Persistent neuronal apoptosis and synaptic loss induced by multiple but not single exposure of propofol contribute to long-term cognitive dysfunction in neonatal rats

Bo Chen¹,², Xiaoyuan Deng¹, Bin Wang³ and Hongliang Liu¹

¹Department of Anesthesiology, Chongqing Cancer Institute, Chongqing 40030, China
²Guangxi Medical University, Nanning, Guangxi 530021, China
³Institute of Life Science, Chongqing Medical University, Chongqing 400016, China

(Received May 20, 2016; Accepted July 27, 2016)

ABSTRACT — Propofol can induce acute neuronal apoptosis or long-term cognitive dysfunction when exposed at early age in rodents, but it is unclear how the neurotoxicity including neuronal apoptosis and synaptic loss will change in a dynamic manner with brain development after multiple or single exposure of propofol, and the role of neuronal apoptosis and synaptic loss in propofol-induced long-term cognitive impairment needs to be elucidated. In this study, we investigated dynamic changes of neuronal apoptosis, neuronal density, synaptic density in hippocampal CA1 region and the prelimbic cortex (PrL), and long-term cognitive function after multiple or single exposure of propofol in neonatal rats. Results showed that single exposure of propofol only induced great neuronal apoptosis and deficit at postnatal day 9(P9); while multiple exposures of propofol could induce significant neuronal apoptosis, neuronal deficit and synaptic loss at P9, P14, P21, or P35 compared with intact, and spatial learning and memory impairment from P36 to P41. Results suggest that single exposure of propofol only induces transient neuronal apoptosis and deficit, while multiple exposures of propofol induce persistent neuronal apoptosis, neuronal deficit, synaptic loss, and long-term cognitive impairment. Furthermore, persistent neuronal deficit and disturbances in synapse formation but not transient neuronal apoptosis may contribute to long-term cognitive impairment.

Key words: Propofol, Neurotoxicity, Developing brain, Exposure times

INTRODUCTION

Developing brain is extremely sensitive and vulnerable to general anesthetics (Jevtovic-Todorovic et al., 2003; Yon et al., 2005). Accumulating studies have demonstrated that clinically used general anesthetics, which are N-methyl-D-aspartate (NMDA) receptor antagonists and/or γ-aminobutyric acid type A (GABA A) receptor agonists, can cause developmental neurotoxicity in a variety of animal species (Yon et al., 2005; Cattano et al., 2008; Rizzi et al., 2010; Zou et al., 2011). The factors contributing to developmental neurotoxicity include dosage of general anesthetic agents, the duration or frequency of exposure, and age (Jevtovic-Todorovic et al., 2013; Lei et al., 2012). Retrospective clinical studies and the latest prospective study have demonstrated that children at an early age receiving multiple but not single general anesthetic exposure have an increased risk to develop learning disabilities, attention-deficit and hyperactivity disorders (Wilder et al., 2009; Flick et al., 2011; Sprung et al., 2012; Davidson et al., 2016), similar results have been observed in animal studies (Murphy and Baxter, 2013; Gonzales et al., 2015; Yu et al., 2013; Karen et al., 2013).

It has been confirmed that the developing brain is susceptible to anesthetic-induced neuronal apoptosis at the peak of synaptogenesis, and the greatest vulnerability in rodents occurs predominantly from postnatal day (P) 7 to P10 (Yon et al., 2005; Rizzi et al., 2010), it was considered that anesthetic-induced acute neuronal apoptosis may be a critical contributing factor to long-term cognitive and behavioral impairment, but recent studies have demonstrated that anesthetic-induced acute neuronal apoptosis does not cause long-term cognitive impairment (Yang et al., 2014; Loepke et al., 2009). Therefore, the role of neu-
ronal apoptosis in long-term cognitive impairment needs to be clarified. Synapse formation is the hallmark of synaptogenesis, which is in a crucial stage of brain development (Colón-Ramos, 2009). Appropriate neuronal density and synapse formation determine the formation and function of neuronal networks, and can ultimately affect long-term cognitive function (Jevtovic-Todorovic, 2012). Synapse formation can be disturbed by general anesthetics (Lunardi et al., 2010; Amrock et al., 2015), but the role of anesthetic-induced disturbance of synapse formation in long-term cognitive impairment remains unclear.

