CHARLES UNIVERSITY IN PRAGUE
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Thesis summary

DOES PRENATAL METHAMPHETAMINE EXPOSURE INDUCE CROSS-SENSITISATION TO DRUGS IN ADULT MALE AND FEMALE RATS?

Vyvolává prenatální expozice metamfetaminu zkříženou citlivost k drogám u dospělých samců a samic laboratorního potkana?

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Content

SUMMARY

For many years, methamphetamine (MA) has dominated the abused drug market in the Czech Republic. Exposure to MA *in utero* was shown to impair reward circuits in the brain of developing offspring in such a way that it increases the predisposition for drug addiction later in life. Sensitisation is defined as an increased reaction to a drug, which could be observed after drug re-administration following discontinuation of repeated drug exposure. It can be developed after repeated drug administration in adulthood, as well as after chronic prenatal exposure.

The aim of my PhD thesis was to determine if prenatal MA exposure can cause cross-sensitisation to different drugs administrated in adulthood.

Pregnant dams were injected daily with MA (5 mg/kg) or saline subcutaneously (s.c.) during the entire gestation period. In adulthood, female and male rats were administrated s.c. - (a) the same drug (MA), (b) drugs with the same mechanism of action as MA (amphetamine- AMP, cocaine- COC, N-methyl-3,4-methylenedioxyamphetamine- MDMA), and (c) drugs with different mechanisms of action (morphine- MOR, delta9-tetrahydrocannabinol- THC), and tested using the Conditioned Place Preference (CPP), the Laboras test, the Elevated Plus Maze test (EPM), the Morris Water Maze test (MWM) and the Social Interaction test (SIT).

Our results showed the sensitising effect of prenatal MA exposure to other drugs that were presented during adulthood, as seen using the Laboras test. Increased locomotion after prenatal MA exposure was found in females and males treated with AMP, and in females treated with COC and MDMA. There was no interaction between prenatal MA exposure and an adult drug treatment either on the CPP, SIT, EPM, or MWM tests. As far as gender differences are concerned, it seems that in some test situations, females were more sensitive to drug effects than males.

In conclusion, our study showed that prenatal MA exposure can increase sensitivity to effects of some drugs challenges in adulthood; however, cross-sensitisation cannot be simplified to general drug addiction, since it seems that the mechanism by which a drug impaired neurotransmitter systems plays an important role. So it seems that although the offspring of MA-addicted mothers have altered sensitivity to drugs in adulthood, they do not display increased active drug-seeking behaviour.

SOUHRN

Už několik let dominuje metamfetamin (MA) drogovému trhu v České republice. Vystavení MA *in utero* nezpůsobuje jenom poruchy ve vývoji centrálního nervového systému, ale i takové změny ve vyvíjejícím se systému odměny mozku, které zvýší pravděpodobnost k rozvoji drogové závislosti později v životě. Senzitizace je definována jako zvýšená reakce po jednorázové aplikaci drogy, když dříve došlo k návyku na tuto drogu. Tento fenomén byl nejenom pozorován po opakovaném podávání drogy v dospělosti, ale také po chronickém prenatálním vystavení droze.

Cílem této dizertační práce bylo otestovat vliv prenatální expozice MA na citlivost k různým drogám aplikovaným v dospělosti.

Samicím laboratorního potkana byl po celou dobu březosti aplikován subkutánně (s. c.) MA (5 mg/kg/den) nebo fyziologický roztok. Dospělým potomkům, samcům i samicím, byla aplikována s. c. a) stejná droga (MA), b) příbuzné drogy (amfetamin- AMP, kokain- COC, N-methyl-3,4-methylenedioxyamfetamin- MDMA), c) nepříbuzné drogy (morfin- MOR, delta9-tetrahydrocannabinol- THC). K zjištění sensitizujícího účinku prenatálního MA byli použity behaviorální testy: sledujících vliv na aktivní vyhledávání drog („Conditioned place preference“ - CPP), na spontánní lokomoční aktivitu v neznámém prostředí (Test Laboras), na anxietu (Vyvýšené křížové bludiště - EPM), a na učení a paměť (Morrisovo vodní bludiště - MWM) a test na vzájemné sociální chování dvou jedinců (Test sociální interakce - SIT).

