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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].

Table 1
Assignment of the animals to the individual female and male groups according to the type of prenatal treatment versus treatment in adulthood. Total number of male rats used in experiment was 72, individual group of females accounted 10 animals, males – 8 animals. Adult treatment started on the day of the beginning of the MWM tests and continues for subsequent 12 days. MA – methamphetamine, SA – isotonic saline solution, AMP – amphetamine.

		Prenatal exposure, *females*		Prenatal exposure, *males*	
		MA (5 mg/kg/day)	SA (1 ml/kg/day)	MA (5 mg/kg/day)	SA (1 ml/kg/day)
Adult treatment (12 days)	AMP (5 mg/kg) SA (1 ml/kg)	MA/AMP, $n = 10$ MA/SA, $n = 10$	SA/AMP, $n = 10$ SA/SA, $n = 10$	MA/AMP, $n = 8$ SA/MA, $n = 8$	SA/AMP, $n = 8$ SA/SA, $n = 8$
Total number of animals	$n = 72$				

The other groups (females: MA, $n = 10$; SA, $n = 10$; males: MA, $n = 8$; SA, $n = 8$) were exposed to an injection of SA (1 mg/kg) following protocol of AMP injections. The AMP and SA injections continued even during the days when no tests were performed. The timing of injections was the same as in the days of testing.

Therefore, the following experimental groups for each sex were formed based on Prenatal exposure/Adult treatment: (1) SA/SA, (2) SA/AMP, (3) MA/SA, (4) MA/AMP (see Table 1).

2.3. The MWM testing

The battery of tests in the MWM was used in this study to analyze changes in cognition: the Place Navigation Test, the Probe Test and the Memory Recall Test. Before each experiment the animals were left to acclimatize to the laboratory conditions, in which the experiments were performed.

The water maze consisted of a blue circular tank (2 m in diameter), filled with water ($22.5 \pm 2.5\,^{\circ}$C). The maze was divided into 4 quadrants in respect to start positions (north – N, south – S, east – E and west – W). A transparent circular platform was placed into the NE quadrant of the maze, 1 cm below the water surface. The maze was surrounded by various extra-maze cues on the walls. The trials were tracked using a video-tracking system EthoVision XT6 (Noldus Information Technology, Netherlands).

2.3.1. The Place Navigation Test

This test was aimed on the evaluation of animals' spatial learning ability. During 6 days animals were trained to locate the hidden platform within the limit of 60 s. If the animal did not reach the platform within the time limit, it was gently guided by the experimenter to the platform. Eight trials were performed per day. The position of the platform was the same throughout the period of learning. After each trial, the animal remained on the platform for 30 s prior to the next trial to have a chance to orientate and learn its position in the room. After the trials on each day of the experiment, the animal received the *s.c.* injection of AMP or SA as appropriate and was returned to the home cage.

The following parameters were evaluated with use of EthoVision program: the latency of platform acquisition [s], distance traveled (the length of the swim-path) [cm], search error ("cumulative distance" from platform throughout a trial) [cm] and the speed of swimming ("velocity") [cm/s].

2.3.2. The Probe Test

The Probe Test was used to clarify animals search strategy and memory retention after the position of the platform had been learned by an animal. During the Probe Test, which was conducted on the 8th day, the platform was removed, and the animal was left to swim in the maze for 60 s. The start position in this test was north (N) for each animal. The following parameters were recorded: distance traveled [cm], the speed of swimming [cm/s], number of crossings of the former placement of the platform, number of crossings of the quadrant where the platform was located and the duration of presence in the quadrant where the platform was located [s], number of crossings of the opposite quadrant and the duration of presence in the opposite quadrant [s].

2.3.3. The Memory Recall Test

Long-term memory recall, in another words its retrieval, was evaluated in this test. The test was performed on the 12th day. Every animal was subjected to eight trials to find the platform located at the same position as during the Place Navigation Test within 60 s. The same parameters were analyzed as in the Place Navigation Test: latency of platform acquisition [s], distance traveled [cm], search error [cm], and the speed of swimming [cm/s].

2.4. Analyzing of the search strategies

Search strategies (swimming pathways) of individual animal were manually analyzed after track acquisition on days 1, 3, 6 and 12. The following search strategies were assigned [16]: (1) thigmotaxis – predominant swimming along the wall of the pool; (2) random search – swimming over the entire area of the pool in straight swims or in wide circular swims; (3) scanning – swimming over the central area of the pool; (4) search in incorrect quadrants – direct swim to an incorrect quadrant of the pool followed by loops and turns there; (5) search in the correct quadrant – direct swim to the goal quadrant of the pool followed by loops and turns there, (6) spatial search – direct swim path to the platform. Analysis of search strategies was aimed at a better understanding of differences in the process of learning in animals exposed to either MA prenatally or to AMP in adulthood.

2.5. Estrous cycle determination

Every day prior to testing each female was smeared with vaginal lavage. The smear was examined by light microscopy. According to Turner and Bagnara [51] two phases of the estrous cycle were recognized in the present study: proestrus/estrus (P/E) with predominance of large nucleated and some cornified epithelial cells; diestrus (D) with predominance of leukocytes. The estrous cycle of a female rat lasts for 4–5 days. We did not find any differences in the estrous cycle between prenatally MA-exposed and prenatally SA-exposed females.

2.6. Statistical analysis

The data from the Place Navigation Test were analyzed by an ANOVA where between factors were: Prenatal exposure × Treatment in adulthood × Sex and within factors were: days × trials/day. The Probe Test data were analyzed by an ANOVA where between factors were: Prenatal exposure × Treatment in adulthood × Sex/Estrous cycle. An ANOVA, with between factors: Prenatal exposure × Treatment in adulthood × Sex/Estrous cycle and within factors: trials, was used to analyze the data from the Memory Recall Test. The Bonferroni test was used for *post-hoc* comparisons. The χ^2 test was used to analyze the pattern of the search

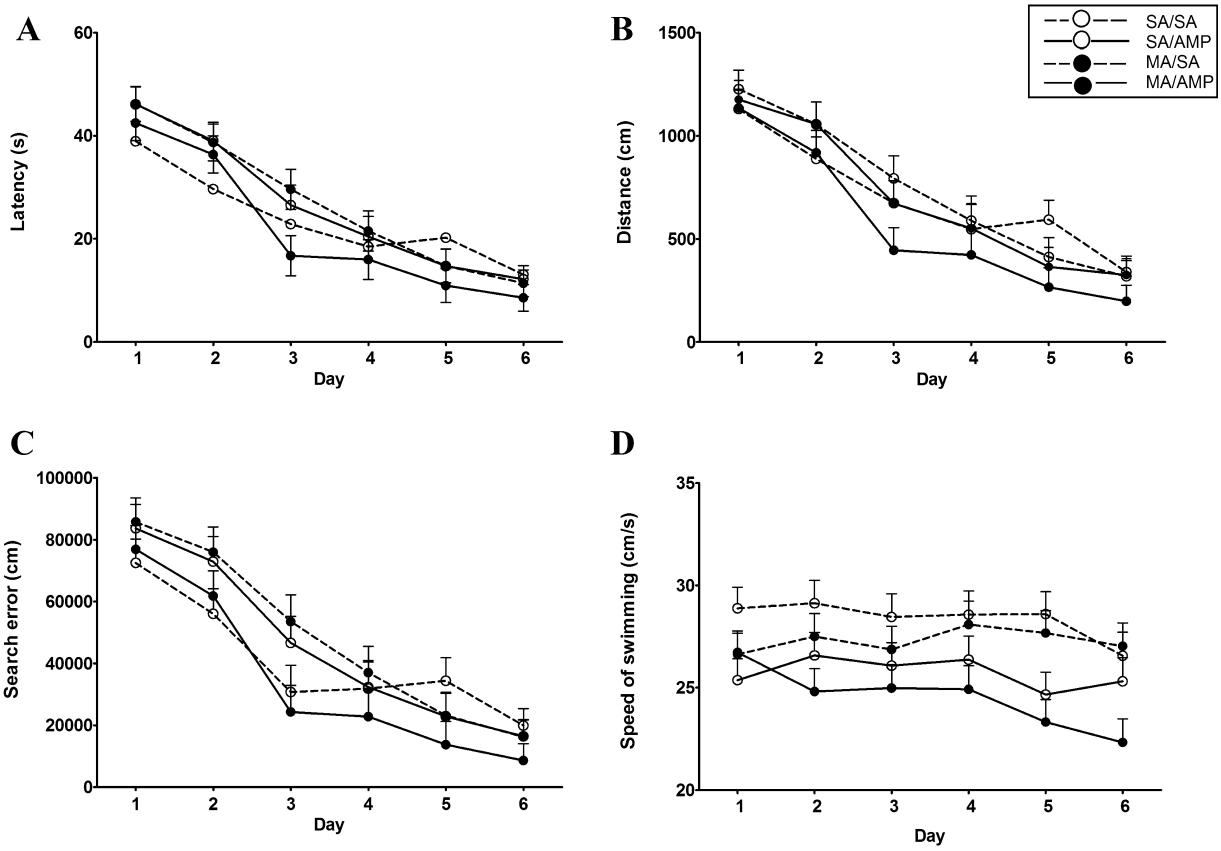

Fig. 1. Effect of prenatal MA exposure and adult AMP treatment on performance of male rats in the Place Navigation Test. (A) Latency of platform acquisition. (B) Distance traveled. (C) Search error. (D) Speed of swimming. Values are presented as mean + SEM, $n = 8$. Figure legend means – Prenatal exposure/Adult treatment: prenatal saline/adult saline; prenatal saline/adult amphetamine; prenatal methamphetamine/adult saline; prenatal methamphetamine/adult amphetamine.

strategies. In all tests, the differences were considered significant if $p < 0.05$.

3. Results

3.1. The Place Navigation Test

Prenatal MA exposure did not induce changes in learning abilities during the Place Navigation Test, nor did it induce changes in the sensitivity to adult AMP treatment (Figs. 1 and 2). Only the speed of swimming during the Place Navigation Test [$F(1, 64) = 5.76$; $p < 0.05$] was increased in prenatally MA-exposed females relative to prenatally SA-exposed females ($p < 0.05$) and relative to prenatally MA-exposed males ($p < 0.01$).

Adult AMP treatment increased the distance traveled [$F(1, 64) = 4.06$; $p < 0.05$], search error [$F(1, 64) = 3.80$; $p < 0.05$] and latency to reach the hidden platform [$F(1, 64) = 6.24$; $p < 0.05$] relative to SA-treated rats. This effect of AMP was, however, sex-specific (Figs. 1 and 2). The distance traveled [$F(1, 64) = 12.79$; $p < 0.001$], search error [$F(1, 64) = 9.09$; $p < 0.01$] and the latency to reach the hidden platform [$F(1, 64) = 10.11$; $p < 0.01$] was increased in AMP-treated females relative to SA-treated females as well as relative to AMP-treated males (Fig. 3A–C). The speed of swimming (see Fig. 3D) was lower in AMP-treated males relative to all the other groups (amphetamine females and saline males or females) [$F(1, 64) = 8.66$; $p < 0.01$]. Moreover, as the time of Place Navigation Test progressed, males displayed faster learning than females, with the effect being mostly apparent in AMP-treated animals (Fig. 3A–C). The speed of swimming did not change during the Place Navigation Test (Fig. 3D).

3.2. The Probe Test

Neither the distance traveled nor the speed of swimming during the Probe Test was affected by prenatal MA exposure, adult AMP treatment or sex. Also the number of crossings of the former placement of the platform and the duration of presence in the quadrant where the platform was located [s] were not affected by any of the factors. However, there was a sex difference in the number of crossings of the quadrant where the platform was located [$F(1, 64) = 7.63$; $p < 0.01$] showing an increase in males compared to females.

