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Do the effects of prenatal exposure and acute treatment of methamphetamine on anxiety vary depending on the animal model used?

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HIGHLIGHTS

- Prenatal methamphetamine exposure has complex effects on development of anxiety.
- The effect of prenatal methamphetamine exposure on anxiety is modified by drug treatment in adulthood.
- The approach-avoid conflict seems to be the most prominent parameter describing fear that is affected by methamphetamine treatment.

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ABSTRACT

The aim of the present study was an evaluation of prenatal exposure to acute methamphetamine (MA) treatment on manifestations of anxiety. Anxiety was evaluated in adult animals in three different experimental models: the Elevated plus-maze (EPM), Social interaction test (SIT) and Ultrasound vocalization (USV). Female rats were administered saline (S) or MA (5 mg/kg) daily throughout their entire gestation period. The male progeny, in adulthood, were administered with challenge dose of S or MA (1 mg/kg) prior to evaluation of anxiety. The study demonstrated that prenatal MA exposure increased the anxiogenic effect on evaluated behaviour patterns in the USV model and to a lesser degree in the EPM model. In addition, the acute MA challenge in adulthood decreased the time spent during social interaction suggesting an anxiogenic effect in the SIT model as well. On the other hand, some of the evaluated parameters (e.g. the number of head-dipping in the EPM and number of dropped boluses in the SIT) also suggest MA-induced anxiolytic effects. Sensitization to a MA challenge was apparent in several parameters of the EPM (e.g. increased number of entries to the closed arms, increased stretched attend postures and increased approach-avoid conflicts) and SIT (total social interaction and following). The present data demonstrate that prenatal MA exposure and adult challenge of the same drug have diverse effects on animal behaviour that depends on the type of anxiety model used.

1. Introduction

In general, stress and drug addiction are closely connected. Either acute or chronic stress can be a reason for drug abuse and drug abuse can act as a chronic stressor [57]. Psychostimulants have been shown to affect a variety of behaviour patterns in humans [58,59] as well as in behaviour patterns in laboratory animal models of psychostimulant abuse [23,25]. Long-lasting alterations in emotional states such as fear, anxiety, social receptivity, depressive symptoms, as well as memory deficits have been demonstrated in laboratory rats repeatedly given psychostimulants [24,33,40,43,53,65,71]. These results match long-term changes reported in human studies [10].

Methamphetamine (MA) is one of the most addictive psychostimulant drugs, which is linked to a high potential for abuse. It is also one of the most frequently used "hard" drugs in the Czech Republic [68] and due to its anorectic effects, it is

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one of the most commonly abused drugs among women, even during pregnancy [32]. Previous studies [1,53,54,64,70] demonstrated that prenatal MA (5 mg/kg) exposure changes behaviour in animals and reduces adaptability to new environments in adulthood. In addition, it has been shown that prenatal MA exposure increases sensitivity to the same drug in adulthood [53,54]. Specifically, prenatally MA-exposed animals that received challenge doses of MA in adulthood displayed higher locomotion and exploratory rearing activity relative to control animals; this was found to correspond with dopamine levels in the nucleus accumbens [8]. These findings may be considered as prenatally induced long-term sensitization similar to that described in adulthood [49].

Experimental models of anxiety as well as animal models of other psychiatric disorders have been classified into five categories, by Gerlai et al. [21], based on induced changes in CNS functions: (1) models of anxiety induced by the presence of anxiogenic stimulus from the surroundings, e.g. presence of a predator (or a stimulus resembling a predator), pain stimuli or stimuli linked to an unknown environment; (2) models of anxiety induced by chemicals or hormones; (3) models created by genetic manipulations; (4) models of anxiety resulting from spontaneous mutations – inbred animals strains; and (5) models that use invasive techniques, e.g. electric stimulations of certain brain structures or other surgical techniques.

The most widely used category is the first, in which anxiety is induced by external stimuli from the surroundings. Three of the models from this category were used in the present study: (1) Geller-Seifter conflict – Elevated plus maze test (EPM) [44,50]; (2) Conditioned emotional response – Social interaction test (SIT) [19]; and (3) Fear potentiated startle reaction – Ultrasound vocalization test (USV) [20,51].