Naše výsledky ukázaly, že prenatální expozice MA zvýšila citlivost k některým drogám aplikovaným v dospělosti, což bylo zejména pozorováno v testu Laboras. Zvýšená lokomoce po prenatální expozici MA byla zjištěna u samců a samic s akutní aplikací AMP, a u samic s akutní aplikací COC a MDMA. V ostatních testech (CPP, EPM, SIT a MWM) interakce mezi prenatální aplikací MA a aplikací ostatních drog v dospělosti nebyla prokázána. Co se pohlavních rozdílů týče, ukázalo se, že za některých testovacích podmínek byly samice citlivější k akutní nebo chronické aplikaci drogy v dospělosti nežli samci.

Výsledky této dizertační práce ukazují, že prenatální expozice MA zvyšuje citlivost k účinku aplikace drog v dospělosti, ale že vznik zkřížené citlivosti nemůže být chápán jako vznik obecné závislosti. Je pravděpodobné, že mechanizmus účinku drogy na neurotransmiterový systém sehrává v senzitizaci klíčovou roli. Zdá se, že potomci matek závislých na MA mají sice změněnou citlivost k drogám v dospělosti, ale neprojevují zvýšený zájem o jejich aktivní vyhledávání.

LIST OF ABBREVIATIONS

5-HT- serotonin

AMP- amphetamine

COC- cocaine

CPP- the Conditioned Place Preference test

DA- dopamine

EPM- the Elevated Plus Maze test

M/D- metestrus/diestrus

MA- methamphetamine

MDMA- N-methyl-3,4-methylenedioxyamphetamine

MOR- morphine

MWM- the Morris Water Maze test

NT- neurotransmitter

P/E- proestrus/oestrus

PD- postnatal day

s. c.- subcutaneously

SIT- the Social Interaction test

THC- delta9-tetrahydrocannabinol

1 INTRODUCTION

For some years, methamphetamine (MA) has dominated the illegal drug market in the Czech Republic (World Drug Report 2015), because of its relatively uncomplicated production and low price compared to other psychostimulants (Marwick 2000). Moreover, almost half of women of a reproductive age, who take drugs, replace those other drugs with MA during pregnancy (Vavřínková *et al.* 2001), exposing not just themselves but also their developing foetus to a substance with potentially harmful and even long-lasting effects (Thompson *et al.* 2004). There is mounting evidence that exposure to MA *in utero* impairs the brain reward circuits of developing offspring in such a way, that it might increase the predisposition for drug addiction later in life. Animals studies have shown that offspring of mothers exposed to MA prenatally are more sensitive to MA administration in adulthood (Schutová *et al.* 2009b, Schutová *et al.* 2010, Šlamberová *et al.* 2011b, Šlamberová *et al.* 2011c).

To better describe the phenomenon of increase sensitivity, the term "sensitisation" has been established (Suzuki *et al.* 2004). It is defined as an augmented psychomotor activity, which could be observed after drug re-administration following discontinuation of repeated drug exposure, and has been demonstrated not only after repeated drug administration in adulthood, but also after chronic prenatal exposure (Robinson and Berridge 1993). In humans, the choice of drug abused in adulthood is contingent on various factors. In these cases, the increased cross-sensitisation (Shuster *et al.* 1977) developed after prenatal exposure depends on the drug abused in adulthood and has been reported between drugs of similar mechanisms of action (Bonate *et al.* 1997, Horger *et al.* 1992, Valvassori *et al.* 2007), as well as between drugs with different mechanisms of action (He and Grasing 2004, Leri *et al.* 2003, Vela *et al.* 1998). Prenatal MA has also been shown to increase reactions to the effect other drugs [anxiety-related behaviour (Schutová *et al.* 2010), cognitive deficits (Schutová *et al.* 2009a)]. These findings have lead us to extend the methodological part using various test models, which have been used for examining different forms of behaviour in reaction to acute or chronic drug treatment in animals with prenatal MA exposure.

