioral sequelae, including sports and cognitive deficits, in the neonatal brains of both rodents and primates.¹⁴ This study investigated the mechanism for long-term function of learning and memory after the administration of propofol, assessing effects on behavior, and morphological and synaptic levels, under hypoxia conditions.

In our study, we used rats at P7 during the spurt brain-growth period (i.e. synaptogenesis), which occurs during the first two weeks of life in rats.¹⁵ Yon et al found that the immature rat brain was the most vulnerable to anesthesia-induced neuroapoptosis at the peak of synaptogenesis.^{16,17} Rats at P7 are frequently used to research cognitive-function impairment. We determined the dosage of propofol based on the results of previous studies.^{18,19} Children who have multiple congenital diseases need repeated treatment and anesthesia. In a single operation, propofol often pumps continuously for a few hours, and propofol is often used as an ICU sedative for many days.

We found that propofol at 50 mg/kg induced significant respiratory depression, accompanied by significantly

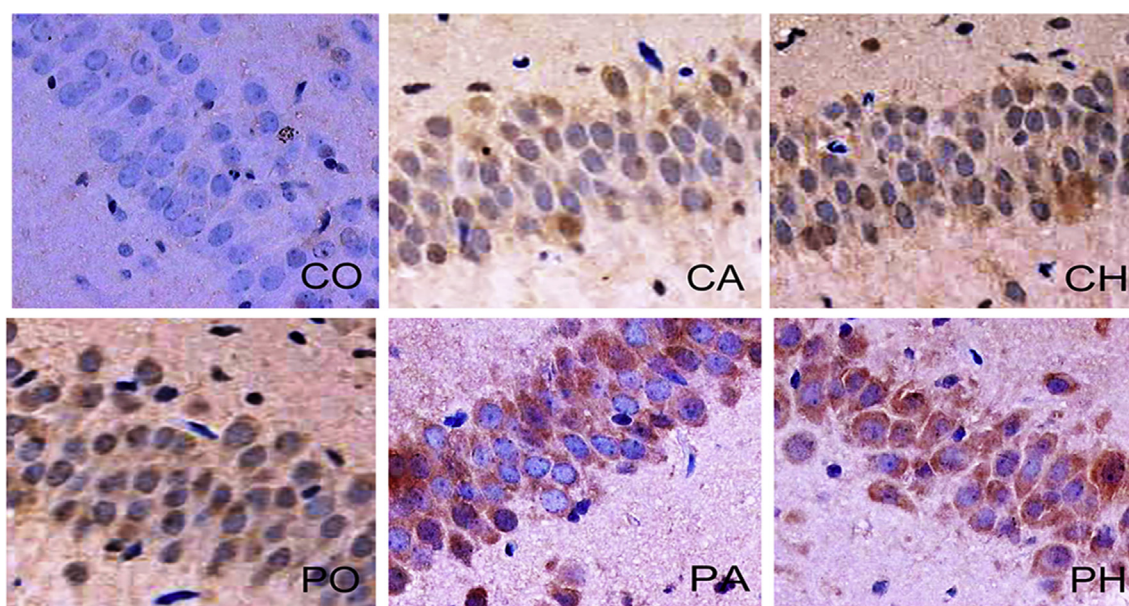


Figure 2 Caspase-3 protein expression in each group. Caspase-3 protein level was determined by immunohistochemistry.