Propofol, the most commonly used intravenous general anesthetic in the clinic, potentiates GABA_A receptor functioning or even directly activates GABA_A receptor at higher concentration. It is known that propofol can induce acute neuronal apoptosis in neonatal animals (Yang et al., 2014; Pesić et al., 2009), or long-term cognitive impairment (Gonzales et al., 2015; Yu et al., 2013; Karen et al., 2013), but it is unclear how neuronal apoptosis and synapse formation will change in a dynamic manner with brain development after neonatal exposure to propofol, and the role of neuronal apoptosis and synapse formation in propofol-induced long-term cognitive impairment needs to be elucidated. Thus, in the present study, we evaluated the effects of different propofol exposures in neonatal rats on neuronal apoptosis, neuronal or synaptic density and long-term cognitive function.

MATERIALS AND METHODS

Animals

P7 (postnatal day 7, n = 148) Sprague-Dawley male rat pups with their lactating dams (n = 13) were purchased from the Animal Center of Chongqing Medical University, China (permission number: SCXK 2012-0001). All rats were housed in a 12/12 hr light-dark cycle at 22°C and allowed *ad libitum* access to food and water. Rat pups were weighed every day and weaned on P21. All animal experiments were approved by the Ethics Committee of Chongqing Cancer Institute, and followed the Guidance Suggestions for the Care and Use of Laboratory Animals, formulated by Ministry of Science and Technology of China, every effort was made to minimize the number of animals used and their suffering.

Anesthesia

The study design is summarized in Fig. 1. P7 pups from different litters were randomly divided into 4 groups. P(repeated): pups received three intraperitoneal injections of 50 mg/kg propofol (10 mg/mL, Sigma-Aldrich Chemical Co., St. Louis, MO, USA), from P7 to P9, with 24-hr intervals; P(single): pups received two intraperitoneal injections of 5 mL/kg 0.1% DMSO at P7 and P8, followed by 50 mg/kg propofol at P9; Control: pups received equal numbers and volumes of intraperitoneal injections of 0.1% DMSO; Intact: pups underwent the same environmental conditions and maternal separation as other groups did, but did not experience any procedure. The dosage of propofol was based on the published data (Pesić et al., 2009) and our preliminary experimental results. The duration of loss of the righting reflex after each intraperitoneal injection of 50 mg/kg propofol was approximately 120 min. During anesthesia, all pups were placed on a heating pad, and rectal temperature was maintained at 37 ± 1°C, oxygen saturation was monitored using pulse oximetry and maintained at 95% or so. After recovery, the pups not intended to be sacrificed immediately were allowed to return to their dams. Some pups were used for transcardial arterial blood gas analysis before full recovery (n = 5), some were sacrificed at P9 (5 hr after recovery), P14, P21, or P35, their brains were used for activated caspase-3 immunohistochemistry, Nissl staining and

![Fig. 1](image1.png)
Developmental neurotoxicity of propofol

Synaptophysin immunofluorescence (n = 5). The others were used for Morris Water Maze (MWM) tests from P36 to P41 (n = 12).

Tissue preparation

Pups were deeply anesthetized with 4% sevoflurane for 3 min and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer saline (PBS). The brains were removed, postfixed in 4% PFA solution for 24 hr at 4°C. The right half-brains were embedded in paraffin and cut into 4 μm-thick sagittal sections for immunohistochemistry and Nissl staining. The left half-brains were cryoprotected with 25% sucrose solution, and cut into 10 μm-thick sagittal frozen sections for immunofluorescence. Three sections corresponding to Figures 164-166 in Atlas of the Rat Brain (Paxinos and Watson, 2006) were chosen from each pup.

Immunohistochemistry

Paraffin sections were deparaffinized in xylene and rehydrated in graded ethanol, followed by antigen retrieval with 0.1 M sodium citrate buffer pH 6.0 in a microwave oven for 20 min. After blocking in 0.3% hydrogen peroxide and 3% normal goat serum, sections were incubated with the primary rabbit polyclonal anti-activated caspase-3 antibody (1:100; Abcam Inc., Cambridge, MA, USA) overnight at 4°C, then incubated with the second goat anti-rabbit IgG antibody and streptavidin-biotin complex (Histostain-plus kit, Boster, Wuhan, China) for 30 min at 37°C respectively, and then colorized with DAB reagents (Beijing Zhongshan Biotechnology Co. Ltd., Beijing, China). Hematoxylin was used for nuclei staining. Negative sections were incubated with PBS instead of primary antibody. Activated caspase-3 positive cells were counted from five microscopic areas in hippocampal CA1 pyramidal cell layer or layers II-V of the prelimbic cortex (PrL) under 40X magnification by an investigator who was blinded to the experiments. The density of activated caspase-3 positive cells was obtained with the number of activated caspase-3 positive cells divided by the defined area (210 x 140 μm).

