Does Cross-Fostering Modify the Prenatal Effect of Methamphetamine on Learning of Adult Male Rats?

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Abstract: Our previous studies demonstrated that methamphetamine administered during gestation and lactation periods impairs maternal behavior, alters the functional development of rat pups and affects behavior in adulthood. The aim of our study was to investigate the effect of prenatal methamphetamine exposure and cross-fostering on learning tested in Morris water maze (MWM) in adult male rats.

Mothers were daily exposed to injection of methamphetamine (MA) (5 mg/kg) or saline (S): prior to impregnation and throughout gestation and lactation periods. On postnatal day 1, pups were cross-fostered so that each mother received some of her own and some of the pups of mother with the opposite treatment. Based on the prenatal and postnatal treatments 4 experimental groups (S/S, S/MA, MA/S, MA/MA) were tested in MWM. Two types of tests were used: (1) "Place navigation test" (Learning) and (2) "Probe test" (Probe).

In the test of learning, all animals fostered by methamphetamine-treated dams had longer latencies and trajectories, and bigger search error than the animals fostered by saline-treated control mother, regardless of prenatal exposure. Further, the animals prenatally exposed to methamphetamine swam slower than the animals prenatally exposed to saline, regardless of postnatal exposure in the test of learning and in the Probe test. Our results showed that neither prenatal nor postnatal methamphetamine exposure affected the Probe test.

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Our results showed that prenatal exposure to methamphetamine at dose of 5 mg/kg does not impair learning in the MWM, while postnatal exposure to methamphetamine from mothers' breastmilk and maternal care of mother exposed to methamphetamine impairs learning of adult male rats. On the other hand, the maternal care of control mothers does not impair learning of rat pups prenatally exposed to methamphetamine. The present study demonstrates that cross-fostering may affect learning in adulthood.

Introduction

One of the most serious problems of the current and last century is drug-, alcohol- and nicotine-abuse. Most notably, drug-abuse has been getting more serious during the last few decades. Methamphetamine (MA) is one of the most frequently used "hard" drugs in the Czech Republic [1] and due to its anorectic effects it is one of the most common drugs abused by pregnant women addicted to drugs [2]. Further, MA crosses the placental barrier easily [3] and therefore it may affect the development of the fetus.

There are studies demonstrating that MA exposure during pregnancy can impair the development of neonatal central nervous system [4, 5]. Increased creatine metabolism in striatum [6] and deficiencies in visual recognition task, which are thought to rely upon hippocampal function [7] have been demonstrated after prenatal MA exposure in humans [8]. Both hippocampus and striatum are regions important in spatial learning and memory in humans and rodents [9, 10].

In rats, Acuff-Smith et al. [11] investigated the effect of MA administered at different times during gestation on cognitive functions of the progeny. The same authors found that higher doses (15 and 20 mg/kg) administered in early days of gestation impair spatial memory in Morris water maze (MWM), while lower doses (5 and 10 mg/kg) did not have any effect on cognition in adult offspring.

When injected to the mother postnatally, the newborns may receive this drug in mothers' breastmilk [12]. The hippocampus in rats is still developing during the PD 11–20 and this development is analogous to human hippocampal development during the third trimester of pregnancy [13]. The neonatal period may be more critical for the effects of MA on cognitive functions in rats than the prenatal period.

There are studies showing that postnatal maternal care plays an important role in the emotional and cognitive development of the offspring. Maternal care in early life is associated with differences in spatial learning and memory that endured even into later phases of aging [14]. This study showed that the maternal care during the first week of postnatal life influence hippocampal development and function. It has been investigated that the offspring of mothers, who exhibit a higher frequency of licking and grooming over the first week of postnatal life, show increased hippocampal synaptic density and enhanced spatial learning and memory [14].

Our previous study [15] demonstrated that impairing effect or prenatal MA exposure may be partially improved by good postnatal maternal care of control

"adoptive" mother. It is, however, unknown, whether this effect of maternal care is long-lasting. Because there are no studies available that would be investigating the effect of cross-fostering on cognitive functions in adulthood, the present study is the first to test the hypothesis that the cross-fostering modifies the prenatal effect of MA on learning of adult male rats.

Methods

Drugs

Physiological saline (0.9% NaCl) was purchased from Sigma (Prague, Czech Republic), d-Methamphetamine HCl was provided from Faculty of Pharmacy of Charles University in Hradec Králové (Czech Republic).