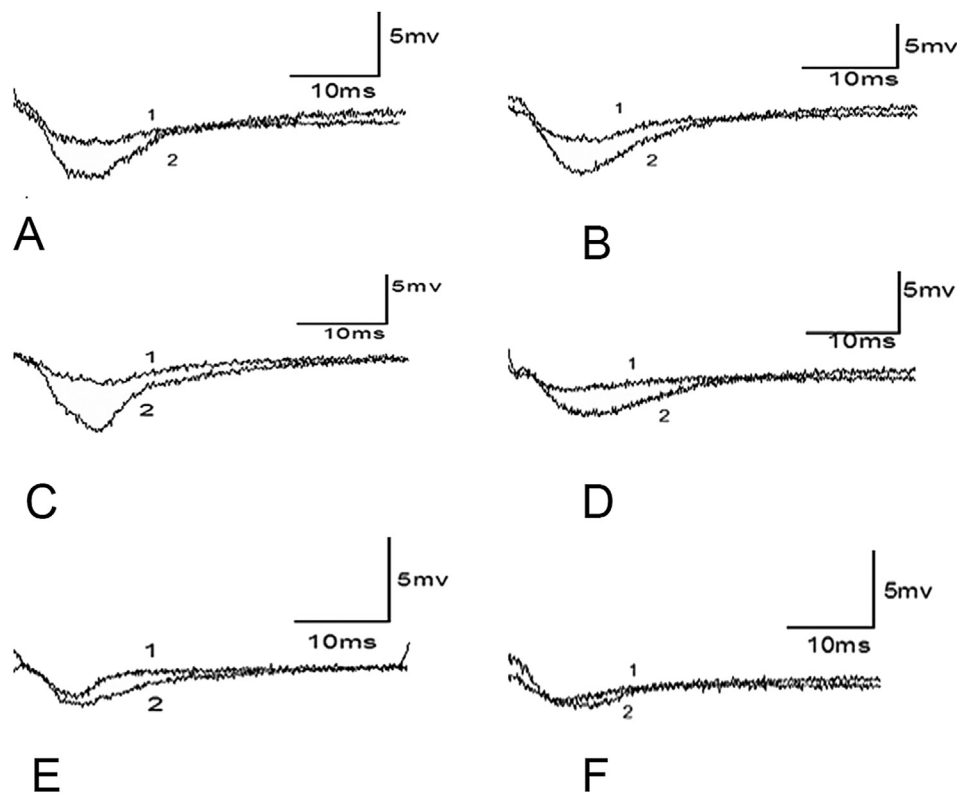


Figure 3 fEPSP changes on P7 rat hippocampus in each group. A represents CO group, B represents CA group, C represents CH group, D represents PO group, E represents PA group, F represents PH group; 1: Before stimulation; 2: After stimulation.

Table 3 The average time of escape latency in each group ($n = 8$, $\bar{x} \pm s$).

Time point	CO	CA	CH	PO	PA	PH
T1	51 $\pm$ 5	50 $\pm$ 4	53 $\pm$ 5	62 $\pm$ 6 ^a	71 $\pm$ 6 ^{bd}	75 $\pm$ 8 ^{cd}
T2	43 $\pm$ 4	42 $\pm$ 3	44 $\pm$ 4	56 $\pm$ 5 ^a	68 $\pm$ 6 ^{bd}	70 $\pm$ 7 ^{cd}
T3	34 $\pm$ 4	31 $\pm$ 4	34 $\pm$ 6	36 $\pm$ 3	57 $\pm$ 6 ^{bd}	61 $\pm$ 6 ^{cd}
T4	24 $\pm$ 3	24 $\pm$ 3	25 $\pm$ 5	27 $\pm$ 3	55 $\pm$ 6 ^{bd}	57 $\pm$ 6 ^{cd}
T5	16 $\pm$ 3	16 $\pm$ 3	18 $\pm$ 6	18 $\pm$ 3	49 $\pm$ 5 ^{bd}	52 $\pm$ 6 ^{cd}
T6	12 $\pm$ 4	13 $\pm$ 4	14 $\pm$ 4	13 $\pm$ 3	42 $\pm$ 5 ^{bd}	47 $\pm$ 9 ^{cd}

^a $P < 0.05$, compared with CO; ^b $P < 0.05$ compared with CA; ^c $P < 0.05$, compared with CH; ^d $P < 0.05$, compared with PO.

decreased RR and SaO₂ (hypoxia) under air and mild hypoxia conditions. Also, four of our anesthetized rats died from apnea after propofol anesthesia. This was very similar to Pascoe's report that anesthesia with propofol was accompanied by a moderate but significant respiratory depression in cats.¹⁶ Perioperative hypoxemic episodes resulting from altered pulmonary mechanics, drug-induced physiological changes, or technical errors are common during administration of anesthesia.^{20,21}

Mild-to-moderate hypoxemia in the perioperative period has been implicated as one of the factors

contributing to postoperative central nervous system impairment,^{22–24} and animals subjected to moderate hypoxia display a combination of cognitive and neurochemical changes. On the aspect of behavior, they are less responsive to their environment, explore less, cannot learn as readily, and appear debilitated.²⁵ Also, exposure to hypobaric hypoxia at a simulated altitude of 5950 m (approximately 10% oxygen) for eight hours or 6100 m for three or seven days produced decrements in memory function.²⁴ In our study of infant rat brains, hypoxia in combination with propofol triggers longer-

Table 4 The number of platform location crossings in each group (bpm, $n = 8$, $\bar{x} \pm s$).

	CO	CA	CH	PO	PA	PH
Number of platform location crossing	9.9 $\pm$ 1.9	10.2 $\pm$ 1.4	10.6 $\pm$ 2.3	9.8 $\pm$ 1.7	3.7 $\pm$ 1.0 ^{ac}	3.1 $\pm$ 1.1 ^{bc}

^a $P < 0.05$, compared with CA; ^b $P < 0.05$, compared with CH; ^c $P < 0.05$, compared with PO.

