Impact of repeated methamphetamine pretreatment on intravenous self-administration of the drug in males and estrogenized or non–estrogenized ovariectomized female rats

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Abstract

OBJECTIVE: The female animals were already recorded to respond differently to methamphetamine (MET) abuse than males. This gender dissimilarity may be caused by the influence of estral cycles and different susceptibility to behavioural sensitization.

METHODS: Influences of gender and pre-exposure to MET were studied in the rat model of MET intravenous self-administration (IVSA). The fixed ratio (FR) paradigm was employed in male rats (M) and estrogenized (F-ESTR) and non-estrogenized ovariectomized female rats (F-OVX) either pre-exposed or not-exposed to MET pretreatment.

RESULTS: In rats that were not pre-exposed to MET, F-ESTR self-administered more MET infusions than each of the other groups, but F-OVX self-administered less than each of the other groups; the same trend was apparent in the MET pre-treated groups. MET pre-exposure decreased subsequent MET IVSA in all groups except F-OVX.

CONCLUSION: Thus, pre-exposure to MET and the loss of inherent estrogen in females notably decreased the intake of MET by rats, suggesting that abuse liability was reduced. Estrogen's effects on MET self-administration here correspond with accumulating evidence of stronger behavioural responses of females to drugs of abuse.

Abbreviations :

MET	- methamphetamine
SAL	- saline
M	- males
OVX	- ovariectomy
F-OVX	- ovariectomized females
F-ESTR	- estrogenized ovariectomized females
IVSA	- IV self-administration

ORIGINAL ARTICLE

INTRODUCTION

Although the role of gender in the mechanisms of drug action remains unclear, both preclinical and clinical studies indicate that ovarian hormones, particularly estrogen, play a role in producing sex differences in drug abuse (Lynch *et al.* 2002). These differences in the behavioural reactivity to drugs in men and women (Brecht *et al.* 2004, Munro *et al.* 2006) will probably require different strategies in the prevention and treatment of addiction depending on gender. So far, most experimental studies have been conducted with male subjects; many researchers have chosen to ignore the influences of the estrous cycle, which is more difficult to study, but has an important impact on animal behaviour. The reasons for differences in female reactivity to drugs can be due to pharmacokinetic specifics (Milesi-Halle *et al.* 2007) such as distinct metabolising enzyme activities, distribution volume and other parameters. Pharmacodynamic effects of drugs can also be dependent on structural changes induced in early life by the physiological hormonal levels in the brain or on particular receptor gene expression modulated by sex hormones (Hu *et al.* 2004). Experiments in laboratory rodents showed that estrogen levels can regulate behavioural responses to drugs of abuse, especially psychomotor stimulants, including methamphetamine (MET). During estrus, the effects of abused drugs are more pronounced, and this is reflected in gender differences in general patterns of drug abuse (Sell *et al.* 2002, Becker & Hu, 2008)

Behavioural sensitization to drugs and the adaptations in striatal neurotransmission that are associated with this sensitization are thought to play an important role in certain aspects of addiction (Ohmori *et al.* 2000). Dopaminergic activity in the brain is enhanced by estrogen in positive correlation with behavioural effects (Thompson & Moss, 1994, Thompson *et al.* 2000, White *et al.* 2002). However, positron emission tomography (PET) scans performed after amphetamine administration in humans showed increased reactivity of the striatal dopamine system in men compared with women (Munro *et al.* 2006). The interactions between ovarian hormones, dopamine, and drugs of abuse are not clear yet, and more studies are necessary for further elucidation. Nevertheless, in a number of studies females were more prone to develop sensitization than males (Phillips *et al.* 1997, Becker *et al.* 2001, Sell *et al.* 2002, Hu & Becker, 2003, Kawakami *et al.* 2007, Kucerova *et al.* 2008).

Intravenous drug self-administration by laboratory animals is a model for testing dependence potential and abuse liability of drugs (Collins *et al.* 1984). Compared to males, females are reported to become addicted more rapidly (and subsequently to relapse more readily following abstinence) when drugs of abuse are offered in the drug self-administration model at lower doses (Becker & Hu, 2008). The acquisition rate was found to be faster in female compared to male rats self-administering nicotine, alcohol, heroin, cocaine or MET (Lynch *et al.* 2002), presumably indicating that the reinforcing effects of these drugs are stronger in females. Rates of acquisition are also dependent on variables such as drug dose, circadian variability in access to drug and previous drug exposure (potentially leading to behavioural sensitization) (Roth & Carroll, 2004).

