

## Swimming exercise attenuates psychological dependence and voluntary methamphetamine consumption in methamphetamine withdrawn rats

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### ARTICLE INFO

**Article type:**  
Original article

**Article history:**

Received: Oct 30, 2015

Accepted: Jan 7, 2016

**Keywords:**

Anxiety  
Craving  
Depression  
Methamphetamine-abstinence  
Obsessive-compulsive disorder  
Swimming

### ABSTRACT

**Objective(s):** This study evaluated the effect of swimming exercise during spontaneous methamphetamine (METH) withdrawal on the anxiety, depression, obsessive-compulsive disorder (OCD) and voluntary METH consumption in METH-dependent rats.

**Materials and Methods:** Male Wistar rats were repeatedly administered with bi-daily doses of METH (2 mg/kg, subcutaneous) over a period of 14 days. Exercised rats were submitted to swimming sessions (45 min/day, five days per week, for 14 days) during spontaneous METH-withdrawal. Then, all animals were tested for the assessment of anxiety by using the elevated plus-maze (EPM), the grooming behaviors (OCD), and depression using forced swimming test (FST) and voluntary METH consumption using a two-bottle choice (TBC) paradigm for the assessment of craving.

**Results:** The results showed that the swimmer METH-withdrawn rats exhibited an increase in EPM open arm time and entries and a reduction of immobility and grooming behaviors compared with the sedentary METH groups. Also, voluntary METH consumption was less in the swimmer METH-withdrawn rats than the sedentary METH groups throughout 5–8 days.

**Conclusion:** This study showed that regular swimming exercise reduced voluntary METH consumption in animal models of craving by reducing anxiety, OCD, and depression in the METH-withdrawn rats. Thus, physical training may be ameliorating some of the withdrawal behavioral consequences of METH.

► *Please cite this article as:*

Damghani F, Bigdeli I, Miladi-Gorji H, Fadaei A. Swimming exercise attenuates psychological dependence and voluntary methamphetamine consumption in methamphetamine-withdrawn rats. *Iran J Basic Med Sci* 2016; 19:594-600.

### Introduction

Chronic methamphetamine (METH) use produces synaptic plasticity in the brain (1), which includes dependence, withdrawal syndrome, drug craving (2-4), cell death via apoptosis (5), and neurotoxicity in dopaminergic, serotonergic, glutamatergic, and noradrenergic pathways (6-8). These changes, in turn, lead to behavioral changes such as anxiety, depression, impulsiveness, obsessive-compulsive disorder (OCD), drug craving and relapse to METH-seeking (9-14). Previous studies have shown a positive correlation between METH-withdrawal signs such as anxiety, depression and OCD with relapse and drug craving (15, 16). Thus, the reversal or prevention of the synaptic alterations induced by METH dependence could be a useful method for the treatment of compulsive drug seeking and craving years after cessation of the drug. We have previously shown that the voluntary exercise diminished the

severity of physical dependence and anxiety behavior in both morphine-dependent and withdrawn rats and voluntary consumption of morphine in the rat pups born from exercising morphine-dependent mothers (17-19). Recent studies have shown that swimming exercise reduces anxiety-depressive-like behavior and HPA activity (20-22) and the level of reactive oxygen species (23), activates opioidergic and serotonergic pathways (24), and increases the level of the brain-derived neurotrophic factor (BDNF) (23) in rodents. Previous studies have also shown that swimming exercise improved performance, muscle strength and endurance (25), METH-induced cerebrovascular toxicity (26) in METH-dependent individuals, and also decreased amphetamine-induced conditioned place preference (CPP) (27), the severity of physical dependence, the anxiety and depressive-like behaviors, and voluntary morphine consumption in

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morphine-dependent rats (28). Also, access to chronic wheel running reduced self-administration of METH (29), extinction and cue-induced reinstatement of methamphetamine-seeking (30) in methamphetamine dependent rats. Thus, a more important question would be whether regular swimming exercise as stress training could blunt the deleterious effects of chronic methamphetamine exposure during withdrawal. Therefore, in the present study, we assessed the effect of regular swimming exercise on the anxiety-depression and obsessive-compulsive-like behaviors and also, voluntary consumption of METH in animal models of craving during spontaneous withdrawal of METH-dependent rats.

