Impact of Methamphetamine Administered Prenatally and in Adulthood on Cognitive Functions of Male Rats Tested in Morris Water Maze

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Abstract: Studies showed that stimulant drugs that affect the monoaminergic system alter both behavioral and cognitive processes. The aim of the present study was to investigate the impact of prenatal and acute methamphetamine (MA) exposure on cognitive functions of adult male rats tested in Morris water maze (MWM). We tested adult male rats prenatally exposed to MA (5 mg/kg), saline or no injection. Half of the animals were injected daily with MA (1 mg/kg) after finishing the testing. All injections were administered subcutaneously. Three types of tests were used: (1) “Place navigation test” (Learning), (2) “Probe test” (Probe) and (3) “Retention memory test” (Memory). Our results showed that prenatal MA exposure did not affect the test of learning and the Probe test. In the test of memory prenatally MA-exposed rats had lower latencies than animals prenatally exposed to saline. Further, acute MA administration increased the speed of swimming in all rats regardless of prenatal drug exposure and the type of test and, however, the increase in the speed was significantly greater in rats prenatally exposed to MA than in rats without any prenatal exposure. In addition, acute MA application significantly prolonged trajectories in the Place navigation test. The present study thus demonstrates that: (1) Prenatal MA exposure does not affect learning in the MWM. (2) Prenatal MA exposure increases the sensitivity to acute drug injection. (3) Acute MA application impairs learning in the MWM.

Introduction
In recent years, methamphetamine (MA) is becoming a more “popular” street drug in many countries of the world because of its relatively uncomplicated production and low price compared to cocaine or heroin [1]. Approximately half of MA users are women, mostly of reproductive age, and consequently some percentage of them become pregnant while using the drug. Since MA readily crosses the placenta, such cases will result in intrauterine exposure. The long-term consequences of these exposures are relatively unknown [2]. Clinical data suggest that transplacental exposure to MA may be detrimental to developing embryo, particularly the central nervous system since altered neonatal behavioural pattern, reduced alertness, tactile-elicited dystonia, and visual and motor difficulties have been found in neonates [3, 4]. Experimental studies shown that prenatal MA exposure results in increased offspring mortality, decreased growth rate, delayed development of physical characters, eye opening and functional reflexes [5, 6].

There are studies demonstrating increased creatine metabolism in striatum [3] and deficiencies in visual recognition task [7], which is thought to rely upon hippocampal function [8], after prenatal MA exposure. Both hippocampus and striatum are regions important in spatial learning and memory in humans and rodents [9–11]. However, studies existing on the impact of MA on cognition are rather inconsistent. Acute MA was shown to produce improvements in cognitive processing when given to drug-naive subjects [12]. In contrast, more recent studies
have shown that long-term MA use is associated with impaired performance on a number of cognitive tasks [13–15].

In rats, Acuff-Smith et al. [5] investigated the effect of MA administered at different times during gestation on cognitive functions of the progeny. They [5] found that high doses (15 and 20 mg/kg) administered in early days of gestation impair spatial memory in the Morris water maze (MWM), while lower doses (5 and 10 mg/kg) did not have any effect on cognition in adult offspring. In addition, Williams et al. [2] demonstrated that repeated administration of MA (5, 10, 15 mg/kg) on postnatal days 11–20 induced impairment in spatial learning and memory in the MWM after the offspring reached adulthood.

There are also studies showing sensitization after repeated administration of psychostimulants (amphetamine, cocaine), such as a progressive increase in their psychomotor activating effects [16–18]. Furthermore, it has been reported that in mice exposed to MA prenatally, there is an intensification of adult MA-induced monoamine neurotoxicity [19], suggesting an increased susceptibility to the drug from prior exposure. Vorhees et al. [20] also showed altered responsivity to later pharmacological challenge in rats exposed neonatally to MA. There are, however, no studies investigating possible sensitizing effect of prenatal MA exposure.