Understanding the influences of gender and sex hormones on drug self-administration in animals may lead to improved strategies for treatment and prevention of drug abuse in humans. While progress is beginning to be made in this area, much remains to be done. In particular, methamphetamine is a widely-abused and highly-addictive drug that has serious health consequences, yet has received less research attention than other major drugs of abuse. Therefore, the present study was designed to evaluate the effects of gender, estrogen, and potential behavioural sensitization expected to be associated with repeated MET pretreatment on MET intake in the intravenous self-administration (IVSA) paradigm in male rats (M) and ovariectomized female rats with (F-ESTR) and without estrogen substitution (F-OVX).

MATERIAL AND METHODS

Animals

The present study with Wistar rats (purchased in the Laboratory Animal Breeding and Experimental Facility, Masaryk University Brno, Czech Republic) consisted of 6 experiments (2 with males – M: n=36, 2 with ovariectomized females – F-OVX: n=36, 2 with ovariectomized females substituted regularly with estrogen – F-ESTR: n=36). Adult male rats weighing 350–400 g and female rats weighing 250–300 g at the beginning of experiment (in order to assure the catheter position stability in animals not growing much to the length anymore) were housed in sections of five in standardized rat plastic cages during the first two weeks of the experiment. After the catheter implantation surgery was performed, rats were housed individually in plastic cages standardized for separate stabling. During the whole experiment the environmental conditions were constant: relative humidity 50%, temperature 23 °C ± 1 °C, inverted 12-hour light-dark cycle (5 a.m. to 5 p.m. darkness). Food and water were available *ad libitum*. All experiments were conducted in accordance with all relevant laws and regulations of animal care and welfare. The animal study protocol was approved by the Animal Care Committee of the Masaryk University Faculty of Medicine, Brno, Czech Republic, and carried out under the European Community guidelines for the use of experimental animals.

Surgery

Under general anaesthesia (xylazine 8 mg/kg + ketamine 50 mg/kg intraperitoneally in combination with

isoflurane inhalation for induction to anaesthesia) a permanent intracardiac silastic catheter (our own production) was implanted through the external jugular vein into the right atrium. The outer part of the catheter exited the skin in the midscapular area. A small nylon bolt was fixed on the skull with dental acrylic to stainless-steel screws embedded in the skull; this served as a tether to prevent the catheter from being pulled out while the rat was in the self-administration chamber. During the surgery all the female animals were gonadectomized. The ovaries were removed and the uterus below was ligated. Access to the ventral cavity was permitted by one central incision. The catheters were flushed daily before all the sessions with 0.2 ml of heparinized cephalosporine (VULMIZOLIN 1.0 inj sic, Biotika a.s., Slovak Republic) solution (0.05 mg/kg in saline with 2.5 I.U./kg) and 0.05 ml of heparin (HEPARIN LECIVA inj. sol. 1x10ml/50 I.U.) solution (5 I.U.) to prevent infection and occlusion of the catheter. During this procedure the blood was aspired daily to assess the patency of the catheter, and changes in general behaviour, weight and other circumstances were recorded. When a catheter was found blocked the animal was excluded immediately from the analysis.

Drugs and treatments

Methamphetamine (MET) from Sigma Chemical, Co., St Louis, MO, USA was used for both intraperitoneal drug pretreatment and IVSA. The administration of MET prior to IVSA was according to the following dosing regimen, which was successfully used in our previous studies (Landa *et al.* 2006, Landa *et al.* 2008) to induce behavioural sensitization: 0.5 mg/kg/day, intraperitoneally, for 14 days, administered in home cages. The identical volume (1 ml/kg/day) and route of administration of saline solution (SAL) were used for all control treatments. The MET dose available for IVSA was 0.08 mg/kg per single infusion with the maximum number of infusions during one session set to 50 (Vinklerova *et al.* 2002).

Estrogen (Estradiol benzoate salt suspension in AGOFOLLIN DEPOT®, Biotika a.s., Slovak Republic dissolved in saline) was administered to ovariectomized females (F-ESTR) once a week intramuscularly as a depot (Shansky *et al.* 2004). The dose of 0.28 mg/kg used is expected to maintain the hormone plasma levels in the physiological range of rat estrous cycle (Mendoza-Rodriguez *et al.* 2003). The other group of ovariectomized rats (F-OVX) received the same volume of saline solution instead.

