Neuroprotective effects of dexmedetomidine on the ketamine-induced disruption of the proliferation and differentiation of developing neural stem cells in the subventricular zone

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Abstract

**Background:** Recently, the number of neonatal patients receiving surgery under general anesthesia has increased. Ketamine disrupts the proliferation and differentiation of developing neural stem cells (NSCs). Therefore, the safe use of ketamine in pediatric anesthesia has been an issue of increasing concern among anesthesiologists and the children's parents. Dexmedetomidine (DEX) is widely used in sedation, as an antianxiety agent and for analgesia. DEX has recently been shown to provide neuroprotection against anesthetic-induced neurotoxicity in the developing brain. The aim of this *in vivo* study was to investigate whether DEX exerted neuroprotective effects on the proliferation and differentiation of NSCs in the subventricular zone (SVZ) following neonatal ketamine exposure.

**Methods:** Postnatal day 7 (PND-7) male Sprague-Dawley rats were equally divided into the following 5 groups: Control group (n=8), Ketamine group (n=8), 1 μg/kg DEX+Ketamine group (n=8), 5 μg/kg DEX+Ketamine group (n=8) and 10 μg/kg DEX+Ketamine group (n=8). The proliferation and differentiation of NSCs in the SVZ were assessed using immunostaining with BrdU incorporation. The levels of Nestin and β-tubulin III in the SVZ were measured using Western blot analyses. Apoptosis was assessed by detecting the levels of the cleaved caspase-3 protein using Western blotting.

**Results:** Neonatal ketamine exposure significantly inhibited NSC proliferation and astrocytic differentiation in the SVZ, and neuronal differentiation was markedly increased. Furthermore, pretreatment with moderate (5 μg/kg) or high doses (10 μg/kg) of DEX reversed the ketamine-induced disturbances in the proliferation and differentiation of NSCs. Meanwhile, neonatal ketamine exposure significantly decreased the expression of Nestin and increased the expression of β-tubulin III in the SVZ compared with the Control group. Treatment with 10 μg/kg DEX notably reversed the ketamine-induced changes in the levels of Nestin and β-tubulin III. In addition, a pretreatment with 10 μg/kg DEX before ketamine anesthesia prevented apoptosis in the SVZ induced by neonatal ketamine exposure.

**Conclusions:** Based on our findings, DEX may exert neuroprotective effects on the proliferation and differentiation of NSCs in the SVZ of neonatal rats in a repeated ketamine anesthesia model.

**Background**

Ketamine is a widely used analgesic and sedative during pediatric examinations and surgical operations [1-2]. Recently, an increasing number of studies have suggested that neonatal ketamine exposure might cause neuroapoptosis and disturb normal neurogenesis in the developing brain and increase the risk of delayed neurocognitive dysfunction [3-6]. The evidence from clinical studies also supported the hypothesis that ketamine exerts long-term adverse effects on the neurocognitive function in children and infants [7-8]. Relevant research conclusions have prompted anesthesiologists to re-evaluate the safety of using ketamine anesthesia in pediatric patients and to search for possible protective measures.

Dexmedetomidine (DEX), a highly selective α2-adrenoceptor agonist, is a widely used anxiolytic, sedative and analgesic in clinical pediatric anesthesia and intensive care [9-10]. DEX exerts protective effects on
the vital organs, including decreases in lung and kidney damage and neural apoptosis [11-12]. In anesthesia models using neonatal animals, DEX has been proven to protect against inhalation anesthetics-induced neurotoxicity in the developing brain [13-14]. In clinical pediatric anesthesia, a medication strategy with DEX is increasingly being accepted as a method to reduce the adverse effects of ketamine [15-16]. However, the potential neuroprotective pathway of DEX requires further investigation.

Neurogenesis in the hippocampus and subventricular zone (SVZ) are important processes in the developing brain [17-18]. Early disruption of these regions has the potential to exert an adverse effect on the formation of neural circuits [19]. Currently, little is known about whether a DEX pretreatment exerts protective effects on the ketamine-induced neurotoxicity in the SVZ. Therefore, the purpose of this study was to investigate the protective effects of DEX on neurogenesis in the SVZ in a repeated ketamine exposure model using neonatal rats.

