Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

The spatial learning and memory performance in methamphetamine–sensitized and withdrawn rats

Imanollah Bigdeli [1], Masomeh Nikfarjam- Haft Asia [1], Hossein Miladi-Gorji [2]*, Atefeh Fadaei [1]

[1] Faculty of Psychology and Educational Sciences, University of Semnan, Semnan, Iran
[2] Laboratory of Animal Addiction Models, Research Center and Department of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran

ARTICLE INFO

Article type:
Original article

Article history:
Received: May 5, 2014
Accepted: Sep 13, 2014

Keywords:
Long -term memory
METH- sensitized rats
Short -term memory
Spatial memory

ABSTRACT

Objective(s): There is controversial evidence about the effect of methamphetamine (METH) on spatial memory. We tested the time- dependent effects of METH on spatial short-term (working) and long-term (reference) memory in METH –sensitized and withdrawn rats in the Morris water maze.
Materials and Methods: Rats were sensitized to METH (2 mg/kg, daily/5 days, SC). Rats were trained in water maze (4 trials/day/ for 5 days). Probe test was performed 24 hr after training. Two days after probe test, working memory training (2 trials/day/ for 5 days) was conducted. Acquisition–retention interval was 75 min. The treatment was continued per day 30 and 120 min before the test. Two groups of METH –sensitized rats were trained in reference memory after a longer period of withdrawal (30 days).
Results: Sensitized rats exhibited significantly longer escape latencies on the training, spent significantly less time in the target zone (all, $P<0.05$), and their working memory impaired 30 min after injection. While, METH has no effect on the spatial learning process 120 min after injection, and rats spent significantly less time in the target zone ($P<0.05$), as well it has no effect on working memory. Also, impairment of reference memory persisted after prolonged abstinence.
Conclusion: Our findings indicated that METH impaired spatial learning and memory 30 min after injection, but spared spatial learning, either acquisition or retention of spatial working, but partially impaired retention of spatial reference memory following 120 min after injection in sensitized rats, which persisted even after prolonged abstinence.

► *Please cite this paper as:*
Bigdeli I, Nikfarjam- Haft Asia M, Miladi- Gorji H, Fadaei A. The spatial learning and memory performance in methamphetamine–sensitized and withdrawn rats. Iran J Basic Med Sci 2015; 18:234-239.

Introduction

Methamphetamine (METH) is a powerful central nervous system (CNS) stimulant (1), which is widely distributed in the human and rat brain (2). Although METH -dependent individuals often claimed the cognitive and attention-enhancing effect following drug use (3), but METH causes dependence and withdrawal syndrome (4), long-term changes in the brain structure and function, changes in synaptic plasticity (2), cell death via apoptosis (5) and neurotoxicity (3).

However, studies existing about the effects of METH on cognitive functioning, learning and memory are rather inconsistent. For example, it has been shown that METH -dependence is associated with neurocognitive impairment, including poor attention, learning and memory, episodic memory, and working memory (6-9). While, other studies in humans (10, 11) and animal (12) models have provided some evidence that METH can improve cognition. Animal studies have also shown that repeated METH exposure impairs learning in the Morris water maze in adulthood; however, prenatal METH exposure improves performance in the retention memory test (13), while lower doses of drug did not have any effect on cognition in adult offspring (14). It appears that these discrepancies are due to total dose and duration of drug exposure (time- dependent effects of METH).

In the present study, enhanced sensitivity to METH occurred in rats but not dependency. Given that, short (acute) and long term (chronic) effects of METH exposure (time- dependent effects of METH) on the spatial memory performance are unknown in sensitized rats. Thus, we examined the spatial short term (working) and long-term (reference) memory of METH –sensitized rats 30 and 120 min after the injection in the Morris water maze. Also, reference memory was evaluated after a 30-day period of withdrawal in METH –sensitized rats in the Morris water maze. Lack of such knowledge therapeutically prevents intervention to reverse METH -induced neurotoxic damage.

