Ketamine affects the functional convergence of developmentally generated granule neurons in the adult stage

Zhanqiang Zhao  
jiangning hospital of traditional Chinese medicine

Bing Li  
jiangning hospital of traditional Chinese medicine

Yuqing Wu  
Jiangsu Province Key Laboratory of Anesthesiology

Xujun Chen  
jiangning hospital of traditional chinese medicine

Yan Guo  
jiangning hospital of traditional chinese medicine

Yang Shen  
jiangning hospital of traditional chinese medicine

He Huang (✉ 353550575@qq.com)  
first affiliated hospital with Nanjing medical university  
https://orcid.org/0000-0003-1573-1204

Research article

Keywords: Ketamine; Dentate gyrus; Functional convergence; Neural circuits; Morris water maze; Learning; Rat

Posted Date: June 20th, 2019

DOI: https://doi.org/10.21203/rs.2.10461/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Version of Record: A version of this preprint was published on December 18th, 2019. See the published version at https://doi.org/10.1186/s12868-019-0542-4.
Abstract

Background Ketamine has been reported to cause neonatal neurotoxicity in a variety of developing animal models. Various studies have been conducted to study the mechanism of neurotoxicity for general anesthetic use during the neonatal period. Previous experiments have suggested that developmentally generated granule neurons in the hippocampus dentate gyrus (DG) supported hippocampus-dependent memory. Therefore, this study aimed to investigate whether ketamine affects the functional integration of developmentally generated granule neurons in the DG. For this purpose the postnatal day 7 (PND-7) SD rats were divided into the control group and the ketamine group (rats who received 4 injections of 40 mg/kg ketamine at 1 h intervals). The BrdU was administered for three consecutive days after the ketamine exposure. To label the dividing cells, NeuN+/BrdU+ cells were observed by using immunofluorescence. To evaluate the developmentally generated granule neurons that support hippocampus-dependent memory, spatial reference memory was tested by using Morris Water Maze at 3 months old, after which the immunofluorescence was used to detect c-Fos expression in the NeuN+/BrdU+ cells. The expression of caspase-3 was measured by western blot to detect the apoptosis in the hippocampal DG. Results The present results showed that the neonatal ketamine exposure did not influence the survival rate of developmentally generated granule neurons at 2 and 3 months old, but ketamine interfered with the integration of these neurons into the hippocampal DG neural circuits and caused a deficit in hippocampal-dependent spatial reference memory tasks. Conclusions In summary, these findings may promote more studies to investigate the neurotoxicity of ketamine in the developing brain.

Background

Every year, millions of children are exposed to a variety of surgeries. Ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, is widely used for sedation in a clinical setting for analgesia in children who are undergoing painful procedures [1-3]. However, current results have demonstrated that ketamine could increase potential risks for brain development. For instance, ketamine that was administered to developing rats resulted in a significant level of neurotoxicity [4]. The intravenous administration of ketamine during the first week of life caused long-lasting cognitive deficits in rhesus monkeys [5]. In vitro experimental evidence has also showed that ketamine could cause apoptosis of neurons that were derived from human embryonic stem cells [6]. Given these data, the safe use of ketamine for pediatric anesthesia has been the subject of concern for both anesthesiologists and the public. The mechanisms by which ketamine induces neurotoxicity in the developing brain remain to be determined.

The brain growth spurt (BGS) lasts from the end of pregnancy to the first 2-3 weeks after birth in rodents, and the corresponding BGS period in humans begins in the last trimester of pregnancy and continues until 2 years after birth [7]. During this period, developmentally generated hippocampal granule neurons normally migrate, survive and become functionally incorporated into pre-existing neural circuits (granule neurons CA3-CA1) [8,9]. Our previous study had suggested that neonatal ketamine exposure could interfere with the postnatal neurogenesis of the hippocampal dentate gyrus (DG), including the inhibition

Page 2/16
of neural stem cell (NSC) proliferation and astrocytic differentiation, the promotion of neuronal differentiation, the inhibition of astrocytic growth and the neuronal migration in the granule cell layer (GCL) [10].