Several preclinical studies have demonstrated that female rodents are more vulnerable than males to treatment with various drugs (Bisagno *et al.* 2003, Cailhol and Mormede 1999, Páleníček *et al.* 2005, Roth *et al.* 2002, Schindler *et al.* 2002, Tseng and Craft 2001), which is probably based on the sexual dimorphism in the neurotransmitter (NT) systems (Andersen and Teicher 2000, Walker *et al.* 2000).

2 HYPOTHESIS AND AIMS

The main **hypothesis** of my thesis is that **prenatal MA increases the sensitivity to:**

(A) to the same drug treatment in adults **(methamphetamine)**

(B) to drug treatment with drugs having a similar mechanism of action **(amphetamine, cocaine, MDMA)**

(C) to drug treatment with drugs having different mechanisms of action **(morphine, THC)**

The main **aims** of my thesis were:

1) To determine sensitising effect of prenatal MA exposure the following tests were used:
   a) for the active drug seeking behaviour;
   b) for locomotor behaviour.
2) To determine if prenatal MA exposure increased the sensitivity to other effects of drugs the following tests were used:
   a) for social behaviour;
   b) for anxiety;
   c) for spatial learning and memory.
3) To determine if sex differences affected drug treatment outcomes, both adult female and male rats were used.

3 MATERIALS AND METHODS

3.1 PRENATAL DRUG ADMINISTRATION

Adult female rats were assigned to two treatment groups through the entire gestation period: half of the females were injected subcutaneously (s.c.) with MA (5 mg/kg/day) and the other half with saline (1 ml/kg). The day of delivery was counted as postnatal day (PD) 0. All litters were adjusted to twelve. To avoid litter bias pups were cross-fostered. On PD 21, the animals were weaned and separated according to sex. They were left undisturbed until adulthood, when they were tested in following behavioural tests.

3.2 BEHAVIOURAL TESTS

3.2.1 The Conditioned Place Preference test

The CPP test is a test used for examining an active drug-seeking behaviour of an animal. The test was performed based on a study by Šlamberová *et al.* (2012).

3.2.2 The Laboras test

The Laboras test is a modified fully automated Open field test used for examining animal's locomotor behaviour in an unknown environment. The test was performed based on a study by Schutová *et al.* (2013).

3.2.3 The Social Interaction test

The SIT is used for examining social interaction of two unfamiliar animals (File and Hyde 1978). The test was performed based on a study by Šlamberová *et al.* (2011a).

3.2.4 The Elevated Plus Maze test

The EPM is a test based on the natural aversion of an animal to high and open spaces, used for measuring anxiety-related behaviour (Rodgers 1997). The test was performed based on a study by Fernandez Espejo (1997) modified by Pometlová *et al.* (2012).

3.3 *The Morris Water Maze test*

The MWM is one of the most widely used ways for testing the spatial navigation skills of an animal (Morris 1984). The test was performed based on a study by Schutová *et al.* (2009a).

3.4 ADULT DRUG TREATMENT

Adult female and male rats (PD 60-90) were tested in different tests. To determine the effect of prenatal MA exposure on the sensitivity to related drugs in adulthood the following drugs and dose were used (Tab. 1).

Table 1: The dose of drugs used in the test

TEST	**Dose (mg/kg)**					
	MA	**AMP**	**COC**	**MDMA**	**MOR**	**THC**
The CPP	5	5	5	5	5	2
The Laboras	-	5	5	5	5	2
The SIT	1	1	5	5	5	2
The EPM	1	1	5	5	5	2
The MWM	1	5	5	5	5	2

3.5 THE OESTROUS CYCLE DETERMINATION

Every day prior to testing each female was smeared with vaginal lavage. According to Turner and Bagnara (1976) two phases of the oestrous cycle were recognized in the present study: proestrus/oestrus (P/E) and diestrus/metestrus (D/M).