In addition, the duration as well as the number of crossings of the opposite quadrant was affected by adult AMP treatment {duration [$F(1, 64) = 66.73$; $p < 0.0001$]; number of crossings [$F(1, 64) = 108.28$; $p < 0.0001$]} and sex {duration [$F(1, 64) = 12.45$; $p < 0.001$]; number of crossings [$F(1, 64) = 14.54$; $p < 0.001$]}. The Bonferroni *post-hoc* test showed that males treated with SA in adulthood swam more often and spent a longer time swimming across the opposite quadrant than males treated in adulthood with AMP or by females treated in adulthood by both, SA or AMP. Moreover, the number of crossings as well as the duration of swimming across the opposite quadrant was increased in females treated in adulthood with SA compared to females treated with AMP (Table 2).

On the day when the Probe Test was performed, all the females in one of the groups were in the same stage of the estrous cycle and therefore it was not possible to run the statistic.

3.3. The Memory Recall Test

Prenatal MA exposure did not affect any of the measures analyzed in the Memory Recall Test, neither induced changes in the sensitivity to adult AMP treatment.

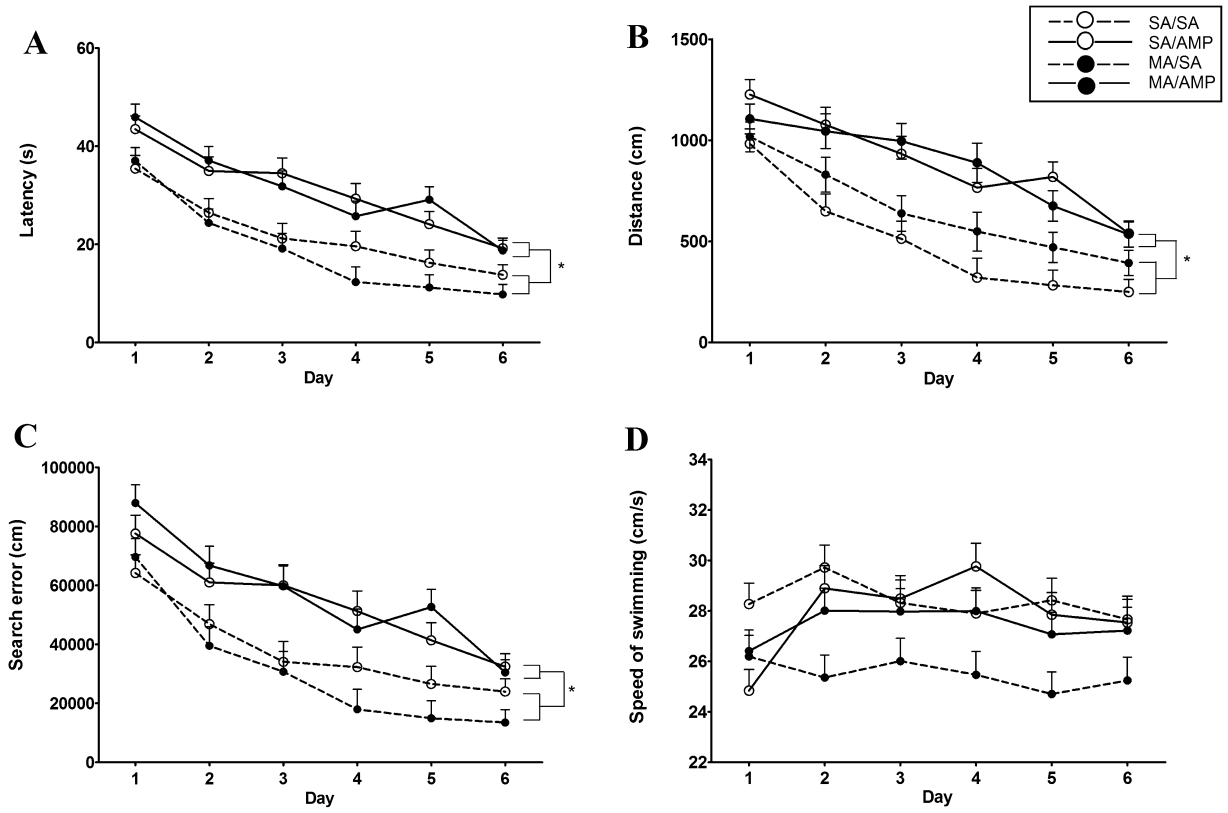

Fig. 2. Effect of prenatal MA exposure and adult AMP treatment on performance of female rats in the Place Navigation Test. (A) Latency of platform acquisition – main effect of adult treatment, $^{*}p < 0.05$. (B) Distance traveled – main effect of adult treatment, $^{*}p < 0.05$. (C) Search error – main effect of adult treatment, $^{*}p < 0.01$. (D) Speed of swimming. Values are presented as mean + SEM, $n = 10$. Figure legend means – Prenatal exposure/Adult treatment: SA/SA = prenatal saline/adult saline; SA/AMP = prenatal saline/adult amphetamine; MA/SA = prenatal methamphetamine/adult saline; MA/AMP = prenatal methamphetamine/adult amphetamine.

There were main effects of adult treatment and sex/estrous cycle in the Memory Recall Test. As shown in Fig. 4, adult AMP treatment increased the distance traveled [$F(1, 60) = 8.10$; $p < 0.01$], search error [$F(1, 60) = 4.48$; $p < 0.05$], and the latency to reach the hidden platform [$F(1, 60) = 9.36$; $p < 0.01$] relative to SA-treated animals. In addition, the distance traveled [$F(2, 60) = 3.33$; $p < 0.05$], the search error [$F(2, 60) = 3.67$; $p < 0.05$], as well as the latency to reach the hidden platform [$F(2, 60) = 3.55$; $p < 0.05$], was shorter in males compared to females.

In addition, there was an interaction between adult treatment and sex/estrous cycle in all of the measures in the Memory Recall Test. Specifically, AMP treatment in adulthood increased the distance traveled, the search error and the latency to reach the hidden platform in females in relation to males treated with AMP as well as in relation to females in the same phase of the estrous cycle treated with SA in adulthood (Fig. 4A–C).

Regarding the speed of swimming (Fig. 4D) AMP treatment in adulthood increased the speed of swimming in females in diestrus ($p < 0.05$), while it decreased it in males ($p < 0.01$). Moreover, AMP-treated diestrous females swam faster than AMP-treated females in proestrus/estrus ($p < 0.05$) or AMP-treated male rats ($p < 0.01$). The speed of swimming did not change within the eight trials of the Memory Recall Test.

Table 2
The effect of prenatal MA exposure, adult AMP treatment, and sex on the parameters analyzed in the Probe Test..

	Prenatal treatment	Treatment in adulthood	Distance [cm]	Speed of swimming [cm/s]	Ex-platform	Quadrant		Opposite quadrant	
					Number of crossing	Duration [s]	Number of crossing[#]	Duration [s]	Number of crossing
Males	SA	SA	1895.80 ± 108.41	31.61 ± 1.81	0.90 ± 0.42	18.27 ± 2.94	6.63 ± 1.04	41.21 ± 4.53[+]	5.5 ± 0.47[+]
		AMP	1617.66 ± 108.41	26.96 ± 1.81	1.50 ± 0.42	23.97 ± 2.94	8.25 ± 1.04	0.61 ± 4.53[*]	1.5 ± 0.47[*]
	MA	SA	1728.76 ± 108.41	28.83 ± 1.81	1.21 ± 0.42	18.23 ± 2.94	7.75 ± 1.04	40.70 ± 4.53[+]	5.75 ± 0.47[+]
		AMP	1704.56 ± 108.41	28.41 ± 1.81	1.75 ± 0.42	21.14 ± 2.94	8.75 ± 1.04	0.55 ± 4.53[*]	1.75 ± 0.47[*]
Females	SA	SA	1552.61 ± 96.96	25.88 ± 1.62	1.20 ± 0.38	17.42 ± 2.63	6.1 ± 0.93	14.31 ± 4.05	4 ± 0.42
		AMP	1599.57 ± 96.96	26.66 ± 1.62	0.90 ± 0.38	19.50 ± 2.63	6.00 ± 0.93	10.40 ± 4.05	0.9 ± 0.42
	MA	SA	1789.46 ± 96.96	29.83 ± 1.62	0.50 ± 0.38	14.68 ± 2.63	5.20 ± 0.93	15.05 ± 4.05	3.4 ± 0.42
		AMP	1739.05 ± 96.96	28.98 ± 1.62	1.40 ± 0.38	15.86 ± 2.63	6.40 ± 0.93	0.39 ± 4.05	1.4 ± 0.42

Values are presented as mean per group ± SEM (females: $n = 10$, males: $n = 8$).
[#] $p < 0.01$, main effect of the sex, males > females.
[*] $p < 0.0001$, males with adult AMP < males with adult SA.
[+] $p < 0.01$, males with adult SA > females with adult SA or AMP.

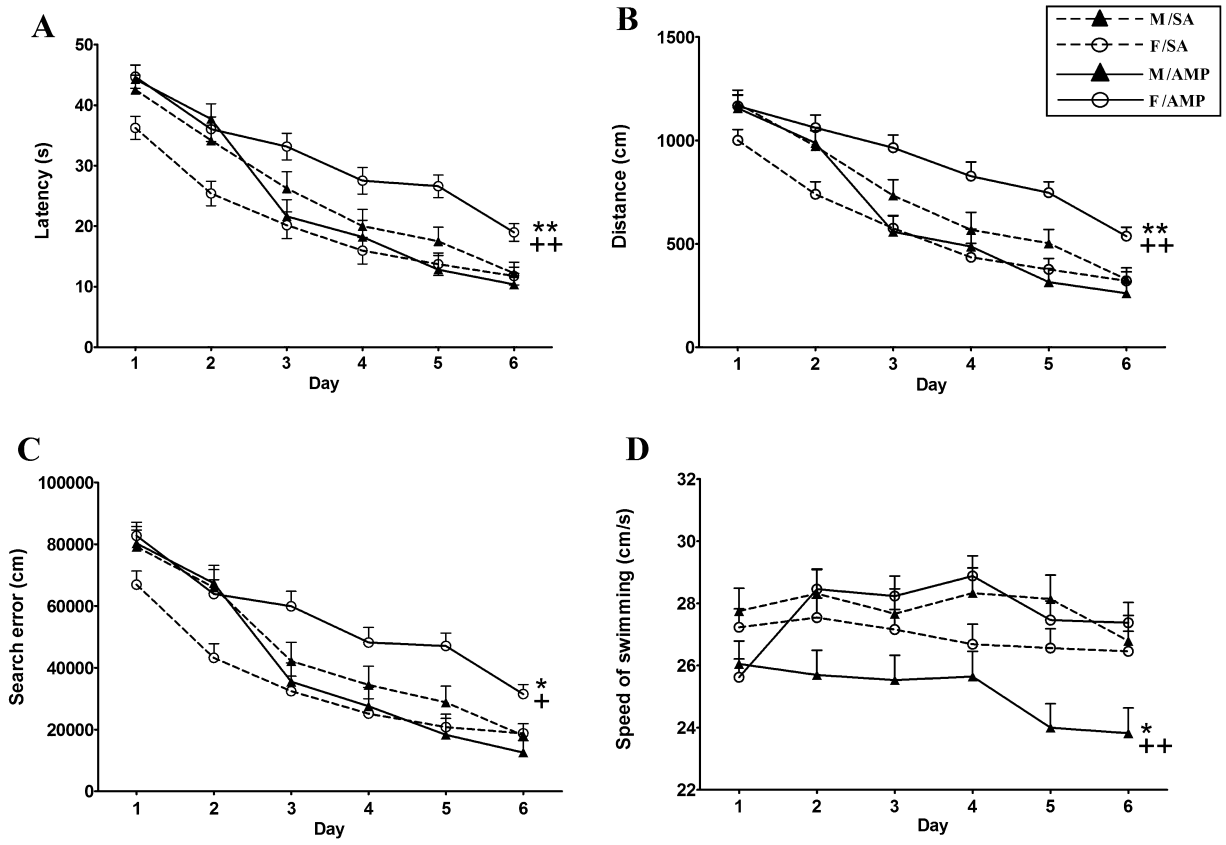

Fig. 3. Effect of adult AMP treatment on performance in respect of sex differences. (A) Latency of platform acquisition. $^{**}p < 0.0001$ AMP females > SA females, $^{++}p < 0.01$ AMP females > AMP males. (B) Distance traveled. $^{**}p < 0.0001$ AMP females > SA females, $^{++}p < 0.01$ AMP females > AMP males. (C) Search error. $^{*}p < 0.001$ AMP females > SA females, $^{+}p < 0.05$ AMP females > AMP males. (D) Speed of swimming. $^{*}p < 0.01$ AMP males < SA males, $^{++}p < 0.01$ AMP males < AMP or SA females. Values are presented as mean + SEM, males: $n = 8$, females: $n = 10$. Figure legend means – sex/adult treatment: M/SA = males with adult saline treatment; F/SA = females with adult saline treatment: M/AMP = males with adult amphetamine treatment: F/AMP = females with adult amphetamine treatment.

