ADHD Symptoms Induced by Prenatal Methamphetamine Exposure

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Summary

Methamphetamine is commonly used psychostimulant in the Czech Republic and is often abused by pregnant women. Methamphetamine may cause abnormalities in placenta and umbilical cord that results in hypoxia and malnutrition. ADHD is a mental disorder with a heterogeneous origin. The number of patients suffering from ADHD is growing. The pathophysiological mechanisms causing ADHD have not yet been clarified. There are very few rat models for ADHD and include genetic models, chemically induced models (ethanol, nicotine, PCBs, 6-hydroxydopamine lesion) or environmentally induced models (anoxia). The aim of the present study was to test prenatal methamphetamine exposure (5 mg/kg) as a potential novel animal model for ADHD. We found that adult male offspring prenatally exposed to methamphetamine presented hyperactivity while exploring novel environments. Together with cognition changes found in our previous studies, these might represent symptoms similar to those seen in ADHD. More experiments are planned to investigate our hypothesis.

Key words

Methamphetamine • ADHD • Prenatal exposure • Hyperactivity • Laboras • Voluntary running

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Introduction

ADHD (Attention Deficit and Hyperactivity Disorder) is a mental disorder with a heterogeneous origin (i.e. a combination of genetic and environmental influences). Environmental risk factors include prenatal exposure to drugs such as alcohol and nicotine, obstetric complications, head injury, and psychosocial adversity (Russell 2007). ADHD is characterized by inattention, hyperactivity, and the inability to control impulses (Smith *et al.* 2016). It has a prevalence rate of 5-5.5 % in young people (Polanczyk *et al.* 2007) and is more common in males (Asherson 2012). The number of patients suffering from ADHD is growing, possibly because of it being underdiagnosed in the past. The estimated prevalence of diagnosed ADHD in US children and adolescents increased from 6.1 % in 1997-1998 to 10.2 % in 2015-2016 (Xu *et al.* 2018). ADHD may affect all aspects of child's life. 30-60 % of affected individuals continuing to show significant symptoms of the disorder into adulthood, often resulting in lower educational levels and employment (Harpin 2005). In the US, medical, and other expenses for ADHD patients are almost $1000 per year (Gupte-Singh *et al.* 2017). To further understand the complexities of the origins of ADHD, there is a critical need to determine early etiological pathways to neurodevelopmental vulnerabilities.

There are few animal models of ADHD. In general, results obtained with animal studies suggest that monoaminergic systems, particularly the dopaminergic systems are functionally altered in ADHD (Russell *et al.* 2007). It means that animal models of ADHD are either genetic, e.g. DAT knock-out mice (dopamine transporter), SNAP-25 mutant mice, SHR (Spontaneously hypertensive rat), NHE mutant mice (Na^+/H^+ exchanger) or involve an insult to the central nervous system during the early stages of development.

Prenatal exposure to ethanol affects mainly dopaminergic transmission and causes hyperactivity (Gibson *et al.* 2000, Gilbertson *et al.* 2005). Prenatal nicotine exposure may increase dopamine reuptake in frontal cortex and fetal oxygen supply (Bush *et al.* 2000, Knopik 2009, Knopik 2010, Zhu *et al.* 2014). Both

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prenatal alcohol and prenatal nicotine exposure are used as animal models for ADHD (Russel 2007, Tiesler *et al.* 2014, Atalar *et al.* 2016).

Methamphetamine (MA) is a psychostimulant drug that is highly addictive. It is the most commonly used street "hard" drug in the Czech Republic (Czech Republic drug report for EMCDDA 2017). MA is relatively uncomplicated to produce with low price compared to cocaine or heroin (Marwick 2000). Addicted pregnant women also often use it to decrease appetite (Šlamberová 2012). MA has been shown to cause abnormalities in the placenta and umbilical cord (Vavřínková *et al.* 2001) and as such, induce hypoxia and lack of prenatal nutrition. Prenatal ischemia-hypoxia, or an insufficient supply of nutrients and oxygen to the developing fetus, is a primary pathway to lower birth weights (Hendriksen *et al.* 2002).

