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How methamphetamine exposure during different neurodevelopmental stages affects social behavior of adult rats?

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ABSTRACT

Social behavior involves complex of different forms of interactions between individuals that is essential for healthy mental and physical development throughout lifespan. Psychostimulants, including methamphetamine (MA), have neurotoxic effect, especially, if they are targeting CNS during its critical periods of development. The present study was aimed on evaluation of changes in social interactions (SI) following scheduled prenatal/neonatal MA treatment in combination with acute application in adulthood. Eight groups of male and eight groups of female rats were tested in adulthood: rats, whose mothers were exposed to MA (5 mg/ml/kg) or saline (SA, 1 ml/kg) during the first half of gestation (ED 1–11), the second half of gestation (ED 12–22) and neonatal period (PD 1–11). To do this, we compared indirect neonatal applications via the exposed dams with group of rat pups that received MA or SA directly through injections. In adulthood, half animals from each group were injected with MA (1 mg/kg), second half with saline 45 min prior to the Social Interaction Test. Females and males were observed for social and nonsocial activities of two unfamiliar individuals of the same sex and treatment in a familiar Open field arena. The present study demonstrated that prenatal/neonatal MA exposure leads to decrease the time spent in genital investigation, following and nonsocial activity. Acute dose of MA leads to a decrease in all SI patterns and to an increase in nonsocial activities relative to acute SA. Females were more active than males. Animals exposed to prenatal/neonatal treatment during the second half of gestation (ED 12–22) and throughout lactation period (PD 1–11 indirect/direct) had fewer SI and greater exploratory behavior than animals exposed during the first half of gestation (ED 1–11).

1. Introduction

Methamphetamine (MA), a potent psychostimulant drug, primarily releases dopamine (DA), serotonin (5-HT), and noradrenalin (NA) by the central nervous system (CNS) and then blocks their reuptake from synapses as well as into synaptic vesicles [28,44]. These monoamines regulate maturation of dendrites and synaptogenesis during brain development, and in adulthood influence brain activity [15,43]. Based on a study by Clancy et al. [8], we created a rat model of MA exposure for the main stages of prenatal development in humans: to simulate exposure during the first and second trimester of a human pregnancy, rats were administered to MA during the first half (ED 1–11) and second half (ED 12–22) of gestation, respectively. To stimulate exposure during the third trimester in humans, rats were administered MA in the lactation period (PD 1–11). Due to these facts, the primary goal was to determine the effects of prenatal/neonatal MA exposure (5 mg/ml/kg) during different neurodevelopmental stages on social and non-social activity of adult male and female rats used the Social Interaction Test (SIT) [14].

Social behavior involves a complex of different forms of interactions between individuals that is essential for healthy mental and physical development throughout the lifespan [47,56]. Lasting alterations have been demonstrated also in a variety of social interactions (SI) in rats given psychostimulants in adulthood [3,52]. Acute and chronic MA abuse has been shown to impair SI in a dose-, stress-, and hormone-specific manner [35,52–54]. MA (0.5, 1.0 and 1.5 mg/ml/kg) dose-dependently reduces SI [53,54]. Environmental conditions (familiarity with the experimental arena and light intensity) influence performance of animals in an Open field (OF). An unfamiliar environment leads to decreased social behavior and increased non-social activities (locomotion and exploratory behavior) in rats [53,54]. For these reasons the secondary goal of the study was to determine how an acute MA treatment (1 mg/ml/kg) in adulthood affects SI and nonsocial activities of rats exposed prenatally/neonatally to the same drug.

Animal behavioral studies have shown sexual dimorphism in social behavior and in response to drug [7,42]. Females are significantly more

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active in OF experiments than males after exposure to psychostimulants [22,46,54]. The tertiary goal of the present study was to determine potential sex dimorphic effects of prenatal/neonatal MA exposure as well as of acute treatment in adulthood.

Different brain region matures at different developmental periods. Therefore the timing of MA exposure during different stages of gravidity may influence different brain structures that develop during the time of drug exposure. Prefrontal cortex is an important regulator of behavior, which is developed between ED 12 to the postnatal period (day 20 in rats) [24,29]. It has been shown that psychoactive drugs differently change neuron connectivity in specific prefrontal cortex regions, which may lead to abnormal functioning. The mesocorticolimbic DA system play a key role in these regions in mediating interactions between drug effects and social activities [9,19]. The first dopaminergic neurons of this system are seen on ED 13 [41]. Both, short-, and long-term changes in these structures may modify animal social behavior [19]. So the novelty of the present study is to investigate which of the trimesters (application period) is the most sensitive to MA on SI of rats.

It is well known, that MA has relatively high lipid solubility, it easily crosses not only blood-brain and placenta barriers, but it is also secreted in maternal breast milk during lactation. It means that the suckling pups may receive MA in mother's breast milk [49] which may result in potential impairing of fetus postnatal development [20,21,30,55]. Therefore, the fourth goal of the present study was investigated the effect on social and non-social behavior of exposed offspring via the breast milk. In order to do so, we compared the results with the group of pups that received MA directly through injection, while mothers were discontinued application of MA after birth.

Thus, eight groups (see Fig. 1) were used to test SI behaviors as well as non-social activities (locomotion and exploratory behavior) between two unknown rats in a familiar environment similarly as in our previous studies [20,21].