*Corresponding author: Hossein Miladi-Gorji. Laboratory of Animal Addiction Models, Research Center and Department of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran. Fax: +98-231-3354186; email: Miladi331@yahoo.com

Materials and Methods

Animals and induction of methamphetamine-induced sensitization

Male Wistar rats (220±10 g) were housed in a 12 hr light/dark cycle at 22 to 24˚C, with food and water *ad libitum*. All of the experimental procedures were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering. Methamphetamine hydrochloride (Sigma–Aldrich, M 8750) was dissolved in 0.9% saline. The rats were chronically treated with subcutaneous injections of METH (2 mg/kg), once a day for 5 days, as described previously (15). Rats become sensitized to METH during 5 days. Normal saline solution was similarly injected into control rats. This dose of METH shows no neurotoxicity but produces behavioral sensitization after repeated treatment in rats (16).

Experimental protocols

Experiment 1: Reference memory and working memory testing using the water maze

This experiment examined spatial memory performance of METH –sensitized rats 30 and 120 min after the injection of drug. In this experiment, 38 male rats were divided into four groups (n=9-10 rats per group) and received saline or METH (2 mg/kg, SC, for 5 days). All training and testing were conducted 30 or 120 min after injection of METH. Considering the half-life of METH in rats approximately 70 min (17, 18), the experimental groups were divided as follows: Group 1: Sal exposed/ Sal 30 min after injection, Group 2: (Sensitive to METH) METH exposed/ METH 30 min after injection, Group 3: Sal exposed/ Sal 120 min after injection, Group 4: METH exposed /METH 120 min after injection.

A detailed description of the apparatus and the tracking system has been given in our previous reports (19). From day 5 to 10, all rats were trained in spatial learning (4 trials per day for 5 consecutive days). Twenty four hours prior to the start of training, rats were allowed to swim 3 min in the pool containing no platform for habituation, as described previously (20, 21). A spatial probe test was performed 24 hr after the last acquisition session, without platform. The rats were allowed to swim for 60 sec, and during this period, we recorded the latency to reach the platform location, the time spent in a zone around the platform (20 cm radius) in each quadrant, and the proximity (the average distance from the center of the platform during the probe test) and velocity of each animal (19).

Two days after probe test, training on working memory version of the water maze task was started. Only two trials per day were given for 5 days to stabilize the performance of the animal in task. Final test was performed on day 6. In the first trial (acquisition), the rat had to find the platform in a new position, the second trial (retrieval) was performed 75 min later, as described previously (20). In this period, METH injection was performed 30 or 120 min before the acquisition phase. The treatment was continued for 13 days of learning and memory testing. Thus, the total duration of the METH injection was 18 days in Experiment 1. (Figure 1A. Time line).

Experiment 2: Reference memory testing after a 30-day period of withdrawal in METH –sensitized rats

This experiment examined the effects of METH withdrawal on the spatial reference memory in METH-sensitized rats. Two groups of rats (saline and methamphetamine-sensitized rats from EXP1) were exposed to 30 days of spontaneous withdrawal after the end of working memory test. The experimental groups were divided as follows: Group 1: Saline (Sal), Group 2: a 30 day period of spontaneous withdrawal (METH/Withd). Rats were trained in spatial learning (2 trials per day for 3 consecutive days). A spatial probe test was performed 24 hr after the last acquisition session, as described above. Methamphetamine injection was discontinued in this period (Figure 1B. Time line).

Statistical analysis

The data expressed as the mean±standard error of the mean (SEM). These data were analyzed using two-way analyses of variance (ANOVA), with repeated measures (day effect, group effect, group×day interaction) and one -way ANOVA as required. *Post-hoc* analyses consisted of Turkey's test. A Student's t-test was used to compare the data between two groups. The statistical differences were considered to be significant at $P< 0.05$.

