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Foster Mother Care but not Prenatal Morphine Exposure Enhances Cocaine Self-Administration in Young Adult Male And Female Rats

ABSTRACT: *The present study was designed to investigate cocaine self-administration in adult male and female rats exposed prenatally to morphine. Pregnant dams were injected two times a day with either saline, analgesic doses of morphine or no drug at all (controls) on gestation Days 11–18. One day after birth, litters were cross-fostered such that control dams were paired with one another and their litters were crossed; saline- and morphine-treated dams were paired and half of each saline litter was crossed with half of each morphine litter. Thus, each mother (control, saline, and morphine) raised half of her own and half of the adopted litter. At the age of 60 days, males and females were trained first to lever press for sucrose pellets and then for cocaine. Once the lever-pressing behavior was learned and baseline level of this activity was established, animals received a cocaine (.5 mg/kg per infusion) reward for each correct response on the active lever during the next 9-day session. The data demonstrate that adult control, saline- and morphine-exposed male rats self-administer cocaine at a similar rate independent of their prenatal treatment. Adult female rats self-administer cocaine at a higher rate than male rats. Further, saline- and morphine-exposed females in diestrus self-administer more than females in proestrus phase of the estrous cycle, while control females show no such differences. In addition, fostering induces increase in cocaine self-administration in all groups of male rats regardless of prenatal drug exposure. In females, the only fostering-induced increase is in prenatally saline-exposed female rats raised by morphine-treated foster mother. Thus, our results suggest that the prenatal drug exposure does not induce changes in lever-pressing behavior for cocaine reward in adult male and female rats, but it sensitizes the animals to postnatal stimuli such as gonadal hormones and/or rearing conditions that result in increased drug self-administration. © 2007 Wiley Periodicals, Inc. Dev Psychobiol 49: 463–473, 2007.*

Keywords: *prenatal morphine exposure; cocaine; self-administration; estrous cycle; foster care; adoption; drugs of abuse*

INTRODUCTION

Preclinical studies have shown that exposure to drugs of abuse (Glatt, Bolanos, Trksak, Crowder-Dupont, & Jackson, 2000; Vathy, 2002) or stress (Clarke & Schneider, 1993; Gue et al., 2004) increase behavioral and neuroendocrine perturbations. The behavioral abnormalities include decreased social interaction in rats

(Takahashi, Haglin, & Kalin, 1992; Wood & Spear, 1998) and altered motivated behaviors including sexual behavior (Vathy, 1999; Vathy, Etgen, & Barfield, 1985). There is also evidence that stress induces neuroendocrine dysregulation in primates (Schneider et al., 1998) and rodents (Vathy, 2002) and alteres hypothalamic-pituitary-adrenal (HPA) axis activity (Choi, Mazzio, Reams, & Soliman, 1998; Dutriez-Casteloot et al., 1999; Rimanóczy, Šlamberová, Riley, & Vathy, 2003; Šlamberová, Rimanóczy, Riley, & Vathy, 2004).

It is well documented that morphine induces its rewarding effects through its action on the μ-opioid receptor (Hall et al., 2003; Matthes et al., 1996). An increase in either μ-opioid receptor density or morphine concentration in the brain may activate the reward circuitry, that is, the nucleus accumbens (NAc) and central amygdala (CeA), implicated in modulating the rewarding effects of drugs of abuse (Koob & Le Moal, 2001; Weiss & Koob, 2001; Wise, 1989). Further, the NAc and the CeA are parts of the mesolimbic system that includes the ventral tegmental area (VTA), which contains dopaminergic pathways involved in the rewarding and reinforcing effects of drugs (Vaccarino, Bloom, & Koob, 1985). In the VTA, there are GABA interneurons, which have a tonic inhibition on the dopaminergic neurons in this area (Steffensen et al., 2006). These interneurons are inhibited by μ-opioid receptor agonists, which in turn increase the dopaminergic activity by disinhibition.

Interestingly, our work showed that although prenatal morphine exposure during gestation Days 11–18 alters the binding characteristics of all three major opioid receptor subtypes; μ, δ, and κ (Rimanóczy, Šlamberová, & Vathy, 2001; Rimanóczy & Vathy, 1995; Vathy, Rimanóczy, & Šlamberová, 2000), it increases μ-opioid receptor density in the NAc and CeA (Vathy, Šlamberová, Rimanóczy, Riley, & Bar, 2003). Thus, the alterations in μ-opioid receptor density induced by prenatal morphine exposure suggest that the sensitivity of the reward circuitry may also be affected and the adult offspring may display a differential sensitivity to drugs of abuse including cocaine (Vathy et al., 2003).

