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Original Investigation

Behavioral Effects of Nicotine Withdrawal Differ by Genetic Strain in Male and Female Adolescent Rats

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Abstract

Introduction: Gender and ethnicity are powerful predictors of initiation and maintenance of cigarette smoking in adults but less is known about their role in smoking in adolescents. Consistent with human studies, rat models also reveal sex and strain differences in response to nicotine administration.

Methods: This research examined nicotine withdrawal behaviors in 96 adolescent, male and female, Sprague Dawley (SD) and Long Evans (LE) rats. Rats received seven days continuous subcutaneous infusion of saline or 3.16 mg/kg nicotine via Alzet osmotic minipumps. Behavioral observations were made before, during, and after saline or nicotine administration. Occurrences of six specific behaviors were quantified: abnormal posture or movement, abnormal grooming, whole-body shakes, ptosis, empty-mouth chewing/teeth chattering, and diarrhea.

Results: SD male and female rats that received nicotine displayed significantly more withdrawal behaviors 1 and 2 days after cessation of nicotine administration compared with rats that had received saline. LE male rats that received nicotine displayed significantly more withdrawal behaviors 1 day but not 2 days after cessation of nicotine administration compared with males that received saline. LE females showed no significant withdrawal behaviors after cessation of nicotine administration.

Conclusion: Results indicate that nicotine withdrawal in adolescent rats depends on sex and strain.

Introduction

Despite the well-known health hazards of tobacco use, roughly 3,600 U.S. adolescents try smoking each day and 1,100 go on to become regular smokers (Substance Abuse and Mental Health Services Administration [SAMHSA], 2008). As 90% of adult smokers initiated and continued smoking behavior before the age of 21 (Mowery, Brick, & Farrelly, 2000), adolescence is a vital period for prevention and cessation efforts. Gender, race, and ethnicity differentially affect smoking behavior in adolescents, suggesting possible genetic differences in the effects of nicotine, the primary addictive agent in tobacco (Kandel, Kiros, Schaffran, & Hu, 2004).

Youth are particularly susceptible to nicotine's addictive effects even after smoking just a few times (DiFranza et al., 2000) or after one cigarette (DiFranza, 2007). Horn et al. (2003) reported that 80% of the 365 adolescent smokers in their study had a physical nicotine dependence but 20% did not, a fact that underscores the role of physical nicotine dependence in adolescent smoking. Several models exist to explain the mechanisms of addiction to nicotine and why individuals might differ in response to this substance (e.g., Abreu-Villaça et al., 2003). Pomerleau, Collins, Shiffman, and Pomerleau (1993) suggested that vulnerability to nicotine addiction is a matter of individual sensitivity to nicotine. Audrain-McGovern, Lerman, Wileyto, Rodriguez, and Shields (2004) suggested that individual sensitivity to the effects of nicotine might result from genetic variants of dopamine transporters and dopamine receptors. In addition, exposure to nicotine causes an increase in nicotinic cholinergic receptors in the brain structures associated with the reward pathway (Schwartz and Kellar, 1985). Individual differences in nicotinic cholinergic receptor upregulation may underlie individual differences in nicotine sensitivity and dependence.

Gender and ethnicity also are related to cigarette smoking behavior (Blitstein, Robinson, Murray, Klesges, & Zbikowski, 2003; Mermelstein, 1999). About 22.3% of adult men smoke cigarettes, whereas 17.4% of adult women smoke (Center for Disease Control and Prevention [CDC], 2008). In terms of ethnicity, the CDC (2008) reported markedly different smoking prevalence by ethnic group: American Indians/Alaska Natives: 36.4%; Caucasians: 21.4%; African Americans: 19.8%; Hispanics: 13.3%; Asians: 9.6%. Ethnic and racial differences in nicotine metabolism have been reported in adults (Derby et al., 2008; Pérez-Stable, Herrera, Jacob, & Benowitz, 1998) and adolescents (Moolchan, Franken, and Jasyzna-Gasior, 2006).