## Materials and Methods

### **Animals and induction of methamphetamine dependence**

Adult male Wistar rats (220±30 g) were group housed (n=7-8 rats per cages) in a temperature-controlled room at 22 ± 2 °C with a 12 hr light/dark cycle and had *ad libitum* access to food and water. The experimental procedures were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (were approved by the university ethics committee). Additionally, the number of animals used per each group was the least possible. Methamphetamine hydrochloride (Sigma-Aldrich, M 8750) was dissolved in saline. The rats were made dependent on subcutaneous injections of METH (2 mg/kg, in a volume of 1 mL/kg), twice per day at 12 hr intervals, for 14 days, as described previously (31). Control rats similarly received saline.

### **Regular swimming exercise**

The exercise protocol was conducted as described previously (28, 32) for 14 days. The swimming pool was a blue circular pool (140 cm in diameter and 50 cm high) filled to a 25 cm depth with 31±1°C water. To acclimate to the new environment, all rats were adapted to water before beginning the experiment and took 4 days. First, rats were placed for 5 min into the swimming pool with shallow water only for standing. On the second day, rats spent 5 min in head-high water, able to start swimming. On the third day, they had to swim for 5 min in water that was deep enough. On the fourth day of adaptation, the animals had to swim for 15 min. The adaptation to this experience was started 3 days before the end of METH dependence. On the second day of METH-withdrawal, the rats swam for 30 min. Then, exercising rats were submitted to swimming sessions (45 min/day, five days per week, until the end of the 14-day protocol). The exercise protocol used in this study was a moderate-intensity exercise (24, 32). Non-exercised rats remained sedentary for 14 days.

### **Assessment of locomotor activity patterns**

To control for differences in rat performance in swimming pool, we also recorded each animal's swimming velocity, as index of motor activity for 1 min during the last day of swimming, on day 28 using a tracking system (EthoVision, Noldus, The Netherlands) as described previously (19, 28).

### **Anxiety measurement in the EPM**

To assess the level of anxiety, the rats were individually placed in the center of the EPM with two open (50 cm × 10 cm) and closed (50 cm×10 cm×40 cm) arms, and a central platform (10 cm×10 cm), and allowed to explore the apparatus for 5 min as described previously (18). The following variables were measured during each 5 min test; (1) time spent in open and closed arms as a percentage of the total time spent exploring both the open and closed arms; (2) the number of entries into the open and closed arms. The apparatus was cleaned after each trial with water. The maze was dimly illuminated by a 100 W bulb in a soundproof room along one wall of the room such that the open arms were illuminated from the side.

### **OCD measurement**

In these experiments, animals were individually placed in a Plexiglas box (41 cm length × 33 cm height × 41 cm width) with moderate illumination as described previously (33). After 10 min of habituation period in the test cage, the number and duration of grooming events were recorded for 20 min. Vibration, face and head washing, body grooming, scratching, paw licking, head shaking, and genital grooming were included as components of grooming behavior by a blind observer. The total number and duration of grooming behaviors were considered as an index of obsessive-compulsive disorder.

### **Forced swim test (FST)**

The FST was used to assess the depression. The test was done in a Plexiglas cylinder with a diameter of 20 cm and a height of 45 cm; the cylinder was filled with 25°C water to a height of 30 cm, under normal light conditions. Rats were forced to swim in two trials. The first trial lasted 15 min and was followed 24 hr later by a 5 min test. The following factors were evaluated: swimming or diving time, escaping time (toward the cylinder wall), immobility time (floating in the water, only necessary movements to keep head above water) as described previously (31). On the test day, swimming sessions were videotaped from a lateral angle using a Nikon Camcorder, and behavioral assessments were accomplished by observers blind about the experimental groups. For each rat, water was exchanged. After each session, the rats were immediately removed from the water, dried with a towel and kept in a heated room until completely dry

before being returned to their home cages. More immobility was considered as an index of depressive-like behavior.