Nissl staining

Nissl staining was performed as previously described (Wang et al., 2012). The deparaffinized sections were incubated with Nissl staining solution containing cresyl violet (Beyotime Institute of Biotechnology, Shanghai, China) for 30 min at 37°C. The density of neurons was achieved with the method mentioned in immunohistochemistry experiment.

Immunofluorescence

After antigen retrieval, frozen sections were blocked with 3% normal goat serum and 0.1% Triton-X 100 in PBS for 1 hr at 37°C and incubated with the primary monoclonal rabbit anti-synaptophysin antibody (1:250; EMD Millipore, Darmstadt, Germany) for 24 hr at 4°C, then incubated with the second Alexa Fluor 550 goat antirabbit (1:200; Invitrogen, Eugene, OR, USA) for 2 hr in the dark at 37°C and counterstained with 0.5% DAPI (Sigma) for 15 min at 37°C. Synaptophysin positive puncta in hippocampal CA1 radiation layer and layers II-V of PrL were determined under a confocal microscope (Leica TCS SP8, Wetzlar, Germany). Images stacks were collected using a 63 × objective (NA 1.4), with a × 2 optical zoom, a Z-stack size of 1 μm, format 1024 × 1024 pixels, and a field size of 92.06 × 92.06 μm. Analysis of synaptophysin images was performed using the spot module of Imaris 8.0.2 (Bitplane, South Windsor, CT, USA).

Morris Water Maze

Spatial learning and memory test was performed using the MWM according to the protocol (Vorhees and Williams, 2006). A round pool (150 cm in diameter, 60 cm in height) was filled with water (22 ± 1°C) containing ink to hide the platform. The platform (9 cm in diameter) was submerged 1.0 cm below the water surface and four visual cues were placed on the wall of the pool. A continuous video tracking system recorded the swimming motions of each rat and data were analyzed using MWM motion-detection software (Zhenghua Biotech Co., Ltd., Huabei, China). In the acquisition trials, each rat received 4 trials per day for 5 days. Rats were placed into the water facing the wall in a random starting position and allowed to search for the platform within 90 sec. The time for each rat to reach the platform (escape latency) was recorded. If a rat did not find the platform within 90 sec, it was guided to the platform and allowed to stay on it for 15 sec, and the escape latency was recorded as 90 sec. On day 6, the platform was removed and probe trial was performed. Rats were placed in the farthest starting point from the previous platform location and allowed to swim for 90 sec. The number of times that rats crossed the previous platform position (platform crossing times) was recorded.

Statistical Analysis

Statistical analysis was performed using SPSS (version 22, IBM, New York, NY, USA). Normality and homogeneity of variance were detected by Kolmogorov-Smirnov and Levene’s tests respectively. Data of blood gas analysis were analyzed using one-way ANOVA followed by
Bonferroni test. The acquisition trial data of MWM were analyzed using a repeated-measures general linear model of ANOVA. Greenhouse-Geisser corrections were used for deviation from sphericity. Post hoc analysis for multiple comparisons was performed using Bonferroni test for data with equal variance and Games-Howell test for data without equal variance. The probe trial data of MWM were expressed as median (interquartile range) and analyzed using Kruskal-Wallis test. Other data were expressed as mean ± S.E.M. and were analyzed using two-way ANOVA, with treatment group, time as the main effects and treatment group × time as the interaction. To further evaluate differences between groups within each time point, post hoc pairwise comparisons with Bonferroni correction (simple main effects tests) were performed. P value less than 0.05 was considered statistically significant.

RESULTS

Effects of propofol on body weight, respiratory or metabolic function

We did not find any differences in weight gain among the groups during the experiments (Fig. 2). The results of the blood-gas analysis on P9 showed that propofol of either single exposure or multiple exposures with 50 mg/kg ip. did not cause respiratory or metabolic distress (Table 1). No rat pups died during propofol anesthesia.