Study of nicotine withdrawal is a useful way to compare an important aspect of addiction (United States Department of Heath and Human Service, 1988) and is relevant to develop effective treatment strategies (American Psychiatric Association,

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2000; Fagerström and Schneider, 1989). Nicotine withdrawal symptoms in humans include irritability, cigarette craving, cognitive and attentional deficits, sleep problems, depression, restlessness, and increased appetite (National Institute of Drug Abuse, 2006) symptoms that have been categorized by Leventhal et al. (2007) into subjective and objective measures. Human studies have established that adult and adolescent smokers display symptoms of withdrawal after cessation of nicotine administration (Colby, Tiffany, Shiffman, & Niaura, 2000; DiFranza et al., 2000, 2007; Horn, Fernandes, Dino, Massey, & Kalsekar, 2003; Hurt et al., 2000; Moolchan et al., 2005; O'Loughlin, Kishchuk, DiFranza, Temblay, & Paradis, 2002; Panday, Reddy, Ruiter, Bergström, & de Vries, 2007). Nicotine withdrawal in daily adolescent smokers was strongly and prospectively associated with level of nicotine dependence (Bailey et al., 2009). However, Smith et al. (2008) reported that adolescent smokers experience few symptoms of nicotine withdrawal while abstinent. With regard to sex differences in adolescents, it has been reported that adolescent female smokers experience higher levels of nicotine withdrawal upon cessation than males (Panday et al., 2007), but it also has been reported that levels of nicotine withdrawal symptoms are similar in male and female adolescent smokers (Dickman et al., 2009). It is difficult to tease apart biobehavioral effects of nicotine from psychosocial effects and expectations in human smokers. It also is logistically and ethically challenging to conduct invasive experiments with nicotine in adolescent humans.

Malin et al. (1992) developed a rodent model of nicotine withdrawal in which behavioral signs of withdrawal are observed upon cessation of continuous nicotine administration. Observed withdrawal behaviors included whole-body shakes, abnormal grooming, abnormal posture or movement, and empty-mouth chewing or teeth chattering. Abnormal grooming was vigorous grooming that lasted for 10 second, with each 10-second session of abnormal grooming counted as one episode. Abnormal posture or movement included writhing or twisting of the body while in a sitting or standing position. All the nicotine withdrawal symptoms observed in this rat model were somatic behavioral signs, which limited the model's external validity as nicotine withdrawal in humans involves many subjective symptoms (Hughes, 2007; Malin, 2001). However, the internal validity of this model is strong—nicotine withdrawal behaviors occur in response to cessation of continuous nicotine administration and are attenuated by administration of nicotine and buproprion (Cryan, Bruijnzeel, Skjei, & Markou, 2003; Hamilton, Berger, Perry, & Grunberg, 2009; Malin et al., 1992). Additionally, this model has produced consistent results across a number of experiments of nicotine withdrawal from the Malin group (Malin, 2001; Malin, 1993, 1994, 1998) and other laboratories (Carboni, Bortone, Guia, &Chiara, 2000; Epping-Jordan, Watkins, Koob, & Markou, 1998; Hamilton et al., 2009; Hildebrand, Nomikos, Bondjers, Nisell, & Svensson, 1997; Hildebrand, Nomikos, Hertel, Schilstrom, & Svensson, 1998; Kota, Martin, & Damaj, 2008; Kota, Martin, Robinson, & Damaj, 2007; O'Dell, Bruijnzeel, Ghozland, Markou, & Koob, 2004; Phillips, Schechter, & Grunberg, 2004; Watkins, Stinus, Koob & Markou, 2000). An animal model is particularly valuable to examine adolescent nicotine withdrawal because administering nicotine and tobacco products to children would be potentially dangerous and unethical. Also, the animal model focuses on biobehavioral aspects of nicotine's actions and limits psychosocial variables and cognitive expectations.

Using the rodent model, nicotine withdrawal has been examined in male Wistar rats (Epping-Jordan et al., 1998; O'Dell et al., 2004; Hildebrand et al., 1997, 1998; Watkins et al., 2000), male Sprague Dawley (SD) rats (Carboni et al., 2000; Hamilton et al., 2009; Phillips et al., 2004; Watkins et al., 2000), female SD rats (Hamilton et al., 2009), and male and female mice (Kota et al., 2007, 2008). The rodent model of nicotine withdrawal has been used to examine age differences in male rats (O'Dell et al., 2004, 2006) and male and female mice (Kota et al., 2007, 2008) and sex differences in male and female adult rats (Hamilton et al., 2009). Strain differences in nicotine's effects on performance have been reported in SD and Wistar adult male rats (Semenova et al., 2007). However, no published studies have compared sex and strain differences in nicotine withdrawal in male and female adolescent rats.

