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Prenatal methamphetamine exposure affects the mesolimbic dopaminergic system and behavior in adult offspring

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ABSTRACT

Methamphetamine is a commonly abused psychostimulant that causes addiction and is often abused by pregnant women. Acute or chronic administration of methamphetamine elevates the levels of the extracellular monoamine neurotransmitters, such as dopamine.

The aim of the present study was to show whether prenatal exposure to methamphetamine (5 mg/kg, entire gestation) or saline in Wistar rats induces changes in dopamine levels and its metabolites in the nucleus accumbens, and in behavior (locomotor activity, rearing, and immobility) after the administration of a challenge dose of methamphetamine (1 mg/kg) or saline in male offspring.

We found that adult offspring prenatally exposed to methamphetamine had higher basal levels of dopamine (about 288%), dihydroxyphenylacetic acid (about 67%) and homovanillic acid (about 74%) in nucleus accumbens. An increased basal level of dopamine corresponds to lower basal immobility in offspring prenatally exposed to methamphetamine. The acute injection of methamphetamine in adulthood increased the level of dopamine in the nucleus accumbens, which is related to an increase of locomotion and rearing (exploration). In addition, prenatally methamphetamine-exposed rats showed higher response to the challenge dose of methamphetamine, when compared to prenatally saline-exposed rats.

In conclusion, rats exposed to methamphetamine *in utero* have shown changes in the mesolimbic dopaminergic system and were more sensitive to the administration of the acute dose of methamphetamine in adulthood.

1. Introduction

Methamphetamine (MA) is a commonly abused psychostimulant that causes addiction and is often abused by pregnant women. Acute or chronic administration of MA elevates the levels of the extracellular monoamine neurotransmitters, such as dopamine, serotonin, and norepinephrine. It has been published that acute administration of MA releases dopamine (DA), but decreases dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Di Chiara, 2002; Gough et al., 2002; Lan et al., 2008; Pereira et al., 2006). The possible mechanism of MA inducing the release of DA involves the redistribution of neurotransmitters from synaptic vesicles via the vesicular monoamine transporter, VMAT2, to the neuronal cytoplasm, and the reverse transport of dopamine through the plasma membrane transporter into the extracellular space. In addition, MA inhibits monoamine oxidase, which catabolizes dopamine into DOPAC (Kish, 2008; Sulzer et al., 2005) and DA reuptake. Both mechanisms decrease DOPAC levels after the administration of MA (Shimada et al., 1996).

The acute administration of MA increases hyperlocomotion (Landa et al., 2005; Shoblock et al., 2003), and induces chewing and sniffing behavior (Izawa et al., 2006). Chronic treatment with MA induces behavioral sensitization, which is typically characterized by the augmentation of locomotor activity (Suzuki et al., 2004; Niwa et al., 2008). Behavioral sensitization has been postulated to result from the enhanced dopamine release in the mesolimbic and nigrostriatal dopaminergic terminals, in response to a challenge injection of psychostimulants. Microdialysis studies demonstrated that a challenge dose of MA, subsequent to daily MA pretreatment, produced an increase in the dopamine level in the nucleus accumbens (Fukakusa et al., 2008; Narita et al., 2004).

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When methamphetamine is abused during pregnancy, because the drug crosses the placental barrier (Dattel, 1990), it is possible that MA changes the development of the central nervous system. In humans isolated cases were found where *in utero* MA exposure lead to cardiac defects, cleft lip, and biliary atresia (Plessinger, 1998). However, relatively little is known about the potential long-term effects of *in utero* MA exposure. Some evidence suggests that prenatal MA exposure may result in long-term cognitive deficits (Cernerud et al., 1996), including impairments in hippocampus-dependent spatial learning and memory (Chang et al., 2004), and in the reduction of the volume of several brain structures, including the hippocampus (Chang et al., 2004).

In rats, our previous studies demonstrated that MA administration during gestation and/or lactation affects maternal behavior in rats (Slamberova et al., 2005a) and induces a delay in the sensory–motor development of pups exposed to MA during prenatal or pre-weaning periods (Hruba et al., 2009; Slamberova et al., 2006). Adult rats exposed to MA prenatally had decreased performance in place navigation tests, with a stable platform (Slamberová et al., 2005b); and the learning ability of prenatally MA-exposed animals is further affected by a challenge dose of MA in adulthood (Schutová et al., 2008).