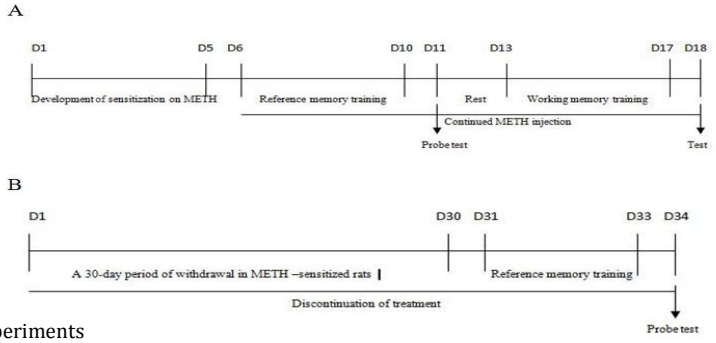

Figure 1. Timelines of experiments

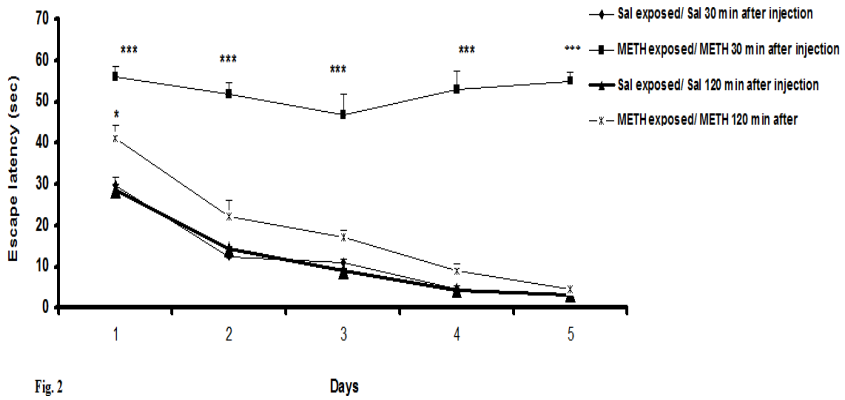

Figure 2. Effect of Methamphetamine (METH) in acquisition phase of spatial learning in METH-sensitized rats 30 and 120 min after the injection as measured by the WM task. All groups learned platform location during the 5-day training, except group 30 min after injection of METH. Result showed group of 30 min after injection of METH is not able to learning. *** represents a significant different between 30 min after injection of METH and saline groups (P= 0.0001). * represents a significant difference between120 min after injection of METH and saline groups on the first day of training (P= 0.002)

Results

Spatial learning

The acquisition data during the 5 days of training in the water maze (WM)are illustrated in Figure 2. Two-way analyses of variance (ANOVA), with repeated measures were used to analyze the escape latencies during training. All groups learned to locate the platform during 5 days of training, as indicated by decreasing escape latencies as training progressed ($F_{4, 170}$=62. 8, P=0.0001), except METH group 30 min after injection. Statistical analysis showed a significant group effect ($F_{3, 34}$= 206.8, P= 0.0001) and significant interaction between factors (group ×day) ($F_{12, 170}$ = 6.4, P= 0.0001) in the acquisition phase of learning. The METH groups exhibited significantly higher escape latencies in all 5 days training than those of control group (P= 0.0001) 30 min post injection. These findings indicated that exposure to METH decreased the learning rate in sensitized groups 30 min post injection.

Data related to the distance swam to reach the platform followed similar to the same pattern as the latency. All groups traveled shorter distances to reach the platform as training progressed ($F_{4, 170}$=42. 63, P=0.0001), except METH group 30 min after injection (Data not shown).

