Charles University in Prague
Third Faculty of Medicine

Department of Normal, Pathological and Clinical Physiology

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?

Thesis

Eva Macúchová, Mgr (MS)

Supervisor: prof. Romana Šlamberová, MD, PhD

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Author's disclosure

I, Eva Macúchová, certify that all personal, professional and financial relationships with other people and organizations that pose a conflict of interests, that could personally be perceived as posing a conflict of interests, or that could potentially influence or bias my work described in this thesis have fully and truthfully been disclosed in the Acknowledgements and Reference sections of this thesis.

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Mgr. EVA MACÚCHOVÁ

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List of abbreviations

5-HT- serotonin

6-OHDA- 6-hydroxydopamine

AMP- amphetamine

AMPA- α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

BS- behavioural sensitisation

CA- the closed arms

CART peptides- cocaine-and-amphetamine-regulated-transcript

CB1 and CB2- cannabinoid receptors 1 and 2

CBC- cannabichromene

CBD- cannabidiol

CBG- cannabigerol

COC- cocaine

CPP- the Conditioned Place Preference test

D1 and D2- dopamine receptors 1 and 2

DA- dopamine

DAT- the dopamine transporter

EMCDDA- The European Monitoring Centre for Drugs and Drug Addiction

EPM- the Elevated Plus Maze test

GABA- Gamma-Amino Butyric Acid

GD- gestation day

GLU- glutamate

L-PAC- phenylacetylcarbinol

M/D- metestrus/diestrus

MA- methamphetamine

MAOs- monoamine oxidases

MDMA- N-methyl-3, 4-methylenedioxyamphetamine

MOR- morphine

MWM- the Morris Water Maze test

NA- noradrenaline

NAc- the nucleus accumbens

NET- the noradrenaline transporter

NMDA- N-methyl-D-aspartate receptor

NTs- neurotransmitters

OA- the open arms

OFC- the orbitofrontal cortex

OVX- ovariectomized

pSAP- protected stretched approach posture

P/E- proestrus/oestrus

PD- postnatal day

PET studies- positron emission tomography studies

PFC- the prefrontal cortex

ROS- Reactive Oxygen Species

SA- saline

s. c.- subcutaneously

SERT- the serotonin transporter

SI- social interaction

SIT- the Social Interaction test

SN- the substantia nigra

THC- delta9-tetrahydrocannabinol

VMAT 2- vesicular monoamine transporters 2

VTA- the ventral tegmental area

ABSTRACT

Women, who abuse drugs during pregnancy, expose not just themselves but also their developing foetus to impairing effects, which can have potentially harmful and even long-term effects on the exposed children. For some years, methamphetamine (MA) has dominated the illicit drug market in the Czech Republic and Slovakia; additionally this drug is on the rise worldwide. It is one of the most accessible drugs, and in many cases the first choice drug for many drug-addicted pregnant women; in part due to its anorectic and stimulant effects. These women are rarely aware of the consequences of their behaviour and their pregnancy is hardly ever a good enough reason for giving up drug use.

These findings are supported by many experimental studies that show the damaging effects of maternal MA exposure on their offspring. There is growing evidence that exposure to MA *in utero* not only causes birth defects and delays in infant development, but also impairs the brain reward neural pathways of a developing offspring in such a way, that it could increase the predisposition for drug addiction later in life. Previously published animal studies have shown that offspring of mothers exposed to MA during pregnancy are more sensitive to MA when they encounter this drug later in adulthood. With respect to increased sensitivity, the term of behavioural sensitisation (BS) has been introduced. It is defined as augmented psychomotor activity, which can be observed after drug re-administration following discontinuation of repeated drug exposure, and has been demonstrated to develop not only after repeated drug administration in adulthood, but also 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 (at a dose of 5 mg/kg) or saline subcutaneously (s. c.) over the entire length of the gestation period. To test the sensitivity after prenatal exposure, rats were administered s. c. with (a) the same drug (MA), (b) drugs with the same mechanism of action to MA (amphetamine- AMP, cocaine- COC, MDMA), or (c) drugs with different mechanisms of action (morphine- MOR, delta9-tetrahydrocannabinol- THC). The dose of the drug administered as well as the regimen of administration depended on the behavioural test used. In adulthood, males and females rats were tested using five different test situations. Conventionally, the Conditioned Place Preference (CPP) and the Laboras test are used for testing BS. Firstly, active drug-seeking behaviour tested using the CPP is thought to be a model of cue-induced craving seen in human addicts. Secondly, enhanced locomotor activity as seen in the Laboras test (after a single drug injection) models drug-induced hyperactivity and euphoria seen in drug users. Additionally, because drugs of abuse have been

shown to affect various forms of behaviour as well as cognition, the following tests were also used: the Elevated Plus Maze test (EPM) for testing anxiety, the Morris Water Maze test (MWM) for testing spatial learning and memory, and the Social Interaction test (SIT) for testing social behaviour in male rats only. In adult female rats, phases of the oestrous cycle were observed and compared.

Our results showed that there was a sensitising effect that could be attributed to prenatal MA exposure to other drug treatment in adulthood, which was best demonstrated using the spontaneous locomotor activity component of the Laboras test. Specifically, increased locomotion after prenatal MA exposure was found in females and males with an adult AMP treatment, and in females with adult COC and MDMA treatment. There was no interaction between prenatal MA exposure and adult drug treatment observed using the CPP test, so that it seems that *in utero* MA exposure does not cause changes that could increase drug-seeking behaviour later in adulthood. Interestingly, prenatal MA exposure sensitised male rats to the social interaction-decreasing effect of MA, AMP, and MDMA.

As far as other tests were concerned, the study found sex differences with regard to various drugs in behaviour and cognition. It seems that in some test situations and adult drug treatment, females were more sensitive than males. Based on sex differences we observed the following: (1) In the EPM test, MA, AMP, and COC induced anxiolytic-like effect, but only in females, while MDMA induced anxiogenic-like effects. (2) In the MWM, chronic treatment with MA, AMP, COC, MDMA, MOR, and THC lowered learning abilities and memory recall in female rats. (3) Additionally, female memory recall was shown to be worse in contrast to males, regardless of the adult drug treatment; (4) moreover, females relative to males demonstrated increased locomotion and decreased anxiety, especially in the phase of proestrus/oestrus when hormone levels were high.

In conclusion, our study showed that prenatal MA exposure can influence the sensitivity to the effects of some drugs, given as a challenge, in adulthood, specifically to those with a similar action mechanism. Our findings indicate that cross-sensitisation between prenatal MA exposure and adult drug treatment cannot be simply termed as a general drug addiction, since it seems that the mechanism by which a drug impairs specific neurotransmitter systems plays an important role. The study findings show that although the offspring of MA-addicted mothers have altered sensitivity to certain drugs in adulthood, they do not display increased active drug-seeking behaviour. Therefore, if we extrapolate the results to humans, it appears that there is a relatively little risk that a person, whose mother abused MA during pregnancy, will actively seek out drugs.

ABSTRAKT

Na drogách závislé těhotné ženy vystavují negativním účinkům drog nejen sebe, ale i své vyvíjející se potomky, což je může dlouhodobě negativně ovlivnit. Už několik let dominuje metamfetamin (MA) drogovému trhu jak v České republice, tak na Slovensku, avšak stále rostoucí je i jeho spotřeba celosvětově. Je stále jednou z nejvíce dostupných drog, a v mnohých případech drogou první volby pro těhotné ženy závislé na drogách, kvůli jeho anorexigennímu a únavu potlačujícímu účinku. Tyto ženy jsou si zřídkakdy vědomy důsledků svého chování, a jejich těhotenství je pro ně málokdy důvodem k ukončení užívání drog.

Tato zjištění byla potvrzena celou řadou experimentálních studií sledujících vliv mateřské aplikace MA na potomstvo. Stále rostoucí počet studií poukazuje na fakt, že vystavení MA *in utero* nezpůsobuje jenom vývojové vady a poruchy ve vývoji centrálního nervového systému, ale může vést k takovým změnám ve vyvíjejícím se systému odměny mozku, které zvýší pravděpodobnost k rozvoji drogové závislosti později v životě. Dostupné studie na animálních modelech poukázaly na fakt, že potomci matek, kteří byli vystaveni prenatálně účinkům MA, jsou citlivější k aplikaci MA v dospělosti. Pro zvýšenou sensitivitu na účinky drogy byl zaveden termín behaviorální senzitizace (BS). BS je definována jako zvýšená psychomotorická aktivita po jednorázové aplikaci drogy, když dříve došlo k návyku na tuto drogu. BS byla pozorována nejen po opakovaném podávání drogy v dospělosti, ale také po chronické prenatální expozici účinkům drogy.

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

Dospělým samicím laboratorního potkana byl po celou dobu březosti aplikován subkutánně (s. c.) MA (v dávce 5 mg/kg/den) nebo fyziologický roztok. Abychom otestovali citlivost dospělých potomků po prenatální expozici, zvířatům byla aplikována s. c. (a) stejná droga (MA), (b) příbuzné drogy (amfetamin-AMP, kokain-COC, MDMA), (c) nepříbuzné drogy (morfin-MOR, THC). Dávka aplikované drogy, jako i systém dávkování závisel na použitém behaviorálním testu. V dospělosti, samci a samice byli testováni v pěti různých behaviorálních testech. Tradičně, test aktivního vyhledávání drog („Conditioned place preference“ - CPP) a test na spontánní lokomoční aktivitu v neznámém prostředí (Test Laboras) jsou používány k testování BS. Zaprvé, aktivní vyhledávání drogy v CPP testu je považováno za model podmiňovaného bažení po droze u závislých jedinců. Zadruhé, zvýšená lokomoční aktivita v testu Laboras modeluje situaci drogou zvýšené hyperaktivity a euforie. Protože aplikace drog ovlivňuje různé formy chování a taky kognitivní schopnosti, použili jsme v naší studii i následující testy: Vyvýšené křížové bludiště- EPM, k testování anxiety, Morrisovo

vodní bludiště- MWM, k testování prostorového učení a paměti, a Test sociální interakce- SIT, k testování vzájemných sociálních interakcí jenom u samců. Byly zjišťovány případné pohlavní rozdíly a vliv ženských pohlavních hormonů v různých fázích estrálního cyklu na měřené parametry u jednotlivých experimentů.

Naše výsledky ukázaly, že prenatální expozice MA zvýšila citlivost k některým drogám aplikovaným v dospělosti, což bylo zejména pozorováno na spontánní lokomoční aktivitě v testu Laboras. Konkrétně, 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 testu CPP nebyla zjištěna interakce mezi prenatální aplikací MA a aplikací ostatních drog v dospělosti. Zdá se tedy, že vystavení MA *in utero* nezpůsobuje takové změny, které by zvýšily zájem o vyhledávání drogy v dospělosti.

Pokud se ostatních testů týká, naše studie demonstrovala pohlavní rozdíly v účinku různých drog na chování a kognitivní schopnosti. Ukázalo se, že za určitých testovacích podmínek byly samice citlivější k akutní nebo chronické aplikaci drogy v dospělosti nežli samci. Konkrétně, v testu EPM, MA, AMP a COC měly anxiolytický účinek, ale pouze u samic, zatímco MDMA měl účinek anxiogenní. Chronická aplikace MA, AMP, COC, MDMA, MOR a THC zhoršila schopnost učení a vybavitelnost paměťové stopy u samic. Navíc, prostorové učení bylo horší u samic a to nezávisle na aplikaci drogy. Pozorovali jsme také zvýšenou lokomoci a sníženou anxietu u samic v porovnání se samci, a to zvláště ve fázi proestrus/estrus s vysokou hladinou pohlavních hormonů.

Výsledky této dizertační práce ukazují, že prenatální expozice MA zvyšuje citlivost k účinku aplikace drog v dospělosti, konkrétně k těm s podobným mechanizmem účinku. Avšak, naše výsledky naznačují, že vznik zkřížené citlivosti mezi prenatálním MA a akutní aplikací drogy nemůže být chápán jako vznik obecné závislosti. Zdá se, že mechanizmus účinku drogy na neurotransmiterové systémy sehrává pravděpodobně klíčovou roli ve vzniku senzitizace. Nadějným je zjištění, že potomci matek závislých na MA mají sice změněnou citlivost k drogám v dospělosti, ale neprojevují zvýšený zájem o jejich aktivní vyhledávání. Takže pravděpodobnost, že by osoba, jejíž matka užívala MA během těhotenství, vyhledávala aktivně drogu, je relativně nízká.

CONTENT

I. INTRODUCTION

1 THE CURRENT SITUATION REGARDING DRUG ABUSE

The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) defined 'high risk drug use' as injecting drugs use or long duration/regular use of opioids, cocaine and/or amphetamines. This definition specifically includes regular or long-term use of prescribed opioids such as methadone but excludes their rare or irregular use and the use of other drugs, such as ecstasy or cannabis. Globally, it is estimated that 246 million people between the ages 15 and 64 (that is 1 out of 20) used any kind of illicit drug in 2013. According to the most recent data available, by estimation 12.2 million out of these people injected drugs (World Drug Report 2015). In Eastern and South-eastern Europe from 1.8 to 4.8 million of people injected some illicit drug in 2013. Based on the annual statistics, the top four most misused illicit drugs in 2013 globally were cannabinoids (with 181 million users), opioids (32.4 million users), cocaine (17 million users), ecstasy (18.8 million users) and amphetamine-types stimulants (33.9 million users). Because of the increasing availability of methamphetamine in some markets the use of methamphetamine has continued to rise since 2012 (World Drug Report 2015). Globally, the number of amphetamine-type drug laboratories (including methamphetamine) that were dismantled increased from 12 571 in 2011 to 14 322 in 2012. The increase in amphetamine-type drug seizures from 2002 is primarily attributable to the growing amount of methamphetamine seized, which increased from 34 tons in 2009 to 88 tons in 2013 (World Drug Report 2015). In 2012, methamphetamine accounted for 114 tons of a total 144 tons of amphetamine type drug seizures (World Drug Report 2014).

For some years, methamphetamine has dominated the market in the Czech Republic and Slovakia. However, in 2013, methamphetamine seizures not only accounted for the largest share of amphetamine-types substance seizures reported in the Czech Republic and Slovakia, but also in some countries in the Baltics and Eastern Europe, such as Belarus, Latvia, Lithuania and in addition Greece and Portugal (World Drug Report 2015). Two main European areas of methamphetamine production can be identified. The first one is in the Baltic States, which mainly export to Norway and to the United Kingdom. In this region benzyl methyl ketone is used as a principal precursor. In the second area, around the Czech Republic, Slovakia and Germany, production of methamphetamine, known also as pervitin, is mainly based on ephedrine and pseudoephedrine, and takes place in small-scale so-called kitchen laboratories, and from here the output is destined primarily for distribution within these countries (European Drug Report 2015). In 2011, out of 350 reported small kitchen laboratories 338 were found in

the Czech Republic. After the introduction of restrictions on the sale of medicines containing pseudoephedrine in the Czech Republic in 2009, an increase in imports of other pharmaceutical products from neighbouring countries has been reported, mainly from Poland. A new production method has been reported from Serbia, where ephedrine and pseudoephedrine are produced from L-PAC (phenylacetylcarbinol) (Exploring methamphetamine trends in Europe 2015).

The Annual Report on the Drug Situation in the Czech Republic (2013) stated that the four most abused drugs in the population of people aged 15-64 are cannabinoids, ecstasy, hallucinogenic mushrooms (e.g., *Psilocybe bohemica, Psilocybe semilanceata* and others) and methamphetamine. In 2013, 261 small kitchen laboratories were found in the Czech Republic and 69.1 kg of methamphetamine seizures was reported the same year. This number represents a twofold increase since 2012. It has been estimated that there were 44.9 thousand of 'high risk drug users' in 2013, out of those 34.2 thousand used methamphetamine. As far as the regional differences are concerned, an increase in the number of high risk methamphetamine users was reported in Prague, Central and South Bohemian Region, Liberec and Vysočina Region (2013 Annual Report: the Czech Republic Drug Situation 2013). It has also been shown that young Czechs underestimate the risks connected to drug use more than young people from other European countries (European Drug Report 2015).

Another growing problem of recent years is of drug abuse during pregnancy. Women using drugs during pregnancy expose not just themselves but also their developing foetus to the substance and this can have potentially harmful and long-term effects on the exposed children. Over the past several years the number of infants with drug-related birth defects has increased dramatically. Almost half of women of a reproductive age, who take drugs, replace another drug with methamphetamine during pregnancy. The reasons they do so is, because this drug has an anorectic effect, as well as providing an increase in alertness (Marwick 2000). Moreover, it is difficult to reason with drug-addicted woman, as their pregnancy is hardly ever a reason for giving up drug use. In addition to drug use, drug abusing pregnant women often consume alcohol and smoke cigarettes. Bad social conditions including unemployment and prostitution are also common problems which worsen their life situation (Vavříková *et al.* 2001).

It is clear from this data that the research of harmful effects which can be caused by methamphetamine abuse deserves some attention. Although many studies examining the effects of methamphetamine administration during pregnancy have been reported, findings from this research are still inconclusive.

2 THE DRIVING FORCE FOR TAKING DRUGS

Drug addiction is a relapsing disorder in which compulsive drug-seeking and drug-taking persist despite serious negative consequences (Koob and Moal 2006a). What plays the key role in the process of a drug dependence development has always been discussed. One of the most important findings for understanding the motivation of drug users in general, was made in 1954 by James Olds and Peter Milner. They used the intracranial electrical stimulation of the hypothalamus and associated structures and found out that this stimulation can act as a reinforcement of reward for behaviour. The discovered reward pathway is an evolutionary old and stable system, which is essential for survival (Kupfermann *et al.* 2000). The key role of this system is to find out important reinforcing stimuli, associate it with some value, predict a reward's response and initiate a motivational reply which leads to some kind of behaviour resulting in feelings of satisfaction and reward. The previously mentioned brain stimulations in that experiment act in many respects like natural reinforcers, but with one important difference. While natural reinforcers are effective only if the animal is in a particular drive state (e.g. searching for food is reinforced in a hungry animal), electrical stimulation of the brain works regardless of the animal's drive state. While in the experiments of Olds and Milner an electrical stimulation made an animal pull a lever more frequently, in the life of an addicted person, the pleasure after taking drugs and the craving without that drug drives him to search for it. A feeling of satisfaction after taking a drug arises more quickly and with a higher intensity than the pleasure which arrives after natural reinforcers (Kupfermann *et al.* 2000).

Imaging studies have provided evidence that multiple brain circuits are involved in the development of addiction. These circuits are connected to one another by direct or indirect innervations that are either glutamatergic (GLU) or GABAergic (Volkow *et al.* 2004). The fundamental role in the reward system is played by dopamine (DA) - the predominant catecholamine neurotransmitter (NT) in the brain, which is synthesized by mesencephalic neurons in the substantia nigra (SN) and the ventral tegmental area (VTA). The neurons of VTA form most of the mesolimbic and mesocortical projections involved in the reward pathway (see Fig. 1). It has been demonstrated that drugs of abuse induce large increases in DA in the nucleus accumbens (NAc) and the prefrontal cortex (PFC) (Koob and Bloom 1988, Shoblock *et al.* 2003a) and this DA increase is linked to the reinforcing effects of stimulants as assessed by the subjective reports of “high” and “euphoria” in addicted as well as non-addicted subjects (Volkow *et al.* 2004). Additionally, a lower level of D2 receptors in the striatum in a wide variety of drugs addictions (cocaine, methamphetamine, heroin) is believed to make the drug

addicts less sensitive to natural reinforces and thus give them a higher predisposition to drug addiction (Volkow *et al.* 2004).

While the mesolimbic reward pathway (the VTA to core of the NAc) is necessary for the 'pleasure principle' of drug taking, the development of drug addiction cannot be fully understood without looking beyond this principle. The repeated stimulation of the shell of the NAc activates the NAc core, which is connected to the dorsal striatum (nucleus caudatus and putamen) and leads to an increase in synaptic plasticity and shifts from recreational drug taking to uncontrolled behaviour and compulsive drug searching. At this time, PFC regions including the orbitofrontal cortex (OFC) and the anterior cingulate gyrus, which are connected to decision making and emotions, get involved. The OFC plays an important role in attributing salience to rewards (Everitt and Robbins 2005) (Fig. 1). Decreased activity of these brain areas has been documented to affect the motivational process and to lead to a loss of control over the drug use of an addicted person (Volkow *et al.* 2001a, Volkow *et al.* 2001b).

It is also well known that the drug effects are modulated by non-pharmacological variables like a subject's expectation of the effects of a drug, which in turn modify responses to the drug. It was shown in the positron emission tomography studies (PET) that in the brains of subjects who received methylphenidate intravenously, the DA concentration in mediodorsal and paraventricular nuclei of thalami was 50 % higher when people were expecting the drug. The thalamus receives direct projections from DA cells and from the OFC and also indirect projections from the NAc, and sends projections back to these regions, forming cortico-striatal-thalamic loops, through which thalamus modulates the drug response by expectations (Deutch *et al.* 1998). Also limbic regions (e.g. amygdala, ventral striatum, and ventral cingulate) and multiple memory systems are traditionally linked to reinforcing stimuli. Additionally, conditioned-incentive learning (mediated in part by the NAc and the amygdale), habit learning (mediated by the caudate and putamen) and declarative memory (by hippocampus) contribute to setting up addiction (White 1996) (Fig. 1).

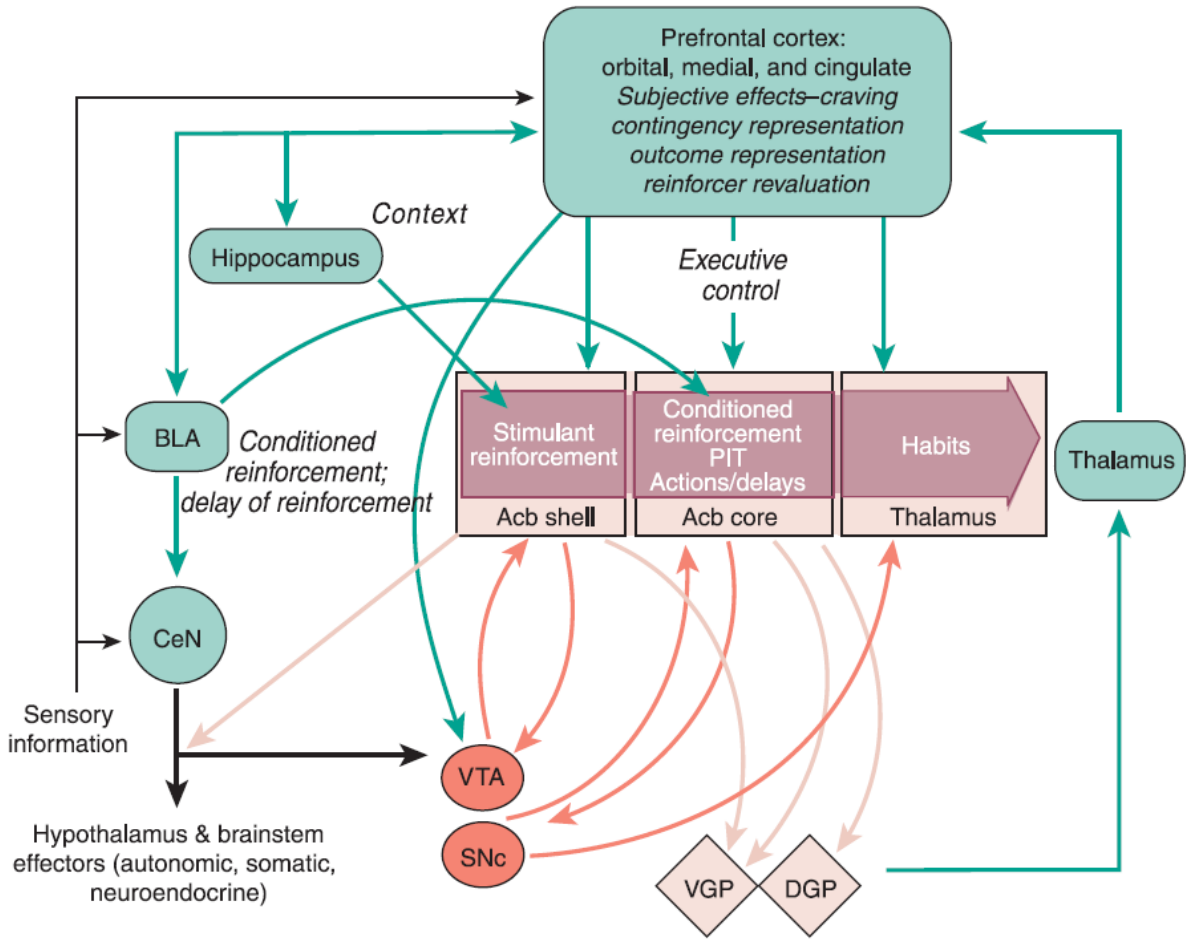

Figure 1: Cortico-striatal-thalamic loop involved in drug addiction. VTA- ventral tegmental area, SNc- substantia nigra pars compacta, Acb shell- shell of nucleus accumbens, Acb core- core of nucleus accumbens, VGP- ventral globus pallidus, DGP- dorsal globus pallidus, BLA- basolateral complex of amygdala, CeN- central nucleus of amygdala. Narrows indicate neurotransmitter systems: green- glutamatergic; red- dopaminergic; pink- GABAergic. From: Everitt and Robbins (2005).

3 AN OVERVIEW OF PSYCHOSTIMULANT TYPES OF DRUGS

3.1 AMPHETAMINES TYPE OF DRUGS

Amphetamine type drugs are produced by a chemical synthesis and can be divided into two categories: legal amphetamine derivatives (methamphetamine- MA, amphetamine- AMP and its isomers and analogues- ephedrine, phenmetrazine, methylphenidate, phentermine and chlorphentermine) and illegal amphetamine derivatives (MDMA= N-methyl-3,4-methylenedioxyamphetamine, MDM= 3,4-methylenedioxyamphetamine, etc.) (Bečková and Višňovský 1999a).

3.1.1 METHAMPHETAMINE

MA is one of the most widely abused amphetamine type drugs worldwide, including the Czech Republic (Marwick 2000, Vavřínková *et al.* 2001). The main reason for its popularity is because of its relatively uncomplicated production and low price when compared to other psychostimulants (Marwick 2000).

3.1.1.1 PHARMACOKINETICS AND PHARMACODYNAMICS OF METHAMPHETAMINE

MA is a powerful addictive psychostimulant drug with a high potential for addiction, which exists in two forms: base and salt. The pure base is a clear, volatile oil, which is insoluble in water and can be readily converted into MA hydrochloride (the most prominent salt form). The hydrochloride salt form is a crystalline solid, which is soluble in water. In powder form MA granulated crystals can be mixed with other ingredients such as lactose, dextrose or caffeine. Powder MA is either inhaled intra-nasally (snorted) or dissolved and injected. The MA euphoric effect lasts for a long time (from 8 to 24 hours) because of the slow drug metabolism. Bioavailability, the time to the peak effect and the time to reach peak plasma concentration, differ based on the route of administration (Cruickshank and Dyer 2009), with the terminal plasma half-life of MA being approximately 10 hours. It has got a high bioavailability: 62.7% after oral, 79% after nasal, 90% after smoking, and 100% after intravenous administration. The metabolism of MA largely takes place in the liver via a) N-demethylation to produce AMP (catalysed by cytochrome P450 2D6); b) aromatic hydroxylation (via cytochrome P450 2D6) producing 4-hydroxymethamphetamine; and c) beta-hydroxylation to produce norepinephrine. Inter-individual variability in MA metabolism might involve the polymorphic cytochrome P450 2D6 (Lin *et al.* 1997). Approximately 70% of MA leaves the body via urine within 24 hours (30-50% as MA, 10% as AMP and up to 15% as 4-hydroxymethamphetamine (Bečková and Višňovský 1999a, Harris *et al.* 2003).

MA is an indirect agonist at DA, noradrenaline (NA) and serotonin (5-HT) receptors. Its main action is to increase the concentration of these three NT. Thanks to its structural similarity, MA substitutes for monoamines in two places: a) cell surface integral membrane proteins-transporters, namely the DA transporter (DAT), the NA transporter (NET) and the serotonin transporter (SERT); and b) vesicular monoamine transporters (VMAT 2). Physiologically, membrane transporters help to pump the amines back to the synapse, where they are, with the help of vesicular transporters, stored back into vesicles (Sulzer *et al.* 2005).

In vitro studies showed that MA is twice as potent at releasing NA as DA, and has a 60 times greater effect on a NA than a 5-HT release (Rothman and Baumann 2003). MA's effect on NTs overflow is primarily due to a reverse transport of them from the cytosol into the synapse, and the uptake inhibition also contributes to the total effect (Sulzer *et al.* 2005). As far as the storage of the NTs in the presynaptic terminals is concerned, MA redistributes monoamines from storage vesicles into the cytosol by reversing the function of VMAT 2, and also by disturbing the pH gradient which normally drives the accumulation of monoamines in the vesicles. With the help of these two mechanisms, monoamines are available to stimulate postsynaptic monoamine receptors. MA also increases the quantity of biogenic amine available for release by inhibiting monoamine oxidases (MAOs; greater selectivity for MAO A over MAO B), key enzymes of amine catabolism located in the outer mitochondrial membrane (Sulzer *et al.* 2005). There are other properties that contribute to the effects of MA, which are, however, still under discussion. Some of them are the effect of MA on DA synthesis by enhancing tyrosine hydroxylase activity or the increase of CART peptides („cocaine-and-amphetamine-regulated-transcript“) after the MA administration, in the brain areas connected to the reward system (Kimmel *et al.* 2000). It should be noted that the interaction of DA with other NTs such as GLU and GABA plays an important role in modulating the magnitude of the DA response to drugs (Cornish and Kalivas 2001).

3.1.1.2 THE EFFECT OF METAMPHETAMINE

In humans, with the low to moderate doses used in clinical experiments, the main MA responses include a reduction in fatigue, euphoria, positive mood and arousal. Subjects also describe higher self-confidence, decreased fear, reduced appetite, and increased alertness. Because of its peripheral sympathomimetic effect, even a small amount of MA can result in many physical effects, which include a rapid heart rate, irregular heartbeat, elevated blood pressure, increased respiration, increased body temperature and pupil dilatation. Cardiovascular and subjective effects appear to increase depending on the dose (Cruickshank and Dyer 2009). A high-dose of MA administrated intravenously (55-640 mg) evoked psychotic symptoms, aggressive behaviour, confused speech and motor restlessness. Long-term use of MA was shown to be connected with anxiety, confusion, insomnia and mood disturbances. Symptoms of psychosis, such as hallucinations, paranoia and delusions (for example, the sensation of insects crawling under the skin) were also reported among chronic users (Nordahl *et al.* 2003). Several case studies revealed that prolonged use of MA is also associated with an eight-fold

increased risk of developing Parkinson's disease (Garwood *et al.* 2006). MA overdose is often recognised by tachycardia, hypertension, chest pain, shivering and an altered mental status including suicidal intensions and acute psychosis (Nordahl *et al.* 2003). In terms of MA withdrawal the most prominent symptoms are disturbed sleep, depressed mood, anxiety, craving, cognitive and concentration impairment, and anhedonia (McGregor *et al.* 2005). The MA withdrawal effects are thought to be originated from the depletion of presynaptic monoamine stores and down-regulation of the receptors and neurotoxicity (Barr *et al.* 2006).

It has been demonstrated in animal studies that the physical effects of MA administration (a low dose ≤5 mg/kg) are similar to those found in humans. Those which can be seen the most are higher locomotion and vertical activity (Schutová *et al.* 2010, Šlamberová *et al.* 2011c). Stereotypical behaviour including repetitive motion, cage sniffing and licking, and nail biting have been also reported (Frohmader *et al.* 2010). It has been found that an increased DA neurotransmission in the NAc is responsible for the induced locomotion, while the stereotypical behaviour relies on an increase of DA in SN (Kelly *et al.* 1975).

Studies indicate that repeated MA exposure leads to a long-lasting depletion of striatal DA and 5-HT, as well as damage to the striatal DA and 5-HT nerve terminals. The mechanisms of neurotoxicity are not yet fully understood. The initial study was done on rhesus monkeys, which received MA in low doses eight times a day for a period of four to six months (total of 52 mg/kg of MA a day). After another period of six months without the drug they were sacrificed and a regional brain assay of transmitter levels was conducted. It was observed that MA-treated monkeys had significantly reduced regional DA levels (Fischman and Schuster 1974, Seiden *et al.* 1976). In two other different species- rats and Guinea pigs, the repeated administration of MA was shown to cause long-lasting depletions of central DA (Wagner *et al.* 1979). Experiments on primates also demonstrated that the MA induced neurotoxicity may require more than a year for complete recovery (Harvey *et al.* 2000). Since the first experiments, several hypotheses regarding the mechanism of MA-neurotoxicity have been proposed. One explanation is the auto-oxidation of cytosolic DA and 5-HT to 6-hydroxydopamine (6-OHDA) and 5, 6-dihydroxytriptamine. 6-OHDA is extremely unstable and hydrogen peroxide is generated during auto-oxidation of DA (Kita *et al.* 2003). The formation of DA-related reactive oxygen species (ROS) such as superoxide and hydroxyl radicals appears to play an important role in MA-induced neurotoxicity. It has also been shown, that the administration of antioxidants, such as ascorbic acid or vitamin E, decreased MA-induced neurotoxicity (Wagner *et al.* 1986). Other factors, which are thought to contribute to the neurotoxic effect, are an elevated cerebral temperature and DA-induced secondary release of GLU in the striatum via

the cortico-striathothalamo-cortical negative feedback loop (Carlsson and Carlsson 1990). Interestingly, it appears that despite the structural similarities of DAT, SERT and NET, NA transporters are less vulnerable to oxidative inactivation (Haughey *et al.* 1999). Similarly to experimental studies, clinical studies with the help of PET and magnetic resonance imaging data also show brain abnormalities which persist further than the period of MA consumption, including inflammation, reduced density of DA markers such as DAT, D2 receptors in prefrontal cortex and basal ganglia, and reduced of VMAT2 and SERT. Striatal abnormalities, which correlate with different psychotic symptoms, impaired psychomotor coordination and memory deficits, persisted for years after the period of MA administration, but recovered partially after 6-12 months of abstinence (Sekine *et al.* 2003).

