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Thesis summary

Paternal methamphetamine exposure - effect on the development of offspring

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Summary

Drug addiction and its effect on the behavior and development of children has become a serious problem in our society. Methamphetamine (MA) is one of the most abused psychostimulants in the Czech Republic, and its abuse is rising worldwide. Previous studies have demonstrated the adverse long-term effects of maternal drug abuse on rat offspring. However, the father's contribution as a parent and donor of half the genetic information is unclear.

First, the present study aimed to examine the effect of MA administration on male sexual behavior, locomotor activity, spermatogenesis, and testosterone level. Second, the impact of paternal MA exposure on behavioral development, locomotor activity, and social interaction in rat offspring was examined.

MA was administrated for 30 days at a dose of 5 mg/kg s.c. to adult male rats (PD 90). The control group was exposed to saline (SA). During the experiments, 6–8 individuals from each group were tested. The sensorimotor development of rat pups was examined during PD 1–23. The Social play experiment was conducted with juvenile rats (PD 30). The sexual behavior, spermatogenesis, and locomotor activity of fathers and offspring were tested in adulthood. Prior to testing, adult offspring were exposed to an acute challenge dose of MA (1 mg/kg) to examine the possible sensitizing effect of the paternal treatment.

Our results demonstrated that MA exposure did not affect the sexual behavior of male rats. Moreover, MA administration did not influence testosterone levels or spermatogenesis in adult males compared to the control group. The data from the Laboras test showed that chronic MA administration impairs locomotor activity in fathers. Further, our results demonstrated a significant increase in locomotor activity on the Laboras test after acute MA application. Regarding the paternal administration effect on offspring, there were no significant differences in behavioral development or locomotor activity in adulthood. Our data showed that paternal and acute MA administration significantly impaired the social interaction of adolescent offspring. When comparing sex differences, males were more active during development whereas females showed more activity in adolescence and in adulthood. These results suggest that drug addiction in fathers may not have the same serious consequences for their offspring as drug addiction in mothers. However, our study is critical because it is the first to assess the effect of MA on the male's role as a parent and donor of half the genetic information of their offspring.

Souhrn

Drogová závislost a její vliv na chování a vývoj potomstva se v naší společnosti stává závažným problémem. Metamfetamin (MA) je považován za jednu z nejčastěji zneužívaných drog v České republice, jehož zneužívání roste i celosvětově. Předchozí studie prokázaly negativní účinky zneužívání drog matkami, a především jejich nepříznivý vliv na jejich potomky. Ovšem podíl otce jako rodiče a dárce poloviny genetické informace zůstává dosud neobjasněný.

Prvním cílem této studie je prozkoumat vliv dlouhodobého podávání MA na sexuální chování samců, jejich lokomoční aktivitu, spermatogenezi a hladinu testosteronu. Druhým cílem je prozkoumat vliv paternitní expozice MA na vývoj, chování, lokomoční aktivitu a sociální interakci u potkaních potomků.

MA byl podáván dospělým samcům denně po dobu 30 dní v dávce 5 mg/kg subkutánně. Kontrolní skupině byl aplikován fyziologický roztok (SA) ve stejným objemu a ve stejný čas. Během pokusů bylo testováno 6-8 jedinců z každé skupiny. Potkaní mláďata byla testována na senzomotorický vývoj v průběhu postnatálních dnů (PD) 1-23. Adolescentní mláďata (PD 30) byla dále testována v testu sociální hry. V dospělosti byl testován vliv MA na hladinu testosteronu, spermatogenezi a sexuální chování samců. Lokomoční aktivita (Laboras test) byla zjišťována u potkaních otců i jejich potomků. Před testováním lokomoční aktivity byli dospělí potomci vystaveni akutní dávce MA (1 mg/kg) na možný senzitizační účinek paternitní aplikace drogy.

Naše výsledky ukázaly, že expozice MA neovlivnila sexuální chování potkaních samců. Podávání MA navíc neovlivnilo hladinu testosteronu ani spermatogenezi u dospělých samců ve srovnání s kontrolní skupinou. Údaje z testu Laboras ukázaly, že chronické i akutní podávání MA významně ovlivňuje lokomoční aktivitu dospělých samců. Pokud jde o vliv paternitní expozice MA na potomstvo, nebyly zjištěny žádné významné rozdíly ve vývoji chování nebo lokomoční aktivitě v dospělosti. Naše výsledky ukázaly, že paternitní a akutní podání MA významně snížilo sociální hru u dospívajících potomků. Při porovnání rozdílů mezi pohlavími byli samci aktivnější během vývoje, zatímco samice vykazovaly větší aktivitu v adolescenci a dospělosti než samci. Tyto výsledky naznačují, že drogová závislost u otců nemusí mít pro jejich potomky stejně závažné důsledky jako drogová závislost matek. Naše studie je unikátní, jelikož jako první hodnotí vliv podávání MA z pohledu muže jako rodiče na potomstvo.

INTRODUCTION

Methamphetamine (MA) is a psychotropic stimulant that affects the body on a biological, behavioral, and psychological level. In many countries, it is one of the most widely used illicit drugs. Psychostimulant drugs such as MA activate the dopaminergic and serotoninergic pathways of the central nervous system (CNS), which are mainly associated with reward circuits, affective states, sexual behavior, and also in control of motor function and cognition (Frost and Cadet, 2000). MA is known to be a powerfully addictive drug, and due to its low price and relatively simple production, it has become increasingly popular in our society. MA was first synthesized from ephedrine in 1893 by the Japanese scientist Nagai Nagayoshi. In the Czech Republic (previously Czechoslovakia), MA has been illicitly produced since the mid-1970s. The annual report of the EMCDDA in 2019 considered MA the 4th most abused illegal drug in the Czech Republic after cannabinoids, ecstasy, and hallucinogenic fungus (psilocybin). Currently, most MA users are reproductive-age men between 15 and 34 years.