### Two-bottle choice (TBC) paradigm

The present study, an 8-day protocol of two-bottle free choice procedure, slightly modified (31, 34) used in animal models of craving, examined the preference or voluntary consumption of oral METH after 2 weeks of withdrawal. One day prior to testing, rats were kept in individual cages. In one bottle, METH was dissolved in water, on days 1–4 of the test, (20 mg/l METH) and on days 5–8 of the test, (40 mg/l METH), and the control bottle contained only water. The rats had access to both bottles for 18 hr to avoid anorexia associated with METH consumption. To minimize effects related to learning, the position of the bottles was changed at the time of daily bottle weighing. The contents of both bottles were measured between 8:00 and 9:00 am daily. Body weights of the rats were measured every day. The daily consumption of METH was measured based on mg/kg/18 hr. The average of METH and water consumption and preference ratios (ml METH solution consumed/total ml consumed from both bottles) were evaluated during a 4-day period.

### The experimental protocol

This experiment examined the effects of swimming exercise on the anxiety-depression and OCD behaviors and voluntary consumption of METH over the spontaneous withdrawal in METH-dependent rats. Rats were divided into four groups ( $n=7-8$  rats per group): saline-sedentary (Sal/No Swim), saline-swimming exercise (Sal/Swim), METH-sedentary (METH/No Swim), and METH-swimming exercise (METH/Swim). In each of the four groups, saline or METH injection was performed for 14 days. Then, rats were maintained in their home cages without any METH injection (drug abstinence) for 14 days. However, the exercising rats were simultaneously exposed to a regular swimming exercise for 14 days. On day 29, rats rested. All animals were tested in the EPM on day 30, followed by evaluation of OCD on days 31, and also the forced swim test (FST) on days 32–33. Rats were tested in different rooms. On days 34–41, METH-withdrawn rats were housed individually in standard cages with two bottles for 8 days to evaluate the voluntary consumption of METH. Swim training was discontinued from day 29 to day 41 (Figure 1).

### Statistical analysis

The data is expressed as the mean  $\pm$  standard error of the mean (SEM). These data were analyzed using two-way analyses of variance (ANOVA), with treatment (saline and METH) and groups (No swim and Swim) fixed factors, and with repeated measures as required. *Post hoc* analyses included Tukey's test. A Student's t-test was used to compare the data from

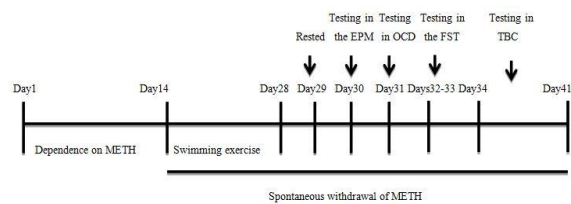


Figure 1. Timeline of experiments

two groups. Statistical differences were considered significant at  $P<0.05$ .

## Results

### Anxiety-like behavior

The results of the EPM are illustrated in Figure 2A. Two-way ANOVA revealed significant effect of group ( $F_{1,26} = 18.7, P<0.001$ ), treatment ( $F_{1,26} = 4.72, P<0.038$ ), and interactions between treatment and group ( $F_{1,26} = 6.3, P<0.018$ ) for open arms entries. Also, two-way ANOVA revealed significant effect of group ( $F_{1,26} = 48.66, P<0.001$ ) and treatment ( $F_{1,26} = 14.6, P<0.001$ ) for open arm time. Comparisons between groups showed that the number of open arm entries ( $P<0.009$ ) and time spent ( $P<0.002$ ) were more in swimmer METH-withdrawn rats compared with the controls (METH/No swim), respectively (Figure 2A). Also, swimmer saline groups spent more time in the open arms compared with the control group ( $P<0.05$ ). The number of open arm entries was less in the sedentary METH-withdrawn rats compared with the control group ( $P<0.04$ ).