Effects of propofol on the dynamic changes of cellular apoptosis and neuronal deficit

In the hippocampal CA1 region, a two-way ANOVA analyses revealed that the main effects of treatment group ($F_{(3,64)} = 104.10, P < 0.0001$), time ($F_{(3,64)} = 156.96, P < 0.0001$) and interaction of treatment group × time ($F_{(9,64)} = 40.06, P < 0.0001$) were statistically significant. Post hoc pairwise comparisons revealed that the density of activated caspase-3 positive cells increased only at P9 compared with intact ($P < 0.0001$), but not at P14 ($P > 0.05$), P21 ($P > 0.05$) or P35 ($P > 0.05$) in P(single) group. However, it increased significantly in P(repeated) group at different time points from P9 to P35 compared with intact ($P < 0.0001$). P0.0001, $P = 0.006$, $P = 0.009$, respectively; Fig. 3D, E). In the PrL region, the main effect of treatment group ($F_{(3,64)} = 25.59, P < 0.0001$) and interaction of treatment group × time ($F_{(9,64)} = 2.19, P = 0.035$) were statistically significant. Similarly, the density of activated caspase-3 positive cells in P(single) group increased only at P9 ($P = 0.003$); while in P(repeated) group, it increased significantly at P9, P14, P21 or P35 compared with intact ($P = 0.015, P = 0.001, P = 0.002, respectively; Fig. 3D, F). Vehicle (0.1%DMSO) was not associated with alterations in activated caspase-3 positive cellular density in the two brain regions at different time points ($P > 0.05$, Fig. 3E, F).

Then we tested the effect of propofol on neuronal deficit. In the hippocampal CA1 region, there were significant main effects of treatment group ($F_{(3,64)} = 23.30, P < 0.0001$) and time ($F_{(3,64)} = 20.43, P < 0.0001$), and interaction of treatment group × time ($F_{(9,64)} = 2.36, P = 0.023$). Compared to intact, neuronal density in P(single) group decreased only at P9 ($P < 0.0001$); but it significantly decreased at P9, P14, P21 or P35 in P(repeated) group ($P < 0.0001$, $P = 0.039, P = 0.009$, $P = 0.022$, respectively, Fig. 4A, B). Similar changes were observed in the PrL region, with significant main effects of treatment group ($F_{(3,64)} = 19.46, P < 0.0001$) and time

Table 1. Arterial blood physiological parameters in P9 pups.

|                     | Intact (n = 5) | Control (n = 5) | P(single) (n = 5) | P(repeated) (n = 5) |
|---------------------|---------------|-----------------|-------------------|---------------------|
| pH                  | 7.30 ± 0.04   | 7.32 ± 0.04     | 7.22 ± 0.04       | 7.25 ± 0.03         |
| pCO2 (mm Hg)        | 49.4 ± 4.6    | 49.3 ± 3.4      | 54.3 ± 6.1        | 52.5 ± 3.8          |
| pO2 (mm Hg)         | 91.1 ± 3.6    | 89.6 ± 3.5      | 83.3 ± 5.2        | 85.0 ± 4.4          |
| Glucose (mmol/L)    | 8.3 ± 0.2     | 8.4 ± 0.2       | 8.1 ± 0.4         | 8.5 ± 0.2           |
| Lactate (mmol/L)    | 3.1 ± 0.3     | 3.4 ± 0.2       | 4.5 ± 0.6         | 4.2 ± 0.4           |

Data are expressed as mean ± S.E.M.
(F(3,64) = 16.79, P < 0.0001), and interaction of treatment group × time (F(9,64) = 2.07, P = 0.046). Neuronal density in P(single) group decreased only at P9 (P < 0.0001); while it significantly decreased at different time points from P9 to P35 in P(repeated) group compared with intact (P < 0.0001, P = 0.009, P = 0.011, P = 0.033, respectively; Fig. 4A, C).

**Effects of propofol on the dynamic changes of synaptic density**

To determine how the synaptic density changed in a dynamic manner after propofol exposure, we examined the density of synaptophysin positive puncta by immunofluorescence. In the hippocampal CA1 region, no interaction of treatment group × time (F(9,64) = 0.62, P = 0.78) was observed, but the main effects of treatment group (F(3,64) = 26.02, P < 0.0001) and time (F(3,64) = 4.10, P = 0.01) were significant. Bonferroni’s pairwise comparisons revealed that synaptic density in P(single) group did not decrease at P9 (P > 0.05), P14 (P > 0.05), P21 (P > 0.05), or P35 (P > 0.05). However, it significantly decreased in P(repeated) group at different time points from P9 to P35 compared with intact (P < 0.0001, P = 0.009, P = 0.011, P = 0.033, respectively; Fig. 4A, C).
points from P9 to P35 compared with intact ($P = 0.038$, $P < 0.0001$, $P = 0.002$, $P = 0.001$, respectively, Fig. 5A, B). Similar changes were observed in the PrL region, no interaction of treatment group × time ($F_{1,64} = 0.59$, $P = 0.81$) was observed, however, the main effects of treatment group ($F_{1,64} = 28.09$, $P < 0.0001$) and time ($F_{1,64} = 15.01$, $P < 0.0001$) were significant. Bonferroni’s pairwise comparisons revealed that there were no differences in synaptic density between P(single) and intact from P9 to P35 ($P > 0.05$, respectively); while in P(repeated) group, synaptic density significantly decreased at different time points from P9 to P35 compared with intact ($P = 0.006$, $P < 0.0001$, $P = 0.008$, $P < 0.0001$, respectively, Fig. 5A, C).

