Tropical Journal of Pharmaceutical Research June 2023; 22 (6): 1255-1261
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i6.15

Original Research Article

Effect of maternal anesthesia with ketamine in the second trimester of pregnancy on cognitive memory and neuron development in hippocampus of rat offspring

Dehong Gao[1], Xin Liu[1], Fan Zhang[2], Mingyan Ding[1]*
[1]Department of Anesthesia, Hanchuan People's Hospital, Hanchuan, [2]Department of Anesthesia, Renmin Hospital of Wuhan University/Hubei General Hospital, Wuhan, PR China

For correspondence:* ***Email:* *s125fk@163.com*

Sent for review: 24 July 2020 *Revised accepted: 28 May 2023*

Abstract

Purpose: *To investigate the effect of maternal anesthesia with ketamine in the second trimester of pregnancy on cognitive memory and neuronal development in rat offspring hippocampus.*
Methods: *Twenty female Sprague-Dawley rats, were randomly divided into ketamine and control groups with 10 rats each. Rats in control group were fed normal diet, while ketamine group was injected ketamine (10 mg/mL) and kept till they littered. Rat offspring at birth were designated as T0, while offspring aged 15, 20, and 25 days were designated T15, T20 and T25, respectively. After 20 days, (T20) rats were subjected to Morris water maze test, olfactory memory test, and other behavioral tests, Nissl staining and Golgi staining were done and expression of BrdU-positive cells was determined using immunofluorescence.*
Results: *Escape latency period of ketamine group was significantly higher and the number of original platform crossings was significantly lower than that of control group ($p < 0.05$). However, there was no significant difference in swimming speed between the two offspring groups ($p > 0.05$). The number of stagnations in fear conditioning test was significantly lower in ketamine group ($p < 0.05$). Nissl and Golgi staining showed that cell density in hippocampal CA1 and CA3 areas in T25 offspring as well as the number of pyramidal cells in hippocampus of T20 offspring were significantly lower in ketamine group than in control group ($p < 0.05$). Proliferation of neurons in DG and SVZ regions of hippocampus in ketamine group was lower than in control group.*
Conclusion: *Maternal ketamine anesthesia in the second trimester of pregnancy decreases the density of hippocampal neurons, and affects the cognitive and memory functions of rat offspring.*

Keywords: *Second trimester of pregnancy, Ketamine, Maternal anesthesia, Cognitive memory function, Hippocampal neuronal development*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The brain functions on which humans and lower animals depend are learning and memory. Cognitive function refers to the process in which the brain receives external information and acquires or applies knowledge after processing it. Cognitive disorder results from the impairment

of one or more cognitive functions such as memory, language, and visual space, which negatively affects the daily life of an individual [1]. Through a number of rodent and primate experiments, it has been revealed that routinely used anesthetics (e.g. ketamine) are neurotoxic to the developing brain, and interfere with the development of the central nervous system, resulting in cognitive impairment [2]. Pregnancy is an important period of fetal growth and development at which time the proliferation and differentiation of brain nerves and synaptic growth are very sensitive to external stimulants [3].

Previous studies on the toxic effects of general anesthesia on fetuses during gestation period were mainly focused on teratogenicity in the early stages of pregnancy, as well as the effects of cesarean section anesthesia on the basic functions and organs of fetuses. In most cases, surgeries that can be performed in the early stages are generally postponed to the second trimester of pregnancy, in order to avoid surgical complications and adverse reactions from stress. Moreover, most cesarean sections in the third trimester of pregnancy are not done under general anesthesia, and due to the short duration of anesthesia, the fetus is not adversely affected. It is generally believed that the safest period of operation during pregnancy is the second trimester of pregnancy, at which period embryonic development is already completed. However, the second trimester of pregnancy is a busy period for fetal brain development. The proliferation and migration of human neurons begin to accelerate in the second trimester of pregnancy, and they peak at the end of pregnancy. The period from the second trimester of pregnancy to a few years after birth is considered a "vulnerable period" of synaptic formation [4,5].