3.1.1.3 PRENATAL METHAMPETAMINE EXPOSURE

MA is one of the most frequently abused drugs by female addicts, especially during pregnancy. It is mostly taken because it decreases appetite and food intake and therefore helps women to control their weight, while increasing energy (Marwick 2000). Since MA is a lipophilic drug it can easily cross the blood-brain barrier (one of the most resistant barriers of the body), the placental barrier is even more easily permeable. Thus, if pregnant women don't quit taking MA during pregnancy, they expose not only themselves but also their foetuses to the danger of the drug, and it might lead to causing harm to the developing foetus (Greenhill 2006, Nordahl *et al.* 2003).

Clinical studies have revealed that exposure to MA during pregnancy induces birth defects such as heart defects or cleft lift, small head circumference, undescended testicles and also lowers the birth weight (Oro and Dixon 1987). Additionally, increased muscle tone, tremor, irregular sleep and impaired adaptability to stress have also been shown (Wouldes *et al.* 2014). Quantitative morphological analysis showed a reduction in the volume of subcortical structures of the brain (putamen, globus pallidus and hippocampus) in children with prenatal MA exposure (Thompson *et al.* 2004). Not only structural abnormalities, but also delays in child development have been reported. Volume decrease in the affected brain areas correlated with a worse performance of attention and verbal memory (Chang *et al.* 2004). It should be noted, that the developmental impairment of the children of drug-abusing women might be affected by other factors, e.g. combining alcohol or smoking at the same time as taking drugs, or less careful prenatal as well as postnatal care of their children (Sowell *et al.* 2010, Vavřínková *et al.* 2001).

Because clinical trials are restricted to statistical comparisons, the scientific research in humans is quite limited. Therefore experimental studies on animals' models are very useful.

It has been proven that prenatal MA exposure has harmful effects on both mothers and their offspring. Acuff-Smith *et al.* (1996) showed that repeated administration of pregnant rats with MA resulted in a higher incidence of delivery failure and the mother's death. It also shortened the gestation period, decreased the number of pups in the litter, and lowered the weight gain during pregnancy (Martin 1975, Martin *et al.* 1976, Šlamberová *et al.* 2006). In addition to growth restriction structural eye defects, delayed motor development, and learning impairments are also consistent findings in animals exposed to prenatal MA exposure (Acuff-Smith *et al.* 1996). Prenatal MA exposure has been shown to affect development of postural movements of the pups in the first three week of postnatal life, which was shown in different tests (righting reflex in mid-air, righting reflex on surface, rotarod test and bar-holding test) (Šlamberová *et al.* 2006, Šlamberová *et al.* 2007). Also the time of the drug administration has been demonstrated to be crucial in the final effects of the drug. It has been shown that MA exposure during the first half of gestation hinders the early locomotion, while exposure during the second half of gestation leads to reduction in sensorimotor development (Acuff-Smith *et al.* 1996).

There is a growing number of studies which show that changes in the brain caused by prenatal and neonatal MA exposure might persist into adulthood. Problems in adapting to a new environment, long-term cognitive deficits, as well as changes in locomotor activity have been previously shown (Acuff-Smith *et al.* 1996, Schutová *et al.* 2013, Šlamberová *et al.* 2005, Šlamberová *et al.* 2011c, Weissman and Caldecott-Hazard 1993, Williams *et al.* 2003). On the other hand, there are studies showing that exposure to MA *in utero* does not induce such changes, which would persist until adulthood as a reflexion of disturbance in various forms of behaviour (Schutová *et al.* 2008, Schutová *et al.* 2009)

3.1.2 METHAMPHETAMINE VS. AMPHETAMINE

MA and AMP are structurally similar drugs that are reported to share several pharmacokinetic and pharmacodynamic properties. Both belong to phenylethylamines, while MA is the N-methylated analogue of AMP (Melega *et al.* 1995). There is no consensus in literature as to which analogue is more potent. The commonly accepted opinion is that MA is more addictive and preferred by drug addicts than AMP and, despite structural similarities, MA has been suggested to be a more potent central stimulant with less peripheral activity (Peachey *et al.* 1977). However, disagreements over the effect of these two drugs at the key neurotransmitter pathways have been shown. Using *in vivo* microanalysis (Shoblock *et al.* 2003b) showed no differences between the effect of intraperitoneal MA and AMP

administration on DA levels in the NAc. On the other hand, in the same study, AMP was shown to be more effective at rising DA levels in the PFC than MA, and also AMP raised GLU levels in the NAc while MA didn't. Based on these findings, Shoblock *et al.* (2003b) suggested that AMP and its effect on the GLU release in the NAc might have a modulatory role in locomotor-stimulating effect of this drug. Additionally, this increase in GLU and DA levels after AMP may activate other pathways that inhibit reward and thus cause a lower reinforcing effect of the drug. On the other hand, some other authors didn't show any differences in the potencies of AMP and MA in either inducing locomotor activity (Milesi-Halle *et al.* 2007) or inducing release of DA (Melega *et al.* 1995). Moreover, MA was shown to have a three-fold greater potency than AMP in releasing 5-HT (Kuczenski *et al.* 1995). Because of the fact, that the PFC is connected to the performance of a working memory, a higher impact of AMP on this structure might be, according to Shoblock *et al.* (2003b), capable for causing deficits in working memory.

3.1.3 3, 4-METHYLENEDIOXYMETHAMPHETAMINE

N-methyl-3,4 -methylenedioxyamphetamine (MDMA) is a 'club' drug widely popular among young people in social situations thanks to its unique psychoactive effects, including mood elevation, evocation of feelings of empathy to others, mild hallucinations, increase of readiness and change of sensory perception (Parrott and Lasky 1998).

3.1.3.1 PHARMACOKINETICS AND PHARMACODYNAMICS OF MDMA

MDMA is usually taken orally, as a capsule or tablet and commonly in a combination with other drugs (cocaine, MA, ketamine). The drug's effect lasts approximately 3 to 6 hours, although it is common for users to take a second dose of the drug as the effects of the first dose begin to decline (Váchová *et al.* 1999). MDMA taken by humans is a mixture of (+) an (-) stereoisomers, and (+) MDMA is a stronger monoamine releaser than (-) MDMA (Baumann *et al.* 2007). Similarly to MA, MDMA interacts with monoamine transporters to reverse the normal direction of transmitter flux and thus cause a non-exocytotic release of three NTs (5-HT, DA and NA) (Johnson *et al.* 1986, Spanos and Yamamoto 1989). MDMA exhibits somehow a greater affinity to SERT versus DA transporters and recent *in vitro* experiments suggested that MDMA is a stronger 5-HT releaser than DA in the nervous system (Verrico *et al.* 2007).

3.1.3.2 THE EFFECT OF MDMA

Acute 5-HT release after MDMA contributes to the unique subjective effects described by humans, which have been mentioned before (especially euphoria with mild hallucinations and feelings of closeness to others). Of the physical effects, irregular heartbeat, dehydration, hyperthermia and reduced appetite have been documented (Liechti and Vollenweider 2001). Additionally, some negative consequences for heavy MDMA users have been experienced, including confusion, depression, sleep problems, drug craving, reductions in social interactions, anxiety and problems with attention and memory (Bull *et al.* 2004, Morley *et al.* 2001, Parrott and Lasky 1998). Although, similarly to MA, MDMA-induced long-lasting reductions in basal levels of 5-HT, substantial loss of 5-HT reuptake transporters and an irreversible degeneration of 5-HT nerve terminals in rats (Baumann *et al.* 2007) and in humans (McCann *et al.* 2000, Quinton and Yamamoto 2006) have been reported, it is still unclear if MDMA-induced neurotoxic effects contribute to long-lasting changes.

In experimental studies, MDMA causes different pattern of locomotion as those seen after MA administration. Typically, forward locomotion is presented by thigmotaxis and reduction in vertical activity, and stereotypic movements are presented by head weaving and forepaw treading (Hiramatsu *et al.* 1989, Spanos and Yamamoto 1989). It should be noted that forward locomotion relies on both, a release of DA and 5-HT. The NAc and striatal DA release after a microinjection of MDMA was shown to be connected to forward locomotion, and this process required both, D1 and D2 receptors (Bubar *et al.* 2004). Additionally, pre-treatment with selective 5-HT reuptake inhibitors attenuated an MDMA-induced 5-HT release as well as forward locomotion, and through the effect on 5-HT_{1B} and 5-HT_{2A} receptors MDMA facilitates this effect (Callaway *et al.* 1990, Gudelsky and Nash 1996). MDMA-induced stereotypic behaviour was shown to rely on a DA release in the striatum and a 5-HT release in the NAc, PFC and striatum (Baumann *et al.* 2008). The increased social interaction after acute MDMA treatment was shown to be linked to 5-HT_{1A} receptors, which play a role in the control of the neurohormone oxytocin release (Morley and McGregor 2000).

3.2 COCAINE

Cocaine (COC) is a powerfully addictive psychostimulant drug, which causes a euphoric effect like that of MA and MDMA. However, it differs from these drugs in the production mechanism. It is derived from a plant *Erythroxylon coca* which grows in the

mountains of Latin America. It has been chewed by members of Indian tribes for more than 5000 years, and firstly isolated and introduced to other countries in 1859 (Dixon 1989).

3.2.1 PHARMACOKINETICS AND PHARMACODYNAMICS OF COCAINE

There are more ways by which COC can be used. The most popular ones are intranasal and intravenous. It can also be made into hydrolysed crystal “crack”, which can be smoked. The intensity and duration of COC's high effects depend on the way it is administrated. Injecting or smoking COC delivers the drug rapidly into the bloodstream producing a quick, strong and brief effect, while the ‘high’ from snorting might last 15 to 30 minutes (Dixon 1989). The plasma half-life of the drug is about 30-40 minutes and it is metabolised to benzoylecgonine and ecgonine and a positive result of using the drug can be identified by immunoassay of the urine five days after use. It was firstly shown in 1960 by Whitby *et al.* (1960) that COC blocks re-uptake of catecholamine, which is now acknowledged to be its primary mean of increasing extracellular levels. COC binds with comparable affinity to NETs, DATs and SERTs and the addictive qualities appear to be dependent on the blockade of DAT function (Rothman and Baumann 2003).

3.2.2 THE EFFECT OF COCAINE

The powerful euphoric effect of COC depends largely on DA release and it is represented by increased self-esteem and vigour, decreased fatigue and appetite and increased sexual prowess (Dixon 1989). The mostly described physical effects after cocaine use are: an increase in body temperature, heat rate and blood pressure and sometimes nausea. Some additional adverse effects of the drug have been described after chronic COC use, including psychosis, insomnia, depression, mood disturbances, loss of appetite and aggressive behaviour (Williamson *et al.* 1997). Because of its powerful vasoconstrictive effect, the drug is often connected to sudden death caused by heart attacks and strokes (Dixon 1989). It has been stated that chronic use of COC has a neurotoxic effect on the dopaminergic system and this hypothesis has been shown by clinical findings showing a lasting decrease in DA in the brains of COC addicts (Dackis and Gold 1985, Wilson *et al.* 1992).

In animal models COC administration was shown to be connected to psychomotor sensitisation, which relies on excitatory neurotransmission in the VTA (Ungless *et al.* 2001). Ungless *et al.* (2001) demonstrated a long-lasting synaptic potentiation in VTA after a single COC injection. Other studies reported that increased DA neurotransmission plays a crucial role

in a COC-induced self-administration (Caine and Koob 1994, Thomas *et al.* 2008). The reinforcing effect of COC has been demonstrated by attenuation of COC self-administration after a selective lesion of DA terminals with 6-hydroxy DA (Caine and Koob 1994).

3.3 OTHER DRUGS OF ABUSE

3.3.1 OPIOIDS

The term 'opiates' describes all agents which are originally derived from opium (extracted from the opium poppy *Papaver somniferum* L). While opioids are defined as all drugs, natural and synthetic, with a morphine-like action, such as diacetylmorphine- heroin, codeine and dihydrocodeine, oxycodone, hydrocodone and buprenorphine. Some other synthetic opioids are methadone, fentanyl, naloxone, levorfanol and many others (Bečková and Višňovský1999b). They have a major medical use in the treatment of diarrhoea and pain. Out of these MOR has been used for pain relief for a long period of time. However, their beneficial medical effects are accompanied by significant side effects, the most devastating being opioid addiction which comes with chronic uncontrolled use (Koob and Moal 2006b).

3.3.1.1 PHARMACOKINETICS AND PHARMACODYNAMICS OF MORPHINE

Morphine (MOR) was first isolated from opium in 1804, and it was named after Morpheus, the God of Dreams, or Morphina, the God of Sleep (Koob and Moal 2006b). It is one of the most powerful and effective drugs for pain relief (Bečková and Višňovský1999b, Koob and Moal 2006b). However, its use within or outside of medical situations leads to an intractable physiological dependence and addiction. Intramuscular and subcutaneous administrations are the most common routes of administration with MOR-addicted people. Additionally, MOR injected intravenously is a sign of a strong MOR-addiction (Bečková and Višňovský1999b, Martin 1983). The liver is probably the major site of MOR metabolism with morphine 6-β-glucuronide and 3-β-glucuronide being the most dominant metabolites. 3-β-glucuronide has no analgesic activity and it is thought to be rather toxic, having also some excitatory effects. By contrast 6-β-glucuronide is believed to have similar analgesic qualities compared with MOR (Osborne *et al.* 1988, Penson *et al.* 2000). The plasma concentration differs based on the type of application, with peak plasma levels 20 minutes after intramuscular injection ranging from 51 to 62 ng/ml (Stanski *et al.* 1978).

MOR interacts predominantly with the opioid mu (μ)-receptor. These μ -binding sites are distributed in different areas of the human brain, on the terminal axons of primary afferents

within laminae I and II (substantia gelatinosa) of the spinal cord and in the spinal nucleus of the trigeminal nerve. They also show a high concentration in the posterior amygdala, hypothalamus, thalamus, nucleus caudatus, putamen, and certain cortical areas (Koob and Moal 2006b). As discussed later, it is still not fully understood which structures play a preliminary role in the neurobiology of the acute reinforcing effect of opioids.

3.3.1.2 THE EFFECT OF MORPHINE

Intoxication with MOR following an intravenous injection has been described as having four different phases. Firstly, there is a profound euphoria (sometimes termed as a rush) including visceral sensations. Secondly, euphoria is then followed by a feeling of well-being which can extend for several hours. Thirdly, a state of nods is described as an escape from reality to virtual unconsciousness. In the last phase the user is no longer experiencing the rush but not yet experiencing withdrawal. This state can last for up to 8 hours usually followed by another injection of the drug. However, how long the final effect lasts for depends on, if the drug user takes the drug chronically or if it is their first contact with the drug (Dole 1980). An overdose might be connected to an increased risk of depressed respiration leading to coma and death. The symptoms of MOR withdrawal were well described by and include elevation in temperature and blood pressure, perspiration, yawning, diarrhoea, goose bumps, muscle spasms, restlessness and insomnia. Anxiety and depressive-like symptoms have also been described (Bečková and Višňovský 1999b).

In animal models MOR administration increased locomotor activity in a dose dependent manner (Babbini and Davis 1972, Vezina *et al.* 1987). Accordingly to Nader and van der Kooy (1997) two separate motivational systems are involved in the reinforcing effect of opioids. The Mesocorticolimbic DA system is only important in mediating the motivational effects when an animal is in a deprived state (i.e., opiate-dependent) and the pedunculopontine tegmental nucleus of the brain stem mediates MOR's rewarding properties only when an animal is in a nondeprived state (not in a state of withdrawal - previously drug-naive rats). The self-administration of intravenously delivered MOR was first shown in 1960' by Weeks (1962) and by Thompson and Schuster (1964) in the Rhesus monkey, and since then studies have shown the lateral hypothalamus, the NAc, the amygdala and the VTA to be involved in MOR self-administration (Bozarth and Wise 1981). There are more ways how MOR administration affects DA neurotransmission. Firstly, MOR increases DA release through activity on μ receptors on GABA neurons leading to their hyperpolarization and inhibition of GABA release, and thus in

turn disinhibiting DA neurons (Johnson and North 1992). In addition, MOR increases burst firing of DA neurons in VTA (Nowycky *et al.* 1978).

3.3.2 CANNABINOIDS

Originally, the term cannabinoids referred to the phytocannabinoids of a plant *Cannabis sativa* L., but today the term includes all ligands of cannabinoid receptors and related compounds, comprising of endogenous ligands and a large number of synthetic cannabinoids ligands (Grotenhermen 2004). To present, 66 phytocannabinoids have been identified: cannabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), delta9-tetrahydrocannabinol (THC), delta8-THC and other types (Elsohly and Slade 2005). The most important cannabinoids present in the plant are delta9-THC, CBD, CBG and CBC, however delta9-THC is thought to be the primary active one in the resins of the marijuana plant. Some of the other ones produce similar behavioural and physiological effects of THC, some others only alter the effect of THC and contribute to its subjective outcome (Wachtel *et al.* 2002).

3.3.2.1 PHARMACOKINETICS AND PHARMACODYNAMICS OF DELTA9-TETRAHYDROCANNABINOL

Cannabis products are commonly either inhaled by smoking a cigarette, or taken orally as capsules or in cooked foods and liquids. Some other routes of administration include intravenous, eye drops or aerosols and inhalation with vaporisers (Grotenhermen 2004). The plasma concentration differs based on the type of application, with a peak 3-10 minutes after the onset of smoking, 20-30 minutes after intravenous administration, and 60-120 minutes with oral use. Metabolism of THC mainly takes place in the liver by microsomal hydroxylation and oxidation, and at least 100 metabolites have been identified, out of which 11-OH-THC is one with a similar action to its parent molecule (Harvey and Brown 1991). One single dose of THC might be detectable in the urine for usually 3-5 days, and sometimes up to 12 days (Schwartz *et al.* 1985).

The delta9-THC receptors have been identified and cloned in 1990 as the cannabinoid CB receptors (Matsuda *et al.* 1990). Subsequently after the identification of the CB1 receptor the CB2 receptor was discovered, but only the CB1 receptors are normally found in the brain, the spinal cord and the peripheral nervous system (Pertwee 1997). Activation of CB1 receptors produces a marijuana-like effect on behaviour and circulation, while activation of CB2 receptors does not. The delta9-THC has approximately equal affinity to the both, CB1 and CB2

receptors, however its effectiveness is less at CB2 than at CB1 (Grotenhermen 2004). Several endocannabinoids, which naturally bind to CB1 receptors, have recently been discovered, from which the well-known ones are anandamide and 2-arachidonylglycerol (Mechoulam *et al.* 1998).

3.3.2.2 THE EFFECT OF THC

Numerous effects have been reported after THC use. The effect is characterised by a unique psychological mixture of depressant and stimulant effects, which can be divided into four groups: affective (euphoria, enhanced well-being, anxiety), sensory (increased perception of external stimuli), somatic (feeling of the body floating) and cognitive (disturbed memory, difficulty in concentration). Apart from the effect on the central nervous system, the circulatory system is also affected. Tachycardia, vasodilatation and enhanced heart activity are commonly seen, sometimes leading to fatal consequences (Grotenhermen 2004). It is still under discussion whether heavy regular use may impair cognition, however, a disruption of sensory processing and impaired learning and memory have already been reported in humans after THC administration (D'Souza *et al.* 2004, Ramaekers *et al.* 2006). A cessation of long-term administration of THC has been shown to lead to withdrawal effects including insomnia, sweating and inner unrest, though symptoms are mild and a risk of physical and psychic dependence is low when compared to other drugs of abuse (Grotenhermen 2004).

As with other drugs of abuse, THC is believed to induce a rewarding effect on the central VTA-NAc circuit, however, the specific mechanism of the DA release after cannabinoids has not yet been identified (Lupica *et al.* 2004). It has been demonstrated using the CB1 receptor agonist that the increase of DA release in VTA might be caused by a local disinhibitory mechanism, in which inhibition of a GABA release via activation of the CB1 receptors leads to a higher activity of DA neurons (Szabo *et al.* 2002). Rewarding properties similar to those found after other drugs of abuse have been supported using preclinical studies. Braida *et al.* (2004) showed a reinforcing effect of a low THC administration in a self-administration test as well as in the conditioned place preference test. THC has also been shown to have an antinociceptive, hypothermic and motor activity-decreasing effect on laboratory mice and rats (Schramm-Sapyta *et al.* 2007, Varvel *et al.* 2005).

3.4 GENDER DIFFERENCES IN DRUG ABUSE

Traditionally, drug abuse is considered to be primarily a problem specific to men (World Drug Report 2015). However, numerous sex differences found in recent years have brought attention to drug abuse in women, and thus the need to consider drug abuse from different biological basis. Although the role of sex in the mechanisms of drug action remains unclear, clinical, as well as preclinical studies, indicates that ovarian hormones, oestrogen specifically, play a key role in producing sex differences in drug abuse (Lynch *et al.* 2002). The following chapters are focused on preclinical and clinical findings of sex differences and possible mechanisms that might underline these differences.

3.4.1 PRECLINICAL STUDIES

Several preclinical studies demonstrated that female rodents are more vulnerable than male rodents following treatment with AMP (Bisagno *et al.* 2003, White *et al.* 2002), cocaine (Cailhol and Mormede 1999, Lynch and Carroll 1999), MA (Roth and Carroll 2004, Schindler *et al.* 2002), MDMA (Páleníček *et al.* 2005), cannabinoids (Tseng and Craft 2001) and heroin (Lynch and Carroll 1999, Roth *et al.* 2002). In particular, locomotor activity and stereotypical behaviour were shown to be higher in female rats compared to males following acute and chronic AMP treatment (Bisagno *et al.* 2003) and acute and chronic MA treatment (Schindler *et al.* 2002, Schutová *et al.* 2013). Females were also reported to have an increased motivation for self-administration of cocaine and MA (Kučerová *et al.* 2009, Lynch and Carroll 1999). Additionally, female rats showed more problems with spatial memory after an acute dose of AMP (Bisagno *et al.* 2003). The most current opinion is that sex-related differences in the behavioural effect of drugs are based on sexual dimorphism in the NT system. A higher density in D1 receptors in the NAc was shown in female rats when compared to male rats (Andersen and Teicher 2000). Moreover, Walker *et al.* (2000) using a fast-scan cyclic voltammetry in anesthetized rats provided evidence that DA release and an uptake in the striatum is greater in female rats than in male rats. Variations in levels of cytochrome P-450 and other enzymes are also thought to play a critical role in different drug eliminations in females and males (Kato and Yamazoe 1992). Recently, higher concentrations of MA were revealed in a female rat's brain and plasma compared to a male's, following a single dose of MA (Rambousek *et al.* 2014).

It has been reported that females show a greater response to drugs in the oestrus when compared to other phases of the oestrous cycle (Becker 1990). It is well known, that during the rat oestrous cycle, ovarian hormones fluctuate and induce a variation of neurochemical and

behavioural responses to psychostimulants. Figure 2 shows the oestrous cycle of a female rat divided into four phases: 1) proestrus (oestradiol rises to the highest level and progesterone level is low at the beginning and rapidly rises and decreases at the end), 2) oestrus (oestradiol and progesterone levels rapidly decline), 3) metestrus (oestradiol level is low and progesterone level begins to rise), 4) diestrus (oestradiol rises and progesterone level falls) (Lynch *et al.* 2002).

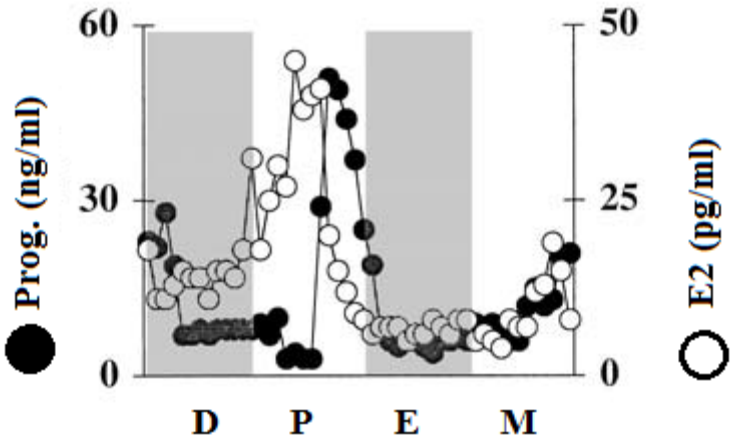

Figure 2: Changes in levels of oestrogen and progesterone throughout the phases of the rat oestrous cycle. The shaded bars separate the successive oestrous cycle phase 1) D- diestrus, 2) P- proestrus, 3) E- oestrus, 4) M- metestrus to identify the start and end of each phase. Prog- progesterone, E2- oestradiol. From: Lynch *et al.* (2002).

Oestrogen appears to have a dominant role in the enhanced responsiveness to psychostimulants in female rodents. This statement has been supported by studies using ovariectomized (OVX) females treated with oestrogen. Oestrogen treatment in OVX females has been shown to enhance behavioural responsiveness to COC (Sell *et al.* 2000) and AMP (Becker 1990) when compared to OVX females with no hormone treatment. Moreover, acute administration of oestrogen to OVX females was shown to induce a rapid increase in AMP-induced striatal DA release (Becker and Cha 1989, Becker 1990). Less is known about the mechanisms through which oestrogen acts in the striatum to enhance DA release in female rats. Two hypotheses have been stated by Becker (1999). Firstly, oestrogen acts on intrinsic medium spiny striatal neurons, which are primarily GABA neurons. This effect results in a decreased firing of recurrent collaterals that synapse on GABA receptors found on DA terminals. This, in turn, results in a decreased response to GABA at the DA terminals and an increased DA release.

Secondly, oestrogen acts directly on DA terminals and downregulate the D2 DA autoreceptors, which also results in DA release (Fig. 3).

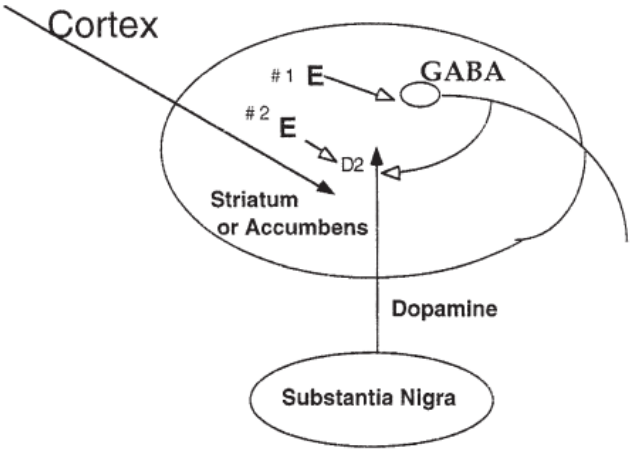

Figure 3: Two mechanisms postulated to contribute to the effect of oestrogen (E) on stimulation of DA release. #1: Oestrogen acts to inhibit intrinsic GABA neurons that have recurrent collaterals onto DA terminals. This results in a greater DA release. # 2: Oestrogen acts on DA terminals to enhance DA release by downregulating presynaptic D2 DA receptors. From: Becker (1999).

3.4.2 CLINICAL STUDIES

It should be noted that based on the epidemiological data from the American National Survey on Drug Use and Health (2014), adult men are more likely, compared to adult women, to be illicit substance users (11.5 % to 7.3%). Additionally, it has been shown that men are 2-3 times more likely to develop some type of drug dependence disorder than women (Brady and Randall 1999). It has also been shown that men differ in their biological response to drugs when compared to women. Results from a study investigating the effects of intranasal COC use indicate that women report weaker subjective effects compared to men (Lukas *et al.* 1996). As far as the pattern of use is concerned (Hser *et al.* 1987) reported no differences between the sexes in the time spent using the illicit drug, amount of substance abused or abstinence periods. Moreover, for women it takes a shorter period of time to progress from recreational user to drug addict (Hser *et al.* 1987, Westermeyer and Boedicker 2000). On the other hand, it is not clear whether women are more vulnerable than men to relapse as there are studies supporting both

sides, however, females were shown more likely to attribute relapse to a stressful event (Lynch *et al.* 2002).

Gender differences in four major determinants of pharmacokinetic variability have been revealed - bioavailability, distribution, metabolism and elimination. Changes in bioavailability depend on the route of drug administration and differences in the organs of absorption. Especially in the case of drugs with an oral route administration, gastrointestinal motility which has been shown to be affected by sex hormones plays a significant part in a drug's bioavailability. The distribution of a drug is influenced by numerous factors including mass index, body composition and plasma levels as well. As far as metabolism is concerned, the leading role in determining gender differences is thought to be played by the CYP450 superfamily (Franconi *et al.* 2007).

Similarly to preclinical studies, sex differences in the striatal DA system have been observed in humans (Kaasinen *et al.* 2001, Munro *et al.* 2006). For example, women have been reported to exhibit higher concentration of D2 receptors than men in the frontal cortex (Kaasinen *et al.* 2001). Additionally, a higher concentration of DA transporters in the striatum has been shown in women compared to men (Mozley *et al.* 2001). Interestingly, the reverse effect following a single administration of AMP on DA release in healthy adult women and men was reported in a study by Munro *et al.* (2006). They showed using PET studies greater DA release in the ventral striatum, the anterior putamen, and anterior and posterior caudate nuclei of men compared to women. Additionally, greater DA release in men was associated with greater subjective responses to AMP and COC in men compared to women (Oswald *et al.* 2005). As with animals, in humans, the ovarian hormones are also important in the way the different genders respond drugs of abuse. Three main phases of the menstrual cycle are presented: 1) the follicular (the oestrogen level is low at the beginning and moderate later, the progesterone level is low), 2) the peri-ovulatory (the oestrogen level peaks and declines, progesterone level begins to increase) and 3) the luteal (the oestrogen level is moderate and progesterone level is high (Lynch *et al.* 2002). Positive correlation between increased plasma level of oestrogen and increased positive subjective effects were found in females as a response to AMP and COC treatment in the follicular phase compared to the luteal phase (Justice and De Wit 2000, Sofuoglu *et al.* 1999).

4 THE SENSITISATION

In the context of the study of drug addiction, two important terms are defined. The first one is tolerance, which refers to the decreased effectiveness of a drug with repeated

administration, when the drug is being exposed continuously. On the other hand, behavioural or psychomotor sensitisation (BS) is defined as a progressive and enduring response produced by repeated intermittent drug administration with the same or lower dose (Suzuki *et al.* 2004). Other terms that refer to the BS are reverse tolerance, behavioural augmentation or facilitation (Robinson and Becker 1986). The phenomenon of BS to the effects of various drugs has been observed in several preclinical studies [for COC (Estelles *et al.* 2006), MA (Schutová *et al.* 2009, Schutová *et al.* 2010, Šlamberová *et al.* 2011b, Šlamberová *et al.* 2011c), and MOR (Valjent *et al.* 2010)] and others. It should be noted that the interval between drug applications is an essential variable. The closer together in time injections are, the greater likelihood that tolerance will develop, and the sensitisation is less likely (Post 1980). It was found that this enhanced drug sensitivity persists for very long periods of time. Even though only one single injection might be sufficient for its development, repeated administration produces more enhanced effect (Robinson and Becker 1986). For example, Magos (1969) reported that in rats two injections of AMP (6 mg/kg), given 2-5 weeks apart, enhanced the behavioural response produced by the third injection given 4 weeks later.

Robinson and Berridge (1993) claim that with a repeated intermittent drug administration, brain regions involved in a reward system become hypersensitive to a specific drug effect, which results in a pathological drug craving. Despite numerous studies investigating sensitisation as a complex process arising from different cellular changes in many brain regions, the neural basis of behavioural sensitisation has not been thoroughly characterized. To answer the question 'what is the locus of the neural changes underlie behavioural sensitisation ', different hypothesis have been proposed. According to the neural hypothesis, two phases of BS can be defined. The initiation of BS occurs in the VTA and it is defined by a transient sequence of cellular and molecular changes caused by drug administration. While the neuronal events associated with expression of BS are distributed among the interconnected nuclei of the motivation circuit and are defined as enduring neural alterations from the initiation process (Kalivas and Stewart 1991, Robinson and Becker 1986). The development of BS after repeated intermittent psychostimulant administration is specifically based on changes in the DA system- nigrostriatal, mesolimbic and mesocortical systems (Robinson and Becker 1986). This is to be expected because psychostimulants cause striatal DA release, and much of the behaviour which is sensitised by them is thought to be caused by increased DA release (Fukakusa *et al.* 2008, Sulzer *et al.* 2005, Vanderschuren and Kalivas 2000). While the increase in extracellular DA at terminals (NAc) following repeated injections of AMP is responsible for behavioural activation and expression of BS, an increased

extracellular DA level in VTA is sufficient for the induction of BS (Kalivas and Stewart 1991). Similar to animal models, repeated intermittent administration of AMP was reported to cause sensitisation of DA release in humans, even when an one dose of acute drug is given a year later (Boileau *et al.* 2006). Figure 4 schematically illustrates some of the changes in brain DA neurons that occur following repeated intermittent AMP administration. Apart from enhanced DA release, some other cellular changes are suggested to accompany BS (Robinson and Becker 1986). Although the essential role of D1 receptors in the induction of BS has been declared in previously published studies using D1 receptors antagonists, the involvement of D2 receptors in this process is still less clear (Ujike *et al.* 1989, Vezina and Stewart 1989). Additionally, not only DA but also other neurotransmitters (NTs) have been shown to be needed for BS induction after psychostimulants treatment (Kalivas and Alesdatter 1993, Wolf 1998). Specifically, increased GLU transmission in the NAc, striatum and VTA was reported after repeated intracranial AMP administration (Wolf 1998, Xue *et al.* 1996). Moreover, using pre-treatment with non-competitive NMDA antagonist, MK-801, the induction of BS was inhibited, indicating the NMDA and AMPA receptors to be involved (Stewart and Druhan 1993, Wolf and Jeziorski 1993).