3.4. Search strategies

The strategies of swimming to find the hidden platform differed based on the prenatal or adult drug exposure as well as based on the sex. They changed as the time of the experiment was progressing.

On Day 1 of the experiment, mostly sex differences were demonstrated. While males used more thigmotaxis than females {males 32.8%, females 22.8% [$\chi^2 = 24.94$; $p < 0.001$]}, females swam more in the quadrant of the hidden platform than males {males 2.3%, females 9.1% [$\chi^2 = 15.41$; $p < 0.05$]}.

On Day 3 of the experiment, differences induced by adult drug treatment were apparent in females, but not in male rats. Specifically, while females injected with AMP in adulthood used thigmotaxis {MA/AMP females 8.8%, SA/AMP females 16.3% [$\chi^2 = 41.78$; $p < 0.0001$]} or a random strategy {MA/AMP females 26.3%, SA/AMP females 12.5% [$\chi^2 = 44.75$; $p < 0.0001$]}, females injected in adulthood with SA, used scanning {MA/SA females 30%, SA/SA females 32.5% [$\chi^2 = 22.77$; $p < 0.05$]}.

On Day 6 of the experiment, females swam more in the opposite quadrant than males {males 16.8%, females 23.8% [$\chi^2 = 27.37$; $p < 0.001$]}; moreover, males injected in adulthood with AMP swam less in the opposite quadrant than males injected in adulthood with SA {MA/AMP males 7.8%, SA/AMP males 7.8%, MA/SA males 25%, SA/SA males 26.6%}. On the other hand, males more often used a direct strategy than females {males 67.8%, females 55.3% [$\chi^2 = 33.26$; $p < 0.0001$]}.

On Day 12 of the experiment, regardless of the drug treatment (prenatal or adult), males more often used a direct strategy {males 68.8%, females 56.6% [$\chi^2 = 57.83$; $p < 0.001$]} or swimming in the quadrant with the hidden platform than females {males 7.0%, females 4.7% [$\chi^2 = 14.99$; $p < 0.05$]}. On the other hand, the scanning strategy was more often used by females than by males {males 10.9%, females 23.1% [$\chi^2 = 33.84$; $p < 0.0001$]}. Moreover, in females there was a difference in random strategy induced by AMP injection in adulthood: females with AMP used a random strategy more often than females {MA/AMP females 6.3%, SA/AMP females 5%, MA/SA females 1.3%, SA/SA females 0%, or than males regardless of the treatment {0% [$\chi^2 = 20.6$; $p < 0.01$]}

4. Discussion

The main goal of the present study was to increase the understanding of the sex differences in the effect of psychostimulant drugs administrated prenatally (MA) and in adulthood (AMP) and the possible cross-sensitization between these drugs. The effect of psychostimulant drugs was used to analyze allocentric learning, a type of hippocampally dependent spatial navigation that relies on distant cues [52]. The results did not show any effect of prenatal MA exposure on spatial abilities of male or female rats, while AMP treatment in adulthood induced changes in learning and memory in a sex-specific manner. Learning and memory of female rats seemed to be more affected by adult AMP treatment. Some sex differences were also found in the search strategies of swimming used in the test. Moreover, prenatal MA exposure and AMP treatment in adulthood increased the speed of swimming in females compared with males.

The first goal was to examine the effect of prenatal MA exposure on the learning abilities of male and female rats. The prenatal MA

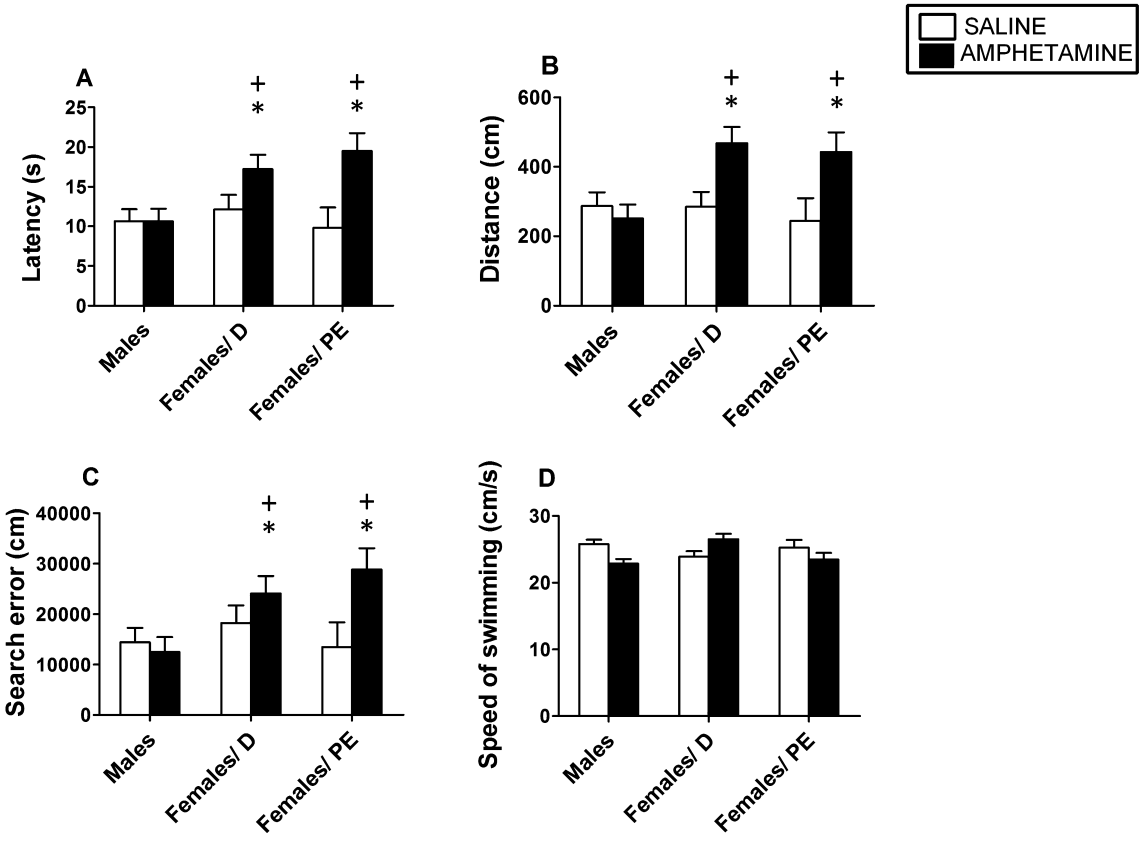

Fig. 4. Effect of adult AMP treatment on performance of male and female rats in the Memory Recall Test. (A) Latency of platform acquisition. (B) Distance traveled. (C) Search error. (D) Speed of swimming. Values are presented as mean + SEM (males: $n = 8$, females: $n = 10$), $^{*}p < 0.05$ females with adult SA < females with adult AMP, $+ p < 0.05$ males with adult AMP < females with adult AMP. D = diestrus, P/E = proestrus/estrus.

treatment had no effect on learning of both genders. In the present study the dose of 5 mg/kg of MA for the prenatal exposure was chosen as it was used in our previous studies [16,49]. However, accordingly to Acuff-Smith et al. [6], the impact of prenatal MA exposure on cognitive functions is dose dependent. They showed that while a lower dose of MA (5–10 mg/kg) administrated prenatally does not have any effect on spatial learning and memory in the MWM, exposure to a higher dose (15–20 mg/kg) affects spatial orientation. The application of a relatively low dose of the drug can explain why we did not find any effect of prenatal MA administration on spatial learning and memory in adulthood. It is well known that the effect of the exposure on the exogenous factors relies on the stage of brain development when the exposure occurs [53]. For instance, the hippocampus, structure involved in the process the spatial learning, begins to develop in rats on embryonic day 14 and continues until postnatal day 19. Rodents exposed to MA during the period of hippocampal development showed impaired spatial learning in the MWM, which persists into adulthood [54]. However, in the study of Vorhees et al. [54], the drug was administrated repeatedly at the higher doses (10–20 mg/kg) from PD 11–20. So it seems, that not only the dose of the drug, but also the period of the administration, need to be taken into consideration, when analyzing the spatial learning impairments.

The second goal of the present study was to determine the effect of AMP treatment in adulthood on spatial learning of female and male rats and the possible cross-sensitization effect of prenatal MA exposure on the AMP treatment. It has been shown, that chronic psychostimulants use in humans leads to cognitive deficiencies [55,56]. To the best of our knowledge, there is a lack of studies investigating the effect of AMP treatment in adulthood on allocentric learning of the rats. We showed in our previous studies that females and males with MA exposure in adulthood had diminished learning abilities in the MWM [16,17]. Vorhees et al. [57] showed that repeated AMP treatment at a higher dose of 25 mg/kg impairs egocentric learning. However, in comparison to the allocentric learning, this type of learning relies upon proprioceptive self-movement cues, change of direction and velocity [58].

Our present study showed that adult AMP treatment affects females' performance in the MWM (by increasing latencies, distances traveled and search errors). However, this effect of AMP was not apparent in males. This finding of the effect of AMP treatment on the learning abilities of female rats was supported by the results of the analyzed strategies after 6 days of the learning test. The expected trend of using the swimming strategies for finding the hidden platform by naïve animals is as follows. At the beginning of the test an animal prefers non-spatial strategies such as thigmotaxis, which is swimming around the maze's rim or randomly swimming across. As the test continues, the animal more often uses scanning. The most efficient strategy is a direct swim to the hidden platform, or so called spatial search, and it is used when the animal learns the platform's location [59]. In our present study, females with AMP treatment used more often non-spatial strategies than females with no drug treatment or males, as the test progressed. However, the percentage of animals in each of the tested group adopting the specific search strategy was quite low, so that the results should be taken indicatively.

Search error is another analyzed parameter in our present study showing that AMP treatment in adulthood affects the females' spatial abilities more markedly than it does in males. Our previous study [16] suggested search errors to be a better reflectance of

the accuracy of spatial learning than latency, as this parameter describes the total distance to the platform during the trials. Two animals may have similar latencies although their lengths of swimming paths might differ markedly. While one animal searches for the platform in the quadrant, in which the platform is placed, the other may search more within the opposite quadrant [60]. The data of the present study demonstrate that AMP treatment in adulthood increased search errors in a group of AMP-treated females when compared with AMP-treated males.

Unexpectedly, in the Probe Test we found that SA- and AMP-treated animals had comparable spatial abilities, as they did not differ in any of the parameters. These findings are in agreement with our previous results of the studies with adult MA treatment tested in female rats [17]. This type of test, in which the animal swims without the platform's presence, provides information about the memory retention after the position of the platform has been learned by an animal. However, the effect of AMP treatment on adult females was shown in the Memory Recall Test, which was performed on the last day of the testing. So it seems that even though the effect of the drug was not apparent in the control test, AMP treatment impaired the female's ability to recall the spatial map formed during the learning phase.