Studies have shown that 3-h anesthesia with 1.5 % sevoflurane in the second trimester of pregnancy resulted in impairments of spatial learning and memory in rat offspring [6]. It has been suggested that these impairments may occur through a mechanism related to ketamine-induced dysfunction in N-methyl-D-aspartic acid receptor (NMDA) in hippocampus, and the abnormal development of hippocampal neurons caused by inhibition of mammalian target of rapamycin (mTOR) signal transduction pathway [6].

The present study was aimed at investigating the effect of maternal ketamine anesthesia on cognitive memory and the development of hippocampal neurons in rat offspring.

EXPERIMENTAL

Animals

Healthy adult Sprague Dawley (SD) of both sexes (mean weight = 213 ± 37 g) were obtained from the Nanjing Junke Bioengineering Co. Ltd (product license: SCXK (Ning) 2017-0001). The rats were fed adaptively for 7 days in an environment with a 12-h light/12-h dark cycle, with *ad libitum* access to feed and water. This research protocol was approved by the Animal Ethical Committee of Department of Anesthesia, Hanchuan People's Hospital, Hanchuan, PR China, (approval no. 201829371) and was conducted according to the Principles of Laboratory Animal Care [7].

Reagents

The reagents used, and their suppliers (in brackets) were xylene (Zhangjiagang Free Trade Zone Zerunxin Chemical Trade Co. Ltd), anhydrous ethanol (Traditional Chinese Medicine Group Chemical Reagent Co. Ltd), reverse transcription kit (Dalian Takara Bioengineering Co. Ltd), skim milk powder (Zhengzhou Longsheng Chemical Products Co. Ltd), phosphate buffer (Shanghai Thermo Fisher Technology Co. Ltd), Hematoxylin (Shanghai Ruisai Biotechnology Co. Ltd) and ketamine (Xi'an Hanfeng Pharmaceutical Co. Ltd, production batch number: 20164748, specification: 2 mL: 0.1 g).

Mating

The male and female rats were put together in the same cage during estrus. After pregnancy, the female rats were kept separately for 2 weeks (20 female rats were mated).

Establishment of animal models and grouping

The pregnant rats were separated and kept in a cage for 2 weeks, after which they were randomly divided into ketamine group and control group with 10 female rats in each group. Rats in the control group were given normal feed only, while those in the ketamine group received ketamine injection (10 mg/mL in normal saline) via the tail vein at a rate of 50 mg/kg/h for 1.5 h. The infusion rate was adjusted to keep the pregnant rats in a state of muscle relaxation at surgical depth of anesthesia, without autonomic movement and eyelid reflex. In the ketamine group, rat body temperature was maintained at 37 - 37.5 ℃ during anesthesia. The respiration and heartbeat of rats were monitored, and oral

and nasal secretions were treated promptly. On recovery from anesthesia, the rats were returned to their original cages, and feeding was continued. When the rats littered, the offspring immediately after birth was designated T_0, while offspring aged 15, 20, and 25 days were designated T_{15}, T_{20}, and T_{25}, respectively. The T_{15} rats were raised in separate cages with the mother rats after weanling. Twenty-six rat offspring were selected from each group.

Evaluation of parameters/indices

Morris water maze test

This is a well-known test for measuring the learning and memory abilities of rats. Usually, the water maze is divided into four areas, and a mark for water entry point is set in each area, while a hidden circular escape platform is in the third area. In the directional navigation test (3 times a day for 4 days), one area was randomly selected for water entry each time. The time taken by a rat to locate the circular platform (within 100 sec) is termed the escape latency period. In this study, the time it took the rats to find and climb the platform was observed and recorded. On the 5th day, the platform was removed for space exploration experiments. The rats entered the water from the original area, and the time used in exploration to find the original platform area (within 100 sec) was recorded. If the platform was not found within 100 sec, the rats were led to the platform. After 48 h, the experiment was repeated to note the exploration time for locating the platform area, the number of times of crossing the original platform, and the swimming speed of the rats within 100 sec.