**Materials And Methods**

**Animals and drug administration**

All animal procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University. Timed-pregnant Sprague-Dawley rats were housed at 24°C on a 12-h light/dark cycle with free access to food and water. Postnatal day 7 (PND-7) male rats (11-14 g) were selected from all the pups and used in the experiments.

Ketamine and dexmedetomidine (DEX, HengRui Pharma, China) were dissolved in 0.9% normal saline. The rat pups were randomly divided into 5 groups: (1) Control group (CON): the rats received an equal volume of saline (n=8); (2) Ketamine group (KET): the rats were administered four intraperitoneal injections of 40 mg/kg ketamine at 1 h intervals (40 mg/kg × 4) (n=8); (3) KET+DEX 1 group: the rats were pretreated with an intraperitoneal injection of 1 μg/kg DEX 30 min prior to ketamine anesthesia (40 mg/kg × 4) (n=8); (4) KET+DEX 5 group: the rats were pretreated with an intraperitoneal injection of 5 μg/kg DEX 30 min prior to ketamine anesthesia (40 mg/kg × 4) (n=8); (5) KET+DEX 10 group: the rats were pretreated with an intraperitoneal injection of 10 μg/kg DEX 30 min prior to intraperitoneal ketamine anesthesia (40 mg/kg × 4) (n=8). Custom-made temperature probes were used to control the temperature at 36.5 ± 1°C using computer-controlled heater/cooler plates integrated into the chamber floor. Between each injection, animals were returned to their chamber to help maintain their body temperature and reduce stress. Four injections of 40 mg/kg ketamine administered at 1 h intervals produced a satisfactory level of anesthesia, and all animals in each group survived after the anesthesia.

Arterial blood (0.25 ml) gas levels were measured by cardiac puncture with a 30-gauge needle immediately after the end of anesthesia through (6 animals per group) to ensure that hypoxia did not occur in any newborn animal during anesthesia. Arterial blood gases were measured with a portable clinical analyzer (GEM Premier 3000, USA).

**BrdU injections**
Immediately after the treatment of each group, the rats received a single intraperitoneal injection of BrdU (5-bromo-2-deoxyuridine; Sigma, 100 mg/kg) in a 0.9% NaCl solution. At 24 h after the BrdU injection, the animals were intraperitoneally injected with 4% chloral hydrate (0.1 ml/10g) and fixed by perfusion. The detailed experimental protocol is listed in Table 1.

| Targeted Process                  | BrdU Injections | Interval to Perfusion | IF Stain             |
|-----------------------------------|-----------------|-----------------------|----------------------|
| NSC proliferation                 | PND-7/100       | 24 h (PND-8)          | BrdU                 |
| Neuronal differentiation           | PND-7/100       | 24 h (PND-8)          | β-tubulin III/BrdU   |
| Astrocytic differentiation         | PND-7/100       | 24 h (PND-8)          | GFAP/BrdU            |

The interval to perfusion refers to the time from the BrdU injection to transcardiac perfusion. IF = immunofluorescence.

**Tissue preparation and immunofluorescence staining**

Double immunofluorescence staining with BrdU were conducted as described in our previously reported methods [5]. The rat brain was isolated, removed and fixed with 4% paraformaldehyde fixative for 6 h. Then, the brain was embedded in medium on ice and stored at -80°C. Coronal brain sections were cut using a microtome at a thickness of 30 μm. When the SVZ was initially exposed in the slice, five consecutive coronal sections were cut and discarded, three successive brain slices were selected for the BrdU single-label immunofluorescence staining and double-label immunofluorescence staining for β-tubulin III/BrdU and GFAP/BrdU. The sections were incubated with 50% formamide in PBS for 2 h at 65°C prior to an incubation with 2 N hydrochloric acid for 30 min at 45°C and 3 washes with PBS for 10 min. Nonspecific epitopes were blocked with 10% donkey serum in PBS with 0.3% Triton-X for 2 h prior to an overnight incubation at 4°C with the appropriate primary antibody (Table 2) in PBS containing 0.3% Triton-X. After 3 washes with PBS, the sections were incubated with suitable fluorescent dye-conjugated secondary antibodies (Alexa Fluor 488-labeled donkey anti-rabbit and Alexa Fluor 594-labeled donkey anti-mouse; 1:200; Invitrogen) for 2 h at room temperature. The sections were observed and image stacks were captured by a skilled pathologist who was blinded to the groups using a laser scanning confocal microscope (Fluoview 1000, Olympus). For each brain, we selected three sections for immunofluorescence staining for BrdU, three sections for immunofluorescence staining for β-tubulin III/BrdU and three sections for immunofluorescence staining for GFAP/BrdU, respectively. The fluorescently labeled area was measured using ImageJ software (from the National Institutes of Health, Bethesda, MD, USA). The number of BrdU+ cells per SVZ from each animal, the ratio of β-tubulin III+/BrdU+ cells to BrdU+ cells and the ratio of GFAP+/BrdU+ cells to BrdU+ cells were calculated (n = eight animals/group).
### Table 2