In the present research, nicotine withdrawal was compared in adolescent male and female SD and Long Evans (LE) rats. These two strains were selected because they are both commonly used laboratory rat strains, but LEs differ substantially from the albino SDs because they are "hooded" rats with pigmented fur around the head and withers. Further, SD and LE rats differ in responses to chronic nicotine (Faraday, Blakeman, & Grunberg, 2005) but have not been examined for differences in nicotine withdrawal. The purpose of the present experiments was to determine whether strain and sex differences exist in adolescent nicotine withdrawal using a rat model.

Methods

Overview

The present research was conducted to examine nicotine withdrawal-related behaviors in male and female adolescent rats. The technique to measure withdrawal in the present work was based on Malin et al. (1992), but there were several key differences. In the Malin paradigm, rats are observed by observers who were blind to experimental conditions in a well-lit room in cages containing no bedding. Bright lights can be stressful for the albino rat because rats are nocturnal animals and albino rats are sensitive to bright lights (Lawlor, 2002; Russell, 2002). The empty cages in the Malin paradigm (i.e., no bedding) also may act as an environmental stressor and potentiate effects of cessation of nicotine administration. The paradigm used in the present experiments was modified from the Malin approach and was based on Phillips et al. (2004) and Hamilton et al. (2009). Specifically, rats were observed by observers who were blind to experimental conditions in a dimly lit room in cages with wood-chip bedding to mimic, as closely as possible, the animals' home cage environment. Data for the two strains were collected at two timepoints, with the SD data collected first, and the LE data collected in a replication. Because all conditions and procedures in the replication were identical to the initial experiment, it is unlikely that time-dependent effects altered the ability to detect strain differences.

Procedure

This experiment was conducted in three phases: baseline (predrug administration), drug administration, and withdrawal (postdrug administration).

Baseline Phase

The baseline phase consisted of 7 days predrug administration during which time there was no substance administered to the

animals. On Day 1, the animals arrived at the laboratory animal facility and were placed into their cages. Each rat was handled for approximately 5 minute on the first 4 days after arrival to minimize any stress of handling during experimental procedures (Chapillon, Patin, Roy, Vincent, & Caston, 2002; Levine, 2005; Tuli, Smith, & Morton, 1995). Open-field locomotor activity was measured by placing rats into individual electronic physical activity monitoring chambers of the Omnitech/Accuscan ElectronicsDigiscan infrared photocell system (Test box model RXYZCM [16 TAO]; Omnitech/Accuscan Electronics, Columbus, OH) for 1 hr. All animals were acclimated to the open-field chambers on Day 4 to minimize any stressful effects of exposure to a novel environment (Faraday & Grunberg, 2000) and were tested in the open-field chambers on Day 5. Observers were trained to recognize withdrawal behaviors during a pilot study; interrater reliability was ≥90%.

On Day 6 of the baseline phase, each animal was observed by two independent raters for 15 minutes in low-light conditions. Occurrences of six specific types of behavior were quantified by observers: abnormal posture or movement, abnormal grooming, whole-body shakes, ptosis, empty-mouth chewing/teeth chattering, and diarrhea. Abnormal postures or movements could include writhing or twisting of the body while in a sitting or standing position. Abnormal grooming is especially persistent (10 s without interruptions) or rough grooming behavior that may include chewing of the forepaws or other body parts and vigorous washing of the face and body. Every 10-s episode of abnormal grooming was recorded as a discrete occurrence of the behavior. In rats, ptosis is a slackening or relaxing of the facial muscles that can include a drooping of the eyelids. Empty-mouth chewing or teeth chattering is rapid chattering of the teeth or empty-mouth chewing. Each uninterrupted sequence of teeth chattering or empty-mouth chewing was recorded as a single event, even if it included several mouth movements. The behaviors counted in the nicotine withdrawal scale in the present experiment were among the range of behaviors captured by withdrawal scales used in previous studies (i.e., Hildebrand et al., 1997; Malin et al., 1992). Similar to the present study, most scales recorded five to six categories of withdrawal behaviors, including empty-mouth chewing/teeth-chattering, abnormal posture, and whole-body shakes (Hamilton et al., 2009; Hildebrand et al., 1997; Malin et al., 1992), though one scale recorded only four behavioral categories (Epping-Jordan et al., 1998) and some scales recorded additional behavioral categories such as escape attempts, sniffing, and foot licks (Carboni et al., 2000; O′Dell et al. 2004; Watkins et al., 2000).