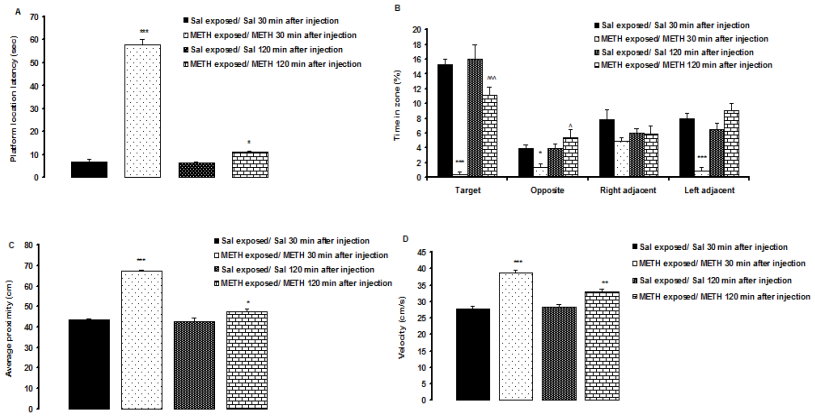

Figure 3. Effect of Methamphetamine (METH) on spatial references memory in METH-sensitized rats using the probe trial during the WM task 30 and 120 min after the injection. (A) The mean latency to reach the platform location. (B) The mean percentage of total time spent in within a zone, with a radius of 20 cm in the target zone or in other zone (C) The proximity (D) Swimming speed of each rat. Results showed that the METH sensitized rats took significantly more time to reach the platform location (A), spent significantly less time in the target zone (B), and had significantly further proximity values (C) and also with increased swimming speed compared to their controls (D). In A: *** and * Indicates a significant different between METH groups compared to their controls (P=0.0001, and P=0.045, respectively). In B: *** and ^^^Indicates a significant different between METH groups compared to their controls (P=0.0001, both) in the target zone. *and ^ Indicates a significant different between METH groups compared to their controls (P=0.024, and P=0.034, respectively) in the opposite zone. *** Indicates a significant different between 30 min after injection than those control (P=0.0001) in the left zone. In C: *** and * Indicates a significant different between METH groups compared to their controls (P=0.0001, and P = 0.037, respectively). In D: *** and ** Indicates a significant different between METH groups compared to their controls (P=0.0001, and P = 0.003, respectively)

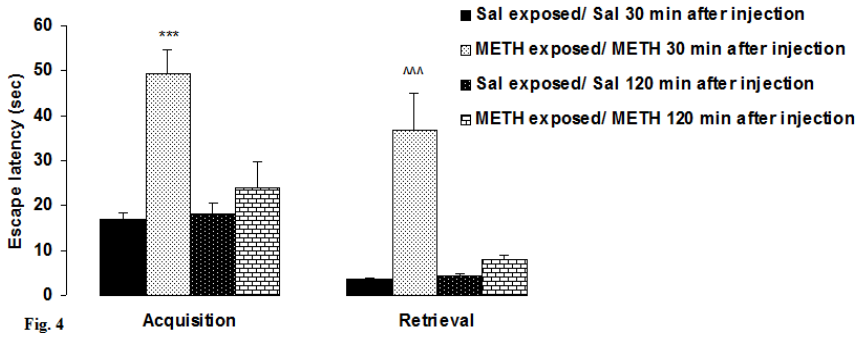

Figure 4. Effect of Methamphetamine (METH) on acquisition and retention of spatial (working) memory in METH-sensitized rats 30 and 120 min after the injection. In acquisition and retention: *** and ^^^ Indicates a significant different between 30 min after injection than those control (P=0.0001, both)

Spatial reference memory

The data for the spatial reference memory test are shown in Figure 3. One -way analyses of variance (ANOVA) showed significant effects on the platform location latency between groups ($F_{3, 34}$=410.9, P=0.0001) (Figure 3A). Comparisons between groups showed that the mean latency to reach the platform location in sensitized groups were significantly more than those of control groups (P=0.0001, P=0.045; respectively) 30 and 120 min after injection of METH, representing the impairment of the spatial reference memory in both groups. There are also significant differences between METH groups (P=0.0001). Analysis (Figure 3B) indicate significant difference between groups in time spent in the target ($F_{3, 34}$=35, P=0.0001), opposite ($F_{3, 34}$=10.34, P=0.0001) and left zones ($F_{3, 34}$=20.9, P=0.0001). Comparisons between groups showed that the METH groups spent significantly less time in the target zone than those of control groups (P=0.0001 and P=0.036, respectively) 30 and 120 min after injection. Also, METH group spent significantly more time in the opposite zone than those of control groups (P=0.034) 120 min after injection.

Figure 3C represents the average proximity to the platform. A one way ANOVA revealed significant difference between groups ($F_{3, 34}$=78.9, P=0.0001). The METH groups had significantly larger average proximity value than control groups (P=0.0001, P=0.037; respectively). Figure 3D showed that both groups had significantly more swim speed than of control groups (P=0.0001, P=0.003, respectively).

Spatial working memory

Figure 3. Illustrates the mean escape latencies on the acquisition and retention trials for control and 30 and 120 min after injection of METH. A one way ANOVA revealed a significant difference between groups in the acquisition ($F_{3, 34}$=13.76, P=0.0001) and retention trials ($F_{3, 34}$=7.95, P=0.0001). Comparisons between groups showed that the METH groups exhibited significantly higher escape latencies in the acquisition and retention trials than those of control group (P=0.0001, both) 30 min after injection. While, there was not significant difference in the acquisition and retention trials 120 min after injection in the METH groups. This finding indicates that 120 min after the injection of METH has no effect on either acquisition or retention of working memory in METH -sensitized rats in the water maze.

