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Female rats display dose-dependent differences to the rewarding and aversive effects of nicotine in an age-, hormone-, and sex-dependent manner

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Abstract

Introduction—The objective of this study was to examine age-, hormone-, and sex-dependent differences to the behavioral effects of nicotine using place-conditioning procedures in female rats.

Methods—Animals received nicotine in their initially non-preferred side and saline on alternate days in their initially preferred side. Following four conditioning trials, rats were retested for their preference. To examine developmental differences, we compared the effects of various nicotine doses in female and male adolescent and adult rats. To examine whether our developmental differences are specific to nicotine, we included adolescent and adult females that were conditioned with various amphetamine doses. To examine the influence of hormones on the behavioral effects of nicotine, we compared the effects of various nicotine doses in intact females that were tested during different phases of the estrous cycle and in separate females that were ovariectomized.

Result—The rewarding effects of nicotine were observed at a lower nicotine dose in adolescents versus adults. Amphetamine produced similar rewarding effects across age groups in females. The shifts in preference produced by nicotine were similar across the different phases of estrous. Females lacking ovarian hormones did not display rewarding effects of nicotine at any dose. The rewarding effects of nicotine were enhanced in adult female versus male rats. An intermediate nicotine dose produced rewarding effects in adolescent male but not female rats, suggesting that developmental differences to nicotine may be enhanced in males.

Conclusion—In females, nicotine reward is enhanced during adolescence and is facilitated by the presence of ovarian hormones.

Keywords

Conditioned place preference; Conditioned place aversion; Adolescence; Adolescent ovariectomy; Development; Estrous

Introduction

Most clinical reports have suggested that females are more vulnerable to tobacco abuse than men. This is based on the finding that females consume more tobacco products and have more difficulty quitting relative to males (Pauly 2008; Perkins et al. 1999; Perkins and Scott 2008;

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Pogun and Yararbas 2009; Schnoll et al. 2007). Also, females display a lower probability of quitting smoking if they initiated smoking during adolescence relative to males that initiated smoking at a young age (Chen and Millar 1998). Studies examining sex differences among adolescent smokers report that females are more likely to use tobacco products and are less likely to quit smoking relative to their male adolescent counterparts (Cropsey et al. 2008; Hebert 2003). Collectively, these studies suggest that females are particularly susceptible to tobacco abuse.

Pre-clinical animal studies have supported the notion that there are sex-dependent differences to the rewarding effects of nicotine. For example, female adult rats display faster acquisition rates of intravenous self-administration (IVSA) of low doses of nicotine relative to male adults (Donny et al. 2000). This study also demonstrated that female adults display higher motivation for nicotine intake, as they reach a higher break point for nicotine infusions on a progressive ratio schedule of reinforcement relative to males. This report also demonstrated that nicotine IVSA does not differ with respect to which phase of the estrous cycle the females are tested in, suggesting that the rewarding effects of nicotine are not influenced by hormonal fluctuations across the 4-day estrous cycle in adult female rats. More recent reports have further demonstrated that sex differences to the rewarding effects of nicotine are also age-dependent. For example, adolescent female rats display an increase in nicotine IVSA as they enter adulthood (Levin et al. 2003). However, a subsequent report from the same laboratory demonstrated that male adolescent rats display reduced nicotine intake as they enter adulthood (Levin et al. 2007). The hypothesis that adolescent females are more sensitive to the rewarding effects of nicotine is also consistent with the finding that adolescent female rats acquire nicotine IVSA more readily and at lower doses relative to male adolescents (Chen et al. 2007).

The goal of this study was to examine the effects of age, sex, and hormonal fluctuations on the rewarding and aversive effects of nicotine using place-conditioning procedures. Although previous studies have found the adolescent rats demonstrate enhanced conditioned place preference (CPP) than adult rats, these studies either examined effects only in male rats (Belluzzi et al. 2004; Shram et al. 2006; Torres et al. 2008) or did not distinguish the effects between male and female rats (Vastola et al. 2002). A recent report showed that both male and female mice displayed enhanced CPP to nicotine when conditioned as adolescents than as adults (Kota et al. 2007, 2008). The present study systematically examined sex- and age-dependent effects of nicotine in female rats by comparing CPP produced by various doses of nicotine in adolescent and adult female rats. Subsequent studies compared the effects of nicotine with those of amphetamine, examined the influence of gonadal hormones by determine the effects of ovariectomy on nicotine-induced CPP, and compared the effects nicotine between female and male rats.

Materials and methods

Animals

Subjects were adolescent (postnatal day (PND) 28–43) and adult (PND 60–75) male and female Wistar rats. All rats were fully outbred and maintained in the animal vivarium of the Psychology Department at the University of Texas at El Paso (UTEP). Rats were weaned on PND 21 and paired with two to three same-sex siblings in Plexiglas cages ($46 \times 20 \times 19$ cm^3) with wood-chip bedding (Harlan Teklad). Rats had ad libitum access to standard rodent chow and water, except during conditioning and preference testing. All rats were housed in a humidity- and temperature-controlled (22°C) vivarium on a 12-hour light/dark cycle (lights on 8:00 a.m.–8:00 p.m.). Rats were handled 2–3 min for 3 days prior to experimentation, and the animals' weight was recorded every day. Adolescent rats were 28 days old, and adult rats were at least 60 days old at the beginning of each experiment. All procedures and experimental protocols were approved by The UTEP Institutional Animal Care and Use Committee and were

conducted in adherence to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Each experimental group consisted of two to three animals from different litters. To avoid an artificial reduction in variance in any experimental condition with an excess of siblings, we ensured that each condition consisted of only two to three siblings from the same litter. In order to achieve this, we maintained ten to 12 separate lines of outbred breeding pairs so that the number of siblings was equalized across experimental conditions, and individual doses did not have an overrepresentation of siblings from any single litter. Great care was also taken to avoid a potential timing confound produced by running rats from different age groups at separate points in time. To avoid this confound, we coordinated our breeding such that each experimental cohort consisted of rats from each age group and experimental condition. In this way, adolescent and adult rats were represented each time animals were conditioned. Also, control rats from each condition were run each time the experiments were repeated in order to verify that the baseline values were consistent across each cohort of animals. All of the female rats were tested in an adjacent room using a separate set of conditioning chambers from males.