There are, however, few studies investigating the possible effects of prenatal MA exposure on the development of the mesolimbic dopaminergic system. The aim of the present study was to show whether prenatal exposure to MA induces changes in the mesolimbic dopaminergic system after the administration of a challenge dose of MA in male offspring. In addition, we would like to show if a change of the dopaminergic system has been related to locomotor and exploratory behavior in adult male rats after the administration of a challenge dose of MA.

2. Experimental procedures

2.1. Animals and drug administration

Adult female Wistar rats (250–300 g) from Anlab farms (Prague, Czech Republic) were randomly assigned to either a MA-treated or saline-treated control group. They were smeared by vaginal lavage to determine the phase of their estrous cycle (Turner and Bagnara, 1976). At the onset of the estrous phase, each female was housed overnight with a sexually mature stimulated male; always one pair of animals per one cage. The next morning the female was smeared again for the presence of sperm and returned to her previous home cage. The day after impregnation was counted as day 1 of gestation. MA-treated females were injected daily, subcutaneously (s.c.) with D-methamphetamine HCl (provided by the Faculty of Pharmacy of The Charles University in Hradec Králové, Czech Republic) at a dose of 5 mg/kg throughout the entire gestation period (i.e. from the first to the last day of gestation) (Slamberova et al., 2005a). The date of delivery was the 22nd or 23rd day of gestation, thus the amount of injection did not differ more than ±1 injection (one injection more was always preferred rather than to induce withdrawal in pregnant rats prior to natural delivery). At the same time, control females were treated with the same volume of saline by s.c. injection. The day of birth was counted as postnatal day (PD) 0.

On PD 1, MA-exposed pups were injected intra-dermally with black India ink in the left foot pad and saline-exposed control pups were injected in the right. 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 PD 1, so that one mother usually raised 6 saline-exposed and 6 MA-exposed pups. Two male offspring from each litter were used, one from each of the prenatally treated groups (either saline-exposed or MA-exposed). The rest of the animals were used in other studies. On PD 21, animals were weaned and housed in groups, separated by sex. The male offspring were left undisturbed until adulthood PD 90.

Each rat was experimentally naive and tested only once. All manipulations respected the Guidelines of the European Union Council (86/609/EU) and followed the instructions of the National Committee for the Care and Use of Laboratory Animals.

2.2. Microdialysis in conscious rats

Before surgery, the male rats were anaesthetized with a mixture of ketamine hydrochloride (Narkamon® 5%; 2 mg/kg) and xylazine (Rometar® 2%; 0.5 mg/kg) intraperitoneally (i.p.) Each rat was placed in a stereotaxic apparatus (Stoelting, Stoelting Co., Illinois, USA) and a guide cannula (MAB 4.15.IC; Agn Tho's AB, Sweden) was implanted through a burr hole 2 mm above the NAc (AP +1.6 mm; ML −1.2 mm; DV −7.3 mm from Bregma). Seven days after the surgery, dialysis probes (MAB 4.15.1.Cu; Agn Tho's AB, Sweden; cuprophane 2 mm membrane; cut-off 6 kD) were placed into the NAc under a light halothane anaesthesia (Narcotan® 0.01%). The probes were connected to a syringe pump (Univentor 864, Agn Toho's AB) and perfused with artificial cerebro-spinal fluid with a flow rate of 2 μl/min. After a 60 min wash-out period, the dialysates were collected at 20 min intervals in plastic vials, containing 15 μl of 0.1 M HCl to reduce decomposition of the analytes. The challenge dose of MA (1 mg/kg; i.p.) was injected after the 3rd sample, Fig. 1.

The placement of microdialysis probes was verified using Nissl staining. After the microdialysis study, rats were sacrificed by cervical dislocation; brains were removed and stored in 10% formaline. To determine the exact probe implantation in

