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Prenatal MDMA exposure delays postnatal development in the rat: A preliminary study

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ABSTRACT

3,4-methylenedioxymethamphetamine or MDMA (ecstasy) is a synthetic illicit drug which is widely consumed throughout the world. Drug abuse during pregnancy may have an impairing effect on the progeny of drug-abusing mothers. The purpose of the present study was to assess the effect of prenatal MDMA exposure on the progeny development, using a rat model. Pregnant animals were injected daily with MDMA (10 mg/kg) between the 13th and 20th days of gestation. Male and female pups were then tested throughout the lactation period on the appearance and improvement of physical and sensory motor parameters. Appearance of some physical features (eyes opening and incisor eruption) and neurological reflexes as well as improving performances in negative geotaxis, gait and inclined board tests were delayed in pups prenatally exposed to MDMA compared to saline-treated pups. In contrast, functions that are necessary for survival such as forelimb reflex (that enables suckling) were present in both groups. At four weeks of age, MDMA animals recovered to normal level in all studied parameters. The delay in physical and neurological reflex development could be interpreted as alterations in maturation of some neuronal circuitries induced by prenatal MDMA exposure.

1. Introduction

The use of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is constantly increasing among young adults [2,34,48]. Consequently, there is a sensible risk of fetal exposure, all the more so as 80% of pregnancies among women who consume MDMA are unplanned [31]. The few available studies have reported that infants exposed to MDMA in utero had significantly higher incidences of congenital defects [18,43]. Thus, determining whether MDMA influences neurodevelopment of a child is still a critical public health issue. Interpretation of clinical data is difficult because women using MDMA during pregnancy are often multiple drug users [25,31]. In addition, numerous preparations may be sold as MDMA or as ecstasy but do not exclusively contain MDMA [11,56]. Therefore, studies from animal models appear to be necessary to evaluate strict effects of prenatal MDMA exposure on pups.

MDMA administration results in a massive immediate release of serotonin (5-HT), dopamine (DA) and norepinephrine (NE) and subsequently induces 5-HT depletion in multiple brain regions [28,41,60]. As monoaminergic systems have a key role in regulating brain development [4,6,8,37,38,62], exposure to MDMA during prenatal life may affect fetus maturation and have long-term consequences on the functions regulated by these systems. Indeed, some brain monoaminergic system alterations have already been demonstrated [23,36,64] as well as behavioral modifications [23,57] in adult rats intoxicated in prenatal period to MDMA.

Some of these long-term modifications could be related to alterations occurring during the early postnatal development period. Indeed, it has already been reported that prenatally MDMA-exposed rats showed a two-fold decrease of whole brain levels of 5-HT at birth [23] and a decrease in the DA metabolite homovanillic acid on postnatal day P3, persisting through P21 in the striatum [36]. Based upon these studies, we hypothesized that these modifications of monoaminergic systems in neonate rats produced by prenatal MDMA exposure would lead to neurobehavioral alterations during the pup's postnatal development.

Therefore, we explored the consequences of a chronic administration of MDMA to pregnant rats on the postnatal development of their progeny by neurobehavioral evaluations which are widely used to examine the potential neurotoxicity of drugs. We chose to expose pregnant rats to MDMA from the 13th to 20th gestational days. With regard to human brain development, this corresponds approximately to the period from the second month to the end of the first half of pregnancy which is characterized by the appearance and establishment of DA and 5-HT system in the brain [30,54]. The administered dose, i.e. 10 mg/kg/day, referred to the method of interspecies scaling allowing estimating it equivalent to that used by humans [39]. Postnatal development is reflected among others by maturation of neurological reflexes, physical development and motor coordination, which are all hallmarks of nervous system development [1]. We quantified the physical development and the sensory motor maturation with a Fox's battery adapted for rats [22]. Further, we assessed the mothering

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behavior of the dams, in order to assess the hypothesis that developmental changes observed in the pups could be linked to postnatal-related maternal effects.

2. Methods

2.1. Animals and protocol of drug exposure

Timed pregnant Wistar rats purchased from CERJ (Le Genest, France) were received at the laboratory on gestational day 6 (G6). Animals were housed in a temperature-controlled room (22 ± 2 °C) under a 12 h light/dark cycle (lights on at 6:00 a.m. and off at 6:00 p.m.) and had free access to food and water. Drug exposure was started after an acclimatization period of 7 days. At G13, the dams were semi-randomly assigned to the treatment or the control group, in order to have homogenous groups in terms of body weight. The experimental group comprised 7 females which were subcutaneously injected with 10 mg/kg (±)-3,4-methylenedioxymethamphetamine HCl (MDMA; Sigma Aldrich) per day from G13 to G20. The control group (saline) was constituted of 7 females subcutaneously injected with saline (NaCl 0.9%) at the same time and within the same period. Injection sites were varied to minimize local cutaneous inflammation. Dams were weighted daily and data were expressed in percent of weight gain during treatment period [i.e. $(G20 - G13)/G13 \times 100$]. At birth (P0), progeny was counted, weighted, sex typed and culled to 10 per litter.

