Diminished food-related motivation in adult rats treated with methamphetamine during adolescence

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Drug use among adolescents continues to be an area of concern because of the possibility of long-lasting physical and mental changes. The aim of this study was to determine whether methamphetamine exposure during adolescence results in long-lasting neurobehavioral alterations in adulthood. Sprague–Dawley rats were injected with methamphetamine (4 mg/kg/day) during postnatal days 28–37. Once rats reached postnatal days 150, they were placed in standard operant chambers, where they were trained to respond to a lever for sucrose pellets, the experimental reinforcement. Methamphetamine exposure during adolescence did not result in a noteworthy impairment in the development of the correct lever touch response in the autoshaped learning test with 4 seconds delayed reinforcement. These rats were also tested for the motivation to obtain sucrose pellets under a progressive ratio schedule of the reinforcement on postnatal days 170. Decreased lever-pressing response was noted in male rats exposed to methamphetamine during adolescence, but not in female rats. These results indicate that methamphetamine exposure during adolescence results in a decrease in the motivation for a natural reinforcer later in adulthood, particularly in male rats. From our data, we suggest that male brains are less capable of facilitating recovery than female brains after methamphetamine-induced perturbation of brain function during the adolescent period. *NeuroReport* 30: 1143–1147 Copyright © 2019 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

Methamphetamine is a central nervous system (CNS) stimulant that has been frequently abused in Asian countries since the 1940s. Abuse of methamphetamine is now widespread throughout western countries. Long-term or high-dose treatment with methamphetamine results in long-lasting damage to monoaminergic neuron terminals in experimental animals and in humans. Methamphetamine treatment reduces the dopamine (DA) and serotonin (5-HT) content in multiple brain regions, inhibits presynaptic DA and 5-HT reuptake, reduces tyrosine and tryptophan hydroxylase activities, and can cause cell apoptosis [1,2]. Cognitive and motor impairments also occur after methamphetamine abuse [3]. In some studies, neurochemical and cognitive deficits in human brains are irreversible and only partially recover after a long period of abstinence [3,4].

Historically, methamphetamine was mostly used by adults. However, adolescent methamphetamine use became common in the early 2000s [5]. Although reports have shown that the overall rate of adolescent methamphetamine use decreased [6], the admission rate for methamphetamine use in the adolescent population rose in the past decade [5]. Common reasons for the start of methamphetamine use include reducing fatigue, sustaining attention, and reducing weight. The literature also describes several additional reasons for methamphetamine use, including the improvement and enjoyment of life [7].

During adolescence, the CNS is still developing, making this an especially vulnerable period during which the developing brain is susceptible to permanent damage. For example, remodeling or refinement occurs in the cortical, limbic, and reward areas of the adolescent brain [8]. In a recent study using MRI, chronic methamphetamine use was found to induce more widespread structural alterations in adolescent than adult brains [9]. DA innervation to the prefrontal cortex (PFC) continues to increase during adolescence [10]. Prefrontal functions closely related to the DA system, including working memory and reward, are still maturing during adolescence [11]. A recent study showed that adolescent methamphetamine use can reduce DA connections in PFC [10].

Most of the current studies on adolescent methamphetamine use subscribe to experimental paradigms that are designed to investigate the short-term effects of either a

high dose or prolonged use of methamphetamine. There is a noticeable lack of research regarding the long-term irreversible effects of the recreational use of methamphetamine during adolescence, underscoring the need for further research.

The aim of the present study was to determine the long-term effects of recreational methamphetamine use restricted to the adolescent period. Adolescent rats were repeatedly exposed to a low dose of methamphetamine, far below the dose that elicits obvious toxicity [12], and their learning ability and the motivation to a natural reinforcer were measured. Various autoshaping paradigms have been applied to study the effects of pharmacological agents on a learning behavior [13], and the progressive ratio (PR) is a useful technique for evaluating changes in the motivation for natural reinforcing stimuli [14]. Behavioral changes in autoshaping paradigms and a PR test are sensitive to the pharmacological alteration of a DA system activity [15,16]. Some previous studies evaluated the effects of adolescent methamphetamine exposure using autoshaping paradigms and a PR technique [17,18]. Therefore, the behavioral effects were evaluated using an autoshaping and a PR paradigm.

Methods
Drug
Methamphetamine hydrochloride was provided by the National Institute of Scientific Investigation, Republic of Korea.