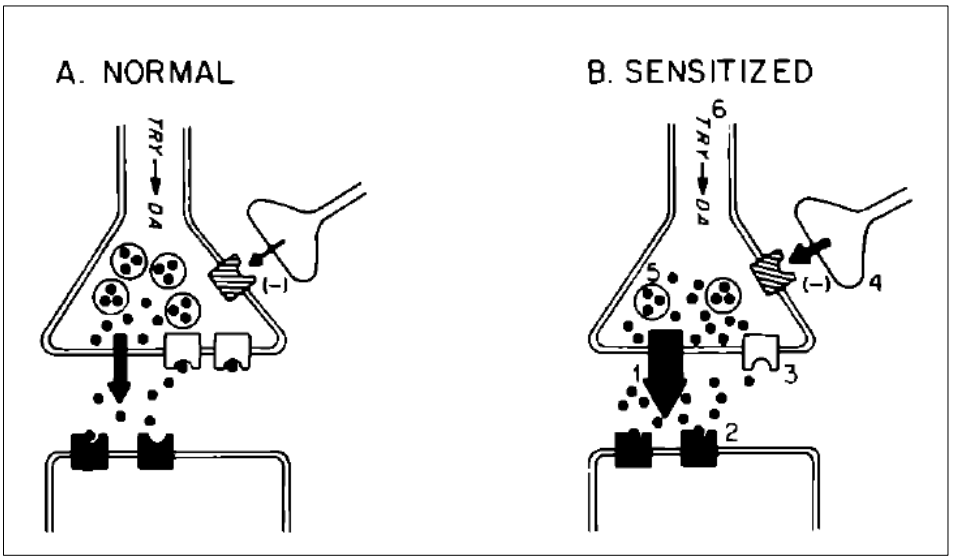

Figure 4: ***A:*** An illustration of DA release from dopamine terminals after the first time of AMP administration. ***B:*** An illustration of DA release from dopamine terminals after the animal has been sensitised to AMP (1- enhanced DA release, 2- changes in postsynaptic DA receptors, 3- DA autoreceptors sensitivity, 4- presynaptic facilitation by hyperpolarization of the DA terminals via a presynaptic input, 5- a shift in the distribution of DA from a storage pool. Black dots represent DA. Postsynaptic DA receptors are black, presynaptic DA autoreceptors are white, and presynaptic receptor receiving a hyperpolarizing input from another cell is striped. From: Robinson and Becker (1986).

There are an increasing number of studies which show that abuse of one drug leads to an increased sensitivity to another drug. This effect of a developed general drug sensitivity is called cross-sensitisation (Shuster *et al.* 1977) and has been reported between drugs of similar mechanisms of action like AMP and cocaine (Horger *et al.* 1992, Shuster *et al.* 1977) or between methylphenidate and AMP (Valvassori *et al.* 2007). Repeated AMP pre-treatment was first shown to sensitised animals to the locomotor activating effect of COC (Shuster *et al.* 1977). In another study, pre-treatment with AMP enhanced the acquisition of COC self-administration (Ferrario and Robinson 2007). Moreover, cross-sensitisation has also been demonstrated between drugs with different mechanisms of action, e.g. between opioids and COC (He and Grasing 2004, Leri *et al.* 2003) and between endocannabinoids and opioids (Fattore *et al.* 2005, Vela *et al.* 1998),

Furthermore there are studies which show that the exposure to a drug of abuse *in utero* causes such differences in the brain of a developing animal, which results in a development of a higher predisposition to drugs of abuse in adulthood (Malanga and Kosofsky 2003). Increased tendency of drug abuse in adulthood has been shown in prenatally MA-exposed (Schutová *et al.* 2010, Šlamberová *et al.* 2011c), COC-exposed (Keller *et al.* 1996, Rocha *et al.* 2002) cannabinoid-exposed (Vela *et al.* 1998) and MOR-exposed (Gagin *et al.* 1997) offspring compared to controls. In a study by Bubeníková-Valešová *et al.* (2009) offspring with prenatal MA exposure had increased brain levels of DA after a challenge dose of MA in adulthood, which suggests increased sensitivity to MA after prenatal treatment. The effect of the drug administrated prenatally has been documented to be dose dependent. A low dose of MA (2 mg/kg) decreased the expression of DA transporters in the striatum and 5-HT transporters in the hippocampus, striatum and hypothalamus. On the other hand, a high dose (10 mg/kg) increased the concentration of binding sides for the uptake of DA and 5-HT suggesting a stimulating growth effect of the particular axon terminals (Weissman and Caldecott-Hazard 1993), while Heller *et al.* (2001) showed MA at a toxic dose of 40 mg/kg not affecting the basal level of DA, but to increase the DA level in the striatum and tegmentum after the challenge dose of MA. MA at a dose of 5-20 mg/kg is used in experimental studies because it leads to such drug concentrations in the brain that correspond to the amount in the foetuses of the drug-dependent mothers (Acuff-Smith *et al.* 1996,Martin *et al.* 1976). Although there is still little known about how the MA exposure *in utero* interacts with the neurotransmitter systems of the developing brain and how this interaction affects the development of predisposition for addiction in prenatally exposed offspring.

4.1 TESTING OF SENSITISING DRUG'S EFFECT

Traditionally, there are three test models used for testing behavioural or locomotor sensitisation (Malanga and Kosofsky 2003). Firstly, there is an intravenous self-administration, which measures drug-seeking behaviour, in which the reward depends on the animal's operant behaviour. In anthropomorphic terms, it represents how much the animal “likes” or “wants” the drug. Then, there are the Conditioned Place Preference test (the CPP test) and the test for examining spontaneous locomotor activity of an animal in an unknown environment (the Laboras test, Open field test). In these two tests the reward doesn’t depend on the animal's behaviour. Specifically, in the CPP test, an animal demonstrates preference for an environment which has been paired with a drug, and this is thought to be a model of cue-induced craving seen in human addicts. Last but not least, the Laboras test is conducted to test augmented locomotor activity produced by repeated drug administration, in anthropomorphic terms, drug-induced euphoria (Malanga and Kosofsky 2003). The general pattern of induction of locomotor stimulation in a psychostimulant addict and in an animal model is illustrated in Fig. 5. The augmented motor activity is observed after readministration the drug following discontinuation of the repeated injection regimen (Pierce and Kalivas 1997). Different types of animal behaviour have previously been reported as a response to repeated intermittent psychostimulants administration (e.g. more intense stereotyped behaviour including repetitive head movement, increased forward locomotion, rotational behaviour, acoustic startle behaviour, cage climbing and others) (Malanga and Kosofsky 2003). It was discovered that the expression of BS is strengthened by the association of drug injection with environmental cues. BS was not manifested if animals were tested in a context where drugs have never been experienced (Anagnostaras and Robinson 1996, Duvauchelle *et al.* 2000).

There are fewer studies researching the behavioural expression of sensitisation in humans, however, eye-blink responses, increased vigour and energy ratings was shown to be caused by repeated administration of amphetamines in humans (Strakowski and Sax 1998). Also, drug readministration were shown to be followed by paranoia and psychosis (Pierce and Kalivas 1997) (Fig. 5).

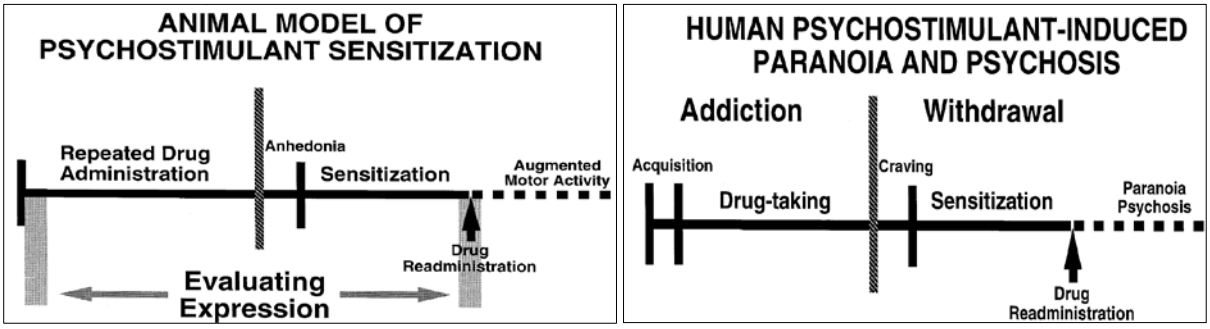

Figure 5: Induction of psychostimulant-induced sensitisation in animal and human models. From: Pierce and Kalivas (1997).

Previous studies have shown that prenatal MA exposure might sensitise the animals not only to the locomotor-stimulating effect of drugs administrated later in adulthood, but could be responsible for a modified reaction to the other drugs' effect. For example, (Schutová *et al.* 2010) found that prenatal MA altered the responsiveness of adult male rats to acute MA administration. Specifically, they found that prenatally MA-exposed males demonstrated increased anxiolytic behaviour in the Elevated Plus Maze (EPM) test when compared to prenatally saline-exposed males. This result indicated that prenatal MA exposure might sensitise the animals to the anxiogenic behaviour of an acute MA treatment. In another study by Schutová *et al.* (2009) the effect of prenatal MA exposure on spatial learning in the Morris Water Maze test after chronic treatment with MA was examined. Contrary to the EPM study, this study revealed that prenatal MA exposure did not sensitise animals to the worsening effect of chronic MA on the parameters of spatial learning. Moreover, in a study by Šlamberová *et al.* (2008) prenatal MA was shown to increase the sensitivity to a challenge dose of MA in a model of seizures induced by kainic acid.

These are interesting findings which highlight the fact, that sensitisation doesn't have to be only understood as a classical concept of augmented locomotor reaction after treatment with various drugs. These findings have lead us to extend the methodological part of various test models, which were used for examining different forms of behaviour as a reaction to acute or chronic drug treatment in animals with prenatal MA exposure.

II. EXPERIMENTAL PART

5 HYPOTHESIS AND AIMS

Previous works, using drugs, have shown that prenatal MA exposure increases sensitivity to acute drug treatment in adulthood. Not only has sensitisation to the same drug been shown, but also "cross-sensitisation" between drugs with different mechanisms of action. Moreover, evidence shows that female rats tend to react differently to the effect of psychostimulants, which might be related to changes in gonadal hormones during the oestrous cycle.

HYPOTHESIS

Regarding the above mentioned findings the following hypothesis were set up:

Prenatal methamphetamine increases the sensitivity:

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)

AIMS

1) To determine the sensitising effect of prenatal MA exposure using the following tests:
   a) for active drug seeking behaviour (the Conditioned Place Preference test),
   b) for locomotor behaviour (the Laboras test).

2) To determine if prenatal MA exposure increases sensitivity to any of the other known effects of the tested drugs, the following tests were used:
   a) for social behaviour (the Social Interaction test),
   b) for anxiety (the Elevated Plus Maze test),
   c) for spatial learning and memory (the Morris Water Maze test).

3) To determine if sex differences affected drug treatment outcomes, tests were carried out using both adult female and male rats.

6 MATERIALS AND METHODS

All procedures were performed in accordance with the Ethical Guidelines of the Third Faculty of Medicine, Charles University in Prague, Czech Republic and reviewed and approved by the Institutional Animal Care and Use Committee and in agreement with the Czech Government Requirements under the Policy of Humans Care of Laboratory Animals (No. 246/1992) with the subsequent regulations of the Ministry of Agriculture of the Czech Republic (as Project of the Experiment No. 79).

6.1 ANIMALS AND PRENATAL DRUG ADMINISTRATION

Adult female and male Wistar rats were delivered by Anlab (Prague, the Czech Republic) from Charles River Laboratories International, Inc. They were housed for 4 females - 5 males respectively per cage and left undisturbed for a week in a temperature-controlled colony room (22-24°C) with free access to food and water on 12 h (light):12 h (dark) cycle with lights on from 6:00. After the acclimatization period females were smeared with vaginal lavage to determine the phase of their oestrous cycle. When the oestrous phase was reached females were housed overnight with sexually mature males. There were always two female rats and one male rat per cage. The following morning females were smeared for the presence of sperms and returned to their home cages. The day when sperms were detected was designated as day 1 of gestation (GD 1). Animals were randomly assigned to two treatment groups through the entire gestation period: half of the females were injected subcutaneously (*s. c.*) with MA (5 mg/kg) and the other half with saline (1 ml/kg). The dose chosen was based on the previous studies (Šlamberová *et al.* 2005, Šlamberová *et al.* 2006). Females were injected daily throughout the entire gestation period (GD 1-22).

The day of delivery was counted as postnatal day (PD) 0. On PD 1, pups were weighted and tattooed for father identification. Prenatally MA-exposed pups were injected intradermally with black India ink in the left foot and prenatally saline-exposed pups in the right foot. All litters were adjusted to twelve. To avoid litter bias pups were cross-fostered so that each mother had six prenatally MA-exposed pups (3 males and 3 females) and six prenatally saline-exposed pups (3 males and 3 females). 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. Always one prenatally saline-exposed and one prenatally MA-exposed female and male, respectively, per group and test were used from each litter to avoid litter effects. Animals were housed for 4 females - 5 males respectively per cage on 12 h (light):12 h (dark) cycle with lights

on from 6:00 (the Morris Water Maze test and the Elevated Plus Maze test) or on reversed cycle with lights on from 18:00 (the Conditioned Place Preference test, the Laboras test, and the Social Interaction test).

6.2 BEHAVIOURAL TESTS

6.2.1 THE CONDITIONED PLACE PREFERENCE TEST

The Conditioned Place test (CPP) is a test used for examining an active drug-seeking behaviour of an animal. As mentioned before, the CPP test reflects a preference for an environment due to the contiguous association between the environment and a drug-associated stimulus based on the Pavlovian conditioning principles (Šlamberová *et al.* 2012).

In our experiment, the Conditioned Place Preference apparatus was made of Plexiglas, with two main compartments [25x25x25 cm (l x w x h)] and one central (neutral) compartment (15x25x25 cm) (Fig. 6). The central compartment was detached from the main chambers by removable doors. Walls of one of the main chambers were painted with 2.5-cm-wide alternating black and white horizontal lines; walls of the other main chamber were painted with 2.5-cm-wide alternating black and white vertical lines. The central compartment was made of a grey opaque Plexiglas. The central compartment had a smooth Plexiglas floor, while the floor of both main compartments was made of wire mesh with different size of the meshes. The CPP apparatus dimensions and a general procedure were modified accordingly to the work by Sanchez *et al.* (2003).

The CPP test was divided into three phases: pre-exposure, conditioning and the CPP test accordingly to Mueller and Stewart (2000) and Šlamberová *et al.* (2011b). Both, adult male and female rats were tested in the CPP test.

1) ***The Pre-exposure:*** On the Day 1, animals received a single pre-exposure test in which they were placed in the centre compartment with the doors open, so they were allowed to access to the entire apparatus for 15 min. The total time spent in each chamber and the amount of entries was measured and used to assess unconditioned preferences.
2) ***The Conditioning:*** The following conditioning phase lasted for 8 days. Each day during this phase rats were assigned to receive drug pairings with one of the two chambers in a counterbalanced fashion (the 'unbiased' procedure). Half of each group started the experiment on the drug-paired side and the other half on the saline-paired side. On alternate days, rats received either saline (1.0 ml/kg) or drug *s. c.* prior to being placed in the other chamber (Tab. 3, 4, 5). After administration of drug or saline, animals were allowed to

explore the specific chamber for 1 hour. Half of each treatment group received drug injections on the 2nd, 4th, 6th and 8th day; the remaining subjects on the 3rd, 5th, 7th, 9th day. The central compartment was not used during this phase of the test and was blocked by the doors.

3) ***The CPP test:*** On the Day 12, a test for the CPP was given. Animals were placed in the central compartment with the doors opened and thus allowed them a free access to the entire apparatus for 15 min. The time spent in each chamber and the number of entries was recorded to assess individual preferences. No injections were given during the CPP test, maintaining the same procedure as that used during the pre-exposure test.

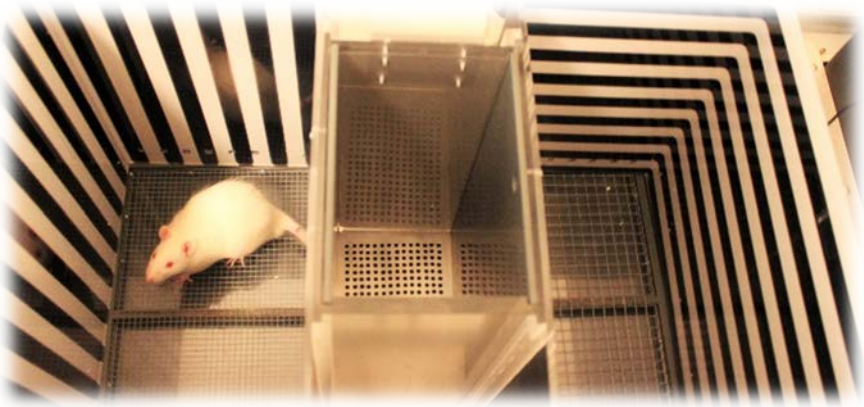

Figure 6: Animal in the Conditioned Place Preference apparatus.

6.2.2 THE LABORAS TEST

The Laboras test is a modified fully automated Open field test used for examining animal's locomotor behaviour, exploratory behaviour and general activity in an unknown environment. The Laboras test is an advanced and completely non-invasive system that automatically recognizes several normal and special behaviours of rats by analysis of the forces that are induced by the activities of the animal (Animal behaviour research, 2015a).

In our experiment, the Laboras apparatus was a triangular shaped cage (45 x 25 x 30cm) located in a dark room, and with walls made of Plexiglass (Fig. 7). It stood on a sensor platform connected to a computer. When the animal moved in the cage, platform recorded vibrations evoked by an animal's movements. Each behaviour had its own unique signal signature which was detected and identified by the software.

Rats were injected either with saline (1.0 ml/kg) or drug *s. c.* and placed in the centre of the Laboras cage (Tab. 3, 4, 5). There was no habituation to the apparatus before the testing, so it means that the rats were exposed to a novel environment on the day of the testing. The 1h period of testing was divided into six 10-minute intervals, to see how the behaviour of a rat was changing during the time spent in the Laboras apparatus. Both, adult male and female rats were tested in the Laboras test

The following parameters were automatically evaluated in the Laboras test:

1) The time spent in locomotion [s];
2) The distance travelled (trajectory length) [m];
3) The time spent rearing [s];
4) The speed of movement [mm/s].

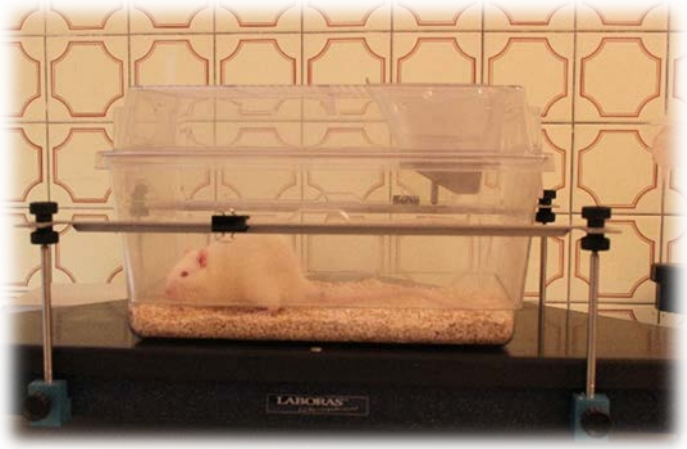

Figure 7: Animal in the Laboras apparatus.

6.2.3 THE SOCIAL INTERACTION TEST

The Social interaction test (SIT) is used for examining the situation when two animals are placed into a familiar open field arena in which neither has established territory and engage in social interaction (SI), which include a variety of behaviours excluding aggressive and sexual behaviour (File and Hyde 1978).

In our experiment, the SIT was performed in the open field arena (45x45x30cm) located in a dimly lit room (Fig. 8). Before the experiment, animals were habituated individually in the

open field on two consecutive days for 10 minutes (File and Hyde 1978). The habituation was performed in the same conditions as the experiment. On the third day, a pair of unfamiliar animals (each from different cage) of similar weight and the same treatment was tested for social interactions. The injection of drug or saline (1.0 ml/kg) was administered *s. c*. 45 minutes prior to SIT (Tab. 3, 4, 5). The behaviour of each pair of animals was recorded for 5 minutes. Only adult male rats were tested in the SI test.

Subsequently, the video recordings were evaluated by using the ODLog program (Macropod software). Behaviour was scored by typing pre-set keys on the keyboard of a computer. The ODLog software registered the number of pressings and the time in seconds between each pressing. Firstly, the total time spent in social interactions (SI; including time spent by mutual sniffing, following, climbing over, crawling under and allogrooming) was calculated. Secondly, the number (occurrence) and the time spent in various patterns of social behaviours, and non-social patterns of behaviour were scored separately to calculate the locomotion and exploration for each pair (Tab.1).

Table 1: Ethogram of rat behaviour in SIT test

Category	Pattern	Description
Social behaviour	Mutual sniffing	Mutual sniffing of different body parts including genital investigation
	Following	The pursuit of one animal by another
	Climbing over	Climbing over the other animal
	Crawling under	Crawling under the other animal
	Allogrooming	Grooming performed by one animal upon the another animal
Non-social behaviour	Locomotion	Several steps in a forward direction
	Rearing	Vertical activity, regardless whether it occurred on or off the walls

Figure 8: Two animals in social interactions. One is performing “mutual sniffing” the other one is performing “rearing”.

6.2.4 THE ELEVATED PLUS MAZE TEST

The Elevated Plus Maze test (EPM) is one of the most widely used models in contemporary preclinical research on anxiety. It is based on the natural aversion of the animal to high and open spaces and on the fact, that in mazes consisting of open and closed arms, rats show higher level of exploration of closed arms and avoidance of open arms (Rodgers *et al.* 1997).

In our experiment, the EPM apparatus consisted of two opposite arms enclosed by brown plastic walls (30 cm high) and two opposite open arms and surrounded by transparent Plexiglas ledges (0.5 cm high). All the arms were 10 cm wide and joined in the centre of the maze (10x10 cm), so the animal could freely move from one arm to another (Fig. 9). The apparatus was elevated 40 cm above the floor. The room with the EPM apparatus was illuminated by dim lighting (Pometlová *et al.* 2012).

All of the animals were handled according to the protocol by Geyer and Swerdlow (2007) during three days prior to the EPM test. The animals were moved in their home cages into the testing room for at least a 60 minutes acclimation period. The testing was conducted between 8:00 a.m. and 13:00 p.m. They were tested in a randomized order, starting the test in the central square, facing one of the open arms. An animal received an injection of saline (1.0 ml/kg) or drug *s. c.* 45 minutes prior to the test (Tab. 3, 4, 5) and its behaviour was video-recorded for 5 minutes. In between the individual testing, the maze was cleaned and dried. Both, adult male and female rats were tested in the EPM test.

The video recordings were evaluated by using the ODLog program (Macropod software). Four categories were introduced with the parameters chosen based on the study by Espejo (1997) modified by Pometlová *et al.* (2012) (Tab.2).

Table 2: Ethogram of rat behaviour in the EPM

Category	**Pattern**	**Description**
Anxiogenic behaviour	Time spent in closed arms (CA) [s]	Total time spent in closed arms
Anxiolytic behaviour	Time spent in open arms (OA) [s]	Total time spent in open arms
Approach/avoid conflict	Protected stretched approach posture (pSAP) [number]	Forward elongation of the front quarter of the body followed by retraction occurring in the central platform/closed arm
Locomotor and exploratory behaviour	All arm entries [number]	Moving from the central platform into the closed arms and open arms
	Rearing [number]	Vertical activity in the central platform and open arms
	Sniffing [time]	Mobile or quiet olfactory exploration of the environment

Figure 9: Animal exploring the open arm of the Elevated Plus Maze.

6.3 COGNITIVE TEST

6.3.1 THE MORRIS WATER MAZE TEST

The Morris Water Maze test (MWM) is one of the most widely used ways for testing the spatial navigation skills of an animal. The concept behind it is that the animal must learn to use distal cues to navigate from the start points around the perimeter of an open arena to locate the hidden escape platform (Morris 1984, Stuchlík 2003).

In our experiment, three test settings were used in this MWM test: the Place Navigation test, the Probe test and the Retention Memory test (Schutová *et al.* 2009). Before each experiment the animals were left to acclimatize to the laboratory conditions, in which the experiments were performed. Both, adult male and female rats were tested in the MWM test.

The water maze consisted of a blue circular tank (2m in diameter), filled with water (22.5 ± 2.5°C). The maze was divided into 4 quadrants in respect to start positions (north-N, south-S, east-E and west-W). A transparent circular platform was placed into NE quadrant of the tank, 1 cm below the water surface. The maze was surrounded by various extra-maze cues on the walls. The trials were tracked using a video-tracking system EthoVision XT6 (Noldus Information Technology, Netherlands) (Fig. 10).

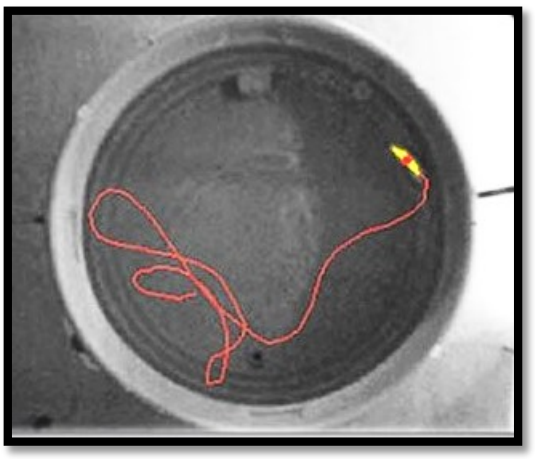

Figure 10: Animal in the Morris Water Maze test. From: Animal behaviour research (2015b)

1) The Place Navigation test

During 6 days of spatial learning (Fig. 11) animals were trained to locate the hidden platform within the limit of 60 s. If the animal did not reach the platform within the time limit, it was gently guided by the experimenter to the platform. Eight trials per day were performed. The position of the platform was the same throughout the period of learning. After each trial, the animal remained on the platform for 30 s prior to the next trial to have a chance to orient and learn its position in the room. After the trials on each experimental day, the animal received the injection of drug or saline (1.0 ml/kg) *s. c.* and was placed into the home cage (Tab. 3, 4, 5). The following parameters were evaluated with use of EthoVision program: the latency of platform acquisition [s], the distance travelled (the length of the swim-path) [cm], the search error (cumulative distance) [cm] and the speed of swimming [cm/s].

2) The Probe test

During the Probe test (Fig. 11), which was conducted on the 8th day, the platform was removed, and the animal was left to swim in the maze for 60 s. The start position in this test was for each animal north. The following parameters were recorded: the distance travelled [cm], the number of crossing of the quadrant where the platform was located and the duration of presence in the quadrant where the platform was located [s], and the speed of swimming [cm/s]. After the trials the animal received the injection of drug or saline *s. c.* and was placed into the home cage (Tab. 3, 4, 5).

3) The Memory Recall test

The memory test was performed on the 12th day (Fig. 11). An animal was expected to find the platform located at the same position as during the learning test within 60 s. Each animal was subjected to 8 trials. The same parameters were analysed as in the Place Navigation test: the latency of platform acquisition [s], the distance travelled (the length of the swim-path) [cm], the search error (cumulative distance) [cm] and the speed of swimming [cm/s].

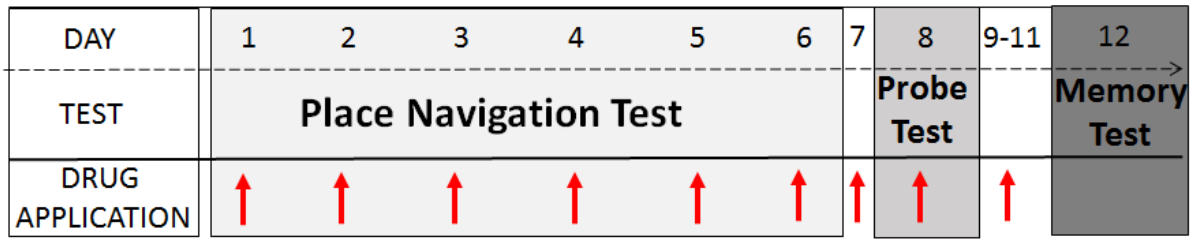

Figure 11: The setting of the Morris Water Maze test with the drug application

6.4 ADULT DRUG TREATMENT - EXPERIMENTAL GROUPS

Adult female and male rats (PD 60-90) were tested in different tests. From 8 to 16 animals (or pairs of animals) per group, per sex and per prenatal and adult drug treatment were used in each test. The experimental groups are shown in the Table 3. To determine the effect of prenatal MA exposure on the sensitivity to related drugs in adulthood the following drugs were used (Tab. 4):

1) Methamphetamine (MA)

- In the CPP test the dose of 5 mg/kg was chosen because it induces similar foetal brain drug concentrations and similar behavioural changes to those found in humans (Acuff-Smith *et al.* 1996, Šlamberová *et al.* 2011b).
- In the EPM and SIT the effect of MA at a dose of 1 mg/kg was chosen based on our preliminary data showing that these doses do not induce stereotypy behaviour that would affect the behaviour of animals.

- The effect of MA (1 mg/kg) on the spontaneous locomotor activity of females and males was not tested in the Laboras test, as this was previously published in a study by Schutová *et al.* (2013) (*).
- The chronic effect of MA (1 mg/kg) on the on the spatial learning of males was not tested in the MWM test, because this was previously published in a study by Schutová *et al.* (2009). The same dose (5 mg/kg) was used to test the chronic effect of MA on the spatial learning of females.

2) *Drugs with a similar mechanism of action to MA:*

a) Amphetamine (AMP):

- In the CPP test and the Laboras test the dose of 5 mg/kg was chosen based on a work by Timar *et al.* (1996) showing developed positive place preference conditioning by using this dose of AMP.
- In other tests AMP at a dose of 1 and 5 mg/kg was chosen based on our preliminary data showing that these doses do not induce stereotypy behaviour. 1 mg/kg of AMP used in the EPM tests was chosen based on a study by Dawson *et al.* (1995) showing an anxiolytic effect of AMP.

b) Cocaine (COC):

- In all of the tests the dose of 5 mg/kg was chosen based on a work by Heyser *et al.* (1992) showing developed positive place preference conditioning by using this dose of COC and at the same time not inducing stereotypy behaviour.

c) MDMA („ecstasy“):

- In all of the tests the dose of 5 mg/kg was chosen based on a work by Bubeníková *et al.* (2005) showing increased acoustic startle response by using this dose of MDMA and at the same time not inducing stereotypy behaviour.

3) Drugs with different mechanism of action to MA

a) Morphine (MOR):

- In all of the tests the dose of 5 mg/kg was chosen based on a work by Riley and Vathy (2006) showing developed positive place preference conditioning by using this dose of MOR and at the same time not inducing stereotypy behaviour.

b) THC

- In all of the tests the dose of 2 mg/kg was chosen based on a work by Cheer *et al.* (2000) showing developed positive place preference conditioning by using this dose of THC and at the same time not inducing stereotypy behaviour.

Tab. 3: The experimental groups used in the behavioural tests

GROUP	PRENATAL EXPOSURE	DRUG TREATMENT IN ADULTHOOD
SA/SA	saline	saline
MA/SA	methamphetamine	saline
SA/MA	saline	methamphetamine
MA/MA	methamphetamine	methamphetamine
SA/AMP	saline	amphetamine
MA/APM	methamphetamine	amphetamine
SA/COC	saline	cocaine
MA/COC	methamphetamine	cocaine
SA/MDMA	saline	MDMA
MA/MDMA	methamphetamine	MDMA
SA/THC	saline	THC
MA/THC	methamphetamine	THC
SA/MOR	saline	morphine
MA/MOR	methamphetamine	morphine

Tab. 4: The dose of drugs used in the tests

TEST	Dose (mg/kg)					
	MA	**AMP**	**COC**	**MDMA**	**MOR**	**THC**
The Conditioned Place Preference test	5	5	5	5	5	2
The Laboras test	- (*)	5	5	5	5	2
The Social Interaction test	1	1	5	5	5	2
The Elevated Plus Maze test	1	1	5	5	5	2
The Morris Water Maze test	1	5	5	5	5	2

Tab. 5: Drug treatment regimen in different tests

TEST	TREATMENT
The Laboras test	Before testing (see 6.2.1)
The Conditioned Place Preference test	Depended on the testing day (see 6.2.2)
The Social Interaction test	45 minutes prior to the test (see 6.2.3)
The Elevated Plus Maze test	45 minutes prior to the test (see 6.2.4)
The Morris Water Maze test	On each day of 12 days period of testing (immediately after testing) (see 6.3.1)

6.5 THE OESTROUS CYCLE DETERMINATION

Every day prior to testing each female was smeared with vaginal lavage. The smear was then examined by light microscopy. According to Turner and Bagnara (1976) two phases of the oestrous cycle were recognized in the present study: proestrus/oestrus (P/E) with predominance of large nucleated and some cornified epithelial cells in the smear; diestrus/metestrus (D/M) with predominance of leukocytes in the smear.