In addition, an interaction with the classical reward circuitry of the brain taught to be a critical downstream mediator of the rewarding properties of intracranial self-stimulation (ICSS) (Wise & Bozarth, 1984; You, Chen, & Wise, 2001; You, Tzschentke, Brodin, & Wise, 1998). It has also been shown that the most effective site to alter reward threshold with ICSS is in the medial forebrain bundle (MFB). Morphine (Hand & Franklin, 1986) and cocaine (Mague, Andersen, & Carlezon, 2005) administration potentiates ICSS responding in adult animals. Interestingly, our prenatal morphine exposure, on gestation Days 11–18, also enhances ICSS in the presence of a

single 5 mg/kg cocaine injection in adult male rats (Vathy, 2002).

It was shown (Malanga & Kosofsky, 2003) that rodents exposed to various abused drugs in utero, become sensitized in adulthood to the rewarding effects of drugs. For example, they respond to lower doses of drug than control animals. Increased predisposition to abuse drugs in adulthood has been shown in prenatally cocaine-exposed (Rocha, Mead, & Kosofsky, 2002), cannabinoid-exposed (Vela et al., 1998), and morphine-exposed offspring (Gagin, Kook, Cohen, & Shavit, 1997b) relative to controls. These studies suggest that susceptibility to drug-taking behaviors may be the result of structural and functional changes in the brain reward system during gestation (Rocha et al., 2002; Schneider, 1992; Vela et al., 1998). Most of the studies (Gagin et al., 1997b; Rocha et al., 2002) examined the effect of prenatal drug exposure on drug seeking behavior and sensitivity in the adult offspring, to the same drug they had been exposed prenatally. Others like Vela at al. (Vela et al., 1998) showed that prenatal drug exposure may alter predisposition to abuse drugs with different mechanisms of action such as delta9-tetrahydrocannabinol and morphine. We (Vathy, 2002), in our previous work demonstrated that prenatal morphine exposure enhances ICSS in the presence of a single 5 mg/kg cocaine injection in adult male rats. Therefore, the present work examines the hypothesis that prenatal morphine exposure enhances cocaine self-administration in adulthood.

In addition, some studies showed sex differences in drug-taking behavior (Becker, Rudick, & Jenkins, 2001; Lynch & Carroll, 1999, 2000; Lynch, Roth, & Carroll, 2002). Moreover, behavioral responses of females to psycho-stimulants fluctuate across the estrous cycle despite constant brain levels of drug (Becker, Robinson, & Lorenz, 1982). However, the self-administration literature is limited and inconsistent with a large body of evidence on the effect of ovarian hormones on behavioral responses to psychostimulants. Study of Roberts, Bennett, and Vickers (1989) suggests that female rats responding on a progressive ratio schedule of cocaine reinforcement during the estrus phase of the estrous cycle have reached break points significantly higher than when they are not in estrus. Interestingly, estrous cycle has no effects on female's responding on the fixed ratio (FR) 1 schedule of reinforcement (Roberts et al., 1989). Others showed (Mello et al., 1995; Mendelson et al., 1999) that the effects of estradiol may facilitate the initial acquisition of cocaine habit, but sustained use of cocaine can disrupt and even block the normal cycle that leads to decreased levels of circulating estradiol. In ovariectomized (OVX), females estrogen administration enhances cocaine acquisition relative to OVX, vehicle-treated females (Grimm & See,

1997; Lynch & Carroll, 2001; Sell, Scalzitti, Thomas, & Cunningham, 2000).

Because the rate and the timing of brain development as well as the hormonal background are different in the two sexes (Beyer & Feder, 1987), prenatal drug exposure could produce different responses in male and female offspring as had been shown by us and others (Gagin, Cohen, & Shavit, 1997a; Johnston, Payne, & Gilmore, 1992, 1994; Kashon, Ward, Grisham, & Ward, 1992; Rimanóczy & Vathy, 1995; Siddiqui & Gilmore, 1988; Siddiqui, Haq, & Shah, 1997; Siddiqui, Haq, Shaharyar, & Haider, 1995; Vathy & Kátay, 1992; Vathy, Rimanóczy, Eaton, & Katay, 1994; Vathy et al., 1985). Based on the above, the present study was designed to test the hypothesis that prenatal morphine exposure sex-dependently alters the propensity to abuse cocaine in adult progeny.