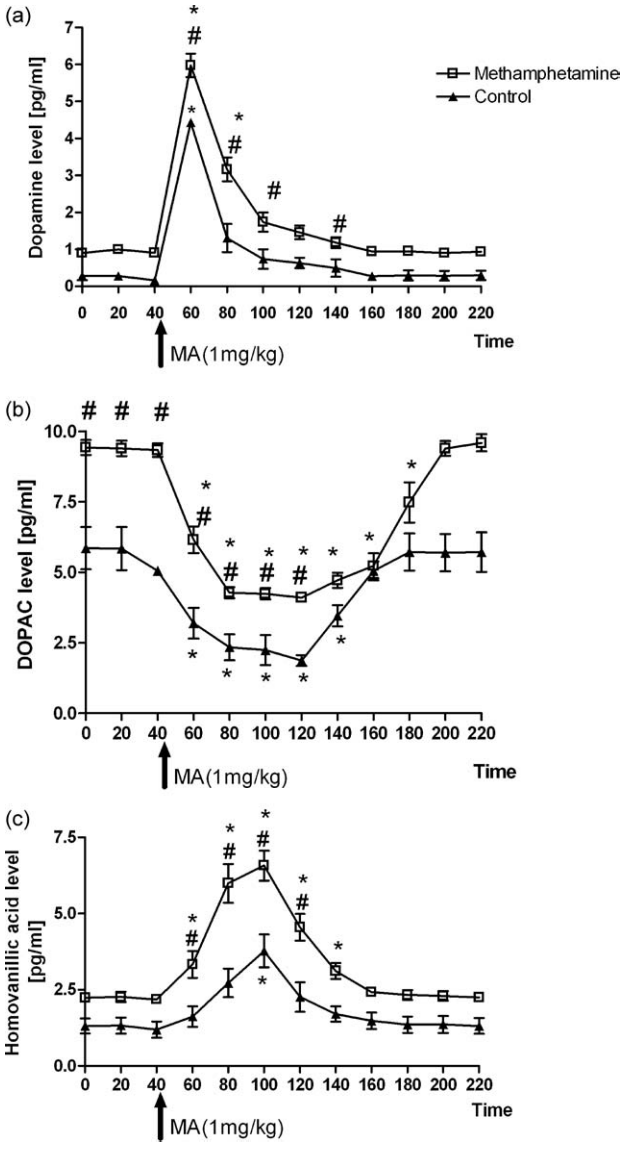

Fig. 1. The effect of prenatal methamphetamine exposure on dopamine, DOPAC, and HVA levels in the nucleus accumbens, after the injection of the challenge dose of methamphetamine (1 mg/kg) in adult rats. *Dopamine level* (a): The challenge dose of methamphetamine increased the level of dopamine in both prenatally saline-exposed and methamphetamine-exposed rats (*$P < 0.05$ vs. baseline). The level of dopamine was about 35% higher in prenatally methamphetamine-exposed rats compared to prenatally saline-exposed rats, after the administration of the challenge dose of methamphetamine (1 mg/kg) (#$P < 0.05$). *3,4-dihydroxyphenylacetic acid (DOPAC) level* (b): In both groups the level of DOPAC was decreased after the administration of the challenge dose of methamphetamine (*$P < 0.05$ vs. baseline). We found a higher level of DOPAC in prenatally methamphetamine-exposed rats before and after the challenge dose of MA (#$P < 0.05$). *Homovanillic acid (HVA) level* (c): The challenge dose of methamphetamine increased the level of HVA in both saline and methamphetamine-exposed groups (*$P < 0.05$ vs. baseline). We found a higher level of HVA in the methamphetamine-exposed group, when compared to the saline-exposed, after administration of the challenge dose of methamphetamine (#$P < 0.05$).

the NAc, brains were placed in a cryostat and coronal frozen sections of the brain were sliced serially at 40 μm intervals. Slices were investigated under a Zeiss AxioVision Imaginer Z1; and the appropriate placement of the probe estimated in comparison to the corresponding slices obtained by the atlas (Paxinos and Watson, 2003). After the verification, a total of 14 male offspring were used for the determination of dopamine and its metabolites (8 animals in the MA-exposed group and 6 animals in the saline-exposed group).