Drugs

The drugs used in these experiments were (−)nicotine hydrogen tartrate and d-amphetamine sulfate (Sigma Aldrich, Inc.). All drugs were dissolved in 0.9% sterile saline and administered via subcutaneous (s.c.) injections in a volume of 1 ml per kg of body weight using a 27-gauge 1/2 in. needle. All doses of nicotine refer to the base of the compound. Nicotine solutions were made fresh every 2 days and administered at pH=7. The doses of nicotine and amphetamine were selected based on previous studies from our laboratory demonstrating robust CPP in male rats (Torres et al. 2008). Control rats received physiological saline vehicle during conditioning.

Apparatus

Our conditioning apparatus consisted of two rectangular Plexiglas® chambers (76 × 24 × 30 cm) with one-way mirrors on the front walls to allow for behavioral observations. Each chamber was divided into two distinct compartments of equal proportions that were separated by a removable solid partition. One compartment had pine bedding beneath a smooth Plexiglas® floor with small holes. The other compartment had green-tinted pine bedding beneath a textured Plexiglas® floor with small holes. Both compartments were equally illuminated during the conditioning and testing procedures. White noise was used throughout conditioning and testing to minimize any disturbances from outside noise. The conditioning room was dimly lit in order to decrease outside variability in the conditioning sessions and maximize behavioral observations through the one-way tinting on the front walls of the conditioning apparatus. Separate chambers were used for male and female rats, and they were kept in different behavioral test rooms.

General conditioning procedures

This study employed a biased CPP procedure consisting of three phases: an initial preference test, four conditioning trials, and a final preference test. A biased conditioning procedure was used because these procedures have been shown to be more sensitive at detecting rewarding effects of drugs with mild reinforcing effects, such as nicotine (O'Dell and Khroyan 2009). During preference testing, the solid partition separating the two compartments of the apparatus was removed and replaced by a similar partition with an opening in the center to allow free access to both sides simultaneously. Rats were allowed to shuttle freely between the two compartments for 15 min during the preference test. The rats were considered to have entered a compartment if the two front paws were placed on the floor of that compartment. Preference testing was conducted in order to determine the initially non-preferred side of the apparatus for each animal. Only six female rats with an initial preference greater than 65% for either

compartment were eliminated from the study. This criterion was employed based on our previous observations that it is difficult to detect nicotine-induced CPP in animals with a strong initial bias for either compartment.

Conditioning began 6 days after the initial preference test in order to minimize latent inhibition that could weaken the association between the drug and external environmental cues. During conditioning, a solid partition separated the chambers so that the rats could be confined to only one side of the conditioning apparatus. Conditioning was conducted during the light phase of the animal's light/dark cycle. Rats received one of various doses of nicotine or amphetamine and were placed immediately into their initially non-preferred side for 30 min. On alternate days, rats received saline and were confined to their initially preferred side for 30 min. Control groups received saline injections in both sides of the conditioning apparatus. The order of drug treatment was counterbalanced within treatment groups such that some rats received drug on the first day of conditioning and the other half of the rats received drug on the second day of conditioning. Each animal was assigned to only one treatment condition (e.g., a single dose of nicotine or d-amphetamine).

The day after the last conditioning session, rats were retested for their preference for 15 min in a drug-free state. The order of exposure to the paired versus unpaired chambers was counterbalanced, such that half of the animals in each group were tested 24 h after their last nicotine injection and the other half were tested 48 h after the last nicotine injection. At the end of each study, all animals were euthanized using CO_2 inhalation.

Specific details of study 1: developmental differences in nicotine place conditioning

Study 1 compared place conditioning produced by various doses of nicotine (0, 0.2, 0.4, 0.6, 0.8, 1.2, and 1.8 mg/kg; base) in female adolescent and adult rats. Adolescent rats received the initial preference test on PND 28, the conditioning procedures began 6 days later on PND 34, and the final preference test was conducted on PND 43. Adult rats received the initial preference test on PND 60, the conditioning procedures began 6 days later on PND 66, and the final preference test was conducted on PND 75.

After the final preference test was completed, the adult female rats underwent vaginal lavage procedures to determine the phase of the estrous cycle the rats were in during the final test (i.e., proestrus, estrus, metestrus, or diestrus). Adolescent female rats were not subjected to vaginal lavage procedures due to the undifferentiated nature of their epithelial cells. A sterile and disposable plastic pipette was filled with 0.9% saline and was used to collect epithelial cells. Epithelial cells were then transferred to a labeled glass microscope slide. Microscope slides were fixed with methylene blue stain (Sigma, Inc.) and viewed under a light microscope at ×40 to examine the shape of the cells and determine the phase of the estrous cycle by the following criteria: proestrus=presence of round nucleated epithelium cells, estrus=presence of cornified un-nucleated epithelium cells, metestrus=presence of leukocytes, and diestrus=limited presence of epithelium cell and leukocytes. The vaginal lavage procedures were repeated the following day to verify the estrous results from the previous day, and rats that did not proceed to the next phase of estrous were omitted from this study.

Specific details of study 2: developmental differences in amphetamine place conditioning

Study 2 examined the specificity of our developmental differences in nicotine reward by comparing place conditioning produced by various doses of d-amphetamine (0, 0.25, 0.5, and 1.0 mg/kg) in adolescent and adult female rats. The conditioning procedures used with amphetamine were the same as the nicotine studies.

Specific details of study 3: hormonal fluctuations on nicotine place conditioning

Study 3 examined the effects of hormonal fluctuations on place conditioning produced by a single dose of nicotine (0.6 mg/kg) in separate groups of adult female rats that were tested on different days of the estrous cycle. We chose to examine fluctuations across estrous in adult females using this dose of nicotine because it produced robust CPP in adolescent female animals. The animals in this study were allowed to freely cycle, and they received vaginal lavage procedures after the preference test to determine what phase of the estrous cycle they were in during the final test. Thus, a large number of rats were used in order to achieve a minimum of at least n=9 per group. This naturalistic approach was used based on previous wok showing that ethanol intake is different in freely cycling rats versus female rats that received gonadotropin-releasing hormone to synchronize the estrous cycle (Roberts et al. 1999).