6.6 STATISTICAL ANALYSIS

First, data were tested for normality of distribution. Data with normal (Gaussian) distribution were analysed using the Analysis of variance (ANOVA) and presented as [F (N-1,

n-N) = xx.xx; p<0.0x], where F is test criterion of ANOVA, N-1 degrees of freedom of groups, n-N=degrees of freedom of individual subjects, p is probability level.

THE CONDITIONED PLACE PREFERENCE TEST

Three-Way ANOVA (factors: prenatal exposure x chamber with drug x sex/oestrous cycle) with Repeated Measure (time: before vs. after conditioning) was used to analyse differences in the number of entries to chamber and the total time spent in the chamber associated with the drug. 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.

THE LABORAS

Three-way ANOVA (factors: prenatal exposure x adult drug treatment x sex/oestrous cycle) with Repeated Measure (time: 10-minute intervals) was used to analyse differences. 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.

THE SOCIAL INTERACTION TEST

Two-way ANOVA (factors: prenatal treatment x acute treatment) was used to analyse differences in male rats. When appropriate, comparisons between treatment groups were conducted by the Bonferroni post-hoc test. In all tests, the differences were considered significant if
$p < 0.05$.

THE ELEVATED PLUS MAZE TEST

Three-way ANOVA (factors: prenatal treatment x acute treatment x sex/oestrous cycle) was used to analyse differences. When appropriate, comparisons between treatment groups were conducted by the Bonferroni post-hoc test. In all tests, the differences were considered significant if $p < 0.05$.

THE MORRIS WATER MAZE TEST

The data from the Place Navigation test were analysed by a Three-Way ANOVA (factors: prenatal exposure x treatment in adulthood x sex) with Repeated Measure (6 days of

the test x 8 trials per day). The Probe test data were analysed by a Three-Way ANOVA (factors: prenatal exposure x treatment in adulthood x sex/oestrous cycle). A Three-Way ANOVA (factors: prenatal exposure x treatment in adulthood x sex/oestrous cycle) with Repeated Measure (8 trials per day) was used to analyse the data from the Retention Memory test. The Bonferroni post-hoc test was used for post-hoc comparisons. In all tests, the differences were considered significant if $p < 0.05$.

7 RESULTS

7.1 The Conditioned Place Preference test

7.1.1 METHAMPHETAMINE

As shown in Figure 12 A neither males nor females, showed MA-induced increase in number of entries to the chamber associated with the drug [F (1,88)=0.09; p=0.87], however MA conditioning increased time spent in the chamber associated with the drug [F (1,88)=15.13; p<0.01], regardless of sex and prenatal drug exposure. Moreover, males regardless of prenatal drug exposure spent more time in the chamber associated with the drug than females [F (1,44)=7.85; p<0.01].

7.1.2 AMPHETAMINE

As shown in Figure 12 B neither males nor females, showed AMP-induced increase in number of entries to the chamber associated with the drug [F (1,56)=0.42; p=0.52], and in the time spent in the chamber associated with the drug [F (1,56)=3.42; p=0.07], regardless of prenatal drug exposure.

7.1.3 COCAINE

As shown in Figure 13 A neither males nor females, showed COC-induced increase in number of entries to the chamber associated with the drug [F (1,56)=0.01; p=0.93], and in the time spent in the chamber associated with the drug [F (1,56)=0.04; p=0.84], regardless of prenatal drug exposure.

7.1.4 MDMA

As shown in Figure 13 B neither males nor females, showed MDMA-induced increase in number of entries to the chamber associated with the drug [F (1,56)=1.29; p=0.26]. MDMA conditioning increased the time spent in the chamber associated with the drug in females, while it decreased in males [F (1,56)=57.93; p<0.05], regardless of prenatal drug exposure. Additionally, prenatally-saline exposed females spent more time in the chamber associated with the drug than prenatally-saline exposed males [F (1,28)=10.66; p<0.05].

7.1.5 MORPHINE

As shown in Figure 14 A neither males nor females, showed MOR-induced increase in number of entries to the chamber associated with the drug [F (1,56)=1.23; p=0.27]. MOR

conditioning increased the time spent in the chamber associated with the drug [F (1,56)=57.93; p<0.05], regardless of sex and prenatal drug exposure. Moreover, saline-exposed females preferred the chamber associated with the drug more than saline-exposed males [F (1,56)=8.39; p<0.05].

7.1.6 THC

As shown in Figure 14 B neither males nor females, showed THC-induced increase in number of entries to the chamber associated with the drug [F (1,47)=0.81; p=0.37], and in the time spent in the chamber associated with the drug [F (1,47)=0.04; p=0.85], regardless of prenatal drug exposure.

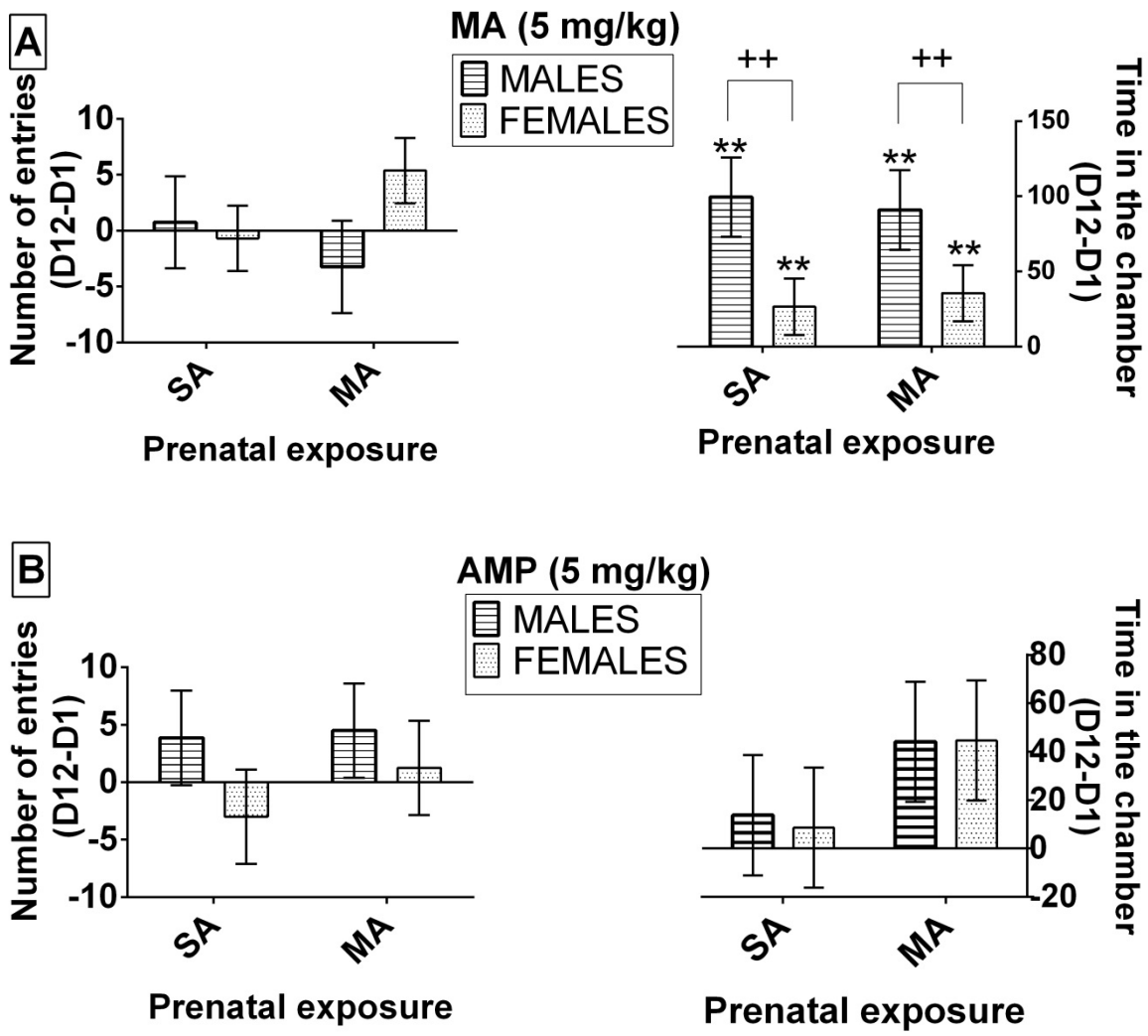

Fig. 12: The effect of **MA** (**A**) and **AMP** (**B**) conditioning on the drug-seeking behaviour in prenatally MA-exposed and saline (SA)-exposed male and female rats. **Lef graph:** number of entries to the chamber associated with the drug; **Right graph:** time spent in the chamber associated with the drug. Data are presented as differences between experimental day 12 (CPP test) and experimental day 1 (pre-exposure). Values are means ± SEM. n (MA)= 8 (males), 16 (females); n (AMP)= 8.

**p < 0.01 difference vs. chamber without drug (possitive number means preference and negative means avoidance of the chamber associated with the drug).

++ p< 0.01 females vs. males (time in the chamber associated with the drug).

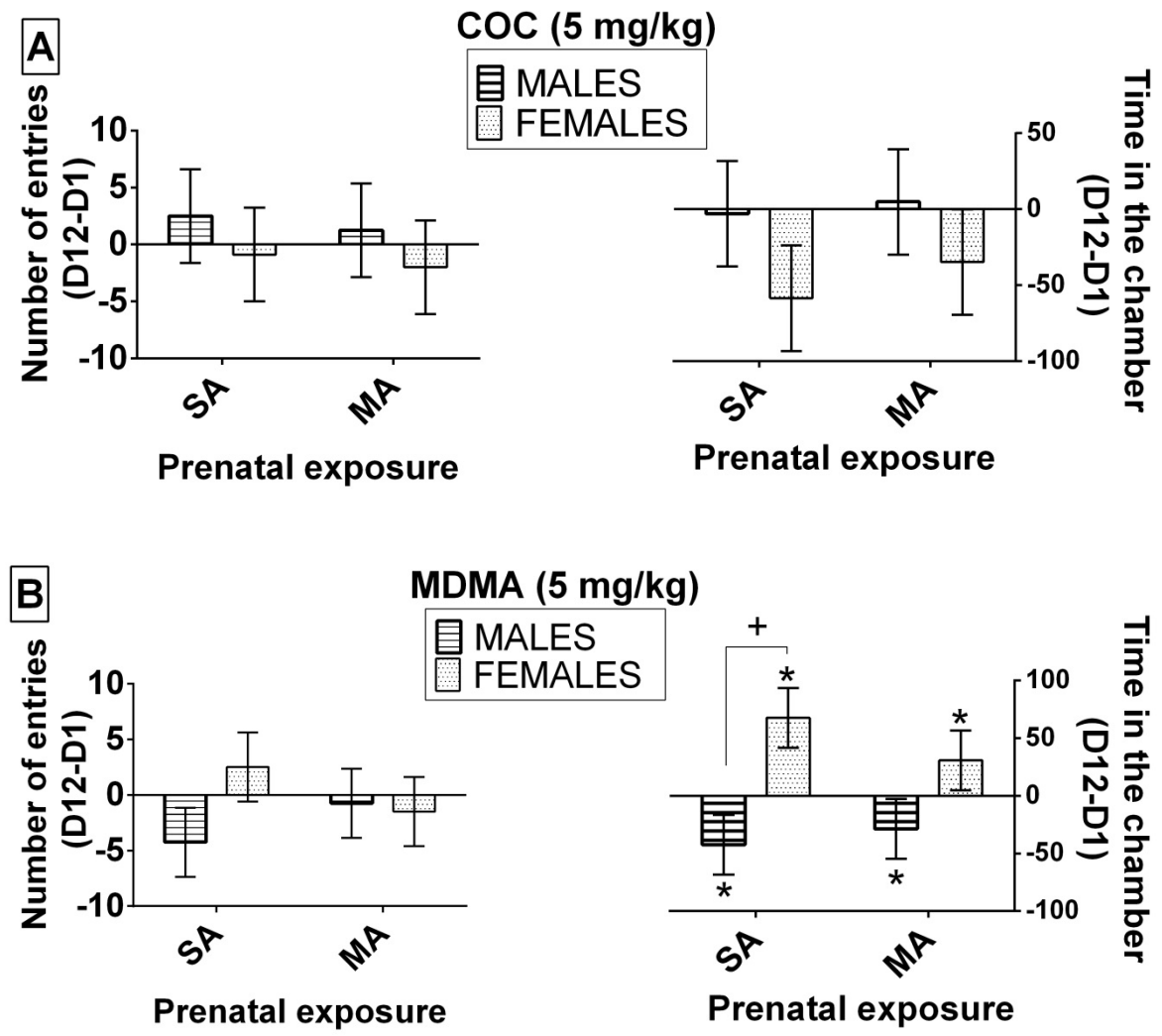

Fig. 13: The effect of **COC** (**A**) and **MDMA** (**B**) conditioning on the drug-seeking behaviour in prenatally MA-exposed and saline (SA)-exposed male and female rats. **Lef graph:** number of entries to the chamber associated with the drug; **Right graph:** time spent in the chamber associated with the drug. Data are presented as differences between experimental day 12 (CPP test) and experimental day 1 (pre-exposure). Values are means ± SEM. n=8.

*p < 0.05 difference vs. chamber without drug (possitive number means preference and negative means avoidance of the chamber associated with the drug).

+ p< 0.05 females vs. males (time in the chamber associated with the drug).

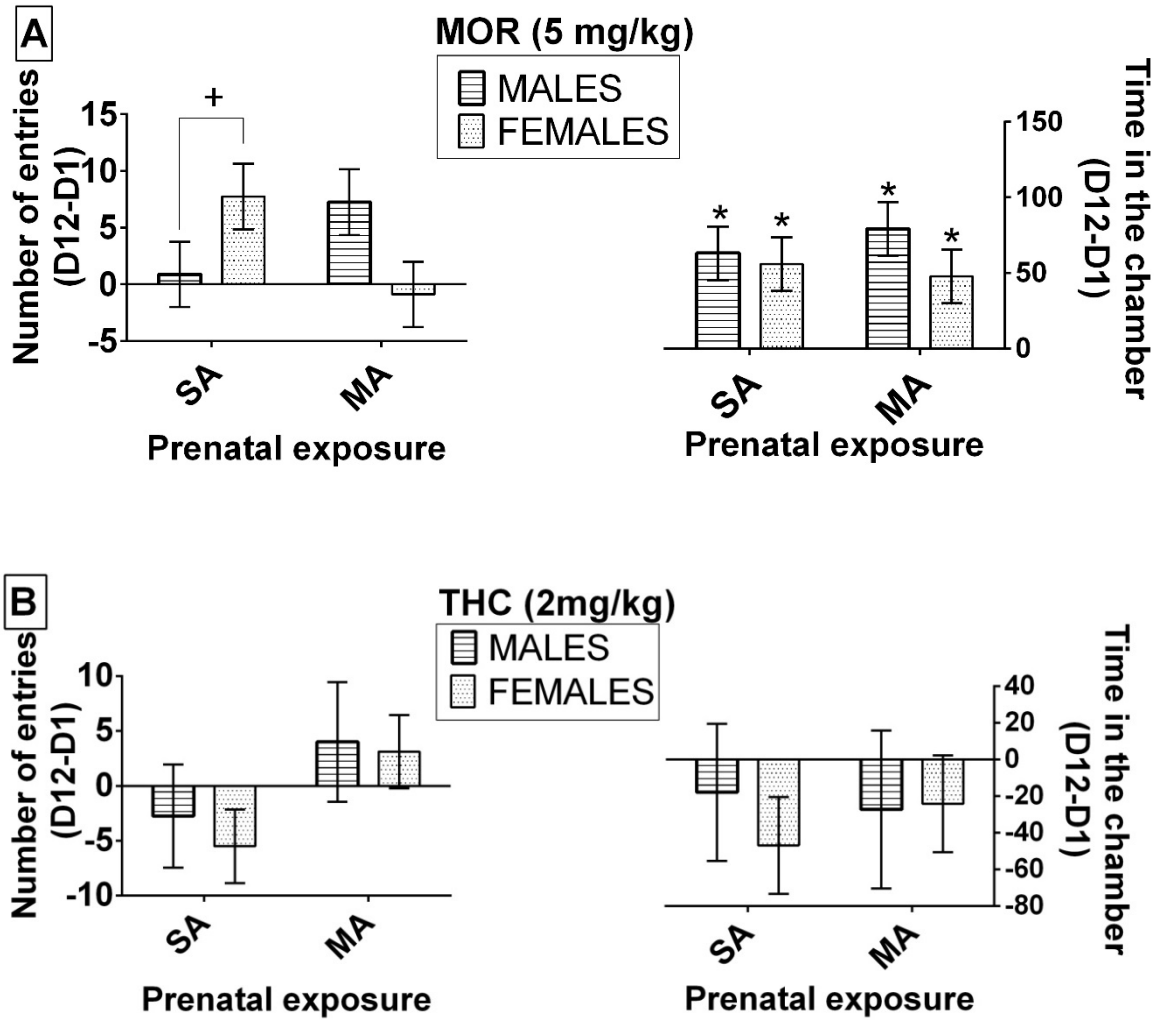

Fig. 14: The effect of **MOR** (**A**) and **THC** (**B**) conditioning on the drug-seeking behaviour in prenatally MA-exposed and saline-exposed male and female rats. **Lef graph:** number of entries to the chamber associated with the drug; **Right graph:** time spent in the chamber associated with the drug. Data are presented as differences between experimental day 12 (CPP test) and experimental day 1 (pre-exposure). Values are means ± SEM. n=3-8.

*p < 0.05 difference vs. chamber without drug (possitive number means preference and negative means avoidance of the chamber associated with the drug).

+ p< 0.05 females vs. males (time in the chamber associated with the drug).

7.2 The Laboras test

7.2.1 METHAMPHETAMINE

Data with acute MA were published previously by dr. Schutová (Schutová *et al.* 2013), therefore these experiments are not part of the present PhD Thesis.

7.2.2 AMPHETAMINE

AMP treatment in adulthood increased in both sexes the time spent in locomotion {males: [F (1,33)=15.24; p<0.001]; females [F (1,59)=4.64; p<0.05]} and the distance travelled {males: [F (1,33)=20.06; p<0.0001]; females [F (1,59)=5.66; p<0.05]}. AMP treatment did not affect speed of movement in males [F (1,33)=0.0003; p=0.99] while decreased in females [F (1,59)=5.36; p<0.05] (Table 6 and 7; Figure 15 I). In both genders, prenatal MA exposure sensitised the animals to AMP, which was mostly seen in the time spent rearing [F (1,92)=5.21; p<0.05]. Specifically, prenatally MA-exposed males [F (1,33)=5.10; p<0.05] and females [F (1,59)=4.18; p<0.05] injected with AMP spent more time rearing than prenatally saline-exposed rats with the same drug administration.

7.2.3 COCAINE

COC treatment in adulthood did not affect behaviour in the Laboras Test in males. In females, COC increased the time spent in locomotion [F (1,55)=9.29; p<0.01], the distance travelled [F (1,55)=6.97; p<0.05], the time spent rearing [F (1,55)=14.66; p<0.001], as well as the speed of movement [F (1,55)=15.62; p<0.001] (Table 6 and 7; Figure 15 II). In females, prenatal MA exposure sensitised the animals to COC, which was mostly seen in the time spent rearing [F (1,55)=1.89; p<0.05] and the speed of movement [F (1,55)=1.34; p<0.05]. Specifically, prenatally MA-exposed females injected with COC spent more time rearing and demonstrated increased speed of movement than prenatally saline-exposed rats with the same drug administration.

7.2.4 MDMA

MDMA treatment in adulthood increased in both sexes the time spent in locomotion {males: [F (1,33)=198.15; p<0.0001]; females [F (1,56)=181.70; p<0.0001]}, the distance travelled {males: [F (1,33)=81.97; p<0.0001]; females [F (1,56)=96.55; p<0.0001]} and the speed of movement {males: [F (1,33)=29.36; p<0.0001]; females [F (1,56)=41.69; p<0.0001]}. MDMA treatment did not affect time spent rearing in males [F (1,33)=3.85; p=0.06] but

increased in females [F (1,56)=41.69; p<0.0001] (Table 6 and 7; Figure 16). In addition, prenatal MA exposure sensitised females to adult MDMA treatment, when prenatally MA-exposed females with MDMA treatment spent more time rearing than prenatally saline-exposed females [F (1,56)=4.55; p<0.05].

7.2.5 MORPHINE

MOR treatment in adulthood decreased in both sexes the time spent in locomotion {males: [F (1,28)=20.29; p<0.0001]; females [F (1,56)=30.21; p<0.0001]}, the distance travelled {males: [F (1,28)=15.44; p<0.0001]; females [F (1,56)=27.99; p<0.0001]}, the time spent rearing {males: [F (1,28)=41.63; p<0.0001]; females [F (1,56)=76.93; p<0.0001]} and the speed of movement {males: [F (1,28)=28.26; p<0.0001]; females [F (1,56)=22.28; p<0.0001]} (Table 6 and 7; Figure 17 I). The effect of adult MOR treatment was seen regardless of prenatal drug exposure.

7.2.6 THC

THC treatment in adulthood did not influence behaviour in the Laboras test in males. In females, THC increased the time spent rearing [F (1,58)=2.73; p<0.05] and the speed of movement [F (1,58)=3.38; p<0.05] only in a group of prenatally saline-exposed rats (Table 6 and 7; Figure 17 II).

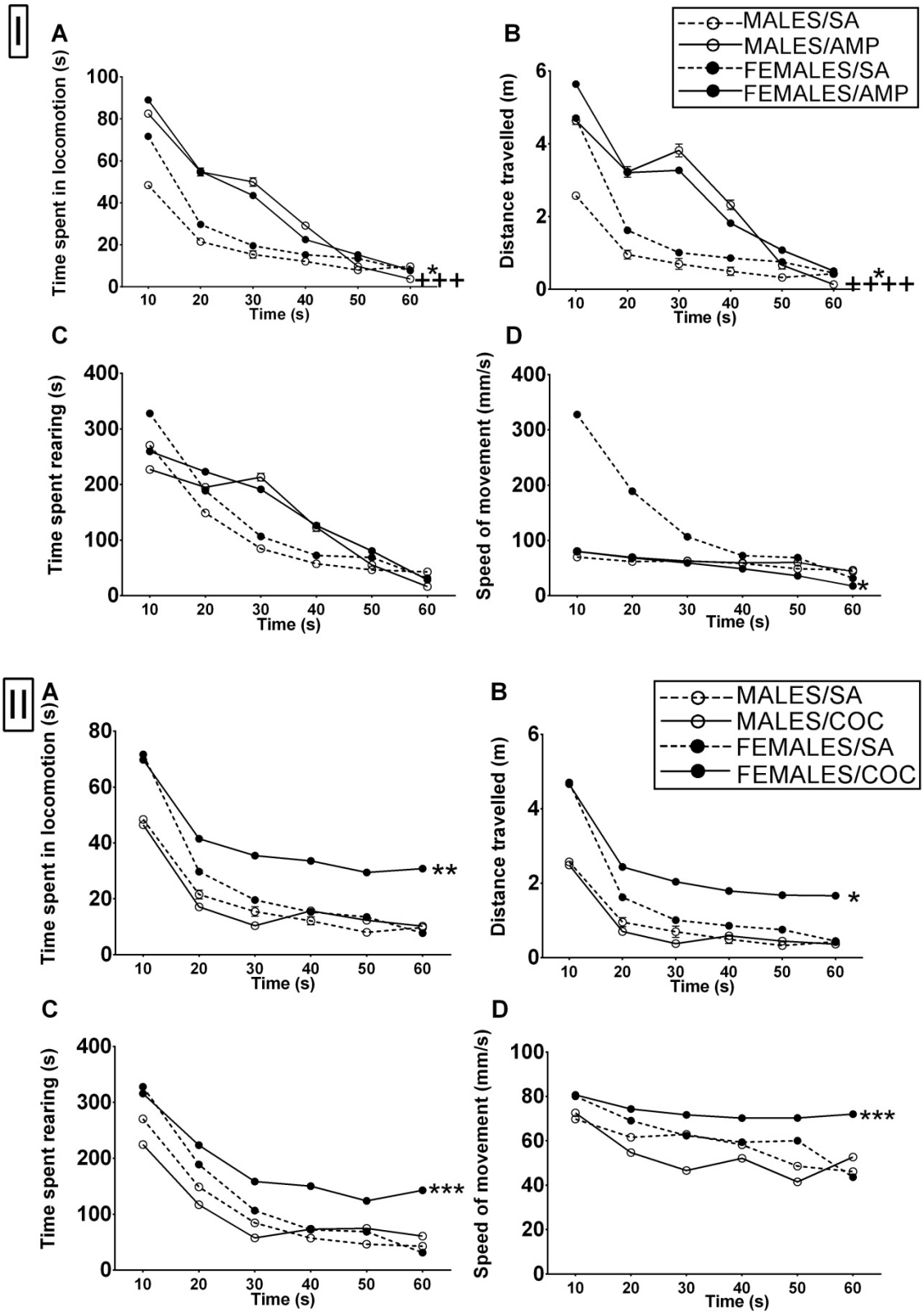

Fig. 15: The effect of acute **AMP (I)** and **COC (II)** treatment on locomotion of male and female rats in the Laboras test. **A**- Time spent in locomotion, **B**- Distance travelled, **C**- Time spent rearing, **D**- Speed of movement. Values are means ± SEM. n (males) = 16-20; n (females) = 25-32. Females AMP/COC vs. females SA (saline) *p<0.05, ** p<0.01; males AMP/COC vs. males SA +++ p<0.001, ++++ p<0.0001.

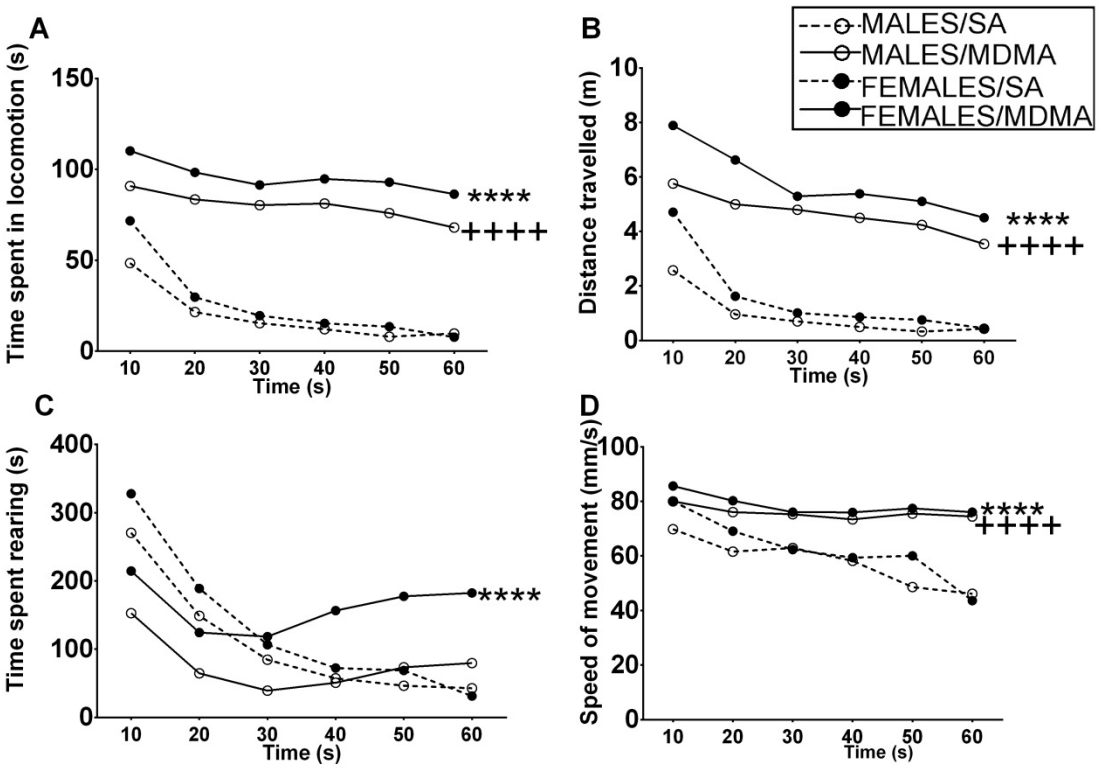

Fig. 16: The effect of acute **MDMA** treatment on locomotion of male and female rats in the Laboras test. **A**- Time spent in locomotion, **B**- Distance travelled, **C**- Time spent rearing, **D**- Speed of movement. Values are means ± SEM. n (males) = 17-20; n (females) = 32. Females MDMA vs. females SA (saline) **** $p<0.0001$; males MDMA vs. males SA ++++ $p<0.0001$.

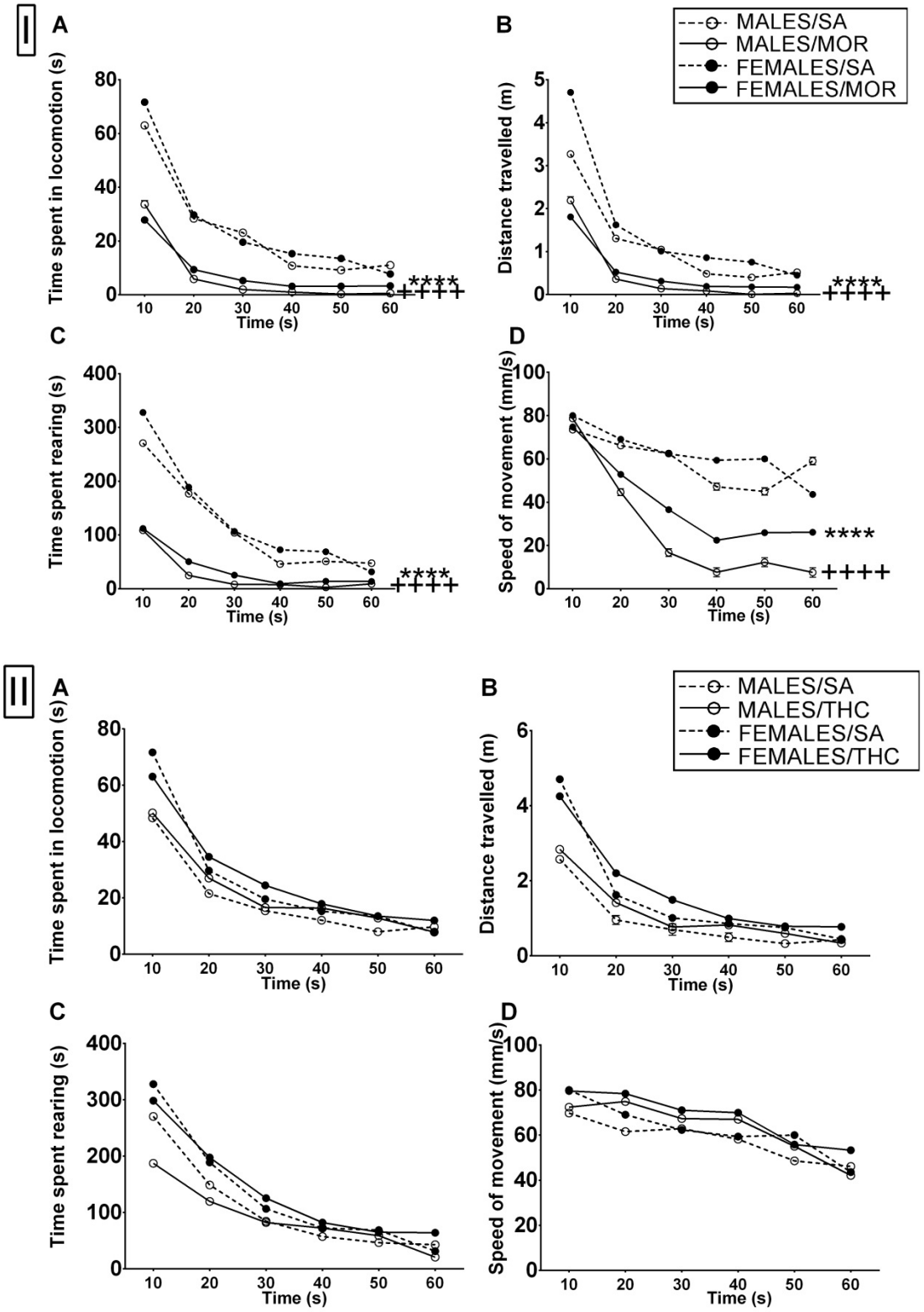

Fig. 17: The effect of acute **MOR (I)** and **THC (II)** treatment on locomotion of male and female rats in the Laboras test. **A**- Time spent in locomotion, **B**- Distance travelled, **C**- Time spent rearing, **D**- speed of movement. Values are means ± SEM. n (males) = 15-22; n (females) = 32-34. Females MOR vs. females SA (saline) **** $p<0.0001$; males MOR vs. males SA ++++ $p<0.0001$.

