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Sex differences in conditioned nicotine reward are age-specific

Magalie Lenoir, Amy K. Starosciak, Jennifer Ledon, Caitlin Booth, Elena Zakharova, Dean Wade, Beatrice Vignoli, Sari Izenwasser *

Department of Psychiatry and Behavioral Sciences, University of Miami Miller School of Medicine, 1600 NW 10th Avenue, Rm 4113A (D-80), Miami, FL 33136, USA

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ABSTRACT

Women constitute half of all smokers and many studies suggest that adult males and females differ in factors that maintain tobacco smoking, yet there is limited information about sex differences in nicotine reward during adolescence. Limited studies suggest that adolescent male rats self-administer more nicotine than adults, suggesting that drug administration during adolescence leads to different behavioral effects than during adulthood. In the present study, male rats developed a significant conditioned place preference (CPP) to lower doses of nicotine than females, regardless of age. In addition, adolescents were more sensitive than adults. In female rats, adolescents exhibited a CPP of greater magnitude than adult females. In males, the magnitude of the CPP did not differ as a function of age, but adolescents exhibited CPP to lower doses than adults. There also were differences in nicotinic acetylcholinergic receptor binding in nucleus accumbens and caudate putamen in response to nicotine across age and sex. These findings suggest that it is necessary to consider sex- and age-specific effects of drugs such as nicotine when developing strategies for improving smoking cessation treatments.

1. Introduction

Cigarette smoking is the leading cause of preventable death in the United States and approximately 20% of American adults are current cigarette smokers, with males (23%) having a slightly higher rate than females (18%) (CDC, 2010). The rate of cigarette smoking in high school students in the United States is only slightly less (17%) than that of American adults, with boys having a slightly higher rate than girls (20% vs. 15%, respectively) (NSDUH, 2010). These statistics are concerning because it is well known that approximately 80% people who start smoking before the age of 18 go on to become regular smokers as adults (CDC, 2003). In fact, children and adolescents may be especially susceptible to nicotine addiction as symptoms of dependence can emerge as early as the first time or first few times tobacco is used (DiFranza et al., 2000) and can even develop in adolescents not classified as daily smokers (i.e., weekly or monthly smokers) (Panday et al., 2007).

Although more males than females smoke cigarettes in both age groups, there is some evidence that female cigarette smokers may be more susceptible to the negative health consequences of tobacco use. For example, females metabolize nicotine faster (and, thus, must dose themselves accordingly), may be more likely to develop chronic obstructive pulmonary disorder (COPD), and have more difficulty with tobacco cessation (reviewed in Rahmanian et al., 2011) than male cigarette smokers. Further, nicotine craving may be more severe in adolescent females than in adolescent males (Panday et al., 2007). Therefore, it is important to understand sex and age differences in behaviors related to nicotine and tobacco, as well as nicotine reward.

Preclinical animal models are useful to examine whether biological sex differences and age differences have considerable effects on behaviors related to nicotine use and dependence (Carroll and Anker, 2010; Carroll et al., 2009; Lynch et al., 2002; O'Dell and Khroyan, 2009). During adolescence, subjects exhibit a unique pattern of behavioral and neurochemical responses to nicotine that are different in males and females. Examples of these age- and sex-specific behaviors and responses are described below.

Female adult rats acquire nicotine self-administration faster than males at the lowest training doses (Chaudhri et al., 2005; Donny et al., 2000; Lanza et al., 2004), and self-administer more intravenous infusions of nicotine per session than males (Rezvani et al., 2008). Although these sex differences seem to be diminished at the end of acquisition and during maintenance (Chaudhri et al., 2005; Donny et al., 2000; Lanza et al., 2004), females are more motivated to initially obtain nicotine as compared to males (Donny et al., 2000). If these findings extrapolate to humans, then perhaps women are more sensitive to lower doses of nicotine, and are more likely to continue tobacco use after fewer "tries" than are men. In adolescence, males administer more nicotine than during adulthood, after which rates decline to adult rates (Levin et al., 2007). In females, adolescents also administer more nicotine than adults; however, this difference is maintained into adulthood (Levin et al., 2003). Thus, starting nicotine during adolescence leads to different use patterns in adults compared to initiating administration in adulthood, and this is sex-dependent.

* Corresponding author. Tel.: +1 305 243 2032; fax: +1 305 243 5475.
E-mail address: sizenwasser@med.miami.edu (S. Izenwasser).

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As sex differences in nicotine self-administration occur in adults, there also are sex differences in behavioral actions of nicotine in adolescents. Data from our laboratory have shown that adolescent females rapidly become sensitized to the locomotor-activating effects of nicotine, with significant effects seen on day 2 of treatment (Collins and Izenwasser, 2004). This finding is in contrast to adult female and male rats that exhibited significant sensitization beginning on day 5 of treatment. Further, adolescent male rats did not become sensitized to the locomotor-activating effects of nicotine within a 7-day treatment period, a finding that has been shown in our laboratory (Collins and Izenwasser, 2004; Collins et al., 2004a) and others (Schochet et al., 2004). Although the adolescent male and female rats were tested during the periadolescent period (postnatal days 28–40; Spear and Brake, 1983) in these studies, males and females have different rates of maturation (Ojeda et al., 1980, 1983) that could contribute to sex differences in nicotine self-administration and sensitization during adolescence. Similar to adults, adolescent female rats more easily acquire nicotine self-administration and express greater motivation to earn nicotine than adolescent male rats (Lynch, 2009).