Previous studies have demonstrated that granule neurons that are generated in the early postnatal days have numerically dominated the adult hippocampal DG [11] and have played important roles in the formation of hippocampal-dependent spatial learning and memory function [12,13]. In addition, Guzowski, et al. found that the expression of immediate early genes (IEGs, such as c-Fos) was regulated by neuronal activity (such as memory testing), and immunofluorescence approaches have made it possible to discover the functional convergence of developmentally generated granule neurons that support hippocampus-dependent memory [14]. According to these results, the purpose of this experiment was to investigate the effect of ketamine on the fates of developmentally generated granule neurons during the adult stage by using the in vivo neonatal ketamine exposure model.

Methods

Animal Treatment

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 (No: 15030254). Sprague-Dawley (SD) dams with pups were bred in our colony in a temperature-controlled (22-23°C) room on a 12 h light/dark cycle (lights on at 8:00 AM) with free access to food and water. Forty PND-7 SD male rat pups (11-14 g) were used in our experiment and were randomly assigned to ketamine-treated and sham-treated groups. The grouping method was performed by using the methods described in our previous study [10]. In the anesthesia group, ketamine was diluted in 0.9% normal saline, and PND-7 rats were intraperitoneally administered with 40 mg/kg doses of ketamine in four injections at 1 h intervals (40 mg/kg×4 injections). In the sham-treated group, rats received an equal volume of normal saline. Temperature probes were used to facilitate the control of temperature at 36.5±1°C by using computer-controlled heater/cooler plates that were integrated into the floor of the chamber. Between each injection, animals were returned to their individual chambers to help in maintaining body temperature and to reduce stress.

Morris Water Maze Test (MWM)

The apparatus and behavioral procedures of the MWM test have been previously described [10]. Behavioral testing was conducted in a circular, black painted pool (180 cm diameter, 50 cm deep). The water temperature was maintained at 25±1°C. An invisible platform (10 cm diameter) was submerged 1 cm below the water surface and was placed in the center of the quadrant III, which was determined by using four starting locations (defined as A, B, C and D) at equal distances on the edge of the pool. During five consecutive days, the experiments were conducted in a dark and quiet laboratory setting, and all of the rats were trained four times per day, with the starting positions being randomized for each rat. When
each of the rats found the platform, the rat was allowed to stay on it for 30 s. If a rat did not find the platform within 120 s, the rat would be gently guided to the location and allowed to stay on the platform for 30 s, and the latency time in finding the hidden platform was recorded as 120 s. The average time from the 4 trials was represented as the daily result for each of the rats. Following the completion of the training, spatial memory was assessed in the probe tests, the hidden platform was removed and each of the rats was placed in the opposite quadrant. The rats were allowed to swim freely for 120 s. The number of times that each of the rats swam to cross the previous platform area and the number of times that each of the rats stayed in the target quadrant within 120 s were recorded. The paths of each of the animals were tracked by using a computerized video system. After every trial, each rat was placed in a heater plate for 1 to 2 min until they were dry, after which they were returned to their chambers. The data were analyzed by using software for the MWM (Jiangsu Province Key Laboratory of Anesthesiology, Xuzhou Medical university, Xuzhou, China.).

**Experimental Methods**

The experimental design that was used in the present study is outlined in Fig. 1. To label the dividing neurons in the early postnatal DG, we injected BrdU (5-bromo-2-deoxyuridine; Sigma), at a dosage of 100 mg/kg, into the rats. In experiments 1 and 2, animals received three consecutive BrdU injections on PND-7, 8 and 9 after exposure to the treatment.

Experiment 1 evaluated the survival rate of developmentally generated granule neurons in the adult DG. The animals were weaned at PND-35, after which they were housed in cages with free access to food and water for up to 2 months. Then, a portion of the animals were deeply anesthetized with 40 mg/kg ketamine and transcardially perfused with 0.9% normal saline, followed by a transfusion with 4% paraformaldehyde. The NeuN+/BrdU+ cells were examined by using double-immunofluorescence staining (n=5).

Experiment 2 evaluated the integration rate of developmentally generated granule neurons into the hippocampus-dependent memory networks in the DG. The animals were raised to an age of 3 months old, after which they were trained in the MWM task. Hippocampus-dependent memory was assessed following the training period (n=6). The expression of c-Fos was induced by neural activity, such as memory testing, all animals were deeply anesthetized with 40 mg/kg ketamine and sacrificed immediately after the completion of the MWM test. The experimental protocol is shown in Fig. 1. The c-Fos expression in NeuN+/BrdU+ cells was examined by triple-immunofluorescence staining (n=5). The integration rate of developmentally generated granule neurons into the hippocampal memory networks was estimated by calculating the proportion of c-Fos+/NeuN+/BrdU+ cells in the hippocampal DG.