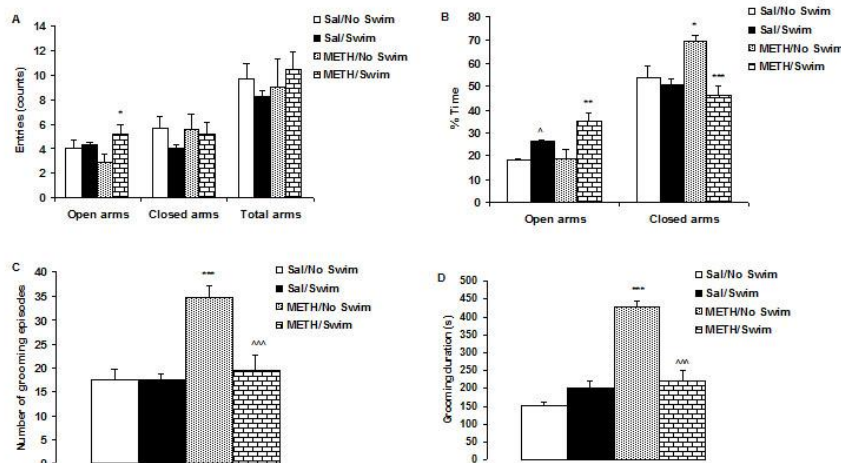
### OCD-like behavior

Two-way ANOVA revealed significant effect of group ( $F_{1,26} = 9.7, P<0.005$ ), ( $F_{1,26} = 15.54, P<0.001$ ), treatment ( $F_{1,26} = 14.18, P<0.001$ ), ( $F_{1,26} = 50.42, P<0.001$ ), and interactions between treatment and group ( $F_{1,26} = 9.14, P<0.006$ ), ( $F_{1,26} = 37.07, P<0.001$ ) for the number and duration of grooming behaviors, respectively. Comparisons between groups showed that the number and duration of grooming behaviors in swimmer METH-withdrawn rats were less than sedentary METH-withdrawn groups (both,  $P<0.001$ ). Also, the number and duration of grooming behaviors in the sedentary METH-withdrawn rats were more than the sedentary control rats ( $P<0.001$ ) (Figures 2 B and C).

### Depression-like behavior

The results of the FST using a two-way ANOVA revealed significant effect of group ( $F_{1,26} = 80.49, P<0.001$ ) and ( $F_{1,26} = 4, P<0.05$ ) for immobility and swimming time, respectively, and significant effect of treatment ( $F_{1,26} = 11.13, P<0.003$ ) for swimming time, and significant interaction between both factors ( $F_{1,26} = 36.21, P<0.001$ ) and ( $F_{1,26} = 4.04, P<0.05$ ) for immobility and swimming time, respectively (Figure 3). Comparisons between groups showed that the duration of immobility and diving





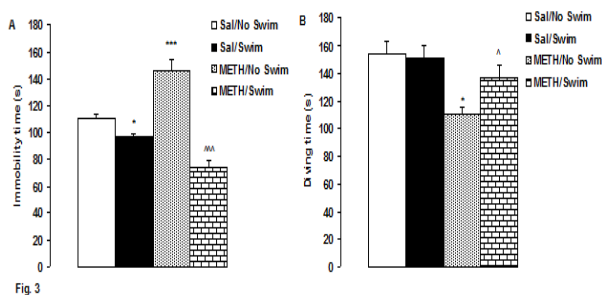
**Figure 2.** Effect of regular swimming exercise on the anxiety and obsessive-compulsive-like behaviors in METH-withdrawn rats. A) The percentage of time spent and entries into the open arms of the EPM. B) The number of grooming behaviors. C) The total duration of grooming behaviors in OCD test. The percentage of time spent in the closed arms was more in the sedentary METH-withdrawn rats than the sedentary control. Swimmer rats spent significantly respectively more and less time in the open and closed arms than the sedentary control rats. The sedentary METH-withdrawn rats exhibited more grooming behaviors than the sedentary control. Whereas, the swimmer METH-withdrawn rats groomed less than those control. In A) \* $P < 0.04$  vs. Sal/No Swim, \*\* $P < 0.009$  vs. METH/No Swim, ^ $P < 0.05$  vs. Sal/No Swim, ^^ $P < 0.002$  vs. METH/No Swim. In B and C) \*\*\* $P < 0.001$  vs. Sal/No Swim, ^^ $P < 0.001$  vs. METH/No Swim.