Spatial learning and reference memory after a withdrawal period of METH

The data for the spatial reference memory test after prolonged abstinence are shown in Figure 5. Student's t-test indicated that METH -withdrawn rats spent significantly less time in the target zone than those of control group (t_{17}=6.48, P=0.0001).

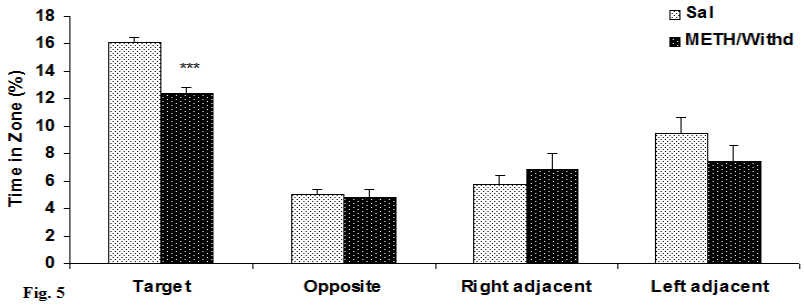

Figure 5. Effects of Methamphetamine (METH) withdrawal on spatial reference memory in METH-sensitized rats using the probe trial during the WM task. The mean percentage of total time spent in within a zone or in other zone. Results showed that the METH withdrawn rats spent significantly less time in the target zone. *** Indicates a significant different between METH/Withd group compared to saline (P=0.0001)

Discussion

We found that METH-sensitized rats showed spatial working and reference memory impairment 30 min after injection of METH. Furthermore, rats did not show impairment of learning ability in reference memory and the acquisition and retention of working memory 120 min after drug injection in METH- sensitized rats, but the retention of spatial reference memory in the WM task is impaired. So far, similar studies have not found in line with this study. Thus, the spatial learning and memory deficits observed in the sensitized rats 30 min after injection could potentially be interpreted as an acute effect of METH, which is associated with increased levels of anxiety and aggressive behavior (13, 22, 23). In our study, aggressive behavior was evident 30 min after injection, whereas it is clearly decreased 120 min after injection of the drug in the sensitized rats; probably because of the half-life of METH that is 70 min in rats (17, 18). Thus, drug sensitivity -induced anxiety and aggressive behaviors were increased after 30 min of injection in METH- sensitized rats and decreased during 120 min after injection. It seems that stress is reduced 120 min after injection during training of water maze, which facilitated spatial learning. Also, we observed that almost all rats spend most of their time during trials training in the peripheral regions of the quadrant 30 min after injection. Thus, despite the high swimming speed 30 min after injection, the learning has not occurred and rats failed to find the hidden platform. This finding is consistent with previous results showing that methamphetamine increases locomotor activity (24). The mechanism(s) underlying the spatial learning and memory deficits following METH (30 min after the drug injection) are unknown. Although it seems, stress-related pathways, the creatine system and monoamine levels in brain may be involved. Since it was shown that acute administration of METH after a single dose increased corticosterone, and hyperthermia and decreased hippocampal 5-HT levels and the brain creatine that affect cognitive function (25).

Also, we observed that the intensity of impairment is low 120 min after injection compared to 30 min after injection of drug (This was statistically significant). Therefore, another explanation is that 120 min after injection may be interpreted as residual drug effects of chronic and rats developed tolerance to the methamphetamine and may have resulted in a reduction in drug efficacy; so, the intensity of impairment was low; however, there was statistically significant difference as compared to the saline group.

In line with our study, it was shown that neonatal treatment with METH had no effect on working memory (26). Another study has shown that prenatal exposure to higher doses of METH (15, 20 mg/kg) induced impairments of spatial memory in the MWM tested in adulthood (14). While, in our study dose of 2 mg/kg induced impairment of spatial long-term memory. This difference may be due to the duration and timing of drug injection during pregnancy (27). Also, we found that after prolonged abstinence, rats exhibited a deficit in spatial reference memory. Our findings is in accordance with those reported in previous studies (28, 29). Although the mechanism that underlies the impairing effects of METH after prolonged abstinence is unknown, it has been suggested that chronic METH may lead to the neurodegeneration (30) apoptosis (5) and reduction of LTP (2) in neurons of the hippocampus. METH administration results in long lasting dopamine depletion in humans and animals (31). Given that the hippocampus is more sensitive to METH (25). It is probable that the degenerative effects of METH have been sustained even after prolonged abstinence in the hippocampus. Therefore, we found that rats showed spatial memory deficits after 30 days of withdrawal.