MA also readily crosses the placental and blood-brain barrier which allows MA to potentially induce changes in the development of unborn progeny (Šlamberová *et al.* 2006). Our previous studies have shown that prenatal MA exposure can induce effects that are similar to ADHD symptoms, e.g. memory and cognitive functions impairment (Fialová *et al.* 2015) as well as affecting the dopaminergic system (Bubeníková-Valešová *et al.* 2009). Prenatal MA exposure leads to lower birth weight (Šlamberová 2012), which is also a risk factor of ADHD (Asherson 2012). Other studies have shown, that prenatal MA exposure impairs postnatal development (Šlamberová *et al.* 2006), causes cognitive deficits (Chang *et al.* 2004) and leads to behavior problems (LaGasse *et al.* 2012). The present study (Fialová *et al.* 2015) looks at prenatal MA exposure as possible experimental model for ADHD.

Our goal was to examine whether prenatal MA exposure induces hyperactivity, which is one of the most distinct symptoms of the ADHD. The hyperactivity was tested by using two methods (1) voluntary running during development and in adulthood and (2) locomotor activity in novel (unknown) environment.

Methods

The procedures used in this study were reviewed and approved by the Institutional Animal Care and Use Committee, and 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.

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 a 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 acclimatization period, the females were weighed and smeared (vaginal lavage) to determine the phase of the estrous cycle. At the onset of the estrous phase of the estrous cycle, females were housed overnight with adult males. One female was paired with one male in each cage. The following day females were smeared again to check for the presence of sperm, and returned to their previous home cages. The day after impregnation was counted as day 1 of gestation.

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, females were placed individually in maternity cages (one female per cage). The day female gave birth was counted as postnatal day (PD) 0. Mothers and offspring were left undisturbed until the day of testing.

A total of 20 litters were used in the experiment. The number of pups in each litter was adjusted to 12. 8 male and 4 female pups were kept in each litter. 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. 160 male rats were used in this study. The rest of the animals were used in other studies. On PD 21, pups were moved to cages with a running wheel for voluntary running experiment.

Behavioral experiments

Voluntary running

Voluntary running is a method for measuring activity in rats (Persson *et al.* 2004).

Male rat offspring were tested for their activity in a cage (45×36×20 cm, type 765-423-1505 INDIANA 47903) with wheel attached to the inside of the cage. The voluntary running apparatus was purchased from Lafayette instrument Co, IN, USA. The wheel only rotated when animal(s) were running. Wheel rotation was automatically monitored using customized computer

software. All animals were weighed at the beginning and at the end of the running test. The experimental protocol involved rats being tested three times for 5 consecutive days starting on PD 21 (i.e. PD 21-PD 25), PD 30 (i.e. PD 30-PD 34), and PD 90 (i.e. PD 90-PD 94).

On PD 21, pups were weaned and housed in groups of 8 together with their male siblings in the testing cage (large cage with a running wheel) with free access to wheel, food and water.

The second trial was conducted with the same groups of animals on PD 30. In each younger developmental period 10 nests with 8 animals was used. On PD 90, in adulthood, only one animal from each group was tested for the voluntary running (one animal in cage). All animals were weighed at the beginning and at the end of the five-day trial. The amount of food and water consumed was also measured within each trial period. Animals were left in standard housing cages in groups of four between experiments.

Laboras test

Some of the animals from the litter tested for running activity on PD 21-25 and 35-40 were tested on PD 90, for activity testing in novel environment. The test was conducted in the dark and during the dark phase of the reverse light/dark cycle. The light/dark cycle was changed with lights on at 18:00 three weeks before testing.

LABORAS (Metris B. V., Netherlands) is a fully automated device for the recording and analysis of the behavior of individually housed mice or rats. A covered Plexiglass cage (45×30×25 cm) filled with bedding material is equipped as normal home cage with food and water available *ad libitum*. Single animal was placed into the cage. The activity was analyzed using Laboras software for 1 h, which was divided into six 10-minute intervals. The time spent and the frequency of each activity was recorded for each 10-minute interval. The following parameters were analyzed in all animals during the 1-hour period of testing: time spent in locomotion, immobility, rearing (exploratory behavior), distance travelled and average speed.

Statistical analyses

Two way ANOVA was used for the voluntary running experiment. The average value for each group was used for PD 21 and PD 30. A One-way ANOVA (Prenatal treatment) with Repeated Measure (Intervals) was used for LABORAS experiment. Simple comparisons were used in *post-hoc* tests. Differences were considered significant if $p<0.05$.