Specific details of study 4: gonadal hormone effects on nicotine place conditioning

Study 4 examined the influence of gonadal hormones on place conditioning produced by various doses of nicotine (0, 0.2, 0.4, 0.6, 0.8, and 1.2 mg/kg; base) in adult female rats that had received ovariectomy procedures. The ovariectomized females in this study were compared to intact females from study 1, some of which were conditioned and tested at the same time as animals in study 4. To address a potential confound related to the animals' history (i.e., surgery), we also included a group of control females (n=7) that received sham surgery and were conditioned later using a dose of nicotine (1.2 mg/kg) that produced robust CPP in intact females. This difference in the animals history does not appear to be confound, since the magnitude of CPP was similar in intact and sham ovariectomized female rats (difference scores=99±29 and 102±33, respectively).

The ovariectomy procedure was conducted in young female rats (PND 40–43) that were sedated using isoflurane gas. Once the animals were anesthetized, they were placed in a prone position on the surgical table. An incision extending approximately 5–8 mm long was made at a point about 1 cm medial to the knee and 2 cm lateral to the spinal cord. The tissue was separated through the inner layers of connective tissue and finally into the body cavity through the muscle layers of the ventral abdomen. Probing gently with forceps, the fat bodies in which the ovary is embedded were located. The tissue was then retracted through the incision onto the body surface. The oviduct was then ligated, and the ovary was cut away from the oviduct distal to the ligature. These procedures were then repeated for the ovary on the other side. Then ends of the oviduct were then placed inside the body cavity, and the skin was sutured. The animals then received flunixin (2.5 mg/kg, s.c.) for 2 days and were allowed to recover for 15 days prior to conditioning with various doses of nicotine. After the final preference test, ovariectomized females underwent vaginal lavage procedures to verify that the animals were not cycling in an estrous-dependent manner.

Specific details of study 5: sex differences to nicotine place conditioning

Study 5 examined sex differences by comparing place conditioning produced by various doses of nicotine (0, 0.2, 0.4, 0.6, 0.8, 1.2, and 1.8 mg/kg; base) in adult female and male rats. The male data were compared to the effects produced by nicotine in female rats from study 1. The males in this study were compared to intact females from study 1, some of which were conditioned and tested at the same time as animals in study 5.

Statistical analysis

Difference scores were computed to reflect the amount of time spent in the initially non-preferred compartment after conditioning minus before conditioning such that positive values reflect a positive shift in preference for the drug-paired compartment. CPP was operationally defined as a significant increase in the difference score obtained from drug-treated rats relative

to their respective controls that received saline during conditioning. In contrast, conditioned place aversion (CPA) was operationally defined as a significant decrease in the difference score of drug-treated rats relative to controls.

Our statistical analyses included overall analysis of variances followed by post hoc tests where appropriate. Specifically, the ability of various doses to produce CPP or CPA was compared across age (adolescent versus adult), sex (male or female), treatment groups (intact adult females versus ovariectomized females), or estrous cycle (proestrus, estrus, metestrus, or diestrus). In our experience with place-conditioning procedures, significant overall interactions are not usually observed, and group differences are most evident at individual doses rather than overall shifts in dose–response curves (O'Dell et al. 1996, 2007). This is because of the "all-or-none" nature of the results with certain doses producing CPP or CPA in some but not all experimental groups. Further, it is difficult to compare the magnitude of preference shifts across groups because ceiling effects may conceal group differences. Thus, individual post hoc comparisons were also conducted between the various groups at individual doses (and phases of the estrous cycle) using the Fisher's least significant difference test ($p \leq 0.05$). The type of analysis that was performed is denoted by different symbols on the figures. Asterisks (*) denote a significant difference relative to their respective saline controls and daggers (†) reflect a significant difference between age or treatment groups at each individual drug dose.

Results

Study 1 compared place conditioning produced by various doses of nicotine in naïve adolescent and adult female rats (see Fig. 1). Overall, the results revealed that nicotine produced CPP at a lower dose in female adolescent versus adult rats that displayed CPA at the highest nicotine dose. The statistical analyses revealed a significant main effect of dose ($F_{6, 213}=3.76$; $p<0.003$) with nicotine producing either CPP or CPA in a dose-dependent manner. Specifically, post hoc comparisons revealed that adolescent rats displayed CPP at a lower dose of nicotine (0.6 mg/kg) relative to adult rats that displayed CPP at a higher dose (1.2 mg/kg) relative to their respective saline controls ($p \leq 0.05$). Also, a significant CPA was only observed in adult rats conditioned with 1.8 mg/kg of nicotine relative to saline controls ($p \leq 0.05$). Regarding developmental differences, the post hoc comparisons revealed a significant difference between adolescent and adult female rats conditioned with the 0.6 mg/kg dose of nicotine ($p \leq 0.05$). Although adolescent female rats did not display CPA, toxic effects were observed in these young animals. Specifically, some of the adolescent females experienced seizure-like behavior, and one of them died following administration of the highest nicotine dose. In order to compare fluctuations across the estrous cycle, we collapsed across behaviorally equivalent doses of nicotine (0.2–0.8 mg/kg) and compared shifts in preference during each of the four phases of the estrous cycle (see Fig. 3b). The results revealed that there were no significant differences in this effect across the four phases of the estrous cycle ($F_{4, 105}=0.43$; p =ns).

Study 2 compared place conditioning produced by various doses of amphetamine in naïve adolescent and adult female rats (see Fig. 2). Overall, the results revealed that amphetamine produced CPP in both age groups in a similar manner. The statistical analyses revealed a significant main effect of dose ($F_{3, 78}=6.43$; $p<0.001$) with amphetamine producing CPP in an inverted U-shaped dose-response manner. Post hoc comparisons revealed that adolescent and adult rats displayed CPP at the 0.25, 0.5, and 1.0 mg/kg dose of amphetamine relative to their respective controls ($p \leq 0.05$). There were no developmental differences in the magnitude of CPP produced by amphetamine across these age groups ($F_{1, 78}=0.004$, p =ns).

Study 3 compared place conditioning produced by one dose of nicotine (0.6 mg/kg) in separate groups of adult female rats that were tested in various stages of the estrous cycle (see Fig. 3). There were no significant differences in the shifts in preference produced by nicotine in animals

that were tested in the various phases of the estrous cycle relative to saline controls ($F_{4, 69}$=1.25, p=0.3). Post hoc comparisons revealed there were no significant differences in the magnitude of CPP produced by the four phases of the estrous cycle relative to saline controls.