2.3. Determination of dopamine and its metabolites by LC–ESI-MS/MS

A total amount of 14 animals were used for the determination of dopamine and its metabolites by HPLC. A Varian ProStar HPLC system, consisting of a dual pump ProStar 210, a degasser, and a Varian 410 autosampler (Varian, USA) equipped with a Gemini C18 column (5 μm × 150 mm × 2 mm; Thermo Electron Corporation, USA), was used. The vehicle (solvent A = water, pH = 2, adjusted by acetic acid; B = methanol) was used for gradient elution (elution program: 0–3 min, 95% solvent A; 3–5 min, 75% solvent A; 5–16 min, 75% solvent A; 16–20 min, 95% solvent A) at the flow rate of 150 μl/min. The column was heated to 25 °C. The injection volume was 10 μl. The HPLC system was directly coupled to the Varian 1200L triple quadrupole mass spectrometer (Varian, USA) equipped with an electrospray ion source, operated in the alternating ionization mode (ESI^+ for dopamine hydrochloride (0.0–6.0 min) and ESI^- for HVA and DOPAC (6.0–20.0 min)). The multiple reaction monitoring mode (MRM) was used (dopamine hydrochloride 137 → 120 (−17.5 eV); HVA 181 → 122 (17.0 eV); DOPAC 167 → 122 (8.5 eV)) for its extremely high degree of selectivity and the stable-isotope-dilution assay for its high precision of quantification ([1,1′,2,2′-$^2H_4$] dopamine hydrochloride 141 → 124 (−17.5 eV)). CID was performed under an argon pressure of 2.2 mTorr, capillary voltage of −70 V, and needle voltage of −4500 V. The temperature of the ESI ion source was 250 °C. Air was used as the nebulizing gas in ESI^+ mode (50 psi), but nitrogen was used as the drying gas (17 psi), and the nebulizing gas in ESI^- mode (50 psi). Data were acquired and evaluated using ProStar software version 6.52 (Varian, USA).

Hydrochloric acid was added to the withdrawn sample in order to stabilize the dopamine, by transforming it to a more stable form of dopamine hydrochloride. The internal standard of dopamine hydrochloride-*d4* (1 PG) was added to the sample, which was immediately frozen at −80 °C and submitted to lyophilization for 12 h at −47 °C and 9 kPa pressure (freeze dryer, Labconco Free Zone, USA). The lyophilized residue was dissolved in 50 μl of methanol and analyzed by LC–ESI-MS/MS. The sample was kept in the dark throughout the procedure to prevent any light-induced disintegration.

2.4. Behavioral test—open field

A Laboras apparatus (Metris B.V., Netherlands) was used to test behavior in adult male rats. The Laboras is a fully automated system for continuous behavior recognition and tracking in small rodents. Based of the vibration and force changes in the animal's behavior it distinguishes several types of normal behavior (duration, speed, and frequency), as well as some abnormal behaviors (seizures and stereotypy). The animal is placed into the covered plexiglass cage (45 cm × 30 cm × 30 cm), equipped as a normal home cage with food and water available *ad libitum*, and filled with bedding material. The cage is placed on a triangular sensor platform (95 cm × 75 cm × 75 cm), making up the basis of the computer connected system. All movements are analyzed by the Laboras software that records different types of activities during the time of open field testing. Data can be saved as an Excel file and used for statistical analysis.

Male offspring ($n = 32$) were tested in adulthood (PD 80–90) during the dark phase of the light/dark cycle in a darkroom. To determine the effect of MA in adulthood, half of the animals from each prenatally exposed group (i.e. MA or saline) received a low dose of MA (1 mg/kg) s.c. The dose of 1 mg/kg was used because it does not cause stereotypy, unlike the dose of 5 mg/kg used in gestation, and therefore should not affect the ability to walk. Thus, we examined four groups of adult male rats: prenatally saline-exposed with a challenge dose of either MA (saline/MA) or saline (saline/saline), and prenatally MA-exposed with a challenge dose of either MA (MA/MA) or saline (MA/saline) (in each group were eight animals). Immediately after either saline or MA s.c. injection, the rat was placed into the Laboras testing cage. Their behavior was monitored for 1 h in the Laboras open field apparatus, with the experiment divided into six 10-min intervals. The time spent by each and the frequency of each behavior was recorded for each 10-min interval.

The following parameters were analyzed in each animal in the 1-h period of testing: the duration spent by locomotion, immobility, rearing (exploratory behavior), grooming (comfortable behavior), the distance traveled, and the average and maximal speed of locomotion. The number of boluses was recorded at the end of each test.

2.5. Data analysis and statistics

Statistical analysis was performed using Statistica 5.5 software. Dialysate dopamine levels and its metabolites were calculated as the quantity of DA, DOPAC, or HVA in pg/ml. In both, microdialysis and behavior tests, Two-way ANOVA (prenatal drug × challenge injection) with repeated measurement (minutes) was performed with the Newman–Keuls *post hoc t*-test when appropriate. Average behavioral data evaluated within 60 min was measured by two-way ANOVA with Newman–Keuls *post hoc* test. The significance level was set at $P < 0.05$.