GENERAL METHOD

Drugs

Morphine sulfate and cocaine hydrochloride were obtained from the National Institute on Drug Abuse (Research Technology Branch, Rockville, MD) and dissolved in saline. Saline (.9% NaCl) was purchased from Abbott Laboratories (North Chicago, IL).

Prenatal Treatment

Forty, timed-pregnant Sprague–Dawley female rats were purchased from Taconic Farms (Germantown, NY) on gestational Day 8. Upon arrival, pregnant rats were housed individually in maternity cages with food and water available ad libitum, and maintained in a temperature-controlled colony room on a 10 hr (light):14 hr (dark) cycle with lights off at 0800 hr. Pregnant rats were randomly assigned to receive morphine (14 dams), saline (13 dams), or no injections (13 dams). Beginning on gestation Day 11 through gestation Day 18, pregnant female rats that were assigned to receive morphine or saline were injected two times daily with subcutaneous (sc) morphine sulfate or physiological saline at 0800 hr and at 2,000 hr, respectively. The timing of morphine injection was chosen to evaluate whether this drug affects the development of μ-opioid receptors in the offspring. Critical period for the appearance of μ-opioid receptors is gestation Day 14–15 in rats (Clendeninn, Petraits, & Simon, 1976). The same injection paradigm was used as in our previous work (Rimanóczy et al., 2003; Šlamberová, Hnatczuk, & Vathy, 2004; Vathy, 2002).

Morphine sulfate was dissolved in sterile .9% saline to a final injection volume of .1 ml. The dose of the first three morphine injections was 5 mg/kg, and the dose of all subsequent morphine injections was 10 mg/kg (Vathy et al., 1985). These doses of morphine are used in clinical practice to relieve pain, and the twice daily injection may resemble the fluctuating morphine levels in the fetal/maternal circulation in pregnant women, who take opiates for analgesia or abuse it (Wang, Traub, & Murphy, 2006). These doses of morphine do not induce physical dependence or tolerance (Riley & Vathy, 2006).

Postnatal Treatment

The day of birth was designated as postnatal Day (PND) 0. On PND 1, pups were weighed and sexed. Morphine- and saline-exposed pups were tattooed with black India ink on one rear and front foot-pad, respectively. The number of pups in each litter was adjusted to 10. Litters were cross-fostered such that control dams were paired and half of each control litter was crossed with another control litter; saline- and morphine-treated dams were paired and half of each saline litter was crossed with half of each morphine litter. Thus, each mother {control (C), saline (S), and morphine (M)} raised half of her own {C/C; S/S; and M/M} and half of the adopted {C/C; M/S; and S/M} litter. Because we kept a record of which pup was raised by biological or foster mother, we knew at any given time the origin of pups. Whenever possible, equal numbers of males and females were raised by each mother. On PND 25, animals were weaned, ear-punched for identification, and housed individually until adulthood.

During the time of the experiments, the age of the animals was PND 60–95. To avoid litter effect, one male and one female rat was used from each litter of each prenatal treatment. The remaining animals of each litter were assigned to other studies. Both males and females were weighted daily. Females were smeared by vaginal lavage every morning through the entire experimental period. The stage of estrous cycle was determined by microscopic examination of slides containing vaginal smears, and each female was entered into the appropriate group of diestrous or proestrus. The procedures for animal experimentation utilized in this report were reviewed and approved by the Institutional Animal Care and Use Committee.

Drug Self-Administration

Operant Conditioning. Operant conditioning was performed with young adult male and female rats (PND 60–95 at the start of conditioning). Because all experiments were conducted at the same age and only one female and one male was used from each groups of control, saline- and morphine-exposed, there were equal number of control, saline- and morphine-exposed females and males at any age. Therefore, the mean age and range of each experimental group were the same. All behavioral experiments were conducted during the dark phase of the light-dark cycle in a sound-attenuated operant chamber (Med Associates, Inc., St. Albans, VT) with two retractable levers located on the front wall, equidistant from the floor. Rats were trained to press one of the two levers for a reward. Each response on the active, reward-producing lever turned on a stimulus light mounted directly above the lever, and resulted in delivery of food or drug reward. Responses on the inactive lever did not turn on the stimulus light and were not rewarded. The number of responses on both the active and inactive levers was recorded by an automated MED-PC system (Med Associates, Inc.).