| Raised Against | Supplier       | Raised in          | Dilution |
|----------------|---------------|--------------------|----------|
| BrdU           | Sigma         | Mouse, monoclonal  | 1:1000   |
| GFAP           | Millipore     | Rabbit, monoclonal | 1:200    |
| β-tubulin III  | Abcam         | Rabbit, polyclonal | 1:100    |

List of primary antibodies, their suppliers, the animal used to raise the antibodies in, and the working dilution.

**Western blot analysis**

The levels of the Nestin (proliferation marker) and β-tubulin III (neuronal differentiation marker) proteins were detected using Western blot analyses. We decapitated rat pups from each group to extract SVZ tissues at 24 h after the end of anesthesia. The level of cleaved caspase-3 (apoptosis marker) was detected 12 h after the end of anesthesia. The immunoblotting procedure was performed as described in our previous study [5]. Briefly, the brain tissues from the SVZ were homogenized in lysis buffer containing protease inhibitors (Beyotime, China). The lysates were centrifuged at 14000 rpm for 15 min at 4°C. Equal amounts of the proteins (25 μg) were resolved on a 10% or 12% sodium dodecyl sulfate-polyacrylamide gel, and the separated proteins were transferred to nitrocellulose membranes. The membranes were incubated with blocking buffer for 2 h at room temperature and then incubated with the primary antibodies against Nestin (1:1000, Abcam), β-tubulin III (1:1000, Abcam), cleaved caspase-3 (1:500, Abcam) and GAPDH for 24 h. Then, the membranes were incubated with the appropriate secondary antibodies for 1 h. The immunoreactive bands were visualized with a chemiluminescence detection system. The band intensity was quantified using ImageJ software (n = three animals/group).

**Statistical analysis**

The data obtained from the analysis of the proliferation and differentiation of NSCs are reported as medians and interquartile ranges (IQRs). The blood gas levels are presented as means ± SD. Differences among the treatment groups were evaluated using one-way ANOVA. The statistical analysis and the graphs were generated using GraphPad Prism 5 software. Significant differences between the groups were analyzed using one-way ANOVA. \( P<0.05 \) was considered statistically significant.

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Ketamine and dexmedetomidine (DEX, HengRui Pharma, China) were dissolved in 0.9% normal saline. The rat pups were randomly divided into 5 groups: (1) Control group (CON): the rats received an equal volume of saline (n=8); (2) Ketamine group (KET): the rats were administered four intraperitoneal injections of 40 mg/kg ketamine at 1 h intervals (40 mg/kg × 4) (n=8); (3) KET+DEX 1 group: the rats were pretreated with an intraperitoneal injection of 1 μg/kg DEX 30 min prior to ketamine anesthesia (40 mg/kg × 4) (n=8); (4) KET+DEX 5 group: the rats were pretreated with an intraperitoneal injection of 5 μg/kg DEX 30 min prior to ketamine anesthesia (40 mg/kg × 4) (n=8); (5) KET+DEX 10 group: the rats were pretreated with an intraperitoneal injection of 10 μg/kg DEX 30 min prior to intraperitoneal ketamine anesthesia (40 mg/kg × 4) (n=8). Custom-made temperature probes were used to control the temperature at 36.5 ± 1°C using computer-controlled heater/cooler plates integrated into the chamber floor. Between each injection, animals were returned to their chamber to help maintain their body temperature and reduce stress. Four injections of 40 mg/kg ketamine administered at 1 h intervals produced a satisfactory level of anesthesia, and all animals in each group survived after the anesthesia.