Animals
Male and female Sprague–Dawley rats were supplied by the Division of Laboratory Animal Medicine, Yonsei University College of Medicine. All animal experiments were approved by the Committee for the Care and Use of Laboratory Animals at Yonsei University (Project license number #00062). Animals were cared for as directed by The Guide for Animal Experiments consistent with the NIH Guideline for the Care and Use of Laboratory Animals. Rats were cared for, and the experiments were conducted in a specific pathogen free barrier area under constant temperature (22 ± 0.5°C), humidity (55%), and a 12 hours light: 12 hours dark cycle (light on at 07:00 a.m.).

We mated and bred all experimental animals to minimize stress during their developmental period. Pups were divided by sex and weight, and litters were culled on postnatal day (PND) 2 to groups of five males and five females each. Pups were individually weighed every day and weaned on PND 21. For behavioral studies, we used two pups of each sex (four in total, two male pups and two female pups) in a litter taken from 10 litters. In a litter, one pair of pups (one male pup and one female pup) was assigned to the saline-injected control group and the other pair was assigned to the methamphetamine-injected group. In this way, littermates were evenly distributed between the saline and methamphetamine groups.

Pups were injected with 4 mg/kg/day methamphetamine over the duration of PND 28–37. Their learning ability and food-related motivation were tested using two behavioral tests, an autoshaping with delayed reinforcement [8] and a modified PR schedule of reinforcement [10], beginning from PND 150.

Before the test, the pups were allowed at least 7 days to acclimate to the housing conditions. The body weights of the animals were gradually reduced to 85% of their free-feeding weights and maintained at that level with a restricted feeding schedule. Water was available *ad libitum* throughout the experiment. All testing was performed between 9:30 a.m. and 1 p.m.

Behavioral tests
The animals were tested in standard operant chambers (ENV-007; MED Associates, St. Albans, Vermont, USA), enclosed in sound-attenuating cubicles with built-in C.C.T.V. systems. Behavioral boxes were 30.5 cm high, 29 cm wide, and 24 cm long, with grid floors of stainless steel bars (4.8 mm diameter, spaced 1.6 cm apart). Each box was equipped with a retractable lever (ENV-112B; MED Associates), dispenser (ENV-203; MED Associates) for the delivery of 45 mg food pellets (Improved Formula A, P. J. Noyes, Lancaster, New Hampshire, USA), and a speaker for the introduction of white masking noise. The boxes were controlled and data were collected using the MED-PC program for IBM computers (MED Associates).

During each autoshaping trial, the lever was extended into the chamber for 15 seconds at random intervals, each intermission ranging from 22 to 68 seconds, with an average interval of 45 seconds. One food pellet was delivered 4 seconds after the lever was retracted regardless of whether the rat made a lever touch response.

A daily session consisted of 12 trials and the procedure continued up to the 12th session. The extended lever touch was defined as a correct lever touch, and the maximum number of touches in a session was 12. In this learning task, animals learned to associate food reinforcement with the correct extended lever touch.

Following the autoshaping trials, rats were subjected to the modified PR schedule. For the daily session, the lever was extended for 15 minutes, during which food pellets were delivered contingent upon the completion of a fixed number of lever presses. Each lever press needed 20 g of pressing power. For the first session, rats were automatically adjusted to a progressive fixed ratio (FR) (PFR) 1 (PFR 1; one pellet for one lever press) schedule. A small piece of white cloth (1.5 × 2.5 cm) was attached to the lever to allow the rats to recognize the lever. The rats were maintained on the PFR 1 schedule for 2 days to allow for stabilization of the lever press response. Each day thereafter, the number of lever presses required for the reinforcement was doubled until animals were responding under a PFR 128 (one pellet for 128 lever presses) schedule.

Statistical analysis

The data are presented as the means ± standard error (SE). Differences between means were analyzed with a mixed-measures analysis of variance (ANOVA) in which sessions were included as the within-subjects factor and conditions (saline, methamphetamine) as the between subjects factor. The number of animals used for final statistical analyses was 33; 17 in the saline group (male pups, n = 8; female pups, n = 9) and 16 in the methamphetamine group (male pups, n = 8; female pups, n = 8). Differences were considered significant when $P < 0.05$.