Food-Reinforced Lever Pressing. To assess activity and learning behavior in general, rats were food-deprived to 85% of free-feeding body weight before the initiation of food-reinforced lever pressing. Each response on the active lever resulted in the delivery of a 45 mg sucrose pellet (Research Diets, Inc., New Brunswick, NJ). Rats were allowed to lever press for sucrose pellets for 5 consecutive days, 30 min per day, until they achieved stable responding (less than 10% variability over three consecutive days). Animals were food deprived 2 days out of the 5-day lever-pressing session for sucrose pellets. All animals learned to lever press for food in 2 days and were given free access to food after Day 2 of food training. All rats established stable baseline responding in the next consecutive 3 days.

Surgical Procedure. Micro-renathane catheters (ID .025 mm, OD .040 mm; Braintree Scientific, Inc., Braintree, MA) were surgically implanted into the external jugular vein under general anesthesia (50 mg/kg sodium pentobarbital, i.p.; Sigma Chemical, St. Louis, MO) 3–5 days following the completion of food training. The catheter was inserted through the jugular vein to the level of the right atrium and ligated to the muscle adjacent to the vein as described previously (Krinke, 2000). The distal end of the catheter was passed s.c. between the scapulae, through a small incision on the scalp and attached to an L-shaped pedestal (Plastics One, Roanoke, VA). Triple antibiotic ointment (Taro Pharmaceuticals, Inc., Bramalea, Ontario) was applied to the incision points. The pedestal was secured to the skull with stainless steel screws and dental cement. Animals were allowed to recover about a week prior to the start of cocaine self-administration. Catheters were flushed daily with heparinized saline (20 IU/ml).

Cocaine Self-Administration. Cocaine self-administration was conducted for 9 consecutive days, 1 hr per day. Drug delivery was controlled by an automated syringe pump (model PHM-100; Med Associates) connected to the L-shaped pedestal by a cannula connector (Plastics One). The cannula connector was suspended from a swivel attached to a counterbalanced arm that allowed the animals to move freely within the operant chamber. There were no time-outs and no priming injections during cocaine self-administration. Each active lever press produced a cocaine reward while inactive lever presses produced no cocaine rewards (FR = 1).

Statistical Analysis

To see expected differences in groups of prenatal drug exposure, data were first analyzed by a three-way ANOVA on 9 days of cocaine self-administration, where prenatal drug exposure (morphine, saline, or no drug), sex (male or female), and trials (9 days of testing) were factors. Separate statistical analysis was used for the 5 days lever-pressing behavior for sucrose. Because we did not find any differences between prenatal treatment groups in males or females collapsed over raising (biological and foster) mothers (Fig. 1) we analyzed our data on cocaine self-administration taking into account maternal care (foster vs. biological; Fig. 2). That is, each group of animals (control, saline-, and morphine-exposed) was divided into two groups:

(a) raised by biological mother, and (b) raised by foster mother. A four-way ANOVA (prenatal drug exposure × rearing status × sex × trials) was used to assess the effect of biological/foster maternal care. In addition, lever-pressing behavior of females was assessed as a function of ovarian hormone fluctuation (Fig. 3). Lever-pressing behavior during diestrus and proestrus phase of the estrous cycle was compared. A two-way ANOVA (prenatal drug exposure × estrous cycle) was conducted to assess these differences in female rats. Newman-Keuls post hoc test was used in all statistical analyses. Differences were considered significant if $p < .05$.

RESULTS

There were no differences in lever pressing for sucrose pellets during the 5-day acquisition period on the active $\{F(2,43) = 2.5; \ p = .09\}$ or inactive $\{F(2,43) = 1.67; \ p = .2\}$ lever. All animals, regardless of prenatal drug exposure (morphine, saline, none), have learned lever pressing at a similar rate.

The daily cocaine self-administration was analyzed to establish whether prenatal morphine exposure affected total drug intake. As shown in Figure 1A, prenatal morphine exposure did not alter lever-pressing behavior in male or female rats on an FR1 schedule of reinforcement $\{F(2,42) = .36; p = .7\}$, or lever-pressing behavior (Fig. 1B) on the inactive lever $\{F(2,42) = 2.18; p = 1.26\}$ producing no cocaine reward. However, there were sex differences in both active $\{F(1,42) = 65.45; \ p < .0001\}$ and inactive $\{F(1,42) = 29.77; \ p < .0001\}$ lever pressing. All female rats regardless of prenatal drug exposure pressed on the active (Fig. 1A) as well as inactive (Fig. 1B) lever more than male rats.