Each animal group (M, F-ESTR, and F-OVX) was randomly divided into four subgroups ($n_{1,2,3,4}$) for the following treatments: a) n_1=6: 14 days of daily pretreatment with saline (SAL), 1.0 ml/kg, intraperitoneally + the 15th day surgery procedure + 14 days of recovery and drug washout + 21 days of IVSA of SAL; b) n_2=12: 14 days of daily pretreatment with SAL, 10.0 ml/kg, intraperitoneally + the 15th day surgery procedure + 14 days of recovery and drug washout + 21 days of IVSA of methamphetamine (MET); c) n_3=6: 14 days of daily pretreatment with MET, 0.5 mg/kg, intraperitoneally + the 15th day surgery procedure + 14 days of recovery and drug washout + 21 days of IVSA of SAL, 1.0 ml/kg, intraperitoneally; d) n_4=12: 14 days of daily pretreatment with MET, 0.5 mg/kg, intraperitoneally + the 15th day surgery procedure + 14 days of recovery and drug washout + 21 days of IVSA of MET, 0.5 mg/kg, intraperitoneally. The MET or SAL pretreatment was given in the home-cage daily at the same time within the dark period of the light cycle.

Self-administration apparatus and procedure

Standard experimental chambers (with all accessories provided by Coulbourn Instruments, USA) with two nose-poke holes allocated on one side of the cage were programmed by software L2T2 (Coulbourn Instruments, USA). IVSA sessions were initially conducted under the fixed ratio (FR) schedule of reinforcement starting at FR1 (each correct response reinforced). Fixed-ratio requirements were raised (e.g. FR2 – two correct responses required, FR3 – three correct responses required, etc.) when the animal fulfilled the following conditions for three consecutive sessions: a) at least 70% preference for the active nose-poke; b) minimum intake of 10 infusions per session; c) stable intake of the drug (maximum 10% deviation). Active nose-pokes led to the activation of the infusion pump and administration of a single infusion paired with a 2-s light cue, followed by a 30-sec time-out. Nose-pokes in the other (non-active) hole were recorded but had no programmed consequences. The cage was illuminated by a house light, which was off during the time-out. There were 21 daily sessions in 21 consecutive days, each lasting 120 minutes and taking place regularly between 7 a.m. and 4 p.m. during the dark period of the reversed light cycle. After the session the animals were returned to the home-cage.

Statistical Data analysis

For statistical analysis of differences in either saline or MET IVSA the Mann-Whitney U test was applied (comparing nose-poke responses on the active lever to those on the inactive lever), and for evaluation of the IVSA acquisition rates a Survival Data Analysis (Peto-Peto-Wilcoxon test) was used. Level of statistical significance was determined to $p<0.05$.

RESULTS

Table 1 demonstrates the reinforcing properties of the dosing IVSA MET schedule as all groups of rats (M, F-ESTR, F-OVX) regardless of repeated pretreatment (SAL or MET) exhibited preference for active (reinforced) nose-poke over the inactive (non-reinforced) nose-poke when nose-poking was reinforced by MET (0.08 mg) infusions. In each of these groups the number

Tab. 1. The table shows the mean number of nose-pokes in the 21 IVSA sessions (non-active: not associated with IVSA; active: associated with IVSA) exhibited during the whole experiment by rat males (M) and ovariectomized females with presence (F-ESTR) or absence (F-OVX) of estrogen substitution (depot suspension of estradiol benzoate, 0.28 mg/kg/week) after 14 days of withdrawal from 14 day intraperitoneal pretreatment with either saline (SAL) or methamphetamine (MET – 0.5 mg/kg/day). Statistical evaluation was processed by the Mann-Whitney U test.

Group	Pretreatment	IVSA	Mean No. Of nose-pokes per session		Mann Whitney U-test result
			active	non-active	
Males (M)	saline	saline	**9.44**±3.01	**6.95**±1.94	**NS**
	saline	MET	**48.34**±10.76	**8.39**±2.47	**p=0.0001**
	MET	saline	**8.38**±1.37	**3.92**±0.76	**NS**
	MET	MET	**40.67**±9.80	**7.66**±1.81	**p=0.0001**
Female estrogenized castrates (F ESTR)	saline	saline	**9.73**±3.91	**7.22**±1.69	**NS**
	saline	MET	**51.32**±8.88	**13.29**±1.96	**p=0.0001**
	MET	saline	**9.16**±2.70	**5.49**±1.61	**NS**
	MET	MET	**44.86**±8.51	**9.68**±3.18	**p=0.0001**
Female castrates (F OVX)	saline	saline	**12.92**±4.04	**3.40**±0.83	**NS**
	saline	MET	**20.71**±2.47	**8.34**±1.47	**p=0.0001**
	MET	saline	**19.41**±5.86	**4.60**±1.83	**NS**
	MET	MET	**25.16**±9.84	**11.56**±3.11	**p=0.0147**