Mothers

Adult female albino rats (250–300 g) were purchased from Anlab farms (Prague, Czech Republic). Animals were housed in groups (4–5/cage) and left undisturbed for a week in a temperature-controlled (22–24 °C) colony room with a 12 h (light): 12 h (dark) cycle (lights on at 0600 h). Access to food and water was *ad libitum*. The procedures for animal experimentation utilized in this report was reviewed and approved by the Institutional Animal Care and Use Committee and is in agreement with the Czech Government Requirements under the Policy of Humans Care of Laboratory Animals (No. 246/1992) and with the regulations of the Ministry of Agriculture of the Czech Republic (No. 311/1997).

Drug administration

Females were randomly assigned to MA-treated (MA) or saline-treated (S) groups. Subcutaneous (s.c.) injection of MA (5 mg/kg) was administered daily approximately for nine weeks: about three weeks prior to impregnation, throughout the entire gestation period and for 23 days of lactation period (until the weaning) (for details see [16]). Saline (S) was injected s.c. at the same time and volume as MA. All females were weighed daily to see possible effects of MA treatment on weight gain during the period prior to impregnation and gestation.

Fertilization

Approximately three weeks after the drug administration, females were smeared by vaginal lavage to determine the phase of estrous cycle. At the onset of the estrus phase of the estrous cycle female rats were housed overnight with sexually mature stimulus males. There was always one female and one male in each cage. The next morning females were smeared again for the presence of sperms and returned to their previous home cages. The day after impregnation was counted as day 1 of gestation (see [17]). On day 21 of gestation, females were separated to maternity cages. The day of delivery was counted as postnatal day (PD) 0.

Pups and cross-fostering

On PD 1, litter sizes were adjusted to 12 pups. Pups were cross-fostered so that 6 pups (usually 3 males and 3 females) remained with their biological mother and the other (usually 3 males and 3 females) were assigned to the mothers with the opposite treatment. We obtained 4 experimental groups based on biological and fostering mother: (1) Group S/S: prenatally saline-exposed control animals fostered by their natural mother. (2) Groups MA/MA: prenatally MA-exposed offspring fostered by their natural mother. (3) Groups S/MA: prenatally saline-exposed control rats fostered by mother injected with MA. (4) Groups MA/S: prenatally MA-exposed rats fostered by saline-treated control mother. The offsprings fostered by MA-treated mothers were exposed to the effect of MA also postnatally from mothers' breast milk. On PD 1, prenatally MA-exposed pups were injected intradermally with black India ink in left foot pad and prenatally saline-exposed pups in right foot pad for identification. On PD 23, pups fostered by MA-treated mothers were ear punched in the left ear and pups fostered by saline-treated mothers in the right ear. Only one saline and one MA male were used from each litter to prevent litter effect. The rest of the animals were assigned for other experiments.

Morris water maze

In total 32 male offspring (8 per each treatment group) were tested in adulthood (PD 60–90) for learning in the MWM (blue circular tank, 2 m in diameter) filled with opaque water. On the rim of the pool, four starting positions were marked north (N), south (S), east (E), west (W), thus dividing the pool into four quadrants. A transparent circle platform (13 cm in diameter) 1 cm below the water surface was used for learning and memory tasks. The platform (placed in the N-E quadrant of the pool) was invisible for the swimming rats. Various pictures hanging on the walls were available to the rats as extra-maze cues. Rats' performance was tracked automatically using a video tracking system EthoVision 3.1 (Noldus Information Technology, Netherlands). Rats were tested over a 6 day period. Two types of tests were used in the present study: "Place navigation test" (Learning) and "Probe test".

In the learning test, which was performed on the first 5 consecutive days, an animal was supposed to find the platform within the limit of 60 seconds. The animal which was unable to find the platform within the time limit was guided to the platform manually. Each rat was exposed to 8 trials daily starting from 4 different positions. The position of the platform was the same in all trials. The rat remained on the platform for 30 seconds prior to next trial to have a chance to orient itself in the room. Latency to reach the hidden platform, length of the trajectory, search error (a measure of proximity to the escape platform) and speed of swimming were recorded. After finishing all trials in the experimental day, animal was dried by towel, placed in a dry holding cage for approximately 2 min and finally returned to its home cage.

In the probe test, which was administered on the 6th day, the platform was removed and the animal was left to swim in the pool for 60 seconds. The start position was north (N) for the probe test, thus in the location nearest to the platform. The following measures were recorded: speed of swimming; frequency and duration of presence in the quadrant where the platform in the learning test was located.