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List of primary antibodies, their suppliers, the animal used to raise the antibodies in, and the working dilution.

**Western blot analysis**

The levels of the Nestin (proliferation marker) and β-tubulin III (neuronal differentiation marker) proteins were detected using Western blot analyses. At 24 h after the end of ketamine anesthesia, the animals were intraperitoneally injected with 4% chloral hydrate (0.1 ml/10g) and all of them were anaesthetised and unconscious. Then, we decapitated rat pups from each group to extract SVZ tissues. The level of cleaved caspase-3 (apoptosis marker) was detected 12 h after the end of anesthesia. The immunoblotting procedure was performed as described in our previous study [5]. Briefly, the brain tissues from the SVZ were homogenized in lysis buffer containing protease inhibitors (Beyotime, China). The lysates were centrifuged at 14000 rpm for 15 min at 4°C. Equal amounts of the proteins (25 μg) were performed electrophoresis on a 10% or 12% sodium dodecyl sulfate-polyacrylamide gel. At the end of
electrophoresis, the gel were cropped depending on the molecular weight range of target protein. Next we made the 8 cm\*1.5 cm PVDF membrane and covered it on the cropped gel for electrotransfer. Then, the membranes were incubated with blocking buffer for 2 h at room temperature and then incubated with the primary antibodies against Nestin (1:1000, Abcam), β-tubulin III (1:1000, Abcam), cleaved caspase-3 (1:500, Abcam) and GAPDH for 24 h. Then, the membranes were incubated with the appropriate secondary antibodies for 1 h. The immunoreactive bands were visualized with a chemiluminescence detection system (Bio-Rad, Hercules, CA, USA). The band intensity was quantified using ImageJ software (n = three animals/group).

Statistical analysis

The data obtained from the analysis of the proliferation and differentiation of NSCs are reported as medians and interquartile ranges (IQRs). The blood gas levels are presented as means ± SD. Differences among the treatment groups were evaluated using one-way ANOVA. The statistical analysis and the graphs were generated using GraphPad Prism 5 software. Significant differences between the groups were analyzed using one-way ANOVA. $P<0.05$ was considered statistically significant.

Results

The arterial blood gas analyses

In the experiment, all rats in each group survived to the end of the experiment. The animals’ skin was ruddy and the respiration was smooth. No significant changes in the pH, pCO$_2$ and pO$_2$ were observed between the groups (Table 3).

Table 3
The arterial blood gases analyse (6 animals per group)

| Parameter  | group I | group II | group III | group IV | group V |
|------------|---------|----------|-----------|----------|---------|
| pH         | 7.39 ± 0.02 | 7.41 ± 0.03 | 7.41 ± 0.03 | 7.42 ± 0.04 | 7.42 ± 0.04 |
| P$_{CO2}$  | 39.3 ± 1.4 | 39.1 ± 1.7 | 38.0 ± 2.1 | 40.0 ± 2.3 | 39.8 ± 2.6 |
| P$_{O2}$   | 168.0 ± 3.1 | 166.0 ± 4.4 | 163.3 ± 4.5 | 164.8 ± 5.1 | 162.2 ± 4.0 |

a P$_{CO2}$ pressure carbon dioxide.

b P$_{O2}$ pressure oxygen.

The effects of ketamine on the proliferation and differentiation of NSCs in the SVZ
BrdU immunofluorescence staining was performed to evaluate the proliferation of NSCs in the SVZ. When 100 mg/kg BrdU was injected immediately after anesthesia in PND-7 rats, we observed a substantial reduction in the number of BrdU+ cells in the SVZ of the ketamine group (median [IQR]: 28900 [26500-30750]) compared to the Control group (median [IQR]: 44435 [42450-47408], shown in Fig. 1A and 1B, n = 8, *p*<0.01). Thus, neonatal ketamine exposure inhibited NSC proliferation in the SVZ.