Study 4 compared place conditioning produced by various doses of nicotine in intact adult females from study 1 versus ovariectomized adult female rats (see Fig. 4). Overall, the results revealed that nicotine did not produce CPP at any dose in ovariectomized rats. However, high doses of nicotine that produced CPP in intact adult females produced CPA in ovariectomized adult females. The statistical analyses revealed a significant interaction between treatment and dose ($F_{5, 170}$=2.27, p≤0.05). Post hoc comparisons revealed that intact rats displayed CPP at a dose of nicotine (1.2 mg/kg) that produced CPA in ovariectomized females relative to saline controls (p≤0.05). Intact rats did display aversive effects when the dose of nicotine was increased (1.8 mg/kg) relative to saline controls (p≤0.05).

Study 5 compared place conditioning produced by various doses of nicotine in naïve adult male and adult female rats from study 1 (see Fig. 5). Overall, the results revealed that nicotine produced a larger upward shift in preference in female versus male rats. The statistical analyses revealed a significant interaction between sex and dose ($F_{6, 173}$=2.22, p ≤0.05), with nicotine producing either CPP or CPA in a dose-dependent manner. Specifically, post hoc comparisons revealed that adult male rats displayed a significant CPP at a low dose of nicotine (0.2 mg/kg) relative to saline controls. However, this dose did not produce significant CPP in females, and this was likely due to a higher shift in preference in the female controls. In general, each dose of nicotine produced a larger shift in preference in females versus males. This effect that was significant across male and female rats that received the 0.8 and 1.2 mg/kg dose of nicotine (p≤0.05). CPA was observed in both male and female rats that received the 1.8 mg/kg dose of nicotine relative to saline controls (p≤0.05).

Discussion

Most animal studies comparing age- and sex-dependent differences to nicotine reward have utilized IVSA procedures. However, one limitation of this procedure is that IVSA does not assess drug-environment associations in the absence of nicotine, and these environmental factors are important in the maintenance and relapse to smoking behavior during abstinence (Caggiula et al. 2001; Ferguson and Shiffman 2009; Sohn et al. 2003). Thus, this report contributes to the existing literature examining age- and sex-dependent differences to the rewarding effects of nicotine using CPP procedures. The major findings of this report are adolescent female rats display nicotine-induced CPP at a lower nicotine dose relative to adults (study 1), adolescent and adult female rats display similar CPP produced by amphetamine (study 2), freely cycling adult females display similar shifts in preference produced by nicotine across the estrous cycle (study 3), ovariectomized adult females did not display CPP at any nicotine dose (study 4), and adult females display enhanced rewarding effects of nicotine relative to males (study 5). Our studies also revealed that adult females display a lack of aversive effects produced by a high nicotine dose as compared to ovariectomized adult females and adult males. Also, male (but not female) adolescents display enhanced CPP produced by an intermediate nicotine dose versus their adult male counterparts.

Study 1 revealed that adolescent female rats displayed CPP at a lower dose of nicotine than adult female rats that did not display CPP until a high dose of nicotine was used during conditioning. Our findings are consistent with previous reports in mice demonstrating that adolescent female mice display CPP at a lower nicotine dose versus adult mice that only display CPP at higher doses of this drug (Kota et al. 2008). Furthermore, IVSA studies have demonstrated that adolescent female rats display higher levels of nicotine intake relative to their adult female counterparts (Levin et al. 2003). Previous work in our laboratory using

similar conditioning procedures and nicotine doses also demonstrated that in male rats, nicotine-induced CPP is also enhanced in adolescent versus adult rats (Torres et al. 2008). Taken together, these studies provide converging lines of evidence that the rewarding effects of nicotine are enhanced during the adolescent period of development, consistent with CPP reports from other laboratories in rats (Belluzzi et al. 2004; Shram et al. 2006; Vastola et al. 2002) and mice (Kota et al. 2007; 2008). Thus, the present study suggests that adolescence is a period of enhanced vulnerability to the rewarding effects of nicotine in females.

Study 2 revealed that our observed developmental differences may be specific to nicotine, since adolescent and adult female rats display a similar magnitude of CPP produced by various doses of amphetamine. This is consistent with a report from another laboratory demonstrating that adolescent and adult female rats display similar CPP produced by amphetamine (Mathews and McCormick 2007). Furthermore, IVSA studies have demonstrated that adolescent and adult rats display similar levels of amphetamine intake (Shahbazi et al. 2008). Collectively, these studies suggest that enhanced developmental sensitivity to nicotine is specific to this drug and not other stimulant compounds, such as amphetamine. Also, the lack of developmental differences in amphetamine-induced CPP suggests that our age-dependent differences to nicotine are not due to differences in learning since adolescent and adult rats display similar acquisition of CPP produced by amphetamine.

Study 3 revealed that female adults that were tested during different phases of the estrous cycle displayed a similar shift in preference produced by a single dose of nicotine (see graph A in Fig. 3). Furthermore, there were still no differences in preference behavior across the estrous cycle when we collapsed across several behaviorally equivalent doses of nicotine (see graph B in Fig. 3). Taken together, these analyses reveal that hormonal fluctuations do not likely influence the expression of the conditioned behavioral effects of nicotine. This is consistent with previous reports demonstrating similar levels of nicotine intake (Donny et al. 2000) and locomotor activity (Kuo et al. 1999) in female rats tested during different stages of the estrous cycle.

Study 4 revealed that female rats that lacked ovarian hormones did not display rewarding effects at any dose of nicotine that was administered. These results suggest that ovarian hormones are necessary for the expression of the rewarding effects of nicotine. Estrogen is one likely candidate hormone that facilitates the rewarding effects of nicotine in the mesolimbic reward pathway. Estrogen receptors are located on inhibitory GABAergic terminals in the dopamine cell body region of the ventral tegmental area. Estrogen inhibits calcium currents and this causes a decrease in GABAergic transmission that normally inhibits dopamine release in the nucleus accumbens (NAcc). Thus, estrogen facilitates dopamine release in the terminal region of the NAcc that plays a critical role in mediating the rewarding effects of drugs of abuse (see Becker 1999). Estrogen has also been shown to enhance nicotine-induced increases in striatal dopamine levels in female, but not male rats (Dluzen and Anderson 1997). Also, ovariectomized female rats lacking estrogen display a decrease in dopamine in the ventral tegmental area (Russo et al. 2003). Thus, it is possible that estrogen may be necessary for the expression of the rewarding effects of nicotine based on estrogen's anatomical position to facilitate dopamine transmission in the mesolimbic reward pathway.