The present study was therefore designed to investigate 3 objectives: (1) to find the effect of prenatal MA exposure on cognitive functions of adult male rats, (2) to find the effect of acute MA application on cognitive functions of adult male rats, (3) to determine whether prenatal MA exposure changes sensitivity to acute MA injection in adulthood.

**Materials and methods**

Adult female Wistar rats (250–300 g) from Anlab farms (Prague, Czech Republic) were randomly assigned to MA-treated, saline-treated or control group. MA-treated females were injected subcutaneously (s.c.) with D-methamphetamine HCl in a dose of 5 mg/kg through the entire gestation (i.e. from the first to the last days of gestation) [21]. Saline-treated dams were administered saline s.c. at the same time and volume as MA. Control females were not exposed to any injection. Two control groups (i.e. saline and control) were used to differentiate the possible effect of injection-induced stress. The day of delivery was counted as PD 0. On PD 1, MA-exposed pups were injected intradermally with black India ink in the left foot pad and saline-exposed pups in the right foot pad for identification. Control pups were not tattooed. The number of pups in each litter was adjusted to 12. Whenever possible, the same number of male and female pups was kept in each litter. On PD 21, animals were weaned and housed in groups, separated by sex. Animals were left undisturbed until adulthood.

Adult male rats (PD 60–90) were tested for learning and memory 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. Various pictures hanging on the walls were available to the rats as extra-maze cues. Rats were tested for 12 consecutive days.

To determine the effect of acute MA half of the animals from each prenatally exposed group (i.e. MA, saline, control) were administered MA (1 mg/kg) s.c. after finishing testing each day, while the other half were not exposed to any acute drugs. On the days 7–11 when no tests were performed, MA was administered at approximately the same time as in the other days. Three types of tests were used in the present study: “Place navigation test” (Learning), “Probe test” (Probe) and “Retention memory test” (Memory). 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 unable to find the platform within the limit was placed on the platform manually. Each rat was exposed to 8 trials daily starting from 4 different positions with intertrial interval (ITI) of 30 seconds. In the probe test the platform was removed and the animal was left to swim in the pool for 60 seconds. In the memory test the rat should find the platform located in the same position as in the learning test. Each rat was exposed to 8 trials starting from 4 different positions with ITI of 30 seconds.

Rats’ performance was tracked automatically using a camera located above the pool and video tracking software EthoVision (Noldus). Latency to reach the hidden platform, length of the trajectory and speed of swimming were recorded in the tests of learning and memory. Frequency and duration of presence in the quadrant where the platform was located in the learning and memory tests and speed of swimming were recorded in the “Probe test”.

The procedures for animal experimentation utilized in this report were 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).

Two-way ANOVA (Prenatal treatment × Acute treatment) with multilevel repeated measure was used to analyze the data from the test of learning. Two-way ANOVA (Prenatal treatment × Acute treatment) repeated measure was used to analyze the data from the test of memory. Two-way ANOVA (Prenatal treatment × Acute treatment) was used to analyze the data from the “Probe test”. Fisher LSD 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

For latency to reach the hidden platform, no main effects of Prenatal treatment and Acute treatment were found. For the length of the trajectory the main effect of Acute treatment was demonstrated \( [F (1,65) = 5.37; p<0.05] \), the animals with acute MA application had longer trajectory than the animals without acute MA application, regardless of prenatal treatment as shown in Figure 1. All animals, regardless of treatment, demonstrated learning over the 5-day test period as represented by a decrease in latency \( [F (4,260) = 202.80; p<0.0001] \) and trajectory length \( [F (4,260) = 162.18; p<0.0001] \). Main effect of Acute treatment for swimming speed was found \( [F (1,65) = 10.58; p<0.01] \), such that acute MA-treated animals were faster than the animals without acute MA. Although for the swimming velocity the interaction between Prenatal treatment and Acute treatment was not significant, post-hoc test revealed, that the increase of swimming speed after acute MA application was significantly greater in the rats prenatally exposed to MA than in the rats without any prenatal exposure \( [F (2,65) = 0.74; p<0.05] \) as shown in Figure 2.