Conclusion

Our findings indicate that drug sensitivity was increased 30 min after injection in methamphetamine -sensitized rats, thus leading to the destruction of learning and memory. So that 120 min after injection of METH in sensitized rats did not impair learning ability and working memory, but partially impaired retention of spatial reference memory, which persisted even after prolonged abstinence of drug.

Acknowledgement

We would like to thank the Research Center of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran for providing research facilities.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Cherner M, Suarez P, Casey C, Deiss R, Letendre S, Marcotte T, *et al*. Methamphetamine use parameters do not predict neuropsychological impairment in currently abstinent dependent adults. Drug Alcohol Depend 2010; 106:154-163.
2. Swant J, Chirwa S, Stanwood G, Khoshbouei H. Methamphetamine reduces LTP and increases baseline synaptic transmission in the CA1 region of mouse hippocampus. PloS One 2010; 5:e11382.
3. Johnson BA, Roache JD, Ait-Daoud N, Wells LT, Wallace CL, Dawes MA, *et al*. Effects of topiramate on methamphetamine-induced changes in attentional and perceptual-motor skills of cognition in recently abstinent methamphetamine-dependent individuals.

Prog Neuropsychopharmacol Biol Psychiatry 2007; 31:123-130.
4. Kitanaka N, Kitanaka J, Tatsuta T, Tanaka K, Watabe K, Nishiyama N, *et al*. Withdrawal from fixed-dose injection of methamphetamine decreases cerebral levels of 3-methoxy-4-hydroxyphenylglycol and induces the expression of anxiety-related behavior in mice. Neurochem Res 2010; 35:749-760.
5. Stumm G, Schlegel J, Schäfer T, Würz C, Mennel H, Krieg JC, *et al*. Amphetamines induce apoptosis and regulation of bcl-x splice variants in neocortical neurons. FASEB J 1999; 13:1065-1072.
6. Scott JC, Woods SP, Matt GE, Meyer RA, Heaton RK, Atkinson JH, *et al*. Neurocognitive effects of methamphetamine: a critical review and meta-analysis. Neuropsychol Rev 2007; 17:275-297.
7. Simon SL, Dean AC, Cordova X, Monterosso JR, London ED. Methamphetamine dependence and neuropsychological functioning: evaluating change during early abstinence. J Stud Alcohol Drugs 2010; 71:335-344.
8. Woods SP, Rippeth JD, Conover E, Gongvatana A, Gonzalez R, Carey CL, *et al*. Deficient strategic control of verbal encoding and retrieval in individuals with methamphetamine dependence. Neuropsychology 2005;19:35-43.
9. Kalechstein AD, De La Garza R 2nd, Newton TF. Modafinil administration improves working memory in methamphetamine-dependent individuals who demonstrate baseline impairment. Am J Addict 2010; 19:340-344.
10. Mahoney JJ 3rd, Jackson BJ, Kalechstein AD, De La Garza R 2nd, Newton TF. Acute, low-dose methamphetamine administration improves attention/information processing speed and working memory in methamphetamine-dependent individuals displaying poorer cognitive performance at baseline. Prog Neuropsychopharmacol Biol Psychiatry 2011; 35:459-465.
11. Simon SL, Dacey J, Glynn S, Rawson R, Ling W. The effect of relapse on cognition in abstinent methamphetamine abusers. J Subst Abuse Treat 2004; 27:59-66.
12. Kennedy CD, Houmes SW, Wyrick KL, Kammerzell SM, Lukowiak K, Sorg BA. Methamphetamine enhances memory of operantly conditioned respiratory behavior in the snail Lymnaea stagnalis. J Exp Biol 2010; 213:2055-2065.
13. Schutová B, Hrubá L, Pometlová M, Deykun K, Šlamberová R. Cognitive functions and drug sensitivity in adult male rats prenatally exposed to methamphetamine. Physiol Res 2009; 58:741-750.