Study 5 revealed that both female and male rats displayed nicotine place conditioning in an inverted U-shaped dose-response manner. This is presumably due to rewarding effects at intermediate nicotine doses and the emergence of aversive effects at high doses of this drug. An examination of the intermediate dose range (i.e., rewarding doses) shows that there is a larger upward shift in the CPP dose–response curve in female versus male rats. The finding that female rats display a larger shift in preference produced by nicotine suggests that the rewarding effects of nicotine are enhanced in female versus male rats. This interpretation of

our dose–response data is consistent with nicotine place-conditioning studies conducted in female and male mice (Kota et al. 2007, 2008). In consideration of the full dose–responses curves, we also observed that the nicotine curve was flatter in female versus male rats. This appears to be related to the onset of the aversive effects of nicotine at lower doses in males versus female rats who only displayed aversive effects at the highest dose.

To address the influence of female hormones on the aversive effects of nicotine, we also compared place conditioning produced by a high dose of nicotine (1.2 mg/kg dose) in male, female, and female rats lacking ovarian hormones. This comparison revealed that female rats that lacked ovarian hormones displayed aversive effects at a high dose of nicotine that produced rewarding effects in intact females. Interestingly, nicotine-induced place aversion was similar in adult males and adult ovariectomized female rats. These findings suggest that the presence of gonadal hormones may protect against the aversive effects of nicotine in females. Future studies are needed to examine which ovarian hormones protect against the aversive effects of nicotine.

The sex difference produced by high nicotine doses appears to be hormone-dependent. Both male and ovariectomized female adult rats display aversive effects at high nicotine doses that are rewarding in intact females. The notion that drugs of abuse are more aversive in males is consistent with a previous report demonstrating that male rats are more sensitive to the toxic effects of 3,4-methylenedioxymethamphetamine relative to females (Fonsart et al. 2008). The possibility exist that our observed sex differences are due to metabolic differences that are hormone-dependent. This is based on a previous study demonstrating that nicotine administration produces higher plasma levels of nicotine in female versus male rats, and this sex difference is obliterated in ovariectomized females (Harrod et al. 2007).

Our results from study 1 revealed that the developmental differences in nicotine reward in female rats appeared to be smaller than what we had observed in previous studies in male rats (Torres et al. 2008). Thus, we compared CPP produced a dose of nicotine (0.4 mg/kg) that produced robust developmental differences in previous studies in male rats in a group of adolescent and adult male and female rats. The results revealed that an intermediate dose of nicotine produced enhanced CPP in adolescent versus adult males, and this effect was absent in females. These data at a single dose suggest that developmental differences to nicotine are enhanced in male versus female rats. This interpretation of our data is consistent with an overview of our previously published dose–response data in adolescent and adult male rats (Torres et al. 2008) with the dose–response data that was collected in this study in females. Specifically, the magnitude of nicotine-induced CPP in our recent report in males is larger and significant across a wider nicotine dose range in adolescent versus adult male rats compared to females in the present study who only display developmental differences at an intermediate dose of nicotine. The finding that the magnitude of developmental differences is larger in males versus female rats is also consistent with previous reports in mice (see Kota et al. 2007, 2008). Thus, the possibility exists that male adolescent rodents are most sensitive to the rewarding effects of nicotine versus adult males and females of both age groups. Consistent with this, nicotine IVSA is higher during adolescence in male (Levin et al. 2007) versus female (Levin et al. 2003) rats, with male adolescents displaying threefold higher levels of nicotine intake versus female adolescent rats. Interestingly, the latter studies also show that adolescent males decrease their nicotine intake as they enter adulthood; whereas, female adolescents increase their nicotine intake as they approach adulthood. Thus, developmental differences in nicotine reward may be more pronounced in male versus female rats. Future work is needed to determine the mechanisms by which sex differences alter developmental sensitivity to the behavioral effects of nicotine.

In summary, our finding that females are less sensitive to the aversive effects of nicotine relative to males and that ovarian hormones facilitate the rewarding effects of nicotine may have clinical implications. Specifically, enhanced tobacco use in females may be due to enhanced rewarding effects in combination with a lack of aversive responses to nicotine that may limit tobacco use in males. Thus, females may be more vulnerable to tobacco abuse based on enhanced rewarding effects and reduced aversive effects of nicotine. We have previously suggested that adolescents experience enhanced reward and reduced aversive effects of nicotine that contribute to enhanced vulnerability to tobacco abuse during adolescence (O'Dell 2009). Thus, heightened vulnerability to tobacco abuse in females may involve enhanced rewarding effects of nicotine in combination with the absence of factors that limit tobacco use, such as aversive effects. It should be noted, however, that a recent clinical report demonstrated that female smokers report enhanced aversive effects produced by an acute nicotine injection versus males (Sofuoglu and Mooney 2009). Thus, future work may be needed to fully examine whether pre-clinical studies are homologous between rodent and human models. Also, future work is needed to explore whether our hypothesis regarding the contribution of rewarding and aversive effects fully explains enhanced vulnerability to tobacco abuse across different populations of tobacco users.