Table 6: Effect of drugs on behaviour of adult male rats tested in the Laboras test

	Locomotion (s)	**Distance travelled (m)**	**Rearing (s)**	**Speed of movement (mm/s)**
AMP (5 mg/kg)	↑	↑	P	0
COC (5 mg/kg)	0	0	0	0
MDMA (5 mg/kg)	↑	↑	0	↑
MOR (5 mg/kg)	↓	↓	↓	↓
THC (2 mg/kg)	0	0	0	0

↑= increasing drug effect; ↓= decreasing drug effect; P = effect dependent on prenatal drug exposure; 0 = no effect

Table 7: Effect of drugs on behaviour of adult female rats tested in the Laboras test

	Locomotion (s)	**Distance travelled (m)**	**Rearing (s)**	**Speed of movement (mm/s)**
AMP (5 mg/kg)	↑	↑	P	↓
COC (5 mg/kg)	↑	↑	↑	↑
MDMA (5 mg/kg)	↑	↑	↑	↑
MOR (5 mg/kg)	↓	↓	↓	↓
THC (2 mg/kg)	0	0	P	P

↑= increasing drug effect; ↓= decreasing drug effect; P = effect dependent on prenatal drug exposure; 0 = no effect

7.3 The Social Interaction test

7.3.1 METHAMPHETAMINE

Social interaction in total

Acute MA treatment in adulthood decreased total time spent in SI only in prenatally MA-exposed male rats [F (1,28)=8.05; p<0.05] [Figure 18 I (A)] but did not influence occurrence of SI [F (1,28)=0.01; p=0.97].

Particular patterns of social interaction

Mutual sniffing (including genital investigation)

As shown in Table 8, time of mutual sniffing was decreased after MA treatment only in prenatally MA-exposed rats [F (1,28)=17.26; p<0.01]. Occurrence of mutual sniffing was not influenced by MA treatment [F (1,28)=0.63; p=0.44].

Following

As shown in Table 8, MA treatment did not influence duration of following [F (1,28)=0.58; p=0.45]. Occurrence of following was increased by MA treatment only in prenatally saline-exposed rats [F (1,28)=12.23; p<0.05].

Climbing over

As shown in Table 8, MA treatment neither influenced duration [F (1,28)=0.00; p=1.00] nor occurrence of climbing over [F (1,28)=0.00; p=1.00].

Crawling under

Because of a very low duration and occurrence of crawling under in each group, this activity could not be statistically analysed.

Allogrooming

As shown in Table 8, MA treatment neither influenced duration [F (1,28)=1.47; p=0.24] nor occurrence of allogrooming [F (1,28)=1.92; p=0.18].

Particular patterns of non-social behaviour

Locomotion

As shown in Fig. 18 I (B), MA treatment in adulthood did not influence time of locomotion [F (1,28)=0.44; p=0.51].

Rearing

As shown in Fig. 18 I (C), MA treatment in adulthood increased occurrence of rearing in prenatally SA-exposed rats [F (1,28)=36.89; p<0.001] and prenatally MA-exposed rats [F (1,28)=36.89; p<0.05] .

7.3.2 AMPHETAMINE

Social interaction in total

AMP treatment in adulthood did not influence occurrence of SI in total between groups [F (1,28)=4.63; p=0.04]. Only time spent in SI [F (1,28)=3.23; p=0.08] was decreased after AMP treatment in prenatally MA- exposed rats [F (1,28)=3.23; p<0.05] [Figure 18 II (A)].

Particular patterns of social interaction

Mutual sniffing (including genital investigation)

As shown in Table 9, AMP treatment neither influenced duration [F (1,28)=4.4; p=0.59] nor occurrence of mutual sniffing [F (1,28)=1.58; p=0.22].

Following

As shown in Table 9, AMP treatment decreased time of following relative to saline-treated groups [F (1,28)=5.26; p<0.05] regardless of prenatal treatment, and occurrence of following was decreased only in the group of prenatally MA-exposed rats [F (1,28)=5.40; p<0.05].

Climbing over

As shown in Table 9, AMP treatment decreased duration of climbing over relative to saline-treated rats [F (1,28)=6.59; p=0<0.05] regardless of prenatal exposure. AMP treatment did not influence occurrence of climbing over [F (1,28)=2.10; p=0.16].

Crawling under

Because of a very low duration and occurrence of crawling under in each group, this activity could not be statistically analysed.

Allogrooming

As shown in Table 9, AMP treatment neither influenced duration [F (1,28)=1.47; p=0.24] nor occurrence of allogrooming [F (1,28)=1.92; p=0.18].

Particular patterns of non-social behaviour

Locomotion

As shown in Fig. 18 II (B), AMP treatment in adulthood increased time of locomotion in saline-exposed rats [F (1,28)=27.27; p<0.0001].

Rearing

As shown in Fig. 18 II (C), AMP treatment increased occurrence of rearing only in prenatally saline-exposed rats [F (1,28)=10.958; p<0.001].

Table 8: Effect of MA on particular patterns of social interaction in adult male rats

Social interaction pattern	**SA/SA**	**MA/SA**	**SA/MA**	**MA/MA**
Mutual sniffing Duration	26,13±4.67	37.25±4.67**	15.50±4.67	9.13±4.67**
Occurrence	29.13±3.07	30.75±3.07	28.25±3.07	26.75±3.07
Following Duration	22.00±5.19	17.50±5.19	19.75±5.19	11.88±5.19
Occurrence	11.50±3.22#	21.38±3.22	25.88±3.22#	13.25±3.22
Climbing over Duration	0.75±0.54	1.5±0.54	1.50±0.54	1.5±0.54
Occurrence	0.38±0.26	0.75±0.26	0.75±0.26	0.75±0.26
Crawling under Duration	LO	LO	LO	LO
Occurrence	LO	LO	LO	LO
Allogrooming Duration	0.25±0.52	1.00±0.52	0	0
Occurrence	0.25±0.23	0.38±0.23	0	0

Values are mean ± SEM (n=8 pairs).
**P<0.01
#P<0.05
LO= “low occurrence”

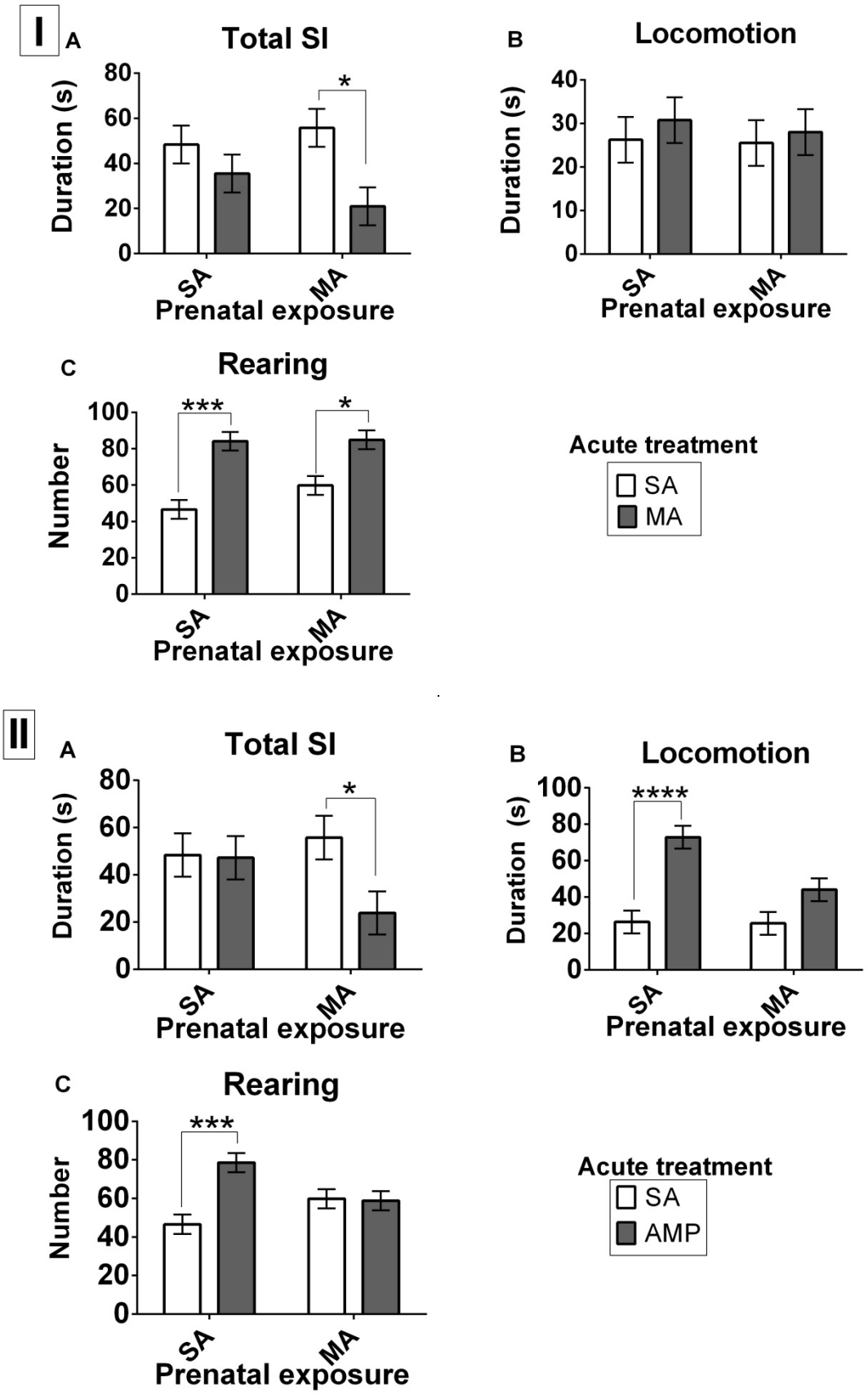

Fig. 18: The effect of **MA (I)** and **AMP (II)** on the behaviour of male rats in the SIT. **A**- total time spent in SI (social interactions), **B**- time of locomotion, **C**- number of rearing, Values are means±SEM. n=8 (pairs). *p < 0.05, *** p< 0.001, **** p< 0.0001.

Table 9: Effect of AMP on particular patterns of social interaction in adult male rats

Social interaction pattern	SA/SA	MA/SA	SA/AMP	MA/AMP
Mutual sniffing Duration	26.13 ± 5.78	37.25 ± 5.78	33.88 ± 5.78	20.63 ± 5.78
Occurrence	29,13 ± 3.04	30,75 ± 3.04	27,13 ± 3.04	25,13 ± 3.04
Following Duration	22,00 ± 5.07	17,50 ± 5.07	13,13 ± 5.07 +	3,13 ± 5.07 +
Occurrence	11,50 ± 2.69	21,38 ± 2.69*	10,13 ± 2.69	10,25 ± 2.69 *
Climbing over Duration	0.75±0.42	1.5±0.42	0 +	0.13 ± 0.42 +
Occurrence	0,38 ± 0.3	0,75 ± 0.3	0,75 ± 0.3	1,25 ± 0.3
Crawling under Duration	LO	LO	LO	LO
Occurrence	LO	LO	LO	LO
Allogrooming Duration	0,25 ± 0.52	1.00 ± 0.52	0	0
Occurrence	0,25 ± 0.23	0,38 ± 0.23	0	0

Values are mean ± SEM (n=8 pairs).
*P<0.05
+ P<0.05 (acute AMP< acute SA)
LO= “low occurrence”

7.3.3 COCAINE

Social interaction in total

COC treatment in adulthood neither influenced time spent in SI [F (1,28)=0.22; p=0.64] [Figure 19 I (A)] nor occurrence of SI in total between groups [F (1,28)=1.48; p=0.23].

Particular patterns of social interaction

Mutual sniffing (including genital investigation)

As shown in Table 10, COC treatment neither influenced duration [F (1,28)=0.59; p=0.45] nor occurrence of mutual sniffing [F (1,28)=0.03; p=0.88].

Following

As shown in Table 10, COC treatment neither influenced duration [F (1,28)=2.68; p=0.11] nor occurrence of following [F (1,28)=7.69; p=0.06].

Climbing over

As shown in Table 10, COC treatment decreased duration of climbing over relative to saline-treated rats [F (1,28)=6.48; p=0<0.05] regardless of prenatal drug exposure. COC treatment did not influence occurrence of climbing over [F (1,28)=2.10; p=0.16].

Crawling under

Because of a very low duration and occurrence of crawling under in each group, this activity could not be statistically analysed.

Allogrooming

As shown in Table 10, COC treatment neither influenced duration [F (1,28)=1.17; p=0.29] nor occurrence of allogrooming [F (1,28)=0.53; p=0.47].

Particular patterns of non-social behaviour

Locomotion

As shown in Fig. 19 I (B), COC treatment in adulthood increased time of locomotion in prenatally saline-exposed rats [F (1,28)=28.78; p<0.05] and MA-exposed rats [F (1,28)=28.78; p<0.001].

Rearing

As shown in Fig. 19 I (C), COC treatment in adulthood increased occurrence of rearing only in prenatally MA-exposed rats relative saline-exposed group [F (1,28)=0.06; p<0.05].

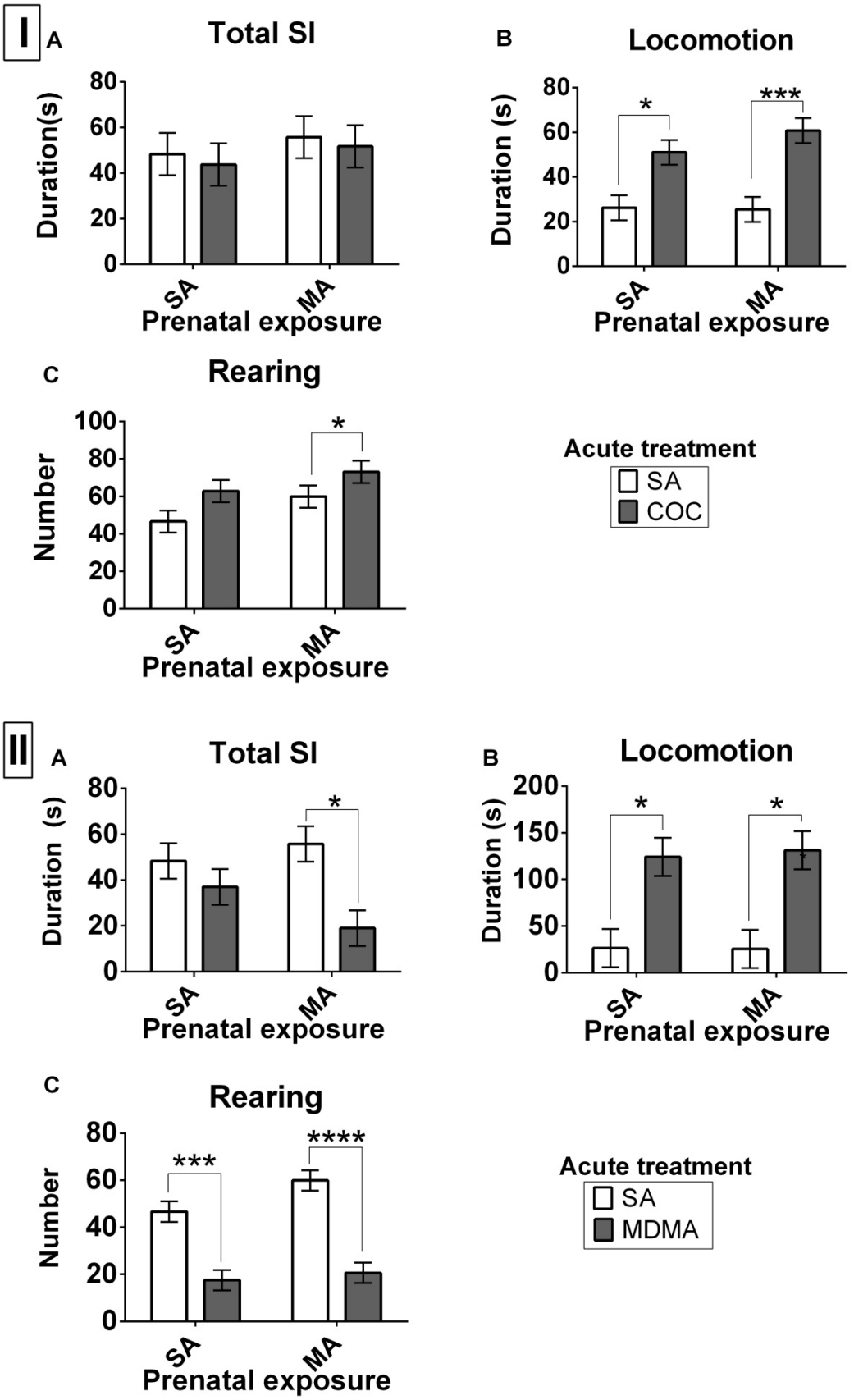

Fig. 19: The effect of **COC (I)** and **MDMA (II)** on the behaviour of male rats in the SIT. **A**- total time spent in SI (social interactions), **B**- time of locomotion, **C**- number of rearing, Values are means±SEM. n=8 (pairs). *p < 0.05, *** p< 0.001, **** p< 0.0001.

Table 10: Effect of COC on particular patterns of social interaction in adult male rats

Social interaction pattern	SA/SA	MA/SA	SA/COC	MA/COC
Mutual sniffing Duration	26,13±5.72	37,25±5.72	34,13±5.72	38,00±5.72
Occurrence	29,13±3.53	30,75±3.53	28,00±3.53	33,00±3.53
Following Duration	22,00±5.18	17,50±5.18	9,13±5.18	13,75±5.18
Occurrence	11,50±2.57	21,38±2.57	7,38±2.57	11,25±2.57
Climbing over Duration	0.75±0.04	1.5±0.04	0.25±0.01^{+}	0^{+}
Occurrence	0,38±0.42	0.75±0.42	1.75±0.42	0.75±0.42
Crawling under Duration	LO	LO	LO	LO
Occurrence	LO	LO	LO	LO
Allogrooming Duration	0,25±0.52	1.00±0.52	1,00±0.52	0
Occurrence	0,25±0.26	0.38±0.26	0,25±0.26	0

Values are mean ± SEM (n=8 pairs).
+ P<0.05 (acute COC< acute SA)
LO= “low occurrence”

7.3.4 MDMA

Social interaction in total

MDMA treatment in adulthood decreased time spent in SI only in prenatally MA-exposed rats [F (1,28)=9.65; p<0.05] [Figure 19 II (A)], but did not affect occurrence of SI [F (1,28)=0.82; p=0.37].

Particular patterns of social interaction

Mutual sniffing (including genital investigation)

As shown in Table 11 time of mutual sniffing was decreased after MDMA treatment only in prenatally MA-exposed rats [F (1,28)=14.44; p<0.05]. Occurrence of mutual sniffing was not influenced by MDMA treatment [F (1,28)=1.54; p=0.22].

Following

As shown in Table 11, MDMA treatment neither influenced duration [F (1,28)=1.17; p=0.29] nor occurrence of following F (1,28)=0.26; p=0.61].

Climbing over

As shown in Table 11, MDMA treatment decreased duration of climbing only in prenatally MA-exposed rats [F (1,28)=8.53; p=0<0.05]. MDMA treatment did not influence occurrence of climbing over [F (1,28)=0.000; p=1.00].

Crawling under

Because of a very low duration and occurrence of crawling under in each group, this activity could not be statistically analysed.

Allogrooming

As shown in Table 11, MDMA treatment neither influenced duration [F (1,28)=1.47; p=0.24] nor occurrence of allogrooming [F (1,28)=1.92; p=0.18].

Particular patterns of non-social behaviour

Locomotion

As shown in Fig. 19 II (B), MDMA treatment in adulthood increased time of locomotion in prenatally saline-exposed [F (1,28)=24.79; p<0.05] and prenatally MA-exposed rats [F (1,28)=24.79; p<0.05].

Rearing

As shown in Fig. 19 II (C), MDMA treatment in adulthood decreased occurrence of rearing in prenatally saline-exposed [F (1,28)=62.65; p<0.001] and prenatally MA-exposed rats [F (1,28)=62.65; p<0.0001].

Table 11: Effect of MDMA on particular patterns of social interaction in adult male rats

Social interaction pattern	SA/SA	MA/SA	SA/MDMA	MA/MDMA
Mutual sniffing Duration	26.13±4.77	37.25±4.77*	15.88±4.77	11.25±4.77*
Occurrence	29.13±4.03	30.75±4.03	32.00±4.03	37.88±4.03
Following Duration	22.00±4.92	17.5±4.92	21.13±4.92	17.75±4.92
Occurrence	11.50±2.57	21.38±2.57	20.13±2.57	15.38±2.57
Climbing over Duration	0.75±0.40	1.5±0.40*	0	0*
Occurrence	0.38±0.27	0.75±0.27	0	0
Crawling under Duration	LO	LO	LO	LO
Occurrence	LO	LO	LO	LO
Allogrooming Duration	0.25±0.52	1±0.52	0	0
Occurrence	0.25±0.23	0.38±0.23	0	0

Values are mean ± SEM (n=8 pairs).
*P<0.05
LO= “low occurrence”

7.3.5 MORPHINE

Social interaction in total

As shown in Fig. 20 I (A), acute MOR treatment in adulthood decreased time spent in SI relative to groups of saline-treated rats [F (1,28)=9.42; p<0.01], regardless of prenatal drug exposure, as well as decreased occurrence of SI, regardless of prenatal exposure [F (1,28)=33.92; p<0.05].

Particular patterns of social interaction

Mutual sniffing (including genital investigation)

As shown in Table 12, MOR treatment did not influence duration of mutual sniffing [F (1,28)=3.58; p=0.07], but decreased occurrence of mutual sniffing in prenatally saline- [F (1,28)=34.74; p<0.05] and MA- [F (1,28)=34.74; p<0.0001] exposed rats.

Following

As shown in Table 12, MOR decreased duration of following relative to group of saline-treated rats [F (1,28)=10.47; p<0.01] regardless of prenatal treatment, as well as decreased occurrence of mutual sniffing only in prenatally MA-exposed rats [F (1,28)=14.90; p<0.01].

Climbing over

As shown in Table 12, MOR treatment only decreased time in climbing over in prenatally MA-exposed rats [F (1,28)=9.61; p<0.05], but did not influence occurrence of climbing over [F (1,28)=0.78; p=0.39].

Crawling under

Because of a very low duration and occurrence of crawling under in each group, this activity could not be statistically analysed.

Allogrooming

As shown in Table 12, MOR treatment neither influenced duration of allogrooming [F (1,28)=1.47; p=0.24] nor occurrence of allogrooming [F (1,28)=1.92; p=0.18].

Particular patterns of non-social behaviour

Locomotion

As shown in Fig. 20 I (B), MOR treatment in adulthood decreased time of locomotion relative to groups of saline-treated rats [F (1,28)=4.53; p<0.05] regardless of prenatal drug exposure.

Rearing

As shown in Fig. 20 I (C), MOR treatment decreased occurrence of rearing in prenatally saline-exposed [F (1,28)=46.24; p<0.01] and prenatally MA- [F (1,28)=46.24; p<0.0001] exposed rats.

7.3.6 THC

Social interaction in total

THC neither influenced time spent in SI [F (1,28)=0.48; p=0.49] [Figure 20 II (A)], nor occurrence of SI [F (1,28)=0.75; p=0.05].

Particular patterns of social interaction

Mutual sniffing (including genital investigation)

As shown in Table 13, THC treatment neither influenced duration [F (1,28)=1.34; p=0.26] nor occurrence of mutual sniffing [F (1,28)=0.95; p=0.76].

Following

As shown in Table 13, THC treatment did not influence time spent in following [F (1,28)=4.33; p=0.05], but increased occurrence of following in prenatally saline-exposed [F (1,28)=36.88; p< 0.001] and MA-exposed rats [F (1,28)=36.88; p< 0.001].

Climbing over

As shown in Table 13, THC treatment increased time in climbing over relative to group of saline-treated rats regardless of prenatal treatment [F (1,28)=5.39; p<0.05], as well as increased number of climbing over in prenatally saline- [F (1,28)=21.1; p<0.05] and MA- [F (1,28)=21.1; p<0.01] exposed rats.

Crawling under

Because of a very low duration and occurrence of crawling under in each group, this activity could not be statistically analysed.

Allogrooming

As shown in Table 13, THC neither influenced duration [F (1,28)=0.92; p=0.35] nor occurrence of allogrooming [F (1,28)=0.00; p=1.00].

Particular patterns of non-social behaviour

Locomotion

As shown in Fig. 20 II (B), THC treatment in adulthood did not influence time in locomotion [F (1,28)=3.14; p=0.09].

Rearing

As shown in Fig. 20 II (C), THC treatment in adulthood did not influence number of rearing [F (1,28)=1.54; p=0.70].

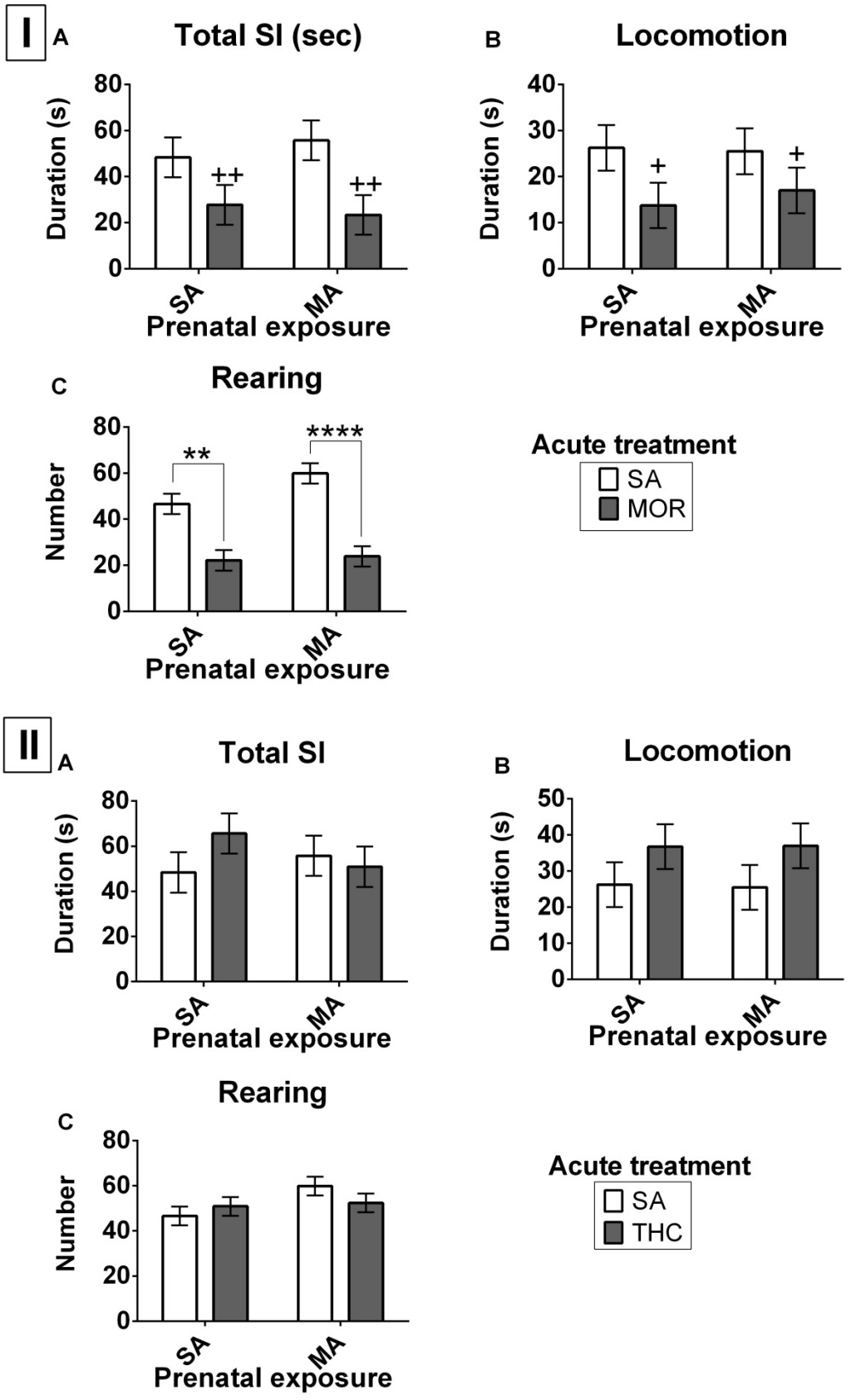

Fig. 20: The effect of **MOR (I)** and **THC (II)** on the behaviour of male rats in the SIT. **A**- total time spent in SI (social interactions), **B**- time of locomotion, **C**- number of rearing, Values are means±SEM. n=8 (pairs). ** $p< 0.01$, **** $p< 0.0001$; acute SA vs. acute MOR + $p< 0.05$, ++ $p< 0.01$.

Table 12: Effect of MOR on particular patterns of social interaction in adult male rats

Social interaction pattern	**SA/SA**	**MA/SA**	**SA/MORF**	**MA/MORF**
Mutual sniffing Duration	26.13±5.78	37.25±5.78	23.25±5.78	18.25±5.78
Occurrence	29.13±2.45#	30.75±2.45****	18.38±2.45#	12.63±2.45****
Following Duration	22.00±4.81	17.5±4.81	4.25±4.81++	4.123±4.81++
Occurrence	11.50±2.69	21.38±2.69**	5.13±2.69	7.00±2.69**
Climbing over Duration	0.75±0.41	1.5±0.41*	0	0*
Occurrence	0.38±0.62	0.75±0.62	0.13	0.63
Crawling under Duration	LO	LO	LO	LO
Occurrence	LO	LO	LO	LO
Allogrooming Duration	0.25±0.52	1.00±0.52	0	0
Occurrence	0.25±0.23	0.38±0.23	0	0

Values are mean ± SEM (n=8 pairs).
#P<0.05
* P<0.05
** P<0.01
****P<0.0001
++ P<0.01 (acute MOR< acute SA)
LO= “low occurrence”

Table 13: Effect of THC on particular patterns of social interaction in adult male rats

Social interaction pattern	**SA/SA**	**MA/SA**	**SA/THC**	**MA/THC**
Mutual sniffing Duration	26.13±5.35	37.25±5.35	43.38±5.35	32.38±5.35
Occurrence	29.13±2.44	30.75±2.44	32.00±2.44	26.38±2.44
Following Duration	22.00±5.80	17.50±5.80	30.00±5.80	36.63±5.80
Occurrence	11.50±2.89###	21.38±2.89***	29.38###	38.63±2.89***
Climbing over Duration	0.38±0.54	1.5±0.54	2.00±0.54+	2.75±0.64+
Occurrence	0.38±0.62#	0.75±0.62**	2.88±0.62#	3.88±0.62**
Crawling under Duration	LO	LO	LO	LO
Occurrence	LO	LO	LO	LO
Allogrooming Duration	0.25±0.52	0.00±0.52	0.00±0.52	0.25±0.52
Occurrence	0.25±0.24	0.38±0.24	0.25±0.24	0.38±0.24

Values are mean ± SEM (n=8 pairs).
#P<0.05
###P<0.001
** P<0.01
*** P<0.001
+ P<0.05 (acute THC> acute SA)
LO= “low occurrence”

7.4 The Elevated Plus Maze test

7.4.1 METHAMPHETAMINE

Anxiolytic and anxiogenic behaviour

As shown in Figure 21 I (A,B) females with MA treatment spent more time in the OA than males with saline treatment [F (1,56)=0.20; p<0.05]. Acute MA treatment decreased time spent in the CA in a sex specific manner. Female with MA treatment spent less time in the CA than males with the same drug treatment [F (1,56)=1.69; p<0.01]. Number of pSAP was not affected by acute MA treatment [F (1,56)=2.25; p=0.14]. The effect of an acute MA treatment was seen regardless of prenatal drug exposure.

Locomotor and exploratory behaviour

As shown in Figure 21 I (C,D) locomotion was increased in MA-treated females {[number of all arm entries [F (1,56)=7.57], number of rearing [F (1,56)=2.86]} compared to MA-treated males [number of all arm entries (p<0.001), number of rearing (p<0.05)] and saline-treated females [number of all arm entries (p<0.05), number of rearing (p<0.05)]. MA treatment in adulthood also decreased [F (1,56)= 1.28] time spent sniffing in both, females (p<0.0001) and males (p<0.05). The effect of an acute MA treatment was seen regardless of prenatal drug exposure.

7.4.2 AMPHETAMINE

Anxiolytic and anxiogenic behaviour

As shown in Figure 21 II (A, B), females with AMP treatment [F (1,56)=1.12] spent more time in the OA relative to SA treated females (p<0.05) as well as relative to saline-treated males (p<0.01). AMP did not have any effect on time spent in the CA [F (1,56)=1.47; p=0.23]. Number of pSAP was decreased in females regardless of prenatal drug exposure [F (1,56)=3.07; p<0.05]. The effect of an acute AMP treatment was seen regardless of prenatal drug exposure.