The effects of MA depend on the route of administration. About 80% of MA users prefer injection (Mravcik et al., 2007) because when MA is injected subcutaneously, the drug enters the bloodstream faster, and its effect occurs more quickly. MA is a lipophilic molecule that rapidly penetrates membrane structures (Dattel, 1990). In addition, MA has been shown to cross the placenta and enter the breast milk of mothers, which may affect the prenatal development of the fetus or postnatal development of the newborn during lactation, respectively (Smith et al., 2001). Consequences of MA exposure include a reduction in the volume of several brain structures, such as the hippocampus, dentate gyrus, and striatum (Bubeníková-Valešová et al., 2009, Chang et al., 2004, Šlamberová et al., 2006), followed by long-term cognitive deficits, e.g., impairment in spatial learning and memory (Chang et al., 2004, Hrebíčková et al., 2014). Elevated MA levels are associated with increased motor activity and a reduced sense of fatigue. MA abusers experience euphoria, joy, well-being, and a sense of self-confidence (Iversen et al., 2013, Mravčík et al., 2007), which often involves the loss of social restraints. MA also has an anxiogenic effect, which can lead to anxiety, fear, panic, paranoia, and hallucinations.

Psychostimulants affect the behavior of addicts, increase aggression and disrupt social and maternal behavior (Šlamberová et al., 2013, Holubová et al., 2019). These facts highlight the very serious worldwide problem of women abusing MA during pregnancy (Marwick, 2000). Since MA crosses the placenta, maternal MA exposure may cause prenatal hypoxia and fetal

malnutrition, resulting in irreversible fetal changes. Previous studies showed that MA administration to pregnant rats impairs postnatal sensorimotor development of pups during the pre-weaning period (Hrubá et al., 2009, Šlamberová et al., 2006, Ševčíková et al., 2020) affects learning abilities (Macúchová et al., 2013) and evokes anxiety-like behavior in offspring (Schutová et al., 2009, Macúchová et al., 2016). While the effect of MA on mothers and offspring has been the subject of detailed research over the past few decades, the effect of MA on fathers and offspring has received far less attention.

There are few studies examining the impact of paternal drug exposure on rat pup development and behavior in adulthood. A study by Abel et al. (1989) showed that paternal cocaine administration leads to increased hyperactivity and behavioral changes in rat pups. Studies by Bielawski et al. (1997, 2002) found that paternal alcohol abuse results in offspring malformations and reduced fetal weight. Another study by Dalterio et al. (1984) showed that paternal THC (delta-9-tetrahydrocannabinol) exposure significantly impairs the development of rat pups. The above few studies present serious findings about the effect of drugs administered to male rats and the effect on their offspring. There are no published studies examining the effects of paternal MA on the postnatal development of rat offspring and persistence into adulthood. Our study attempts to fill a gap in second-generation drug addiction research. In addition, our study is exceptional because examine also the effect of MA abuse on reproductive system, sexual behavior and locomotor activity of fathers. Psychostimulant drugs may adversely affect the quality and quantity of sperm and result in infertility of drug users (Verstegen et al., 2020). Previous experiments have demonstrated the adverse effect of cocaine abuse on reproduction and spermatogenesis in males (George et al., 1996). Other substances such as nicotine, cannabis, and amphetamines also alter spermatogenesis by inducing oxidative stress and subsequent apoptosis in testicular tissue. Study by Alavi et al. (2008) showed that MA administration significantly decreases cell proliferation and increases apoptosis in rat spermatogonia and primary spermatocytes. Previous studies also indicate that MA exposure could influence the sexual behavior in male rats, since MA alters the neurotransmitter systems involved in genital reflexes, copulatory patterns, and sexual motivation. Moreover, based on previous findings, psychostimulants could negatively impact the physiological testosterone levels in rats.

HYPOTHESES AND AIMES

Based on current findings regarding MA abuse and its consequences, we hypothesize that:

1) The long-term application of MA (30 days) to adult male rats should induce changes in their reproductive system. Hypothetically, we expect increased sexual and locomotor activity after MA application. However, it is questionable whether long-term MA administration will induce stereotypic behavior and rejection of a sexual partner. Another unanswered question is whether the MA application will affect spermatogenesis and testosterone levels in males. Since this effect has been demonstrated in other psychostimulants, we expect reduced sperm production and a negative impact on reproductive functions after long-term MA exposure.
2) Paternal MA administration (30 days) could also lead to changes in rat pup functional development, social behavior, and locomotor activity, as occurs with maternal MA administration.

Based on our hypotheses, we set the following aims for the dissertation:

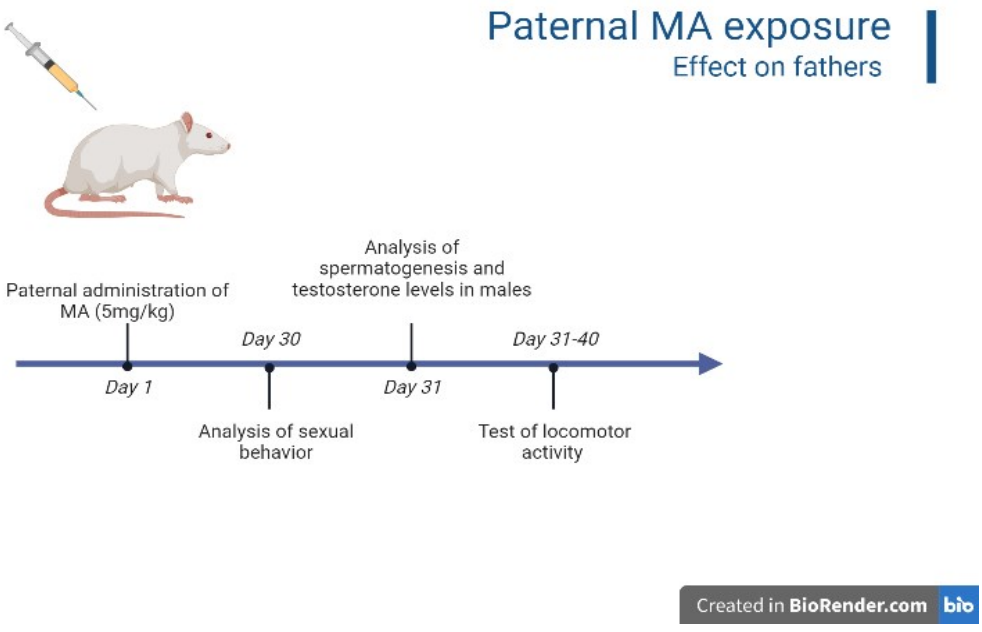
1. Investigate the impact of MA administration on **male sexual behavior**.
2. Investigate the effect of MA exposure on **male spermatogenesis** and analyze testosterone concentrations.
3. Investigate the impact of paternal MA exposure on the functional and **morphological development of rat pups**.
4. Investigate the effect of paternal MA exposure on the **social behavior of adolescent progeny** and the **locomotor activity of offspring in adulthood**.

METHODS

D-methamphetamine hydrochloride was administrated subcutaneously (s.c.) in a dose of 5 mg/ml/kg for 30 days to adult male rats (PD 90). This administration period was chosen in accordance to adverse impact of cocaine as also a psychostimulant on sexual behavior and spermatogenesis in male rats (Li et al.,1999). Moreover, this dose of MA induces similar behavioral changes that correspond to those found in humans and which is standardly use in all our experiments (Šlamberová et al., 2005). Control group was exposed to saline s.c. injection (1 mg/kg) at the same time in the same volume as MA group.

After 30 days of MA administration, male rats were mated with non-treated females. As behavior in females can differ depending on phase of the estrous cycle, the phase of the cycle was determined by vaginal lavage smears subsequently 2-3 days before mating. The smears were examined by light microscopy (Turner and Bagnara, 1976). After a week, females were separated from males and left undisturbed until the day of delivery, postnatal day (PD) 0.

BEHAVIORAL TESTS OF FATHERS



Sexual behavior testing

The experiment has conducted after 5 pm in the same laboratory under dim light, when animals are becoming more active. The occurrence and disappearance of phases of sexual mating were determined by parameters of mating behavior (Agmo et al., 1995, Zanoli at al., 2005). The observed parameters were recorded up to 2 hours of pairing and used for further analysis.

Analysis of testosterone levels

Adult male rats (n=18) after 30 days of MA administration were used to determine the testosterone levels measured by competitive ELISA method. ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the principle of competitive binding (Microsoft Word - IFU AA E-1300 V9.0 (ldn.de)). The plasma samples were collected in morning hours (8-10 am), when the testosterone levels are increased.

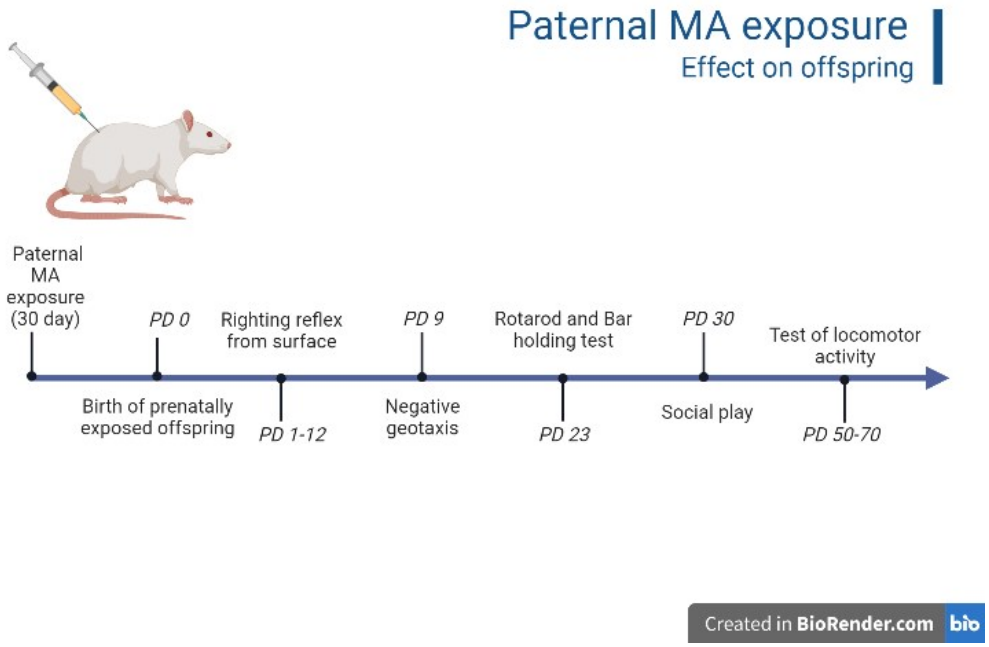
Analysis of spermatogenesis - sperm count

6 MA-treated and 6 SA-treated adult male rats were used to examine the effect of the long-term abuse of MA on spermatogenesis. The total sperm amount per milliliter (mL) was evaluated by using a bright field microscope. A Bürker hemocytometer was used for counting of spermatozoa. The physiological sperm concentration in rats is ranged from 152.5 to 230.0 x 10^7 spermatozoa/ml (Kempinas and Carvalho, 1988).