3.6 STATISTICAL ANALYSIS

First, data were tested for normality of distribution. Data with normal (Gaussian) distribution were analysed using the Analysis of variance (ANOVA). Three-way ANOVA with Repeated Measure was used to analyse differences in the Laboras, the CPP test and the MWM. Three-way ANOVA was used in the EPM test and Two-way ANOVA was used to analyse differences in male rats in the SIT test. When appropriate, comparisons between treatment groups were conducted by the Bonferroni post-hoc test. Differences were considered significant if $p<0.05$ in all statistical analyses.

4 RESULTS

4.1 The Conditioned Place Preference test

Sensitisation: Prenatal MA exposure did not sensitise animals to the preference of an environment associated with any of the drugs administrated.

Sex differences: In both sexes, MA conditioning increased the time spent in the chamber associated with the drug. MDMA after conditioning decreased the time spent in the drug-paired chamber in males, while it was increased in females. Both sexes demonstrated increased time spent in the chamber associated with MOR and no preference for a chamber associated with AMP, COC, and THC.

4.2 The Laboras test

Sensitisation: Prenatal MA exposure sensitised animals to the locomotor-stimulating effect of AMP in both sexes, while the effect of COC and MDMA was only seen in females. There was no cross-sensitisation found between prenatal MA exposure and MOR or THC administrated in adulthood.

Sex differences: Both sexes, after AMP and MDMA, demonstrated increased locomotion. Females, but not males, demonstrated increased locomotion after COC. After

MOR, both sexes demonstrated decreased locomotion. THC did not influence time spent in locomotion in either sex.

4.3 The Social Interaction test

Sensitisation: Prenatal MA exposure sensitised males to the social interaction-decreasing effect of MA, AMP, and MDMA.

Males exposed to MA prenatally demonstrated decreased total time spent in social interactions after MA, AMP, and MDMA administration. MA did not influence locomotion, while AMP, COC, and MDMA increased locomotion. MOR decreased, while THC did not have any effect on total time spent in social interactions.

4.4 The Elevated Plus Maze test

Sensitisation: Prenatal MA exposure did not sensitise animals to the anxiogenic and anxiolytic effect of any of the drugs.

Sex differences: In females, MA, AMP, and COC produced anxiolytic effects and locomotor-stimulating effects. Females, after MDMA demonstrated anxiogenic and locomotor-stimulating effects. In both sexes, THC and MOR produced anxiogenic and locomotor-inhibiting effects.

4.5 The Morris Water Maze test

Sensitisation: Prenatal MA exposure did not sensitise animals to the impairment effects of any of the drugs relative to spatial learning.

Sex differences: In females only, chronic treatment with MA worsened spatial learning; however, it did not have any effect on memory recall. In females only, chronic treatment with AMP and COC worsened both learning and memory recall. On the other hand, MDMA given to females worsened both learning and memory recall, while it only worsened memory in males. In females, chronic treatment with both, THC and MOR, worsened learning and memory.

5 DISCUSSION

5.1 Sensitisation

5.1.1 MA and drugs with similar mechanism of action as MA

Results from the Laboras test showed that in both sexes prenatal MA exposure induced sensitisation, but only to the psychostimulant effects of acute AMP, specifically, prenatally MA-exposed males and females demonstrated increased time spent rearing after AMP treatment. This result is in agreement with results from a study by Schutová *et al.* (2013) showing an increased sensitivity to MA in female and male rats prenatally exposed to another psychostimulant drug- MA. In contrast to the results from the Laboras test, our data from the CPP test did not demonstrate any significant increase in active AMP-seeking behaviour induced by prenatal MA exposure, which agrees with a CPP study by Šlamberová *et al.* (2011c) showing no MA conditioning in prenatally MA-exposed animals. The Laboras test showed that only females displayed sensitisation induced by prenatal MA exposure to COC and MDMA. The most likely explanation of this sex- specific effect in females might be based on a sexual dimorphism in the development of the mesolimbic dopaminergic system. Prenatal MA exposure might affect the female and male brain development differently and as a result, females respond more sensitively when exposed to other drugs in adulthood (Bisagno *et al.* 2003, Cailhol and Mormede 1999). Interestingly, the SIT showed that prenatally MA-exposed males with the acute MA, AMP, and MDMA treatment decreased the amount of time spent in social interactions compared to saline-exposed animals treated in adulthood with the same drugs. It appears, that prenatal MA might sensitise animals to effects drugs; however, no other interactions were found using the EPM or MWM test.