Fear conditioning test

This experiment is used to evaluate the ability of experimental animals to develop fear memory. The rats were first subjected to conditional training. The two groups of offspring were placed in an electric shock box under the same conditions but without any stimulation. After 3 min, 100 decibels of sound prompt were delivered for 25 sec. Thereafter, the rats were exposed to 4 sets of 0.4 mA plantar electrical stimulation, each for 3 sec, at 1 min intervals. After the 4th stimulation, the rats were observed for 1 min and then put back into their original cages. For environment-related training, after 48 h, the offspring were put back into the shock box for 8 min, and the number of stagnations was recorded in the absence of any hint or stimulation. In condition-related training, after 2 h, the rats were placed in triangular plastic boxes in a completely different environment, and the light intensity in the boxes was changed. After 3 min, 100 decibels of sound prompts were given for 25 sec. The number of stagnations in the offspring was recorded in each group.

Four T_{25} rats were randomly selected from each group and were sacrificed. The hippocampal tissue of each rat was peeled off and kept in a refrigerator. Nissl staining and Golgi staining were used to determine the development of hippocampal neurons (CA1 and CA3) in the rat offspring.

The proliferation of SVZ and DG cells in offspring hippocampus was observed under a microscope, while the expression of BrdU-positive cells was determined using immunofluorescence method.

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical analysis was done using SPSS 20.0 statistical software. Measurement data were compared between the two groups using an independent sample *t*-test. Values of $p < 0.05$ were considered indicative of statistical significance.

RESULTS

Effect of maternal ketamine anesthesia on venous blood gas

As shown in Table 1, there was no significant difference in venous blood gas between the control group and the ketamine group ($p > 0.05$).

Table 1: Effect of maternal ketamine anesthesia on venous blood gas in maternal rats in the second trimester of pregnancy (mean ± SD)

Index	Group		*t*	*P*-value
	Control	Ketamine		
K^+	135.47±1.43	134.66±1.27	1.339	0.197
pH	7.42±0.05	7.39±0.26	0.358	0.724
PO_2	47.14±5.46	48.27±4.85	0.489	0.631
PCO_2	51.26±3.43	50.65±5.88	0.283	0.781
Ca^{2+}	1.26±0.24	1.37±0.17	1.183	0.252
HCO_3^-	30.64±0.76	28.46±3.69	1.829	0.084

Morris water maze test results

The results of Morris water maze test showed that the escape latency period of the ketamine group was significantly higher than that of the control group, and the number of original platform crossings was significantly lower than that of the control group ($p < 0.05$). However, there was no significant difference in swimming speed between the two groups of rat offspring (*p*

> 0.05). These results are shown in Table 2.

Fear conditioning

As shown in Table 3, the number of stagnations in the fear conditioning test in the ketamine group was significantly lower than that in the control group ($p < 0.05$).

Density of hippocampal neurons

As shown in Figure 1, in the results of Nissl staining, cell density in hippocampal CA1 area and CA3 area in the ketamine group of rat offspring was significantly lower than that in the control group on day 25. Figure 2 shows the results of Golgi staining. The number of pyramidal cells in the hippocampus of the ketamine group of rat offspring (T_{25}) was significantly lower than that in the control group.

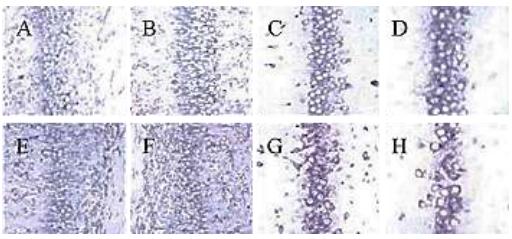

Figure 1: Nissl staining showing loss of hippocampal neurons in rat offspring. (A) Neuronal cells of hippocampal CA1 area in T_0 offspring in the control group, (B) Neuronal cells of hippocampal CA1 area in T_0 offspring in the ketamine group, (C) Neuronal cells of hippocampal CA1 area in T_{25} offspring in the control group, (D) Neuronal cells of hippocampal CA1 area in T_{25} offspring in the ketamine group, (E) Neuronal cells of hippocampal CA3 area in T_0 offspring in the control group, (F) Neuronal cells of hippocampal CA3 area in T_0 offspring in the ketamine group, (G) Neuronal cells of hippocampal CA3 area in T_{25} offspring in the control group, (H) Neuronal cells of hippocampal CA3 area in T_{25} offspring in the ketamine group