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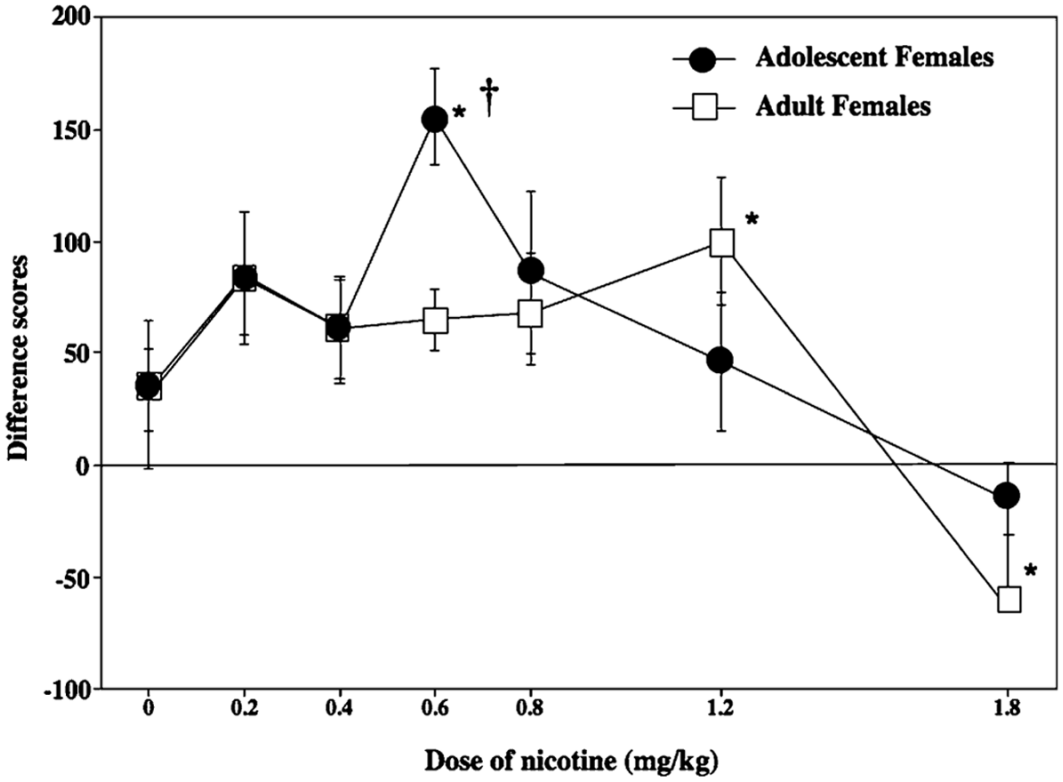

Fig. 1.

This graph reflects place conditioning produced by various doses of nicotine (0, 0.2, 0.4, 0.6, 0.8, 1.2, or 1.8 mg/kg, base, s.c.) in adolescent versus adult female rats. The group numbers are as follows for adolescent females (0 dose *n*=14, 0.2 dose *n*=12, 0.4 dose *n*=20, 0.6 dose *n*=13, 0.8 dose *n*=12, 1.2 dose *n*=12, or 1.8 dose *n*=5) and adult females (0 dose *n*=18, 0.2 dose *n*=12, 0.4 dose *n*=13, 0.6 dose *n*=55, 0.8 dose *n*=11, 1.2 dose *n*=22, or 1.8 dose *n*=8). These data are presented as difference scores (±SEM), which reflect time spent in the initially non-preferred side after conditioning minus before conditioning such that values above "0" reflect a positive shift in preference (i.e., conditioned place preference); whereas, values below "0" represent an a negative shift in preference (i.e., conditioned place aversion). The *asterisks* denote a significant difference from their respective saline controls, and the *dagger* denotes a significant difference between age groups ($p \leq 0.05$)

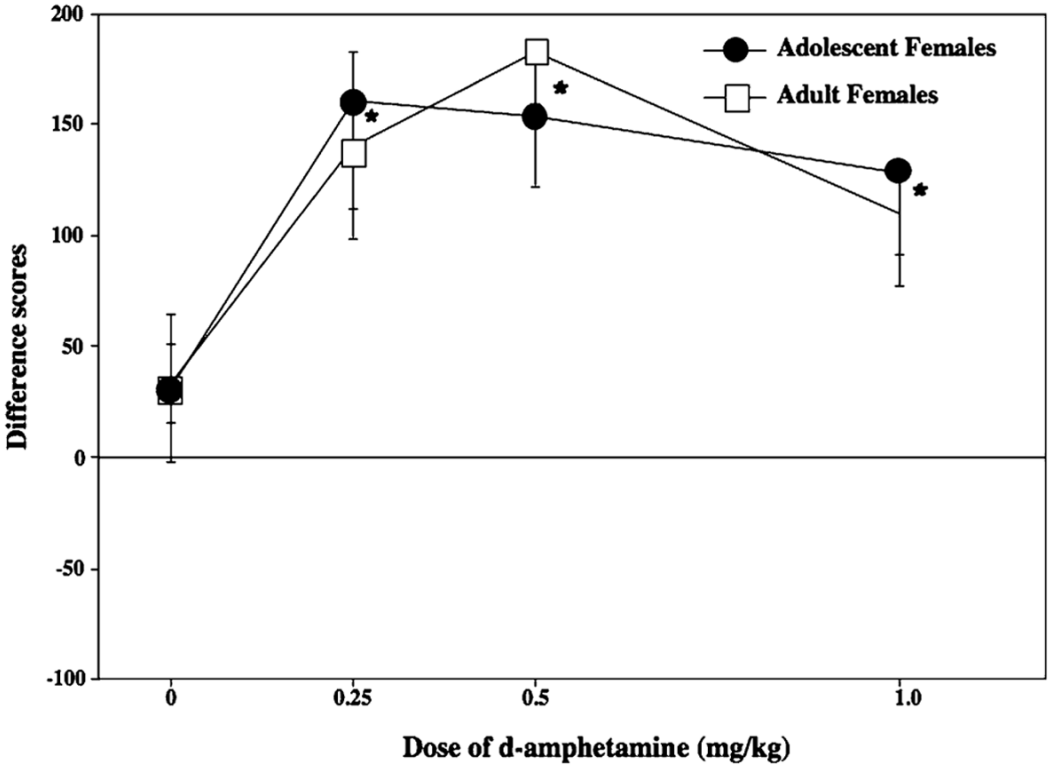

Fig. 2.