3. Results

3.1. The basal levels of dopamine, DOPAC, and HVA in saline or MA-exposed rats

The values for basal levels of dopamine, DOPAC, and HVA were calculated as a mean ± SEM for three time points for each animal. The basal level of dopamine in prenatally saline-exposed rats was 0.241 ± 0.04 pg/ml and in prenatally MA-exposed rats was statistically increased about 288% to a value of 0.936 ± 0.03 pg/ml (unpaired two-tailed *t*-test $P < 0.001$). The basal level of DOPAC in prenatally saline-exposed rats was 5.62 ± 0.37 pg/ml and in prenatally MA-exposed rats was statistically increased about 67% to a value of 9.38 ± 0.14 pg/ml (unpaired two-tailed *t*-test $P < 0.001$). The basal level of HVA in prenatally saline-exposed rats was 1.28 ± 0.14 pg/ml and in prenatally MA-exposed rats was statistically increased about 74% to a value of 2.23 ± 0.08 pg/ml.

3.2. The effect of a challenge dose of MA on dopamine, DOPAC, and HVA levels in adulthood, in prenatally saline or MA-exposed rats

The repeated ANOVA measurement showed a difference between prenatally MA-exposed rats and saline-exposed rats ($F(1, 10) = 130.4$; $P < 0.001$), and between time-intervals ($F(1, 10) = 728.6$; $P < 0.001$), in their dopamine levels. In both groups the level of dopamine was increased 20–60 min after administration of the challenge dose of MA by about 1000% in the control group and 600% in the prenatally MA-exposed group ($^*P < 0.05$ vs. baseline). The level of dopamine was about 35% higher in prenatally MA-exposed rats compared to saline-exposed rats, after the administration of the challenge dose of MA ($^\#P < 0.05$); Fig. 1a.

The repeated ANOVA measurement showed a difference between prenatally MA-exposed rats and saline-exposed rats ($F(1, 10) = 106.2$; $P < 0.001$), and between time-intervals ($F(1, 10) = 1399.8$; $P < 0.001$), in their DOPAC levels. In both groups the level of DOPAC was decreased after the administration of the challenge dose of MA ($^*P < 0.05$ vs. baseline). We found a higher level of DOPAC in prenatally MA-exposed rats both before and after the challenge dose of MA ($^\#P < 0.05$); Fig. 1b.

The repeated ANOVA measurement showed a difference between prenatally MA-exposed rats and saline-exposed rats groups ($F(1, 10) = 29.2$; $P < 0.001$), and between time-intervals ($F(1, 10) = 261.8$; $P < 0.001$), in their HVA levels. The challenge dose of MA increased the level of HVA in both saline and MA-exposed group ($^*P < 0.05$ vs. baseline). We found a higher level of HVA in the MA-exposed group, as compared to the saline-exposed control group, 20–80 min after the administration of the challenge dose of MA ($^\#P < 0.05$); Fig. 1c.

3.3. The effect of prenatal MA exposure on behavior in the open field

3.3.1. Locomotory activity

There was a significant effect of prenatal drug exposure ($F(1, 28) = 4.80$; $P < 0.05$), adult treatment ($F(1, 28) = 18.29$; $P < 0.001$), and an interaction between prenatal drug exposure and adult treatment ($F(1, 28) = 5.32$; $P < 0.05$). The Newman–Keuls *post hoc* test showed that acute administration of MA increased locomotor activity in prenatally MA-exposed rats (MA/saline vs. MA/MA; $^+P < 0.0001$), while not affecting locomotion in prenatally saline-exposed rats (saline/saline vs. saline/MA; $P = 0.17$). Moreover,

Table 1
Averages of behavior activities within 1 h of open field testing.

Prenatal drug	Challenge	Locomotion	Immobility	Rearing
Saline	Saline	25.16 ± 4.82	276.62 ± 26.47	159.82 ± 22.58
	MA	35.98 ± 4.25	5.11 ± 10.20*	170.78 ± 21.42
MA	Saline	24.53 ± 3.73	137.26 ± 24.24^{x}	139.48 ± 19.46
	MA	60.67 ± 5.11$^{+,\#}$	0.54 ± 8.62^{+}	315.04 ± 24.42$^{+,\#}$

Values are averages for the entire 1-h testing (means ± SEM).
$^{+}$ $P < 0.05$ in MA/saline vs. MA/MA.
$^{\#}$ $P < 0.05$ in MA/MA vs. saline/MA.
* $P < 0.05$ in saline/saline vs. saline/MA.
^{x} $P < 0.05$ in MA/saline vs. saline/saline.

prenatally MA-exposed rats administered with a challenge dose of MA in adulthood spent more time with locomotion, relative to prenatally saline-exposed rats with a challenge dose of MA ($^{\#}P < 0.05$) (Table 1 and Fig. 2).