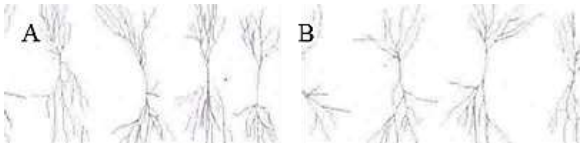

Figure 2: Golgi staining showing developmental disorders in rat offspring neurons. (A) Neuronal cells of hippocampal CA3 area in T_{25} offspring in the control group, (B) Neuronal cells of hippocampal CA3 area in T_{25} offspring in the ketamine group

Expression of BrdU-positive cells in rat offspring

As shown in Figure 3, the proliferation of neurons in the DG and SVZ regions of the hippocampus in the ketamine group of rat offspring was significantly lower than that in the control group.

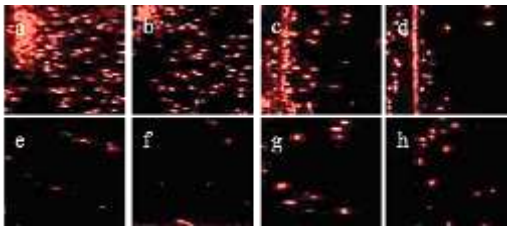

Figure 3: Expression of BrdU-positive cells in two groups of offspring. (A) BrdU-positive cells in hippocampal DG region of T_0 in the control group, (B) BrdU-positive cells in hippocampal DG region of T_0 in the ketamine group, (C) BrdU-positive cells in hippocampal SVZ region of T_0 in the control group, (D) BrdU-positive cells in hippocampal SVZ region of T_0 in the ketamine group, (E) BrdU-positive cells in hippocampal DG region of T_{25} in the control group, (F) BrdU-positive cells in hippocampal DG region of T_{25} in the ketamine group, (G) BrdU-positive cells in hippocampal SVZ region of T_{25} in the control group, (H) BrdU-positive cells in hippocampal SVZ region of T_{25} in the ketamine group

Table 2: Comparison of Morris water maze test results between the two groups (mean ± SD, n = 26)

Group	Escape latency period (sec)				Number of platform crossings	Swimming speed (cm/sec)
	First day	*Second day*	*Third day*	*Fourth day*		
Control	78.54±2.52	61.71±6.67	52.47±6.54	40.79±7.72	4.02±0.48	25.01±1.97
Experimental	89.43±2.98	74.09±7.41	61.08±6.72	49.21±7.79	2.82±0.36	26.02±1.65
t	14.228	6.332	4.682	3.915	10.198	2.004
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.051

Table 3: Comparison of the results of fear conditioning test between the two groups (mean ± SD, n = 26)

Group	Conditioned training	Environment-related training	Condition-related training
Control	23.49 ± 5.38	45.72 ± 8.63	51.18 ± 8.98
Experimental	17.68 ± 4.41	36.07 ± 5.14	32.74 ± 5.01
t	4.259	4.899	9.144
P-value	<0.001	<0.001	<0.001

DISCUSSION

Pregnancy is a period of fetal growth and development. During this period, there is rapid development of fetal nervous system, and the proliferation and differentiation of cerebral nerves and synaptic growth are easily compromised by external stimulation. At present, the second trimester of pregnancy is still considered to be a relatively safe surgery period for the pregnant mother and fetus. However, the second trimester of pregnancy is the peak period for the development of fetal nervous system. Moreover, the period from the second trimester of pregnancy to a few years after birth is the most vulnerable stage in synaptic formation [8,9]. Gamma-aminobutyric acid (GABA) and glutamic acid play important roles in brain nutrition and brain development. Most of the frequently used general anesthetics such as N-methyl-D-aspartic acid (NMDA) receptor antagonists and GABA receptor agonists are fat-soluble drugs that readily pass through the placenta [10]. Thus, maternal anesthesia in the second trimester of pregnancy may enter the fetal blood circulation and brain tissue through the placenta, thereby exposing the fetal brain to prolonged anesthesia. This may affect the proliferation and migration of fetal brain neurons, leading to neuronal apoptosis [11].