This graph reflects place conditioning produced by various doses of amphetamine (0, 0.25, 0.5, or 1.0 mg/kg, s.c.) in adolescent versus adult female rats. The group numbers are as follows for adolescent females (0 dose *n*=14, 0.25 dose *n*=11, 0.5 dose *n*=8, or 1.0 dose *n*=10) and adult females (0 dose *n*=18, 0.25 dose *n*=10, 0.5 dose *n*=8, or 1.0 dose *n*=7). These data are presented as difference scores (±SEM), which reflect time spent in the initially non-preferred side after conditioning minus before conditioning such that values above "0" reflect a positive shift in preference (i.e., conditioned place preference); whereas, values below "0" represent a negative shift in preference (i.e., conditioned place aversion). The *asterisks* denote a significant difference from their respective saline controls ($p \leq 0.05$)

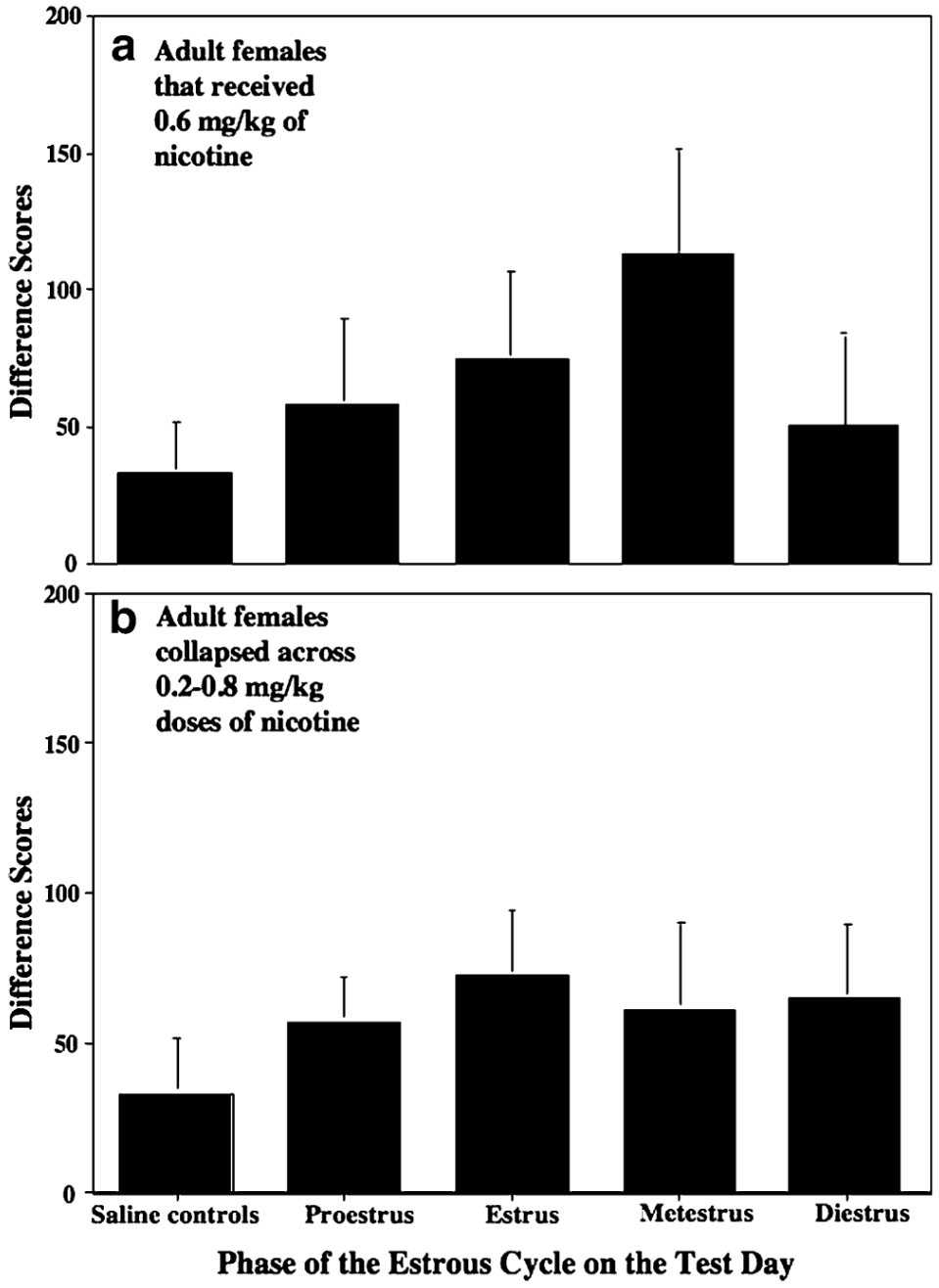

Fig. 3.

a The top graph reflects place conditioning produced by one dose of nicotine (0.6 mg/kg) in freely cycling adult females that were tested during various phases of the estrous cycle. **b** The bottom graph reflects place conditioning collapsed across behaviorally equivalent doses of nicotine (0.2–0.8 mg/kg) in freely cycling adult females that were tested during various phases of the estrous cycle. The group numbers are as follows for females in panel *A* (saline *n*=18, proestrus phase *n*=20, estrus phase *n*=16, metestrus phase *n*=11, or diestrus phase *n*=9) and panel *B* (saline *n*=32, proestrus phase *n*=27, estrus phase *n*=18, metestrus phase *n*=15, or diestrus phase *n*=18). There was no difference in place conditioning produced by nicotine across any phase of the estrous cycle. It should be noted that there was no difference in place

conditioning produced by the two doses of nicotine (0.8 and 1.2 mg/kg) that did produce significant shifts in conditioned place preference