NSCs have the ability to differentiate into neurons, and the early neuronal marker associated with differentiation is β-tubulin III. BrdU+ cells coexpressing the neuronal marker β-tubulin III were analyzed to assess the neuronal differentiation of NSCs. In the present study, the proportion of β-tubulin III+/BrdU+ cells among BrdU+ cells in the ketamine group (median [IQR]: 19.05% [18-19.725%]) was increased compared to control animals (median [IQR]: 13.15% [12.475-13.7%]) at 24 h after the BrdU injection (Fig. 2A and 2B, n = 8, *p*<0.01).

The proportion of BrdU+ cells coexpressing GFAP was calculated to assess the astrocytic differentiation of NSCs. The proportion of GFAP+/BrdU+ cells among BrdU+ cells at 24 h after the BrdU injection was significantly greater in control rats (median [IQR]: 16.3% [15.925-16.925%]) than in ketamine-anesthetized rats (median [IQR]: 11.35% [10.775-11.975%], Fig. 3A and 3B, n = 8, *p*<0.01). Based on these findings, neonatal ketamine exposure significantly promoted neuronal differentiation and attenuated the astrocytic differentiation of NSCs in the SVZ.

**Dexmedetomidine protects the proliferating and differentiation of NSCs from ketamine-induced injury**

Next, we investigated the effect of dexmedetomidine (DEX) on the ketamine-induced disruption of the proliferation and differentiation of NSCs in the SVZ using immunofluorescence staining. As shown in Fig. 1D and 1E, a pretreatment with 5 μg/kg DEX or 10 μg/kg DEX before ketamine anesthesia significantly increased the number of BrdU+ cells in the SVZ compared to the ketamine group (median [IQR]: 35900 [35025-36950] in the 5 μg/kg DEX plus ketamine group, median [IQR]: 41100 [38925-43368] in the 10 μg/kg DEX plus ketamine group, and median [IQR]: 28900 [26500-30750] in the ketamine group, n = 8, *p*<0.01).

Moreover, compared with the ketamine group, the pretreatment with 5 μg/kg DEX or 10 μg/kg DEX before ketamine anesthesia significantly decreased the proportion of β-tubulin III+/BrdU+ cells in the SVZ compared with the ketamine treatment (median [IQR]: 15.6% [14.85%-16.05] in the 5 μg/kg DEX plus ketamine group, median [IQR]: 13.85 [13.125-14.175] in the 10 μg/kg DEX plus ketamine group, and median [IQR]: 19.05% [18%-19.725%] in the ketamine group, n = 8, *p*<0.01, Fig. 2A and 2B). In addition, the DEX pretreatment dose-dependently ameliorated the reduction in the proportion of GFAP+/BrdU+ cells in the SVZ caused by ketamine anesthesia (median [IQR]: 13.7% [12.88%-13.98] in the 5 μg/kg DEX plus ketamine group, median [IQR]: 15.35 [14.93-15.68] in the 10 μg/kg DEX plus ketamine group, and median [IQR]: 11.35% [10.775%-11.975%] in the ketamine group, n = 8, *p*<0.01, Fig. 3A and 3B). However, the 1 μg/kg DEX pretreatment did not exert a protective effect on the ketamine-induced disruption of proliferation and differentiation of NSCs (*p*>0.05, Figs. 1F, 2B and 3B).
In summary, moderate- and high-dose DEX pretreatments alleviated the ketamine-induced disturbance in the proliferation and differentiation of NSCs in the SVZ.

**The dexmedetomidine pretreatment reversed the ketamine-induced changes in the levels of neural stem cell and neuronal markers**

As shown in our previous study, the levels of the Nestin protein (Neural stem cell marker) are significantly reduced in the SVZ after neonatal ketamine anesthesia and the level of the β-tubulin III protein (newly differentiated neuron marker) is significantly increased in the ketamine group. The results of the present study are consistent with our previous observations. Compared with the Control group, four injections of 40 mg/kg ketamine significantly decreased the Nestin level (0.45 ± 0.06 vs. 1 ± 0.04, \( p<0.01 \)) and increased the β-tubulin III level (2.15 ± 0.16 vs. 1 ± 0.07, \( p<0.01 \)) in the SVZ of neonatal rats (Fig. 4A and 4B).