The observation room was illuminated at 4.30 lx (Advanced Light Meter, Model No. 840022; Sper Scientific Ltd.) by a 60-watt light bulb. The raters observed and recorded spontaneous behaviors that are often seen during nicotine withdrawal. These ratings were used to establish baseline activity levels for each group of rats.

Drug Administration Phase

Twelve animals of each sex matched by body weight and baseline locomotor activity were assigned to the saline or nicotine conditions. On day 7 of the experiment, the animals were anesthetized individually in a plastic chamber with a continuous flow of oxygen (flow rate: 0.5 to 1.0 l/minute) and 2%–4% isoflurane gas into the chamber to induce anesthesia. The rats' anesthesia-induced unconscious state was maintained during the implant via a nose cone and tube that delivered a combination of 0.25%–3% isoflurane and oxygen from the induction chamber. ALZET osmotic minipumps (Model 2001; DURECT Corporation, Cupertino, CA) filled with nicotine hydrogen tartrate (bitartrate) solution or 0.9% NaCl (physiological saline) were implanted SC between the withers, based on procedures of Grunberg (1982). The order of the surgical procedures was counterbalanced in order to alternate nicotine and saline minipump implantation.

The minipumps delivered 3.16 mg/kg of nicotine bitartrate or saline and remained in the animals for 7 days. A nicotine dose of 3.16 mg/kg for 1 week followed by explant or accompanied by the administration of a nicotine pharmacologic antagonist reliably produces withdrawal behaviors in rats (Malin et al., 1992; O′Dell et al., 2004; Phillips et al., 2004). On the morning of the drug administration phase Day 7, behavioral observations were conducted in an identical manner to behavioral observations during the baseline phase. After behavioral observations, the drug administration phase ended with the surgical explant of the minipumps on Day 14 of the experiment (7 days after implant), following a procedure similar to the implant.

Withdrawal Phase

After 7 days of nicotine or saline administration, animals were anesthetized (following the procedure described above) and minipumps were explanted. The withdrawal phase began immediately after pump explant and open-field activity was measured 17.5 hr after pump removal for a period of 1 h to monitor general locomotion. Withdrawal behavior observations occurred 20 hr after pump removal, an optimal time to observe nicotine withdrawal behaviors in rats (Malin et al., 1992; O′Dell et al., 2004; Phillips et al., 2004). Withdrawal phase observations were conducted in a manner that was identical to that in which the baseline and drug administration phase observations were conducted. The procedure was repeated 24 hr later for the second day of withdrawal.

Experiment 1: Male and Female Adolescent SD Rats

The subjects were 48 SD adolescent male ($n = 24$) and female ($n = 24$) rats obtained from Charles River Laboratories (Wilmington, MA). Rats were approximately 21–28 postnatal days old at the beginning of the experiment and were approximately 28–35 days postnatal when nicotine pumps were implanted. Adolescence in rats spans postnatal days 28–42 (Spear, 2000). Each animal was housed singly in a standard polycarbonate rat cage (42 × 20.5 × 20 cm) with hardwood chip bedding (Pine-Dri) and unrestricted access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained at 23°C and 50% relative humidity on a 12-hr reversed light/dark cycle (lights on at 0700 and off at 1900 hr). Because the rat is a nocturnal animal, the reversed light cycle was maintained to match the animals' high-activity period with the researchers' daytime observation period. This experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (National Institutes of Health Guide for Care and Use of Laboratory Animals, 1996).

Experiment 2: Male and Female Adolescent LE Rats

The subjects were 48 LE male ($n = 24$) and female ($n = 24$) adolescent rats, obtained from Charles River Laboratories. Rats were approximately 21–28 days postnatal at the start of the experiment and were approximately 28–35 days old at the start of the nicotine phase. LE rats were used because they differ from the commonly used SD strain in their reactivity to stress (Faraday, 2002) and nicotine administration (Faraday et al., 2005) and their acquisition of nicotine self-administration (Shoaib, Schinder, and Goldberg, 1997). While rat strain differences are not analogous to human ethnic differences, research using different rat strains may reveal the role of genetic differences in drug actions and responses. Housing conditions and experimental procedures in Experiment 2 were identical to those in Experiment 1.

Results

Data Analytic Strategy

Behavioral signs of withdrawal were analyzed by repeated measures analyses of variance (ANOVAs) and by analyses of covariance (ANCOVAs) accounting for baseline behaviors, with baseline behaviors as the covariate. Additionally, repeated measures ANOVAs and ANCOVAs were conducted within sex. ANOVAs also were used to analyze open-field locomotor activity before and after drug administration. All statistical analyses were two tailed, with an α level of $p < .05$.

