Ketamine Interferes with the Proliferation and Differentiation of Neural Stem Cells in the Subventricular Zone of Neonatal Rats

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Abstract
Background: Previous studies have shown ketamine can alter the proliferation and differentiation of neural stem cells (NSCs) in vitro. However, these effects have not been entirely clarified in vivo in the subventricular zone (SVZ) of neonatal rats. The present study was designed to investigate the effects of ketamine on the proliferation and differentiation of NSCs in the SVZ of neonatal rats in vivo. Methods: Postnatal day 7 (PND-7) male Sprague-Dawley rats were administered four injections of 40 mg/kg ketamine at 1-h intervals, and then 5-bromodeoxyuridine (BrdU) was injected intraperitoneally at PND-7, 9 and 13. NSC proliferation was assessed with Nestin/BrdU double-labeling immunostaining. Neuronal and astrocytic differentiation was evaluated with β-tubulin III/BrdU and GFAP/BrdU double-labeling immunostaining, respectively. The expressions of nestin, β-tubulin III and GFAP were measured using Western blot analysis. The apoptosis of NSCs and astrocytes in the SVZ of neonatal rats was evaluated using nestin/caspase-3 and GFAP/caspase-3 double-labeling immunostaining. Results: Neonatal ketamine exposure significantly reduced the number of nestin/BrdU and GFAP/BrdU double-positive cells in the SVZ. Meanwhile, the expressions of nestin and GFAP in the SVZ from the ketamine group were significantly decreased compared to those in the control group. Still, no double-positive cells for nestin/caspase-3 and GFAP/caspase-3 were found after ketamine exposure. In addition, the neuronal differentiation of NSCs in the SVZ was markedly promoted by ketamine with an increased number of β-tubulin III/BrdU double-positive cells and enhanced expression of β-tubulin III. These effects of ketamine on the NSCs in the SVZ often lasted at least 1 week after ketamine anesthesia. Conclusion: In the present study, it was demonstrated that ketamine could alter neurogenesis by inhibiting the proliferation of NSCs, suppressing their differentiation into astrocytes and promoting the neuronal differentiation of the NSCs in the SVZ of neonatal rats during a critical period of their neurodevelopment.
Introduction

Ketamine, a N-methyl-D-aspartate receptor (NMDA-R) ion channel blocker, is widely used in anesthesia, analgesia and sedation during the neonatal period [1, 2]. However, ketamine has been found to cause neuronal apoptosis and neurological function deficits. The conclusions of animal and clinical research have generated worry regarding the safety of using ketamine in the pediatric population [3-9].

The developing brain experiences a critical period called the brain growth spurt (BGS), which begins at the end of pregnancy in rodents and extends to the first 2-3 weeks after birth [10]. In humans, the corresponding period starts at the last trimester of pregnancy and continues to 2 years after birth. Substantial neurogenesis occurs in this period, which is coordinated by NSC proliferation, differentiation, migration, survival and synaptogenesis. This critical phase is essential to normal brain structure and functional development [11]. Neurogenesis occurs in several regions during brain development, especially in the SVZ, a restricted neurogenesis region that exists throughout life in the brain beside the hippocampal dentate gyrus (DG). Neurogenesis in the SVZ and DG can be stimulated by many factors, including stroke [12]. One or more of the events that are required for neurogenesis, such as NSC proliferation and neuronal differentiation, play an important role in compensating for lost neurons.

Although the neonatal neuroapoptosis induced by ketamine has been demonstrated by increasing number of studies, the effects of ketamine on neurogenesis have not been completely clarified. A recent in vitro study showed that ketamine altered the proliferation and differentiation of rat cortical neural stem progenitor cells [13]. However, the effects of ketamine on neonatal neurogenesis in vivo remain to be investigated. Given the importance of SVZ neurogenesis to the structural and functional development of the brain, the effects of ketamine on the proliferation and differentiation of the NSCs in the SVZ of neonatal rats were investigated in this study.