Results

Autoshaping learning test results

In the autoshaping trials, all rats showed a gradually rising learning curve. Therefore, a 4 seconds delay of reinforcement was appropriate. From the first session, rats could touch the lever correctly (4.5 ± 0.4, n = 33), and no differences were found between groups. The difficulty of the task (4 seconds delay of reinforcement) seemed appropriate because almost all rats (30 out of 33) showed perfect correct lever touch behavior during the 12th session. Based on this task, we did not find alterations in the acquisition ability of the autoshaped behavior in rats, in either sex, that had been exposed to methamphetamine during adolescence (Fig. 1a and b).

Progressive ratio test results

Following the acquisition of autoshaping, rats were tested on a modified PR schedule starting at PND 170. The number of lever presses for the reinforcement was gradually increased along with daily PFR doubling, which plateaued at PFR 16, 32, and 64, and then declined. We found sexual dichotomy in this task. The number of lever presses in male rats that had been exposed to methamphetamine during adolescence was lower than that in saline-injected male rats. In male rats, a lower mean PFR response was noted in all PFR sessions. Among them, the lever pressing in first three PFR sessions showed significant treatment effect ($F_{(1,14)}$ = 7.267, $P < 0.05$) and treatment by sessions interaction ($F_{(1,14)}$ = 4.895, $P < 0.05$) based on mixed measures ANOVA (Fig. 2a). Interestingly, there were no significant differences in the mean PFR responses of methamphetamine-treated and saline-treated female rats (Fig. 2b).

Discussion

According to the present study, adolescent methamphetamine exposure at a recreational dose decreased the motivation of adult male rats, as exemplified by the decrease in lever pressing in the PR test. However, adult female rats did not show altered motivation after the same exposure. The autoshaping test results showed that adolescent recreational methamphetamine use does not hinder the ability to learn or acquire lever pulling responses to reinforcement.
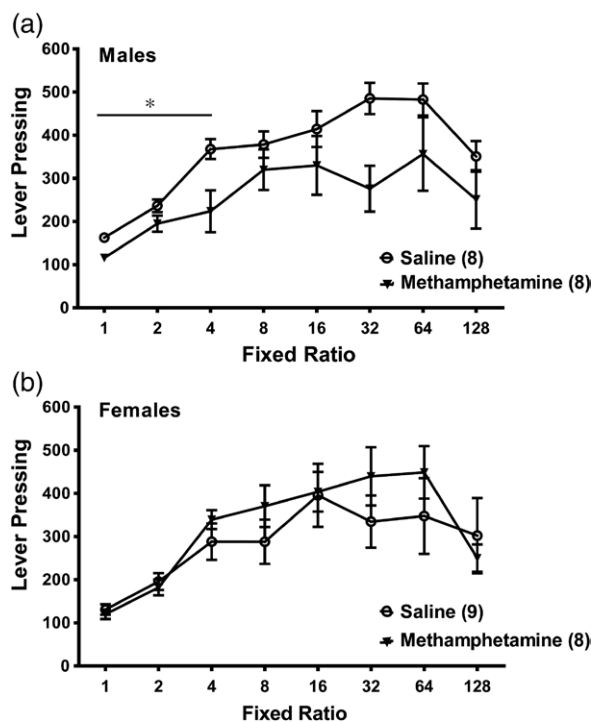
Fig. 1
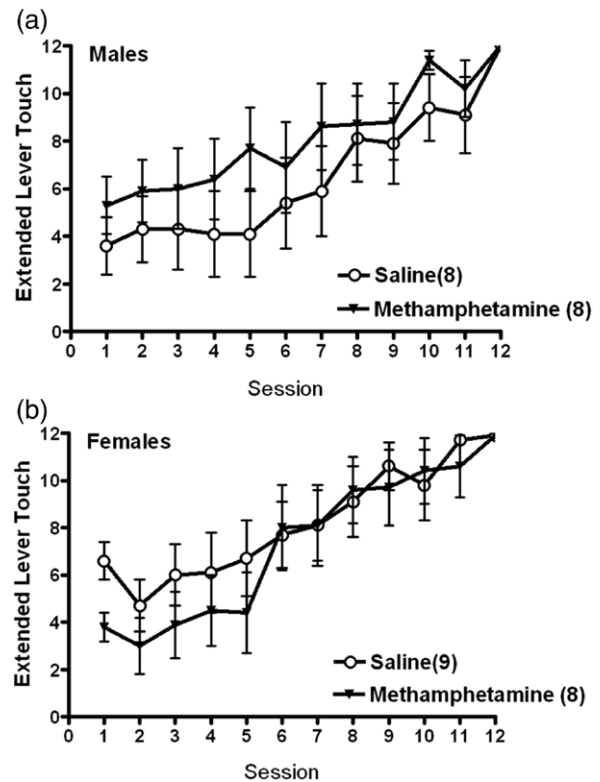
Autoshaped learning test results starting from PND 150 of (a) male and (b) female rats exposed to methamphetamine. Methamphetamine 4 mg/kg/day was intraperitoneally injected on PND 28–37 and 45. The lever was randomly presented 12 times/daily sessions with a 45 seconds interval schedule and retracted when the animal made a lever touch response or after 15 seconds. There was no retardation in the acquisition of autoshaped behavior compared with the control group. PND, postnatal day.