Test of locomotor activity - Laboras test

The Laboras test is a modified fully automated Open field test used for examining animal's locomotor behavior in an unknown environment. The test was performed based on a study by Schutová et al. (2013). Each animal was tested and recorded for 1 hour, lately divided into six consecutive 10-minute intervals. The same method was used to test fathers and their offspring.

BEHAVIORAL TESTS OF OFFSPRING



Behavioral experiments during development

Righting reflex on surface

The surface righting reflex was tested daily within PD 1- 12 (Altman and Sudarshan, 1975, Hrubá et al., 2009, Ševčíková et al., 2017). Each pup was turned to supine position and the time that it took for the pup to right itself with all four paws contacting the surface of the testing table was recorded.

Negative geotaxis

The Negative geotaxis was performed on PD 9 (Altman and Sudarshan, 1975, Hrubá et al., 2009, Ševčíková et al., 2017). Each animal was given three trials and the best latency of turning their face upward (180º rotation) was recorded.

Bar holding test

The Bar holding test on PD 23 was performed to examine vestibular function and sensorimotor coordination to achieve the maintenance of the balance on the narrow bar (Murphy et al., 1995, Hrubá et al., 2009, Ševčíková et al., 2017). Animals were subjected to three consecutive trials.

Rotarod test

Rotarod test was performed on PD 23 to examine the sensorimotor coordination and dynamic postural reaction necessary for active moving to maintain balance on a rotating cylinder (Šlamberová et al., 2006, Hrubá et al., 2009, Ševčíková et al., 2017). The duration of balance on rotarod was determined for 120 s. Trials were repeated until the rats successfully accomplished the task, or until there were 6 failures.

Social play

Behavioral procedures of social play used in my study were provided according to previous studies (Ševčíková et al., 2020, Achterberg et al., 2014, Trezza et al., 2009, Vanderschuren et al., 2008). On PD 30, an acute dose of MA (1 mg/kg) or SA (1 ml/kg) was administered to pairs of rats approximately 45 min before testing. Rats were paired with a similar body weight and with rats that were not cage mates to minimalize the influence of dominant behavior. The 15-minutes video recordings were analyzed to assess the specific patterns of social play behavior by using the ODLog (Macropod Software) program.

Statistical analysis

Data with normal (Gaussian) distribution were analyzed using the Analysis of variance (ANOVA). Two-way ANOVA with Repeated Measure was used to analyze differences in the tests of development in rat pups. Three-way ANOVA with Repeated Measure was used to analyze differences in the Laboras test and Social play. When appropriate, comparisons between treatment groups were conducted by the Bonferroni post-hoc test. Data from sexual behavior, levels of testosterone and spermatogenesis were analyzed separately by *t-test.* Differences were considered significant if $p < 0.05$ in all statistical analysis.

RESULTS

BEHAVIORAL TESTS OF FATHERS

Sexual behavior testing

Our results demonstrate that chronic MA application (30 days) did not induced any statistically significant differences between MA males relative to parameters of sexual behavior compared to saline controls.

Analysis of testosterone level

Our data show that chronic MA administration did not significantly decrease the testosterone levels compared to control group.

Analysis of spermatogenesis

The analysis of spermatogenesis in adult male rats exposed to MA did not show any significant differences in sperm production compared to control group. However, our data indicate that both treated groups had lower sperm levels compared to physiological level of non-treated healthy male rats.

Laboras test in fathers

Experimental data of Laboras test show that 30-day MA administration has impact on locomotory activity of male rats. Our results showed that acute MA application increased locomotion more in animals treated 30-days with SA than in animals with chronic MA treatment. This finding indicates that chronic MA administration decreased the baseline level of locomotor activity in male rats. Additionally, there was a significant effect of acute MA application in all measures showing increase of locomotor activity.

BEHAVIORAL TESTS OF OFFSPRING

Behavioral experiments during development

Our data did not show any significant effects of paternal MA exposure on sensorimotor development in the Righting reflex on a surface, Negative geotaxis, Bar holding test, or Rotarod test. In addition, our data indicate sex differences in tests performed during development. Based on our data, males are faster in righting, while females are better at maintaining balance in Bar-holding test.

Social play

Our results demonstrate that paternal MA administration significantly decreased social play and social exploration in juvenile rat offspring (PD 30) by decreasing the frequency of pouncing in males and by decreasing the time spent mutual sniffing in both genders. Moreover, acute dose of MA significantly decreased all social behavior patterns and social exploration of offspring. In our experiment juvenile male rats show decreased activity during social play in frequency of pouncing compared to females.

Laboras test in offspring

Our results do not demonstrate any significant differences in the parameters of Laboras test regarding the effect of paternal MA exposure on locomotor activity of offspring. However, acute MA exposure significantly increased all parameters of locomotor activity.

DISCUSSION

BEHAVIORAL TESTS OF FATHERS

Sexual behavior testing

Our results demonstrate that chronic MA exposure did not influence sexual behavior of adult male rats. An animal study by Bolin and Akins (2009) demonstrates that chronic pre-exposure to MA impairs sexual motivation but not sexual performance. However, MA is also associated with decreased sexual function, as chronic MA abuse results in an inability to reach full erection, delayed ejaculation, and orgasm (Frohmader et al., 2010). Studies by Frohmader et al. (2010) found that MA administration in male rats impairs sexual motivation and performance in a dose-dependent matter by showing that low doses of MA did not disrupt sexual functions. Moreover, they (Frohmader et al., 2011) found that MA pretreatment did not affect the expression of sexual behavior. Thus, our results showed no effect of MA on sexual activity and correlated with previous findings that MA exposure does not affect sexual motivation and performance in pre-treated male rats (Mihalčíková et al., 2019).