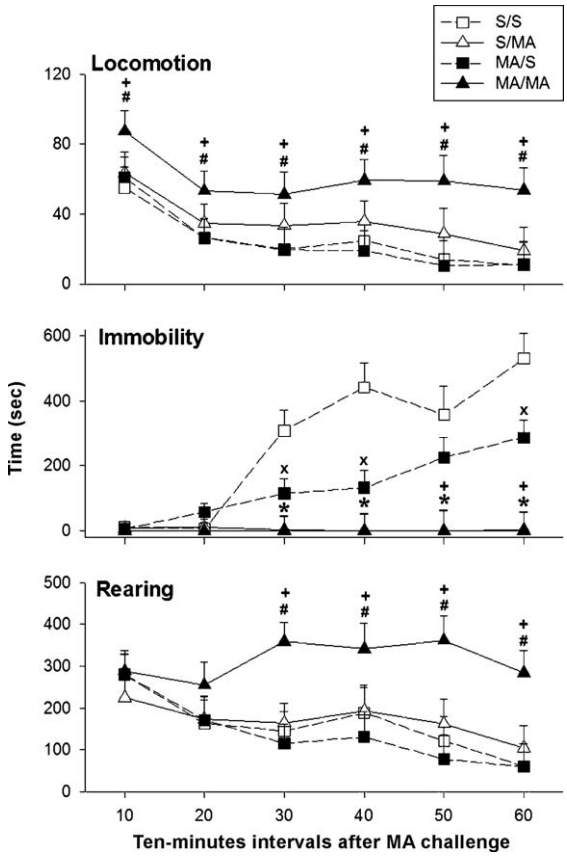

Fig. 2. The effect of prenatal methamphetamine exposure on locomotor activity, immobility, and exploratory activity in an open field. *Locomotion*: Acute administration of methamphetamine increased locomotion only in offspring prenatally exposed to methamphetamine ($^{+}P < 0.05$ methamphetamine/saline vs. methamphetamine/methamphetamine). Locomotion was increased in rats prenatally methamphetamine-exposed, as compared to saline-exposed rats, after the administration of the acute methamphetamine ($^{\#}P < 0.05$ saline/methamphetamine vs. methamphetamine/methamphetamine). *Immobility*: Immobility was decreased in prenatally methamphetamine-exposed rats compared to prenatally saline-exposed rats, without the administration of the challenge dose of methamphetamine ($^{x}P < 0.05$ saline/saline vs. methamphetamine/saline). Acute administration of methamphetamine decreased immobility only in prenatally saline-exposed rats ($^{*}P < 0.05$ saline/saline vs. saline/methamphetamine). *Rearing*: Acute administration of methamphetamine increased rearing only in offspring prenatally exposed to methamphetamine ($^{+}P < 0.05$ methamphetamine/saline vs. methamphetamine/methamphetamine). Rearing was increased in rats prenatally methamphetamine-exposed compared to saline-exposed rats after the administration of the acute methamphetamine ($^{\#}P < 0.05$ saline/methamphetamine vs. methamphetamine/methamphetamine).

In addition, all animals, regardless of prenatal drug exposure or the challenge dose in adulthood, displayed decreased locomotor activity during the 1-h testing ($F(5, 140) = 48.83$; $P < 0.0001$) (Fig. 2).

3.3.2. Immobility

There was a significant effect of prenatal drug exposure ($F(1, 28) = 9.10$; $P < 0.01$), adult treatment ($F(1, 28) = 73.20$; $P < 0.0001$), and an interaction between prenatal drug exposure and adult treatment ($F(1, 28) = 7.98$; $P < 0.01$). The Newman–Keuls *post hoc* test showed that acute administration of MA decreased immobility in either prenatally saline or MA-exposed rats (saline/saline vs. saline/MA; $^{*}P < 0.01$ and MA/saline vs. MA/MA; $^{+}P < 0.001$). Immobility did not differ between prenatally saline and MA-exposed rats, treated with the challenge dose of MA (MA/MA vs. saline/MA; $P = 0.89$), but immobility was lower in prenatally MA-exposed rats treated in adulthood with saline, relative to prenatally saline-exposed rats (MA/saline vs. saline/saline; $^{x}P < 0.001$) (Table 1 and Fig. 2).