Results

Voluntary running

As shown in Figure 1, there was a tendency toward an MA-induced increase in distance travelled in Voluntary running experiment PD 30 and in PD 90. In two development stages (PD 21 and PD 30) we used group data instead of individual data. It was impossible to use ANOVA for repeated measures, so we used arithmetic averages of measured data instead. When two-way ANOVA (factors Prenatal treatment and Days) was used in the analysis, group differences were found to be significant [$F_{(1,54)}=8.05$, $p<0.01$].

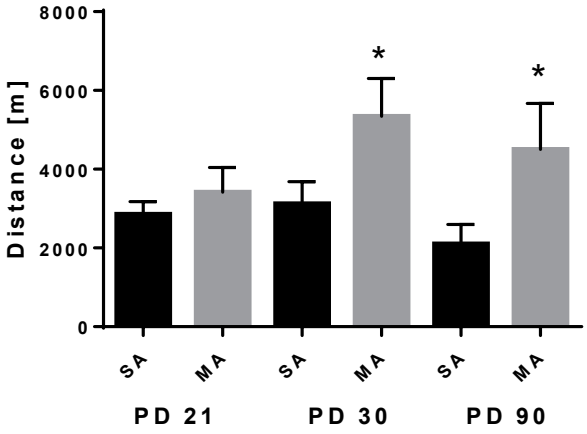

Fig. 1. The effect of prenatal MA and SA on behavior in the Voluntary running test. MA=methamphetamine; SA=saline; Values are mean ± SEM (n=10). * $p<0.01$, MA>SA of the same postnatal day.

Laboras

As shown in Figure 2, methamphetamine increased locomotion [$F_{(1,58)}=6.16$; $p<0.01$], additionally, there was a tendency toward an increased average speed ($p=0.07$) and distance traveled during the third 10-minute interval. There were no significant differences in the time spent by immobility, rearing and grooming in general.

Discussion

The present study was conducted to better understand the effect of prenatal MA exposure on locomotor activity of laboratory rats in different life stages and to test if the prenatal MA exposure could be used as a animal model for ADHD.