As to the possible sensitizing effects of prenatal MA exposure to AMP administrated in adulthood, contrary to our expectations, we did not prove a higher sensitivity. Despite the same doses of prenatal MA (5 mg/kg) and adult AMP treatment (5 mg/kg) used in this study and study of Šlamberová et al. [32], the effect of cross-sensitization was not confirmed. We suggest that the discrepancy might be caused by different tests used in these studies. The cross-sensitization between MA and AMP was confirmed in the Conditioned Place Preference Test and in the test for spontaneous locomotor activity and exploratory behavior in the Laboras apparatus [32]. Thus, further tests need to be done to clarify the question of cross-sensitization.

The third and last goal of the present study was to examine gender differences in the effect of the adult AMP treatment in respect of the prenatal MA treatment. First of all, as mentioned above, AMP-treated females presented marked changes in cognitive functions examined in the MWM in comparison to males.

In the present study, we found the sex differences in the effect of the prenatal and adult drug treatment on the speed of swimming, which reflects an increase in locomotion. Prenatally, MA-exposed females swam faster in the Place Navigation Test than prenatally MA-exposed males, i.e. females were more active. Our findings are supported by the results of the previous studies [16,17]. Thus, we suggest that the higher speed of swimming in a group of prenatally MA-exposed females might have been caused by their higher sensitivity to MA exposure during the period of the brain development. Additionally, AMP-treatment in adulthood also increased the speed of swimming in a group of female rats compared with AMP-treated males. Some animal studies showed that females are more sensitive than males in tests of spontaneous locomotor activity after acute or chronic treatment with AMP [13,36,61]. According to Milesi-Halle et al. [62] intrinsic variations in dopaminergic and serotoninergic systems could partially account for the greater activity seen in females after AMP treatment. Additionally, higher AMP-induced dopamine release in the striatum has been found in females, followed by a greater locomotor response [63].

Some authors claim that sex differences in the response to psychostimulants are also due to the dynamic effect of gonadal hormones, which act via regulation of the dopamine and serotonin systems [35,64,65]. However, contrary to our expectations, AMP-treated females in diestrus swam in the test of memory faster than AMP-treated females in proestrus. It is well known that during the estrous cycle ovarian hormone fluctuations cause variation in behavioral and neurochemical responses to psychostimulants [34,66]. In the female rat, levels of estrogen and progesterone fluctuate over a 4–5 days estrous cycle, and they both reach their maximum during proestrus. Both of these hormones decline during estrus and reach the lowest levels during diestrus [67]. A higher behavioral response to AMP in female rats is shown when the striatal dopamine system is affected by a higher level of estrogen during the behavioral proestrus [34,68,69].

Moreover, it seems that male rats were able to memorize the location of the platform more effectively than females, as they displayed faster learning during the 6-day period of the first test. These differences were even apparent in the Probe Test, when males swam more often across the former platform position and in the Memory Recall Test, as well. Additionally, the strategies of swimming also show a better ability of males to memorize the platform position. Generally in humans, males tend to be better at spatial orientation than females and this difference seems to be due to the organizational and activating effect of gonadal hormones, particularly testosterone [70].

5. Conclusions

The study does not show any effect of the prenatal MA treatment (5 mg/kg) on the allocentric learning and memory in males or in females. On the other hand, AMP application in adulthood (5 mg/kg) affects learning and memory in the MWM in a sex specific manner. Females were more sensitive to the effect of AMP treatment. Finally, data showed no sensitizing effect of the prenatal MA treatment to the AMP application in adulthood. Further experiments need to be done to verify the possible sex differences in the effect of different psychostimulant drugs.

Acknowledgments

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References

[1] Nesse RM, Berridge KC. Psychoactive drug use in evolutionary perspective. Science 1997;278:63–6.
[2] Marwick C. NIDA seeking data on effect of fetal exposure to methamphetamine. J Am Med Assoc 2000;283:2225–6.
[3] Vavřínková B, Binder T, Živný J. Characteristics of a population of drug dependent pregnant women in the Czech Republic. Ceska Gynekol 2001;66:285–91.
[4] Smith LM, Lagasse LL, Derauf C, Grant P, Shah R, Arria A, et al. Prenatal methamphetamine use and neonatal neurobehavioral outcome. Neurotoxicol Teratol 2008;30:20–8.
[5] Rambousek L, Kačer P, Syslová K, Bumba J, Bubeníková-Valešová V, Šlamberova R. Sex differences in methamphetamine pharmacokinetics in adult rats and its transferral to pups via placental membrane and breast milk 2014, http://dx.doi.org/10.1016/j.drugalcdep.2014.03.023, pii: S0376-8716(14)00799-6. [Epub ahead of print].
[6] 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.
[7] Martin JC, Martin DC, Radow B, Sigman G. Growth, development and activity in rat offspring following maternal drug exposure. Exp Aging Res 1976;2:235–51.
[8] Šlamberová R, Pometlová M, Charousová P. Postnatal development of rat pups is altered by prenatal methamphetamine exposure. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:82–8.

[9] Hrubá L, Schutová B, Šamberová R, Pometlová M, Rokyta R. Effect of methamphetamine exposure and cross-fostering on sensorimotor development of male and female rat pups. Dev Psychobiol 2009;51:73–83.
[10] Šlamberová R, Rokyta R. Seizure susceptibility in prenatally methamphetamine-exposed adult female rats. Brain Res 2005;1060:193–7.
[11] Yamamotová A, Hrubá L, Schutová B, Rokyta R, Šlamberová R. Perinatal effect of methamphetamine on nociception in adult Wistar rats. Int J Dev Neurosci 2011;29:85–92.
[12] Kelley AE. Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 2004;44:161–79.
[13] Camp DM, Robinson TE. Susceptibility to sensitization II. The influence of gonadal hormones on enduring changes in brain monoamines and behavior produced by the repeated administration of D-amphetamine or restraint stress. Behav Brain Res 1988;30:69–88.
[14] Thompson PM, Hayashi KM, Simon SL, Geaga JA, Hong MS, Sui Y, et al. Structural abnormalities in the brains of human subjects who use methamphetamine. J Neurosci 2004;24:6028–36.
[15] Chang L, Smith LM, LoPresti C, Yonekura ML, Kuo J, Walot I, et al. Smaller subcortical volumes and cognitive deficits in children with prenatal methamphetamine exposure. Psychiatry Res 2004;132:95–106.
[16] 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;58:741–50.
[17] Macúchová E, Nohejlová-Deykun K, Šlamberová R. Effect of methamphetamine on cognitive functions of adult female rats prenatally exposed to the same drug. Physiol Res 2013;62:89–98.
[18] Suzuki T, Fukuoka Y, Mori T, Miyatake M, Narita M. Behavioral sensitization to the discriminative stimulus effects of methamphetamine in rats. Eur J Pharmacol 2004;498:157–61.
[19] Šlamberová R, Yamamotová A, Schutová B, Hrubá L, Pometlová M. Impact of prenatal methamphetamine exposure on the sensitivity to the same drug in adult male rats. Prague Med Rep 2011;112:102–14.
[20] Šlamberová R, Schutová B, Hrubá L, Pometlová M. Does prenatal methamphetamine exposure affect the drug-seeking behavior of adult male rats. Behav Brain Res 2011;224:80–6.
[21] Bubeníková-Valešová V, Kačer P, Syslová K, Rambousek L, Janovský M, Schutová B, et al. Prenatal methamphetamine exposure affects the mesolimbic dopaminergic system and behavior in adult offspring. Int J Dev Neurosci 2009;27:525–30.
[22] Bernášková K, Matějovská I, Šlamberová R. Postnatal challenge dose of methamphetamine amplifies anticonvulsant effects of prenatal methamphetamine exposure on epileptiform activity induced by electrical stimulation in adult male rats. Exp Neurol 2011;229:282–7.
[23] Schutová B, Hrubá L, Pometlová M, Deykun K, Šlamberová R. Responsiveness to methamphetamine in adulthood is altered by prenatal exposure in rats. Physiol Behav 2010;99:381–7.
[24] Schutová B, Hrubá L, Rokyta R, Šlamberová R. Gender differences in behavioral changes elicited by prenatal methamphetamine exposure and application of the same drug in adulthood. Dev Psychobiol 2013;55:232–42.
[25] Bonate PL, Swann A, Silverman PB. Behavioral sensitization to cocaine in the absence of altered brain cocaine levels. Pharmacol Biochem Behav 1997;57:665–9.
[26] Ferrario CR, Robinson TE. Amphetamine pretreatment accelerates the subsequent escalation of cocaine self-administration behavior. Eur Neuropsychopharmacol 2007;17:352–7.
[27] Horger BA, Giles MK, Schenk S. Preexposure to amphetamine and nicotine predisposes rats to self-administer a low dose of cocaine. Psychopharmacology (Berl) 1992;107:271–6.
[28] Leri F, Flores J, Rajabi H, Stewart J. Effects of cocaine in rats exposed to heroin. Neuropsychopharmacology 2003;28:2102–16.
[29] He S, Grasing K. Chronic opiate treatment enhances both cocaine-reinforced and cocaine-seeking behaviors following opiate withdrawal. Drug Alcohol Depend 2004;75:215–21.
[30] Arnold JC. The role of endocannabinoid transmission in cocaine addiction. Pharmacol Biochem Behav 2005;81:396–406.
[31] Šlamberová R, Pometlová M, Schutová B, Hrubá L, Macúchová E, Nová E, et al. Do prenatally methamphetamine-exposed adult male rats display general predisposition to drug abuse in the conditioned place preference test? Physiol Res/Acad Sci Bohemoslov 2012;61(Suppl. 2):S129–38.
[32] Šlamberová R, Macúchová E, Nohejlová-Deykun K, Schutová B, Hrubá L, Rokyta R. Gender differences in the effect of prenatal methamphetamine exposure and challenge dose of other drugs on behavior of adult rats. Physiol Res 2013;62:99–108.
[33] Carroll ME, Lynch WJ, Roth ME, Morgan AD, Cosgrove KP. Sex and estrogen influence drug abuse. Trends Pharmacol Sci 2004;25:273–9.
[34] Becker JB. Gender differences in dopaminergic function in striatum and nucleus accumbens. Pharmacol Biochem Behav 1999;64:803–12.
[35] Sell SL, Scalzitti JM, Thomas ML, Cunningham KA. Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. J Pharmacol Exp Ther 2000;293:879–86.
[36] Bisagno V, Ferguson D, Luine VN. Chronic D-amphetamine induces sexually dimorphic effects on locomotion, recognition memory, and brain monoamines. Pharmacol Biochem Behav 2003;74:859–67.
[37] Cailhol S, Mormede P. Strain and sex differences in the locomotor response and behavioral sensitization to cocaine in hyperactive rats. Brain Res 1999;842:200–5.
[38] Schindler CW, Bross JG, Thorndike EB. Gender differences in the behavioral effects of methamphetamine. Eur J Pharmacol 2002;442:231–5.
[39] Páleníček T, Votava M, Bubeníková V, Horáček J. Increased sensitivity to the acute effects of MDMA (ecstasy) in female rats. Physiol Behav 2005;86:546–53.
[40] Tseng AH, Craft RM. Sex differences in antinociceptive and motoric effects of cannabinoids. Eur J Pharmacol 2001;430:41–7.
[41] Lynch WJ, Carroll ME. Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology (Berl) 1999;144:77–82.
[42] Roth ME, Casimir AG, Carroll ME. Influence of estrogen in the acquisition of intravenously self-administered heroin in female rats. Pharmacol Biochem Behav 2002;72:313–8.
[43] Peris J, Coleman-Hardee M, Millard WJ. Cocaine in utero enhances the behavioral response to cocaine in adult rats. Pharmacol Biochem Behav 1992;42:509–15.
[44] Fleckenstein AE, Gibb JW, Hanson GR. Differential effects of stimulants on monoaminergic transporters: pharmacological consequences and implications for neurotoxicity. Eur J Pharmacol 2000;406:1–13.
[45] Glatt SJ, Bolanos CA, Trksak GH, Crowder-Dupont C, Jackson D. Prenatal cocaine exposure alters behavioral and neurochemical sensitization to amphetamine in adult rats. Neuropharmacology 2000;39:599–610.
[46] Peris J, Decambre N, Coleman-Hardee ML, Simpkins JW. Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal [3H]dopamine release. Brain Res 1991;566:255–64.
[47] Schutová B, Hrubá L, Pometlová M, Deykun K, Šlamberová R. Impact of methamphetamine administered prenatally and in adulthood on cognitive functions of male rats tested in Morris water maze. Prague Med Rep 2008;109:62–70.
[48] Camarasa J, Rodrigo T, Pubill D, Escubedo E. Memantine is a useful drug to prevent the spatial and non-spatial memory deficits induced by methamphetamine in rats. Pharmacol Res 2010;62:450–6.
[49] Šlamberová R, Pometlová M, Syllabová L, Mančušková M. Learning in the Place navigation task, not the New-learning task, is altered by prenatal methamphetamine exposure. Brain Res Dev Brain Res 2005;157:217–9.
[50] Timar J, Gyarmati Z, Barna L, Knoll B. Differences in some behavioural effects of deprenyl and amphetamine enantiomers in rats. Physiol Behav 1996;60:581–7.
[51] Turner CD, Bagnara JT. Endocrinology of the ovary. In: Turner CD, Bagnara JT, editors. General endocrinology. Philadelphia: WB Saunders Company; 1976. p. 450–95.
[52] D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. Brain Res Brain Res Rev 2001;36:60–90.
[53] Williams MT, Moran MS, Vorhees CV. Refining the critical period for methamphetamine-induced spatial deficits in the Morris water maze. Psychopharmacology (Berl) 2003;168:329–38.
[54] Vorhees CV, Inman-Wood SL, Morford LL, Broening HW, Fukumura M, Moran MS. Adult learning deficits after neonatal exposure to D-methamphetamine: selective effects on spatial navigation and memory. J Neurosci 2000;20:4732–9.
[55] Ornstein TJ, Iddon JL, Baldacchino AM, Sahakian BJ, London M, Everitt BJ, et al. Profiles of cognitive dysfunction in chronic amphetamine and heroin abusers. Neuropsychopharmacology 2000;23:113–26.
[56] Simon SL, Domier C, Carnell J, Brethen P, Rawson R, Ling W. Cognitive impairment in individuals currently using methamphetamine. Am J Addict/Am Acad Psychiat Alcoh Addict 2000;9:222–31.
[57] Vorhees CV, He E, Skelton MR, Graham DL, Schaefer TL, Grace CE, et al. Comparison of (+)-methamphetamine, +/−-methylenedioxymethamphetamine, (+)-amphetamine and +/−-fenfluramine in rats on egocentric learning in the Cincinnati water maze. Synapse 2011;65:368–78.
[58] Clark BJ, Bassett JP, Wang SS, Taube JS. Impaired head direction cell representation in the anterodorsal thalamus after lesions of the retrosplenial cortex. J Neurosci 2010;30:5289–302.
[59] Able JA, Gudelsky GA, Vorhees CV, Williams MT. 3,4-Methylenedioxymethamphetamine in adult rats produces deficits in path integration and spatial reference memory. Biol Psychiatry 2006;59:1219–26.
[60] Gallagher M, Burwell R, Burchinal M. Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. Behav Neurosci 1993;107:618–26.
[61] Forgie ML, Stewart J. Six differences in the locomotor-activating effects of amphetamine: role of circulating testosterone in adulthood. Physiol Behav 1994;55:639–44.
[62] Milesi-Halle A, McMillan DE, Laurenzana EM, Byrnes-Blake KA, Owens SM. Sex differences in (+)-amphetamine- and (+)-methamphetamine-induced behavioral response in male and female Sprague-Dawley rats. Pharmacol Biochem Behav 2007;86:140–9.
[63] Castner SA, Xiao L, Becker JB. Sex differences in striatal dopamine: in vivo microdialysis and behavioral studies. Brain Res 1993;610:127–34.
[64] Callahan PM, De La Garza 2nd R, Cunningham KA. Mediation of the discriminative stimulus properties of cocaine by mesocorticolimbic dopamine systems. Pharmacol Biochem Behav 1997;57:601–7.
[65] Callahan PM, Cunningham KA. Modulation of the discriminative stimulus properties of cocaine: comparison of the effects of fluoxetine with 5-HT1A and 5-HT1B receptor agonists. Neuropharmacology 1997;36:373–81.
[66] Parylak SL, Caster JM, Walker QD, Kuhn CM. Gonadal steroids mediate the opposite changes in cocaine-induced locomotion across adolescence in male and female rats. Pharmacol Biochem Behav 2008;89:314–23.
[67] Smith MS, Freeman ME, Neill JD. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin

and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology 1975;96:219–26.
[68] Becker JB, Cha JH. Estrous cycle-dependent variation in amphetamine-induced behaviors and striatal dopamine release assessed with microdialysis. Behav Brain Res 1989;35:117–25.
[69] Becker JB, Robinson TE, Lorenz KA. Sex differences and estrous cycle variations in amphetamine-elicited rotational behavior. Eur J Pharmacol 1982;80:65–72.
[70] Jarvik LF. Human intelligence: sex differences. Acta Genet Med Gemellol (Roma) 1975;24:189–211.

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Effect of Methamphetamine on Cognitive Functions of Adult Female Rats Prenatally Exposed to the Same Drug

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Summary

The aim of this study was to investigate the effect of prenatal methamphetamine (MA) exposure and application of the same drug in adulthood on cognitive functions of adult female rats. Animals were prenatally exposed to MA (5 mg/kg) or saline (control group). The cognitive function was tested as ability of spatial learning in the Morris Water Maze (MWM). Each day of the experiment animals received an injection of MA (1 mg/kg) or saline. Our results demonstrated that prenatal MA exposure did not affect the latency to reach the hidden platform or the distance traveled during the Place Navigation Test; however, the speed of swimming was increased in prenatally MA-exposed rats compared to controls regardless of the treatment in adulthood. MA treatment in adulthood increased the latency and distance when compared to controls regardless of the prenatal exposure. Neither prenatal exposure, nor treatment in adulthood affected memory retrieval. As far as the estrous cycle is concerned, our results showed that prenatally MA-exposed females in proestrus/estrus swam faster than females in diestrus. This effect of estrous cycle was not apparent in control females. In conclusion, our results indicate that postnatal, but not prenatal exposure to MA affects learning of adult female rats.

Key words

Morris Water Maze • Methamphetamine • Spatial learning • Memory • Sensitization • Estrous cycle

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Introduction

Methamphetamine (MA) is one of the most frequently used illicit drugs in the Czech Republic, due to its relatively uncomplicated production, and its low price in comparison with cocaine or heroin (Vavřínková *et al.* 2001). MA belongs to a group of highly addictive psychostimulant drugs (Marwick 2000). Roughly half of MA users are women in reproductive age, who would continue using the drug also during pregnancy (Williams *et al.* 2003a). Even though, the long-term effects of prenatal exposure of MA remain relatively unclear, some studies suggest that prenatal MA exposure impairs the development of the CNS of the neonate (Šlamberová *et al.* 2006, Williams *et al.* 2003a).

Psychostimulant drugs activate molecular signalization in dopaminergic and glutamatergic pathways, which are widely distributed in the brain (e.g., basal ganglia, nucleus accumbens, and prefrontal cortex) (Kelley 2004). Previously we demonstrated that an acute administration of MA causes an increase in the dopamine level in the nucleus accumbens (Bubeníková-Valešová *et al.* 2009). Dopamine pathways are involved in motor control, reward circuits, sexual behavior, affective state and cognition (Camp and Robinson 1988).

It is known that prenatal MA exposure leads to long-term behavioral effects on spatial learning (Acuff-Smith *et al.* 1996). Brain regions, which are involved in the process of learning and memory, develop relatively late compared to other structures. Substantial remodeling and growth of rat hippocampus and related structures begin on embryonic day (ED) 14 and continue until postnatal day (PD) 19. The brain of neonatal rats (PD 11-20), which approximates human brain development

during the second and third trimester is highly vulnerable to pharmacological effects (Rice and Barone 2000). Repeated exposure of neonates to high doses of MA (15 and 20 mg/kg) lead to long-term cognitive deficits in spatial learning, which are associated with structural changes within the hippocampus (Williams *et al.* 2003b).

The effect of psychostimulants is affected by the stages of CNS development. Vorhees *et al.* (2005) found that MA exposure of adolescent rats (PD 40-60) causes deficits in spatial learning when tested in adulthood. Other study demonstrated that exposure to MA during preadolescence improves spatial learning in male rats (Moenk and Matuszewich 2012). In addition, our previous study (Schutová *et al.* 2008) demonstrated that prenatal MA exposure (5 mg/kg) does not affect learning in the Morris Water Maze (MWM) (Morris *et al.* 1982) and that MA treatment in adulthood (1 mg/kg) impairs learning in terms of changing search strategies. In humans, however, prenatal MA exposure during this critical period of development leads to long-term cognitive deficits (Chang *et al.* 2004, Smith *et al.* 2001).

Some laboratories (Crozatier *et al.* 2003, Stanwood and Levitt 2003) including our own (Šlamberová *et al.* 2011b) have investigated a phenomenon defined as behavioral sensitization or reverse tolerance (Suzuki *et al.* 2004) induced by prenatal drug exposure. The results are rather inconsistent. While Crozatier *et al.* (2003) demonstrated cocaine-induced sensitization in mice to cocaine exposure in adulthood, Stanwood and Levitt (2003) showed behavioral tolerance to the challenge dose of amphetamine in rabbits prenatally (ED 16-25) exposed to cocaine. Our results showed a sensitizing effect of prenatal MA exposure to MA treatment in adulthood (Bubeníková-Valešová *et al.* 2009, Šlamberová *et al.* 2011a, b).

In most of the studies male rats are used for investigation the effects of drug exposure on behavioral patterns. However, as other studies suggest, sexual differences in the drug sensitivity are important to be taken into consideration (Roth *et al.* 2002, Schutová *et al.* 2013). Female rats have been shown to be more sensitive to amphetamine (Becker 1999, Bisagno *et al.* 2003), MA (Schindler *et al.* 2002) and cocaine (Cailhol and Mormede 1999) when compared to male rats. In addition, females differ in spatial learning across the estrous cycle (Berry *et al.* 1997, Warren and Juraska 1997). There are only a few studies available that examined the differences in the drug sensitivity of female rats across their estrous cycle. Most of them are focused on behavioral responses to psychostimulants influenced by ovarian hormones (Becker 1990, Peris *et al.* 1991). For example, Sell *et al.* (2000) reported hyperactivity caused by cocaine in female rats in proestrus and estrus.

We already published in our previous study (Schutová *et al.* 2008), how does the MA (1 mg/kg) administration affect the spatial learning and memory tested in the MWM in adult male rats prenatally exposed to MA (5 mg/kg). To see the possible sex differences, the present study will examine the changes induced by prenatal and adult MA administration in female rats. The study was designed to test the following four objectives: 1) the effect of prenatal MA exposure and 2) the effect of MA administration in adulthood on cognitive functions of female rats; 3) the possible sensitizing effect of prenatal MA exposure; 4) the different drug sensitivity of females across the estrous cycle.