5.1.2 Drugs with different mechanism of action than MA

As far as the sensitising effect of prenatal MA exposure on adult MOR and THC treatment is concerned, we did not find any significant results, on the CPP test or the Laboras test. There was no interaction found using the SIT, the EPM test, or the MWM test. To the best of our knowledge, there is no study investigating increased sensitivity to MOR after prenatal MA exposure. The work by Vela *et al.* (1998) demonstrated that females prenatally exposed to THC during the gestation and lactation period exhibited an increase in the rate of MOR self-administration. On the other hand, prenatal MOR exposure was not shown to affect MOR self-administration in a study by Riley and Vathy (2006). One possible explanation that

we can suggest is that prenatal MA does not sensitise animals to various effects of drug with different mechanisms of action, however, more studies are needed to clarify this issue.

5.2 Sex differences relative to drug effects

5.2.1 The Conditioned Place Preference

MA conditioning increased time spent in the chamber associated with the drug in both, females and males, which is in agreement with a study using male rats by Šlamberová *et al.* (2011c). Our results showed a sex-dependent effect of MDMA conditioning. While males demonstrated aversion to the drug, females showed the opposite. Increased drug seeking after MOR conditioning in both sexes is in accordance with other studies showing rewarding properties of MOR (Bozarth and Wise 1981, Mueller *et al.* 2002). No preference of both sexes for THC-paired chamber; furthermore some aversion to the chamber was in agreement with a study by Cheer *et al.* (2000). Unfortunately, there is a lack of evidence showing sex differences in the drug conditioning, which could be compared with our results. A possible explanation of our results showing sex-differences in the drug conditioning might be based on the gender differences in the reactivity of the NT systems to the different drug treatment (Bisagno *et al.* 2003, Carlsson and Carlsson 1988).

5.2.2 The Laboras test

Our results showed that acute AMP and MDMA increased locomotion comparably in both sexes, which is in contrast to studies showing females to be more vulnerable to locomotor-stimulating effects of drug treatment (Milesi-Halle *et al.* 2007, Páleníček *et al.* 2005). We suggest that no sex differences found after AMP and MDMA treatment are based on dose-dependent reactivity of females and males. COC increased locomotion, but only in females; this agrees with studies showing sex differences in 5-HT and DA-neurotransmission, with female NT systems more vulnerable to COC stimuli (Walker *et al.* 2001). No sex differences were found after treatment with MOR and THC, which is probably explained by the different NT systems involved in mechanisms of drug action.

5.2.3 The Social Interaction Test

The SIT test was only used to examine the effect of acute drug treatment on the social interactions of male rats.

5.2.4 The Elevated Plus Maze test

Results from the EPM test showed that females treated with MA, AMP, and COC spent more time spent in the open arms, which indicates an anxiolytic-like effect of these drugs. No effect of MA on anxiety-related behaviour of males was observed, which was in contrast to a study by Schutová *et al.* (2010) that showed that MA produced anxiolytic-like effect. Contrary to this study, in our study animals were habituated to the experimenter 3 days prior to the test to reduce the stress. So no effect of drug treatment found in males might be simply based on a different stress reactivity of females compared to males. MOR and THC increased time spent the closed arms in both sexes, which indicate an anxiogenic effect. This "MOR" result is in contrast to results of Zarrindast *et al.* (2005) that showed MOR to have an anxiolytic effect. On the other hand, agrees with a "THC" study by Arevalo *et al.* (2001) showing an aversion of male rats to the OA of EPM. However, in these MOR and THC studies females were not examined.