**Tissue Preparation and Immunofluorescence**
The brains were postfixed in 4% paraformaldehyde and the coronal sections of the brains were cut consecutively at a thickness of 30 μm, at the point in which the hippocampus was initially exposed, the fifteenth section was taken and stored in PBS. The position of the hippocampus coronal sections selected in our study was approximately 2.80-2.85 mm posterior to the bregma for the 2 months old rats and approximately 2.90-2.95 mm posterior to the bregma for the 3 months old rats [15,16].

For the NeuN/BrdU double-immunofluorescence staining, the BrdU antigen was exposed by incubating the sections in 2-normal hydrochloric acid for 30 min at 37°C and by performing 3 washes with PBS for 5 min between each of these steps. The blocking of nonspecific epitopes with 10% donkey serum in PBS (which contained 0.3% Triton-X) for 2 h at room temperature preceded an overnight incubation at 4°C with the primary antibodies against NeuN (Mouse anti-NeuN monoclonal antibody; 1:200; Millipore, Massachusetts, USA) and BrdU (Rabbit anti-BrdU monoclonal antibody; 1:500; Abcam, San Francisco, USA). On the next day, the sections were incubated with the appropriate secondary fluorescent antibodies (Invitrogen Carlsbad, USA) for 2 h at room temperature.

For the Fos/NeuN/BrdU triple labeling, identical procedures were performed by using a primary rabbit anti-c-Fos polyclonal antibody (1:200; Abcam), a mouse anti-NeuN antibody (1:200; Millipore) and a rat anti-BrdU monoclonal antibody (1:500; Abcam). On the next day, the sections were incubated with the appropriate secondary fluorescent antibodies (Invitrogen) for 2 h at room temperature.

**Imaging**

The photographs of the stained sections were taken by using a laser scanning confocal microscope (Fluoview 1000, Olympus, Japan), and a skilled pathologist, who was blinded to the study conditions, examined the labeled sections. The numbers of double-positive or triple-positive cells in the hippocampal DG were quantified by using Image-Pro Plus software (Media Cybernetics Inc., Bethesda, USA).

**Brain Tissue Harvest and Western Blot Analysis**

The rats selected in sham group and ketamine group were deeply anesthetized with ketamine and decapitated at 2 months or 3 months old respectively (n=3). The hippocampal DG tissue was dissected carefully with an anatomic microscope (leica EZ4HD). The harvested hippocampal DG tissues were homogenized on ice using lysate buffer plus protease inhibitors. The lysates were centrifuged at 14,000 rpm for 15 min at 4°C and were resolved by 12% polyacrylamide gel electrophoresis, and the target 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 at 4°C with the primary antibodies rabbit anti-caspase-3 antibody (Cell Signaling Technology) and β-tubulin (Abcam). The membranes were then incubated with the appropriate secondary alkaline phosphatase-conjugated antibody (Abcam) for 1 h. The band intensity was quantified using Image J software.

**Statistical Analysis**
The statistical analysis was conducted by using SPSS 13.0 (SPSS Inc., Chicago, USA), and the graphs were created by using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, USA). The data were analyzed by using the Mann-Whitney U test. The interaction between the time and group factors, which was determined by using a two-way ANOVA, was used to analyze the differences in escape latency between the rats in the control group and the rats that were treated with ketamine in the MWM. The data are presented as the mean±SD, and \( P<0.05 \) was considered to be statistically significant.

## Results

### Exposing ketamine to PND-7 rats caused spatial memory impairment at 3 months old in the MWM Test

In the MWM test, and when comparing the time that each rat spent in reaching a platform during the reference training (the escape latency), the latency to find the hidden platform in the two groups of rats had a reduced time as the training progressed. However, the latency in locating the hidden platform in the ketamine group was significantly longer than that in the sham group (Fig. 2A). In the memory retrieval tests, the time spent in the target quadrant (ketamine: 32±4.63 vs. sham: 51±3.54; Fig. 2B) and the numbers of crossovers of the previous platform site (ketamine: 4±0.75 vs. sham: 8±1.47; Fig. 2C) within 120 s were significantly reduced in the ketamine group than that in the sham group. The typical track charts are shown in Fig. 2D. Taken together, these findings suggested that exposing ketamine (40 mg/kg×4 injections) to PND-7 rats could cause hippocampal-dependent learning and memory impairments in the adult stage.