Using immunofluorescence staining, we further observed the effect of the 10 µg/kg dexmedetomidine (DEX) pretreatment on the levels of Nestin and β-tubulin III after ketamine anesthesia. Immunoblots did not reveal a significant difference in Nestin levels between the Control group and 10 µg/kg DEX group (1 ± 0.04 vs. 1.03 ± 0.14, \( p=0.97 \)) (Fig. 4A); the pretreatment with 10 µg/kg DEX prior to ketamine anesthesia resulted in more intense bands representing Nestin (Fig. 4A) than in the Ket group (0.95 ± 0.08 vs. 0.45 ± 0.06, \( p<0.05 \)) (Fig. 4A). Next, an analysis of the immunoblotting results did not reveal a significant difference in the β-tubulin III levels between the Control group and 10 µg/kg DEX group (1 ± 0.07 vs. 1.08 ± 0.19, \( P=0.92 \)) (Fig. 4B), and the quantification of the Western blot showed a reversal of the ketamine-induced increase in β-tubulin III levels by the 10 µg/kg DEX pretreatment (1.33 ± 0.06 vs. 2.15 ± 0.16, \( P<0.05 \)) (Fig. 4B).

**The dexmedetomidine pretreatment alleviated apoptosis in the SVZ after neonatal ketamine exposure**

Repeated ketamine exposure during the neonatal stage has been shown to induce apoptosis in the developing brain. Quantification of the Western blot results suggested that four injections of 40 mg/kg ketamine at 1 h intervals increased the levels of cleaved caspase-3 compared with the Control group (2.94 ± 0.18 vs. 1 ± 0.15, \( p<0.01 \)) (Fig. 5), suggesting that ketamine induced apoptosis in the SVZ. We first measured the levels of cleaved caspase-3 in the 10 µg/kg DEX group to evaluate the neuroprotective effects of DEX, and a significant difference in apoptosis in the SVZ of neonatal rats was not observed after the DEX treatment (1.05 ± 0.08 vs. 1 ± 0.15, \( P=0.9704 \)) (Fig. 5). However, pretreatment with 10 µg/kg DEX prior to ketamine anesthesia significantly decreased the levels of cleaved caspase-3 compared with the ketamine group (1.36 ± 0.08 vs. 2.94 ± 0.18, \( P<0.05 \)) (Fig. 5). Based on these findings, the DEX pretreatment potentially reversed the effects of ketamine on apoptosis in the SVZ.

**Discussion**

Ketamine is a dissociative anesthetic that is commonly used in pediatric anesthesia. Based on increasing preclinical evidence, ketamine may cause neurodegeneration and neuroapoptosis in the developing brain...
and precipitate significant long-term cognitive sequelae in rodents and nonhuman primates. Epidemiological evidence has indicated that long-term neurotoxicity may ensue following prolonged and/or repeated exposure to ketamine in early life. The U.S. Food and Drug Administration (FDA) had issued a warning about prolonged and/or repeated exposure to general anesthetics and their potential negative effects on the developing brains of children[20].

The purpose of this study was to investigate the effect of prolonged and/or repeated exposure to general anesthetics on the developing brain and explore the potential protective approaches. We had performed the dose-dependent studies and selected four doses of ketamine, including 10, 20, 40 and 60 mg/kg. It was found that four injections of 10 or 20 mg/kg ketamine with 1 h intervals only obviously reduced the movement of animals, while the righting reflex still existed in some animals. In addition, only a small part of neonatal rats could survive after the end of four injections of 60 mg/kg ketamine with 1 h intervals. Finally, the four injections of 40 mg/kg ketamine with 1 h intervals could exert the satisfactory anesthesia effect and all animals could survive after the anesthesia. Therefore, the four injections of 40 mg/kg ketamine with 1 h intervals could construct an model of multiple anesthesia during neonatal period in the present experiment.