Locomotor and exploratory behaviour

As shown in Figure 21 II (C, D) locomotion was increased in AMP-treated females {number of all arm entries [F (1,56)=6.95], number of rearing [F (1,56)=2.83] relative to AMP-treated males [number of all arm entries (p<0.001), number of rearing (p<0.05)] and saline-treated females [number of all arm entries (p<0.01)]. Time spent sniffing was increased in females compared to saline-treated females [F (1,56)=5.92, (p<0.001)]. The effect of an acute AMP treatment was seen regardless of prenatal drug exposure.

7.4.3 COCAINE

Anxiolytic and anxiogenic behaviour

As shown in Figure 22 I (A, B), females with COC treatment [F (1,56)=3.94] spent more time in the OA relative to saline-treated females ($p<0.001$), COC-treated males ($p<0.01$), as well as saline-treated males ($p<0.0001$). COC did not have any effect on time spent in the CA [F (1,56)=0.01; p=0.91]. COC treatment also decreased number of pSAP [F (1,56)=14.43] in females relative to saline-treated females ($p<0.01$) and COC-treated males ($p<0.05$). The effect of an acute COC treatment was seen regardless of prenatal drug exposure.

Locomotor and exploratory behaviour

As shown in Figure 22 I (C, D) locomotion was increased in COC-treated females {number of all arm entries [F (1,56)=90.33]} relative to saline-treated females ($p<0.0001$), saline-treated males ($p<0.0001$), as well as COC-treated males ($p<0.0001$). COC treatment decreased time spent sniffing only in males [F (1,56)=10.03; $p<0.05$]. Number of rearing was not affected by acute COC treatment [F (1,56)=1.13; p=0.72]. The effect of an acute COC treatment was seen regardless of prenatal drug exposure.

7.4.4 MDMA

Anxiolytic and anxiogenic behaviour

As shown in Figure 22 II (A, B) MDMA treatment did not influence time spent in the OA in both genders [F (1,56)=0.15; p=0.69]. Time spent in the CA was increased in females after MDMA treatment relative to saline-treated females [F (1,56)=13.31; $p<0.01$]. Number of pSAP was not affected by acute MDMA treatment [F (1,56)=3.43; p=0.07]. The effect of an acute MDMA treatment was seen regardless of prenatal drug exposure.

Locomotor and exploratory behaviour

As shown in Figure 22 II (C, D) MDMA increased number of all arm entries [F (1,56)=5.47; $p<0.05$] as well as decreased number of rearing [F (1,56)=23.16; $p<0.0001$] regardless of sex. Time spent sniffing was not affected by acute MDMA treatment [F (1,56)=0.32; p=0.57]. The effect of an acute MDMA treatment was seen regardless of prenatal drug exposure.

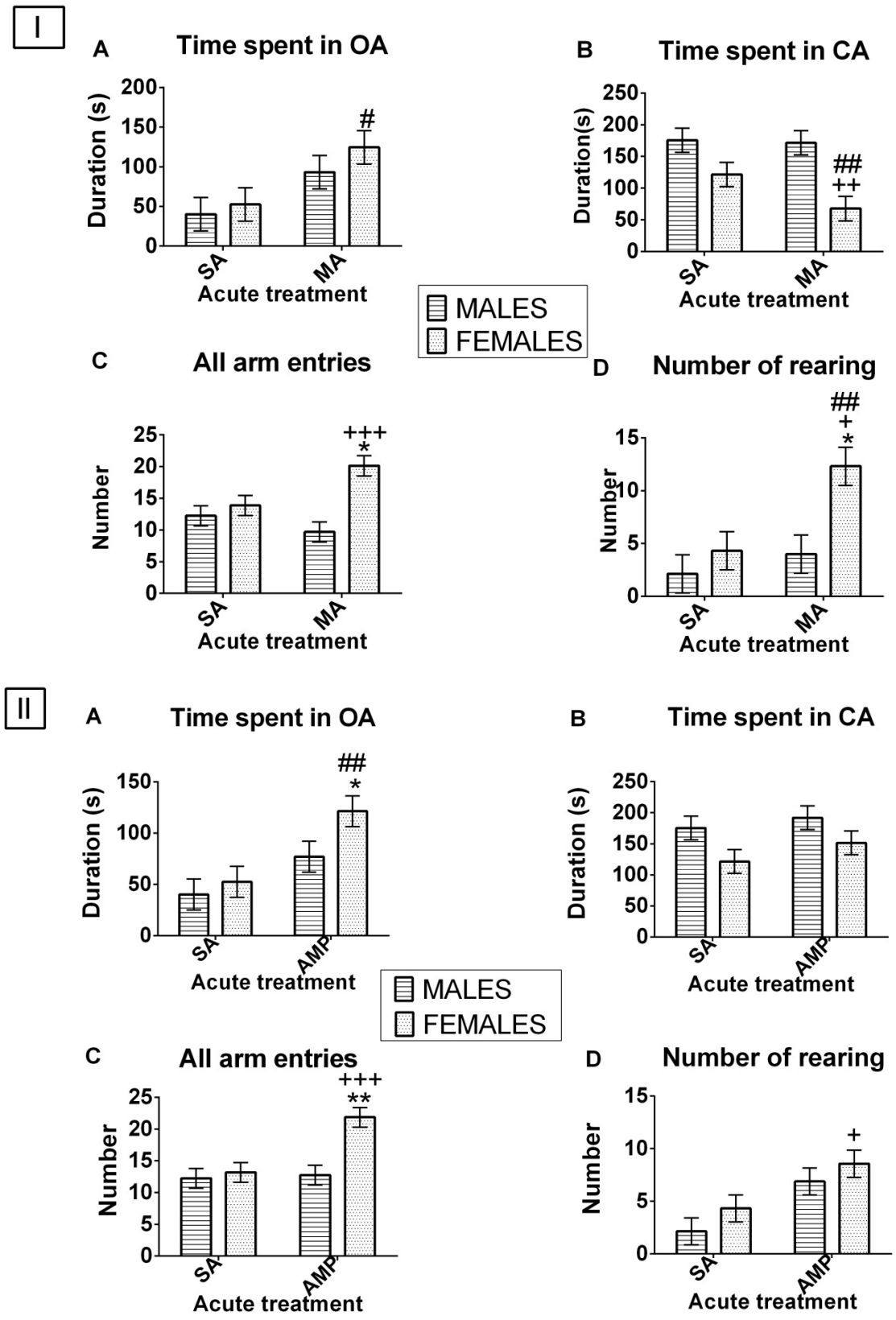

Fig. 21: The effect of **MA (I)** and **AMP (II)** on the behaviour of female and male rats in the EPM. **A**- time spent in OA, **B**- time spent in CA, **C**- number of all arm entries, **D**- number of rearing. Values are means±SEM. n=16. Females MA/AMP vs. females SA *p < 0.05, **p < 0.01; females MA/AMP vs. males MA/AMP +p < 0.05, ++ p < 0.01, +++ p < 0.001; females MA/AMP vs. males SA # p < 0.05, ## p < 0.01.

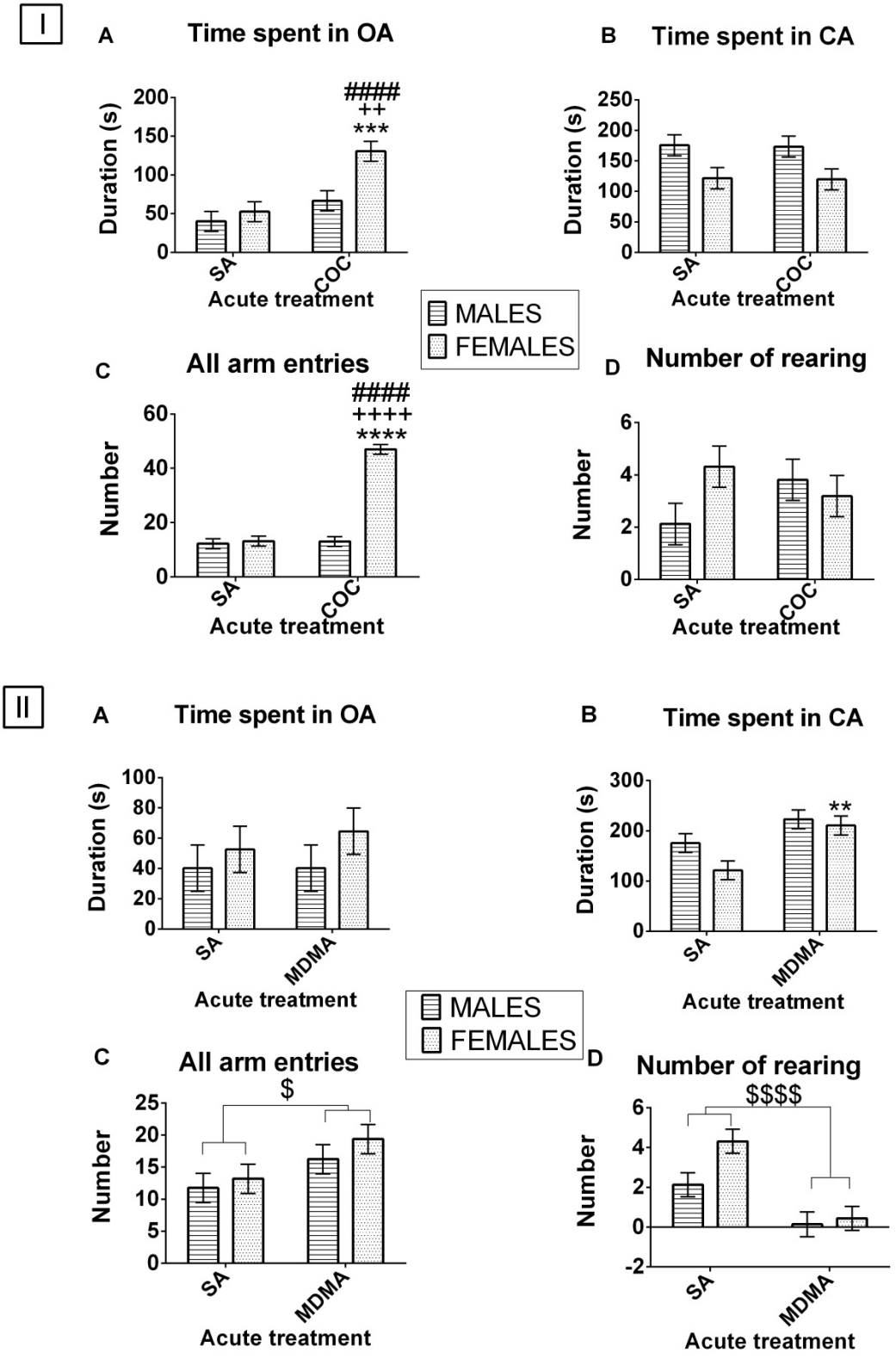

Fig. 22: The effect of **COC (I)** and **MDMA (II)** on the behaviour of female and male rats in the EPM. **A**- time spent in OA, **B**- time spent in CA, **C**- number of all arm entries, **D**- number of rearing. Values are means±SEM. n=16. Females COC/MDMA vs. females SA **p < 0.01, ***p < 0.001, ****p < 0.0001; females COC vs. males COC ++ p < 0.01, ++++ p < 0.0001; females COC vs. males SA #### p < 0.0001; acute MDMA vs. acute SA $ p < 0.05, $$$$ p < 0.0001.

7.4.5 MORPHINE

Anxiolytic and anxiogenic behaviour

As shown in Figure 23 I (A, B) MOR treatment did not influence time spent in the OA in both genders [F (1,56)=1.28; p=0.26]. MOR treatment [F (1,56)=23.82] increased time spent in the CA in males ($p<0.01$) and females ($p<0.05$)] relative to saline-treated animals. MOR treatment [F (1,56)=3.86] also decreased number of pSAP in females ($p<0.0001$) and in males ($p<0.0001$) compared to saline-treated females. The effect of an acute MOR treatment was seen regardless of prenatal drug exposure.

Locomotor and exploratory behaviour

As shown in Figure 23 I (C, D) locomotion was decreased in MOR-treated males {all arm entries [F (1,56)=9.03]} relative to saline-treated males [all arm entries ($p<0.05$)] as well as relative to MOR-treated females [all arm entries ($p<0.0001$)]. Number of rearing was not affected by acute MOR treatment [F (1,56)=2.52; p=0.12]. MOR treatment decreased time spent sniffing in both sexes [F (1,56)=12.64; $p<0.05$]. The effect of an acute MOR treatment was seen regardless of prenatal drug exposure.

7.4.6 THC

Anxiolytic and anxiogenic behaviour

As shown in Figure 23 II (A, B) THC treatment did not influence time spent in the OA in both genders [F (1,56)=1.03; p=0.31]. THC treatment increased time spent in the CA both genders relative to saline-treated animals [F (1,56)=3.04; $p<0.001$]. THC treatment [F (1,56)=4.54] also decreased number of pSAP in females ($p<0.01$) and in males ($p<0.01$) relative to saline-treated females. The effect of an acute THC treatment was seen regardless of prenatal drug exposure.

Locomotor and exploratory behaviour

As shown in Figure 23 II (C, D) THC decreased number of all arm entries [F (1,56)=9.20; $p<0.05$] regardless of sex. Number of rearing [F (1,56)=0.20; p=0.66] and time spent sniffing [F (1,56)=0.6; p=0.05] were not affected by acute THC treatment. The effect of an acute THC treatment was seen regardless of prenatal drug exposure.

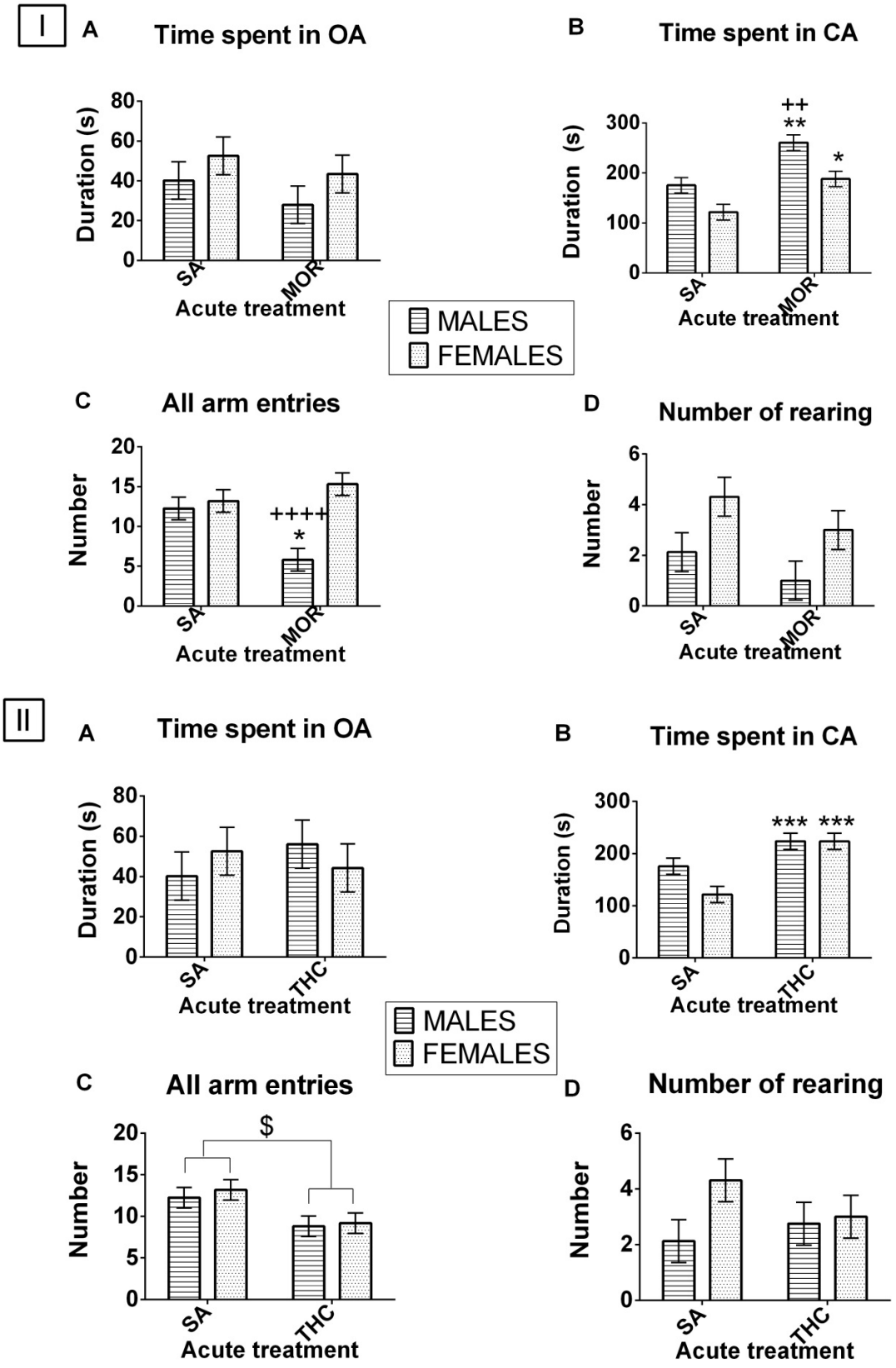

Fig. 23: The effect of **MOR (I)** and **THC (II)** on the behaviour of female and male rats in the EPM. **A**- time spent in OA, **B**- time spent in CA, **C**- number of all arm entries, **D**- number of rearing. Values are means±SEM. n=16. Females MOR/THC vs. females SA (resp. males SA vs. males MOR/THC) *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$; males MOR vs. females MOR ++ $p < 0.01$, ++++ $p < 0.0001$; acute THC vs. acute SA \$ $p < 0.05$.

7.5 The Morris Water Maze test

7.5.1 METHAMPHETAMINE

Data with the effect chronic MA treatment on males were published previously by dr. Schutová (Schutová *et al.* 2009), therefore these experiments are not part of the present PhD Thesis.

The Learning test

Adult MA treatment increased in females the latency [$F(1,36)=6.28$; $p<0.05$], the distance travelled [$F(1,36)=6.33$; $p<0.05$], and the search error [$F(1,36)=8.94$; $p<0.05$] relative to saline-treated females (Figure 24). The effect of adult MA treatment was only seen in a group of prenatally saline-exposed females.

The Probe test

Adult MA treatment in females did not influence any of the parameters.

The Memory Recall test

Adult MA treatment in females did not influence the distance travelled, the latency and the search error.

The speed of swimming

Adult MA treatment in females did not influence the speed of swimming in any of the tests (Table 14, 15, 16).

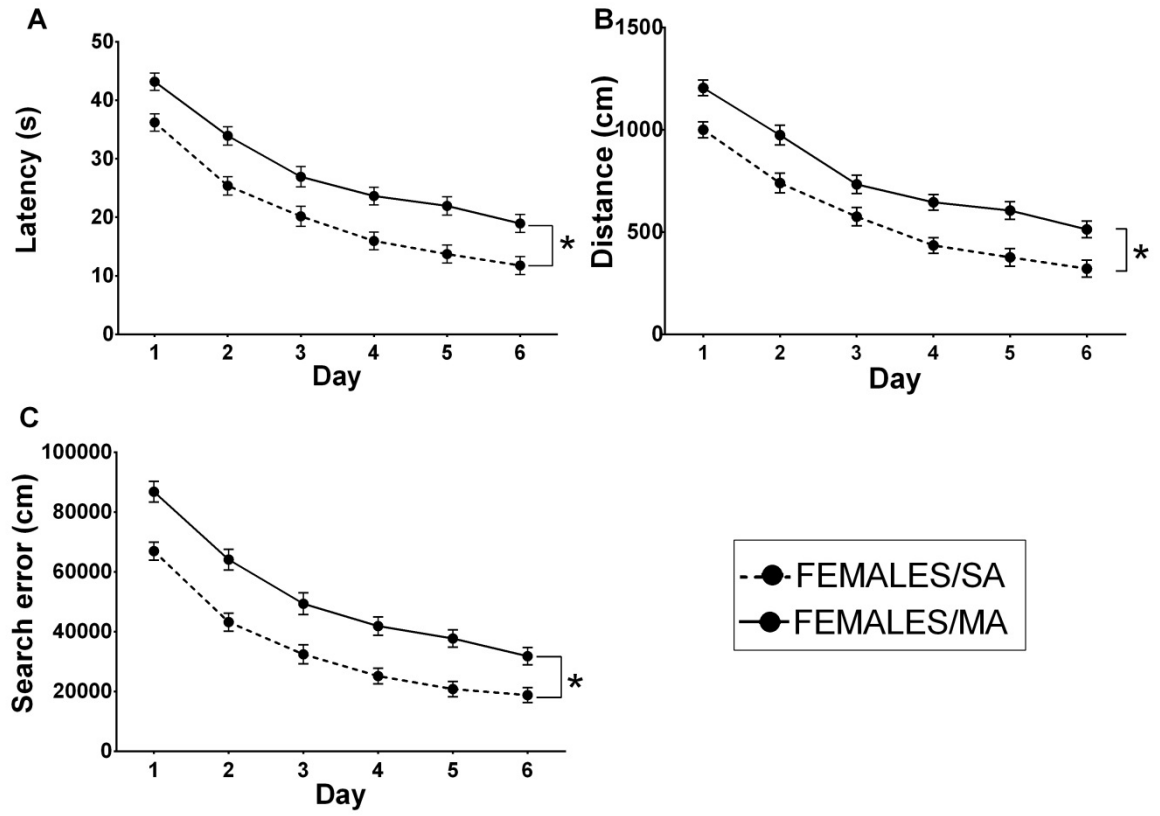

Fig. 24: Effect of adult MA treatment on the performance of female rats in the Place Navigation test: A. Latency of platform acquisition **B.** Distance travelled **C.** Search error. Values are presented as mean ± SEM, n=20. Figure legend means - Sex/Adult treatment females MA vs. females MA: *p<0.05.

7.5.2 AMPHETAMINE

The Learning test

Adult AMP treatment increased in females the latency [F (1,64)=10.11; p<0.001], the distance travelled [F (1,64)=12.79; p<0.001], and the search error [F (1,64)=9.09; p<0.01] relative to saline-treated females, as well as to AMP treated males [the distance travelled (p<0.05)] (Figure 25). The effect of adult AMP treatment was only seen in a group of prenatally saline-exposed females.

The Probe test

In both sexes adult AMP treatment did not influence any of the parameters.

The Memory Recall test

Adult AMP treatment increased in females the latency [F (1,64)=6.38; p<0.01] and the distance travelled [F (1,64)=8.73; p<0.01] relative to saline-treated females, as well as to AMP

treated males [the distance travelled ($p<0.01$), the latency ($p<0.01$), and the search error ($p<0.01$)]. The effect of adult AMP treatment was seen regardless of prenatal drug exposure.

The speed of swimming

In both sexes adult AMP treatment did not influence the speed of swimming in any of the tests (Table 14, 15, 16).

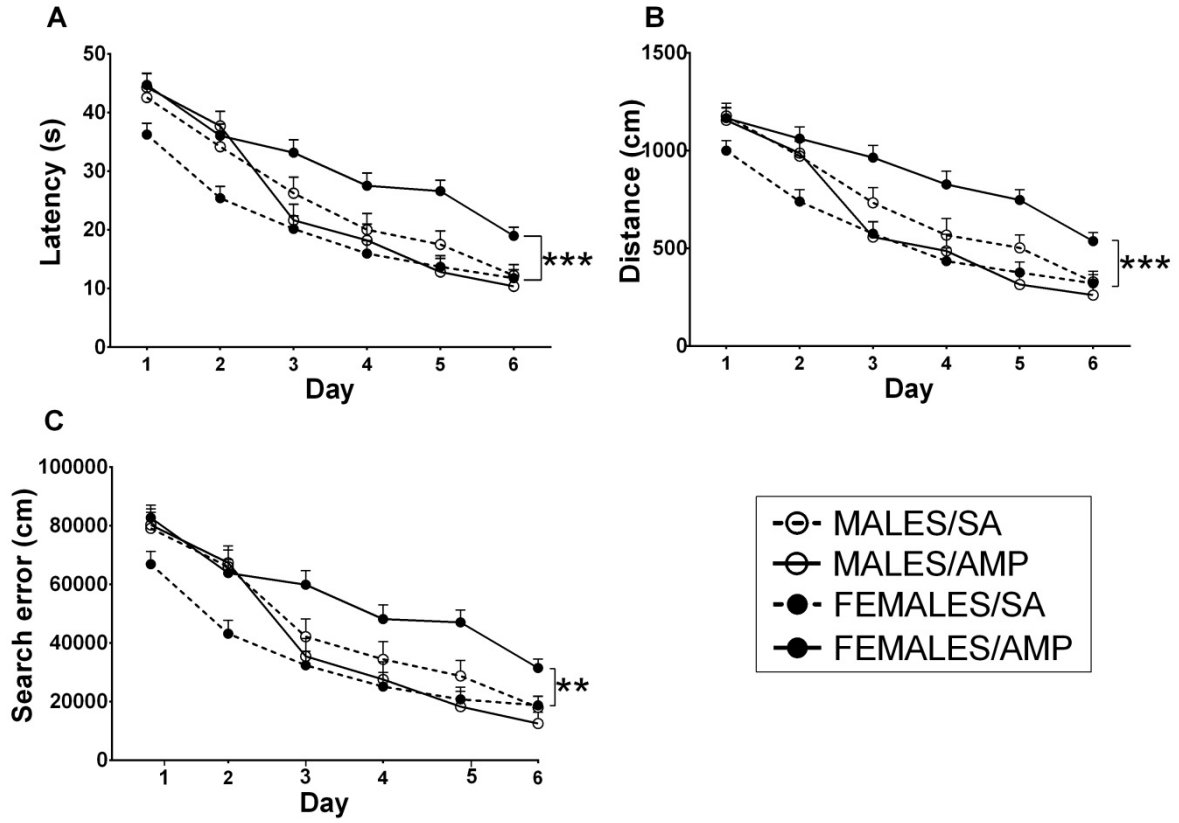

Fig. 25: Effect of adult AMP treatment on the performance of male and female rats in the Place Navigation test: A. Latency of platform acquisition **B.** Distance travelled **C.** Search error. Values are presented as mean + SEM, n (female)= 20; n (male)=16. Figure legend means - Sex/Adult treatment, females AMP vs. females SA: ** $p<0.01$, *** $p<0.001$.

7.5.3 COCAINE

The Learning test

Adult COC treatment increased in females the latency [$F(1,56)=11.65$; $p<0.01$], the distance travelled [$F(1,56)=14.79$; $p<0.001$], and the search error [$F(1,56)=21.64$; $p<0.0001$] relative to saline-treated females, as well as to COC-treated males [the latency ($p<0.01$), the

distance travelled (p<0.01), and the search error (p<0.001)] (Figure 26). The effect of adult COC treatment was only seen in a group of prenatally saline-exposed females.

The Probe test

In both sexes adult COC treatment did not influence any of the parameters.

The Memory Recall test

Adult COC treatment increased in females the latency [F (1,56)=8.37; p<0.01], the distance travelled [F (1,56)=13.29; p<0.001], and the search error [F (1,56)=7.82; p<0.01] relative to saline-treated females, as well as to COC-treated males [the latency (p<0.01), the distance travelled (p<0.001), and the search error (p<0.001)]. The effect of adult COC treatment was only seen in a group of prenatally saline-exposed females.

The speed of swimming

In both sexes adult COC treatment did not influence the speed of swimming in any of the tests (Table 14, 15, 16).

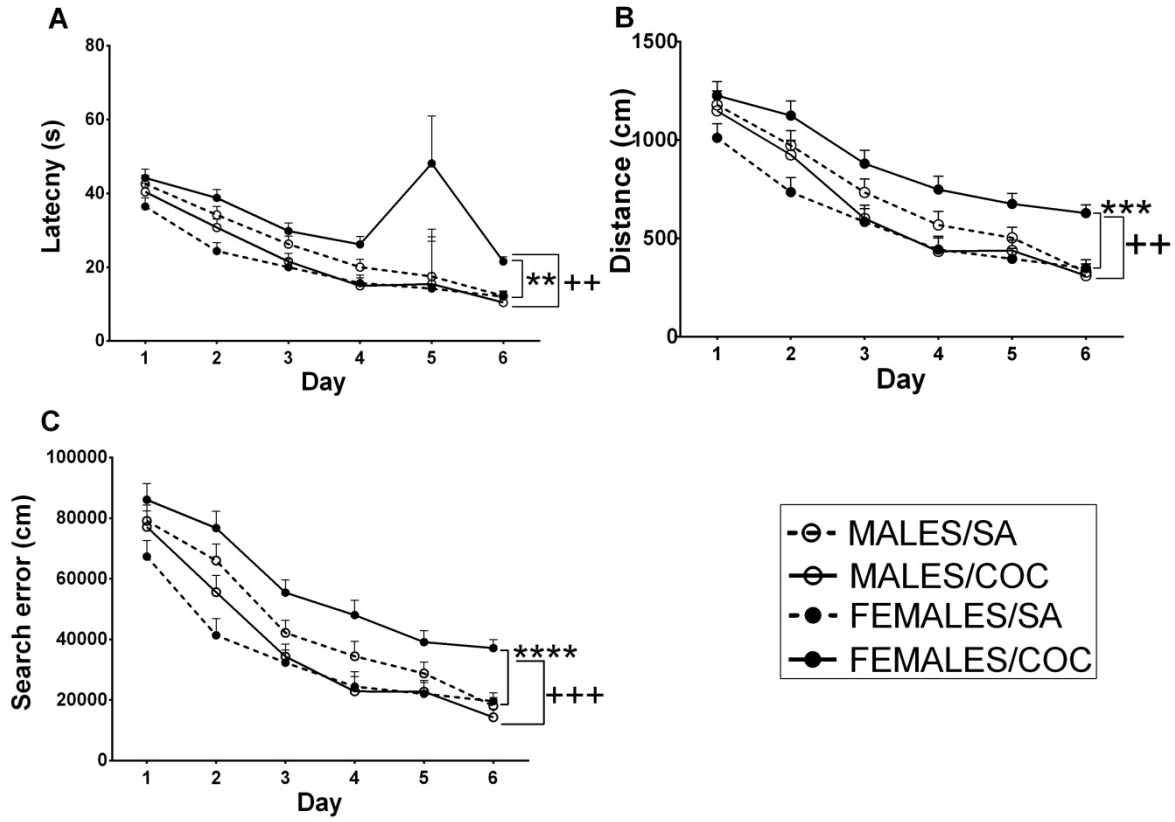

Fig. 26: Effect of adult COC treatment on the performance of male and female rats in the Place Navigation test: A. Latency of platform acquisition **B.** Distance travelled **C.** Search error. Values are presented as mean + SEM, n=16. Figure legend means - Sex/Adult treatment. Females COC vs. females SA: ** p<0.01, *** p<0.001, **** p<0.0001; females COC vs. males COC: ++ p<0.01, +++ p<0.001.

7.5.4 MDMA

The Learning test

Adult MDMA treatment increased in females the latency [F (1,56)=2.93; p<0.001], the distance travelled [F (1,56)=8.34; p<0.0001], and the search error [F (1,56)=3.66; p<0.01] relative to saline-treated females (Figure 27). The effect of adult MDMA treatment was only seen in a group of prenatally saline-exposed females.

The Probe test

In females, adult MDMA treatment increased the distance travelled relative to saline-treated females as well as MDMA treated males [F (1,56)=8.40; p<0.001], and also decreased the number of crossing of the quadrant where the platform was located [F (1,56)=88.92; p<0.0001] in both sexes.

The Memory Recall test

Adult MDMA treatment increased the distance travelled in both sexes [F (1,56)=30.08; p<0.0001]. The latency [F (1,56)=2.97; p<0.001] and the search error [F (1,56)=3.10; p<0.01] were increased after MDMA treatment only in males relative to saline-treated males. The effect of adult MDMA treatment was only seen in a group of prenatally saline-exposed rats.

The speed of swimming

Adult MDMA treatment increased the speed of swimming in females compared to saline treated females tested in the Learning test [F (1,56)=14.61; p<0.01], the Probe test [F (1,56)=8.6; p<0.05], as well as the Memory test [F (1,56)=22.22; p<0.0001] (Table 14, 15, 16). There was no interaction between prenatal exposure and adult drug treatment.