When maternal care (biological vs. foster mother) was considered as a factor, there was a main effect of sex $\{F(1, 36) = 358.924; \ p < .0001\}$ and rearing status $\{F(2, 36) = 9.65; \ p < .002\}$ and an interaction between them $\{F(1, 36) = 10.12; \ p < .0001\}$. Data in Figure 2 demonstrate that all foster mothers' raised male rats displayed significantly higher lever presses than biological mothers' raised male rats. In female rats, this difference was apparent only in prenatally saline-exposed animals raised by morphine-treated mothers.

When ovarian hormone fluctuation at different phases of the estrous cycles was taken into consideration (see Fig. 3), there was a main effect on the stages of estrous cycle $\{F(1, 41) = 12.02; \ p < .01\}$ and an interaction between the prenatal drug exposure and the stages of estrous cycle $\{F(2, 41) = 4.33; p < .05\}$ on lever-pressing behavior. The lever-pressing behavior of control females stayed the same during diestrus and proestrus phases, but saline-exposed and morphine-exposed females exhibited significantly more lever-pressing behavior for cocaine
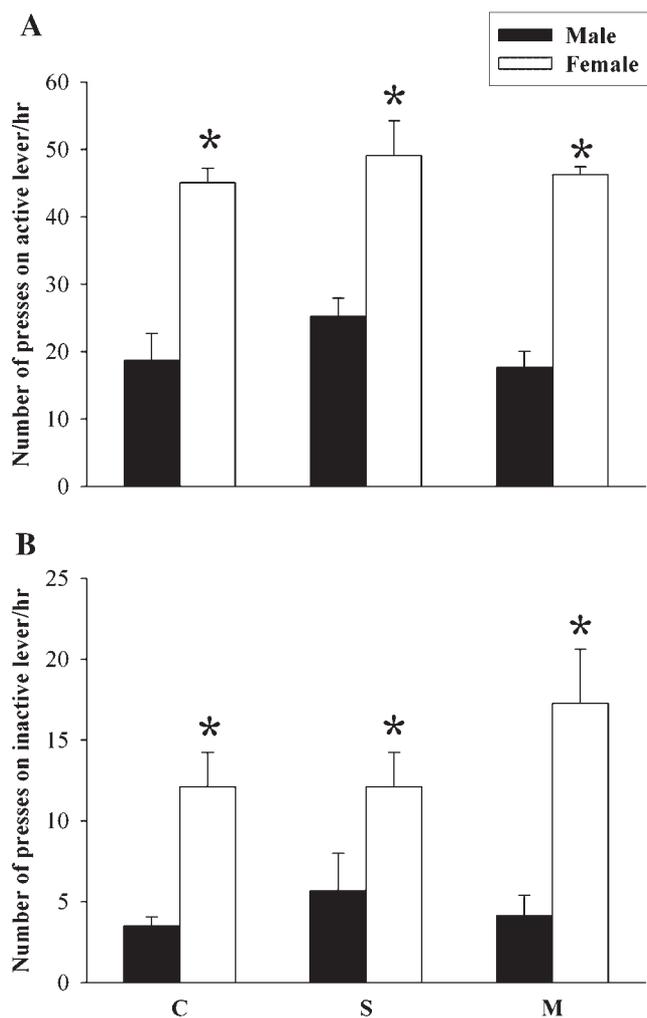
A

B

FIGURE 1   Prenatal morphine exposure does not alter cocaine self-administration in adult male or female rats. Neither prenatal saline- nor morphine-exposure had any effects on active lever-pressing behavior (A) or inactive lever-pressing behavior (B) in adult rats. Values are averages per 9 days of testing (means $\pm$ SEM), $n = 14-16$/group. C, control; S, saline-exposed; M, morphine-exposed. $^*p < .05$ versus males of the same prenatal condition.

reward during the diestrus phase than during proestrus phase of their estrous cycle.

DISCUSSION

The data in the present study demonstrate that prenatal morphine exposure does not affect cocaine self-administration in adult male or female rats using an FR1 schedule of reinforcement, and active lever-pressing behavior is greater than inactive lever-pressing behavior in both sexes in all experimental groups. It is possible that the FR1 schedule of reinforcement may not be the best to

demonstrate subtle differences between morphine-exposed and saline-exposed animals relative to controls. Based on these results, we may conclude that prenatal morphine exposure does not alter cocaine self-administration in adulthood under the limited experimental parameters used in the present study and, thus, cannot be compared to studies demonstrating reinstatement of a previously extinguished drug taking behavior (Chiamulera, Borgo, Falchetto, Valerio, & Tessari, 1996; Shaham & Stewart, 1994).