Probe test

For frequency and duration of presence in the quadrant with the hidden platform, no main effects or interactions were demonstrated. For the speed of swimming, Acute treatment was the only significant main effect \( [F (1,65) = 8.47; p<0.01] \).
Reference Memory test
Analysis of the data showed significant main effect of Prenatal treatment for latency \( [F (2,65) = 3.76; p<0.05] \). Post-hoc test showed, that all animals with prenatal exposure to MA had significantly shorter latencies than the animals prenatally exposed to saline (Figure 3). Further, significant main effect of Acute treatment for the speed of swimming was demonstrated \( [F (1,65) = 21.60; p<0.001] \), such that all animals treated with acute MA, regardless of prenatal exposure, swam faster than the animals without acute MA application. No other main effects or interactions were found for the recorded measures in this test.

Discussion
Our findings from this experiment are as follows. First, we found that prenatal MA exposure at dose as low as 5 mg/kg did not influenced learning in Place navigation task. This finding is in agreement with the work of Acuff-Smith et al. [5] who also showed that low dose of MA did not have effect on spatial memory in the MWM.

Second, surprisingly, rats with prenatal MA exposure had shorter latencies than rats with prenatal exposure to saline in the Retention memory test. It was shown [22, 23] that placebo injection of saline administered to control pregnant mothers may induce mild stress for the rat and that way indirectly affect the development of her pups. Furthermore, high concentrations of glucocorticoids are known to produce cell death in the dentate gyrus of neonatal animals [24]. Therefore the animals after saline application may exhibit alterations in hippocampal function. Moreover, animals with prenatal saline exposure may have changed reactivity to stress later in life. Morris recognized that the water maze task has an aversive component to it [25] and in agreement with this fact, adrenal activation and release of corticosterone following training in the MWM were observed [26, 27]. MA was also shown to protract activation of the hypothalamic-pituitary-adrenal (HPA) system during the neonatal period, which may induce long-term changes in the stress response when the animals are adults [28]. Therefore, to explain the effects

![Figure 3 – Effect of Prenatal MA exposure on latency in Reference memory test. Values are means ± SEM (n = 24). +p < 0.05 vs. prenatally saline-exposed animals.](image-url)
of MA on memory in the MWM, more studies concerning the functioning of HPA system during the tests in the MWM after prenatal exposure to saline and MA are necessary.

Third, we found that acute MA application increased the speed of swimming in all animals regardless of prenatal exposure and the type of test. However, the increase in the speed after acute MA application was significantly greater in rats prenatally exposed to MA than in rats without any prenatal exposure. Thus, rats prenatally exposed to MA were more sensitive to acute MA application than rats without any prenatal exposure. This finding is consistent with studies showing sensitization effects of psychostimulant drugs [18] on central nervous system.

Fourth, rats with acute MA application had longer trajectories than the rats without acute drug administration, regardless of prenatal exposure, although acute MA treatment had no significant effect on the latency in Place Navigation Test. Not only that trajectory length has been shown to be a better index of spatial learning than latency [29], but also the fact that animals with acute MA treatment achieved increased swimming velocities indicates that acute MA application impaired learning in the MWM. The animals with acute MA treatment found the hidden platform as fast as the animals without acute MA, because they swam faster, but their trajectory when searching for the platform was longer. However, this finding is in disagreement with the study of Brown et al. [30] who studied the effects of amphetamine on performance in the MWM and demonstrated that acute amphetamine administered immediately after finishing trial facilitated MWM performance and that this facilitation may be mediated by dopaminergic system and dopamine synthesis in the prefrontal cortex. As MA use has been shown to damage both dopaminergic and serotonergic systems [31, 32], more studies investigating particularly these systems after acute MA application in the MWM are necessary to explain the discrepancies.