In recent years, a large number of studies have shown that general anesthesia triggers apoptosis of cerebral nerve cells, thereby negatively affecting the learning and memory of experimental animals. Ketamine is a derivative of phencyclidine, and it is a non-competitive antagonist of NMDA [12]. It has been found that ketamine impairs memory cognitive function by inhibiting the glutamate neural pathway. The NMDA receptor plays an important role in synaptic plasticity with respect to nerve cell growth and development. The hippocampus is an area of the brain involved in long-term learning and memory, as well as acousto-optics. Ketamine inhibits the generation of long-term potentiation effects by suppressing the transmission of various stimuli, and it affects the memory integration of learning and cognition [13,14].

Animal behavior testing has been applied in many fields of neuroscience, especially in evaluating animal models and studying physiological mechanisms of cognitive impairment-related diseases. Carspecken *et al* have reported that multiple high-dose or long-term persistent low-dose of ketamine injections could damage spatial learning and memory function in developmental phase of the brain in young rats [15]. Ketamine impairs the cognitive function of neonatal rats. In this study, the Morris water maze test and fear conditioning test were carried out on 20-day-old rat offspring to determine the effect of ketamine injection on memory in the second trimester of pregnancy. The results of the study showed that the escape latency of the ketamine group was significantly higher than that of the control group, and the number of original platform crossings was significantly lower in the ketamine group than in the control group. However, there was no significant difference in swimming speed between the two groups of rat offspring. These results show that ketamine anesthesia may damage the cognitive function of rat offspring.

It has been confirmed that ketamine and other general anesthetics produce neurotoxicity in the developing brain. This may lead to neuronal apoptosis, inhibition of the proliferation of neurons and suppression of the formation of bone structure, impairment of synaptic plasticity, and inhibition of long-term potentiation of hippocampus. There is an interdependent relationship between the number of hippocampal nerve cells and cognitive memory [16]. Yao *et al* have reported neuronal apoptosis and necrosis in the offspring of guinea pigs in the early and second trimesters of pregnancy, but there was no obvious pathological change in the offspring of guinea pigs in the third trimester of pregnancy [17]. In the results of Nissl staining, the cell density in hippocampal CA1 area and CA3 area of the ketamine group of T_{25} rat offspring was significantly lower than that in the control group. Moreover, the number of pyramidal cells in the hippocampus, and the proliferation of neurons in the DG region and SVZ region of the hippocampus in the ketamine group were significantly lower than those in the control group. These results suggest that maternal ketamine anesthesia in the second trimester of pregnancy is toxic to the hippocampus of offspring, and may result in decreased dendritic spine density and neurodevelopmental disorders which will further lead to some degree of cognitive impairment.

CONCLUSION

Maternal ketamine anesthesia in the second trimester of pregnancy in rats causes hippocampal neurotoxicity in the offspring, leading to disorders in neuronal development, suppression of synaptic plasticity of neurons, and cognitive and functional impairment in the offspring. Further studies are required to ascertain the implications of these findings in clinical practice.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors, all authors read and approved the manuscript for publication. Mingyan Ding conceived and designed the study, Dehong Gao, Xin Liu, Fan Zhang, Mingyan Ding collected and analysed the data, while Dehong Gao wrote the manuscript.

