Postnatal challenge dose of methamphetamine amplifies anticonvulsant effects of prenatal methamphetamine exposure on epileptiform activity induced by electrical stimulation in adult male rats

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A R T I C L E   I N F O

Article history:
Received 16 July 2010
Revised 6 January 2011
Accepted 15 February 2011
Available online 22 February 2011

Keywords:
Methamphetamine
EEG
Electrical stimulation
Afterdischarges

A B S T R A C T

Administration of psychostimulants is often associated with increased seizure susceptibility. In our previous studies prenatal methamphetamine (MA) exposure increased seizure susceptibility of adult rats in models of primarily or secondarily generalized seizures induced by convulsant drugs. The effect of a single MA challenge dose in adulthood on chemically induced generalized seizures however, depends on the prenatal MA exposure history. Thus, the present study used a model of focal electrical stimulation to determine whether prenatal MA exposure with or without the adult challenge MA dose has the same outcome in a focal seizure model. Total of six groups of adult male rats were tested (prenatally MA-exposed, prenatally saline-exposed and rats without prenatal injections), each of these groups was either postnaturally challenged with MA or with vehicle injection (MA-MA, MA-S; S-MA, S-S; C-MA, C-S). Seizures were induced by repetitive electrical stimulation (15 s/8 Hz) of sensorimotor cortex. Stimulation threshold, duration of afterdischarges (ADs), and presence and duration of spontaneous ADs (SADs) were evaluated. Additionally, behaviors associated with stimulation and ADs, and occurrence of wet-dog shakes (WDS) were analyzed. Our data demonstrate that daily injection of MA (5 mg/kg) within prenatal period decreased the occurrence of WDS and SADs, and shortened the duration of ADs and SADs suggesting anticonvulsant effects. Moreover, the challenge dose of MA (1 mg/kg) increased seizure threshold in all groups of rats, shortened duration of ADs in controls and prenatally saline-exposed animals, shortened duration of SADs in prenatally saline-exposed rats and totally eliminated WDS in all groups. Thus, the present study demonstrates that both chronic prenatal MA exposure and a single dose of MA in adulthood decrease focally induced epileptiform activity in adult male rats.

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Introduction

Drug abuse in pregnant women is a growing public health problem (Gonzales et al., 2010). Methamphetamine (MA) is one of the most commonly abused “hard” drugs during pregnancy (Marwick, 2000), and also one of the top illicit drugs in the Czech Republic (Vavřínková et al., 2001). Because MA crosses both the placental and blood–brain barriers (Greenhill, 2006; Nordahl et al., 2003; Rohanová and Balíková, 2009; Sharma and Ali, 2006), prenatal drug exposure can have devastating effects on fetal development and long-term negative consequences (Hutchings, 1982; McGuinness and Pollack, 2008; Rokyta et al., 2008), We (Hrubá et al., 2009; Pometlová et al., 2009; Šlamberová et al., 2006) and others (Acuff-Smith et al., 1996; Smith et al., 2008; Vorhees and Pu, 1995) have shown that rat maternal MA exposure impairs embryonic development with long-term specific consequences: Those studies demonstrated that prenatal MA exposure prolongs and slows down the sensorimotor and mental development, and induces long-term impairments of behavior, stress response, and cognition, that may last through adulthood.

In addition, clinical evidence shows that maternal exposure to psychostimulants increases seizure susceptibility in human newborns and toddlers and may later result in development of epilepsy (Earnest, 1993; Zagnoni and Albano, 2002). There are however only few studies showing that administration of MA alters EEG activity in humans (Newton et al., 2003; Newton et al., 2004). Similarly, experimental studies (Macedo et al., 2004; Zagnoni and Albano, 2002) with psychostimulant drugs, such as cocaine, reveal proconvulsant or true convulsant effects in rodents. In the rabbit EEG after postnatal MA, Yamamoto (1997) demonstrated a prolonged decrease in the cortical EEG power spectra. Previously we found that prenatal MA exposure is proconvulsant in seizure models induced in adult rats by convulsant drugs, such as flurothyl, bicuculline, N-Methyl-d-Aspartate (NMDA), or kainic acid (Šlamberová, 2005; Šlamberová and Rokyta, 2005). However, long-term effects of prenatal MA exposure on the EEG seizure activity of rats have not been yet investigated.
Because our previous studies (Šlamberová et al., 2008, 2010, 2009) indicate that in chemically induced generalized seizures, the effects of a single MA challenge dose in adulthood depend on the prenatal MA exposure history, the present study used a model of focal electrical stimulation to see if the effect of prenatal MA exposure with or without the adult challenge MA dose has the same consequences in focal seizures.