Laboras test in fathers

Our findings of increased locomotion and exploration, induced by acute MA exposure, agree with other studies that found that psychostimulants, such as MA, increase locomotor activity (Glatt et al., 2000, Hall et al., 2008, Schutová et al., 2013). Dopaminergic neurotransmission in the nucleus accumbens and the caudate nucleus mediates MA-induced hyperlocomotion and stereotypy, respectively (Kelly and Iversen, 1976, Lucot et al., 1980, Wallace et al., 1999). Previous studies demonstrated the negative impact of long-term MA administration on locomotor activity in rats (Nazari et al., 2020). Thus, our results showing decreased baseline level of locomotor activity after chronic MA exposure agree with previous findings.

Analysis of testosterone levels

Our results demonstrated that chronic MA exposure did not influence testosterone concentrations compared to the saline controls, which is in contrast with a study by Lin et al. (2014), showing that chronic MA administration significantly decreased total testosterone secretion compared to the control treatment. Also, other researchers reported that illicit use of

MA decreased plasma testosterone concentrations (Nudmamud-Thanoi and Thanoi 2011). Interestingly, our raw testosterone data show differences in measured values of testosterone concentration among the same treatment group. We suggest that dominance-subordinate relationships between male rats could influence these differences. Animal studies on rats demonstrate that testosterone plays a primary role in intermale social aggression and dominant behavior and that castration, thus the loss of testosterone, is typically accompanied by a loss of social dominance. Therefore, we suggest that variety of measured testosterone levels could have been influenced by animal hierarchy (Albert et al., 1986).

Analysis of spermatogenesis - sperm count

Our results demonstrated that chronic MA exposure did not significantly affect sperm production relative to saline controls. Several studies have reported that MA administration induces apoptosis of spermatogenic cells, lower sperm quality, as well as damage to Leydig cells and their functions (Nudmamud-Thanoi and Thanoi, 2011, Lin et al., 2014, Kaewman et al., 2018). Additionally, MA abuse induce detrimental effects on male reproductive functions, including impairment in sperm parameters and sperm chromatin/DNA integrity (Sabour et al., 2017). Thus, our results are inconsistent with the abovementioned studies. Surprisingly, our MA- and SA-treated data show lower spermatozoa concentrations than physiological levels. If we found reduced sperm concentration only in the MA group, we could argue that this was a drug effect; however, since the same reduction was also seen in the control group exposed to SA injections, then the explanation may be due to the effect of stress induced by repeated injections (Šlamberová et al.,2018).

BEHAVIORAL TESTS OF OFFSPRING

Behavioral experiments during development

Based on our results, it seems that paternal MA exposure does not influence sensorimotor development of rat pups as does maternal MA exposure. The explanation may be that while maternal exposure can directly affect the development of pups (since MA crosses the placenta and enters breast milk during lactation) (Dattel, 1990, Rambousek et al., 2014), paternal exposure would need to change the genetics of the pup, which does not appear to occur. In addition, our data demonstrate that paternal MA administration does not result in such a serious impairment in development of offspring compared to paternal exposure of other

psychostimulants (cocaine and cannabinoids) (Abel et al., 1989, George et al., 1996, Dalterio et al., 1984). Moreover, our data indicate sex differences in tests performed during development. We suggest that the sex differences relative to test performance can be caused by the age at which the test was performed and because males and females differ in developing sensorimotor skills.

Social play

Our results indicate that paternal MA administration alters specific patterns of social play in offspring. Specifically, paternal MA exposure significantly decreased frequency of pouncing in males and duration of mutual sniffing in both genders. In rats, an episode of social play behavior usually starts when a rat approaches a mate and attempts to touch the mate's neck with its own snout (Panksepp and Beatty, 1980, Pellis and Pellis, 1987, Vanderschuren et al., 1997). This behavior, called pouncing or nape contact, is considered the most critical parameter of play initiation, perhaps reflecting the motivational aspect of social play. Mutual sniffing indicates social exploration of a partner and represents a non-playful pattern of social behavior. Since our results showed a decreased frequency of pouncing, we suggest that paternal MA impaired mainly the initiation of social behavior, which also resulted in the suppression of non-playful patterns of social play (mutual sniffing). Based on previous studies, paternal cocaine and nicotine exposure could lead to alterations in the social behavior of offspring, as seen in paternal MA exposure in our experiment. We suggest that psychostimulants might similarly affect neurotransmitter system regulation, which may play a role in altered social behavior. Since psychostimulants, such as MA, directly increase dopamine levels in the nucleus accumbens, the behavioral effects of these drugs are mainly attributed to their impact on dopamine neurotransmission. However, we suggest that chronic paternal MA administration can alter mechanisms of neurotransmission in the CNS, which could lead to impairment of specific social behavior patterns in offspring.

Regarding the effect of acute MA exposure on social play behavior, our results correlate with previous findings of our laboratory that acute MA administration significantly decreased all patterns of social play behavior in juvenile rats compared to controls (Ševčíková et al., 2020). Other experimental studies have also reported significantly decreased social contact after acute MA administration both in adolescence and adulthood (Davidson et al., 2001, Manduca et al., 2014, Šlamberová et al., 2015). It can be hypothesized that the effects of psychostimulants, such as MA, result in enhanced or exaggerated behavioral inhibition (Achterberg et al., 2014).

Because of increased inhibition of behavior, psychostimulant drugs may increase attention toward non-social stimuli in the environment and suppress patterns of social play.

Additionally, in our experiment, juvenile male rats show decreased activity during social play, relative to the frequency of pouncing, compared to females. We suggest that sex differences seen in social play behavior are not affected only by MA exposure but also by complex interactions between neurotransmission and gonadal function. Evidence shows that the dopaminergic system plays a role in the reward aspect of social play behavior. The role of cholinergic, noradrenergic, and opioid systems is also essential to attentional processes, which enhance the expression of social play behavior; androgens are also vital to the sexual differentiation of social play behavior (Vanderschuren et al., 1997).