Conclusion
This study demonstrates that prenatal exposure to MA at dose as low as 5 mg/kg does not impair learning in the MWM, and that rats prenatally exposed to MA perform better in the Retention memory test than rats prenatally exposed to stress. In contrast, we found that acute MA application impairs learning in the MWM. Finally, our study shows that prenatal MA exposure increases sensitivity to acute treatment with the same drug in the adulthood. The last finding is in agreement with the studies concerning behavioural sensitization, which is supposed to produce compulsive patterns of drug-seeking behaviour. As there are no studies investigating the impact of prenatal MA exposure on behavioural sensitization, our present study will contribute to further knowledge of this problem. However, another studies, especially on HPA, dopaminergic and serotonergic systems after prenatal MA exposure must be done to fully understand its long-term effects on cognitive and behavioural processes.
References


Impact of Methamphetamine Administered Prenatally and in Adulthood
Precision of Marginal Adaptation of the Incisor and Molar Procera® AllCeram Crown Copings

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Abstract: The primary objective of this in vitro study was to compare the absolute marginal discrepancy (AMD) of CAD/CAM produced Procera® AllCeram crown copings, fabricated on die stone master models of two different tooth groups, incisor and molar. Two maxillary central incisors and two first molars typodont teeth were prepared with 0.8 mm of circumferential chamfer, duplicated 9 times to obtain 36 die stone models and allotted into three groups of 12 models (incisors = 6 & molars = 6). Procera® AllCeram 0.6 mm copings were fixed with zinc phosphate (AZ), glass ionomer (AG) and resin (AR) cement accordingly under 50 N static finger force. The AMDs were measured using the scanning electron microscope (SEM) on four axial walls with 4 measurements on each wall to obtain 16 readings for one tooth. Statistical analysis of the data was performed using the non-parametric test of Kruskal-Wallis and Mann-Whitney test. The analysis did not find any significant differences in the mean AMD of incisor and molar crown copings, and in different axial surfaces too ($p\leq0.05$). Recorded mean AMD of incisor copings were AZ group 59 µm, AG 37.9 µm, and AR 44.4 µm and molar copings were AZ 48.8 µm, AG 27 µm, and AR 50.2 µm. It can be concluded that AMD of Procera® AllCeram copings were within accepted level of 100 µm. Incisors showed higher AMD than molars. Molars demonstrated the higher AMD on mid-distal and mid-lingual surfaces whereas for incisor it was mid-buccal and mid-lingual surface.

Introduction
Aesthetically oriented modern dentistry frequently utilizes high strength, biocompatible all-ceramic materials to satisfy the clinical demand of a patient. However, all these materials have to meet with three important criteria like high fracture resistance, aesthetics and marginal fit [1] for long-term survival in complex oral environment. The marginal fit of the all-ceramic systems can be a critical factor to its long-term success. Inaccurate marginal adaptation is potentially detrimental to the tooth and the supporting periodontal structures [2, 3, 4]. Recent introduction and popularity of CAD/CAM designing and production of substrutures for fixed partial dentures have minimized the human errors during production stage which can influence the marginal fit of the restorations [5]. A well accepted and popular among a plethora of CAD/CAM based all-ceramic system is Procera® AllCeram system (Procera®, Gothenberg, Sweden) which was introduced in 1993 by Andersson and Oden [6].

Procera® is a versatile CAD/CAM system, with in-office facility to digitisation of tooth preparation model using contact scanner with an accuracy of ± 10 µm [7] and custom designing the substructure of FPD using user friendly software program. These data are transferred to the production centre at Nobel Biocare, Gothenberg, Sweden. In production centre, using CAD/CAM technology, duplicate of die is produced with 12–20 % enlargement to compensate the sintering shrinkage of Al₂O₃. Procera® AllCeram copings are fabricated from 99.9 % densely