In previous research, it has been shown that a moderate dose of nicotine (i.e., 0.6 mg/kg) produces CPP in adolescent rats but not adult rats when male and female data were combined (Vastola et al., 2002). However, the dose used in this study was based on the weight of the salt rather than nicotine as a free base, so the amount of nicotine actually received by these animals was somewhat lower. Others found similar results, in which adolescents developed CPP to the highest tested doses (0.5–0.8 mg/kg nicotine base), but older adolescents and adults did not (Belluzzi et al., 2004; Brielmaier et al., 2008; Shram et al., 2006). Torres et al. (2008) described the same results as mentioned above, but also found that both adolescents and adults developed CPP to 0.2 mg/kg nicotine. These results were specific to males, as females were not tested. In females, adolescents developed maximal CPP to 0.6 mg/kg, whereas adults developed CPP to 1.2 mg/kg nicotine (Torres et al., 2009).

Sex differences in nicotine CPP have been examined in adult animals by several groups. It has been reported that adult male rats developed CPP in response to 0.1 and 0.2 mg/kg nicotine base (but not any higher doses), whereas adult females did not develop CPP to any of the doses tested (0.1–0.6 mg/kg base) (Yararbas et al., 2010). Results reported by Torres et al. (2009) were similar, in which male rats had maximal CPP to 0.2 mg/kg nicotine and females had maximal CPP to 1.2 mg/kg nicotine; males and females developed conditioned place aversion to 1.8 mg/kg. However, Wistar rats were used in the study by Torres, whereas Sprague–Dawley rats were used in the study by Yararbas. Comparisons between the two studies are somewhat limited because of potential strain differences. Further, sex differences in nicotine CPP are not species-dependent; as male and female differences also have been reported in mice. For example, both male and female mice developed significant CPP to 0.32 mg/kg nicotine, but CPP was greater in females than males at this dose (Isiegas et al., 2009). These results give credence to the idea that sex differences in nicotine reward are not specific to rats and that this likely is a general phenomenon. However, the current literature lacks reports in which effects of age and sex on nicotine reward have been addressed by directly comparing male and female adults and adolescents using nicotine conditioned place preference.

In light of this previous literature, it is clear that the rewarding effects of nicotine are different in male and female rats and that these differential responses likely will be age-specific. While males and females and adolescents and adults have been studied, full dose–response curves for nicotine CPP in all four groups have not yet been reported in a single study. In the present study, the rewarding effects of nicotine were measured using CPP to several doses of nicotine, such that full dose–response curves were attained in male and female adult and adolescent rats. In addition, the responsiveness of brain nicotinic acetylcholine receptors (nAChRs) to stimulation by nicotine was measured in each group to explore possible neurobiological correlates underlying any age and sex differences.

2. Methods

2.1. Subjects

Naïve periadolescent male (n = 110), periadolescent female (n = 52), adult male (n = 72), and adult female (n = 68) Sprague–Dawley rats were used (Charles River, Wilmington, MA). All rats were housed in a light- (12 h light/dark cycle with lights on at 7 a.m. and off at 7 p.m.), temperature- (21 ± 2 ºC) and humidity-controlled vivarium (53 ± 13%). At the start of the experiment, male and female periadolescent rats (postnatal day (PND) 34) weighed an average of 126.1 ± 1.9 g and 117.3 ± 1.5 g respectively, and the adult male and female rats (PND 66) weighed an average of 325.7 ± 2.3 g and 221.7 ± 2.3 g respectively. All behavioral tests occurred during the light schedule between 8:30 a.m. and 5:00 p.m., with each group tested at the same hour every day and groups counterbalanced over the day. Food and water were available ad libitum, except during the 30-min conditioning and testing sessions. Male and female rats were studied in separate groups at different times, using identical methods. All experiments were carried out in accordance to the guidelines of the Guide for Care and Use of Laboratory Animals, National Research Council, Department of Health, Education and Welfare, NIH Publication 85-23, revised 1996 and were approved by the Institutional Animal Care and Use Committee.

2.2. Drugs

(−)-Nicotine hydrogen tartrate salt (Sigma-Chemical Co., Saint-Louis, MO) was dissolved in an isotonic saline solution (0.9% sodium chloride in water). Nicotine doses were expressed as the weight of the base. Nicotine was injected intraperitoneally (i.p.) in a volume of 1 ml/kg body weight.

2.3. Nicotine conditioned place preference paradigm

Test chambers (40.64 width × 40.64 length × 30.5 cm height) were located in a dimly lit testing room adjacent to the colony room. A removable center barrier divided each chamber into two equal sized compartments, which were easily distinguishable by distinctive visual and tactile cues. On one side, the walls and the lid were white and the floor was smooth. On the other side, the walls and the lid were black and white striped, and the bottom was covered with a textured metal floor. Initially, a pretest was conducted on PND 34 in periadolescents and PND 66 in adults to determine the initial preference to both sides of the chamber. The amount of time spent on each side of the box was recorded during a 30-minute test session that occurred during the middle of the day. There were no significant differences in initial preference for one side over the other across groups. The following day marked the beginning of the conditioning phase. This phase was carried out over three consecutive days (from PND 35 to PND 37 in periadolescents and from PND 67 to PND 69 in adults), each of which consisted of morning and afternoon training sessions. In the morning, rats were injected with saline and then confined to one side by a dividing barrier for 30 min. In the afternoon, they received an injection of nicotine in the opposite side for 30 min. Morning and afternoon sessions were separated by at least 4 h. A range of nicotine doses was tested (0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg/kg, i.p.) and different groups of rats were used for each dose (n = 8–16/group). This training schedule was chosen instead of training saline and nicotine on separate days because of the constraints involved in doing developmental studies, as has been described elsewhere (Badanich and Kirstein, 2004; Balda et al., 2006; Brenhouse and Andersen, 2008; Zakharova et al., 2009a,c). On day 5, the testing phase occurred in the middle of