Laboras test in offspring

The Laboras test results demonstrate that paternal MA exposure does not affect the locomotor activity and exploratory behavior of offspring in adulthood. Surprisingly, previous studies reporting paternal effect of other psychoactive drug (cocaine, nicotine or THC) are inconsistent in their outcomes. The inconsistent outcomes of previous studies could be the result of different experimental conditions as well as related to varying drug doses, methods of administration, and duration of exposure period.

The increased overall activity in the Laboras test, induced by an acute MA application of 1 mg/kg, was mainly associated with increased levels of dopamine, especially in the nucleus accumbens (Bubeníková-Valešová et al., 2009). Apart from the effects of acute MA administration, our data showed that saline injections significantly decreased locomotor activity relative to SHAM-injected rats. The explanation for this finding may be that the injection itself, regardless of the injected substance, induces behavioral changes in animals in an unknown environment (Šlamberová et al., 2018), which may be associated with stress reaction (Gomez and Garcia-Garcia, 2017). Sex differences were also observed during the Laboras experiment after an acute dose of MA was administrated. In rodents, acute or chronic treatment with psychostimulants results in higher locomotor activity in females than males (Becker, 1999, Bisagno et al., 2003, Páleníček et al., 2005, Milesi-Hallé et al., 2007). Thus, our findings that acute MA exposure increase locomotor activity more in females compared with males agree with previous studies.

CONCLUSION

In conclusion, based on our results, we can answer our hypotheses as follows.

Firstly, our results demonstrate that MA administration in adult male rats does not affect sexual performance and sexual motivation. Thus, our hypothesis that MA exposure may influence the sexual behavior of fathers was not confirmed. Our assumptions were shown to be wrong regarding the effect of MA exposure on spermatogenesis and testosterone levels in male rats. Chronic MA administration (30 days) did not influence sperm production or testosterone levels compared to saline controls. Despite these negative results, more detailed studies are needed to thoroughly investigate dose-dependent responses and other factors that may play a role in the possible effect of MA exposure on male reproductive system.

Secondly, the present study's data did not show any significant effects of paternal MA exposure on sensorimotor development in the offspring. Thus, hypothesis that paternal MA exposure could influence the sensorimotor development of rat pups, as does maternal MA exposure, was not confirmed. Our study demonstrates that paternal MA exposure significantly impaired social play behavior in offspring, which corresponds with our hypothesis that paternal MA exposure could impair social behavior in offspring. However, the mechanism by which paternal MA exposure alters play behavior in offspring remains unknown. More experiments are needed to clarify the mechanisms of drug addiction and its influence on future generations, which appear to involve complex modulations of neurotransmitter systems.

Finally, our results show that MA administration to male rats does not influence the locomotor activity and exploratory behavior of their adult offspring. These findings agree with previous studies showing that maternal MA exposure did not influence the locomotor activity of their adult offspring. In addition, an acute dose of MA significantly increased all parameters of locomotor activity, which confirmed one of the significant effects of MA exposure.

To conclude, our study demonstrated that the effect of paternal MA exposure on offspring was not as significant as that observed after maternal MA exposure. Nevertheless, our results indicated that paternal MA alters specific patterns of social behavior that could seriously impact the social adaptation, mental health, and social life of their offspring.

REFERENCES

ABEL, Ernest L. Paternal behavioral mutagenesis. *Neurotoxicology*, 1989, 10.3: 335-345.

ACHTERBERG, EJ Marijke, et al. Amphetamine and cocaine suppress social play behavior in rats through distinct mechanisms. *Psychopharmacology*, 2014, 231: 1503-1515.

ÅGMO, Anders, et al. Lesions of the medial prefrontal cortex and sexual behavior in the male rat. *Brain Research*, 1995, 696.1-2: 177-186.

ALAVI, Seyed Hassan; TAGHAVI, Mohammad Mohsen; MOALLEM, Seyed Adel. Evaluation of effects of methamphetamine repeated dosing on proliferation and apoptosis of rat germ cells. *Systems biology in reproductive medicine*, 2008, 54.2: 85-91.

ALBERT, D. J., et al. Testosterone removal in rats results in a decrease in social aggression and a loss of social dominance. *Physiology & behavior*, 1986, 36.3: 401-407.

ALTMAN, Joseph; SUDARSHAN, Kiran. Postnatal development of locomotion in the laboratory rat. *Animal behaviour*, 1975, 23: 896-920.

BECKER, Jill B. Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacology Biochemistry and behavior*, 1999, 64.4: 803-812.

BIELAWSKI, Dawn M., et al. Paternal alcohol exposure affects sperm cytosine methyltransferase messenger RNA levels. *Alcoholism: Clinical and Experimental Research*, 2002, 26.3: 347-351.

BIELAWSKI, Dawn M.; ABEL, Ernest L. Acute treatment of paternal alcohol exposure produces malformations in offspring. *Alcohol*, 1997, 14.4: 397-401.

BISAGNO, Veronica; FERGUSON, Deveroux; LUINE, Victoria N. Chronic D-amphetamine induces sexually dimorphic effects on locomotion, recognition memory, and brain monoamines. *Pharmacology Biochemistry and Behavior*, 2003, 74.4: 859-867.

BOLIN, B. Levi; AKINS, Chana K. Methamphetamine impairs sexual motivation but not sexual performance in male Japanese quail. *Experimental and Clinical Psychopharmacology*, 2009, 17.1: 10.