Experiment 1: Behavioral Signs of Withdrawal in SD Rats

Behavioral signs of withdrawal were measured at four time points during the experiment: before drug administration (baseline), during drug administration, and 1 and 2 days after cessation of nicotine or saline (i.e., "Withdrawal Day" 1 or 2).

Rats that received nicotine had more behavioral signs of withdrawal than rats that received saline, $F(1, 44) = 21.53$, $p < .001$, behavioral signs of withdrawal changed across behavioral observation timepoints, $F(1, 44) = 37.45$, $p < .001$, and a drug × time interaction occurred in which behavioral signs of withdrawal changed differentially between drug conditions across observation timepoints, $F(1, 44) = 10.68$, $p < .001$. More specifically, rats that had received nicotine displayed significantly more behavioral signs of withdrawal compared to rats that had received saline on Withdrawal Day 1, $F(1, 44) = 23.72$, $p < .001$, and on Withdrawal Day 2, $F(1, 44) = 20.18$, $p < .001$. Effects of withdrawal after cessation of nicotine remained robust after accounting for baseline behaviors, $F(1, 44) = 21.96$, $p < .001$]. There were significant sex differences in basal withdrawal behaviors both at baseline, $F(1, 46) = 23.586$, $p < .001$, and during the nicotine administration phase, $F(1, 46) = 113.90$, $p < .01$, with males showing more withdrawal behaviors than females. However, the sex difference disappeared during the withdrawal phase. There were no significant sex differences in withdrawal on either withdrawal day. Group differences are depicted in Figure 1.

SD male rats that had received nicotine had more withdrawal behaviors than SD male rats that had received saline, $F(1, 22) = 11.04$, $p < .01$. In addition, withdrawal behaviors varied over time in SD males, $F(3, 66) = 7.27$, $p < .001$. SD males that had received nicotine had more withdrawal behaviors than SD males that had received saline on Withdrawal Day 1, $F(1, 22) = 6.85$, $p < .05$, and Withdrawal Day 2, $F(1, 22) = 16.70$, $p < .001$. Effects of withdrawal after cessation of nicotine remained robust in SD males after accounting for baseline behaviors, $F(1, 21) = 4.22$, $p < .052$.

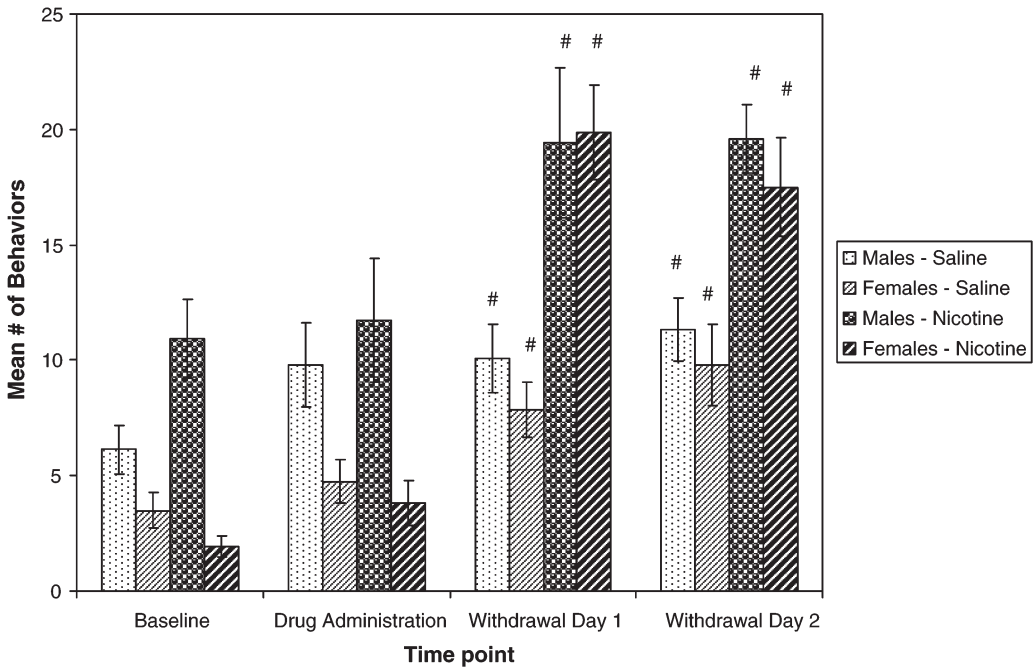

Figure 1. Mean number of total withdrawal symptom behaviors (mean ± *SEM*) observed in all Sprague Dawley rats across four 15-min observation periods. The "#" symbol denotes significance when compared to the same-sex control condition.