Materials and Methods

Animals treatment

All the animal experiments were approved by the Institutional Animal Care and Use Committee of Xuzhou Medical College. Timed-pregnant Sprague-Dawley rats were housed at 24°C on a 12-hr:12-hr light:dark cycle with free access to food and water. The PND-7 male rats (11-14g) selected from all the pups were used in the experiments. These rats were randomly assigned to control groups and ketamine groups. Ketamine was diluted in 0.9 % normal saline. PND-7 rats in treated group were administered intraperitoneally by four injections of 40 mg/kg ketamine with 1h intervals. Animals in control group received equal volume of saline at the same time points. Custommade temperature probes were used to facilitate control of temperature at 36.5 ± 1°C using computer-controlled heater/cooler plates integrated into the floor of chamber. Between each injection animals were returned to their chamber to help maintain body temperature and reduce stress.

BrdU injections

After anesthesia, neonatal rats received a single intraperitoneal injection of BrdU (5-bromo-2-deoxyuridine; Sigma, 100 mg/kg) in 0.9% NaCl solution at PND-7, 9 and 13. The animals were fixed by perfusion at 3 h after BrdU injection to observe the proliferation of matured astrocytes or at 24 h after BrdU injection to observe the proliferation and differentiation of the NSCs. The detailed experimental protocol is listed in Table 1.

Tissue preparation and double-immunofluorescence

The animals were anesthetized and then transcardially perfused after BrdU injection. The coronal sections of the brain were cut on a microtome at a thickness of 30 μm. When the SVZ was initially exposed, the five consecutive coronal sections were cut and discarded, and then the next three consecutive coronal sections were cut and selected for the double-labeled immunofluorescence of nestin/BrdU, β-tubulin III/
Table 1. Experimental Protocol (n=5). The interval to perfusion refers to the time from the BrdU injection to transcardiac perfusion. IF = immunofluorescence

| Targeted Process       | BrdU Injections Timing/Dose (mg/kg) | Interval to Perfusion | IF Stain               |
|------------------------|-------------------------------------|-----------------------|------------------------|
| NSC proliferation      | PND-7/100 24-h (PND-8)              | Nestin/BrdU           |
| Neuronal differentiation| PND-9/100 24-h (PND-10)             | β-tubulin III/BrdU    |
| Neuronal differentiation| PND-13/100 24-h (PND-14)            |                       |
| Astrocytic differentiation| PND-7/100 24-h (PND-8)              | GFAP/BrdU             |
| Astrocyte proliferation| PND-9/100 24-h (PND-10)             |                       |
|                        | PND-13/100 24-h (PND-14)            |                       |

Table 2. Primary Antibodies. List of primary antibodies, their suppliers, the animal used to raise the antibodies in, and the working dilution

| Raised Against | Supplier | Raised in | Dilution |
|----------------|----------|-----------|----------|
| BrdU           | Sigma    | Mouse, monoclonal | 1:1000    |
| Nestin         | Abcam    | Rabbit, polyclonal | 1:100     |
| GFAP           | Millipore| Rabbit, monoclonal | 1:200     |
| β-tubulin III  | Abcam    | Rabbit, polyclonal | 1:100     |
| Caspase-3      | Santa Cruz| Mouse, monoclonal | 1:100     |

BrdU and GFAP/BrdU, respectively. This procedure was repeated five times. The sections were incubated with 50% formamide in PBS for 2 h at 65°C and then in 2 normal hydrochloric acid incubation for 30 min at 45°C, followed by 3 washes with PBS for 10 min. The blocking of nonspecific epitopes with 10% donkey serum in PBS with 0.3% Triton-X for 2 h at RT preceded overnight incubation at 4°C, with the appropriate primary antibody listed in Table 2 in PBS with 0.3% Triton-X. After 3 washes with PBS, the sections were incubated with suitable secondary fluorescent antibodies (Alexa488-labeled donkey anti-rabbit and Alexa594-labeled donkey anti-mouse; 1:200; Invitrogen) for 2 h at room temperature. The sections were observed by a skilled pathologist blinded to this research using image stacks on a laser scanning confocal microscope (Fluoview 1000, Olympus).

Evaluation of cell apoptosis

To evaluate the effect of ketamine on apoptosis in the NSCs and astrocytes, a double-immunofluorescence detection of nestin/caspase-3 and GFAP/caspase-3 was performed. The animals were transcardially perfused with 0.9% saline followed by 4% paraformaldehyde at 12 h after the end of ketamine anesthesia. Then, the brain was removed, postfixed overnight in 4% paraformaldehyde and placed in 30% sucrose until it sunk. Coronal sections of the brain were cut on a microtome. When the SVZ was initially exposed, the coronal sections of the brain were cut consecutively at a thickness of 30 μm. The tenth section was picked up and stored in PBS for double-label immunofluorescence. The sections were blocked with 10% donkey serum in PBS with 0.3% Triton-X for 2 h at RT and then incubated overnight at 4°C with the appropriate primary antibody listed in Table 2 in PBS with 0.3% Triton-X. After being washed with PBS 3 times, the sections were incubated with the suitable secondary fluorescent antibodies for 2 h at room temperature.