**Effects of propofol on spatial learning and memory**

We examined the effects of different propofol exposures on spatial learning and memory using MWM tests. In the acquisition trials, the escape latencies were reduced during daily training in all groups ($F_{2,75,120.64} = 124.61$, $P < 0.0001$), and the between-subjects effect of treatment group ($F_{1,44} = 36.51$, $P < 0.0001$) was significant.

Post hoc Bonferroni test revealed that single propofol exposure did not affect the escape latencies ($P > 0.05$, Fig. 6A); while repeated propofol exposures increased the escape latencies on Day 1 ($P = 0.001$), Day 2 ($P < 0.0001$), Day 3 ($P = 0.009$), Day 4 ($P = 0.025$) and Day 5 ($P < 0.0001$) compared with intact (Fig. 6A). In the probe trial, there were no differences in platform crossing times between P(single) and intact ($P < 0.05$, Fig. 6B), while in P(repeated) group, the platform crossing times were significantly decreased ($P = 0.018$, Fig. 6B).

**DISCUSSION**

Neuronal apoptosis or synaptic loss induced by general anesthetics have been reported in the previous studies, but the results were from only one time point after propofol exposure, such as at P14 or P21 (Yu et al., 2013; Yang et al., 2014; Lunardi et al., 2010; Amrock et al., 2015), and the relationship between neuronal apoptosis or synapse formation and cognitive impairment induced by propofol is inaccurate. The present study demonstrates that multiple exposures to propofol at early age induce persistent neuronal apoptosis, neuronal deficit, synaptic loss and long-term cognitive impairment; while single exposure to propofol only induces transient neuronal apoptosis and deficit. It is clear now that cognitive function is based on normal neuronal circuit, which is determined by appropriate neuronal density and synapse formation (Jevtovic-Todorovic, 2012). Taken together, the present study suggests that propofol-induced persistent neuronal apoptosis, neuronal deficit, and synaptic loss contribute to long-term cognitive impairment. These results are consistent with clinical observation that children at an early age receiving multiple not single general anesthetic exposures have an increased risk to develop learning disabilities in adolescence (Wilder et al., 2009; Flick et al., 2011; Sprung et al., 2012; Davidson et al., 2016).

In the developing brain, neurons that are not involved in synapse formation and neuronal circuit are functionally redundant and eliminated via physiologic apoptosis under normal conditions (Raff et al., 1993). It has been reported that general anesthetic-induced widespread neuronal apoptosis is not simply related to the acceleration of physiologic apoptosis (Sinner et al., 2014; Konno et al., 2016), it can be assumed that apoptotic neurons induced by propofol may be from those both with and without synaptic connections. Moreover, our results indicate that propofol-induced neuronal apoptosis could be the cause of neuronal deficit, and persistent neuronal apoptosis induced by multiple propofol exposures can lead to long-lasting neuronal damage. In addition, propofol can also suppress neurogenesis in the developing brain (Erasso et al., 2013; Huang et al., 2016). Single propofol exposure may not suppress neurogenesis, or may only induce mild suppression of neurogenesis, so that neuronal density can recover rapidly in a compensatory mechanism; while multiple propofol exposures may induce severe suppression of neurogenesis, together with its more profound pro-apoptotic effect, ultimately result in persistent neuronal deficit. Neuronal deficit in pathological conditions has been reported to induce disturbance of synapse formation (Nikzad et al., 2007), so the decrease of synaptic density induced by multiple propofol exposures may attribute to neuronal deficit to some extent. Additionally, propofol with clinically relevant concentrations can cause neurite retraction (Turina et al., 2008), growth cone collapse (Al-Jahdari et al., 2006) and a persistent decrease in dendritic growth (Vutskits et al., 2005), which suggests decreased synaptic density induced by propofol may result from its straight inhibition on synapse formation as well. The present study, for the first time, demonstrates that repeated but not single exposure to propofol during critical periods of synaptogenesis induces persistent synaptic loss both in hippocampus and the PrL, which suggests that the effects of propofol-induced inhibition of synapse formation are exposure times dependent. However, the exact mechanisms underlying propofol-induced neuronal apoptosis and synaptic loss need to be studied in the near future. Furthermore, based on the results that
Fig. 4. Effects of propofol on the dynamic changes of neuronal density. (A) Representative images of Nissl staining at P9 in the hippocampal CA1 and PrL regions of right hemi-brain. Scale bar = 20 μm. (B,C) Quantification of neuronal densities from P9 to P35 in the hippocampal CA1 (B) and PrL (C). Data are expressed as mean ± S.E.M., n = 5. *P < 0.05, **P < 0.01, ***P < 0.0001 vs intact.