**Methods**

**Animals and prenatal MA exposure**

Adult female Wistar rats (250–300 g) were purchased from Anlab (Charles River Laboratories International, Inc., Prague, the Czech Republic). Animals were housed in groups of 4–5 per cage and left undisturbed for a week in a temperature-controlled (22°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. After 1 week, females were smeared by vaginal lavage to determine the phase of estrous cycle. The smear was examined by light microscopy. To ensure successful impregnation, one female rat was housed overnight with one sexually mature male at the onset of the estrus of the estrous cycle (Turner and Bagnara, 1976). The next morning females were smeared for the presence of sperm and returned to their previous home cages. This was counted as gestational day (GD) 1.

Dams were randomly assigned to MA-treated, saline-treated, or a control group. Pregnant rats were injected daily from GD 1 through the day of delivery, which usually occurred on GD 22 (for details see Šlamberová et al., 2005). MA (5 mg/kg; Faculty of Pharmacy of the Charles University in Prague, Hradec Králové, Czech Republic) or saline was injected subcutaneously (s.c.). Females in the control group have not received any injections. All dams were weighed daily yet there was no difference among the groups.

The day of delivery was counted as postnatal day (PD) 0. On PD 1, pups were weighed, identified, and cross-fostered so that each mother received the same number of pups from each of the three treatments. The birth weight was smaller in prenatally MA-exposed rats compared to saline-exposed or control rats (supporting our previous findings; Šlamberová et al., 2006). On PD 21, pups were weaned and group-housed by sex. Animals were left undisturbed until adulthood. Only one male rat per group from each litter was used in the experiments to avoid litter effects. Females entered a different study.

**Surgery**

The surgery was performed on PD 70 under deep combined ketamine/xylazine anesthesia (80/7 mg/kg) (Matějková et al., 1998). Two silver ball stimulating electrodes were implanted epidurally over the right sensorimotor cortex (AP = −1 and +1; L = 2.5 mm). Flat silver recording electrodes were placed over the left sensorimotor cortex (AP = 0; L = 2.5 mm), and over visual cortex of both hemispheres (AP = 6; L = 4 mm). An indifferent electrode was anchored in the nasal bone. All electrodes were fixed by dental cement to the skull. Animals were left to recover for a week.

**Postnatal MA challenge**

One week after surgery, rats were further divided into two groups: (1) those, which have been challenged with a dose of 1 mg/kg MA (the dose often used in sensitization tests; Suzuki et al., 2004) or (2) those without MA challenge. Our pilot data (not shown) indicated that 1 mg/kg of MA does not induce stereotypy that may interfere with behavior changes. Thus, there were six groups (n = 8–11 per group): prenatally MA-exposed rats either with (MA-MA) or without postnatal MA challenge (MA-S), prenatally saline-exposed with (5-MA) or without postnatal MA challenge (S-S) and controls with (C-MA) or without postnatal MA challenge (C-S).

**EEG recordings and electrical stimulation (see Fig. 1—timeline)**

EEG recordings and stimulation were performed in freely moving animals. First, we recorded 5 min of baseline EEG. Then we determined threshold for seizure susceptibility in terms of intensity of stimulation with biphasic rectangular electrical pulses of 1-ms duration at 8 Hz, which was necessary to induce seizures. Seizures were defined as afterdischarges (ADs) longer than 5 s. Then in the challenge dose group, a 1-mg/kg MA was administered (see **Postnatal MA challenge section** above) and the EEG was recorded for 45 min. After the 45-min period, ADs were elicited using double intensity compared to the threshold current stimulus. EEG was recorded for 10 min after the stimulation to register spontaneous ADs (SADs).