### Exposing ketamine to PND-7 rats did not affect the survival rate of developmentally generated granule neurons by using immunofluorescence staining and western blot

To investigate the effect of ketamine on the survival rate of developmentally generated neurons in the hippocampal DG, we used BrdU labeling and tracked the fates of developmentally generated granule neurons during the adult stage via double-immunofluorescence staining. NeuN and BrdU colabeled cells were defined as developmentally generated neurons that were generated at the time of BrdU injections. The experimental protocol is shown in Fig. 1 and 3A. According to our findings, the densities of newborn neurons in the representative hippocampal DG coronal sections were not different between the sham and ketamine groups at 2 months (21±2.56 vs 21±1.91, Fig. 3B) and at 3 months (18±2.42 vs 18±2.70, Fig. 4B).

Next, we detected the expression of caspase-3 in the hippocampal DG by western blot. The result showed that four injections of 40 mg/kg ketamine with 1h intervals did not significantly affect the expression of caspase-3 in the hippocampal DG at 2 months and 3 months old (Fig. 5). These results suggested that 40 mg/kg ketamine×4 injections in PND-7 rats did not affect the survival rate of developmentally generated neurons in the hippocampal DG in the adult stage.

### Neonatal ketamine exposure interfered with the functional integration of developmentally generated neurons in the adult stage by using immunofluorescence staining
The expression of c-Fos has been used as a neuronal activity marker in previous experiments. To investigate the effect of ketamine on the integration of developmentally generated neurons in the hippocampal DG circuits, we observed the c-Fos/NeuN/BrdU triple-positive cells and the NeuN/BrdU double-positive cells by using immunofluorescence staining following the completion of the Morris Water Maze tests. The protocol for this staining is shown in Fig. 1. We observed that ketamine could significantly decrease the density of c-Fos/NeuN/BrdU triple-positive cells in the hippocampal DG compared to that in the sham group (ketamine: 9±1.19 vs sham: 12±1.63, Fig. 4C) and that the percentages of triple-positive cells and double-positive cells were also reduced in the ketamine group (ketamine: 51±6.74% vs sham: 68±5.07%, Fig. 4D). The cell component in the hippocampal DG mainly includes granule neurons in the GCL and interneurons in the polymorphic cell layer (PCL). The granule neurons in the GCL are the foundation for hippocampal function. According to our findings, the ratio of triple-positive cells in the GCL to total triple-positive cells in the DG (ketamine: 46±8.99% vs sham: 65±6.03%, Fig. 4E) was decreased in the ketamine group, compared to the sham group.

The abovementioned results suggest that 4 treatments with 40 mg/kg ketamine in PND-7 rats could interfere with the abnormal localization of developmentally generated neurons in the hippocampal DG and with the functional integration of neurons into the hippocampal-dependent spatial memory circuits. These factors may be associated with cognitive dysfunction that is induced by neonatal ketamine exposure.

Discussion

Ketamine is one of the most commonly used general anesthetics, particularly in the field of pediatric anesthesia. The growing data suggest that, during the period of brain growth spurts, ketamine can induce widespread neuronal apoptosis in parallel with long-term memory and learning abnormalities [4,5]. According to these results, the safe use of ketamine in surgical and intervention procedures has become a major health issue of interest to the public [17,18]. Therefore, it is necessary to clarify the mechanism of the neurotoxicity of ketamine in the developing brain.