Western blot analysis

The expressions of nestin, β-tubulin III and GFAP were measured using Western blot analysis. Briefly, the brain tissues from the subventricular zone (SVZ) were homogenized with lysis buffer and protease inhibitors (Beyotime, China). The lysates were centrifuged at 14000 rpm for 15 min at 4°C. Equal amounts of the proteins (25μg) were resolved on a sodium dodecyl sulfate 10% or 12% polyacrylamide gel, and the separated proteins were transferred to nitrocellulose membranes. The blots were incubated with blocking buffer for 2 h at room temperature and then incubated for 24 h with the primary antibodies against nestin (1:1000, Abcam), β-tubulin III (1:1000, Abcam), GFAP (1:1000, Millipore) and GAPDH. Then, the membranes were incubated with appropriate secondary antibodies for 1 h. The immunoreactive bands were visualized with a chemiluminescence detection system. The band intensity was quantified using Image J software.
Statistical analysis

The data are presented as the means ± SD. The statistical analysis and the graphs were completed using GraphPad Prism 5. The significant differences between the groups were analyzed with an unpaired two-tailed t-test or one-way ANOVA. P<0.05 was considered statistically significant.

Results

Ketamine inhibits the proliferation of NSCs in the SVZ of neonatal rats

As shown in Fig. 1A and B, the density of Nestin+/BrdU+ cells in the ketamine group (36±1/μm²) was significantly decreased compared to that in the control group (107±6/μm²) 24 h (PND-8) after exposure to ketamine. This suppressive effect of ketamine on NSC proliferation was also found at 3 days (PND-10; 39±4 vs 107±8/μm²) and 7 days (PND-14; 41±3 vs 100±13/μm²) after anesthesia. Western blot analysis also showed that the
expression of nestin was significantly reduced at 1, 3 and 7 days after ketamine anesthesia compared to that in the control group (Fig. 1C). There were no significant differences in the NSC proliferation at different time points (PND-8, 10 and 14) in either the control groups or the ketamine-treated groups.

Ketamine promotes the neuronal differentiation of NSCs in the SVZ of neonatal rats

It was shown that the density of β-tubulin III+/BrdU+ cells in the ketamine group (38±3/μm²) was significantly increased compared to that in the control group (34±2/μm²) 24 h (PND-8) after exposure to ketamine. This stimulant effect of ketamine on the neuronal differentiation of NSCs was also found at 3 days (PND-10; 38±1 vs 34±1/μm²) and 7 days (PND-14; 39±1 vs 35±2/μm²) after ketamine anesthesia (Fig. 2A and B). The expression of β-tubulin III also showed a significant increase in the ketamine group compared to the control group at PND-8, 10 and 14 by Western blot analysis (Fig. 2C). There were no significant differences in the neuronal differentiation of the NSCs at different time points (PND-8, 10 and 14) in either the control groups or the ketamine-treated groups.

**Fig. 2.** Effect of ketamine on the neuronal differentiation of NSCs 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, the rats received a single intraperitoneal injection of BrdU (100 mg/kg) immediately after anesthesia or at PND-9 and PND-13. The animals were sacrificed at PND-8, 10 or 14. The newborn neurons were labeled by primary antibodies against neuronal skeleton protein β-tubulin III (Green) and BrdU (Red). The immunoreactive cells were visualized using a laser scanning confocal microscope (A; Magnification 1: ×200, 2: ×400). The arrows pointed to β-tubulin III/BrdU double-labeled cells. The density of β-tubulin III/BrdU double-positive cells was counted (B). The expression level of β-tubulin III was measured using Western blot analysis (C). The data are presented as the means ± SD (n=5). At the same time point: *P<0.05, **P<0.01, vs control group. SVZ = subventricular zone; LV = lateral ventricle.
Fig. 3. Effect of ketamine on the astrocytic differentiation of NSCs 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, the rats received a single intraperitoneal injection of BrdU (100 mg/kg) immediately after anesthesia or at PND-9 and PND-13. The
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Ketamine attenuates the astrocytic differentiation of NSCs in the SVZ of neonatal rats