In addition, there was a significant effect of time ($F(5, 140) = 37.10$; $P < 0.0001$) and an interaction between time, prenatal drug exposure, and adult treatment ($F(5, 140) = 7.85$; $P < 0.0001$) (Fig. 2).

3.3.3. Rearing

The prenatal drug exposure did not show any significant differences in rearing behavior ($F(1, 28) = 4.02$; $P = 0.055$); however, there was a significant effect of adult treatment ($F(1, 28) = 9.11$; $P < 0.01$) and an interaction between prenatal drug exposure and adult treatment ($F(1, 28) = 7.09$; $P < 0.05$). The Newman–Keuls *post hoc* test showed that acute administration of MA increased rearing only in prenatally MA-exposed rats (MA/saline vs. MA/MA; $^{+}P < 0.01$), but not in prenatally saline-exposed rats (saline/saline vs. saline/MA; $P = 0.80$). There was an increase in rearing in prenatally MA-exposed rats after the injection of the challenge dose of MA, compared to prenatally saline-exposed rats with a challenge dose of MA (saline/MA vs. MA/MA; $^{\#}P < 0.01$) (Table 1 and Fig. 2).

In addition, there was a significant effect of time ($F(5, 140) = 26.59$; $P < 0.0001$) and an interaction between time, prenatal drug exposure, and adult treatment ($F(5, 140) = 4.81$; $P < 0.001$) (Fig. 2).

3.3.4. Boluses

There were no statistical differences between dropped boluses that would be induced by prenatal drug exposure ($F(1, 28) = 0.74$; $P = 0.40$), adult drug treatment ($F(1, 28) = 0.45$; $P = 0.51$), or an interaction between both ($F(1, 28) = 4.04$; $P = 0.054$).

4. Discussion

Central dopamine systems have been implicated in mediating reward-related behaviors. In particular, the release of dopamine in the nucleus accumbens is believed to be the main mediator of the reinforcing and locomotor-stereotypy activating properties of psychostimulants (Koob et al., 1998). In adult rats, the acute administration of MA at a dose of 1 mg/kg i.p. increases chewing, sniffing, rearing, and walking up to 80 min after its administration. This MA-induced behavior corresponds with an increased level of dopamine and DOPA (l-3,4-dihydroxyphenylalanine) in the nucleus accumbens (Izawa et al., 2006). In another study, the acute administration of 1 mg/kg of MA increases locomotor activity, which correlates to an increase in dopamine levels in the nucleus accumbens and prefrontal cortex (Shoblock et al., 2003). Also the administration of MA (2 mg/kg) results in an increase of dopamine released in the nucleus accumbens after a challenge dose of MA (0.5 mg/kg) (Narita et al., 2004). In a further study, the

chronic administration of MA (1 mg/kg) increases dopamine levels by about 400% from the baseline in the nucleus accumbens. However, the acute administration of MA increases the level of dopamine by about 130% (Zhang et al., 2001). It has been suggested that MA-induced dopamine release in the striatum and nucleus accumbens is associated with behavioral sensitization (Zhang et al., 2001).

In our study, we investigated the effect of prenatal MA exposure on dopamine levels in the nucleus accumbens in adult rats before and after the injection of a challenge dose of MA (1 mg/kg). The injection of the challenge dose of MA elevated dopamine levels by about 1000%. The difference between prenatally MA-exposed and saline-exposed rats at the maximum effect, after the injection of the challenge dose, was 35%. Interestingly, prenatally MA-exposed rats had higher basal levels of dopamine by about 300%. These data support our hypothesis that prenatally administered MA induces changes in the development of the mesolimbic dopaminergic system. Our results are in accordance with the Tonge study (1973), where *in utero* exposure to MA, elevated the levels of dopamine and noradrenaline in several brain regions in adult rats. However, in a study with a toxic dose of MA (40 mg/kg), prenatal exposure to MA did not affect the basal level of dopamine, but decreased dopaminergic markers in the striatum after the injection of the challenge dose of MA (5–20 mg/kg s.c.) (Hellar et al., 2001).