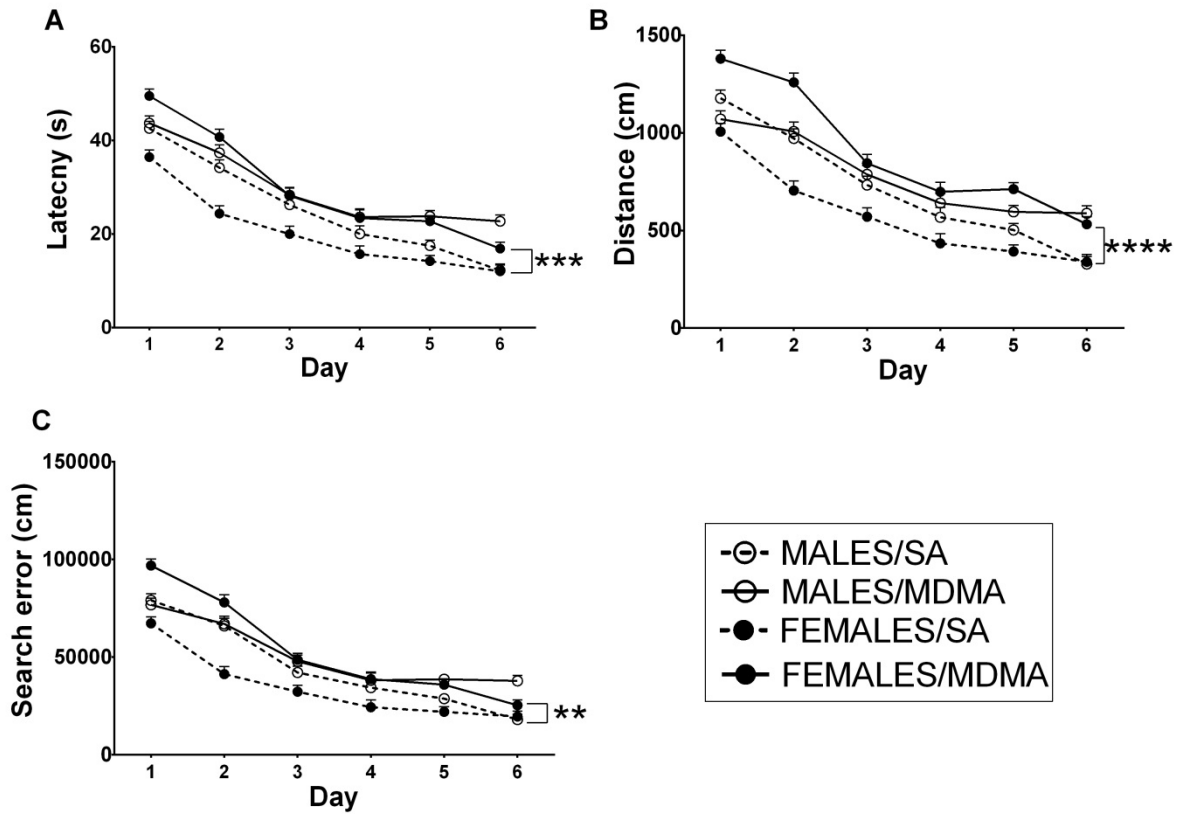

Fig. 27: Effect of adult MDMA treatment on the performance of male and female rats in the Place Navigation test: A. Latency of platform acquisition **B.** Distance travelled **C.** Search error. Values are presented as mean + SEM, n=16. Figure legend means - Sex/Adult treatment. Females MDMA vs. females SA: ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

7.5.5 MORPHINE

The Learning test

Adult MOR treatment increased in females the latency [$F(1, 56)=4.19$; $p<0.001$], the distance travelled [$F(1,56)=10.86$; $p<0.0001$], and the search error [$F(1,56)=5.16$; $p<0.01$] relative to saline-treated females (Figure 28). The effect of adult MOR treatment was only seen in a group of prenatally saline-exposed females.

The Probe test

In both sexes adult MOR treatment did not influence the number of crossing and duration of presence in the quadrant where the platform was located. Adult MOR treatment increased the distance travelled in females compered to MOR treated males [$F(1,56)=8.68$; $p<0.01$], regardless of prenatal drug exposure.

The Memory Recall test

Adult MOR treatment increased in females the latency [F (1,56)=0.10; p<0.05], the distance travelled [F (1,56)=2,37; p<0.001], and the search error [F (1,56)=1.63; p<0.05] relative to saline treated females. The effect of adult MOR treatment was only seen in a group of prenatally saline-exposed females.

The speed of swimming

Adult MOR treatment decreased speed of swimming in males relative to MOR treated females in The Learning test [F (1,56)=12.72; p<0.001] and in the Probe test [F (1,56)=8.81; p<0.01]. Additionally, adult MOR treatment increased the speed of swimming in females relative to saline-treated females tested in the Learning test [F (1,56)=12.72; p<0.01], as well as in the Memory test [F (1,56)=7.94; p<0.01] (Table 14, 15, 16). There was no interaction between prenatal exposure and adult drug treatment.

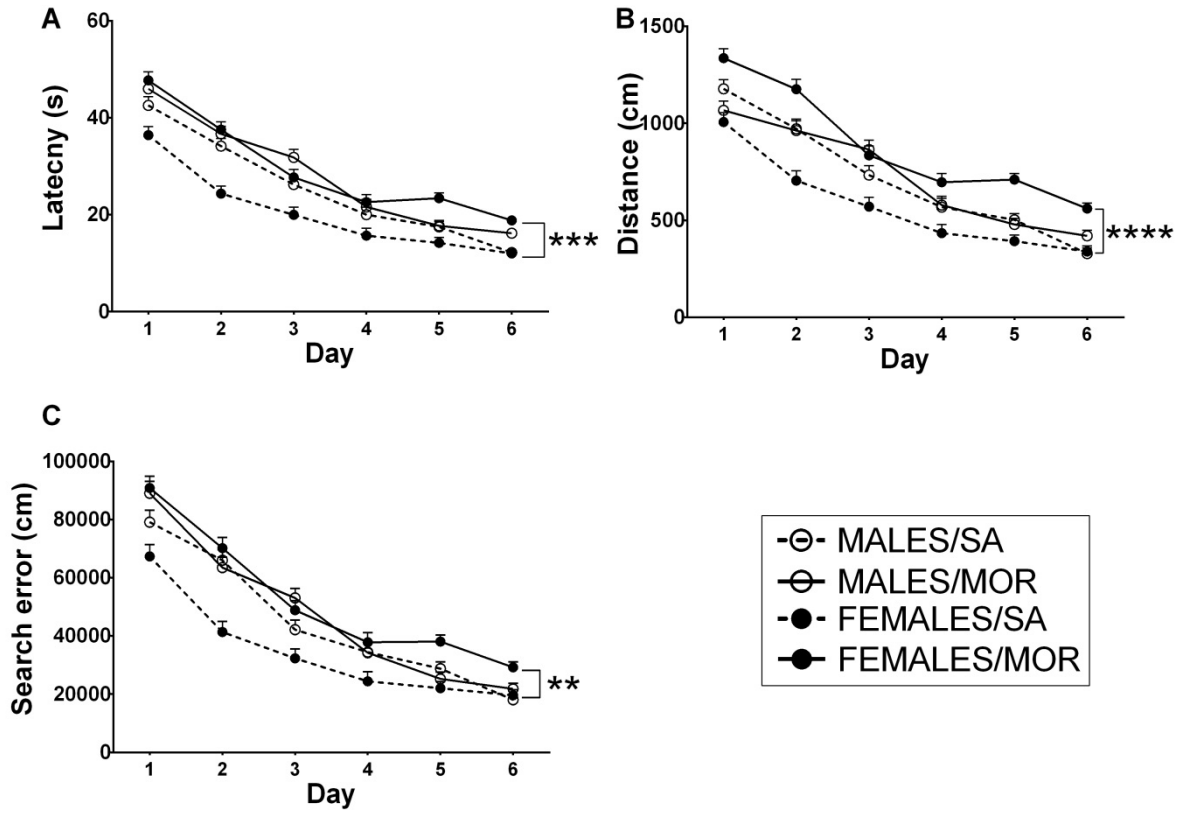

Fig. 28: Effect of adult MOR treatment on the performance of male and female rats in the Place Navigation test: A. Latency of platform acquisition **B.** Distance travelled **C.** Search error. Values are presented as mean + SEM, n=16. Figure legend means - Sex/Adult treatment. Females MOR vs. females SA: ** p<0.01, *** p<0.001, **** p<0.0001.

7.5.6 THC

The Learning test

Adult THC treatment increased in females the latency [F (1,56)=9.39; p<0.001], the distance travelled [F (1,56)=18.16; p<0.0001], and the search errors [F (1,56)=10.57; p<0.001] relative to saline-treated females, as well as to THC-treated males [the distance travelled (p<0.01)] (Figure 29). The effect of adult THC treatment on the duration and the search error was only seen in a group of prenatally saline-exposed females.

The Probe test

In both genders adult THC treatment did not influence the number of crossing of the quadrant where the platform was located. In females adult THC treatment increased the distance travelled [F (1,56)=10.58; p<0.01], as well as decreased the duration of presence in quadrant where the platform was located [F (1,56)=6.78; p<0.05] relative to THC-treated males, regardless of prenatal drug exposure.

The Memory Recall test

Adult THC treatment increased in females the latency [F (1,56)=1.05; p<0.0001], the distance travelled [F (1,56)=9.31; p<0.001] and the search error [F (1,56)=5.06; p<0.05] relative to saline-treated females, as well as to THC-treated males [the distance travelled (p<0.001)] and the search error (p<0.01)]. The effect of adult THC treatment on the duration and the search error was only seen in a group of prenatally saline-exposed females.

The speed of swimming

Adult THC treatment decreased speed of swimming in males relative to THC treated females in The Learning test [F (1,56)=14.48; p<0.001], in the Probe test [F (1,56)=10.87; p<0.01], as well as in the Memory test [F (1,56)=18.75; p<0.0001]. Additionally, adult THC treatment increased speed of swimming in females compared to saline treated females tested in the Learning test [F (1,56)=14.48; p<0.01] and in the Memory test [F (1,56)=18.75; p<0.001] (Table 14, 15, 16). There was no interaction between prenatal exposure and adult drug treatment.

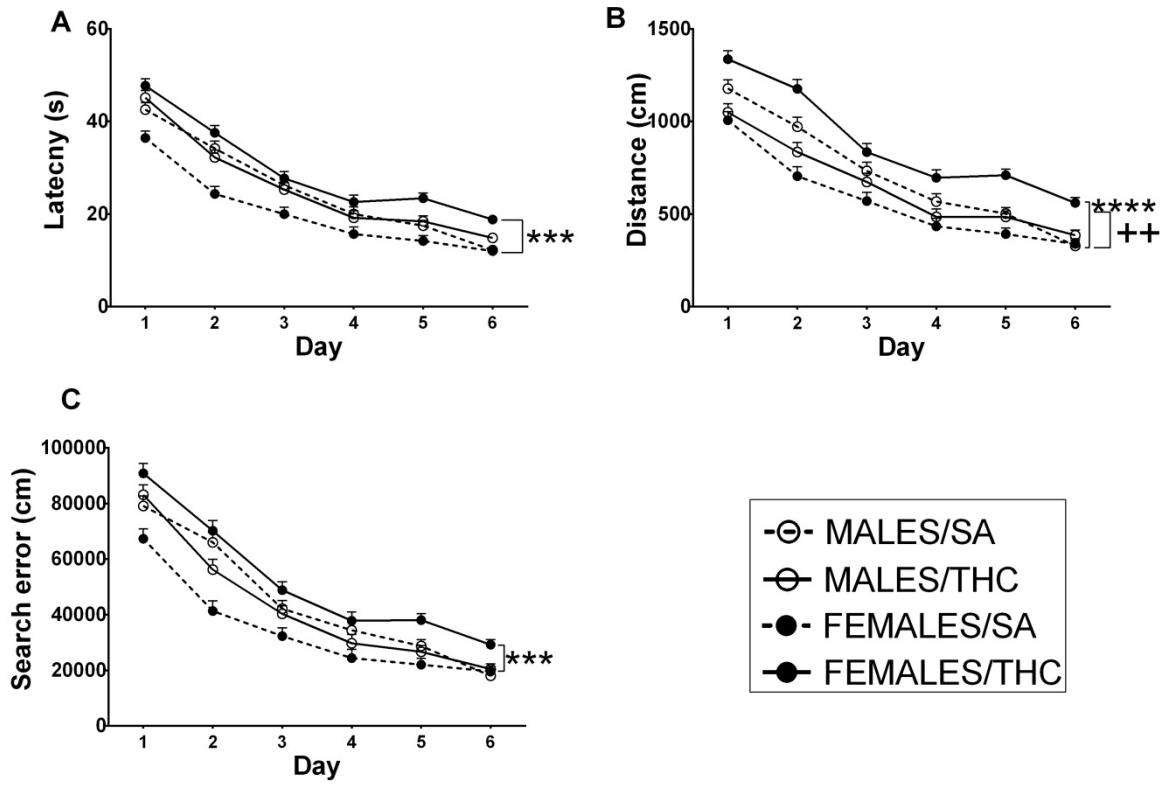

Fig. 29: Effect of adult THC treatment on the performance of male and female rats in the Place Navigation test: A. Latency of platform acquisition **B.** Distance travelled **C.** Search error. Values are presented as mean + SEM, n=16. Figure legend means - Sex/Adult treatment. Females THC vs. females SA: *** $p<0.001$, **** $p<0.0001$, females THC vs. males THC ++ $p<0.01$.

Table 14: The effect of drugs on the speed of swimming on the Learning test

	SA	MA	AMP	COC	MDMA	MOR	THC
MALES	27.83 ±0.62	-	27.12 ±0.62	28.56 ±0.74	26.65 ±0.6	24.46 ±0.58 +++	26.10 ±0.59 +++
FEMALES	27.12 ±0.62	27.47 ±0.59	27.67 ±0.62	29.39 ±0.74	30.53 ±0.6 **	29.9 ±0.58 **	29.90 ±0.59 **

Values are mean ± SEM (n=16-20).
** P<0.01 (females drug vs. females SA)
+++ P<0.001 (males drug vs. females drug)

Table 15: The effect of drugs on the speed of swimming on the Probe test

	SA	MA	AMP	COC	MDMA	MOR	THC
MALES	30.22 ±1.14	-	27.69 ±1.14	28.92 ±1.39	28.59 ±1.14	26.18 ±1.26 ++	24.97 ±1.28 ++
FEMALES	28.80 ±1.14	29.25 ±0.25	27.82 ±1.14	30.06 ±1.39	33.86 ±1.14 *	32.25 ±1.26	31.99 ±1.28

Values are mean ± SEM (n=16-20).
* P<0.05 (females drug vs. females SA)
++ P<0.01 (males drug vs. females drug)

Table 16: The effect of drugs on the speed of swimming on the Memory Recall test

	SA	MA	AMP	COC	MDMA	MOR	THC
MALES	25.75 ±0.67	-	24.85 ±0.67	26.89± 0.93	25.98 ±0.73	25.68 ±0.82	24.13 ±0.82 ++++
FEMALES	24.35 ±0.67	24.85 ±0.68	25.32 ±0.67	28.83± 0.93	29.73 ±0.73 ****	28.54 ±0.82 **	29.86 ±0.82 ***

Values are mean ± SEM (n=16-20).
** P<0.01 (females drug vs. females SA)
*** P<0.001 (females drug vs. females SA)
**** P<0.0001 (females drug vs. females SA)
++++ P<0.0001 (males drug vs. females drug)

7.6 THE PRENATAL DRUG EFFECT

7.6.1 THE LABORAS TEST

Only in two experiments the main effect of prenatal MA exposure was shown and this effect was only seen in females. In the COC experiments, females rats exposed to MA prenatally demonstrated increased time spent in locomotion [$F(1,55)=5.29$; $p<0.05$], longer distance travelled [$F(1,55)=6.06$; $p<0.05$], increased time spent rearing [$F(1,55)=7.31$; $p<0.01$], as well as increased speed of movement [$F(1,55)=4.99$; $p<0.05$], when compared to saline-exposed females, regardless of adult drug exposure. In the MOR experiments females rats exposed to MA prenatally demonstrated increased time spent in locomotion [$F(1,56)=4.78$; $p<0.05$], longer distance travelled [$F(1,56)=4.19$; $p<0.05$], and increased time spent rearing [$F(1,56)=4.19$; $p<0.05$], when compared to saline-exposed females.

7.6.2 THE SOCIAL INTERACTION TEST

In males, prenatal MA exposure neither influenced social interactions {time spent in SI [$F(1,28)=0.69$; $p=0.4$] and occurrence of SI [$F(1,28)=3.36$; $p=0.07$]}, nor influenced the time spent in locomotor activity [$F(1,28)=0.64$; $p=0.43$] and the number of rearing [$F(1,28)=3.69$; $p=0.05$].

7.6.3 THE ELEVATED PLUS MAZE TEST

Prenatal MA exposure did not influence any parameters of anxiogenic and anxiolytic behaviour.

7.6.4 THE MORRIS WATER MAZE TEST

Prenatal MA did not influence any parameters of the Learning, Probe and Memory Recall test.

7.7 THE EFFECT OF THE GONADAL HORMONES

7.7.1 THE CONDITIONED PLACE PREFERENCE TEST

Results from the CPP showed that females were more active than males {higher number of entries to the chamber [$F(1.56)=7.41$, $p<0.01$]}, regardless of prenatal exposure and adult drug treatment.

7.7.2 THE LABORAS TEST

Results from the Laboras test demonstrated that females in P/E were more active than males {spent more time in locomotion [$F(2,87)=12.93$, $p<0.0001$], travelled a longer distance [$F(2,87)=15.26$, $p<0.0001$], spent more time rearing [$F(2,87)=12.63$, $p<0.0001$], and were faster in walking [$F(2,87)=13.29$, $p<0.0001$]}, regardless of the acute drug treatment and prenatal exposure. Additionally, females in P/E were more active than females in M/D {spent more time in locomotion [$F(1,55)=6.07$, $p<0.05$], travelled a longer distance [$F(1,55)=3.75$, $p<0.05$], spent more time rearing [$F(1,55)=5.67$, $p<0.05$], and were faster in walking [$F(1,55)=3.84$, $p<0.05$]}.

7.7.3 THE ELEVATED PLUS MAZE TEST

Results from the EPM demonstrated that females in P/E were less anxious and more active than males {spent more time in open arms [$F(2,52)=4.83$, $p<0.05$], less time in closed arms [$F(2,52)=5.36$, $p<0.01$], and showed higher number of all arm entries [$F(2,52)=38,13$, $p<0.0001$]}, regardless of the acute drug treatment and prenatal exposure. However, females in P/E did not differ in locomotion and anxiety to females in M/D {time in open arms [$F(1,24)=0.05$, $p=0.83$], time spent in closed arms [$F(1,24)=0.47$, $p=0.50$], and all arm entries [$F(1,24)=0.08$, $p=0.93$]}.

7.7.4 THE MORRIS WATTER MAZE TEST

Results from the MWM did not show any differences in the learning abilities between males and females as the Learning test proceeded {the latency [$F(5,280)=1.27$, $p=0.28$], the distance [$F(5,280)=0.47$, $p=0.79$], the search error [$F(5,280)=1.28$, $p=0.27$]}. However, the Memory Recall test revealed a weaker memory recall of females when compared to males {females swam longer distance [$F(1,56)=10.60$, $p<0.01$], for longer time [$F(1,56)=8.03$, $p<0.01$] and showed higher search error [$F(1.56)=8.76$, $p<0.01$]}. On the Probe test females also spent less time in the quadrant where the platform was located than males [$F(1,56)=9.62$, $p<0.01$]. As far as the speed of swimming is concerned, females in different phases of the oestrous cycle did not differ to males {in the Probe test [$F(2,56)=0.48$, $p=0.62$] and in the Memory Recall test [$F(2,56)=0.48$, $p=0.63$]}.

III. DISCUSSION

8 THE SENSITISATION

In this study, using different behavioural models, we tested the hypothesis that prenatal MA exposure sensitises animals to the effect of various drugs administrated to adult rats. We can summarize by saying that a sensitising effect associated with prenatal MA exposure to the psychostimulant effect of some drugs was found, which was mostly observed as increased spontaneous locomotor activity. Specifically, in the Laboras test, prenatally MA-exposed animals demonstrated increased exploration after AMP treatment in adulthood compared to prenatally saline-exposed animals with the same adult treatment. Moreover, in females, prenatal MA exposure sensitised animals to the psychostimulant effects of AMP, COC, and MDMA. We did not find any interaction between prenatal MA exposure and adult drug treatment with regard to active drug-seeking behaviour, which was tested using the CPP test. An interaction was found in the SIT, in which prenatally MA-exposed males demonstrated decreased social interactions after MA, AMP, and MDMA treatment, compared to saline-exposed animals. Prenatal MA exposure did not sensitise animals to the anxiogenic and anxiolytic effect of drugs administrated just prior to testing in the EPM, nor to the effect of chronic administration of these drugs on spatial learning, tested using the MWM test.

To expand the existing knowledge regarding sensitisation, different behavioural test models were used in the present study. Specifically, tests that are traditionally used for testing sensitisation, i.e., the Laboras test for examining augmented locomotor activity produced by repeated drug administration, and the CPP test for examining active drug-seeking in animals. The other tests included the EPM test (for examining anxiety-related behaviour), the SIT test (for examining social interactions of two individuals), and the MWM test (for examining cognitive functions in terms of spatial learning). These tests were used based on the studies of Schutová *et al.* (2009), Schutová *et al.* (2010) and Šlamberová *et al.* (2008) that showed the sensitising effect of prenatal MA not only to the psychomotor-stimulant effect of MA, but also to other drug' effects.

8.1 MA and drugs with similar mechanism of action as MA

Results from the Laboras test showed that in both males and females, prenatal MA exposure induced sensitisation, but only to the psychostimulant effect of an acute dose of AMP, and this sensitising effect was only seen in exploratory activity. Specifically, prenatally MA-

exposed males and females compared to saline-exposed animals demonstrated increased time spent rearing after AMP treatment. In our present study, the effect of an acute MA was not tested, as we wanted to confirm a study of Schutová *et al.* (2013), in which male rats prenatally exposed to MA demonstrated increased sensitivity to adult MA treatment by increased rearing, and in female rats by increased distance travelled. Other studies have demonstrated increased sensitivity to MA in rats exposed to MA *in utero* (using the Laboras test) (Šlamberová *et al.* 2011c) as well as several seizure models (Šlamberová *et al.* 2008, Šlamberová *et al.* 2010b). Moreover, Bubeníková-Valešová *et al.* (2009) showed increased DA release in the NAc after MA challenge in adult rats prenatally exposed to the same drug, which correlated with increased time spent rearing and locomotion. 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. Our results, which showed no sensitising effect resulting from prenatal MA exposure on AMP-seeking in adulthood, are in agreement with a study by Šlamberová *et al.* (2011b) using the CPP test and MA administrated to male rats. According to these results and our results, we suggest that although prenatal MA can sensitise animals to the psychostimulant effect of acute MA and AMP, it does not necessarily increase active drug behaviour relative to these drugs.

Only females, in the Laboras test, displayed sensitisation induced by prenatal MA exposure to COC and MDMA. Specifically, prenatally MA exposed females compared to saline-exposed females, demonstrated increased time spent rearing movements after COC and MDMA treatment. The most likely explanation of this effect, which was found in females but not in males, might be based on sexual dimorphism relative to brain neurotransmitter system development. It has been said by Vathy *et al.* (1993, 1995) that prenatal drug exposure affects the brain of females and males differently (particularly in terms of changes in neurotransmitter levels), and as a result, females might be more sensitive when exposed to other drugs in adulthood. Our data showing sex differences in sensitisation are in agreement with studies of Melnick and Dow-Edwards (2001) and Peris *et al.* (1992) suggesting that these sex differences correspond with dopamine activity. Moreover, Bubeníková-Valešová *et al.* (2009) showed sensitisation induced by prenatal MA exposure to MA challenge in adult male rats corresponding with DA levels in the nucleus accumbens. We suggest more studies to be done to see whether there are also sex differences in the DA concentration after treatment with different drugs that would support our finding showing sex differences in the sensitisation. Additionally, the CPP test did not reveal any sensitising effects, related to sex, of prenatal MA exposure relative to COC and MDMA treatment. Nevertheless, detailed analyses of the "COC

data" in females, revealed avoidance than preference for the chamber associated with COC in animals with prenatal MA exposure. These results indicate some kind of tolerance to COC treatment developed after MA exposure *in utero*. We could only compare our results with the results of Peltier *et al.* (1996), who demonstrated tolerance to the reinforcing effects of COC induced by chronic treatment with MA.

Results showing some kind of interaction between prenatal MA exposure and an acute psychostimulant treatment in the other tests can be described as follows. In the SIT, although there was no interactions found in locomotor activity (non-social behaviour), an interesting result was found with regard to social behaviour in groups of males treated in adulthood with MA, AMP, and MDMA. Specifically, prenatally MA-exposed males with acute MA, AMP, and MDMA treatments showed decreased time spent in social interactions compared to saline-exposed animals treated in adulthood with the same drugs. It appears, that prenatal MA sensitised the animals, such that they have reduced social behaviour when administrated these drugs as adults. As far as we know, there are no studies investigating possible sensitising effects of prenatal MA exposure on disturbances in social interactions after drug treatment later in adulthood. There was a study that investigated prenatal or perinatal exposure to other drugs in rats relative to either decreased social interactions or increased reactivity to stress (Molina *et al.* 1994). Molina *et al.* (1994) also demonstrated that rats prenatally exposed to COC showed increased behavioural responsiveness to stress in adulthood. However, we did not test females (using the SIT), because it has been shown (Šlamberová *et al.* 2011a) that MA at a dose of 1 mg/kg decreased different types of social interaction in both sexes. That is why we could not be sure, if there would be some sensitising effect of prenatal MA in females. Compared to results from the SIT, we did not find any interaction between prenatal drug exposure and acute drug treatment relative to anxiety related behaviour using the EPM test, which is another test for anxiety. We suggest, that the discrepancies might indicate methodological differences between tests that measure anxiety, rather than the effect of the drugs per se. Finally, when analysing data from the MWM, we did not find any sensitising effect of prenatal MA relative to any of the tested drugs with regard to learning abilities in adult female or male rats, which is in agreement with a study by Schutová *et al.* (2009) on males showing that prenatal MA exposure did not increase sensitivity to the same drug in adulthood when tested using the MWM.

8.2 Drugs with different mechanism of action than MA

As far as the sensitising effect of prenatal MA exposure relative to adult MOR and THC treatment is concerned, we did not find any significant result, in the CPP test and in the Laboras test. To the best of our knowledge, there have been no studies investigating increased sensitivity to MOR after prenatal MA exposure. 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); however there was an increase in MOR-conditioned place preference in the study by Gagin *et al.* (1997). Interestingly, in the Laboras test, prenatally saline-exposed females demonstrated increased time spent rearing, as well as increased velocity, after THC treatment compared to prenatally MA-exposed females. Such results indicate tolerance to THC induced by prenatal MA exposure in females, rather than sensitisation. Unfortunately, there are no studies examining the long-term effect of prenatal MA on sensitisation to THC in females, which could be compared to our results.

As far as the other test was concerned, there was no interaction found in the social behaviour tested using the SIT, in anxiety related behaviour tested using the EPM test, or in the spatial learning abilities tested using the MWM test. One possible explanation suggested by us, is prenatal MA does not sensitise the animal to the effect of drugs with different mechanisms of action; however, more studies need to be done to clarify this problem.

Findings from our present study have extended the view of t of sensitisation, developed to different drugs, after prenatal MA exposure. It seems that animals exposed to MA prenatally demonstrate increased sensitivity to MA as well as to drugs with similar mechanisms of action; however, drug effects depend on the behavioural test performed. Moreover, our results also demonstrated that females are more vulnerable to the effect of prenatal MA exposure in terms of developed sensitisation to other psychostimulants administrated in adulthood. However, it is obvious that more tests are needed; we suspect that our results, which show different interactions between prenatal MA exposure and drug treatment in adult rats are probably based on different neurotransmitter pathways. Nestler (2005) has suggested that at least three systems play a key role in development of sensitisation. Firstly, chronic exposure to any of several of commonly abused drugs impairs the VTA-NAc pathway, which was demonstrated by sensitisation of the DA system, with a greater increase in DA transmission occurring in response to the drug. Secondly, chronic exposure to drugs reduces the basal activity of the frontal cortical regions (GLU projections to the NAC and VTA), which decreases self-control and increases

impulsivity. Thirdly, hyperfunction of corticotropin releasing factor systems and their connections to the amygdale have been shown to mediate the negative emotional symptoms that occur during drug withdrawal. At the molecular and cellular level, there has been an increase interest regarding in changes in NMDA and AMPA glutamate receptors in DA neurons after chronic drug use (Šlamberová *et al.* 2014, Thomas and Malenka 2003). It is believed that these receptors are involved in long-term potentiation, which is a key process associated with memory and learning consolidation in the hippocampus (Pu *et al.* 2002). Consequently, similar molecular and cellular mechanisms utilized by the brain to form normal memories and addiction-related memories might play a key role in the reactivity to drugs later in life. The situation relative to development of sensitisation after prenatal drug exposure is even more unclear and our results raised important questions that deserve further attention.

9. EFFECT OF DRUGS ON BEHAVIOUR

9.1 Effect of drugs on active drug-seeking behaviour in the Conditioned Place Preference test

In the CPP test the effect of drug treatment on active drug seeking behaviour of prenatally MA-exposed adult male and female rats was examined. The results demonstrated that MA increased time spent in the chamber associated with MA in both, females and males (independently of prenatal exposure). This result is in agreement with the CPP study on males by Šlamberová *et al.* (2011c). Following conditioning with MA at different dozes (0.25, 0.5, or 1 mg/kg), preference for the MA-paired chamber compared to the saline-paired chamber was also found in a study by Berry *et al.* (2012).There have also been other studies demonstrating MA conditioning in mice (Bryant *et al.* 2012) as well as in humans (Mayo *et al.* 2013). Moreover, in our study, males after MA conditioning spent more time in the drug-paired chamber than females, which is in contrast to the results of a study by Chen *et al.* (2003), who found that gonadal hormones in females (the oestradiol specifically) facilitates MA-induced conditioning. In their study, MA-induced conditioning was shown to be increased in gonadectomised female mice after pre-treatment with the oestradiol compared to gonadectomised male mice.

Neither AMP nor COC conditioning, lead to drug-seeking in females or males. Contrary to our results, COC has been previously shown to induce an increase in drug-seeking after conditioning (Chen *et al.* 2003). We suggest that in this case discrepancy might have been

caused by the use of different models compared our study. Since Chen *et al.* (2003) used a COC dose of 5 mg/kg on mice, our use of the same dose COC on rats might have caused a weaker response. Moreover, Russo *et al.* (2003) showed in their study using male rats, that COC conditioning induces increased drug-seeking only when administrated at a dose of at least 10 mg/kg. Other studies have demonstrated that not only the model and dose of drug used for conditioning, but also the stage of development at which the drug is administrated plays an important factor. Adolescent rats were shown to be more sensitive to the conditioned rewarding properties of COC, MA, and AMP than adult rats exposed to the same dose of drug (Shahbazi *et al.* 2008, Zakharova *et al.* 2009).

The present data also demonstrated sex-dependent effects of MDMA conditioning. While males showed an aversion to the drug, seen as decreased time spent in the drug-paired chamber, females showed the opposite effect, by spending more time in the chamber. These completely different results of MDMA conditioning on males and female were rather surprising, and difficult to compare to other available MDMA studies with ambiguous results on the CPP test. Comparing preference/aversion for the drug-paired chamber required results from studies that used comparable designs to our study. For example, increased drug-seeking after MDMA conditioning was shown in adolescent rats (at a dose of 2.5 mg/kg), as well as in adult rats (at a dose of 5 mg/kg) (Catlow *et al.* 2010). There has been only one study in which MDMA conditioning decreased in males; however, in this study animals were administrated to a neurotoxic dose of MDMA (20 mg/kg) prior to the MDMA CPP testing (Schechter 1991). A possible explanation for the gender differences in drug-searching activity after MDMA conditioning might be based on gender differences in neurotransmitter systems, specifically, a 5-HT. MDMA has been shown to be a strong 5-HT releaser and females tend to show greater 5-HT activity than males (Carlsson and Carlsson 1988, Verrico *et al.* 2007).

Increased drug-seeking after MOR conditioning in both, females and males was found in both prenatally exposed groups, and manifested as increased time spent in the chamber associated with MOR. These results are in accordance with many other reports that found rewarding properties of MOR on the CPP test (Martin *et al.* 2000, Mueller *et al.* 2002), and also self-administration tests (Bozarth and Wise 1981). Mueller *et al.* (2002) showed preferences following conditioning with MOR at the same dose as we used (5 mg/kg), and they found that MOR-induced CPP persisted for at least 12 weeks. As far as we are aware there is no study showing increased drug-seeking behaviour after MOR conditioning in females, so our results provide new information to this research field. We did not find any preferences after THC-conditioning in females or males; however, there was some aversion to the chamber associated

with drug. These results agree with the results of the study of Cheer *et al.* (2000) who also found an aversion to the chamber associated with THC (at a dose of 1.5 mg/kg) as well as to the chamber paired with a synthetic cannabinoid agonist. These findings are also supported by a study from Leite and Carlini (1974) that showed that rats fail to self-administer cannabinoids. Cheer *et al.* (2000) suggested one possible explanation of this aversive effect of cannabinoids. They claimed that the rewarding effects of cannabinoids might be masked by their anxiogenic effects, which was shown in a previous study (Onaivi *et al.* 1990). In addition, our present results from the EPM test support this hypothesis.

9.2 Effect of drugs on the locomotor activity using the Laboras test

Using the Laboras test, we tested the effect of acute drug treatment on the behaviour of prenatally MA-exposed adult male and female rats in an unknown environment. Our results from the Laboras test showed that acute AMP and MDMA increased the time spent in locomotion and the distance travelled, which was comparable in both sexes. AMP (similarly to MA) and MDMA, have been repeatedly shown to increase locomotor activity (Milesi-Halle *et al.* 2007, Páleníček *et al.* 2005, Shoblock *et al.* 2003b, Schutová *et al.* 2010, Schutová *et al.* 2013, Šlamberová *et al.* 2011c). Although, in our study, both drugs (AMP and MDMA) led to equally increased locomotion activity in both sexes, other studies have shown a stronger effect on females than males (Milesi-Halle *et al.* 2007, Páleníček *et al.* 2005). Interestingly, after a detailed analysis of our data we could see that while AMP increased locomotion and distance travelled only at the beginning of the Laboras test, the effect was no longer significant after the 40^{th} minute of testing, while the increased effect of MDMA on these parameters lasted the entire hour of testing. It is possible that the dose of 5 mg/kg of MDMA was too high to return the increased locomotion to the controls prior to the end of the test one hour test period; this is plausible since the effect of MDMA on locomotion has been shown to be dose specific (Páleníček *et al.* 2005). While velocity was increased after MDMA in both genders, AMP did not have any effect on males, and had a decreasing effect on females. This decreasing effect shows, in contrast to previously mentioned studies, the locomotor-stimulating effect of AMP, which might have been caused by the fact that females without any drug treatment show an increased interest in novel environment compared to AMP-treated females or males.