of active nose-pokes reinforced by MET was significantly higher than number of inactive nose-pokes. On the other hand, the number of nose-pokes into active vs. inactive hole was not significantly different in groups that were allowed to self-administer saline, regardless of repeated pretreatment (SAL or MET).

Figure 1 shows the percentage of rats from each group (M, F-ESTR, F-OVX) acquiring MET self-administration after 14 days of withdrawal after 14 day pre-treatment with saline (A) or methamphetamine (B) that met the criteria for increasing FR from initial FR1 to FR2. Differences between acquisition rates of MET IVSA of individual rat groups are shown in both parts of the figure (A and B) were not significant according to a Survival Data Analysis (Peto-Peto-Wilcoxon test). However, there is an apparent trend in both conditions (A-absence or B-presence of MET pre-exposure) showing that non-estrogenized female castrates (F-OVX) exhibited the slowest rate in reaching higher FR conditions of all three groups, and the lowest incidence of meeting this criterion.

This trend is also apparent in Figures 2A and B: at the end of experiment (21st day of consecutive sessions) the highest cumulative percentage of animals staying on FR1 conditions were non-estrogenized female castrates (F-OVX). In contrast, the highest FR7 requirement for MET IVSA was performed only by the group of male rats (M) after MET pre-exposure (Figure 2B). However, there was no significant difference in acquisition rate of MET self-administration between SAL and MET pretreated animals, although some of the latter animals were able to reach one-step higher FR as a more demanding IVSA condition (more nose-pokes needed to obtain one MET infusion) compared to saline pretreated rats (Figure 2).

Figure 3 shows acquisition of MET self-administration over 21 consecutive sessions in all three groups of rats (M, F-ESTR, F-OVX) with (Figure 3B) or without (Figure 3A) repeated pretreatment with MET. The F-OVX group self-administered the lowest while F-ESTR group the highest number of MET infusions over the course of the experiment under both pretreatment conditions (MET or SAL). In the groups that received repeated SAL pretreatment (Figure 3A), the number of MET infusions received was significantly higher in F-ESTR group compared to M (p=0.005) and F-OVX (p=0.0001) groups, and F-OVX animals were consuming significantly (p=0.0001) lower number of MET infusions than both M and F-ESTR animals. The same trend was apparent in the MET pretreated groups (Figure 3B). The number of infusions self-administered by F-ESTR group was significantly higher than in the M (p=0.0001) and F-OVX (p=0.0001) groups while F-OVX animals self-administered significantly (p=0.0001) lower number of MET infusions (Figure 3B).

Figure 4B shows that M and F-ESTR groups repeatedly exposed to MET self-administered a significantly lower number of MET infusions than those to saline (M: p<0.0005 and F-ESTR: p<0.001). The MET pretreatment had no effect on number of infusions self-administered in the F-OVX group (p=0.0849). Figure 4A shows that there were no significant differences in the SAL IVSA in any of experimental groups after both, pretreatment with SAL and MET. The only exception was the group F-OVX which self-administered higher number of SAL infusions after repeated