5.2.5 The Morris Water Maze test

MA, AMP, and COC affected performance on the Learning test as well as the Memory Recall test, but only in females, which indicates that females are more sensitive to chronic treatment with these drugs. Interestingly, MDMA worsened learning and memory in females, but only affected memory recall in males. So it seems that although chronic MDMA did not affect learning processes in males, it caused some long-term consequences, which appeared later on the Memory test. A significant effect was also observed regarding MOR and THC treatment relative to learning and memory in females. To best of our knowledge there are no studies that have investigated the role of sex differences relative to performance in the MWM after chronic treatment with MOR or THC. However, there are studies that showed that chronic treatment with both drugs leads to a reduction of rat hippocampal structures, which play a key role in spatial learning (Pu *et al.* 2002, Rubino *et al.* 2009). We suggest that the worsened spatial learning abilities of females after chronic treatment with different drugs might be either a reflection of higher sensitivity of their NT systems to these drugs, or differences in stress coping between females and males, with the hypothalamic-pituitary axis reacting more robustly in females due the enhancing effects oestrogen.

6 CONCLUSIONS

Results from our study can be summarized as follows:

1) **As far as the effect of prenatal MA exposure on sensitivity to drug treatment in adulthood is concerned:**

a) **The CPP test**: prenatal MA exposure **did not sensitise** animals **to the preference** of an environment associated with any of the tested drugs.

b) **The Laboras test**: prenatal MA exposure **sensitised** animals to the **locomotor-stimulating effect** of AMP in both sexes, and to the effect of COC and MDMA, but only in females. No cross-sensitisation was found between prenatal MA exposure and drugs having different mechanisms of actions (MOR, THC).

c) **The SIT test**: prenatal MA exposure **sensitised** males to the **social interaction-decreasing effect** of MA, AMP, and MDMA. Prenatal MA exposure **did not sensitise** animals to the **anxiogenic and anxiolytic effect** of any of the tested drugs, or to the impairing effects of any of the drugs **on spatial learning**.

2) **The CPP, Laboras, EPM, and the MWM:** There were found some **sex differences on the effect of adult drug treatment**.

Results from our study showed that prenatal MA (at a dose of 5 mg/kg) administrated to mothers during their entire gestational period, can sensitise their offspring to application of other drugs in adulthood. Specifically, it seems that animals after MA exposure *in utero* demonstrated some kind of locomotor augmentation when treated with psychostimulants (e. g. COC, AMP, and MDMA) later in adulthood. Our results suggest that exposure to MA during pregnancy results in changes in neurotransmitter systems that predispose animals to greater responses to other drugs administrated in adulthood. However, an increased locomotor reaction was not seen after application of all drugs. That is why we cannot simply conclude that prenatal MA exposure might lead to an increase sensitivity to different drugs of abuse, and thus lead to development of a general drug addiction. We suggest that although the offspring of MA-addicted mothers have altered sensitivity to drugs in adulthood, they do not display increased active drug-seeking behaviour. In an anthropomorphic language, results from our study show that children of mothers that abused MA during pregnancy might have an increased reaction to other drugs when they encounter them later in life. This situation by itself might intensify their interest in drugs. On the other

hand, prenatal MA might not cause any changes that would lead the individual to actively search for drugs to abuse.

Our study also demonstrated gender differences in the effect of drugs on various forms of behaviour, e. g., drug-seeking behaviour, anxiety-related behaviour, and cognitive functions. It appears that the gonadal hormones of females play an important role in the overall process of drug reactivity.

Our results enhances the knowledge regarding drug addiction from the perspective of the children of women who abuse drugs during pregnancy, and also exemplifies new directions for research into drug addiction.

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