As shown in Fig. 3A and B, the density of GFAP+ /BrdU+ cells in the ketamine treatment group (7±1/μm²) was significantly decreased compared to that in the control group (33±2/μm²) 1 day (PND-8) after ketamine anesthesia. This suppressive effect of ketamine on the astrocytic differentiation of NSCs was also found at 3 days (PND-10; 9±2 vs 32±3/μm²) and 7 days (PND-14; 10±1 vs 33±1/μm²) after ketamine treatment. The Western blot analysis of GFAP also showed a significant decrease in the ketamine group compared to the control group at PND-8, 10 and 14 (Fig. 3C). There were no significant differences in the astrocytic differentiation of the NSCs at different time points (PND-8, 10 and 14) in either the control groups or the ketamine-treated groups. In addition, Fig. 3D and E show that only a small number of GFAP/BrdU double positive cells were found in both the control and ketamine groups at the time points of 3 h after each injection of BrdU. There were no significant differences in the proliferation of matured astrocytes between the ketamine and control groups at different time points (3 h after BrdU injection on PND-7, 9 and 13).
**The NSCs and astrocytes in the SVZ of neonatal rats are resistant to ketamine-induced cell apoptosis**

To evaluate the effects of ketamine on the apoptosis of NSCs and astrocytes in the SVZ of neonatal rats, we measured nestin/caspase-3 and GFAP/caspase-3 double-positive cells by double-labeled immunofluorescence at 12 h after the end of ketamine anesthesia. The result showed that there were no significant nestin/caspase-3 or GFAP/caspase-3 double-positive cells in either the control or ketamine groups. These findings suggest that the present dosage and duration of ketamine was unable to induce apoptosis in the NSCs and astrocytes (Fig. 4).

**Discussion**

Ketamine, a N-methyl-D-aspartate (NMDA) receptor inhibitor, is widely used in infants and children for anesthesia and sedation [14]. Recent studies have reported that ketamine can induce neuronal apoptosis in the neonatal mammalian brain, which is usually considered to contribute to behavioral abnormalities that may arise during adulthood [3-5, 7]. However, some studies have suggested that neonatal anesthesia-induced neuroapoptosis is not the only factor that contributes to sustained cognitive deficits [15].

Neurogenesis is established during gestation in most brain regions and is nearly completed before birth except in two regions: the SVZ and the hippocampal DG. The normal development of these two regions is very important to learning and memory [16-21]. The process of neurogenesis involves the proliferation of NSCs, the neuronal and astrocytic differentiation of NSCs, and their migration and functional integration into the neural circuit. In this complicated process, the proliferation and differentiation of NSCs are not only initial but also extremely crucial.

Numerous studies showed that NMDA-R plays an important role in regulating the proliferation and differentiation of NSCs derived from the hippocampal DG [22-27]. However, the role of NMDA-R in the neurogenesis of SVZ has not been widely studied [28-30] and the consequences of blocking NMDA-R are controversial, partly because of the different animal models and various brain regions studied.

During the S phase of the cell cycle, BrdU is able to substitute for thymine, bearing a critical significance in the study of cellular dynamics. The dosage of BrdU used is based on that of a previous study [31]. Nestin is a commonly used marker of NSCs that is stably expressed from PND-1 to PND-28 in the SVZ [30]. The time points observed in our experiment fell exactly within this period. Of note, GFAP, which is an astrocyte marker, was also able to be expressed in the NSCs. However, the NSCs in this brain region did not initially express GFAP, and later, the NSCs gradually expressed this protein in the SVZ during the adult stage [32, 33]. It is known that mature astrocytes can proliferate after exposure to stimulation, such as a stroke. To exclude the proliferation of other cells, the proliferative NSCs were double-labeled with the neural stem cell marker nestin and the proliferation marker BrdU in the present study. The results indicated that ketamine is able to significantly suppress the proliferation of NSCs in the SVZ of neonatal rats at 1, 3 and 7 days after ketamine anesthesia (PND-8, 10 and 14).