Neurogenesis is an important process that occurs in multiple brain regions, particularly in the hippocampal dentate gyrus (DG) and subventricular zone (SVZ), from the embryonic to adult stages [17-18]. The vast majority of NSCs are in a mitotically active state in the developing brain, and the balance between the mitosis and quiescence of NSCs is crucial to maintain the balance between the types and numbers of cells in the brain, which are the origins of neurons and glial cells. In rodents, a large number of neurons are established during gestation and throughout the first 21 postnatal days, which play critical roles in the formation of neural networks and neurocognitive functions. As shown in our previous study, neonatal ketamine exposure disrupts the proliferation and differentiation of NSCs in the developing hippocampal DG and causes hippocampus-dependent spatial memory dysfunction during the adult stage [5].

During the developmental neurogenesis period in the SVZ, newly generated neurons migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB), where the neurons are incorporated into existing neural circuits and play a crucial role in long-term olfactory recognition memory [21-22]. The early disruption of SVZ neurogenesis has the potential to alter the formation of neural circuits [23] and olfactory cognitive function during adulthood [24]. Based on the findings from the present study, repeated ketamine exposure (40 mg/kg×4) during the developmental stage disrupts neurogenesis, including the significant inhibition of NSC proliferation and astrocytic differentiation and the induction of neuronal differentiation in the SVZ. These results were consistent with our previous findings showing that ketamine affects the fates of NSCs in the hippocampal DG and SVZ [5, 25].

Neuroprotective measures are constantly being explored for general anesthesia administered to pediatric patients, and anesthetics with neuroprotective effects have been widely investigated to avoid the adverse neurological complications of conventional anesthetics. In contrast to ketamine, the α2-adrenoceptor
agonist dexmedetomidine (DEX) is an adjuvant anesthetic that has been extensively studied in recent years, as it produces sedative, analgesic, sympatholytic and anxiolytic effects during the perioperative period. To date, at least three different α₂ receptors (α₂A, α₂B and α₂C) have been identified based on pharmacological and molecular biological probes [26]. The α₂A subtype correlates with the sedative, analgesic, neuroprotective and sympatholytic effects [27]. The role of DEX in neonatal intensive care medicine and pediatric anesthesia has been an interesting research topic in recent years. In preclinical studies, DEX has attracted the attention of researchers and clinicians because it exerts neuroprotective effects on hippocampal neurogenesis and neuronal plasticity in a model of neonatal brain injury [28].

In the field of anesthetic-induced developmental neurotoxicity, DEX has been proven to exert neuroprotective effects on anesthetic-induced neurodegeneration and neuroapoptosis in the developing brain [13, 14, 29-31]. According to a recent in vitro study, DEX protects NSCs in the embryonic cortex from ketamine-induced injury [32]. Although DEX has been extensively studied as a clinical adjuvant anesthetic, little is known about its neuroprotective effects on the proliferation and differentiation of NSCs after neonatal ketamine exposure in animal models. In the clinic, pediatric patients particularly have higher requirements and a better tolerance for DEX [33, 34]. In the present study, the dose of DEX was based on clinical concentrations that had been used in children (1 μg/kg) [35] or had been shown to exert neuroprotective effects on other animal models (5 μg/kg and 10 μg/kg) [36, 37]. In the present study, one key finding was the substantial reversal of the ketamine-induced disruption of the proliferation and differentiation of NSCs in the SVZ by a single pretreatment with DEX at intermediate (5 μg/kg) or high doses (10 μg/kg). However, the administration of low-dose DEX (1 μg/kg) did not produce potential neuroprotective effects. Importantly, the administration of DEX at a concentration of 10 μg/kg in this study did not significantly alter the levels of the Nestin and β-tubulin III proteins in the SVZ, indicating that the higher dose of DEX used in this study did not alter the proliferation and differentiation of NSCs in the SVZ during development. Furthermore, a pretreatment with 10 μg/kg DEX significantly inhibited the apoptosis in the SVZ induced by neonatal ketamine exposure. These findings may provide a basis for the use of a combination of DEX and ketamine in pediatric anesthesia.

**Conclusion**

The neuroprotective effect of DEX has been an interesting topic of neonatological and pediatric anesthetic research in recent years. In conclusion, the present findings preliminarily confirmed that a DEX pretreatment protected against the ketamine-induced disruption of the proliferation and differentiation of NSCs in the SVZ during development in a dose-dependent manner. Moreover, the effects of DEX on the long-term olfactory memory functions require in vivo studies. Before its safe and efficient application in clinical pediatric anesthesia, the potential neuroprotective mechanisms underlying the effect of DEX on ketamine-induced neurotoxicity require further experimental and clinical investigations.