**Data analysis**

Power spectral analysis of baseline EEG activity (Fig. 3) was performed by Fast Fourier Transformation (FFT size 256 point, resolution 2.8 Hz). Differences in seizure threshold were analyzed among the groups. The duration of ADs (see Fig. 2A) and the occurrence and duration of SADs (see Fig. 2B) were evaluated. Motor behaviors accompanying electrical stimulation and the ADs were quantified by the 8-point scale (Mareš and Šlamberová, 2004; Šlamberová and Mareš, 2005). The scale was modified from the Racine’s score (Racine et al., 1972): (0) rest; (1) clonus of facial muscles; (2) clonus of the head; (3) clonus of one forelimb; (4) clonus of both forelimbs together with head in standing posture; (5) loss of balance with clonus of the body and all four limbs; (6) tonic extension of forelimbs; (7) tonic extension of hindlimbs. The score was assigned according to the most advanced behavior. The occurrence of wet-dog shakes (WDS; fast alternating axial head rotations) was also evaluated (Grimes et al., 1988; Lothman and Collins, 1981; Sperk et al., 1985; Velíšek et al., 1994).

**Statistical analysis**

A two-way ANOVA (prenatal exposure × challenge dose of MA) with Fisher LSD post-hoc test was used to analyze seizure threshold and duration of ADs and SADs among the groups. SADs and WDS occurrence and the motor movement score were analyzed by χ² test. Number of rats reaching score 5 points was used for comparison of the seizure severity within electrical stimulation. Number of rats reaching score 4 points was used for comparison of seizure severity within ADs. Level of significance was preset to p < 0.05 in all statistical analyses, adjusted for multiple comparisons if necessary.

**Results**

**EEG analysis**

In the control group, we observed the greatest power in the theta (4–8 Hz) and alpha (8–12 Hz) frequency bands, whereas in the MA-pretreated group, there was a decline in the power in these bands. There was no correlation between the background EEG activity and the threshold or the response to electrical stimulation (see Fig. 3).

**Threshold of the stimulation**

As shown in Fig. 4A, there was no effect of prenatal exposure [F(2,50) = 1.01; p = 0.37]. However, there was a significant effect of the challenge dose of MA on seizure threshold in terms of increased seizure threshold irrespective of prenatal exposure [F(1,50) = 18.64; p < 0.0001]. Table 1 shows that MA-exposed rats had lower occurrence
(27%) of score 5 points (i.e. loss of balance with clonus of the body) \( [\chi^2 = 20.21; p<0.01] \) than any other groups (82–100%).

**Afterdischarges**

In the duration of ADs, we have found the main effect of prenatal exposure \( [F(2,50) = 5.47; p<0.01] \) and the challenge MA dose \( [F(1,50) = 5.08; p<0.05] \). There was also a significant interaction between prenatal exposure and challenge dose in adulthood \( [F(2,50) = 3.43; p<0.05] \). Prenatally MA-exposed male rats without postnatal MA challenge (MA-S) had shorter ADs than controls \( (p<0.001; \text{Fig. 4B}) \) or prenatally saline-exposed animals \( (p<0.0001) \) without postnatal MA challenge (C-S, S-S). In addition, postnatal challenge dose of MA shortened ADs in controls \( (p<0.05) \) (C-MA) and prenatally saline-exposed animals \( (p<0.01) \) (S-MA) compared to the prenatal groups without MA challenge (C-S, S-S). This effect induced by the challenge dose of MA was not seen in prenatally MA-exposed rats.

Seizure severity in ADs is summarized in Table 1, which illustrates the significant differences \( [\chi^2 = 11.79; p<0.05] \) in the occurrence of score 4 points (i.e. clonus of both forelimbs). Specifically, control animals without challenge dose of MA (C-S) had lower occurrence of clonic movements than prenatally saline-exposed animals without MA challenge dose (S-S) \( (p<0.05) \) or than controls with MA challenge (p<0.01) (C-MA) and prenatally MA-exposed animals with MA challenge (p<0.01) (MA-MA).

**Wet-dog shakes (WDS)**

The \( \chi^2 \) test revealed differences in the occurrence of WDS \( [\chi^2 = 23.68; p<0.001] \). Prenatally MA-exposed rats without challenge dose of MA (MA-S) showed lower occurrence than controls and prenatally saline-exposed animals without postnatal MA challenge (C-S, S-S) \( (p<0.01) \). The occurrence of WDS was higher in animals without postnatal MA (especially in controls and prenatally saline-exposed rats (C-S, S-S)), while WDS were not present in animals with postnatal MA challenge regardless of prenatal exposure \( (p<0.01) \).