Neurogenesis in the hippocampal DG plays a crucial role in the formation of the structure and function of the hippocampus. In rodent animals, the granule neurons are continuously generated in the subgranular zone of the hippocampal DG from the 14th day of gestation until the adult stage, and approximately 80% of the granule neurons in the DG are produced postnatally, with a peak at approximately 7 days after birth [19]. Muramatsu et al. found that a good number of postnatally generated granule neurons can numerically dominate the adult hippocampal DG [11]. However, the total number of granule neurons may not be as important for hippocampal function as the efficient integration of these neurons into the neural circuits [20,21]. The key point for estimating how these developmentally generated granule neurons contribute to hippocampal-dependent spatial memory is to explore whether they can functionally migrate into the normal positions of the GCL and whether they can incorporate into neural circuits, in order to meet the functional demand.
BrdU is a classically used tool for the detection of cell fates [22,23]. In our experiment, neonatal rats received 100 mg/kg BrdU injections on PND-7, 8 and 9 after exposures to either saline or ketamine treatments [10]. We defined the NeuN/BrdU colabeled cells that were detected at 2 months and 3 months as the developmentally generated granule neurons that were generated at the time of BrdU injection by using a laser scanning confocal microscope. Our results suggest that the density of NeuN/BrdU colabeled cells in the hippocampal DG was not different between the sham and ketamine groups; ketamine did not significantly affect the expression of caspase-3 in the hippocampal DG at 2 months and 3 months old. Collectively, these results demonstrate that the survival rate of developmentally generated granule neurons in the adult stage was not affected by neonatal ketamine exposure. However, in the MWM test at 3 months old, the latency of rats in locating the hidden platform in the ketamine group was significantly longer than that in the sham group. In the memory retrieval tests that followed the training period, the time spent in the target quadrant and the numbers of crossovers of the previous platform site within 120 s were significantly reduced in the ketamine group than those in the sham group. One key question is whether hippocampal-dependent learning and memory impairment in the adult stage were related to the abnormal integration of developmentally generated DG neurons that was induced by ketamine.

The IEG c-Fos expression has been used as a neuronal activity marker in previous experiments. The c-Fos expression is regulated by neural activity and therefore has been used to map neural activation following, for example, learning and/or memory recall [24]. The c-Fos/NeuN/BrdU triple immunofluorescence labeling makes it possible to estimate the integration rates of developmentally generated granule neurons into the hippocampal-dependent memory networks [13]. In our experiment, BrdU and c-Fos labeling were introduced in order to study the activation of developmentally generated granule neurons following memory recall by using an immunofluorescence procedure. In this study, spatial memory recall induced a relatively small proportion of c-Fos/NeuN/BrdU triple-positive cells in the hippocampal DG in the ketamine group, compared with the sham group. According to these findings, it was suggested that the abnormal integration of developmentally generated granule neurons into the hippocampal neural circuits may be an important determinant for long-term hippocampal-dependent cognitive deficits after neonatal ketamine exposure.

Consistent with a previous study, our earlier findings indicated that the abnormal migration of newborn granule neurons in the hippocampal DG was associated with hippocampal-dependent cognitive deficits [10,25]. Within our present study, it was demonstrated that ketamine could markedly decrease the proportion of triple-positive cells in the GCL, which was similar to the results from our previous study, which demonstrated that ketamine could markedly inhibit the migration of newborn neurons and could affect their normal positions in the hippocampal DG [10].

The mechanisms by which ketamine (40 mg/kg×4 injections) interfered with the functional integration of developmentally generated granule neurons in hippocampal DG circuits remain to be determined. A previous study suggested that the developing mitochondria were exquisitely vulnerable to general anesthesia and may be important early targets of anesthesia-induced developmental neurodegeneration.
We hypothesized that neonatal ketamine exposure may have disturbed the disorder of mitochondrial energy metabolism in the newborn neurons instead of affecting their survival rate in the adult stage. Thus, our future studies will include the exploration of these potential mechanisms.

**Conclusions**

In summary, our findings suggested that neonatal ketamine exposure may not affect the survival rate of developmentally generated granule neurons, but may interfere with their functional integration into the hippocampal circuits. These findings may account for the adult hippocampal-dependent neurocognitive dysfunction that is induced by neonatal ketamine exposure.

**Abbreviations**

**DG:** dentate gyrus  **PND-7:** postnatal day 7

**NMDA:** N-methyl-D-aspartate  **BGS:** brain growth spurt

**NSC:** neural stem cell  **GCL:** granule cell layer

**PCL:** polymorphic cell layer  **MWM:** Morris Water Maze Test

**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.

**Funding**

This work was supported by the National Natural Science Foundation of China (81171013), Key young medical research program of Jiangsu Province (QNRC2016587). Funding bodies played no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.