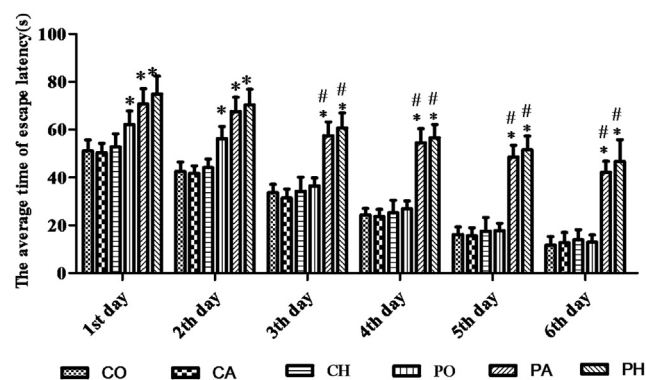


Figure 4 The average time of escape latency in each group. *Represents $P < 0.05$, compared with control groups; #Represents $P < 0.05$, compared with PO group.

lasting learning and memory impairment than propofol alone.

Our experiments indicated that propofol at 50 mg/kg had no overtly neuronal structural changes in P7 rats under oxygen-supplying conditions. Meanwhile, with oxygen supply, propofol anesthesia did not lead to upregulate caspase-3 expression or neonatal neurodegeneration. In Lee's study,²⁶ propofol did not cause neurodegeneration and subsequent neurocognitive disturbances. Hypoxemia leads to neurodegeneration.^{24,27,28} However, we found that propofol – in combination with hypoxia resulting from propofol – increased the activation of caspase-3 and resulted in neuronal apoptosis and degeneration under air conditions.

A series of electrical stimulations can increase excitatory postsynaptic potential (EPSP) amplitude, synaptic transmission efficiency, and a short incubation period, which can last a certain time. Bliss and Gardner-Medwin considered long-term facilitation of synaptic transmission induced by a single synaptic long-range enhancement to be LTP.²⁹ LTP in hippocampal neurons is the performance of central plasticity, which is considered to be the neural basis of learning and memory, and is a model of learning and memory at the synaptic level.^{30,31}

The hippocampus, as an important brain region participating in spatial learning and memory, contains a large number of glutamatergic and GABA neurons. The GABA-A receptor is an important target of propofol. Propofol can

promote or directly activate the GABA-A receptor, make chlorine indrawal, produce super polarization, and reduce the neuron excitability function.³² Simultaneously, propofol has a certain inhibition role on the activation of the NMDA receptor. In addition to sedative and hypnotic effects, propofol has a forgotten role, which is related to the restraining of hippocampal LTP.^{9,33} We found that propofol (20 mg/kg) influences the formation and maintenance of LTP. Takamatsu et al⁹ found that the dose of propofol (50 mg/kg) that inhibits LTP formation through the GABA-A receptor is much higher than the dose of propofol that leads to memory loss. In our study, the fEPSP and the success induction rate of LTP reduced significantly when treated with propofol, and they were lower under air and mild hypoxia conditions than under supplementary oxygen conditions. These results are consistent with previous research.

Conclusion

To sum up, propofol anesthesia per se induces short-term impairment of cognitive performance, but propofol in combination with hypoxia can induce lasting impair of cognitive function in newborn rats. It appears that repeated propofol anesthesia affects LTP more significantly, and further learning and memory impairment is longer under air conditions than under supplementary oxygen conditions. We can draw the conclusion from this result that hypoxia contributes to postanesthesia cognitive-function impairment in newborn rats, which illustrates the importance and necessity of oxygen supply during anesthesia to prevent postoperative cognitive dysfunction in patients, especially in children.

Conflicts of interest

The authors have no conflict of interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gendis.2019.02.001>.

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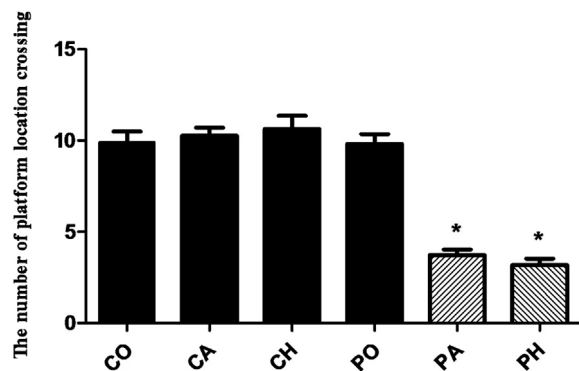


Figure 5 The number of platform location crossings in each group. *Represents $P < 0.05$ compared with PO group.

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