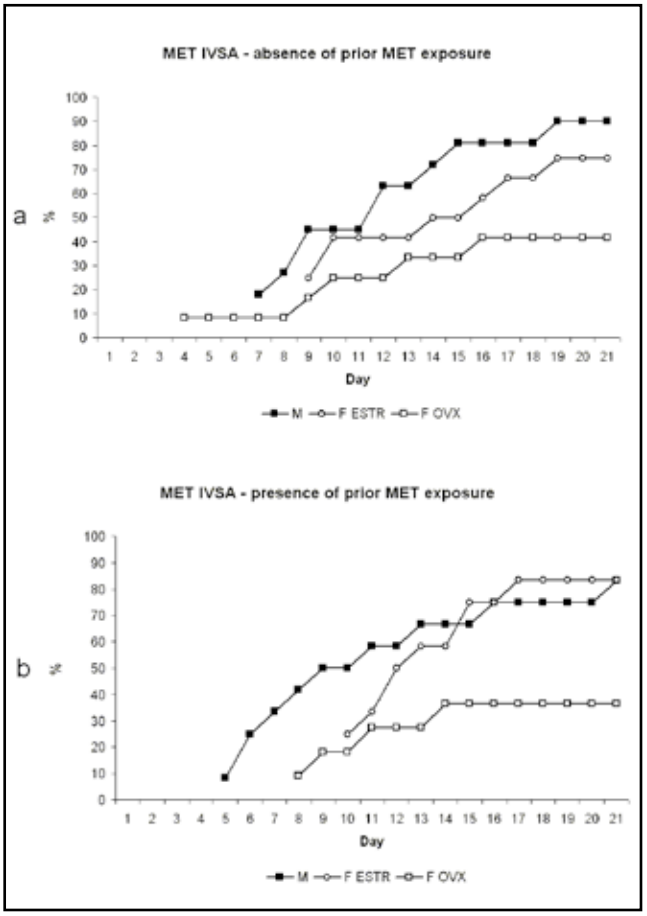

Fig. 1. The cumulative percentage of males (M) and ovariectomized females either with (F-ESTR) or without (F-OVX) estrogen substitution (depot suspension of estradiol benzoate, 0.28 mg/kg/week) after 14 days of withdrawal from 14-day intraperitoneal pretreatment with either (a) saline (SAL) or (b) methamphetamine (MET – 0.5 mg/kg/day) that met the criteria for MET IVSA for switching from FR1 to FR2 conditions in 21 consecutive sessions of the experiment. Evaluation: Survival Data Analysis (Peto-Peto-Wilcoxon test, non-significant).

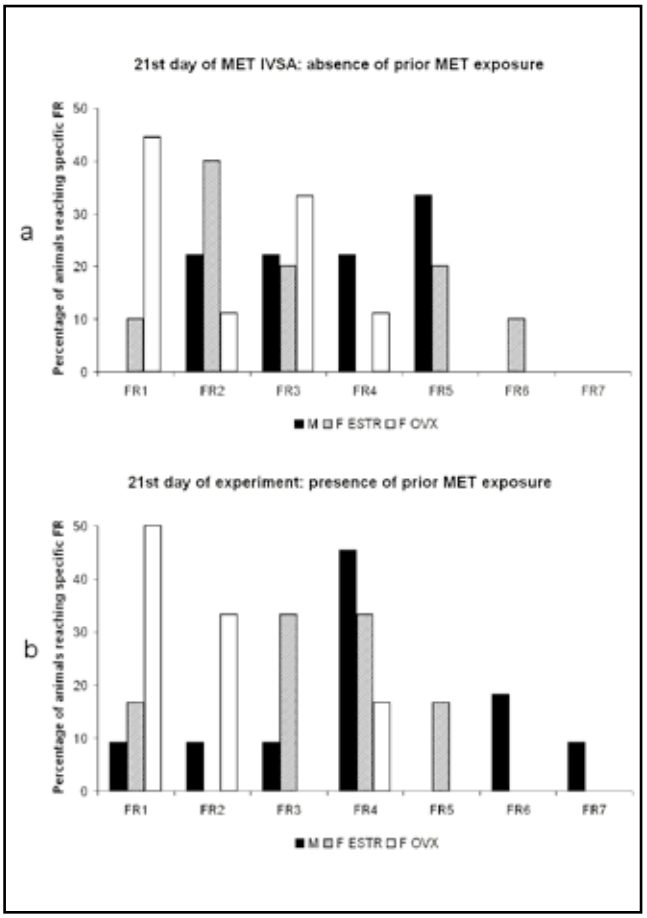

Fig. 2. The percentage of rat males (M) and ovariectomized females either with (F-ESTR) or without (F-OVX) estrogen substitution (depot suspension of estradiol benzoate, 0.28 mg/kg/week) meeting the criteria for MET IVSA under gradually increasing FR requirements after 14 days of withdrawal from 14-day intraperitoneal pretreatment with either (a) saline (SAL) or (b) methamphetamine (MET – 0.5 mg/kg/day).

pretreatment with MET. This difference was significant when compared with matching M and F-ESTR groups (M: $p<0.001$ and F-ESTR: $p<0.001$). In all groups the mean intake was notably lower than 10 infusion/session criterion used as an indicator of reinforcing effects.