REFERENCES

1. *Maruoka H, Yamazoe R, Takahashi R, Yatsuo K, Ido D, Fuchigami Y, Hoshikawa F, Shimoke K. Molecular mechanism of nur77 gene expression and downstream target genes in the early stage of forskolin-induced differentiation in PC12 cells. Sci Rep 2020; 10(1): 6325.*
2. *Sadowska-Krępa E, Domaszewski P, Pokora I, Żebrowska A, Gdańska A, Podgórski T. Effects of medium-term green tea extract supplementation combined with CrossFit workout on blood antioxidant status and serum brain-derived neurotrophic factor in young men: a pilot study. J Int Soc Sports Nutr 2019; 16(1): 13.*
3. *Leyshon BJ, Ji P, Caputo MP, Matt SM, Johnson RW. Dietary Iron Deficiency Impaired Peripheral Immunity but Did Not Alter Brain Microglia in PRRSV-Infected Neonatal Piglets. Front Immunol 2019; 9: 3150.*
4. *Cavus G, Gezercan Y, Ökten AI, Tolunay O, Çelik T, Arslan A, Çelik Ü. Surgical approaches to upper thoracic Pott's disease with spinal instability during childhood: two cases. Childs Nerv Syst 2018; 34(6): 1221-1227.*
5. *Su ZY, Ye Q, Liu XB, Chen YZ, Zhan H, Xu SY. Dexmedetomidine mitigates isoflurane-induced neurodegeneration in fetal rats during the second trimester of pregnancy. Neural Regen Res 2017; 12(8): 1329-1337.*
6. *Igarashi KM, Ito HT, Moser EI, Moser MB. Functional diversity along the transverse axis of hippocampal area CA1. FEBS Lett 2016; 588(15): 2470-2476.*
7. *World Health Organization. Principles of laboratory animal care. WHO Chron 1985; 39: 51-56.*
8. *Benoy A, Dasgupta A, Sajikumar S. Hippocampal area CA2: an emerging modulatory gateway in the hippocampal circuit. Exp Brain Res 2018; 236(4): 919-931.*
9. *McWhorter ES, West RC, Russ JE, Ali A, Winger QA, Bouma GJ. LIN28B regulates androgen receptor in human trophoblast cells through Let-7c. Mol Reprod Dev 2019; 86(9): 1086-1093.*
10. *Huang IJ, Sirotkin HI, Mcelroy AE. Varying the exposure period and duration of neuroactive pharmaceuticals and their metabolites modulates effects on the visual motor response in zebrafish (Danio rerio) larvae. Neurotoxicol Teratol 2019; 72: 39-48.*
11. *Pathirathna ML, Sekijima K, Sadakata M, Fujiwara N, Muramatsu Y, Wimalasiri KMS. Impact of Second Trimester Maternal Dietary Intake on Gestational Weight Gain and Neonatal Birth Weight. Nutrients 2017; 9(6): 627.*
12. *Wanapirak C, Piyamomgkol W, Sirichotiyakul S, Tongprasert F, Srisupundit K, Luewan S, Traisrisilp K, Jatavan P, Tongsong T. Second Trimester Maternal Serum Screening for Fetal Down Syndrome: as a Screening Test for Hemoglobin Bart's Disease: A Prospective Population-based Study. Prenat Diagn 2018; 38(9): 700-705.*
13. *Sun J, Lin J, Feng X, Lu Z, Liu T, Lin L, Chen Y, Hu Y, Li Y, Xu S, et al. Prenatal Exposure to Ketamine Leads to Anxiety-Like Behaviors and Dysfunction in Bed Nucleus of Stria Terminalis. Int J Neuropsychopharmacol 2020; 23(3): 181-191.*
14. *Wang C, Liu F, Patterson TA, Paule MG, Slikker Jr W. Relationship between ketamine-induced developmental neurotoxicity and NMDA receptor-mediated calcium*

influx in neural stem cell-derived neurons. Neurotoxicology 2017; 60: 254-259.

15. *Carspecken CW, Borisovskaya A, Lan ST, Heller K, Buchholz J, Ruskin D, Rozet I. Ketamine Anesthesia Does Not Improve Depression Scores in Electroconvulsive Therapy: A Randomized Clinical Trial. J Neurosurg Anesthesiol 2018; 30(4): 305-313.*
16. *Oliva A, Fernández-Ruiz A, Fermino de Oliveira E, Buzsáki G. Origin of Gamma Frequency Power during Hippocampal Sharp-Wave Ripples. Cell Rep 2018; 25(7): 1693-1700.*
17. *Yao P, Li Y, Yang Y, Yu SC, Chen Y. Triptolide Improves Cognitive Dysfunction in Rats with Vascular Dementia by Activating the SIRT1/PGC-1α Signaling Pathway. Neurochem Res 2019; 44(8): 1977-1985.*