BUBENIKOVA-VALESOVA, Vera, et al. Prenatal methamphetamine exposure affects the mesolimbic dopaminergic system and behavior in adult offspring. *International Journal of Developmental Neuroscience*, 2009, 27.6: 525-530.

CHANG, Linda, et al. Smaller subcortical volumes and cognitive deficits in children with prenatal methamphetamine exposure. *Psychiatry Research: Neuroimaging*, 2004, 132.2: 95-106.

DALTERIO, S., et al. Early cannabinoid exposure influences neuroendocrine and reproductive functions in male mice: I. Prenatal exposure. *Pharmacology Biochemistry and Behavior*, 1984, 20.1: 107-113.

DATTEL, Bonnie J. Substance abuse in pregnancy. In: *Seminars in Perinatology*. 1990. p. 179-187.

DAVIDSON, Colin, et al. Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. *Brain Research Reviews*, 2001, 36.1: 1-22.

FROHMADER, Karla S., et al. Concurrent exposure to methamphetamine and sexual behavior enhances subsequent drug reward and causes compulsive sexual behavior in male rats. *Journal of Neuroscience*. 31(45): 16473-16482, 2011.

FROHMADER, Karla S., et al. Effects of methamphetamine on sexual performance and compulsive sex behavior in male rats. *Psychopharmacology*. 212(1): 93-104, 2010.

FROST, Douglas O.; CADET, Jean-Lud. Effects of methamphetamine-induced neurotoxicity on the development of neural circuitry: a hypothesis. *Brain research reviews*, 2000, 34.3: 103-118.

GEORGE, Valal K., et al. Effects of long-term cocaine exposure on spermatogenesis and fertility in peripubertal male rats. *The Journal of urology*, 1996, 155.1: 327-331.

GLATT, Stephen J., et al. Prenatal cocaine exposure alters behavioral and neurochemical sensitization to amphetamine in adult rats. *Neuropharmacology*, 2000, 39.4: 599-610.

GOMEZ, Francisca; GARCÍA-GARCÍA, Luis. Anxiogenic-like effects of fluoxetine render adult male rats vulnerable to the effects of a novel stress. *Pharmacology Biochemistry and Behavior*, 2017, 153: 32-44.

HALL, Darien A., et al. A comparison of amphetamine-and methamphetamine-induced locomotor activity in rats: evidence for qualitative differences in behavior. *Psychopharmacology*, 2008, 195: 469-478.

HOLUBOVÁ, Anna, et al. Different oxytocin responses to acute methamphetamine treatment in juvenile female rats perinatally exposed to stress and/or methamphetamine administration. *Frontiers in Physiology*, 2019, 10: 305.

HREBÍČKOVÁ, I., et al. Exposure to methamphetamine during first and second half of prenatal period and its consequences on cognition after long-term application in adulthood. *Physiological Research*, 2014, 63.

HRUBÁ, Lenka, et al. Effect of methamphetamine exposure and cross-fostering on sensorimotor development of male and female rat pups. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, 2009, 51.1: 73-83.

IVERSEN, Leslie (ed.). *Drugs, neurotransmitters, and behavior*. Springer Science & Business Media, 2013.

KAEWMAN, Paweena; NUDMAMUD-THANOI, Sutisa; THANOI, Samur. GABAergic alterations in the rat testis after methamphetamine exposure. *International journal of medical sciences*, 2018, 15.12: 1349.

KELLY, Peter H.; IVERSEN, Susan D. Selective 60HDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. *European journal of pharmacology*, 1976, 40.1: 45-56.

KEMPINAS, W. G.; LAMANO-CARVALHO, Teresa Lúcia. A method for estimating the concentration of spermatozoa in the rat cauda epididymidis. *Laboratory animals*, 1988, 22.2: 154-156.

LI, HAIKUN, et al. Cocaine induced apoptosis in rat testes. *The Journal of urology*. 162(1): 213-216, 1999.

LIN, Ji-Fan, et al. Induction of testicular damage by daily methamphetamine administration in rats. *Chin J Physiol*, 2014, 57.1: 19-30.

LUCOT, J. B., et al. The effects of dopaminergic agents on the locomotor activity of rats after high doses of methylamphetamine. *Pharmacology Biochemistry and Behavior*, 1980, 13.3: 409-413.

MACUCHOVA, Eva, et al. DOES PRENATAL AND ADULT METHAMPHETAMINE EXPOSURE AFFECT SPATIAL LEARNING OF FEMALE RATS? *Behavioural Pharmacology*, 2013, 24: e60-e61.

MACÚCHOVÁ, Eva. Does prenatal methamphetamine exposure induce cross-sensitisation to drugs in adult male and female rats? 2016.

MANDUCA, Antonia, et al. Social play behavior, ultrasonic vocalizations and their modulation by morphine and amphetamine in Wistar and Sprague-Dawley rats. *Psychopharmacology*, 2014, 231: 1661-1673.

MARWICK, Charles. NIDA seeking data on effect of fetal exposure to methamphetamine. *Jama*, 2000, 283.17: 2225-2226.

MIHALČÍKOVÁ, L.; OCHOZKOVÁ, A.; ŠLAMBEROVÁ, R. Effect of methamphetamine exposure on sexual behavior and locomotor activity of adult male rats. *Physiological Research*, 2019, 68: S339-S346.

MILESI-HALLÉ, Alessandra, et al. Sex differences in (+)-amphetamine-and (+)-methamphetamine-induced behavioral response in male and female Sprague–Dawley rats. *Pharmacology Biochemistry and Behavior*, 2007, 86.1: 140-149.

MRAVČÍK, Viktor, et al. Drugs and fatal traffic accidents in the Czech Republic. *Cent Eur J Public Health*, 2007, 15.4: 158-162.