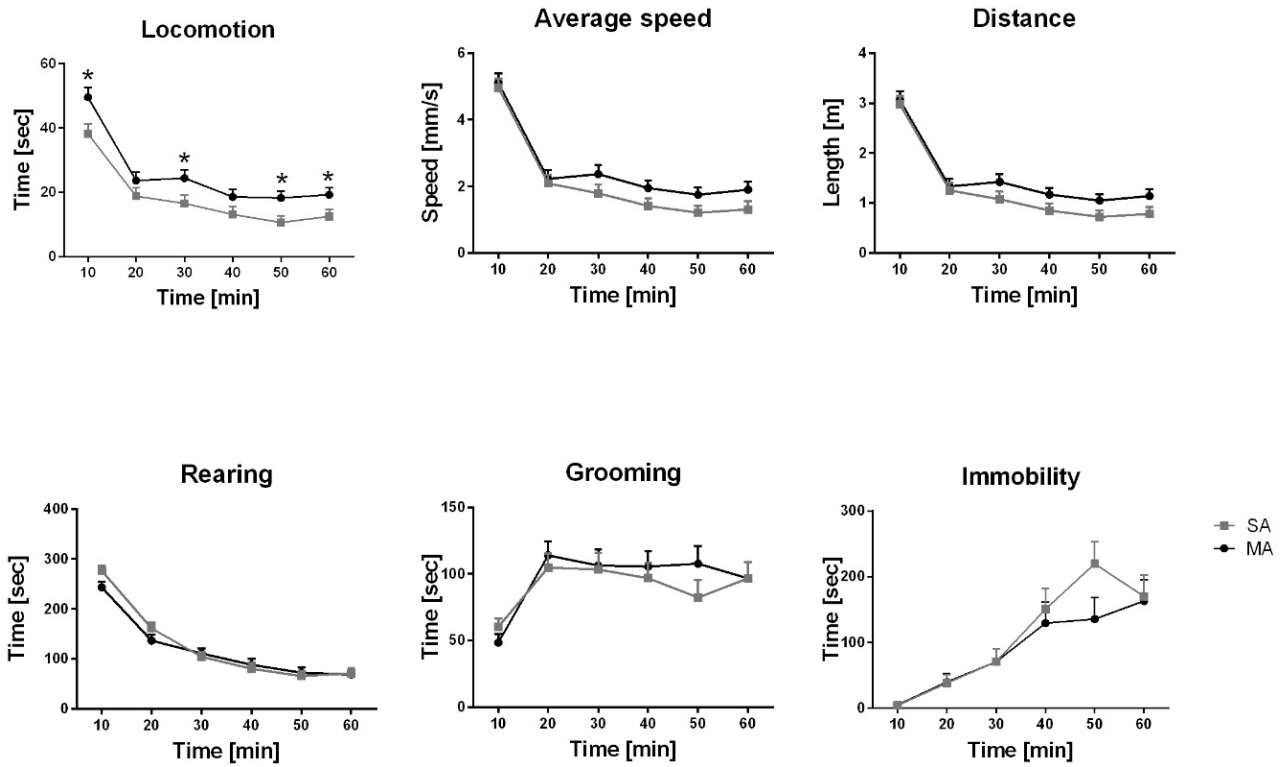

Fig. 2. The effect of MA and SA on behavior in the LABORAS test. MA=methamphetamine; SA=saline; Values are mean ± SEM (n=30). * $p<0.05$, MA>SA of the same time and activity.

We confirmed that when MA is administered at a dose of 5 mg/kg during pregnancy, it resulted in increased locomotor activity (i.e. voluntary running) and also increased locomotor activity of an adult rat in a novel environment (LABORAS test). This finding agrees with other studies showing that amphetamines increase locomotor activity, which seems to be associated with increased levels of dopamine (Bubeníková-Valešová *et al.* 2009).

There are no biological markers for ADHD. Some physical concomitants can be found when individuals are categorized with ADHD (Medin *et al.* 2019). One of them is the decreased function in the brain dopamine reward pathway (Volkow *et al.* 2011). A major function of dopaminergic transmission is to modulate fast, ionotropic synaptic transmission mediated by the neurotransmitter glutamate.

Children with ADHD would experience a delayed dopamine signal, rather than the immediate anticipatory dopamine signal. This is caused by changes in dopamine signaling and sensitivity to positive reinforcement. These conditions would lead to slower learning or even failure to learn (Tripp *et al.* 2008). Other animal models of ADHD, such as SHR or NHE also show changes in the dopamine system. Juvenile SHR has increased levels of dopamine in striatum and prefrontal cortex, with upregulation of D1 receptors in prefrontal cortex and hypofunction of D2 receptors or hyper-expression of the integral plasmalemmal protein dopamine transporter responsible for dopamine clearance (Viggiano *et al.* 2004).

Our previous experiments showed that prenatal MA exposure in rats causes impairment in recognition memory and leads to inability to concentrate, but does not affect the spatial memory (Fialová *et al.* 2015). Moreover, we found that prenatal MA exposure leads to changes in NMDA NR1 receptor subunits in the hippocampus (Šlamberová *et al.* 2014).

In addition, MA acts on serotonergic and dopaminergic neurotransmitter systems and produces not only long-lasting but also permanent changes in the CNS. It leads to a reduction in the volume of subcortical structures in the brain (caudate nucleus, putamen, globus pallidus and hippocampus) in children (Šlamberová 2012). Other studies have found reduced brain volume in ADHD patients, particularly prefrontal cortex, cerebellum, corpus callosum, and basal ganglia (Davids *et al.* 2005, Castellanos *et al.* 2002). A recent study suggests that hyperactivity can be a compensation mechanism to produce more lactate in muscles and

thereby supply brain with energy (Medin *et al.* 2019).

Finally, hyperactivity alone is insufficient to qualify as an animal model of ADHD (Sagvolden *et al.* 2011). However, hyperactivity, together with our previous results, leads us to suggest that prenatal MA exposure causes behavioral changes in adult male rats that are similar to ADHD symptoms. Since the effects of prenatal MA exposure on dopaminergic or glutamatergic transmission in rats are not fully understood, our hypothesis requires further examination. More experiments are planned to investigate our hypothesis. Although we do not know what is the coincidence in the human population in the use of MA during pregnancy and the incidence of ADHD in children of these mothers, in both conditions are observed similar behavioral manifestations and impaired cognitive functions. We cannot completely rule out a similar neurodevelopmental origin.

Conflict of Interest

There is no conflict of interest.

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