DISCUSSION

This study demonstrates that Wistar rats repeatedly exposed to MET (14 daily doses of 0.5 mg/kg) self-administered a lower number of MET infusions under a fixed ratio schedule (FR) of MET infusions (0.8 mg/infusion) compared to animals pretreated with saline. The same trend was observed to some extent in all groups of rats (M, F-ESTR, and F-OVX) but differentially depending on the gender. Both estrogenized ovariectomized female groups (F-ESTR) regardless of prior repeated MET or SAL pretreatment self-administered higher numbers of MET infusions than corresponding male groups. The while non-estrogenized ovariectomized (F-OVX) female groups were self-administering the lowest number of MET infusions regardless of prior MET exposure, and also their acquisition rates were the lowest. Though there was no statistically significant difference, an apparent trend of facilitation of MET IVSA acquisition was present in rats repeatedly pre-exposed to MET. This increased drug-seeking behaviour in this model could be considered a sign of behavioural sensitization (Lorrain *et al.* 2000) , which however can be influenced by the IVSA experimental paradigm itself. A similar trend was described by Lorrain *et al.* (2000) in the rats self-administering amphetamine under a progressive ratio schedule but not under a fixed ratio schedule. The acquisition of IVSA behavior might also be influenced by a previous association of the drug effect and the experimental

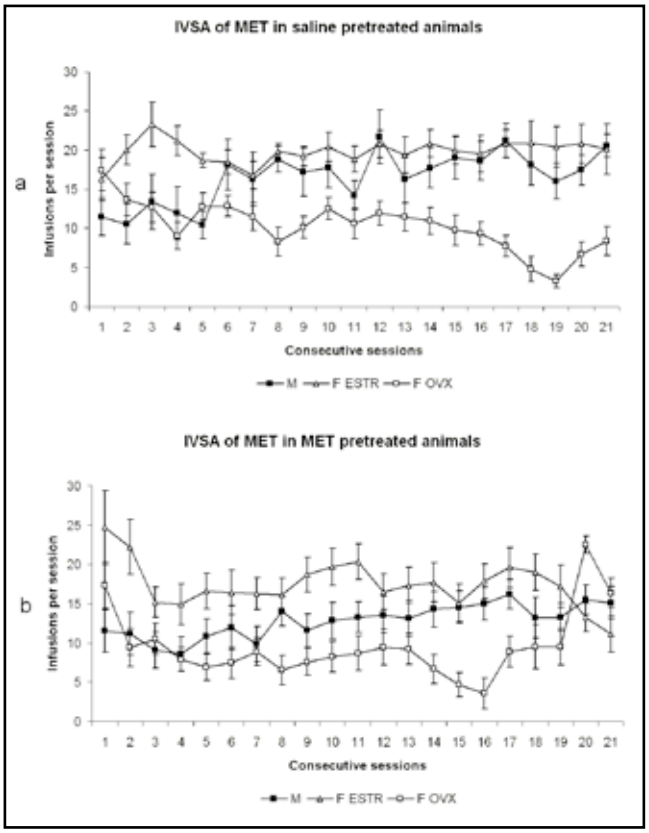

Fig. 3. The acquisition of methamphetamine (MET) infusions (0.08 mg/infusion) in all 21 IVSA daily sessions in the male rats (M - filled squares, A: n1=11, B: n2=12), ovariectomized female rats with estrogen substitution (F-ESTR - open circles, a: n1=11, b: n2=12), and ovariectomized female rats with no hormonal substitution (F-OVX - open squares, a: n1=11, b: n2=11) after 14-day withdrawal from 14 days of intraperitoneal pretreatment with either (**a**) saline (SAL) or (**b**) methamphetamine (MET – 0.5 mg/kg/day). Data are shown as daily means ± SEM.