MURPHY, M. P., et al. A simple and rapid test of sensorimotor function in the aged rat. *Neurobiology of learning and memory*, 1995, 64.2: 181-186.

NAZARI, Azadeh, et al. Age-dependent effects of repeated methamphetamine exposure on locomotor activity and attentional function in rats. *Pharmacology Biochemistry and Behavior*, 2020, 191: 172879.

NUDMAMUD-THANOI, S.; THANOI, S. Methamphetamine induces abnormal sperm morphology, low sperm concentration and apoptosis in the testis of male rats. *Andrologia*, 2011, 43.4: 278-282.

PALENICEK, T., et al. Increased sensitivity to the acute effects of MDMA ("ecstasy") in female rats. *Physiology & behavior*, 2005, 86.4: 546-553.

PANKSEPP, Jaak; BEATTY, William W. Social deprivation and play in rats. *Behavioral and neural biology*, 1980, 30.2: 197-206.

PELLIS, Sergio M.; PELLIS, Vivien C. Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat Rattus norvegicus. *Aggressive behavior*, 1987, 13.4: 227-242.

RAMBOUSEK, Lukas, et al. Sex differences in methamphetamine pharmacokinetics in adult rats and its transfer to pups through the placental membrane and breast milk. *Drug and alcohol dependence*, 2014, 139: 138-144.

SABOUR, Mojdeh, et al. Administration of high dose of methamphetamine has detrimental effects on sperm parameters and DNA integrity in mice. *International journal of reproductive biomedicine*, 2017, 15.3: 161-168.

SCHUTOVÁ, B., et al. Cognitive functions and drug sensitivity in adult male rats prenatally exposed to methamphetamine. *Physiological research*, 2009, 58.5.

SCHUTOVÁ, Barbora, et al. Gender differences in behavioral changes elicited by prenatal methamphetamine exposure and application of the same drug in adulthood. *Developmental psychobiology*, 2013, 55.3: 232-242.

ŠEVČÍKOVÁ, Mária, et al. The influence of methamphetamine on maternal behavior and development of the pups during the neonatal period. *International Journal of Developmental Neuroscience*, 2017, 59: 37-46.

ŠEVČÍKOVÁ, Mária; PETRIKOVA, Ivana; ŠLAMBEROVÁ, Romana. Methamphetamine exposure during the first, but not the second half of prenatal development, affects social play behavior. *Physiological Research*, 2020, 69.2: 319.

ŠLAMBEROVÁ, R., et al. Gender Differences in the Effect of Prenatal Methamphetamine Exposure and Challenge Dose of Other Drugs on Behavior of Adult Rats. *Physiological Research*, 2013.

ŠLAMBEROVÁ, R., et al. What is the role of subcutaneous single injections on the behavior of adult male rats exposed to drugs?. *Physiological Research*, 2018, 67: S665-S672.

ŠLAMBEROVÁ, Romana, et al. Effects of psychostimulants on social interaction in adult male rats. *Behavioural pharmacology*, 2015, 26.8-9: 776-785.

ŠLAMBEROVÁ, Romana; CHAROUSOVÁ, Petra; POMETLOVÁ, Marie. Maternal behavior is impaired by methamphetamine administered during pre-mating, gestation and lactation. *Reproductive toxicology*, 2005, 20.1: 103-110.

ŠLAMBEROVÁ, Romana; POMETLOVÁ, Marie; CHAROUSOVÁ, Petra. Postnatal development of rat pups is altered by prenatal methamphetamine exposure. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 2006, 30.1: 82-88.

SMITH, L. M., et al. Brain proton magnetic resonance spectroscopy in children exposed to methamphetamine in utero. *Neurology*, 2001, 57.2: 255-260.

TREZZA, Viviana; DAMSTEEGT, Ruth; VANDERSCHUREN, Louk JMJ. Conditioned place preference induced by social play behavior: parametrics, extinction, reinstatement and disruption by methylphenidate. *European Neuropsychopharmacology*, 2009, 19.9: 659-669.

TURNER, C. D.; BAGNARA, J. T. Biological effects of the ovarian hormones. *General endocrinology*, 1976, 6: 466-76.

VANDERSCHUREN, Louk JMJ, et al. Methylphenidate disrupts social play behavior in adolescent rats. *Neuropsychopharmacology*, 2008, 33.12: 2946-2956.

VANDERSCHUREN, Louk JMJ; NIESINK, Raymond JM; VAN PEE, Jan M. The neurobiology of social play behavior in rats. *Neuroscience & Biobehavioral Reviews*, 1997, 21.3: 309-326.

VERSTEGEN, Ruud HJ, et al. Paternal exposure to recreational drugs before conception and its effect on live-born offspring: A scoping review. *Birth Defects Research*, 2020, 112.13: 970-988.

WALLACE, Tanya L.; GUDELSKY, Gary A.; VORHEES, Charles V. Methamphetamine-induced neurotoxicity alters locomotor activity, stereotypic behavior, and stimulated dopamine release in the rat. *Journal of Neuroscience*, 1999, 19.20: 9141-9148.

ZANOLI, Paola, et al. Ferula hermonis impairs sexual behavior in hormone-primed female rats. *Physiology & behavior*, 2005, 86.1-2: 69-74.

Author´s publications

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

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Ochozková, A., Mihalčíková, L., Yamamotová, A., & Šlamberová, R. (2021). Can prenatal methamphetamine exposure be considered a good animal model for ADHD? *Physiological Research*, *70*(Suppl 3), S431. **IF 2.139**

Šlamberová, R., Nohejlová, K., Ochozková, A., & Mihalčíková, L. (2018). What is the role of subcutaneous single injections on the behavior of adult male rats exposed to drugs? *Physiological Research*, *67*, S665-S672. **IF 2.139**