Statistical methods

Two-way ANOVA (Prenatal exposure × Postnatal exposure) with multilevel repeated measure (days × trials/day) was used to analyze the data from the "Place navigation test". Two-way ANOVA (Prenatal exposure × Postnatal exposure) was used to analyze the data from the "Probe test". Bonferroni test was used for post-hoc comparisons. Differences were considered significant if $p<0.05$.

Statistical data will be presented as [F (N-1, n-N) = xx.xx; $p<0.0x$], where F = test criterion of ANOVA, N-1 = degrees of freedom of groups, n-N = degrees of freedom of individual subjects, p = probability level.

Results

Place navigation test

In the latency to reach the hidden platform (Figure 1B), the main effect of postnatal exposure was found [F (1,28) = 9.01; $p<0.05$]; the animals postnatally exposed to MA had longer latencies than the animals postnatally exposed to saline, regardless of prenatal exposure. There was no main effect of prenatal exposure

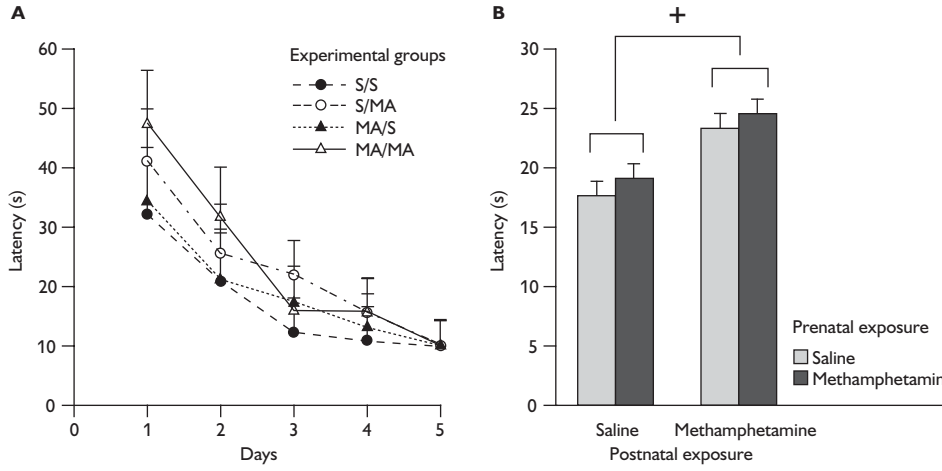

Figure 1 – Effect of prenatal and postnatal MA exposure on latency to reach the platform in Place navigation test. (A) Results are presented as averages of 8 trials per day in days 1–5. (B) Results are presented as average of all trials in five days. Values are means ± SEM (n=8). +p<0.05 Main effect of postnatal exposure; rats postnatally MA-exposed vs. postnatally saline-exposed (ANOVA; Bonferroni post-hoc test).

[F (1,28) = 1.09; p=0.31], no interaction between pre- and postnatal exposure [F (1,28) = 0.06; p=0.81] in the latencies. All animals, regardless of treatment, demonstrated learning ability over the 5-day test period as represented by a decrease in latency [F (4,112) = 111.18; p<0.0001] (Figure 1A).

In the search error (Figure 2B), the main effect of postnatal treatment in adulthood was demonstrated [F (1,28) = 7.53; p<0.05], such that all animals postnatally exposed to MA, regardless of prenatal exposure, had bigger search error than the animals postnatally exposed to saline. No main effect of prenatal exposure [F (1,28) = 1.50; p=0.23] or interaction [F (1,28) = 0.0001; p=0.99] was found in the search error. All animals, regardless of treatment, demonstrated