The astrocytic differentiation of NSCs was also found to be markedly attenuated, reflected by a decreased number of GFAP/BrdU double-positive cells, whereas the neuronal differentiation of NSCs in the SVZ was obviously promoted, reflected by an increased number of β-tubulin III/BrdU double-positive cells. Because the NSCs in the SVZ only gradually express GFAP during the adult stage instead of the neonatal period, the GFAP/BrdU double positive cells in our experiment should not include those proliferative NSCs with a GFAP-positive label. In addition, to exclude the proliferation of mature astrocytes, GFAP/BrdU double-labeling immunostaining was performed 3 h after the BrdU injection, at which time BrdU had been adequately incorporated into the proliferative cells but newly differentiated astrocytes had not been generated. Only a small amount of mature astrocytes were found...
to be capable of proliferating in the SVZ of neonatal rats, and ketamine did not significantly promote or suppress the proliferation of mature astrocytes. Therefore, it was considered that the GFAP/BrdU double-positive cells detected at 24 h after the BrdU injection could represent the newborn astrocytes differentiated from NSCs.

A previous in vitro study showed that ketamine significantly inhibited the proliferation of NSCs isolated from SVZ in the rat fetal cortex and enhanced its neuronal differentiation [13]. Another in vitro study found that the proliferation of NSCs from the SVZ was markedly suppressed by MK-801, an NMDA-R antagonist [34]. These reports were coincident with our present results in vivo.

The toxicity of ketamine may cause cell death rather than directly inhibiting proliferation. The present study showed that neither nestin+/caspase-3+ nor GFAP+/caspase-3+ cells were obviously found in the control or ketamine groups. Although neuronal apoptosis has been demonstrated to be induced by neonatal exposure to ketamine [4, 5], the present dosage and duration of ketamine could not significantly induce the apoptosis of NSCs and astrocytes in the SVZ of neonatal rats. It is thus suggested that the reduced numbers of nestin/BrdU double-positive cells and GFAP/BrdU double-positive cells were not caused by cell death after ketamine exposure.

To explore the duration of the ketamine effect, we observed the proliferation and differentiation of NSCs at 1, 3 and 7 days after ketamine anesthesia. We found that the alterations in the proliferation and differentiation of NSCs induced by ketamine occurred as early as one day after anesthesia (PND-8) and was still obvious at 7 days after ketamine exposure (PND-14). A recent in vivo study reported that, after blocking NMDA-R by MK-801, the number of proliferative cells in the SVZ of neonatal rats markedly decreased at the age of PND-7 and PND-14 [30]. This result was in agreement with our study.

Normally, newly generated neurons in the SVZ migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB), where some of them join in the existing neural circuits; neurogenesis in OB plays a crucial role in long-term olfactory memory [35]. Our study indicated that ketamine significantly inhibited NSC proliferation and astrocytic differentiation while enhancing neuronal differentiation during the development of SVZ, which may result in the abnormalities in the number and proportion of neurons and astrocytes, thus disturbing the structure and formation of neuronal circuits in the cortical layers and olfactory bulb and possibly leading to alterations of the brain functions. Evidence has shown that hippocampus-dependent neurocognitive functions in the adult stage can be affected by neonatal exposure to ketamine [8, 36, 37]. However, the effect of ketamine on olfactory cognitive function has barely been studied. It is unknown whether neonatal ketamine anesthesia could contribute to clinically observed olfactory cognitive impairment in the adult stage. Our findings that ketamine interferes with the activity of NSCs in the SVZ during the postnatal period suggest that neonatal exposure to ketamine may be closely associated with olfactory cognitive disorders.

In summary, this study demonstrated that ketamine is able to inhibit the proliferation of NSCs, to suppress their differentiation into astrocytes, and to promote the neuronal differentiation of NSCs in the SVZ of neonatal rats during the critical period of neurodevelopment. Given the importance of NSC proliferation and differentiation to neurogenesis, it is possible that the neonatal ketamine exposure may interfere with the neurogenic processes of the SVZ in the developing brain.

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References

1. Asadi P, Ghafouri HB, Yasinizadeh M, Kasnavieh SM, Modirian E: Ketamine and atropine for pediatric sedation: a prospective double-blind randomized controlled trial. Pediatr Emerg Care 2013;29:136-139.

2. Guerra GG, Robertson CM, Alton GY, Joffe AR, Cave DA, Dinu IA, Creighton DE, Ross DB, Rebejka IM: Neurodevelopmental outcome following exposure to sedative and analgesic drugs for complex cardiac surgery in infancy. Paediatr Anaesth 2011;21:932-941.