Psychostimulant drugs have been previously tested in these animal models of anxiety, with inconsistent findings. In the EPM test, acute and chronic exposure to psychostimulants has been shown to have both anxiogenic [4,15,17,24,39] as well as anxiolytic effects [13,54]. In the SIT, psychostimulant drugs usually displayed anxiogenic effect in the form of decreased social interaction (SI) indicating increased anxiety [11,19,36,66], which was also observed in our previous studies [62,63] showing that MA administration impairs SI in dose-, stress condition-, and sex-specific manners. As far as the USV test is concerned, there are number of studies showing anxiolytic effects of psychostimulants on USVs in young animals [2,3,35,41], while withdrawal from the drug intake has the opposite, anxiogenic effect [12]. Specifically, a recent study by Manduca et al. [31] demonstrated decreased social play and increased USVs in young rats, suggesting that amphetamine treatment has anxiogenic effects. In adult rats, psychostimulants have been also shown to increase vocalization, thereby to have anxiogenic effects [56]. Based on all mentioned findings, the effect of psychostimulants on anxiety is suggested to be drug-, dose-, timing of administration-, and animal model-specific.

Most of the above cited studies have demonstrated the effect of acute as well as chronic psychostimulant drugs exposure in postnatal life. To the best of our knowledge, ours is the only study examining the long-term effects of prenatal MA exposure on the manifestation of anxiety in adulthood. Therefore, the aim of this study was to investigate the effect of prenatal MA exposure on anxiety in adult offspring (following an acute MA challenge) using three different models of anxiety, the EPM, SIT, and USV. The novelty of the present study is its investigation of prenatal MA exposure on anxiety manifestations in adulthood using three different animal anxiety models.

2. Methods

The procedures for animal experimentation in this study were reviewed and approved by the Institutional Animal Care and Use Committee and were in agreement with the Czech Government Requirements under the Policy of Human Care of Laboratory Animals (No. 246/1992) and with subsequent regulations from the Ministry of Agriculture of the Czech Republic.

2.1. Animals and drug injections

Adult female and male albino Wistar rats (375–400 g) provided by Charles River Laboratories International, Inc. were delivered by AnLab (Prague, the Czech Republic). Animals were housed four per cage by sex and left undisturbed for a week in a temperature-controlled (22–24 °C) colony room with free access to food and water on a 12 h (light):12 h (dark) cycle with lights on at 06:00 h. Females were impregnated as described in our previous study [60]. In total, 24 dams were randomly assigned to either the MA-treated or saline-treated group. On gestational day (GD) 1 the daily injections of MA or saline started and continued until the day of delivery, which usually occurred on GD 22. D-methamphetamine HCl (Sigma–Aldrich, the Czech Republic) was diluted in distilled water in concentration of 5 mg/ml and injected subcutaneously (s.c.) in a volume of 1 ml/kg; saline was injected s.c. at the same time and volume as MA.

The day of the delivery was indexed as postnatal day (PD) 0. On PD 1, pups were weighed, tattooed for identification, and cross-fostered (for detailed information see [30,60]). The pups were cross-fostered in such a way that each of the 24 mothers received and raised 12 pups – 6 of which had been prenatally exposed to MA and 6 to saline. Whenever possible, the number of male and female pups raised by a dam was equal. On PD 21, pups were weaned and group-housed by sex (4 males per a cage and 5 females per a cage). Animals were left undisturbed until adulthood. In total 144 male rats were used in the present study (n = 8–12 per individual experiment). In order to avoid litter effects, one male rat from the MA- or saline-exposed group from each litter was used in individual experiments (EPM, SIT, and USV). The rest of the animals were used in experiments that were a part of another study.

Individual animals were subjected to only one of the three anxiety tests. Forty-five minutes prior to testing, animals were injected with either a challenge dose of MA (1 mg/ml/kg) or saline (1 ml/kg). The dose of MA was chosen based on our previous studies [55,62] because this dose does not induce stereotypical behaviours. The timing of the drug application was also chosen based on our previous study [48] that showed that peak MA level in the brain (not in the blood) occurred between the 45th and 60th minute after administration. Thus, based on prenatal drug exposure and the challenge treatment, the animals were divided to 4 experimental groups: Prenatally MA-exposed rats treated with saline (MA/S) or MA (MA/MA) in adulthood and prenatally saline-exposed rats treated with saline (S/S) or MA (S/MA) in adulthood.

2.2. Elevated plus maze (EPM)

In total, 32 adult male rats were tested in the EPM (n = 8 rats/group). The same method was used as in our previous study [46], which was a modified protocol of Fernández Espejo [16]. All animals were habituated to the laboratory environment and the experimenter during the 3 days prior to the experiment [22]. The EPM test was performed 45 min after the acute MA (1 mg/kg) or saline injection. At the beginning of the test an animal was positioned on the centre square of plus maze with the animal's nose pointing toward one of the closed arms. Animal behaviour in the EPM was video-recorded for five minutes.