**Abbreviations**
Declarations

Ethics approval and consent to participate

All animal experiments were carried out according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (publication no. 85-23, revised 1985). The experiments were approved by the Institutional Animal Care and Use Committee of the Nanjing Medical University.

Consent for publication

Not applicable.

Availability of data and material

The data that support the findings of this study are available from the corresponding author if needed.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

Conceived and designed the experiments: H Huang, HH Sha and PP Peng; Performed the experiments: HH Sha, PP Peng, B Li, GH Wei, J Wang; Data analysis and interpretation: HH Sha, PP Peng, H Huang and YQ Wu; Contributed reagents/materials/analysis tools: YQ Wu; Manuscript preparation: HH Sha, PP Peng and H Huang. All authors read and approved the final manuscript.

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Figures
Figure 1

The effects of ketamine and dexmedetomidine (DEX) on the proliferation of neural stem cells (NSCs) in the SVZ. The neonatal rats were pretreated with different doses of DEX intraperitoneally prior to ketamine anesthesia for 30 min. [A] Representative images of NSCs proliferation (BrdU staining, red) were visualized using a laser scanning confocal microscope (magnification ×200, scale bar: 50 μm). [B] The number of BrdU+ cells per SVZ in the different groups is expressed. Data are shown as the mean ± SD (8 tissue sections per group). **p<0.01 vs. Control group; ##p<0.01 vs. Ket group. SVZ = subventricular zone; LV = lateral ventricle.
Figure 2

The effects of ketamine and dexmedetomidine (DEX) on the neuronal differentiation of neural stem cells (NSCs) in the SVZ. Neonatal rats were pretreated with different doses of DEX intraperitoneally prior to ketamine anesthesia for 30 min. [A] Representative images of neuronal differentiation (BrdU, red; β-tubulin III, green) were visualized using a laser scanning confocal microscope (magnification ×400, scale bar: 50 μm). [B] The proportion of β-tubulin III+/BrdU+ cells to BrdU+ cells per SVZ in the different groups are expressed. Data are shown as the mean ± SD (8 tissue sections per group). **p<0.01 vs. Control group; ##p<0.01 vs. Ket group. SVZ = subventricular zone; LV = lateral ventricle.
The effects of ketamine and dexmedetomidine (DEX) on the astrocytic differentiation of neural stem cells (NSCs) in the SVZ. Neonatal rats were pretreated with different doses of DEX intraperitoneally prior to ketamine anesthesia for 30 min. [A] Representative images of astrocytic differentiation (BrdU, red; GFAP, green) were visualized using a laser scanning confocal microscope (magnification ×400, scale bar: 50 μm). [B] The proportion of GFAP+/BrdU+ cells to BrdU+ cells per SVZ in the different groups are expressed. Data are shown as the mean ± SD (8 tissue sections per group). **p<0.01 vs. Control group; ##p<0.01 vs. Ket group. SVZ = subventricular zone; LV = lateral ventricle.
Figure 4

The effects of ketamine and dexmedetomidine (DEX) on the expression of Nestin and \(\beta\)-tubulin III in the SVZ of neonatal rats. The PND-7 rats were exposed to four injections of 40 mg/kg ketamine at 1-h intervals. Then, SVZ tissues of rat pups in all groups were extracted at 24 h after the end of administration. The expression of Nestin and \(\beta\)-tubulin III were measured using Western blot analysis (A and B). The data are presented as the means ± SD (n=3). **p<0.01 vs. Control group; #p<0.05 vs. Ket group.
Figure 5

The effects of ketamine and dexmedetomidine (DEX) on the expression of caspase-3 fragment in the SVZ of neonatal rats. The PND-7 rats were exposed to four injections of 40 mg/kg ketamine at 1-h intervals. Then, SVZ tissues of rat pups in all groups were extracted at 24 h after the end of administration. The expression of Nestin and β-tubulin III were measured using Western blot analysis (A and B). The data are presented as the means ± SD (n=3). **p<0.01 vs. Control group; #p<0.05 vs. Ket group.

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