3. Liu F, Paule MG, Ali S, Wang C: Ketamine-induced neurotoxicity and changes in gene expression in the developing rat brain. Curr Neuropharmacol 2011;9:256-261.

4. Zou X, Patterson TA, Sadovova N, Twaddle NC, Doerger DR, Zhang X, Fu X, Hanig JP, Paule MG, Slikker W, Wang C: Potential neurotoxicity of ketamine in the developing rat brain. Toxicol Sci 2009;108:149-158.

5. Zou X, Patterson TA, Divine RL, Sadovova N, Zhang X, Hanig JP, Paule MG, Slikker W, Wang C: Prolonged exposure to ketamine increases neurodegeneration in the developing monkey brain. Int J Dev Neurosci 2009;27:727-731.

6. Ikonomidou C, Bosch F, Milksa M, Bittigau P, Vockler J, Dikranian K, Tenkova TI, Stefovska V, Turski L, Olney JW: Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 1999;283:70-74.

7. Wilder RT, Flick RP, Springer J, Katusic SK, Barbaresi WI, Mickleston C, Gleich SJ, Schroeder DR, Weaver AL, Warner DO: Early exposure to anesthesia and learning disabilities in a population-based birth cohort. Anesthesiology 2009;110:796-804.

8. Paule MG, Li M, Allen RR, Liu F, Zou X, Hotchkiss C, Hanig JP, Patterson TA, Slikker W, Wang C: Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. Neurotoxicol Teratol 2011;33:220-230.

9. Pfenniger EG, Durieux ME, Himmelseher S: Cognitive impairment after small-dose ketamine isomers in comparison to equianalgesic racemic ketamine in human volunteers. Anesthesiology 2002;96:357-366.

10. Byrnes ML, Reynolds JN, Brien JP: Effect of prenatal ethanol exposure during the brain growth spurt of the guinea pig. Neurotoxicol Teratol 2001;23:355-364.

11. Muramatsu R, Ikegaya Y, Matsu K, Noyama R: Neonatally born granule cells numerically dominate adult mice dentate gyrus. Neuroscience 2007;48:593-598.

12. Sharp FR, Liu J, Bernabeu R: Neurogenesis following brain ischemia. Brain Res Dev Brain Res 2002;134:23-30.

13. Dong C, Rovnaghi CR, Anand KJ: Ketamine alters the neurogenesis of rat cortical neural stem progenitor cells. Crit Care Med 2012;40:2407-2416.

14. Alletag MJ, Auerbach MA, Baumb CR: Ketamine, propofol, and ketofol use for pediatric sedation. Pediatr Emerg Care 2012;28:1391-1398.

15. Stratmann G, Salo JW, May LD, Bell JS, Magnusson KR, Rau V, Visrodi KH, Alvi RS, Ku B, Lee MT, Dai R: Isoflurane differentially affects neurogenesis and long-term neurocognitive function in 60-day-old and 7-day-old rats. Anesthesiology 2009;110:834-848.

16. Tepavcevic V, Lazarini F, Alfaro-Cervello C, Kerninon C, Yoshikawa K, Garcia-Verdugo JM, Lledo PM, Nait-Oumesmar B, Baron-Van Evercooren A: Inflammation-induced subventricular zone dysfunction leads to olfactory deficits in a targeted mouse model of multiple sclerosis. J Clin Invest 2011;121:4722-4734.

17. Yeung ST, Muczek K, Kang AP, Chabrier MA, Baglietto-Vargas D, Laferla FM: Impact of hippocampal neuronal ablation on neurogenesis and cognition in the aged brain. Neuroscience 2014;259:214-222.

18. So K, Moriya T, Nishitani S, Takahashi H, Shinozuka K: The olfactory conditioning in the early postnatal period stimulated neural stem/progenitor cells in the subventricular zone and increased neurogenesis in the olfactory bulb of rats. Neuroscience 2008;151:120-128.

19. Imayoshi I: Neurogenesis in the postnatal and adult brain. Jpn J Psychopharmacol 2012;32:293-297.

20. Dupret D, Revest JM, Koehl M, Ichas F, De Giorgi F, Costet P, Abrus TN, Piazza PV: Spatial relational memory requires hippocampal adult neurogenesis. PLoS one 2008;3:e1959.