in swimmer METH-withdrawn rats were less ( $P < 0.001$ ) and more ( $P < 0.031$ ) compared with the controls, respectively. Also the immobility ( $P < 0.001$ ) and diving ( $P < 0.004$ ) times in the sedentary METH-withdrawal rats were more and less, respectively compared with the sedentary saline rats. The immobility time was less in the swimmer saline rats than the control group ( $P < 0.046$ ) (Figure 3A and B).

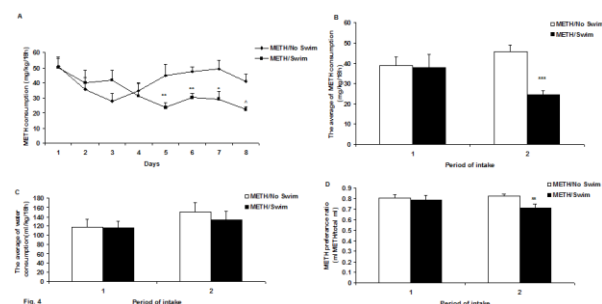
### Assessment of voluntary methamphetamine consumption

Two-way ANOVA with repeated measure (day) for the voluntary methamphetamine consumption during 8 days of intake revealed significant effect of days ( $F_{7, 98} = 4.2$ ,  $P < 0.001$ ), significant effect of groups ( $F_{1, 14} = 6.32$ ,  $P < 0.025$ ) and significant

interaction between day  $\times$  group ( $F_{7, 98} = 3.1$ ,  $P < 0.005$ ). Voluntary consumption of METH during days 5-8 of TBC test decreased significantly in the swimmer METH-withdrawn rats compared to the control group (respectively,  $P < 0.004$ ,  $P < 0.004$ ,  $P < 0.019$ , and  $P < 0.04$ , vs METH/No Swim) (Figure 4A). In general, the average of METH consumption during the second period of testing in METH/swim group was less than METH/No swim group ( $T_{14} = 5.9$ ,  $P < 0.001$ ) (Figure 4B). There were no significant differences in voluntary water intake between groups in both periods ( $P > 0.05$ ), (Figure 4C). In general, METH/No Swim rats exhibited a larger preference ratio compared to METH/Swim rats in the second period ( $T_{14} = 2.87$ ,  $P < 0.012$ ) (Figure 4D).



**Figure 3.** Effect of regular swimming exercise on the immobility (A) and diving (B) times in METH-withdrawn rats using the FST. Time immobility was less in swimmer rats. Also, the immobility and diving times were significantly more and less in the sedentary METH-withdrawn rats than the control group. In A; \* $P < 0.046$ , vs Sal/No swim group. \*\*\* $P < 0.0001$ , vs Sal/No swim group, ^^ $P < 0.001$ , vs METH/No swim. In B; \* $P < 0.004$ , vs Sal/No swim group. ^ $P < 0.031$ , vs METH/No swim



**Figure 4.** Effect of regular swimming exercise on the voluntary consumption of METH in METH-withdrawn rats using a two-bottle choice paradigm. (A) Average of METH consumption during 8 days of intake. (B) The average of METH consumption, (C) water intake and (D) preference ratio in both periods. Swimmer METH-withdrawn rats showed a lower voluntary consumption of METH during days 5-8 of the test. The average of METH consumption was less in the second period in swimmer METH-withdrawn rats compared to METH/No swim. In A, \*\* $P < 0.004$ , \* $P < 0.019$ , ^ $P < 0.04$ , vs METH/No swim. In B; \*\*\* $P < 0.001$ , vs METH/No swim. In D; \*\* $P < 0.012$ , vs METH/No swim

### Locomotor activity patterns

There were no significant differences between groups ( $F_{3, 26}=2.45, P<0.23$ ) in the swimming velocity (locomotor activity) (data not shown).