Authors' contributions

Conceived and designed the experiments: ZQ Zhao, B Li, H Huang; Performed the experiments: ZQ Zhao, B Li, YQ Wu; Data analysis and interpretation: ZQ Zhao, B Li, H Huang; Contributed reagents/materials/analysis tools: XJ Chen; Y Guo; Y Shen; Manuscript preparation: ZQ Zhao; B Li; YQ Wu; XJ Chen; Y Guo; Y Shen; H Huang. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Asadi P, Ghafouri HB, Yasinzadeh M, Kasnavieh SM, Modirian E. (2013) Ketamine and atropine for pediatric sedation: a prospective double-blind randomized controlled trial. Pediatr Emerg Care 29: 136-139.
2. Guerra GG, Robertson CM, Alton GY, Joffe AR, Cave DA, et al. (2011) Neurodevelopmental outcome following exposure to sedative and analgesic drugs for complex cardiac surgery in infancy. Paediatr Anaesth 21: 932-941.
3. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, et al. (2003) Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci 23: 876-882.
4. Zou X, Patterson TA, Sadovova N, Twaddle NC, Doerge DR, et al. (2009) Potential neurotoxicity of ketamine in the developing rat brain. Toxicol Sci 108: 149-158.
5. Paule MG, Li M, Allen RR, Liu F, Zou X, et al. (2011) Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. Neurotoxicol Teratol 33: 220-230.
6. Xiaowen Bai, Yasheng Yan, Scott Canfield, Maria Y. Muravyeva, et al. (2013) Ketamine enhances human neural stem cell proliferation and induces neuronal apoptosis via reactive oxygen species-mediated mitochondrial pathway. Anesth Analg 116(4): 869-880.
7. Byrnes ML, Reynolds JN, Brien JF. (2001) Effect of prenatal ethanol exposure during the brain growth spurt of the guinea pig. Neurotoxicol Teratol 23: 355-364.
8. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, et al. (2002) Functional neurogenesis in the adult hippocampus. Nature 415:1030-1034.
9. Vadodaria KC, Jessberger S. (2014) Functional neurogenesis in the adult hippocampus: then and now. Front Neurosci 8: 55.
10. He Huang, Cun-Ming Liu, Jie Sun, Ting Hao, Chun-Mei Xu, et al. (2016) Ketamine Affects the Neurogenesis of the Hippocampal Dentate Gyrus in 7-Day-Old Rats. Neurotox Res 30:185-198
11. R. Muramatsu, Y. Ikegaya, N. Matsuki, R. Koyama. (2007) Neonatally born granule cells numerically dominate adult mice dentate gyrus. Neuroscience 148: 593-598.
12. Dupret D, Revest JM, Koehl M, Ichas F, De Giorgi F, et al. (2008) Spatial relational memory requires hippocampal adult neurogenesis. PLoS One 3: e1959.
13. Stone SS, Teixeira CM, Zaslavsky K, Wheeler AL, Martinez-Canabal A, et al. (2011) Functional convergence of developmentally and adult-generated granule cells in dentate gyrus circuits supporting hippocampus-dependent memory. Hippocampus 21: 1348-1362.
14. Guzowski JF, Timlin JA, Roysam B, McNaughton BL, Worley PF, et al. (2005) Mapping behaviorally relevant neural circuits with immediate-early gene expression. Curr Opin Neurobiol 15: 599-606.
15. Ashwell KWS; Paxinos G. (2008) Atlas of the developing rat nervous system. Elsevier Academic Press; San Diego.
16. Paxinos G, Watson C. (1986) The rat brain in stereotaxic coordinates, Ed 2.Sydney: Academic.
17. Pfenninger EG, Durieux ME, Himmelseher S. (2002) Cognitive impairment after small-dose ketamine isomers in comparison to equianalgesic racemic ketamine in human volunteers. Anesthesiology 96: 357-366.
18. Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, et al. (2009) Early exposure to anesthesia and learning disabilities in a population-based birth cohort. Anesthesiology 110: 796-804.
19. Altman J, Bayer SA. (1990) Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. J Comp Neurol 301: 365-381.
20. Dupret D, Fabre A, Dobrossy MD, Panatier A, Rodriguez JJ, et al. (2007) Spatial learning depends on both the addition and removal of new hippocampal neurons. PLoS Biol 5: e214.
21. Kee N, Teixeira CM, Wang AH, Frankland PW. (2007) Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. Nat Neurosci 10: 355-362.
22. Guidi S, Ciani E, Severi S, Contestabile A, Bartesaghi R. (2005) Postnatal neurogenesis in the dentate gyrus of the guinea pig. Hippocampus 15: 285-301.
23. Zhang K, Zhao T, Huang X, Wu LY, Wu K, et al. (2014) Notch1 mediates postnatal neurogenesis in hippocampus enhanced by intermittent hypoxia. Neurobiol Dis 64: 66-78.
24. Manning EE, Ransome MI, Burrows EL, Hannan AJ. (2012) Increased adult hippocampal neurogenesis and abnormal migration of adult-born granule neurons is associated with hippocampal specific cognitive deficits in phospholipase C-beta1 knockout mice. Hippocampus 22: 309-319.
25. Sanchez BS, Feinstein BA, Lunardi, Joksovic, Boscolo, et al. (2011) General anesthesia causes long-term impairment of mitochondrial morphogenesis and synaptic transmission in developing rat brain. Anesthesiology 115(5): 992-1002.
Figure 1