In this study, we aimed to determine the long-term effects of recreational methamphetamine use restricted to the adolescent period. To evaluate the effects on learning ability and motivation we used the animal model because human studies are inevitably confounded with social and environmental factors. In several previous animal studies, a high dose (7.5–10 mg/kg) of methamphetamine was injected to investigate the effects of methamphetamine exposure in animals [19,20]. We injected a lower dose (4 mg/kg) of methamphetamine to mimic human recreational use. It is possible that adolescent methamphetamine use induces long-term, irreversible effects. Some functional domains of the rat brain still develop after early adulthood (PND 60) [10]. Thus, to examine permanent life-time disability rather than a plausible temporary developmental lag, we tested behavioral alterations more than 100 days after the last methamphetamine injection, a much longer period of abstinence than in other studies [20].

The first goal of this study was to determine if recreational adolescent methamphetamine exposure would

Fig. 2

Progressive ratio results starting from PND 170 of (a) male and (b) female rats exposed to methamphetamine. Methamphetamine 4 mg/kg/day was injected intraperitoneally on PND 28–37 and 45. Data are the number of times the lever was pressed during a 15 minutes session. PND, postnatal day.

either attenuate or augment learning ability. Previous studies have shown an inconsistent pattern of learning disturbances at various ages in rats after methamphetamine exposure [12,21]. By using the autoshaping paradigm, we were able to test for alterations in learning. In our study, after exposure to recreational methamphetamine during adolescence, the ability to learn was not affected in adulthood.

Interestingly, we found that lever pressing in the PR test in adult male rats, but not in female rats, was reduced after methamphetamine injection during adolescence. A previous study reported no sex difference in the maintenance of cocaine, heroin, or nicotine when a FR schedule was conducted [22]. However, there were sex differences in the motivation to self-administer cocaine or saccharin in a PR schedule [22,23]. The studies described above-investigated sex differences in the motivation to take a self-administered drug, not a natural reinforcer. However, these studies indirectly suggest that a PR paradigm is better for showing sex differences in motivation.

The results of our study suggest that female brains are more robust than male brains after adolescent methamphetamine exposure. Methamphetamine, a substrate for the DA transporter, primarily affects the dopaminergic system which is crucial for learning and motivation. We postulated that the DA system may be the target of methamphetamine at the recreational dose we used in rats during adolescence because the dopaminergic system is still developing during adolescence [10]. In a primate study, it has also been reported that the recreational use of methamphetamine during adulthood induces DA neurotoxicity [24]. Importantly, during the adolescent period, when most illicit drug use begins in humans, there are huge increases in the production of sex hormones. Therefore, sex hormones in females and males may affect the drug-induced perturbation of the CNS during adolescence. Interestingly, female and male sex hormones have opposite effects on the nervous system. In this regard, estradiol acts as a trophic factor after neurotoxicity, while testicular hormones decrease neuronal survival [25]. The sex hormone difference between females and males could explain why adolescent male brains might have been more susceptible to methamphetamine-induced neurotoxicity in this study.

The most salient finding from our study is that recreational methamphetamine use restricted to the adolescent period decreases motivation during adulthood, especially in male rats, with no effects on learning. The results of the present study suggest that the recreational use of methamphetamine during adolescence is dangerous because it can permanently influence motivation, especially in males.

Acknowledgements

Conflicts of interest
There are no conflicts of interest.

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