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Research report

Gender differences in the effect of adult amphetamine on cognitive functions of rats prenatally exposed to methamphetamine

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HIGHLIGHTS

- Prenatal methamphetamine exposure does not have any effect on the learning ability of female and male adult rats.
- Amphetamine treatment in adulthood affects the cognitive functions of adult rats in a sex-specific manner.
- Females are more sensitive to the effect of psychostimulants than males.
- Prenatal MA treatment does not have any sensitizing effect on the AMP application in adulthood.
- Prenatal MA-exposure and adult AMP-treatment increased the speed of swimming in female rats.

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ABSTRACT

Psychostimulants have been shown to affect brain regions involved in the process of learning and memory consolidation. It has been shown that females are more sensitive to the effects of drugs than males. The aim of our study was to investigate how prenatal methamphetamine (MA) exposure and application of amphetamine (AMP) in adulthood would affect spatial learning of adult female and male rats. Mothers of the tested offspring were exposed to injections of MA (5 mg/kg) or saline (SA) throughout the entire gestation period. Cognitive functions of adult rats were evaluated in the Morris Water Maze (MWM) tests. Adult offspring were injected daily with AMP (5 mg/kg) or SA through the period of MWM testing. Our data from the MWM tests demonstrates the following. Prenatal MA exposure did not change the learning ability of adult male and female rats. However, AMP administration to adult animals affected cognitive function in terms of exacerbation of spatial learning (increasing the latency to reach the hidden platform, the distance traveled and the search error) only in female subjects. There were sex differences in the speed of swimming. Prenatal MA exposure and adult AMP treatment increased the speed of swimming in female groups greater than in males. Overall, the male subjects showed a better learning ability than females. Thus, our results indicate that the adult AMP treatment affects the cognitive function and behavior of rats in a sex-specific manner, regardless of prenatal exposure.

1. Introduction

Psychostimulants such as amphetamine (AMP) and its synthetic derivative methamphetamine (MA) induce in humans feelings of pleasure and happiness and suppress negative affective states such as anxiety and depression [1]. MA is one of the most widely abused psychostimulant drugs worldwide [2], including the Czech Republic [3]. Additionally, almost half of women of a reproductive age, who take drugs, replace another drug with MA during pregnancy [2].

It is known that MA crosses placental barriers easily and therefore it might affect the brain development of a fetus [4,5]. However, the research on the long-term effect of prenatal MA exposure remains limited. According to preclinical studies, the administration of MA at a dose of 5 or 10 mg/kg to pregnant mice or rats induces similar fetal brain drug concentrations and similar behavioral changes to those found in humans [6,7]. Therefore, this dose regimen is used for an animal model of *in utero* MA exposure.

Abbreviations: MA, methamphetamine; AMP, amphetamine; SA, saline; MWM, Morris Water Maze; GD, gestation day; PD, postnatal day; P/E, proestrus/estrus; D, diestrus; s.c., subcutaneously; NE quadrant, North-East quadrant.

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Our previous studies showed that administration of MA (5 mg/kg) during gestation affects the sensorimotor development of pups during the preweaning period [8,9], increases seizure susceptibility in both sexes [10] and alters pain sensitivity in a sex-specific manner [11]. Psychostimulant drugs activate molecular signalization in dopaminergic and glutamatergic pathways of the central nervous system, which are greatly involved in reward circuits, affective state, sexual behavior, and also in control of motor function and cognition [12,13]. Cognitive changes in adulthood are expected after intrauterine exposure to MA, because it was found, that this exposure results in the reduction of the volume of the rats' hippocampus, a brain structure involved in the process of learning and memory formation [14]. The data of the studies investigating the effect of prenatal MA on cognition in adulthood are, however, inconsistent. Higher doses (up to 20 mg/kg) of prenatal MA lead to long-term behavioral effects on spatial learning [6,15]. On the other hand, a lower dose of prenatal MA (5 mg/kg) did not result in any impairments of spatial learning tested on male [16] and female rats [17]. Thus, the first goal of the present study was to examine the effect of prenatal MA exposure on cognitive function of rats tested in adulthood.

Suzuki et al. [18] have investigated a phenomenon defined as behavioral sensitization or reverse tolerance, when repeated drug administration induces a higher sensitivity to the same drug in rodents. The long-term sensitizing effect of prenatal MA exposure to MA treatment in adulthood was already demonstrated in our previous studies [19–22]. We demonstrated the sensitizing effect on the changes of spontaneous locomotor activity and exploratory behavior in male rats [23,24]. Moreover, we have shown that increased locomotion and vertical exploratory activity in prenatally MA-exposed rats after the challenge dose of MA is related to an increased level of dopamine in the nucleus accumbens [21]. Additionally, there is growing evidence that the exposure to one drug leads to general sensitivity to other drugs. This effect of cross-sensitization was demonstrated between related drugs [25–27], as well as between unrelated drugs [28–30]. The issue of cross-sensitization, when prenatal MA exposure increases the sensitivity to AMP received in adulthood, has already been studied in our laboratory. Our studies demonstrated changed drug-seeking behavior [20,31], locomotion and exploratory activities [32]. Therefore, the second goal of the present study was focused on the effect of AMP treatment in adulthood on cognition of adult female and male rats and a possible effect of cross-sensitization induced by prenatal MA.