Experimental protocol for the administration of ketamine in test rats.
Anesthesia with ketamine (40 mg/kg×4 injections) in neonatal rats at postnatal day 7 (PND-7) induces learning and memory impairment in the adult stage. Ketamine anesthesia significantly increased the latency times of rats swimming in the Morris Water Maze (MWM), compared with the control group. During the reference training, the latency in locating the hidden platform in the ketamine group was significantly longer than that in the control group (A). In the memory retrieval tests, the time that each rat stayed in the target quadrant within 120 s was significantly reduced in the ketamine group than that in the control group (B). The numbers of crossovers of the previous platform site within 120 s was significantly reduced in the ketamine group than that in the control group (C). Typical path charts of space exploration were exhibited (D). Data are presented as the means±SD (n=6). *P<0.05, **P<0.01 vs. the control group.
Neonatal ketamine (40 mg/kg×4 injections) exposure did not affect the survival rate of developmentally generated neurons in the hippocampal DG during the adult stage. Experimental protocol (A). The Y axis “(/um2)” represents the density of NeuN+/BrdU+ cells in the DG (B). The developmentally generated granule neurons in the hippocampal DG were labeled with primary antibodies against NeuN (Green) and BrdU (Red). The representative images were visualized by using a laser scanning confocal microscope (C; magnification: a and e 20×, b-d and f-h 40×); the scale bar was 50 μm (a and b). The filled arrows point to the NeuN/BrdU double-labeled cells. Data are presented as the means±SD (n=5). GCL=granule cell layer; SGZ=subgranular zone; ML=molecular layer; PCL=polymorphic cell layer.
Neonatal ketamine exposure interfered with the functional integration of developmentally generated neurons into the hippocampal DG circuits during the adult stage. High magnification examples of c-Fos (blue), NeuN (Green) and BrdU (Red) immunofluorescences in the DG were captured by using a laser scanning confocal microscope, following the Morris Water Maze testing (A; magnification: a-h 40×); the scale bar was 50 μm. The arrowheads point to the NeuN/BrdU double-positive cells. The filled arrows...
point to the c-Fos/NeuN/BrdU triple-positive cells. Ketamine could significantly decrease the density of triple-positive cells in the hippocampal DG (C), and the percentages of triple-positive cells and double-positive cells were reduced in the ketamine group (D). The percentage of NeuN/BrdU-positive cells that expressed c-Fos in the GCL was decreased in the ketamine group (E). Data are presented as the means±SD (n=5). *P<0.05, **P<0.01 vs. the control group. GCL=granule cell layer; SGZ=subgranular zone; ML=molecular layer; PCL=polymorphic cell layer.

Figure 5

Neonatal ketamine (40 mg/kg×4 injections) exposure did not affect the expression of caspase-3 in the hippocampal DG at 2 months and 3 months old. The expression of caspase-3 in the hippocampal DG was measured by western blot analysis (A and B). Data are presented as the mean ± SD (n=3).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.pdf