## Discussion

This study provides novel evidence that a period of 14 days of swimming exercise during spontaneous METH-withdrawal, blunted anxiety, OCD, and depression behaviors. No study with the same nature has been conducted thus far. But, this finding is supported further by previous clinical (35-37) and animal (21, 38, 39) studies showing that voluntary or forced exercise decreases anxiety, OCD, or depression behaviors. It may be due to increasing serotonin (40), noradrenalin (41), dopamine (42) and brain-derived neurotrophic factor (BDNF) (43, 44), and decreased oxidative stress (39) and METH-induced cerebrovascular toxicity (26) following the voluntary or forced exercise. Several studies (11, 14) are in agreement with our findings that METH-withdrawal enhances anxiety, depression, and OCD in parallel with drug craving, due to reduction in brain serotonin (45), serum BDNF levels (46, 47) and a further increase in the level of oxidative stress (26) during METH-withdrawal.

There is a correlation between depression and OCD symptoms and craving for METH (12, 14, 48) and also, the most vulnerable time for relapse to METH is likely to be during days 7-14 of abstinence (14, 48), which was 14 days in our study, for METH-withdrawal period. It should be noted that grooming behavior is very sensitive to numerous stressors and psychostimulant drugs and plays an important role in behavioral adaptation to stress. Grooming activity can be generally increased in different situations with low (comfort) and high stress following exposure to the novel environment such as the light-dark box and EPM (49). Therefore, we observed stress-induced self-grooming along with increased anxiety in METH/No swim rats. This finding is supported further by a previous study (49) showing that rostral grooming (forepaws, face, and head) was significantly higher in more anxious light-exposed rats.

As in our previous study (28), we again have found that swimming exercise is associated with anxiety reduction in the saline group. Also, swimming exercise attenuated depression in METH-withdrawn and saline rats. This finding was interesting because other studies suggest that rats must swim as much as 3 (32) or 4 (20) weeks for reducing depression or anxiety, respectively in stressed rats. While the present study showed that only 14 days of swimming exercise is sufficient to reduce anxiety and depression. In this study, we found no difference in speed swimming (locomotor

activity) between groups. Thus, increased immobility in sedentary rats cannot be due to poor performance in the forced swimming test.

Also, our findings have shown that regular swimming exercise during spontaneous METH-withdrawal decreased the voluntary consumption of METH in withdrawn rats. Also, in the present study, no significant difference between groups was observed in water intake. Therefore, the sedentary METH-withdrawal rats preferred METH to water in TBC model, reflecting an incentive demand in higher doses. Thus, we conclude that swimming exercise decreases the rewarding effects of METH which can be explained at least in part, by the anti-anxiety and antidepressant effect of exercise during METH-withdrawal. No similar study has been found thus far. Although, previous studies have shown that exercise by recruiting a common brain pathway prevents amphetamine-seeking behavior (50), psychostimulant drug relapse (27), cocaine seeking (51, 52), self-administration of METH (29), and extinction and reinstatement of methamphetamine-seeking (30). It seems, the dopaminergic and serotonergic dysfunction, negative emotional states such as anxiety, depression, OCD (12, 53, 54), and a reduction of BDNF (46, 55) during spontaneous METH-withdrawal, may play a significant role in relapse of METH-seeking after protracted abstinence. It may be due to an increased BDNF level (32) and normalization of brain neurotransmitters including serotonin (42) following swimming exercise. In a future study, the neurobiological mechanisms should be explored more.

## Conclusion

This study provides novel evidence that regular swimming exercise during spontaneous METH-withdrawal can decrease the severity of the anxiety-depression and obsessive-compulsive behaviors and also the voluntary consumption of METH in animal models of craving during spontaneous withdrawal of METH. Our findings may have a potential therapeutic application for the treatment of METH-induced psychological dependence and relapse in addicts.

## Acknowledgment

This work was supported by University of Semnan, Semnan, Iran. We would like to thank the Physiology Research Center, School of Medicine, Semnan University of Medical Sciences 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.

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