All procedures described here fully comply with French legislation on research involving animal subjects. This research protocol adhered to recommendations by the European Community Council for ethical Treatment of Animals (no. 86/609/EEC).

2.2. Pup development

The Fox's battery permits the assessment of normal and pathological development of the young rat [1,9,40] and is an index of the cerebral maturation. The neurobehavioral tests were performed daily from P2 to P21 between 08:30 and 11:00 p.m., corresponding to the rats' active period.

2.2.1. The maturation of physical characteristics

Observations were made daily to assess maturation of physical characteristics on all pups. The days the animals presented their two eyes opened and their two incisors erupted were noted and results obtained for each litter were averaged.

2.2.2. Reflexes and motor coordination tests

Only one male and one female per litter were randomly chosen at P2. These selected pups were labeled by picric acid and used for all neurobehavioral tests. Pups were taken out of the cage to be tested and returned to the cage with the mother and their sisters/brothers after each test session.

2.2.2.1. Limb grasp. The fore- and hindlimbs were touched with a thin rod, and the first day of grasping onto the rod was recorded.

2.2.2.2. Crossed extensor reflex. The left rear paw was pinched and the animal was observed for the extension of the right leg. The day of disappearance of this crossed extensor reflex was noted.

2.2.2.3. Righting reflex. When the neonates are placed supine, they immediately turn over their longitudinal axis to restore a normal prone position (righting reflex). This test was carried out daily from P1 to P13. It was considered to be fully achieved when the pups turned 180° around their longitudinal axis, their four paws being in contact with the plane surface within the observed 120 s. The time needed to achieve the righting reflex was measured.

2.2.2.4. Negative geotaxis. Negative geotaxis is a postural reaction bringing the animal in the upright position when it is placed downwards. In our experiment, the rat pups were placed on a 20° and 45°-tilted plane with its head pointing down. The animals had to turn around and crawl up the slope for 180° in order to bring their snout upwards and climb up the board with their forelimbs reaching the upper rim. In cases the animals did not succeed at this task within the observed 60 s, the test was considered negative. The day of appearance of geotaxis and the time to reach the upper end of the board were recorded. The tests on 20°- and 45°- titled plane were made daily from P4 to P11 and from P9 to P16, respectively.

2.2.2.5. Inclined board test. Animals were placed on a wooden board, and the board was gradually elevated by 5°. The maximum angle at which the animals could maintain position on the inclined board for 5 s was recorded daily from P10 to P14 and at P17, P21 and P28.

2.2.2.6. Gait. The animals were placed in the middle of a white paper circle of 13 cm in diameter, and we recorded the day they began to move outside the circle with the two forelimbs in less than 30 s. From this day, the time (s) to move off the circle was noted daily until P19.

2.3. Maternal behavior

Observations of maternal behavior were conducted during the light phase of the light/dark cycle, between 01:00 and 6:00 p.m. in the home cage where the dam and her pups left undisturbed. The maternal behavior was recorded on video tape for three periods of 10 min spaced by a 40 min interval during the 2nd, the 6th, the 10th and the 15th postnatal days. The following behaviors were scored: (1) dam's activities directed towards the pups (suckling, carrying, licking the pups, passive contact with pups), and (2) dam's activities non-directed towards the pups (self-grooming, digging the saw-dust, straightening up, immobility with no contact with pups). Using the time-sampling method, the frequency of each behavior was noted every minute during each of the 10 min interval. Results presented here are the sum of behaviors observed during the three sessions.

2.4. Statistical analysis

Data were compiled as means and standard errors. A commercial software program (STATISTICA, Statsoft France) was used for all data analyses.

Percent of body weight gain of pregnant rats, the litter size as well as the male/female ratio at birth were compared between the MDMA and saline groups using an unpaired Student's *t*-test. Maternal behavior testing was analysed using a two-way ANOVA (gestational exposure with repeated measures on postnatal day).

According to a recent review on statistical issues regarding developmental neurotoxicity [32], we used the litters as the statistical unit. Results were first analysed using ANOVAs incorporating prenatal exposure as a between-litters independent factor and gender as a within-litters factor. For righting reflex, negative geotaxis, inclined board and gait, “day” was considered as repeated measures. Thus, a two-way ANOVA was performed for the appearance of physical characteristics and reflexes. For the improvements in daily performance, when an initial three-way ANOVA showed main effects of the factors as well as significant interactions among the factors, we then conducted a nested ANOVA. If a significant *F* value was obtained, Least Significant Difference (LSD) test was conducted. The level of statistical significance was always set at $p \leq 0.05$.