Fig. 5. Effects of propofol on the dynamic changes of synaptic density. (A) Representative images (3D volume rendering) of synapses (red) immunofluorescence at P14 in the hippocampal CA1 and PrL regions of left hemi-brain. Scale bar = 10 μm. (B,C) Quantification of synaptic densities from P9 to P35 in the hippocampal CA1 (B) and PrL (C) regions. Data are expressed as mean ± S.E.M., n = 5. *P < 0.05, **P < 0.01 and ***P < 0.0001 vs intact.
repeated exposures to propofol induces long-term cognitive impairment are consistent with clinical observation, it is reasonable to believe that repeated exposures to propofol in the present study can simulate multiple exposures to general anesthesia or prolonged sedation in pediatric patients in the clinic, and it is of great clinical significance to study the mechanism of multiple propofol exposures-induced developmental neurotoxicity. We used rat pups in this study, but it is unclear whether similar results can be observed in other animal species or with the dosage of propofol changed, so that further studies may be needed in the near future.

It has been confirmed that poor nutrition during early life can result in decreased synapse density in hippocampal formation and cognitive dysfunction (Granados-Rojas et al., 2004; Lister et al., 2005). However, in the present study, there was no significant difference in daily weight gain between the pups in each group. The blood-gas analysis showed that rat pups receiving propofol anesthesia developed mild respiratory acidosis, but there were no significant differences in pCO$_2$ among the groups, and between the two propofol groups. It has been demonstrated neonatal exposure to 4-hr carbon dioxide caused widespread brain cell death but did not lead to long-term cognitive deficits (Stratmann et al., 2009). Hence, it is reasonable to rule out the mild respiratory acidosis as a contributing factor to persistent neuronal apoptosis and deficit, synaptic loss, and long-term cognitive impairment.

There are several limitations in the present study. First, only male rat pups were included, it can not illustrate the sex-related differences. It has been reported that isoflurane exposure in newborn rats induces long-term cognitive dysfunction in males but not females (Lee et al., 2014). Recent studies have demonstrated that there are no sex differences in spatial memory retention performance in Morris Water Maze test after the triple cocktail of isoflurane, nitrous oxide and midazolam exposures in neonatal rats (Boscolo et al., 2013). Second, the effect of propofol on synaptic function was not studied. In addition to synaptic density, synaptic plasticity and synaptic neurotransmissions play an important role in learning and memory as well (Jevtovic-Todorovic et al., 2003; Kato et al., 2013; Sanchez et al., 2011). Further studies are needed to determine whether propofol of different exposure times can affect synaptic function at different time points with brain development. Finally, the rats in the present study did not undergo any operative procedure, and this experimental model could not well simulate clinical situation. Surgical noxious stimulation has been confirmed to cause marked cognitive dysfunction (Chiao and Zuo, 2014). It will be imperative to establish more appropriate experimental animal models to investigate anesthetic-induced neurotoxicity in the developing brain.

In conclusion, single exposure to propofol in neonatal rats only induces transient neuronal apoptosis and deficit, while multiple exposures to propofol induce persistent neuronal apoptosis, neuronal deficit, synaptic loss, and long-term cognitive impairment. Furthermore, permanent neuronal deficit and disturbances in synapse formation may contribute to long-term cognitive impairment induced by multiple propofol exposures.
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ACKNOWLEDGMENTS

This study was supported by the Natural Science Foundation Project of CQ CSTC 2013jjA1005. We would like to thank Lixue Chen, Guangcheng Qing and Xiaoyun Dou for technical assistance.

Conflict of interest--- The authors declare that there is no conflict of interest.

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