21. Coremans V, Ahmed T, Balschun D, D’Hoooge R, De Vriendt A, Cremer J, Antonucci F, Moons M, Baekelandt V, Reumers V, Cremer H, Eisch A, Lagace D, Janssens T, Buzzi Y, Caello M, Conway EM: Impaired neurogenesis, learning and memory and low seizure threshold associated with loss of neural precursor cell survivin. BMC neurosci 2010;11:2.
22 Joo JY, Kim BW, Lee JS, Park JY, Kim S, Yun YJ, Lee SH, Lee SH, Rhim H, Son H: Activation of NMDA receptors increases proliferation and differentiation of hippocampal neural progenitor cells. J Cell Sci 2007; 120:1358-1370.

23 Nacher J, Varea E, Miguel Blasco-Ibanez J, Gomez-Climent MA, Castillo-Gomez E, Crespo C, Martinez-Guijarro FJ, McEwen BS: N-methyl-D-aspartate receptor expression during adult neurogenesis in the rat dentate gyrus. Neuroscience 2007; 144:855-864.

24 Kitayama T, Yoneyama M, Tamaki K, Yoneda Y: Regulation of neuronal differentiation by N-methyl-D-aspartate receptors expressed in neural progenitor cells isolated from adult mouse hippocampus. J Neurosci Res 2004; 76:599-612.

25 Keilhoff G, Bernstein HG, Becker A, Grecksch G, Wolf G: Increased neurogenesis in a rat ketamine model of schizophrenia. Biol Psychiatry 2004; 56:317-322.

26 Winkelheide U, Lasarzik I, Kaeppel B, Winkler J, Werner C, Kochs E, Engelhard K: Dose-dependent effect of S(+)-ketamine on post-ischemic endogenous neurogenesis in rats. Acta anaesthesiol Scand 2009; 53:528-533.

27 Luik KC, Kennedy TE, Sadikot AF: Glutamate promotes proliferation of striatal neuronal progenitors by an NMDA receptor-mediated mechanism. J Neurosci 2003; 23:2239-2250.

28 Faiz M, Acarin L, Castellano B, Gonzalez B: Proliferation dynamics of germinative zone cells in the intact and excitotoxically lesioned postnatal rat brain. BMC neuroscience 2005; 6:26.

29 Xu G, Ong J, Liu YQ, Silverstein FS, Barks JD: Subventricular zone proliferation after alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated neonatal brain injury. Dev Neurosci 2005; 27:228-234.

30 Fan H, Gao J, Wang W, Li X, Xu T, Yin X: Expression of NMDA receptor and its effect on cell proliferation in the subventricular zone of neonatal rat brain. Cell Biochem and Biophys 2007; 47:209-217.

31 Guidi S, Ciani E, Severi S, Contestabile A, Bartesaghi R: Postnatal neurogenesis in the dentate gyrus of the guinea pig. Hippocampus 2005; 15:285-301.

32 Shen Q, Wang Y, Kolovay E, Lin G, Chuang SM, Goderie SK, Roysamb T, Temple S: Adult SVZ stem cells lie in a vascular niche: A quantitative analysis of niche cell-cell interactions. Cell Stem Cell 2008; 3:289-300.

33 Tavazoie M, Van der Velden L, Silva-Vargas V, Louisissaint M, Colonna L, Zaidi B, Garcia-Verdugo J, Doetsch F: A specialized vascular niche for adult neural stem cells. Cell Stem Cell 2008; 3:279-288.

34 Mochizuki N, Takagi N, Kurokawa K, Kawai T, Bessho H, Tanonaka K, Takeo S: Effect of NMDA receptor antagonist on proliferation of neurospheres from embryonic brain. Neurosci Lett 2007; 417:143-148.

35 Sultan S, Mandairon N, Kermen F, Garcia S, Sacquet J, Didier A: Learning-dependent neurogenesis in the olfactory bulb determines long-term olfactory memory. FASEB J 2010; 24:2355-2363.

36 Viberg H, Ponten E, Eriksson P, Gorth T, Fredriksson A: Neonatal ketamine exposure results in changes in biochemical substrates of neuronal growth and synaptogenesis, and alters adult behavior irreversibly. Toxicology 2008; 249:153-159.

37 Fredriksson A, Archer T, Alm H, Gorth T, Eriksson P: Neurofunctional deficits and potentiated apoptosis by neonatal NMDA antagonist administration. Behav Brain Res 2004; 153:367-376.