Materials and Methods

Animals and drug injections

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

When the onset of estrous phase was determined, females were housed overnight with sexually mature males. There were always one female and one male per cage. The following morning they were smeared for the presence of sperms and returned to their home cages. The day when sperms were detected was designated as day 1 of gestation. Most of the females were successful in pregnancy induction. The successful impregnation did not differ between females with MA or saline application. The total number of pregnant females was 20. Animals were randomly assigned to two treatment groups through the entire gestation period: 10 MA-treated (MA, 5 mg/kg) and 10 saline-treated (SA) (Šlamberová *et al.* 2005). The dose 5 mg/kg of methamphetamine (MA) was chosen because it induces similar fetal brain drug concentrations and similar behavioral changes to those found in humans (Acuff-Smith *et al.* 1996). They were injected subcutaneously (*s.c.*). The day of delivery was counted as 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 saline-treated pups

(3 males and 3 females). There were no differences in weights of the pups from prenatally MA- and SA-exposed groups after birth and during lactation period. On PD 21, animals were weaned and housed in groups, separated according to sex (4 males and 5 females, respectively, per cage). They were left undisturbed until adulthood. Always one prenatally SA-exposed and one prenatally MA-exposed female from each litter were used for the experiment to avoid litter effects. The rest of the animals were used in other studies.

Estrous cycle determination

Every day prior to testing each female was smeared by vaginal lavage. The smear was then examined by light microscopy. According to Turner and Bagnara (1976) two phases of the estrous cycle were recognized in the present study: proestrus/estrus (P/E) with predominance of large nucleated and some cornified epithelial cells in the smear; diestrus (D) with predominance of leukocytes in the smear. The estrous cycle of a female rat lasts for 4 to 5 days (Turner and Bagnara 1976). We did not find any differences in the estrous cycle between prenatally MA-exposed and prenatally SA-exposed females.

MWM testing

40 adult female rats (PD 60-90) were tested over a 12-day period. To determine the effect of MA in adulthood half of the females from each prenatal treatment (MA, n=10; SA, n=10) received a low dose of MA (1 mg/kg) *s.c.* after finishing testing each day. The other half (MA, n=10; SA, n=10) was exposed to an injection of saline (1 ml/kg). The dose 1 mg/kg of MA was used because it does not cause stereotypical behavior (unpublished data), unlike the dose of 5 mg/kg, which was used in gestation. This dose is often used as challenge dose in the test of behavioral sensitization (Suzuki *et al.* 2004). The animals also received MA and SA on the days when no tests were performed. The timing of injections was the same as in the days of testing.

Three types of tests were used in this study: the Place Navigation Test, the Probe Test and the Retention Memory Test. Before each experiment animals were left to acclimate to the laboratory conditions, in which the experiments were performed.

Apparatus

The water maze consisted of a blue circular tank (2 m in diameter), filled with water (22.5±2.5 °C). The maze was divided into four quadrants in respect to start positions (north-N, south-S, east-E and west-W). A transparent circular platform was placed into the NE quadrant of the tank, 1 cm below the water surface. The maze was surrounded by various extra-maze cues on the walls. Trials were tracked using a video-tracking system EthoVision 6 (Noldus Information Technology, Netherlands).

The Place Navigation Test

During 6 days of spatial learning animals were trained to locate the hidden platform within the limit of 60 s. If the animal did not reach the platform within the limit, it was gently guided by the experimenter to the platform. Eight trials per day were performed. The position of the platform was the same through the period of learning. After each trial, the rat was remained on the platform for 30 s prior to the next trial to have a chance to orientate and to learn its position in the room. After the trials on each experimental day, the animal received the *s.c.* injection of MA or saline as appropriate and was placed in the home cage.

The following parameters were evaluated with the use of EthoVision program: latency of platform acquisition [s], distance traveled (the length of the swim-path) [cm] and speed of swimming [cm/s].

Search strategies (swimming pathways) of individual animals were manually analyzed after track acquisition on days 1, 3, 6 and 12. The following search strategies were assigned (Schutová *et al*. 2009): 1) thigmotaxis – predominant swimming along the wall of the pool; 2) random search – swimming over the entire area of the pool in straight swims or in wide circular swims; 3) scanning – swimming over the central area of the pool; 4) search in an incorrect quadrants – direct swim to an incorrect quadrant of the pool followed by loops and turns there; 5) search in a correct quadrant – direct swim to a correct quadrant of the pool followed by loops and turns there, 6) spatial search – direct swim path to the platform. Analysis of search strategies was aiming on better understanding of differences in process of learning in animals exposed to MA either prenatal or acute.

The Probe Test

During the Probe Test, which was conducted on the 8th day, the platform was removed, and the animal was left to swim in the pool for 60 s. The start position in

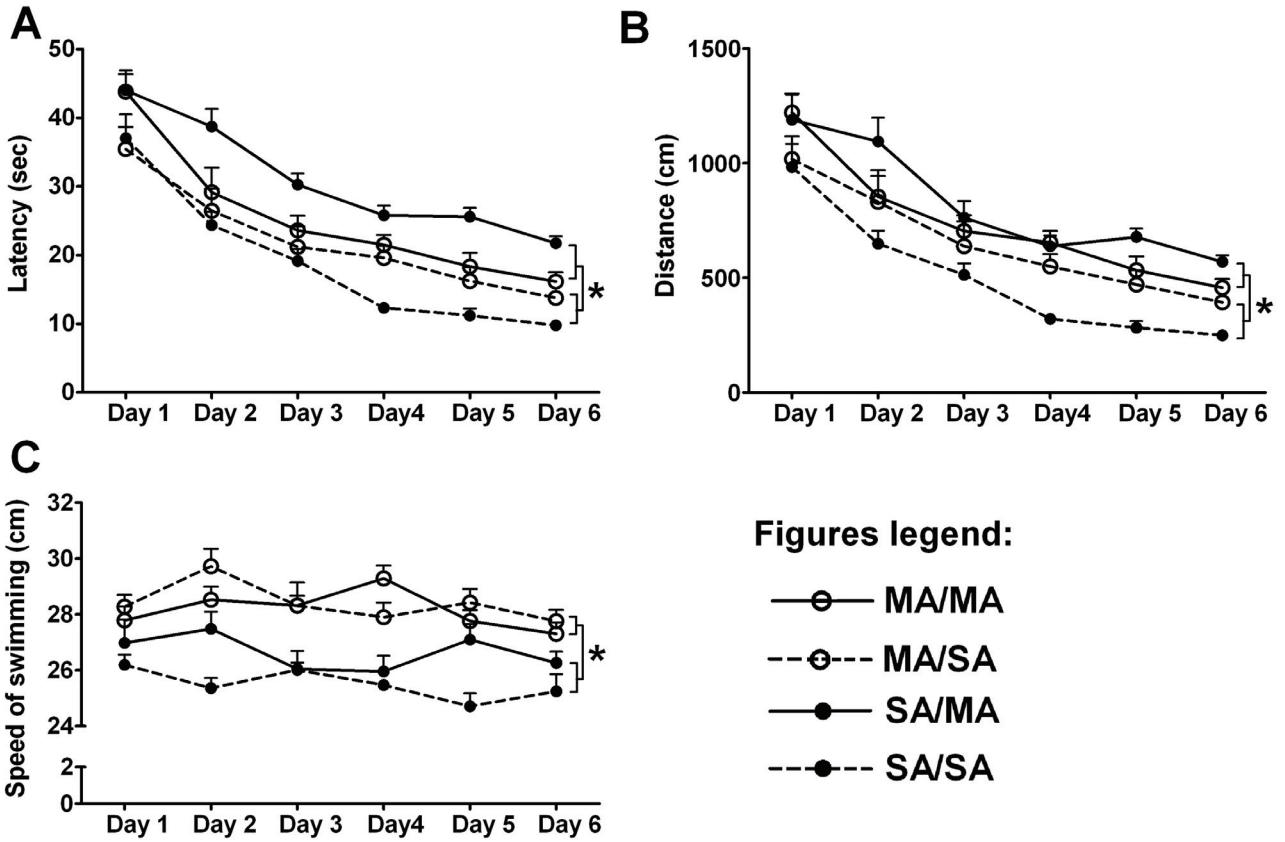

Fig. 1. Effect of prenatal and adult MA exposure on performance in the Place Navigation Test. **A.** Latency of platform acquisition – main effect of MA application in adulthood, * $p<0.05$. **B.** Distance traveled – main effect of MA application in adulthood, * $p<0.05$. **C.** Speed of swimming – main effect of prenatal MA exposure. Results are presented as mean + SEM, n=10.

this test was for each animal north (N). The following parameters were recorded: frequency and duration [s] of presence in the quadrant where the platform was located, frequency of swimming across the former placement of the platform and speed of swimming [cm/s].

The Retention Memory Test

The memory test was performed on the 12th day. An animal was supposed to find the platform located at the same position as in the learning test within 60 s. Each animal was subjected to 8 trials. The same parameters were analyzed as in the Place Navigation Test: latency of platform acquisition [s], distance traveled [cm] and speed of swimming [cm/s].

Statistical analysis

The data from the Place Navigation Test were analyzed by a Two-Way ANOVA (Prenatal exposure x Treatment in adulthood) with multilevel repeated measure (days x trials/day). Probe Test data were analyzed by a Three-Way ANOVA (Prenatal exposure x Treatment in adulthood x Estrous cycle). A Three-Way ANOVA (Prenatal exposure x Treatment in adulthood x Estrous cycle) with repeated measure (trials) was used to analyze the data from the Retention Memory Test. The Bonferroni test was used for post-hoc comparisons. The X^2 test was used to analyze the occurrence of the search strategies. In all tests, the differences were considered significant if $p<0.05$.

Results

The Place Navigation Test

For the latency of platform acquisition (Fig. 1A) and the distance traveled (Fig. 1B) the main effect of MA treatment in adulthood was demonstrated [latency: $F(1, 36)=6.28$, $p<0.05$; distance traveled: $F(1, 36)=6.33$, $p<0.05$]. On average the animals with MA treatment in adulthood had longer latencies and they had longer trajectories when compared to controls, regardless of the prenatal exposure. The speed of swimming was not affected by MA treatment in adulthood [$F(1, 36)=0.52$, $p=0.47$].

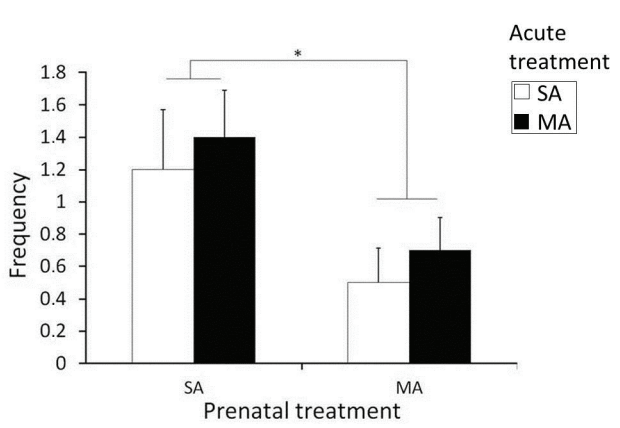

Fig. 2. Effect of prenatal and adult MA exposure on frequency of swimming across the former placement of the hidden platform in the Probe Test. Values are mean + SEM, n=10. * $p<0.05$ – main effect of prenatal exposure: MA group has lower frequency than SA group.

Prenatal MA exposure did not affect the latency to find the hidden platform [$F(1, 36)=0.19$, $p=0.66$] or the distance traveled $F(1, 36)=0.16$, $p=0.69$]. However, the main effect of prenatal MA exposure for swimming speed was found [$F(1, 36)=5.68$, $p<0.05$], animals prenatally exposed to MA, swam faster than animals prenatally exposed to saline (Fig. 1C), regardless of postnatal exposure.

All animals, regardless of prenatal or postnatal treatment, demonstrated learning ability over the 6-day test period as represented by a decrease in latency: [$F(5, 180)=86.69$, $p<0.0001$] and distance traveled: [$F(5, 180)=91.40$, $p<0.0001$]. The speed of swimming did not differ between days [$F(5, 180)=1.28$, $p=0.28$]. There was no interaction between prenatal and adult drug exposure in the latency [$F(1, 36)=2.21$, $p=0.14$], distance traveled [$F(1, 36)=2.09$, $p=0.15$] or speed of swimming [$F(1, 36)=0.61$, $p=0.43$].