One possible explanation is that prenatal morphine exposure is not sufficient to facilitate drug self-administration in adulthood, because these drug-exposed animals have no actual/physical experience with drug intake. It is then likely that the learning/memory component of drug taking is necessary for drug seeking behavior after a long period of abstinence. However, in our ICSS study (Vathy, 2002) a 5 mg/kg systemic cocaine administration in adulthood enabled the prenatally morphine-exposed adult offspring to self-stimulate at a significantly higher rate than controls when the stimulatory electrode was in the MFB. The question then is: do the long-term effects of prenatal morphine exposure predispose the animals to react differently to postnatal stimuli? If so, it might explain another finding of the present work.

Another possible explanation may be that each drug (morphine and cocaine) has different mechanism(s) of action. While morphine binds to opioid receptors and indirectly affects the monoaminergic system, cocaine directly affects the levels of monoamines; dopamine, serotonin, and norepinephrine by blocking the transporters in the CNS. Based on these, any structural changes that is produced by prenatal morphine exposure may not be related to cocaine self-administration in adulthood. It is, therefore, possible that if the same drug would be used prenatally as in adulthood, we would see some change in self-administration. This would support the work of Gagin et al. (1997b) showing that prenatal morphine exposure enhances morphine-conditioned place preference in adult rats, and the work of Rocha et al. (2002) that demonstrates increased vulnerability to self-administration of cocaine in mice prenatally exposed to cocaine. However, the study of Vela et al. (1998) demonstrated that prenatal drug exposure affects sensitivity and predisposition to drugs of abuse in adulthood regardless of the drug used prenatally. Specifically, they (Vela et al., 1998) showed that maternal exposure to delta9-tetrahydrocannabinol facilitates self-administration of morphine in adult female rat offspring. Therefore, our expectation that prenatal morphine exposure could predispose cocaine self-administration in adult rats and as such demonstrate predisposition to abuse cocaine (drug with different mechanism of action) seems reasonable and the results may be considered novel.
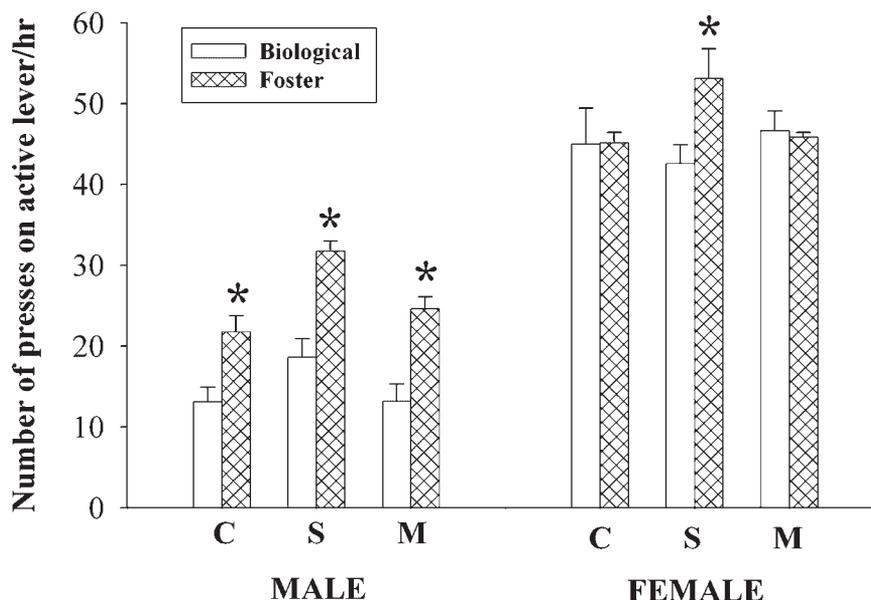
FIGURE 2 Effect of fostering on cocaine self-administration in adult male and female rats. Values are averages per 9 days of testing (means ± SEM), $n = 7-8$/group. C, control; S, saline-exposed; M, morphine-exposed. $^*p < .05$ versus all other groups.