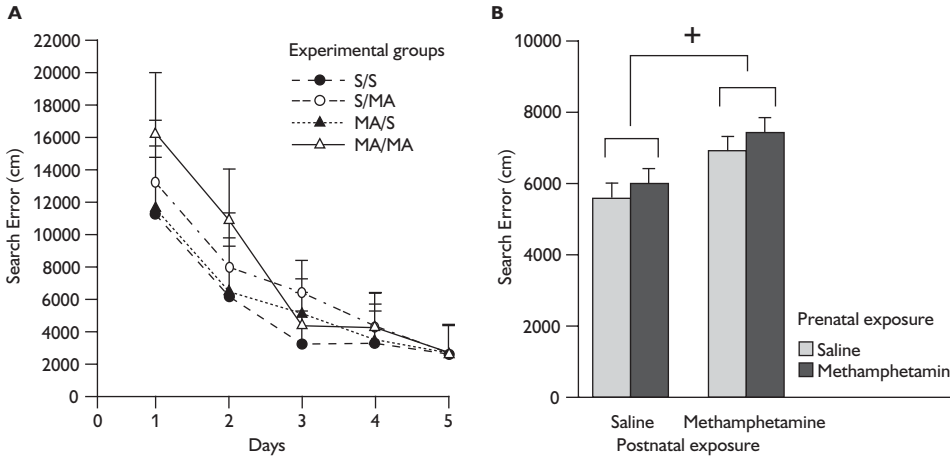

Figure 2 – Effect of prenatal and postnatal MA exposure on search error in Place navigation test. (A) Results are presented as averages of 8 trials per day in days 1–5. (B) Results are presented as average of all trials in five days. Values are means ± SEM (n=8). +p<0.05 Main effect of postnatal exposure; rats postnatally MA-exposed vs. postnatally saline-exposed (ANOVA; Bonferroni post-hoc test).

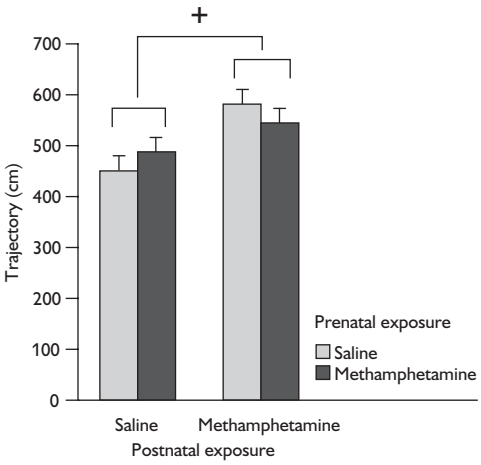

Figure 3 – Effect of prenatal and postnatal MA exposure on trajectory in Place navigation test. Results are presented as average of all trials in five days. Values are means ± SEM (n=8). +p<0.05 Main effect of postnatal exposure; rats postnatally MA-exposed vs. postnatally saline-exposed (ANOVA; Bonferroni post-hoc test).

learning ability over the 5-day test period as represented by a decrease in search error [F (4,112) = 100.48; p<0.0001] (Figure 2A).

In the length of the trajectory (Figure 3), the main effect of postnatal treatment in adulthood was shown [F (1,28) = 4.51; p<0.05]; the animals postnatally exposed to MA had longer trajectories than the animals postnatally exposed to saline, regardless of prenatal exposure as shown in Figure 3. There was no main effect of prenatal exposure [F (1,28) = 0.005; p=0.95], no interaction between pre- and postnatal exposure [F (1,28) = 0.71; p=0.41] in the trajectories. The length of the trajectory did not change with the days of learning [F (4,112) = 0.37; p=0.83].

In the speed of swimming (Figure 4), the main effect of prenatal treatment in adulthood was demonstrated [F (1,28) = 13.47; p<0.05], such that all animals prenatally exposed to MA, regardless of postnatal exposure, swam slower than the animals prenatally exposed to saline. The swimming speed did not change with the days of learning [F (4,112) = 1.35; p=0.26].

Probe test

For the frequency [F (1,28) = 0.98; p=0.43] and duration of presence in the quadrant [F (1,28) = 1.37; p=0.26] with the hidden platform no effects were demonstrated. For the speed of swimming, there was main effect of prenatal treatment [F (1,28) = 5.90; p<0.05]; animals prenatally exposed to MA swam slower than prenatally saline-exposed control animals (data not shown).

Discussion

The aim of the present study was to determine the effect of cross-fostering on learning of adult male rats prenatally exposed to MA tested in the MWM.

First, our results show that prenatal MA exposure at a dose of 5 mg/kg did not affect the latency, search error and length of the trajectory in "Place

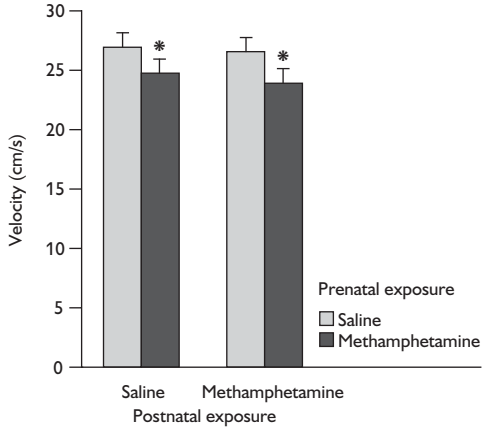

*Figure 4 – Effect of prenatal and postnatal MA exposure on speed of swimming in Place navigation test. Values are means ± SEM (n=8). *p<0.05 vs. Animals prenatally exposed to saline (ANOVA; Bonferroni post-hoc test).*