The Probe Test

Analysis of the data from the Probe Test showed a significant main effect of prenatal MA exposure on the frequency of swimming across the former placement of the hidden platform [$F(1, 32)=4.68$, $p<0.05$]. Female rats prenatally exposed to MA swam less often across the former placement of the hidden platform than prenatal controls (Fig. 2). This measure was not affected by treatment in adulthood [$F(1, 32)=0.65$, $p=0.42$].

There was no significant effect of the frequency or the duration of presence in the quadrant where the hidden platform was placed during the learning phase.

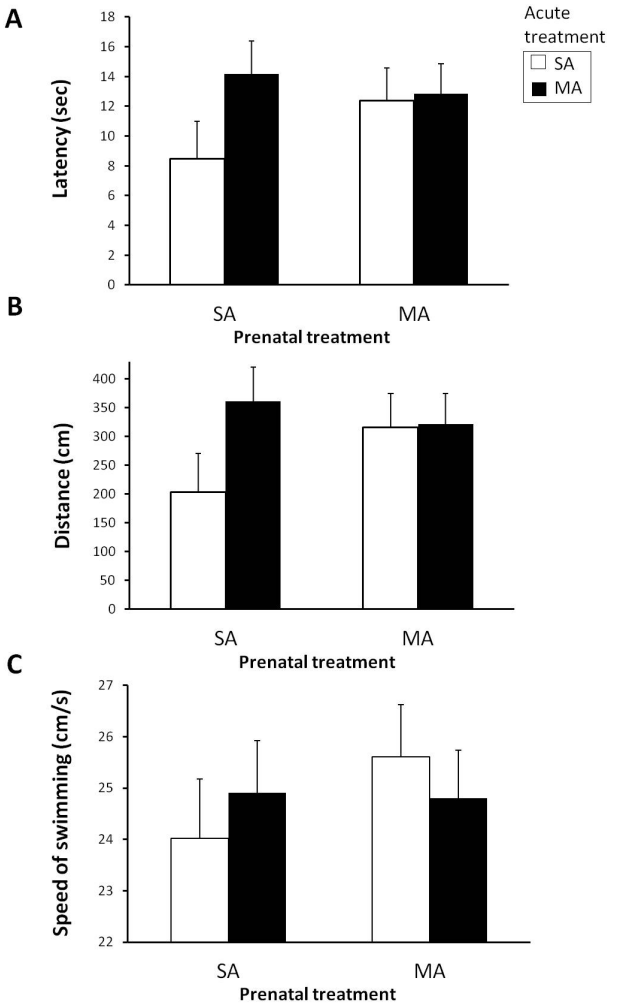

Fig. 3. Effect of prenatal and adult MA exposure on performance in the Retention Memory Test. **A.** Latency of platform acquisition. **B.** Distance traveled. **C.** Speed of swimming. Results are presented as mean + SEM, n=10.

The Retention Memory Test

Statistical analysis of data from Retention Memory Test showed no significant differences between animals exposed to MA with controls in either of the parameters (Fig. 3).

Search strategies

The strategies of swimming to find the hidden platform differed based on the prenatal and adult drug exposure and changed as the time of the experiment progressed.

On Day 1 of the experiment, prenatally MA-exposed rats injected in adulthood with MA preferred thigmotaxis (predominant swimming along the wall of the pool) more than any other groups (SA/SA=12.5 %, SA/MA=30 %, MA/SA=22.5 %, MA/MA=50 %) [$\chi^2=29.54$, $p<0.0001$]. On the other hand, they used at

least from all the groups (SA/SA=12.5 %, SA/MA=20 %, MA/SA=18.75 %, MA/MA=8.75 %) the strategy of scanning (swimming over the central area of the pool) [χ^2=24.95, p<0.0001].

On Day 3, the percentage changed, but the difference in using strategies stayed significant between groups. Thigmotaxis was still used the most (SA/SA=0 %, SA/MA=12.5 %, MA/SA=5 %, MA/MA=37.5 %) [χ^2=53.66, p<0.0001] and scanning the least (SA/SA=30 %, SA/MA=30 %, MA/SA=37.5 %, MA/MA=15 %) [χ^2=10.57, p<0.05] by prenatally MA-exposed rats injected with MA in adulthood.

On Day 6, control group already displayed prevalence of spatial search (direct swim path to the platform) when compared to MA-treated groups (SA/SA=60 %, SA/MA=43.75 %, MA/SA=27.5 %, MA/MA=25 %) [χ^2=26.61, p<0.0001]. On the other hand, prenatally MA-exposed rats injected in adulthood with MA used still the thigmotaxis the most (SA/SA=12.5 %, SA/MA=12.5 %, MA/SA=30 %, MA/MA=43.75 %) [χ^2=29.63, p<0.0001].

On Day 12 (the Retention Memory Test), prenatally saline-exposed rats preferred the spatial search strategy, while prenatally MA-exposed rats did not (SA/SA=63.75 %, SA/MA=60 %, MA/SA=35 %, MA/MA=33.75 %) [χ^2=24.48, p<0.0001]. In contrast, both groups of prenatally MA-exposed rats still used the thigmotaxis (SA/SA=3.75 %, SA/MA=12.5 %, MA/SA=35 %, MA/MA=28.75 %) [χ^2=31.09, p<0.0001].

Effect of the estrous cycle

There were no differences induced by the estrous cycle in any measures in the Place Navigation Test, or in the Probe Test.

In the Retention Memory Test, there were no differences in the latency of platform acquisition or the distance traveled, but there was an interaction between prenatal drug exposure and the estrous cycle in the speed of swimming [F(1, 32)=4.17, p<0.05]. The data showed that prenatally MA-exposed animals in P/E swam faster than prenatally MA-exposed rats in D (p<0.05). In prenatally saline-exposed rats, no such difference was apparent.

Discussion

The dose of MA (5 mg/kg) that was used in the present study for the prenatal exposure is a standard dose used in our experiments (Šlamberová *et al*. 2005). This dose was chosen because it induces fetal brain drug concentrations and behavioral changes similar to those found in humans (Acuff-Smith *et al.* 1996). Even though it was found that daily maternal application of MA in a dose of 5 mg/kg may delay sensorimotor development (Martin *et al.* 1976, Šlamberová *et al.* 2006), the motor skills are not affected in adulthood (Hrubá *et al.* 2010). In addition, the low dose of MA (1 mg/kg) injected in adulthood does not induce stereotypical behavior and, therefore, it should not affect the swimming of the tested animal. To avoid any possible impairing effects of acute MA administration on swimming in the present study MA was injected into the animal each day after the MWM performance. From all of the above, the results that we can see in the present study are clearly due to the effect of both, prenatal MA exposure and MA adult treatment, on cognitive functions of the rat independently from the animals' ability to move or swim.

The first goal of the present study was to determine the effect of prenatal MA exposure on learning abilities of female rats. When analyzing data from the Place Navigation Test we did not find any effect of prenatal MA exposure (5 mg/kg daily) on the latency of platform acquisition or the distance traveled. These results are in accordance with our previous study on male rats (Schutová *et al.* 2009). Data from the Probe Test showed that animals with prenatal MA-exposure had lower frequency of swimming across the former placement of the hidden platform. This finding disagrees with our previous study (Schutová *et al.* 2009), in which no effect of prenatal MA exposure on the performance in the Probe Test was found. This discrepancy may be assigned to sex differences. The Probe Test is a good way of analysis of the memory recall quality. According to Brown *et al.* (2000), if the animal uses a non-spatial strategy during the learning phase, it usually fails in the Probe Test. Therefore, our data from the Probe Test suggest that the prenatal MA exposure may impair learning. These data is in agreement with study on neonates that were exposed to MA and tested in adulthood, showing reduction of performance in the Probe Test (Vorhees *et al.* 2000, Williams *et al.* 2003b). We did not find any effect of prenatal treatment in the Retention Memory Test. Thus, we could say that prenatal MA treatment does not influence memory in the MWM, which is in accordance with study of Acuff-Smith *et al.* (1999).

The second goal of the present study was to determine the effect of MA treatment in adulthood. Data

from the Place Navigation Test showed that animals with MA exposure in adulthood swam longer distances and had longer latencies to reach the hidden platform, regardless of the prenatal treatment. Schutová *et al.* (2008, 2009) reported that MA treatment in adulthood prolonged distance traveled, but did not have any effect on the latency. Our findings are in accordance with the results of Friedman *et al.* (1998), who showed learning impairment in the MWM after acute MA exposure at the neurotoxic MA dose of 12.5 mg. Additionally, Camarasa *et al.* (2010) found out that rats treated with MA (10 mg/kg) in adulthood show increased latencies platform acquisition. The results from the Retention Memory Test did not show any effect of MA exposure in adulthood on the ability of the animal to memorize the platform's position. These results correspond to results of Cao *et al.* (2013) showing no effect of MA (1 mg/kg) on spatial memory in mice exposed to learning for 5 consecutive days and then tested for the memory reconsolidation.

The speed of swimming seems to have the positive correlation to the motivation of the animal to find the platform in the MWM (Lubbers *et al.* 2007). In addition, it has been suggested that motivation is mediated by the meso-accumbens dopamine system (Salamone and Correa 2002) and this system might be affected by MA application in adulthood. We did not find any effect of MA exposure in adulthood on the speed of swimming, which does not coincide with our previous results from MWM (Schutová *et al.* 2008, 2009). On the other hand, the results from the Place Navigation Test showed that prenatal MA-exposure resulted in an increase of speed of swimming. Females prenatally exposed to MA swam faster than animals with prenatal saline exposure, regardless of treatment in adulthood. Thus, it might be that prenatally MA-exposed rats had higher motivation than control rats. Another reason might be that prenatal MA exposure increases locomotor activity (Bubeníková-Valešová *et al.* 2009), similarly as exposure to another psychostimulants, such as cocaine (Peris *et al.* 1992).

The third goal of the present study was to evaluate possible sensitizing effect of prenatal MA exposure to the same drug administered in adulthood. Our previous results showed sensitizing effect of prenatal MA exposure to the same drug treatment in adulthood on locomotion or drug-seeking behavior (Bubeníková-Valešová *et al.* 2009, Šlamberová *et al.* 2011a, b). Additionally, we showed that the sensitizing effect of prenatal MA in males corresponds with dopamine levels in the nucleus accumbens (Bubeníková-Valešová *et al.* 2009). The present study examining cognition in female rats showed no effect of prenatal MA exposure on behavioral sensitization to MA treatment in adulthood that would coincide with results in the report of Schutová *et al.* (2009). The reason might be due to sex differences (Melnick and Dow-Edwards 2001, Peris *et al.* 1992). Nevertheless, even though we did not see any sensitizing effect of prenatal MA exposure in the latency or distance traveled, in search strategies the sensitizing effect was apparent. Specifically, we found that prenatally MA-exposed rats injected in adulthood with MA had higher incidence of thigmotaxis across the days of experiment than any other groups. Search strategies are used as alternative measures to the latency to better recognize the differences in spatial learning (Gallagher *et al.* 1993, Janus 2004). In some cases it may happen that two animals with the same lengths of latencies use different strategies of swimming; further one animal might use a strategy to find the hidden platform without obtaining knowledge of the platform's location (non-spatial strategy). Usually, animals use the non-spatial strategy in the beginning and spatial search at the end of the learning period when the animal remembers the exact location of the platform (Janus 2004).

The last goal of the present study was to investigate how the drug sensitivity of females differs across the estrous cycle. Our results from the Retention Memory Test showed that prenatally MA-exposed animals swam faster in proestrus/estrus than in diestrus. To the best of our knowledge, there is no literature available showing effect of prenatal or acute MA exposure on spatial learning tested in the MWM in female rats. Our unpublished data demonstrated that females (especially in proestrus/estrus) displayed sensitization induced by prenatal MA exposure to adult administration of amphetamine or MDMA. In addition, study of Becker (1999) showed that estrogen and progesterone affect the striatal dopamine function. Female rats show a greater behavioral response when the striatal dopaminergic system is stimulated in the estrus instead of in the diestrus (Becker *et al.* 1982). Thus, future studies are planned to test the sensitizing effect of MA on the striatal dopaminergic system of female rats in respect of their estrous cycle.