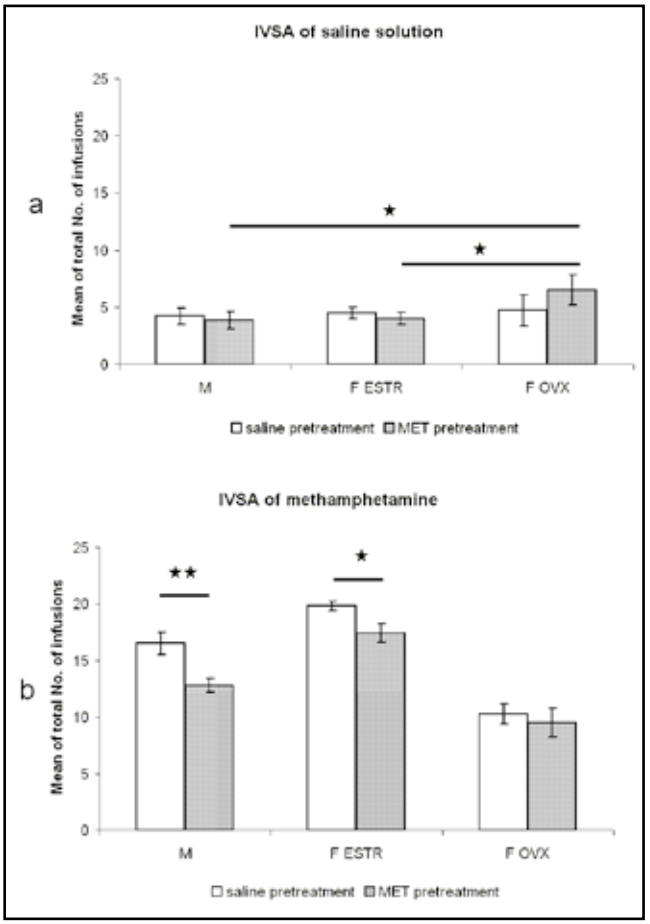

Fig. 4. The effect of methamphetamine (MET) pretreatment (0.5 mg/kg/day, for 14 days, intraperitoneally) on either saline (SAL) (**a**) or MET (**b**) self-administration after 14-day withdrawal from the pretreatment. The graphs show the mean number of IVSA infusions received during the whole experiment in male rats (M) and ovariectomized females rats either with (F-ESTR) or without (F-OVX) estrogen substitution (depot suspension of estradiol benzoate, 0.28 mg/kg/week). Groups of rats after control SAL pretreatment = open bars, groups of rats after MET pretreatment = filled bars. Data shown as means ± SEM. Statistical evaluation was done by using the Mann-Whitney U test (* $p<0.001$, ** $p<0.0005$).

cage environment (Reid *et al.* 1998). In this study, the animals were pre-exposed to MET in their home-cages. In a parallel experiment a different group of male rats were placed for 30 minutes after the intraperitoneal administration of MET pre-treatment into the operant cage used later for IVSA sessions. No significant differences in acquisition rates of MET IVSA were found when compared with the experimental design used in the present study (unpublished data).

The susceptibility of the female organism to effects of drugs of abuse, including the induction of behavioural sensitization, has mostly been reported by both pharmacological experimental studies and clinical trials as being higher and enhanced further by increased estrogen levels: (Lynch *et al.* 2002, Becker & Hu, 2008). The pharmacokinetic and metabolic profiles of a drug have also been suggested as playing significant roles in the differential pharmacological response to MET in male and female rats. Slower MET clearance and lower metabolite (amphetamine) formation were reported in the Sprague-Dawley female rats (Milesi-Halle *et al.* 2005, Milesi-Halle *et al.* 2007). The development of behavioural sensitization has also been reported to vary between rat strains, possibly due to different brain penetration of MET. In Wistar rats, the brain penetration was found to be increased in repeatedly and behavioural sensitization to the effects of MET were observed in MET-treated animals, but these effects were not found to occur in Long-Evans strain (Fujimoto *et al.* 2007). In the present experiment using Wistar rats with the IVSA method, which is the most widely used model for assessing the relative abuse liability of drugs of abuse, we confirmed that estrogen levels can influence intake of MET. The repeated pre-exposure to MET, which was proven to induce behavioural sensitization to stimulatory effects on locomotion in the same rat

strain (Landa *et al.* 2008), lowered the number of MET infusions self-administered during consecutive 21 daily sessions in all groups (M, F-OVX and F-ESTR). This effect is not likely to have been due to habituation as the control SAL-pretreated rats were allowed to self-administer MET at the same IVSA paradigm. It is also unlikely that the lower rates of IVSA seen after MET pre-exposure were actually due to sensitization (comparable to increasing the dose per infusion), since there was evidence that MET exposed rats were less likely to acquire the self-administration response. Under the fixed-ratio procedure used here, the decreased IVSA after MET pre-exposure as well as after ovariectomy were likely due to a reduced motivation to obtain MET or a reduced reinforcing effect of MET.