navigation task". This finding is in agreement with the work of Schutová et al. [18], who showed that low dose of MA (5 mg/kg) administered prenatally did not impair learning in the MWM. There is other study showing that prenatal MA (5 or 10 mg/kg) exposure did not have an effect on spatial memory in the MWM [11]. However, they further demonstrated that prenatal exposure to higher doses of MA (15, 20 mg/kg) did induce impairments of spatial memory in MWM tested in adulthood. We use the low dose of MA (5 mg/kg) in our studies, since application of MA at 5 mg/kg to pregnant female rats induces changes that are comparable with those found in fetuses of drug-abusing women [11].

Second, the data demonstrate that the postnatal MA exposure from mothers' breastmilk in preweaning period (PD 1–23) had long-term effects on spatial learning when assessed in adulthood by using the MWM. The animals fostered by MA-treated dams (MA/MA and S/MA) demonstrated worse spatial learning when compared to animals fostered by saline-treated mothers (MA/S and S/S). The length of trajectory, cumulative distance (search error) from the platform and the latencies in reaching the platform was affected by postnatal exposure. These parameters are indicators of spatial learning ability as shown in the works of Gallagher et al. [19] and Lindner [20].

The reasons may be two: (1) It may be due to no postnatal MA administration during lactation. While MA/MA and S/MA animals received MA postnatally, the MA/S animals only prenatally. (2) It may be that the MA/S animals were fostered by saline-treated control mother. Their maternal care does not impair the prenatal effect of MA.

We have data showing that MA (5 mg/kg) in gestation and/or lactation periods impairs maternal behavior [16]. Specifically, it attenuates active nursing and other maternal activities, such as mother being in the nest, in contact with pups, carrying and grooming pups and a nest building. Accordingly, we showed that control mothers cared about the pups more than MA-treated mothers and this "better care" was independent on the fact that some of the pups were their own and some were adoptive [16].

The fact that better maternal care may improve the development of pups is supported by studies of others [14, 21–23]. There are studies showing that maternal licking and grooming is a major source of tactile stimulation for the developing pup, which affects somatic growth and neural development [24] and during the first week of postnatal life influences hippocampal development and function [14]. It has been investigated that the offspring of mothers, who exhibit a higher frequency of licking and grooming over the first week of postnatal life, show increased hippocampal synaptic density and enhanced spatial learning and memory [14]. It is known that the hippocampus in rats is still developing during the PD 11–20 and this development is analogous to human hippocampal development during the third trimester of pregnancy [13], the neonatal period may be more critical for the effects of MA on cognitive functions in rats than the prenatal period.

Third, we found that all animals prenatally exposed to MA, regardless of postnatal exposure, swam slower than the animals prenatally exposed to saline. There are no studies investigating the effect of prenatal exposure to MA on speed of swimming (locomotion activity) in the MWM. However, there is a study showing the effect of prenatal exposure to MA on locomotion activity tested in the Open field. Weissman et al. [25] demonstrated that chronic in utero MA treatment (10 mg/kg) caused decrease in square crossing and rearing in the Open field. The other reason is, that swimming velocity can also be used to measure motivation to find the hidden platform [26]. Therefore, the decreased swimming velocity suggests that MA/S and MA/MA animals had decreased motivation. To confirm this hypothesis more studies would be necessary.

Fourth, we found no differences between experimental groups during the probe test. The measures commonly used to assess performance in probe test are designed to reflect the spatial bias of an animal's search pattern. These data suggest that all experimental groups had comparable navigational abilities.

Conclusion

In conclusion, the present study investigated the effect of cross-fostering on learning of adult male rats tested in the MWM. Taken together, our results show that prenatal exposure to MA at dose as low as 5 mg/kg does not impair learning in the MWM, while postnatal exposure to MA from mothers' breastmilk and their worse maternal care impairs learning of adult male rats. On the other hand, the maternal care of control mothers does not affect learning of rat pups prenatally exposed to MA. Our hypothesis, that the cross-fostering may affect learning of adult male rats, was confirmed. However, in contrast to our previous study [15] the role of maternal care on learning abilities in adulthood remains unconvincing. More studies are necessary to test the long-term effects of the positive effect of maternal care on prenatal drug exposure.

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