In addition, we measured the levels of metabolites of dopamine, DOPAC, and HVA. We found that the injection of the challenge dose of MA decreased the level of DOPAC in the nucleus accumbens by about 60%. This finding is well documented in existing publications (Pereira et al., 2006; Shoblock et al., 2003). The decrease in extracellular DOPAC is related to the effect of MA on monoamine oxidase, which metabolizes dopamine into DOPAC, and to the inhibition of dopamine reuptake (Sulzer et al., 2005). We also found higher levels (about 82%) of DOPAC in prenatally MA-exposed rats, when compared to controls, after the injection of the challenge dose. Another metabolite of dopamine is HVA, which is catabolized by catechol-O-methyltransferase (COMT) and not blocked by MA (Brannan et al., 1992). After the acute treatment of MA, a decrease of HVA was published, which is explained by the inhibition of extra-neuronal monoamine oxidase by MA (Lan et al., 2008; Tuomainen et al., 1996). Contrary to previous publications, we found higher levels of HVA, by about 190%, after the acute administration of MA. Moreover, we found a higher level (about 120%) of HVA in prenatally MA-exposed rats, as compared to controls.

Altogether our data indicate that prenatally MA-exposed rats have a changed mesolimbic dopaminergic system. Moreover, these adult offspring, which were prenatally MA-exposed have a higher response to the challenge dose of MA. In our study, we were interested whether the elevation of dopamine in the nucleus accumbens is associated with behavior in adult offspring.

We measured behavior which is correlated to the mesolimbic dopaminergic pathway, such as locomotion and exploratory behavior (Fink and Smith, 1980; Piazza et al., 1991; Pijnenburg et al., 1976). Acute administration of MA at a dose of 1 mg/kg did not increase locomotor activity in prenatally saline-exposed rats, but did increase the locomotor activity in prenatally MA-exposed rats. The maximum effect of the challenge dose of MA was 30 min after the administration. This result concurs with our previous study (Schutová et al., 2008) demonstrating that the same challenge dose of MA (1 mg/kg) administered in adulthood, increases the speed of swimming in all animals regardless of prenatal exposure.

Regarding immobility, the injection of the challenge dose of MA decreased immobility in both prenatally saline and MA-exposed rats. The basal level of immobility was lower in rats prenatally exposed to MA compared to prenatally saline-exposed rats. However, immobility did not differ between prenatally saline- and MA-exposed rats treated with the challenge dose of MA. Time spent rearing in a novel environment is the basic parameter of exploratory behavior. We found that the acute administration of the challenge dose of MA increased rearing in MA-exposed rats but not in prenatally saline-exposed rats. Time spent rearing was increased in prenatally MA-exposed rats, as compared to controls, after the challenge dose of MA by about 84%.

The basal value of rearing was similar in prenatally saline and MA-exposed rats. An increase in rearing in prenatally MA-exposed rats after the injection of the challenge dose of MA, might suggest a higher interest for the unknown arena, or in contrast the inability to learn the new arena. Furthermore, it is also possible that increased rearing would indicate some sort of stereotypy behavior. Even though our preliminary data show that the low dose of MA (1 mg/kg) does not induce stereotypy behavior in control rats, this may be not true for prenatally MA-exposed rats, which might have altered sensitivity to MA in adulthood. However, we did not see any typical stereotypy behavior that is usually induced by the administration of psychostimulants, such as sitting in the corner with repetitive head movements and swinging of the body (Segal and Kuczenski, 1997). Based on the above and on our visual observation of the animals, we do not believe that the increased rearing would indicate an increase of stereotypy behavior, but rather suggests higher locomotion, exploration, or even decreased learning abilities.

Our results demonstrate that behavior which is related to the mesolimbic system, as is locomotor activity and exploratory behavior, is increased in MA-exposed offspring after the challenge dose of MA. Similarly, the level of dopamine and its metabolites is elevated in MA-exposed offspring after the injection of the challenge dose of MA. We have suggested that MA-exposed offspring are more sensitive to MA in adulthood. Interestingly, the basal levels of dopamine (without the injection of a challenge dose) and its metabolites are elevated in the nucleus accumbens, which correspond to a decrease in immobility activity.

In conclusion, the present study provides evidence that prenatal exposure to MA has long-term effects on the dopaminergic function of the offspring, which results in changes in behavior (locomotor activity and exploratory behavior). This finding indicates that offspring exposed to MA *in utero* could be more sensitive to MA and potentially to other psychostimulants.

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