The present data further demonstrate that there is a sex difference in all experimental groups (control, saline, and morphine). Females self-administer more cocaine than males regardless of prenatal exposure. This is in agreement with experimental findings of others (Fuchs, Evans, Mehta, Case, & See, 2005; Lynch, Arizzi, & Carroll, 2000; Roberts et al., 1989). Female rats acquire cocaine self-administration quicker than males and a larger proportion of drug-naive females learn to lever press for cocaine than males (Lynch & Carroll, 1999). While female and male rats generally do not differ in
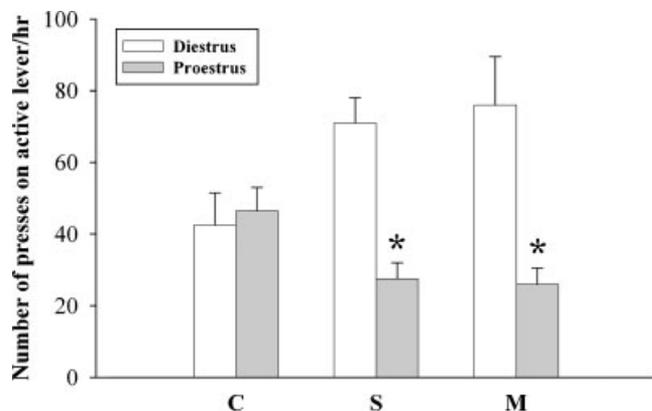

FIGURE 3 Increase in cocaine self-administration in diestrous saline- and morphine-exposed females. Values are averages per day of testing (means ± SEM), $n = 14-16$/group. C, control; S, saline-exposed; M, morphine-exposed. $^*p < .05$ versus diestrous females of the same prenatal treatment.

cocaine intake on an FR1 schedule of reinforcement, females exhibit greater breaking points on a progressive ratio schedule (Roberts et al., 1989). These as well as the results of the present study suggest that females may be more sensitive to the motivational effects of cocaine than males. There are also clinical studies (Brady, Grice, Dustan, & Randall, 1993; Griffin, Weiss, Mirin, & Lange, 1989; McCance-Katz, Carroll, & Rounsaville, 1999; Westermeyer & Boedicker, 2000) showing sex differences in drug-abuse. Men are more likely to have a drug-abuse or drug-dependence disorder than women (Brady et al., 1993), but women progress more rapidly from casual use to drug-dependence than men (McCance-Katz et al., 1999; Westermeyer & Boedicker, 2000). Women also report higher rates of use and shorter drug-free periods (Griffin et al., 1989). In the present work, we found sex differences not only in active lever pressing, but also in inactive lever pressing. There may be two ways to explain this finding: (1) females more readily seek the drug and make more errors in lever pressing; or (2) females display higher general activity than males. Studies (Alonso, Castellano, Afonso, & Rodriguez, 1991; Bisagno, Ferguson, & Luine, 2003; Brotto, Barr, & Gorzalka, 2000; Šlamberová & Vathy, 2002) showing higher activity in females relative to males support the second hypothesis.

Although we did not see any differences in cocaine self-administration as a result of prenatal morphine exposure, the present study shows that fostering increases lever pressing for cocaine reward in all groups of males regardless of prenatal exposure. Even control males that

were raised by control foster mothers press more for cocaine then control males raised by their biological mothers. There are no other preclinical studies that demonstrate an increase/decrease in drug abuse liability in adult, prenatally opiate-exposed offspring as a function of fostering. Therefore, it is difficult to compare similarities and/or differences between our work and the work of others. However, recent studies of Darnaudery et al. (2004) and Barros et al. (2006) showed that early or late fostering (adoption) as well as prenatal stress modify behavior of adult rats and may reverse or enhance the adverse effect of stress of prenatal periods.

In females, the only difference observed is the increased lever pressing in prenatally saline-exposed females raised by morphine-treated mothers but not by those raised by their biological mothers. This difference might be explained by ''undesirable'' maternal behavior of morphine-treated dams as was shown in our previous work (Slamberová, Szilagyi, & Vathy, 2001). It is further possible that the fostered females from saline-injected dams showed increased lever-pressing behavior for cocaine because they are the only group that have had prenatal stress of repeated injections without the effect of morphine and then fostered to a morphine-withdrawn dam. Future studies should include an additional group of control dams to which saline-exposed pups will be fostered to better understand the effects of morphine-withdrawn dams' maternal behaviors toward adopted saline-exposed or control pups. Note, that in male rats, however, the above interpretation does not hold because the differences are clearly due to fostering and is independent of gestational drug treatment of their mothers. Thus, the sex differences on fostering effect needs to be pursued in future studies to elaborate on this important issue of maternal factors in mother–infant relation.