**Spontaneous afterdischarges (SADs)**

The \( \chi^2 \) test revealed differences in the occurrence of SADs \( [\chi^2 = 27.94; p<0.0001] \). Prenatally MA-exposed animals without MA challenge (MA-S) had lower occurrence of SADs than controls \( (p<0.01) \) and prenatally saline-exposed animals \( (p<0.0001) \) without postnatal MA challenge. Similarly, prenatally MA-exposed rats with MA challenge had lower occurrence of SADs than controls with postnatal MA challenge \( (p<0.01) \). In addition, the challenge dose of...
MA decreased the occurrence of SAD in prenatally saline-exposed animals (S-MA) \((p<0.01)\).

Postnatal challenge dose of MA did not affect the duration of SADs \([F(1,50)=3.25; \ p=0.08]\), but prenatally MA-exposed rats, regardless of MA challenge (MA-S, MA-MA), had shorter SADs than controls (C-S, C-MA) \([F(2,50)=7.41; \ p<0.01]\). There was also an interaction between prenatal exposure and MA challenge \([F(2,50)=2.85; \ p<0.05]\). Prenatally MA-exposed rats without MA challenge (MA-S) had shorter duration of SADs compared to prenatally saline-exposed rats (S-S) \((p<0.0001)\). This effect was not present in rats with postnatal MA challenge. In addition, postnatal MA challenge decreased the duration of SADs in prenatally saline-exposed animals (S-MA) \((p<0.01)\).

**Discussion**

The present study demonstrates that both chronic prenatal MA exposure and a challenge dose of MA in adulthood by itself, but not in combination, decrease electrically induced epileptiform activity in adult male rats.

Daily injections of MA (5 mg/kg) within prenatal period decreased seizure susceptibility in terms of decreased occurrence of seizures to 27% of seizing animals and decreased occurrence of WDS (to 18% of seizing animals). Prenatal MA exposure further decreased the occurrence of SADs as well as the duration of ADs and SADs. As there are no studies examining effects of prenatal MA on epileptiform EEG, our results may be compared only to the studies examining the effect of prenatal exposure to other psychostimulant drugs. There is clinical evidence showing changes in EEG activity in children of mothers exposed to cocaine during pregnancy; such as significant excess of relative power in the alpha frequency band, and deficits of absolute and relative power in the delta and theta bands (Doberczak et al., 1988; Legido et al., 1992; Prichep et al., 1995). There is also an experimental work that monitored EEG activity in rats exposed to prenatal MA exposure and a challenge dose of MA in adulthood by itself, but not in combination, decrease electrically induced epileptiform activity in adult male rats.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Occurrence of specific motor movements, wet-dog shakes and spontaneous afterdischarges.</th>
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<tbody>
<tr>
<td>Prenatal exposure dose of MA</td>
<td>Movements within stimulation (level 5)</td>
</tr>
<tr>
<td>Control w/o</td>
<td>91%</td>
</tr>
<tr>
<td>with</td>
<td>100%</td>
</tr>
<tr>
<td>Saline w/o</td>
<td>82%</td>
</tr>
<tr>
<td>with</td>
<td>88%</td>
</tr>
<tr>
<td>MA w/o</td>
<td>27% ***</td>
</tr>
<tr>
<td>with</td>
<td>88%</td>
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Values are percentages of occurrence of the specific movements.  
\[p<0.05; \ \#p<0.01; \ \###p<0.001\] vs. prenatal controls of the same challenge administration.  
\[p<0.01; \ \#\#p<0.001\] vs. animals without challenge dose of MA and the same prenatal exposure.  
\[p<0.01; \ \++++p<0.0001\] vs. prenatally saline-exposed animals without MA challenge.

![Fig. 3](image) Example of the baseline EEG power spectral analysis of a 60-s window of the EEG recording in controls, saline- and MA-pretreated rats. x-axis: Frequency in cycles per second (Hz); y-axis: Power Spectral Density \([μV^2]\).
prenatally to cocaine (Baraban et al., 1997), which demonstrated reduced latency to the first EEG seizure phenomenon after prenatal cocaine. Although this finding (Baraban et al., 1997) seems to contradict our results, it should be noted that the seizures in this experiment were induced chemically and not by electrical stimulation as in the current study. Similar to Baraban et al. (1997) also our previous work using chemical models of seizures showed increased seizure susceptibility after prenatal MA (Šlamberová, 2005; Šlamberová et al., 2008, 2009; Šlamberová and Rokyta, 2005), which is in disagreement with the present results. The differential effects of prenatal drug exposure on seizure activity in chemically and electrically induced seizure models are surprising and suggest distinct mechanism of action.