14. Acuff-Smith KD, Schilling MA, Fisher JE, Vorhees CV. Stage-specific effects of prenatal d-methamphetamine exposure on behavioral and eye development in rats. Neurotoxicol Teratol 1996; 18:199-215.
15. Nagai T, Noda Y, Ishikawa K, Miyamoto Y, Yoshimura M, Ito M, *et al*. The role of tissue plasminogen activator in methamphetamine-related reward and sensitization. J Neurochem 2005; 92:660-667.
16. Nagai T, Takuma K, Dohniwa M, Ibi D, Mizoguchi H, Kamei H, *et al*. Repeated methamphetamine treatment impairs spatial working memory in rats: reversal by clozapine but not haloperidol. Psychopharmacology 2007; 194:21-32.
17. Melega WP, Williams AE, Schmitz DA, DiStefano EW, Cho AK. Pharmacokinetic and pharmacodynamic analysis of the actions of D-amphetamine and D-methamphetamine on the dopamine terminal. J Pharmacol Exp Ther 1995; 274:90-96.
18. Herring NR, Schaefer TL, Gudelsky GA, Vorhees CV, Williams MT. Effect of (+)-methamphetamine on path integration learning, novel object recognition, and neurotoxicity in rats. Psychopharmacology 2008; 199:637-650.
19. Miladi-Gorji H, Rashidy-Pour A, Fathollahi Y, Akhavan MM, Semnanian S, Safari M. Voluntary exercise ameliorates cognitive deficits in morphine dependent rats: the role of hippocampal brain-derived neurotrophic factor. Neurobiol Learn Mem 2011; 96:479-491.
20. Miladi Gorji H, Rashidy-Pour A, Fathollahi Y. Effects of morphine dependence on the performance of rats in reference and working versions of the water maze. Physiol Behav 2008; 93:622-627.
21. Vorhees CV, Skelton MR, Williams MT. Age-dependent effects of neonatal methamphetamine exposure on spatial learning. Behav Pharmacol 2007; 18:549-562.
22. Šlamberová R, Mikulecká A, Pometlová M, Schutová B, Hrubá L, Deykun K. The effect of methamphetamine on social interaction of adult male rats. Behav Brain Res 2010; 214:423-427.
23. Sokolov BP, Schindler CW, Cadet JL. Chronic methamphetamine increases fighting in mice. Pharmacol Biochem Behav 2004; 77:319-326.
24. Wallace TL, Gudelsky GA, Vorhees CV. Methamphetamine-induced neurotoxicity alters locomotor activity, stereotypic behavior, and stimulated dopamine release in the rat. J Neurosci 1999; 19:9141-9148.
25. Herring NR, Schaefer TL, Tang PH, Skelton MR, Lucot JP, Gudelsky GA, *et al*. Comparison of time-dependent effects of (+)-methamphetamine or forced swim on monoamines, corticosterone, glucose, creatine, and creatinine in rats. BMC Neurosci 2008; 9:49.
26. Williams MT, Morford LL, Wood SL, Wallace TL, Fukumura M, Broening HW, *et al*. Developmental D-methamphetamine treatment selectively induces spatial navigation impairments in reference memory in the Morris water maze while sparing working memory. Synapse 2003; 48:138-148.
27. White S, Laurenzana E, Hendrickson H, Gentry WB, Owens SM. Gestation time-dependent pharmacokinetics of intravenous (+)-methamphetamine in rats. Drug Metab Dispos 2011; 39:1718-1726.
28. North A, Swant J, Salvatore MF, Gamble-george J, Prins P, Butler B, *et al*. Chronic methamphetamine exposure produces a delayed, long-lasting memory deficit. Synapse 2013; 67:245-257.
29. Schröder N, O'Dell SJ, Marshall JF. Neurotoxic methamphetamine regimen severely impairs recognition memory in rats. Synapse 2003; 49:89-96.
30. Hopkins KJ, Wang G-J, Schmued LC. Temporal progression of kainic acid induced neuronal and myelin degeneration in the rat forebrain. Brain Res 2000; 864:69-80.
31. Thrash B, Karuppagounder SS, Uthayathas S, Suppiramaniam V, Dhanasekaran M. Neurotoxic effects of methamphetamine. Neurochem Res 2010; 35:171-179.