There is still little known about gender differences related to the effects of psychostimulants. Although abuse of illicit drugs occurs more often in men than in women, there is an evidence-based on animal and clinical studies that females are more vulnerable to the effect of drugs than males [33]. Several studies, including our own demonstrated that females are more sensitive than males in the test of spontaneous locomotion following treatment of D-amphetamine [34–36], cocaine [37], MA [38], MDMA (3,4-methylendioxy-*N*-methylamfetamin) [39] and cannabinoids [40]. Other studies also showed increased motivation for self-administration of MA and cocaine in females than in males [41,42]. Additionally, females seem to be more sensitive to the prenatal effect of MA as well as MDMA [24,32,43]. Some studies suggest that the sex-related behavioral differences in response to psychostimulants are associated with differences in serotoninergic and dopaminergic systems [44,45]. Moreover, females show a greater behavioral response to drugs in the estrus, when the striatal dopaminergic system is stimulated by gonadal hormones [35,46]. As far as cognition is concerned studies demonstrated that MA treatment in adulthood impairs spatial learning of males rats tested in the Morris Water Maze test [47,48] and it seems that the same treatment similarly impairs learning of adult female rats as well [17]. Therefore the last, third goal of the present study was to examine the possible sex differences in cognitive changes as a result of application of AMP in adulthood after prenatal MA exposure.

To summarize, the present study evaluated the effect of prenatal MA exposure, adult AMP treatment on cognition, and the possible sensitizing effect of prenatal MA exposure to adult AMP treatment. The spatial learning was tested in adult male and female rats in the battery of Morris water maze (MWM) tests.

2. Materials and methods

The study was performed on freely moving animals housed under standard conditions.

All procedures were performed in accordance with the Ethical Guidelines of the Third Faculty of Medicine, Charles University in Prague, Czech Republic and reviewed and approved by the Institutional Animal Care and Use Committee and, in agreement with the Czech Government Requirements under the Policy of Humans Care of Laboratory Animals (No. 246/1992) and with the subsequent regulations of the Ministry of Agriculture of the Czech Republic.

2.1. Animals and prenatal drug administration

Adult female Wistar rats were purchased from Anlab (Prague, the Czech Republic, breading Charles River Laboratories International, Inc.). The Animals were left undisturbed to accommodate for a week. After the acclimatization period females were smeared with vaginal lavage to determine the phase of their estrous cycle.

Females in the estrous phase of the cycle were housed overnight with sexually mature males. There were always one female and one male per cage. The following morning the females were smeared for the presence of sperm and returned to their home cages. The day when sperm were detected was designated as day 1 of gestation (GD 1). The total number of pregnant females was 20. Animals were randomly assigned to two treatment groups: half of the females were injected subcutaneously (*s.c.*) with MA (5 mg/kg) and the other half with saline (SA 1 ml/kg) [49]. Females were injected throughout the entire gestation period (GD 1–22). The dose 5 mg/kg of MA was chosen because it induces similar fetal brain drug concentrations and similar behavioral changes to those found in humans [6]. The day of delivery was counted as postnatal day (PD) 0. All litters were adjusted to twelve. To avoid litter bias pups were cross-fostered so that each mother had six MA-treated pups (3 males and 3 females) and six SA-treated pups (3 males and 3 females). There were no differences in the weights of the pups from prenatally MA- and SA-exposed groups after birth and during the lactation period. The animals were weaned on PD 21, housed in a group of 5 or 4 males, respectively, and left undisturbed until adulthood. Always one prenatally SA-exposed and one prenatally MA-exposed female and male, respectively, per group were used from each litter to avoid litter effects. The rest of the animals were used in other studies.

2.2. Experimental groups

In all, 40 adult female rats and 32 adult male rats (PD 60–90) were tested for changes in cognitive functions in the MWM over a 12-day period. Each animal (either female or male) was handled while measuring the weight, so we avoided the impact of the handling stress. To determine the effect of AMP in adulthood animals from each prenatal treatment (females: MA, $n = 10$; SA, $n = 10$; males: MA, $n = 8$; SA = 8) received AMP at a dose of 5 mg/kg *s.c.* daily during the 12 days of the MWM test, on the days of the trials animals received AMP after the last trial. AMP was used at this dose based on the work of Timar et al. and our previous studies [11,32,50].