2. Methods

The procedures used in this study were reviewed and approved by the Institutional Animal Care and Use Committee and they are in agreement with the Czech Government Requirements under the Policy of Humans Care of Laboratory Animals (No. 86/609/EEC) and with the subsequent regulations of the Ministry of Agriculture of the Czech Republic.

2.1. Animals

Adult female (250–300 g) Albino Wistar rats were purchased from Velaz (Prague, Czech Republic, bread by Charles River Laboratories International, Inc.) and housed 4–5 per cage in temperature-controlled (22–24 °C) colony room with a standard 12 h light/dark cycle (lights on at 06.00 h). Prior to testing, animals were left undisturbed for 1 week with food and water ad libitum. After the acclimation period, the females were weighed and smeared by vaginal lavage to determine the phase of their estrous cycle. Females at the onset of the estrous phase of the estrous cycle were housed overnight with a sexually mature male. There was always one female and one male per cage [51]. On the following day, the females were smeared for the presence of sperm and returned to their home cage. Fertilization was designated Day 1 of gestation (ED 1) and was based on the presence of sperm in the vaginal smear. Females were randomly assigned to MA-treated (MA) and saline-treated (SA) groups. Physiological saline solution (0.9% NaCl) and d-Methamphetamine hydrochloride were purchased from Sigma-Aldrich (Czech Republic).

On Day 21 of gestation (ED 21), the females were removed from the group cages and placed into maternity cages (1 female/cage).

A total of 43 litters were used in the experiment. 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. To avoid litter bias pups were cross-fostered on postnatal day 1 (PD). One mother usually raised three male and three female pups exposed to MA and three male and three female pups exposed to saline. At the same time, all prenatally MA-exposed pups were tattooed, with India ink on the left foot and all prenatally SA-exposed pups on the right foot for future identification. 32 male rats (16 with SA prenatal/neonatal exposure and 16 with MA prenatal/neonatal exposure) and 32 female rats (16 with SA prenatal/neonatal exposure and 16 with MA prenatal/neonatal exposure) were used in this study. The rest of the animals were used in other studies. On PD 21, pups fostered by MA-treated mothers were ear-punched in the left ear and pups fostered by SA-treated mothers in the right ear for further identification. After weaning pups were housed in groups separated by sex, and the light/dark cycle of the animals was reversed with lights on at 18:00. Animals were left undisturbed until adulthood.

2.2. Drug administration and experimental groups

Based on prenatal exposure, the pregnant dams were divided into 4 groups: the MA group and SA group exposed throughout the first half of gestation (ED 1–11) and the MA group and SA group exposed throughout the second half of gestation (ED 12–22). Based on neonatal exposure, the pregnant dams and their pups were divided into 4 groups: the MA group and SA group exposed during early neonatal period (PD 1–11), when indirectly pups were exposed to the effect of MA via the maternal breast milk or directly when pups were injected subcutaneously (s.c.) with MA (Fig. 1). The dose of MA was 5 mg/kg/day throughout each period and was administered s.c. (1 ml/kg). The females from the control groups were administered saline at the same time and volume as MA. The dose was chosen based on findings that demonstrated that this dose of MA administered to pregnant female rats corresponds to the levels found in the fetuses of drug-abusing women [1,39,54].

To determine the effect of acute MA in adulthood, half of the rats from each of all experimental groups (MA, SA) were administered 1 mg/ml/kg of MA s.c. and the other half were administered 1 ml/kg of saline s.c. This dose was chosen on the basis of our data showing that this dose does not induce stereotyped behavior [53,54]. MA or SA was injected 45 min prior the SIT. This timing was chosen based on the

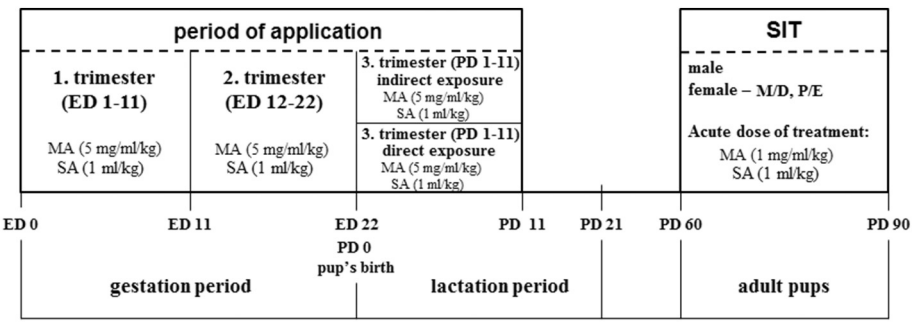

Fig. 1. Timeline of methodological procedures. Assignment of the animals to individual groups according to the schedule and the type of prenatal (ED (embryonic day) 1–11 or ED 12–22) and neonatal (PD (postnatal day) 1–11 indirect and direct) treatment versus acute application in adulthood. Total number of male and female rats used in experiment was 512; individual group accounted: 16 males (n = 8 pairs), 16 females for each phase of estrous cycle (n = 4 pairs for diestrus; n = 4 pairs for proestrus). Application of treatment in adulthood was injected 45 min prior testing in familiar OF arena. MAdose of 5 mg/ml/kg were used during gestation and early lactation period and 1 mg/ml/kg was used for acute application in adulthood.