The effect of adult COC treatment was sex-specific. COC increased all parameters of locomotor activity in the Laboras test, but only in females. Because there have been more studies showing increased behavioural activities after COC treatment in males (Broderick *et al.* 2003, De La Garza and Cunningham 2000), we found this result surprising. On the other hand,

there have also been studies reporting greater behavioural effects of COC on females compared to males (van Haaren and Meyer 1991, Walker *et al.* 2001). A detailed analysis of our data revealed that in females the increased activity induced by COC was not seen until the 20th minute of the test, while in males, increased time spent in locomotion and rearing were not seen until the 40th minute of the test. It is therefore possible that the COC-stimulating effect arises later, specifically, even later in males than in females, and thereby females might be more sensitive than males to COC administration. Additionally, the different effect of COC treatment on males and females could also be linked to the gender differences in the 5-HT system, which has been shown to be more expressed in females (Carlsson and Carlsson 1988), and to the fact, that 5-HT has been suggested as a contributor to the behavioural effects of COC (Rothman and Baumann 2003).

As mentioned before, differences in 5-HT and DA neurotransmission might explain observed gender differences in the locomotor stimulant effects of the psychostimulants used in our study. Gender differences in the brain concentrations of 5-HT have been previously demonstrated, with females showing greater 5-HT activity (Carlsson and Carlsson 1988, Verrico *et al.* 2007). Additionally, greater DA and 5-HT sensitivity to various stimuli have also been reported in females (Robinson *et al.* 1980). It has been shown, that ovarian hormones play an important role in setting the sensitivity and reactivity to these two neurotransmitter pathways. Several studies have reported that the oestradiol plays an important role in inducing increased AMP-stimulated DA release in OVX females. Other studies have shown attenuated SERT and DAT concentrations in OVX females, and these changes were prevented by the oestradiol treatment.

MOR decreased all parameters of locomotor activity, without regard to prenatal exposure or sex. There are studies that have shown dose dependent effects relative to acute MOR treatment on locomotor activity (Babbini and Davis 1972, Patti *et al.* 2005, Vezina and Stewart 1987). For example, Babbini and Davis (1972) demonstrated that a single injection of low-doses of MOR (1.25, 2.5, and 5 mg/kg) administrated intraperitoneally had an excitatory effect, while higher doses (≥10 mg/kg) had an inhibitory effect. In our study we only used MOR at a dose of 5 mg/kg; however, even this dose inhibited locomotor activity. Because we did not use lower doses, we were not able to evaluate if there was a dose-dependent effect of MOR on locomotion. It should be noted that the locomotor-stimulating effect of MOR, shown by Babbini and Davis (1972), was demonstrated 8 hours after the MOR administration, while in our study the animals were tested right after drug administration. THC treatment did not have any effect on the locomotor behaviour of males, which is in contrast to other studies that have shown

motor activity-decreasing effects (Hernandez-Tristan *et al.* 2000, Schramm-Sapyta *et al.* 2007). The observed differences might have been caused by different doses of THC used in our study (2 mg/kg) and the studies by Schramm-Sapyta *et al.* (2007) and Hernandez-Tristan *et al.* (2000) that used 5 mg/kg.

9.3 Effect of drugs on the social behaviour using the Social Interaction test

In the SIT the effect of acute drug treatment on social interaction, as well as locomotor activity of prenatally MA-exposed adult male rats was examined. All psychostimulant drugs tested using the SIT, apart from COC, induced disturbances in social behaviour. Particularly, results from the SIT showed that acute MA, AMP, as well as MDMA decreased total time spent in social interactions (SI), especially in the group of prenatally MA-exposed male rats. In our study we used 1 mg/kg as the acute dose of MA, which did not induce any stereotypical behaviour; however, nonetheless, the dose had still been shown to decrease SI (Šlamberová *et al.* 2010a). There are also other studies that have shown decreases in SI after treatment with MA (Arakawa 1994), AMP (Tikal and Benešová 1972), and MDMA (Bull *et al.* 2004) in a dose-dependent manner. It should be noted, that in the present study [in contrast to the study of Šlamberová *et al.* (2010a)] the effect of acute drug treatment on total time spent in SI was examined in animals exposed to MA *in utero*, and prenatal exposure seemed to have an impact, since the time spent in SI was decreased more in prenatally MA-exposed group than in saline-exposed animals. Our explanation of this result was discussed in more detail Chapter 8 (Sensitisation). Furthermore, we did not find any effect of acute drug treatment for MA, AMP, and COC, with regard to particular patters of SI (specifically, mutual sniffing, allogrooming, and climbing over, were not affected). Only AMP treatment decreased both time and occurrence of following, while time spent in climbing over was decreased after both MDMA and AMP treatment. However, based on these patterns of SI associated with acute drug treatment, no definitive drug effects can be concluded.

As far as the effect of the tested psychostimulants on the patterns of non-social activity was concerned, our results showed that AMP, COC, and MDMA increased the time spent in locomotion, while MA, AMP, and COC also increased the occurrence of rearing, which are in agreement with other studies that have shown increased locomotion after treatment with psychostimulants (Bull *et al.* 2004, Šlamberová *et al.* 2015). It has been previously shown that environmental conditions play a role in social and non-social behaviour, especially, familiarity of the open field arena (File and Hyde 1978, Šlamberová *et al.* 2010a). Šlamberová *et al.* (2010a) showed that animals in an unfamiliar arena demonstrate increased in the exploratory

activity. Because the animals in our present study underwent 2 days of habituation to the open field arena, we could exclude the effect of a novel environment, and conclude that the any increased locomotion would be linked to the effect of the tested psychostimulants. We also noted a correlation between social and non-social behaviour [similar found in a study by Šlamberová *et al.* (2010a)], where an increase in time spent in locomotion correlated with a decrease in SI in rats treated with MA. This trend was found in our present study, showing increased locomotor activity and decreased SI in animals with MA, AMP, and MDMA treatment. We suggest that the locomotor-stimulating effect of these drugs might mask the SI-related behavioural effects, and thus we did not see any drug effects on the particular patterns of SI.

COC treatment neither influenced the total time spent in SI, nor particular patterns of SI. On the other hand, treatment with this drug increased the occurrence of rearing as well as the time spent in locomotion. The COC locomotor-stimulating effect has been previously discussed and is similar to that seen in other studies (Broderick *et al.* 2003, De La Garza and Cunningham 2000). Our results showing no effect of COC (at a dose of 5 mg/kg) on SI disagree with a recent study by Šlamberová *et al.* (2015), who revealed a dose dependent effect of COC on social behaviour, with higher doses (2.5, 5 and 10 mg/kg) decreasing SI and lower doses (1 mg/kg) having no effect.

Our results showed that acute MOR treatment decreased both total time spent in SI as well as the occurrence of SI in both prenatally exposed groups of animals. Additionally, MOR also decreased locomotor activity, specifically it decreased both time spent in locomotion as well as the occurrence of rearing. MOR has been previously shown to inhibit locomotor activity in a dose dependent manner (Babbini and Davis 1972). This inhibiting effect on locomotion also agrees with our present results from the Laboras test. One could conclude that decreased social behaviour in animals was as a consequence of decreased locomotor activity. However, MOR strongly decreased not only total time and occurrence of SI, but also particular patterns of SI, specifically, mutual sniffing, following, and climbing over time. Therefore, it seems that MOR ability to reduce social-interactions was independent of its locomotor-inhibiting effect. Our results are also in agreement with a study by Herman and Panksepp (1978) that showed a separation distress in infant guinea pigs by demonstrating increased vocalization even after low doses of MOR (0.75 mg/kg).

Our results from SIT did not show any effect relative to treatment with THC regarding total time spent in SI, which is in contrast to results of O'Shea *et al.* (2006). The discrepancy might have been caused by different test conditions used. O'Shea *et al.* (2006) demonstrated

decreased SI after chronic treatment with the cannabinoid receptor agonist (CP 55 940) following a 28-day drug-free period before the test. Additionally, Schneider *et al.* (2008) demonstrated that acute cannabinoid administration induced more deficits in social behaviour of pubertal rats than in mature rats. Non-social activities were also not affected by THC treatment, which corresponds to our results from the Laboras test; however, it does not agree with other that have shown that THC decreases locomotor activity (Hernandez-Tristan *et al.* 2000, Schramm-Sapyta *et al.* 2007). Additionally, in our present study, the occurrence of following and climbing over, which are taken as parameters of social behaviour requiring motor activity, were increased after THC treatment. We suggest that the discrepancy might have been cause by a different dose of THC used in our study (2 mg/kg) and the study of Schramm-Sapyta *et al.* (2007), which was 5 mg/kg.

9.4 Effect of drugs on the anxiety in the Elevated Plus Maze test

The EPM test was used to examine the effect of acute drug treatment on anxiogenic and anxiolytic behaviour, as well as locomotor activity of prenatally MA-exposed adult male and female rats. Our results can be summarized as follows: females treated with MA demonstrated increased time spent in the OA and decreased time spent in the CA compared to MA-treated males. Both, AMP and COC treatment increased time spent in the OA in females compared to drug-treated males and saline-treated females and also decreased the number of pSAP. These results indicate an anxiolytic effect of MA, AMP, and COC, which was only seen in the groups of drug-treated females. MDMA increased time spent in the CA in females compared to saline-treated females, which indicates an anxiogenic effect of MDMA shown on females. Anxiogenic-like behaviour was also seen after MOR and THC treatment in females as well as in males; this was demonstrated by increased time spent in the CA and the increased number of pSAP.

It should be noted that studies testing the effect of psychostimulants on anxiety display inconsistent findings. In the EPM test, acute and chronic exposure to psychostimulants has been shown to have both, anxiogenic (Biala and Kruk 2007, Hayase *et al.* 2005, Pometlová *et al.* 2012) and anxiolytic effects (Schutová *et al.* 2010). The disagreements found between different studies might have been caused by different tests settings, as well as by different gender of the animal model used. For example, in the study of Hayase *et al.* (2005) anxiety-related behaviour was observed in males at 3- and 5-day time points after a single dose of MA (4 mg/kg), which was observed to disappear after 10 days. Only the study by Schutová *et al.* (2010) used a similar testing model and the same acute dose of drug; however, the effect of MA was tested only on

the behaviour of male rats. They found that MA (1 mg/kg) decreased anxiety in prenatally MA-exposed males by increasing time spent in the OA, which is in contrast to our results that showed no effect of MA (as well as AMP and COC) on males (Schutová *et al.* 2010). Our explanation of these inconsistencies is as follows: in the present study MA was administrated 45 minutes prior to the test, while in the study of Schutová *et al.* (2010) it was 30 minutes prior to the test. The time of the injection in our study was chosen on the basis of the study of a study by Rambousek *et al.* (2014) showing that MA levels in the brain of adult rats peak from 45 min to 1 h after MA administration. Since testing did not start until 45 minutes after the drug was administrated, we could not see if there was any drug effect in the male rats 30 minutes after drug administration. Moreover, a study by Rambousek *et al.* (2014) also demonstrated that females have higher levels of plasma and brain MA after a single dose of MA (1 mg/kg) compared to males. Because we did not see any effect of MA 45 minutes after drug administration in males, we can speculate, that males are more sensitive to the anxiolytic-like effect at lower brain levels of MA. In the present study animals were habituated to the experimenter 3 days prior to testing to reduce stress. Therefore, another explanation might be nothing more than different stress reactivity of females compared to males. Although no differences in the brain level of COC in rats after an acute COC injection have been found, females react more intensively to COC administration than males (Carroll *et al.* 2004). Additionally, locomotion was increased after MA, AMP, and COC treatment, but only in females compared to drug-treated males, which, again, supports the previous results of higher sensitivity of females to the psychomotor-stimulating effects of these drugs (as previously mentioned). As a result we cannot completely exclude that the anxiolytic effect seen only in females after MA, AMP, and COC treatment was not a consequence of the psychomotor-stimulating effect of these drugs.

Females after MDMA treatment (5 mg/kg) demonstrated increased time spent in the CA, which indicates an anxiogenic effect of MDMA. Similarly to other psychostimulants, both, the anxiogenic and anxiolytic effects of MDMA have been previously shown after acute treatment. A dose-dependent effect was found in the study by Navarro and Maldonado (2002) in rats, with 8 mg/kg producing an anxiogenic-like effect. On the other hand, the anxiolytic effect of MDMA (at a dose of 5 mg/kg) was found in the study of Daza-Losada *et al.* (2009) on mice. In the study by Páleníček *et al.* (2005) the anxiolytic-like effect of MDMA (at a dose of 10 mg/kg) was found, in both, females and males. Moreover, MDMA increased the number of all arm entries by both genders, indicating that MDMA has a locomotor-stimulating effect, which agrees with our present results from the Laboras test. However, contrary to the results of

Páleníček *et al.* (2005) we did not find females to be more sensitive than males to MDMA-induced locomotion. However, in their study they used a different test model (activity cage and open field) for testing locomotor activity. The decreased rearing movement after MDMA was shown in both sexes, which agrees with a study by Spanos and Yamamoto (1989). It has been suggested that the decreased rearing is based on co-activation of the DA and 5-HT systems after MDMA treatment.

In both, females and males, MOR treatment increased the time spent in the CA, but did not significantly affect time spent in the OA and increased the number of SAP. These data indicate the anxiogenic effect of MOR, which is in contrast to results from a study by Zarrindast *et al.* (2005) that showed the anxiolytic effect. Discrepancies might have been caused by different dose regimens and drug administration used in our study and studies of others. While Zhang and Schulteis (2008) used MOR at a dose of 10 mg/kg *s. c.* (compared to our study 5 mg/kg *s.c.*), while Zarrindast *et al.* (2005) administrated 3, 6, and 9 mg/kg, intraperitoneally. Moreover, in the study of Zhang and Schulteis (2008) MOR was administered 2 hours, prior, while in our study it was given 45 minutes prior to the EPM test. As far as the drug effect on locomotion was concerned, we found that MOR decreased locomotion in males, and did not have any effect on females. This locomotor-inhibiting effect corresponds with our results from the Laboras test as well as the SIT; however, it disagrees with results from a study by Babbini and Davis (1972). Similarly to MOR, THC treatment increased time spent in the CA and decreased the number of all arm entries, comparable in both genders, which indicates an anxiogenic and locomotor-inhibiting effect of acute THC treatment. The increased anxiety and decreased locomotion showed in our study agrees with the results of the study by Arevalo *et al.* (2001) that showed an aversion, by rats, to the open arms of the EPM 30 minutes after treatment with a cannabinoid antagonist (CP 55 940).

Rogers and Johnson (1995) recommended incorporating the protected SAP as a parameter of the anxiogenic-like behaviour. Higher numbers on pSAP indicates more anxiogenic-like behaviour (Espejo 1997, Rodgers and Johnson 1995). Even when using this specific parameter, our results from the EPM test were supported. Specifically, the decreased number of pSAP after AMP and COC confirmed the anxiolytic effect, while the increased number of pSAP after MOR and THC confirmed the anxiogenic effect of these drugs.

Additionally, the validity of SIT and EPM for measuring anxiety has been the previous topic of several discussions (File and Hyde 1978, Rodgers *et al.* 1997) based on the different results coming from these tests. These two tests have been suggested for examining different states of fear. While the EPM examines the natural aversion of the test animal to open and high

places, the SIT examines the emotional response to an unknown animal (Rodgers *et al.* 1997). However, the comparison of these two tests regarding their validity in measuring anxiety goes beyond the scope of our discussion.

9.5 Effect of drugs on spatial learning in the Morris Water Maze test

In the MWM test was used to examine the effects of chronic drug treatment on spatial learning, as well as on locomotor activity of prenatally MA-exposed adult male and female rats. Our results are as follows. Firstly, data from the Place Navigation test showed that females with chronic MA treatment in adulthood swam longer distances and demonstrated longer latencies to reach the hidden platform, which indicates reduced learning abilities. In our present study we only tested females relative to the effect of chronic MA treatment, as we wanted to extend the previously published data reported in a study by Schutová *et al.* (2009). They showed prolonged trajectories as well as changes in swimming strategies after MA treatment in males in the same test setup. Furthermore, we found that females with MA treatment, compared to males from a study by Schutová *et al.* (2009), also demonstrated increased search error, which is, according to some authors, a better reflectance of the accuracy of spatial learning than latency, since this parameter describes the total distance to the platform during the trials. Two animals may have similar latencies although the lengths of their swimming paths might differ markedly. While one animal searches for the platform in the quadrant, in which the platform is placed, the other may search more within the opposite quadrant (Gallagher *et al.* 2015). From these results, we might suggest, that the effect of MA treatment has a more potential effect on females than on males. Unexpectedly, in the Probe test, we found that saline- and MA-treated females had comparable spatial abilities, since they did not differ in any of the parameters. This type of test, in which the animal swims without the platform being present, provides information about memory retention after the position of the platform had been learned by the animal. Therefore, it seems that even though the MA treatment reduced learning abilities, it did not have any additional effect on memory recall, which was also supported by the results on the Memory test, which also showed no effects of MA treatment.

Both, AMP and COC treatment affected female rats' performance in the MWM (by increasing latencies, distances travelled and search errors). Moreover, the effect of AMP and COC was not apparent in males. Comparably, MA, AMP, and COC also did not affect any parameters of the Probe test. However, the effect of AMP and COC treatment on adult females was shown on the Memory Recall test, which was performed on the last day of testing. Therefore, it seems that even though the effect of these drugs was not apparent on the control

Probe test, AMP and COC treatment impaired the ability of female rats to recall the spatial map formed during the learning phase. Our COC results are in agreement with a study by Mendez *et al.* (2008) who also showed long-term cognitive deficits in rats, which persisted even 3 months after chronic COC treatment. With respect to our results, there are two thought-provoking outcomes. First, it seems that chronic AMP and COC has more long-term effects on spatial learning abilities than chronic MA, since there was still some memory impairment seen on the last day of testing. Second, females tend to be more sensitive to the memory-impairing effects of AMP and COC. Additionally, there was an interesting gender difference in spatial learning abilities after MDMA treatment. Female rats demonstrated decreased performance on the Place Navigation test, which indicates reduced learning, as well as reduced performance on the Probe test and the Memory test. On the other hand, male rats did not have any impairments in learning skills over the course of the Learning test; however, the Probe test and even more so the Memory test revealed some memory deficiencies in our test animals. Our results are in agreement with studies that showed learning impairments after chronic MDMA treatment (Morley *et al.* 2001), as well as long-term neurotoxicological effects (e.g. depletion of 5-HT), particularly in the hippocampus, a brain region which plays an important role in spatial learning (Aguirre *et al.* 1997).

We suggest that the differences in the drug effects on learning and memory recall found in the MWM are probably based on gender variations in neurotransmitter systems shown in other studies (Robinson *et al.* 1980), and on the fact, that these systems have been shown to be affected by chronic treatment with these drugs in diverse ways (Baumann *et al.* 2007, Wagner *et al.* 1979, Wilson *et al.* 1992). Although, the neurotoxic effect of chronic treatment with these drugs on the different neurotransmitter systems have been shown, the consequences of long-term drug use on the cognitive functions still remain unexplained.

There was also a significant effect of MOR and THC treatment on the learning abilities shown on the Place Navigation test. Females after MOR and THC swam longer distances with increased latency and search error. Moreover, impaired learning skills were revealed on both, the Probe test and the Memory test. To best of our knowledge, there is no study investigating gender differences on performance in the MWM after chronic MOR treatment. Some studies have demonstrated that long-term administration of MOR (Pu *et al.* 2002) leads to reduction in the capacity of male rats' hippocampal LTP, which is a neural mechanism underlying learning and memory. The same authors (Pu *et al.* 2002) reported impaired learning in the MWM in parallel with a reduction of hippocampal long-term potentiation after chronic MOR treatment. Similarly, chronic treatment with THC was reported to produce impairment in spatial working

memory in adult rats accompanied by reduced levels of markers of neuroplasticity in the hippocampus (Rubino *et al.* 2009). Another study showed a dose-dependent relationship regarding the effect of THC on spatial learning in females (Cha *et al.* 2007).

As far as drug treatment effect on the speed of swimming is concerned, we did not find any effect of MA, AMP, and COC treatment in either females or males. Although these results do not correspond with our results from the Laboras test, which showed increased locomotion after AMP treatment, it should be noted that in the MWM test, animals were tested 24 hours after drug administration, when the acute locomotor-stimulating effect might have been diminished. On the hand, chronic MDMA, MOR, and THC treatment increased speed of swimming in females. It should not be marginalise that some authors have suggested that increased speed of swimming in the MWM is positively correlated to an increased motivation of an animal to find a hidden platform (Lubbers *et al.* 2007).

Another explanation for our results showing learning impairments after all drug treatments, but only in females, might be based on different stress-coping mechanisms if females relative to males. It has been reported in a study by Handa *et al.* (1994) that the hypothalamic-pituitary axis in females reacts more robustly to stress, which is in part due to oestrogen having an enhancing effect on it. Moreover, stress conditions in the MWM were shown to increase corticosterone in males (Akirav *et al.* 2001), the level which was shown to have decreased after neonatal MA treatment in males but not in females after MWM testing (Williams *et al.* 2003). Therefore, it seems that increased levels of corticosterone combined with the drug treatment might be responsible for disturbances in spatial learning in females.

10. THE EFFECT OF PRENATAL MA EXPOSURE

The effect of prenatal MA-exposure based on the Laboras locomotor activity test can be summarized as follows: Neither males nor females demonstrated changes in any of the parameters of locomotor activity. Our data are in agreement with a study by Schutová *et al.* (2010) that showed no effect of prenatal MA on males tested in the Open-field test in adulthood. However, the lack of effect prenatal MA exposure on the spontaneous locomotor activity in females demonstrated in our study is in contrast to a study by Schutová *et al.* (2013) that showed prenatal MA exposure decreased locomotion and velocity of females on the Laboras test. Although there are studies showing some impairments of the sensorimotor development in pups after prenatal MA exposure (Acuff-Smith *et al.* 1996, Šlamberová *et al.* 2006), our results

support previous suggestions that these changes do not persist into adulthood. Moreover, female behaviour did not differ from male behaviour after prenatal MA exposure, although, it was reported in a study by Engele *et al.* (1989) that the development of the mesolimbic dopaminergic system in female and male rats displays some gender variances and might be affected differently in response to prenatal drug exposure.

Our results from the SIT did not reveal any effect of prenatal MA exposure on social behaviour, or non-social behaviour. There was also no effect of prenatal MA exposure on anxiogenic, anxiolytic, and locomotor behaviour in EPM, which is in agreement with previous studies of Hrubá *et al.* (2012) and Schutová *et al.* (2010). From these results we suggest that prenatal MA exposure does not cause such changes in the developing brain of rats that would persist into adulthood as a reflexive anxiety-related behaviour. We also did not see any effect of prenatal MA exposure on learning abilities and memory recall in females and males tested in the MWM. This result is in agreement with previously published study by Schutová *et al.* (2009), however it disagrees with the results of other studies that showed prenatal MA reducing spatial learning abilities (Šlamberová *et al.* 2005).

Since several forms of behaviours were tested in this study, and no effect of prenatal MA exposure was found in any of them, we conclude that prenatal MA exposure probably does not impair the development of baseline neurotransmission pathways involved in these forms of behaviour. However, the effect of prenatal MA exposure on different forms of behaviour, tested in adulthood, was not central focus of this study and therefore is we will consider further discussion to be beyond the scope.

11. THE EFFECT OF GONADAL HORMONES

As far as gender differences effecting locomotor activity are concerned, our results from the Laboras test demonstrated that males were generally less active than females, and females in P/E were more active than females in M/D. Additionally, similar results were found on the EPM and the CPP tests, which was shown by increase entries into all arm on the EPM test, and by increase entries into chambers on the CPP test. Similar gender differences in locomotor activity were also found in others studies (Bisagno *et al.* 2003, Hrubá *et al.* 2012). These gender differences in the locomotor activity are probably based on DA metabolism in the striatum, which has been shown to be greater during oestrus than during diestrus in females. Becker

(1999) suggested that a greater behavioural response in oestrus was related to increased stimulation of the striatal dopaminergic system by gonadal hormones.

Our results from the EPM test demonstrated, that females were generally less anxious than males, especially females in proestrus spent more time in the OA and less time in the CA. Decreased anxiety in females in proestrus was not a surprising result, since there are studies reporting that ovarian hormones play an important role, both organizationally and activationally, relative to plus-maze behaviours in females (Mora *et al.* 1996, Zimmerberg and Farley 1993). Furthermore, OVX females have been shown to exhibit anxiogenic behaviour, which was attenuated by the oestradiol treatment (Mora *et al.* 1996). Another possible explanation of our results might be based on differences in ontogenesis of anxiogenic behaviour found in a study by Imhof *et al.* (1993). They showed gender differences in EPM performance in rats at 60 and 120 days. Female rats demonstrated decreased time spent in the OA at the age of 120 days, whereas in males it happened around the age of 90 days. Animals tested in our study were between 60 and 90 days of age.

Results from the MWM did not show any differences in learning abilities between males and females as the Place Navigation test proceeded. However, the Memory test and the Probe test showed that males were able to memorize the location of the platform more effectively than females, which is in agreement with a study by Perrot-Sinal *et al.* (1996). Regarding previously discussed gender differences in the stress reactivity of females, females in a study by Perrot-Sinal *et al.* (1996) demonstrated increased anxiety and aversion-related tigmotaxis behaviour in the MWM compared to males. This behaviour was diminished after familiarization with certain aspects of the water-maze during the pre-training period. There have also been other MWM studies that showed that male animals have an advantage in spatial learning, which were considered to be sex hormones related (D'Hooge and De Deyn 2001).

IV. GENERAL 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 either MA, or drugs with the same mechanism of action to MA (AMP, COC, MDMA), or drugs with different mechanism of action to MA (MOR, THC).

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 in females only. There was no cross-sensitisation found between prenatal MA exposure and drugs with different mechanisms of actions relative to MA (MOR and THC) administrated in adulthood.

c) The SIT test: prenatal MA exposure **sensitised** animals to the social interaction-decreasing effect of MA, AMP, and MDMA.

d) The EPM test: prenatal MA exposure **did not sensitise** animals to the anxiogenic and anxiolytic effect of any of the drugs.

e) The MWM test: prenatal MA exposure **did not sensitise** animals to the impairing effect of any of the drugs relative to spatial learning

2) As far as sex differences on the effect of adult drug treatment is concerned:

a) The CPP test:

✓ There was an increase in time spent in the chamber associated with the drug in both, females and males, after MA conditioning. There was a decrease in time spent in the chamber associated with MDMA after conditioning in males, while females demonstrated an increase in time after MDMA conditioning. AMP and COC conditioning did not lead to preference for a chamber associated with these drugs.

✓ Both, females and males, demonstrated an increase in the time spent in the chamber associated with MOR and no preference for a chamber associated with THC.

b) The Laboras test:

✓ Both, females and males, after AMP and MDMA demonstrated increased time spent in locomotion. MDMA increased speed of movement in both genders, while AMP

decreased speed of movement, but only in females. Females, but not males, after COC demonstrated increased locomotion.

- ✓ Both females and males, after MOR, demonstrated decreased locomotion. THC did not influence locomotor activity in males, while it increased the speed of movement in females.

c) The SIT test

- ✓ Only MA, AMP, and MDMA decreased total time spent in social interactions in males. MA did not influence locomotion, while AMP, COC, and MDMA increased locomotion. MA, AMP, and COC increased rearing; however, MDMA decreased rearing.
- ✓ In males, MOR decreased social interactions (time and occurrence) as well as decreased locomotion and rearing. THC did not influence social interactions and locomotion.

d) The EPM test

- ✓ MA, AMP, and COC showed anxiolytic and locomotor-stimulating effects, but only in females. MDMA demonstrated anxiogenic and locomotor-stimulating effects, in females.
- ✓ Both THC and MOR demonstrated anxiogenic and locomotor-inhibiting effects in both genders

e) The MWM test

- ✓ Chronic treatment with MA reduced spatial learning, in females; however, it did not have any effect on memory recall. Chronic treatment with AMP and COC reduced both learning and memory recall only in females. MDMA reduced both learning and memory recall in females, and reduced memory in males.
- ✓ Chronic treatment with THC and MOR reduced learning and memory in females.
- ✓ The speed of swimming was not affected by chronic treatment with MA, AMP, and COC. On the other hand, chronic treatment with MDMA, MOR, and THC increased speed of swimming in females.

Results from our study showed that prenatal MA (at a dose of 5 mg/kg) administrated to mothers during the entire gestational period can sensitise their offspring to the application of other drugs in adulthood. Specifically, it seems that animals after MA exposure *in utero* demonstrated some kind of locomotor augmentation when exposed to psychostimulants (COC, AMP, and MDMA) later in adulthood. Our results suggest that exposure to MA during pregnancy results in changes in neurotransmitter systems, which predispose the animals to

greater responses to the psychostimulant effects of drugs administrated in adulthood. However, increased locomotor reactions were not seen after application of any of the tested drugs, especially, drugs with different mechanism of action than MA (e.g. MOR, THC). Furthermore, exposure to MA during the gestational period did not cause any changes in the brains of offspring, which would predispose them to increased drug seeking later in life. In addition, other test situations did not reveal any sensitising effect of prenatal MA, apart from the test for social interactions, in which prenatally-MA exposed animals reacted more sensitively to the social-interaction decreasing effects of MA, AMP, and MDMA.

These are interesting findings giving us new insight into the problem of induced-sensitisation after prenatal MA exposure. It should be emphasize that the drugs used to test the sensitising effects after prenatal MA exposure, were all drugs having a similar mechanism of action to MA, and that this sensitising effect was not seen in all test situations. That is why we cannot simply conclude that prenatal MA exposure leads to an increase in sensitivity to different drugs of abuse, and thus causes development of general drug addiction.

Our study also demonstrated gender differences in the effect of drugs on various forms of behaviour, like drug-seeking behaviour, anxiety-related behaviour, as well as cognitive functions. It appears that gonadal hormones in females play an important role in overall response to drug. However, the range and form of behaviours disturbances rely on the type of drug and on its mechanism of action. It is clear, that the interactions between gonadal hormones and the effect of drugs of abuse on neurotransmitter systems have a greater effect on behavioural sensitisation in females than in males.

Despite numerous studies investigating sensitisation as a complex process arising from different cellular changes in many brain regions, the neural basis of it is not fully understood. Moreover, there are increasing numbers of preclinical studies focusing on long-lasting changes in motivational behaviours or the function of brain reward circuits in animals during gestational drug exposure. Since MA is still one of the most accessible drugs in the Czech Republic, and also in many cases, the first drug of choice for many drug-addicted pregnant women, we were faced with the question of whether children born to mothers, who abused MA during pregnancy, have an increased risk of substance abuse or other addictive behaviours as they grew up and entered adulthood. The question is particularly pertinent since changes developed in prenatal life, in many cases, persist until adulthood. This can consequently impair healthy development and future social inclusion of the children as they mature. Since clinical studies are difficult to perform, developing a testable hypothesis through preclinical research can potentially increase the likelihood of finding a neurobiological basis for an increased predisposition for addiction

in prenatally MA-exposed offspring. Our findings are that although the offspring of the MA-addicted mothers have altered sensitivity to various 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 who used 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 such changes that would make an individual more prone to drug search as an adult. In addition, the findings that prenatally MA exposed females are more vulnerable than males when encountering different drugs later in life, need further investigation.

We hope that our results will lead to a better understanding of the factors, which contribute to prenatal MA exposure altering the brain in terms of behaviour, and how these factors enhance the risk for addiction. Our results offer new insights into drug addiction from the perspective of children of women, who abused drugs during pregnancy, and also suggest new directions for research into drug addiction. Hopefully, these new insights will contribute to the continued development of effective drug abuse prevention.

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VI. AUTHOR'S PUBLICATIONS

A) Publications *in extenso* with Impact Factor related to the topic of the thesis

1. Šlamberová R., Pometlová M., Schutová B., Hrubá L., **Macúchová E.**, Nová E., Rokyta R.: Do prenatally methamphetamine-exposed adult male rats display general predisposition to drug abuse in the Conditioned place preference test? *Physiological Research,* 61(Suppl. 2): 129-138, 2012 **(IF_{2012}: 1,531)**.
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B) Publications *in extenso* with Impact Factor related to the thesis methodologically

1. Malinová-Ševčíková M., Hrebíčková I., **Macúchová E.**, Nová E., Pometlová M., Šlamberová R.: Differences in maternal behavior and development of their pups depend on the time of methamphetamine exposure during gestation period. *Physiological Research,* 63(Suppl. 4): 559-572, 2015 **(IF_{2014}: 1,293)**.
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C) Publications *in extenso* without Impact Factor

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D) Presentations and Abstracts

1. **Macúchová E.**: Vliv psychostimulačních drog na učení a paměť samců a samic laboratorního potkana prenatálně exponovaných metamfetaminu. Studentská vědecká konference 3. LF UK, 10. 5. 2012, Praha.
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