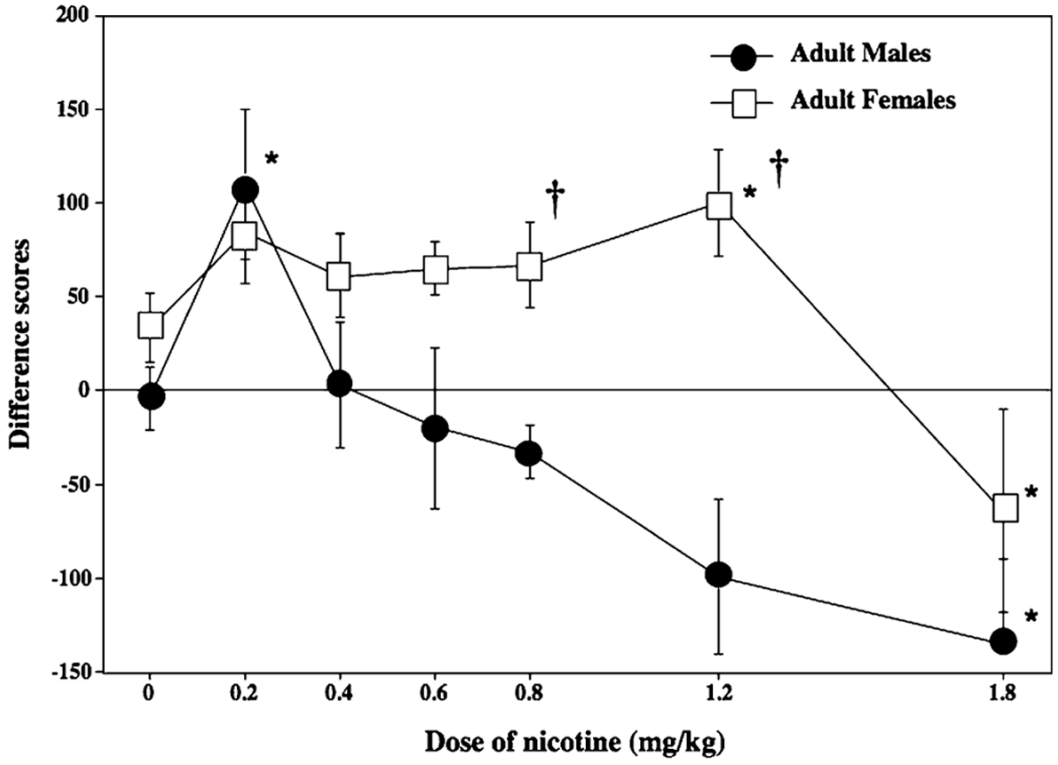

Fig. 4.

This graph reflects place conditioning produced by various doses of nicotine (0, 0.2, 0.4, 0.6, 0.8, 1.2, or 1.8 mg/kg, base, s.c.) in ovariectomized adult female rats and intact female rats from study 1. The data for females is the same as that shown in Fig. 1. The group numbers are as follows for ovariectomized adult females (0 dose n=8, 0.2 dose n=6, 0.4 dose n=7, 0.6 dose n=8, 0.8 dose n=7, or 1.2 dose n=8) and intact females (0 dose n=18, 0.2 dose n=12, 0.4 dose n=13, 0.6 dose n=55, 0.8 dose n=11, 1.2 dose n=22, or 1.8 dose n=8). These data are presented as difference scores (±SEM), which reflect time spent in the initially non-preferred side after conditioning minus before conditioning such that values above "0" reflect a positive shift in preference (i.e., conditioned place preference); whereas, values below "0" represent a negative shift in preference (i.e., conditioned place aversion). The *asterisks* denote a significant difference from their respective saline controls ($p \leq 0.05$), and *daggers* denote a significant difference between treatment groups ($p \leq 0.05$)

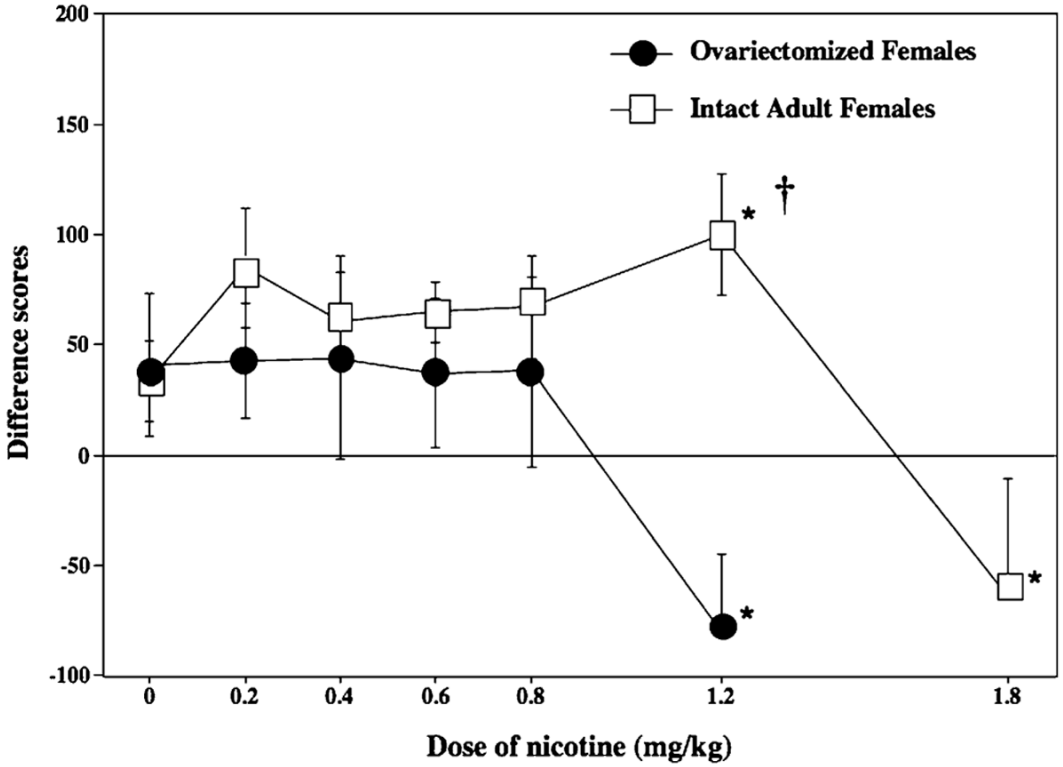

Fig. 5.

This graph reflects place conditioning produced by various doses of nicotine (0, 0.2, 0.4, 0.6, 0.8, 1.2, or 1.8 mg/kg, base, s.c.) in adult male rats and female rats from study 1. The data for females is the same as that shown in Fig. 1. The group numbers are as follows for adult males (0 dose n=5, 0.2 dose n=10, 0.4 dose n=7, 0.6 dose n=6, 0.8 dose n=7, 1.2 dose n=8, or 1.8 dose n=6) and intact females (0 dose n=18, 0.2 dose n=12, 0.4 dose n=13, 0.6 dose n=55, 0.8 dose n=11, 1.2 dose n=22, or 1.8 dose n=8). These data are presented as difference scores (±SEM), which reflect time spent in the initially non-preferred side after conditioning minus before conditioning such that values above "0" reflect a positive shift in preference (i.e., conditioned place preference); whereas, values below "0" represent an a negative shift in preference (i.e., conditioned place aversion). The *asterisks* denote a significant difference from their respective saline controls, and the *dagger* denotes a significant difference between age groups ($p \leq 0.05$)