Reports in the existing literature on the relationship between stimulatory effects of drugs on rodent locomotion and their IVSA are not consistent. Nevertheless, the neurobiological basis of the brain systems underlying both locomotor activation (Schindler *et al.* 2002) and reward are believed to be sexually dimorphic (for review see: (Dluzen & Liu, 2008, Becker *et al.* 2001). The pharmacological mechanism of action of amphetamine and its derivatives (such as MET) involves indirect adrenergic action inducing a massive release of biogenic amines, particularly dopamine and noradrenaline, from the storage sites in nerve terminals to the synapses, and blockade of their reuptake (Kish, 2008). There are confirmed sex differences in changes of dopamine extracellular levels and turnover induced by methamphetamine in rodents (for review see: (Becker & Hu, 2008), as well as in dopamine release in humans (Munro *et al.* 2006). However, in rats, structural differences caused by the sex hormones were also found in the early brain ontogenesis, which was not dependent on the actual hormonal level (Hu *et al.* 2004). According to binding studies, there are sex differences reported in densities of dopamine receptor subtypes in the rat striatum and the nucleus accumbens fluctuating dependently on estrous cycle (Becker & Hu, 2008). The number of dopamine D1 (and to some extent also of D2) binding sites is higher in male rats compared to females and estradiol administration is shown to downregulate D2 receptors in dorsolateral striatum while enhancing basal dopamine extracellular concentrations ("dopaminergic tone") (Xiao & Becker, 1994). Thus, estradiol can elicit changes in dopamine release and dopamine receptor activity leading to greater behavioural response to psychostimulant drugs in intact females in estrus or estrogenized ovariectomized females. This correlates well with a report that under IVSA with FR conditions female rats obtained significantly more MET infusions (0.02 mg/infusion) compared to males (Roth & Carroll, 2004), as well as with the results of the present study in which the estrogenized ovariectomized females (F-ESTR with and without pre-exposure to MET) self-administered the highest number of MET infusions (0.08 mg/infusion). The higher number of MET infusions in M groups compared to F-OVX groups in our experiment corresponds with suggestion that due to higher basal dopamine tone in the male striatum and the nucleus accumbens (Xiao & Becker, 1994), a greater dopaminergic stimulation is required to achieve a rewarding effect (Becker & Hu, 2008).

Our results also showed a significantly higher saline intake in the F-OVX group repeatedly pre-exposed to MET compared to the rest of the SAL self-administering groups. Intravenous SAL is not usually found to have reinforcing effects, but it is known that estrogen can influence electrolyte homeostasis. In the rat model of angiotensin II-induced thirst, the chronic administration of estradiol attenuated water-seeking behaviour (Fregly & Thrasher, 1978). This was confirmed further by other rat experiments and evaluated as central interaction mechanism between this peptide hormone and estrogen on a genomic level (Kisley *et al.* 1999). Estrogens also may influence body fluid regulation by interacting with several neurotransmitters, including serotonin, dopamine and noradrenaline (Kucharczyk, 1984). In rats it was proven that water drinking can be initiated by administration of dopaminergic drugs (Zabik *et al.* 1993). This could be reason for higher IVSA saline intake in the F-OVX rat group lacking estrogen influence and moreover being pretreated with dopaminergically acting MET in the present study.

In summary, the highest spontaneous methamphetamine intake in our model of MET IV self- administration in rats was demonstrated in estrogenized ovariectomized females, with lower intake in males, and the lowest intake in non-estrogenized ovariectomized females. Repeated pre-exposure to MET (potentially inducing behavioural sensitization) produced a significant decrease in the mean number of MET infusions self-administered per sessions in males, as well as, in estrogenized ovariectomized females, but not in non-estrogenized ones. This may indicate that MET infusions self-administered during the sessions produced stronger reinforcing effects in rats previously exposed to MET than in drug naïve animals (perhaps due to behavioural sensitization) and that the lack of estrogen in ovariectomized females may provide protection from the development of such changes in MET effects. Thus, preclinical studies indicate that behavioural and neurobiological responses to psychostimulant drugs are sexually dimorphic and point to a particular role of estrogen, but all mechanisms underlying this dimorphism are not completely clear yet. In humans, it has been demonstrated that higher levels of estrogen are associated with greater subjective stimulation after amphetamine in women (White *et al.* 2002), but amphetamine-stimulated dopamine release can be greater in men (Dluzen & Liu, 2008), which could perhaps increase vulnerability of men to neurotoxic

effects of amphetamines (Munro *et al.* 2006). The findings from the pre-clinical and clinical studies should be taken into an account when creating specific prevention and treatment programs for men and women.

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