When comparing cocaine self-administration in adult female rats at different phases of their estrous cycle, saline- and morphine-exposed females differ from controls. Both saline- and morphine-exposed female rats self-administer cocaine at a much higher rate during diestrus than during proestrus phase of estrous cycle. Control females show no such differences. It seems that females exposed prenatally to injections (morphine or saline), which may be considered as a mild stressor (Drago, Nicolosi, Micale, & Lo Menzo, 2001; Schindler, Šlamberová, & Vathy, 2004), are more sensitive to the fluctuation of gonadal hormones during their estrous cycle than control females. Our data in control females are consistent with the study of Roberts et al. (1989) showing that estrous cycle has no effects on females responding on an FR1 schedule of reinforcement for stimulant. On the other hand, others (Grimm & See, 1997; Lynch & Carroll, 2001; Sell et al., 2000) have demonstrated that OVX

female rats are more likely to show drug-seeking behavior when ovarian hormones were replaced. The difference in the effect of ovarian hormones in intact and OVX females may be due to the stress of ovariectomy and/or to the differences in hormone fluctuation in naturally cycling and OVX females. One cannot compare OVX females, or OVX, hormone-replaced females to naturally cycling females. In cycling female, there is never a hormone free environment as in OVX females. Our results, therefore, are comparable only to those studies using naturally cycling females.

The difference in lever pressing of control females and prenatally saline- or morphine-exposed females in proestrus might be due to different sensitivity to ovarian hormone fluctuation as a function of prenatal stress (injection). It is well known that stress affects estrous cycle as well as plasma levels of gonadal hormones (Smith, Ghuman, Evans, Karsch, & Dobson, 2003). Thus, it is also possible that the injection-induced prenatal stress may be responsible for long-term changes in gonadal hormone levels that persisted into adulthood. This hypothesis is in agreement with findings of others (Harvey & Chevins, 1987; Herrenkohl & Politch, 1978) that prenatal stress alters estrous cycle of adult female rodents. Moreover, prenatal stress affects the level of progesterone that should normally be high during proestrus, and that may be reduced in saline- and morphine-exposed females. This would be in agreement with studies of Ward, Ward, Winn, and Bielawski (1994) and Carter, Saridaki, and Lightman (1988) showing ''defeminization'' in prenatally stressed adult female rats. If it is possible that prenatal stress and/or prenatal morphine-exposure affect progesterone levels and is responsible for increase or decrease of drug-seeking behavior, then it is possible that this is the reason for having opposite results in the present study relative to the work of others (Grimm & See, 1997; Lynch & Carroll, 2001; Sell et al., 2000). This speculation needs to be further investigated in future studies especially, because our early work demonstrates inhibited receptive and proceptive behaviors in prenatally morphine-exposed animals (Vathy et al., 1985; Vathy, Etgen, & Barfield, 1989). Although these early studies (Vathy et al., 1985, 1989) did not include prenatally noninjected control group of females and therefore it is not known how saline-exposed females compare to those of unexposed females when tested for adult sexual behavior. Future study may be necessary to assess this possibility to investigate the importance of defeminization in both prenatally saline- and morphine-exposed females when compared to controls.

Together, our results suggest that analgesic dose of morphine exposure during mid to late gestation does not alter lever-pressing behavior for cocaine reward in adult male and female rats. Although these results do not prove

our hypothesis that prenatal morphine exposure enhance drug abuse liability in adult rats, the present study demonstrates that (1) female rats lever press more for cocaine than male rats confirming the finding of others, (2) estrous cycle plays a role in drug-seeking behavior, and (3) rearing conditions such as foster care enhance drug-seeking behavior, specifically in males. The latter finding could suggest that adopted pups are more sensitive to drugs of abuse than pups rose by biological mothers. However, it is further possible that this effect could depend on altered maternal care if the dam discriminates between foster and biologic pups, which could modify differentially maternal care such as pup retrieving, genital licking (very important in male pups), body stimulation, etc. Possibly, the use of anosmic mothers could help to clarify this point. Thus, stress induced by separation from biological mother may play a role in drug abuse. This is the first report suggesting that fostering sex-dependently alters drug self-administration in adult rats.

NOTE

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