In conclusion, the present study on adult female rats demonstrates that prenatal exposure to MA (5 mg/kg) neither affects learning, nor memory. On the other hand,

we showed that MA treatment in adulthood impairs learning. As a matter of sensitization, the present data demonstrate that the sensitizing effect of prenatal MA exposure to the same drug exposure in adulthood was apparent only in strategies, but not in the time or length that was necessary to find the hidden platform. Furthermore, we suggest that sensitivity to prenatal MA exposure might be related to the hormonal cycle of females. As there are no studies investigating the effect of MA exposure on females' cognitive functions during the estrous cycle, our study might contribute to further understanding of this issue. Our future experiments will test the cognitive functions in animals prenatally exposed to MA in shorter time periods, affecting development during individual trimesters. From this we will try to find the critical period for the long-term consequences caused by MA exposure.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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Abbreviations

MA – methamphetamine; SA – saline; CNS – central nervous system; MWM – Morris Water Maze; PD – postnatal day; P/E – proestrus/estrus; D – diestrus; s.c. – subcutaneously; NE quadrant – north-east quadrant

References

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* **18**: 199-215, 1996.

BECKER JB: Estrogen rapidly potentiates amphetamine-induced striatal dopamine release and rotational behavior during microdialysis. *Neurosci Lett* **118**: 169-171, 1990.

BECKER JB: Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacol Biochem Behav* **64**: 803-812, 1999.

BECKER JB, ROBINSON TE, LORENZ KA: Sex differences and estrous cycle variations in amphetamine-elicited rotational behavior. *Eur J Pharmacol* **80**: 65-72, 1982.

BERRY B, MCMAHAN R, GALLAGHER M: Spatial learning and memory at defined points of the estrous cycle: effects on performance of a hippocampal-dependent task. *Behav Neurosci* **111**: 267-274, 1997.

BROWN RW, BARDO MT, MACE DD, PHILLIPS SB, KRAEMER PJ: D-amphetamine facilitation of morris water task performance is blocked by eticlopride and correlated with increased dopamine synthesis in the prefrontal cortex. *Behav Brain Res* **114**: 135-143, 2000.

BUBENÍKOVÁ-VALEŠOVÁ V, KAČER P, SYSLOVÁ K, RAMBOUSEK L, JANOVSKÝ M, SCHUTOVÁ B, HRUBÁ L, ŠLAMBEROVÁ R: Prenatal methamphetamine exposure affects the mesolimbic dopaminergic system and behavior in adult offspring. *Int J Dev Neurosci* **27**: 525-530, 2009.

CAILHOL S, MORMEDE P: Strain and sex differences in the locomotor response and behavioral sensitization to cocaine in hyperactive rats. *Brain Res* **842**: 200-205, 1999.

CAMARASA J, RODRIGO T, PUBILL D, ESCUBEDO E: Memantine is a useful drug to prevent the spatial and non-spatial memory deficits induced by methamphetamine in rats. *Pharmacol Res* **62**: 450-456, 2010.

CAMP DM, ROBINSON TE: Susceptibility to sensitization. II. The influence of gonadal hormones on enduring changes in brain monoamines and behavior produced by the repeated administration of D-amphetamine or restraint stress. *Behav Brain Res* **30**: 69-88, 1988.

CAO G, ZHU J, ZHONG Q, SHI C, DANG Y, HAN W, LIU X, XU M, CHEN T: Distinct roles of methamphetamine in modulating spatial memory consolidation, retrieval, reconsolidation and the accompanying changes of ERK and CREB activation in hippocampus and prefrontal cortex. *Neuropharmacology* **67**: 144-154, 2013.

CHANG L, SMITH LM, LOPRESTI C, YONEKURA ML, KUO J, WALOT I, ERNST T: Smaller subcortical volumes and cognitive deficits in children with prenatal methamphetamine exposure. *Psychiatry Res* **132**: 95-106, 2004.

CROZATIER C, GUERRIERO RM, MATHIEU F, GIROS B, NOSTEN-BERTRAND M, KOSOFSKY BE: Altered cocaine-induced behavioral sensitization in adult mice exposed to cocaine in utero. *Brain Res Dev Brain Res* **147**: 97-105, 2003.

FRIEDMAN SD, CASTANEDA E, HODGE GK: Long-term monoamine depletion, differential recovery, and subtle behavioral impairment following methamphetamine-induced neurotoxicity. *Pharmacol Biochem Behav* **61**: 35-44, 1998.

GALLAGHER M, BURWELL R, BURCHINAL M: Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. *Behav Neurosci* **107**: 618-626, 1993.

HRUBÁ L, SCHUTOVÁ B, POMETLOVÁ M, ROKYTA R, ŠLAMBEROVÁ R: Effect of methamphetamine exposure and cross-fostering on cognitive function in adult male rats. *Behav. Brain Res.* **208**: 63-71, 2010.

JANUS C: Search strategies used by APP transgenic mice during navigation in the Morris water maze. *Learn Mem* **11**: 337-346, 2004.

KELLEY AE: Memory and addiction: shared neural circuitry and molecular mechanisms. *Neuron* **44**: 161-179, 2004.

LUBBERS ME, VAN DEN BOS R, SPRUIJT BM: Mu opioid receptor knockout mice in the Morris Water Maze: a learning or motivation deficit? *Behav Brain Res* **180**: 107-111, 2007.

MARTIN JC, MARTIN DC, RADOW B, SIGMAN G: Growth, development and activity in rat offspring following maternal drug exposure. *Exp. Aging Res.* **2**: 235-251, 1976.

MARWICK C: NIDA seeking data on effect of fetal exposure to methamphetamine. *JAMA* **283**: 2225-2226, 2000.

MELNICK SM, DOW-EDWARDS DL: Differential behavioral responses to chronic amphetamine in adult male and female rats exposed to postnatal cocaine treatment. *Pharmacol Biochem Behav* **69**: 219-224, 2001.

MOENK MD, MATUSZEWICH L: Juvenile but not adult methamphetamine exposure improves performance in the Morris Water Maze in male rats. *Int J Dev Neurosci* **30**: 325-331, 2012.

MORRIS RG, GARRUD P, RAWLINS JN, O'KEEFE J: Place navigation impaired in rats with hippocampal lesions. *Nature* **297**: 681-683, 1982.

PERIS J, DECAMBRE N, COLEMAN-HARDEE ML, SIMPKINS JW: Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal [3H]dopamine release. *Brain Res* **566**: 255-264, 1991.

PERIS J, COLEMAN-HARDEE M, MILLARD WJ: Cocaine in utero enhances the behavioral response to cocaine in adult rats. *Pharmacol Biochem Behav* **42**: 509-515, 1992.

RICE D, BARONE S Jr: Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect* **108** (Suppl 3): 511-533, 2000.

ROTH ME, CASIMIR AG, CARROLL ME: Influence of estrogen in the acquisition of intravenously self-administered heroin in female rats. *Pharmacol Biochem Behav* **72**: 313-318, 2002.

SALAMONE JD, CORREA M: Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behav Brain Res* **137**: 3-25, 2002.

SCHINDLER CW, BROSS JG, THORNDIKE EB: Gender differences in the behavioral effects of methamphetamine. *Eur J Pharmacol* **442**: 231-235, 2002.

SCHUTOVÁ B, HRUBÁ L, POMETLOVÁ M, DEYKUN K, ŠLAMBEROVÁ R: Impact of methamphetamine administered prenatally and in adulthood on cognitive functions of male rats tested in Morris water maze. *Prague Med Rep* **109**: 62-70, 2008.

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* **58**: 741-750, 2009.

SCHUTOVÁ B, HRUBÁ L, ROKYTA R, ŠLAMBEROVÁ R: Gender differences in behavioral changes elicited by prenatal methamphetamine exposure and application of the same drug in adulthood. *Dev Psychobiol* **55**: 232-242, 2013.

SELL SL, SCALZITTI JM, THOMAS ML, CUNNINGHAM KA: Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. *J Pharmacol Exp Ther* **293**: 879-886, 2000.

ŠLAMBEROVÁ R, POMETLOVÁ M, SYLLABOVÁ L, MANČUŠKOVÁ M: Learning in the Place navigation task, not the New-learning task, is altered by prenatal methamphetamine exposure. *Brain Res Dev Brain Res* **157**: 217-219, 2005.

ŠLAMBEROVÁ R, POMETLOVÁ M, CHAROUSOVÁ P: Postnatal development of rat pups is altered by prenatal methamphetamine exposure. *Prog Neuropsychopharmacol Biol Psychiatry* **30**: 82-88, 2006.

ŠLAMBEROVÁ R, SCHUTOVÁ B, HRUBÁ L, POMETLOVÁ M: Does prenatal methamphetamine exposure affect the drug-seeking behavior of adult male rats? *Behav Brain Res* **224**: 80-86, 2011a.

ŠLAMBEROVÁ R, YAMAMOTOVÁ A, SCHUTOVÁ B, HRUBÁ L, POMETLOVÁ M: Impact of prenatal methamphetamine exposure on the sensitivity to the same drug in adult male rats. *Prague Med Rep* **112**: 102-114, 2011b.

SMITH LM, CHANG L, YONEKURA ML, GROB C, OSBORN D, ERNST T: Brain proton magnetic resonance spectroscopy in children exposed to methamphetamine in utero. *Neurology* **57**: 255-260, 2001.

STANWOOD GD, LEVITT P: Repeated i.v. cocaine exposure produces long-lasting behavioral sensitization in pregnant adults, but behavioral tolerance in their offspring. *Neuroscience* **122**: 579-583, 2003.

SUZUKI T, FUKUOKA Y, MORI T, MIYATAKE M, NARITA M: Behavioral sensitization to the discriminative stimulus effects of methamphetamine in rats. *Eur J Pharmacol* **498**: 157-161, 2004.

TURNER CD, BAGNARA JT: Endocrinology of the ovary. *General Endocrinology*. W. B. Saunders Company, Philadelphia, 1976, pp 450-495.

VAVŘÍNKOVÁ B, BINDER T, ŽIVNÝ J: Characteristics of a population of drug dependent pregnant women in the Czech Republic. (in Czech) *Ceska Gynekol* **66**: 285-291, 2001.

VORHEES CV, INMAN-WOOD SL, MORFORD LL, BROENING HW, FUKUMURA M, MORAN MS: Adult learning deficits after neonatal exposure to D-methamphetamine: selective effects on spatial navigation and memory. *J Neurosci* **20**: 4732-4739, 2000.

VORHEES CV, REED TM, MORFORD LL, FUKUMURA M, WOOD SL, BROWN CA, SKELTON MR, McCREA AE, ROCK SL, WILLIAMS MT: Periadolescent rats (P41-50) exhibit increased susceptibility to D-methamphetamine-induced long-term spatial and sequential learning deficits compared to juvenile (P21-30 or P31-40) or adult rats (P51-60). *Neurotoxicol Teratol* **27**: 117-134, 2005.

WARREN SG, JURASKA JM: Spatial and nonspatial learning across the rat estrous cycle. *Behav Neurosci* **111**: 259-266, 1997.

WILLIAMS MT, BLANKENMEYER TL, SCHAEFER TL, BROWN CA, GUDELSKY GA, VORHEES CV: Long-term effects of neonatal methamphetamine exposure in rats on spatial learning in the Barnes maze and on cliff avoidance, corticosterone release, and neurotoxicity in adulthood. *Dev Brain Res* **147**: 163-175, 2003a.

WILLIAMS MT, MORAN MS, VORHEES CV: Refining the critical period for methamphetamine-induced spatial deficits in the Morris water maze. *Psychopharmacology (Berl)* **168**: 329-338, 2003b.