Our chemically induced seizure models used systemic delivery of convulsant drugs such as NMDA, kainic acid, bicuculline or fluorothyl, most of which either induce primarily generalized seizures or focal seizures with fast secondary generalization (Velišek, 2006). Many of these convulsant drugs act on ubiquitous glutamatergic and GABA-ergic receptors, though some of these receptors prevail in the limbic system (e.g., kainic acid receptors) (Insel et al., 1990; Miller, 1999; Velišek, 2006). Thus, the effect of systemic administration of convulsant drugs is conveyed via limbic structures with rapid spread and secondary occurrence of generalized seizures. On the other hand, local electrical stimulation of sensorimotor cortex used in the present study induces partial myoclonic seizures that generalized only sporadically and their site of origin is therefore better controlled (Mařeš and Kubová, 2006). Thus, the effects of prenatal MA may be depending on the character of the experimental seizures: generalized versus focal.

Postnatal MA challenge was associated with increased seizure threshold in all prenatally manipulated groups of rats. These finding are in agreement with previously published studies (Berridge and Morris, 2000; Culic et al., 1994; Yamamoto, 1997) showing decrease of the EEG power spectra and decrease of Pürkinje cell discharges after administration of psychostimulant drugs in rats or rabbits.

Current data further show that the occurrence of SADs was decreased and the duration of SADs was shortened in both prenatally MA- and saline-exposed animals relative to prenataly naive controls indicating the role of prenatal stress in the developing progeny (Drago et al., 1999; Peters, 1982). This finding of possible prenatal stress-induced sensitization was, however, present only in those animals, which received the MA challenge in adulthood, suggesting either unmasking effect of additional stress or specific effects of MA. This is supported by studies showing that prenatal stress induces long-term changes in seizure susceptibility as well as EEG patterns in rodents and humans (Edwards et al., 2002; Harvison et al., 2009; Rao et al., 1999) that may be further highlighted by administration of amphetamines in adulthood (Henry et al., 1995).

Our finding that prenatal MA decreases the occurrence of WDS and that the challenge dose of MA completely abolishes WDS is also supported by studies of others (Araki et al., 1989; Ervin et al., 1981; Turski et al., 1982) showing the occurrence of WDS in rats. The finding of Turski et al. (1982) showing that selective depletion of brain noradrenalin concentration enhances WDS, while selective depletion of brain dopamine concentration fails to affect it, suggests that the depletion of WDS may be under control of noradrenergic system, which is one of the neurotransmitter CNS systems that is affected by MA.

Conclusion

Current study presents novel findings that prenatal MA exposure induces long-term decreases in electrically induced epileptiform activities and EEG phenomena that may be further modified by a challenge dose of the same drug in adulthood. The mechanisms of the long-term MA effects are not known yet. Culic et al. (1994) suggest that the CNS noradrenergic system is the most likely candidate to be affected. While we have some evidence of catecholaminergic involvement (Bubeniková-Valešová et al., 2009) in prenatal MA effects on seizures, our future studies will investigate levels of catecholamines in brain structures closely associated with seizure propagation (e.g., the hippocampus), in animals prenatally exposed to MA with or without the challenge dose of MA in adulthood.

The main conclusion of this study is that prenatal MA exposure as well as the MA challenge in adulthood decreases epileptiform activity after electrical stimulation of sensorimotor cortex, i.e. lower seizure susceptibility. On the other hand, there is an increased susceptibility to chemically induced seizures after prenatal MA exposure (Šlamberová, 2005; Šlamberová et al., 2008, 2009; Šlamberová and Rokyta, 2005). A possible explanation for such opposing effects may be the differential interference of MA with focal (electrically stimulated) versus generalized (chemically induced) seizure mechanisms.

Acknowledgments

This study was supported by the grant # P303/10/0580 from Grant Agency of the Czech Republic, by the project # CN LC554 and by the Research Goal # MSM 0021620816 from Ministry of Education, Youth and Sports of the Czech Republic to Š. The authors express their appreciation to Dr. Ilona Vathy for critical reading and editing the manuscript. The procedures for animal experimentation utilized in this report were reviewed and approved by the Institutional Animal Care and Use Committee of the Third Faculty of Medicine of the Charles University in Prague and is in agreement with the Czech Government Requirements under the Policy of Humane Care of Laboratory Animals (No. 246/1992) and with the regulations of the Ministry of Agriculture of the Czech Republic (No. 311/1997).

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