SD females that had received nicotine had more withdrawal behaviors than SD females that had received saline, $F(1, 22) = 11.16$, $p < .01$. Nicotine withdrawal behaviors varied across observation times in SD females, $F(3, 66) = 46.05$, $p < .001$. Additionally, there was a time × drug condition interaction in SD females, $F(3, 66) = 14.34$, $p < .001$. Greater withdrawal behaviors occurred in SD female rats that had received nicotine after cessation of nicotine administration than occurred in SD females that had received saline, $F(3, 66) = 46.05$, $p < .001$. SD females that had received nicotine had more withdrawal behaviors on Withdrawal Day 1, $F(1, 22) = 25.79$, $p < .001$ and Withdrawal Day 2, $F(1, 22) = 7.75$, $p < .05$. Effects of withdrawal behaviors changing over time, $F(2, 42) = 20.02$, $p < .001$, more withdrawal behaviors occurring in SD females that had received nicotine, $F(1,21) = 20.27$, and withdrawal behaviors changing differentially between drug conditions over time in SD females, $F(2, 42) = 11.83$, $p < .001$, remained when accounting for baseline behaviors by using them as a covariate in ANCOVA.

Experiment 1: Locomotor Activity in SD Rats

Open-field locomotor activity was similar for rats before saline or nicotine administration. After cessation of drug, rats that had received nicotine were less active than rats that had received saline, $F(1, 44) = 7.08$, $p < .05$. Therefore, the increased behavior displayed for the nicotine cessation rats could not be explained by a change in general motor activity.

Within SD males, there were no baseline locomotor activity differences in rats that would later receive nicotine or saline. After drug cessation, male SDs that had received nicotine had less activity than males that had received saline, $F(1,21) = 4.47$, $p < .05$. Within SD females, there were no drug group differences in locomotor activity in the baseline or withdrawal open-field locomotor activity measurements.

There was a significant sex difference in locomotor activity, with SD females having more locomotor activity than SD males at both the Baseline, $F(1, 44) = 9.521$, $p < .01$, and Withdrawal Day 1, $F(1, 44) = 7.322$, $p < .05$, measurements. Male and female rats also differed in Baseline center time, $F(1, 46) = 7.530$, $p < .01$, with female SD rats having more baseline center time than male SD rats. Male and female SD rats did not differ on Withdrawal Day 1 center time.

In addition, in SD males, there was a significant inverse relationship between Withdrawal Day 1 locomotor activity and Withdrawal Day 2 withdrawal behaviors ($r = -.424$, $p < .05$), although there was no relationship between Withdrawal Day 1 locomotor activity and Withdrawal Day 1 withdrawal behaviors. For SD females, there was a significant inverse relationship between Withdrawal Day 1 locomotor activity and Withdrawal Day 1 withdrawal behaviors ($r = -.438$, $p < .05$), although there was no relationship between Withdrawal Day 1 locomotor activity and Withdrawal Day 2 withdrawal behaviors. There were no sex differences in SD rats in withdrawal behaviors. While correlations occurred, they cannot explain nicotine withdrawal in SD adolescent male and female rats.

Experiment 2: Behavioral Signs of Withdrawal in LE Rats

In LE rats, behavioral signs of nicotine withdrawal varied across observation timepoints, $F(1, 44) = 27.55$, $p < .001$, varied differentially between the sexes across observation timepoints, $F(1, 44) = 4.75$, $p < .05$, and varied differentially among sex and drug condition groups across observation timepoints, $F(1, 44) = 7.43$, $p < .001$. Male LE rats that had received nicotine displayed significantly more withdrawal behaviors than male rats that had received saline on Withdrawal Day 1, $F(1, 22) = 12.66$, $p < .05$. On Withdrawal Day 2, there was no difference between male LE rats administered nicotine and saline. In contrast, for female LE rats, there was no effect of drug condition on withdrawal behavior on Withdrawal Day 1 or Withdrawal Day 2. Group differences are depicted in Figure 2.

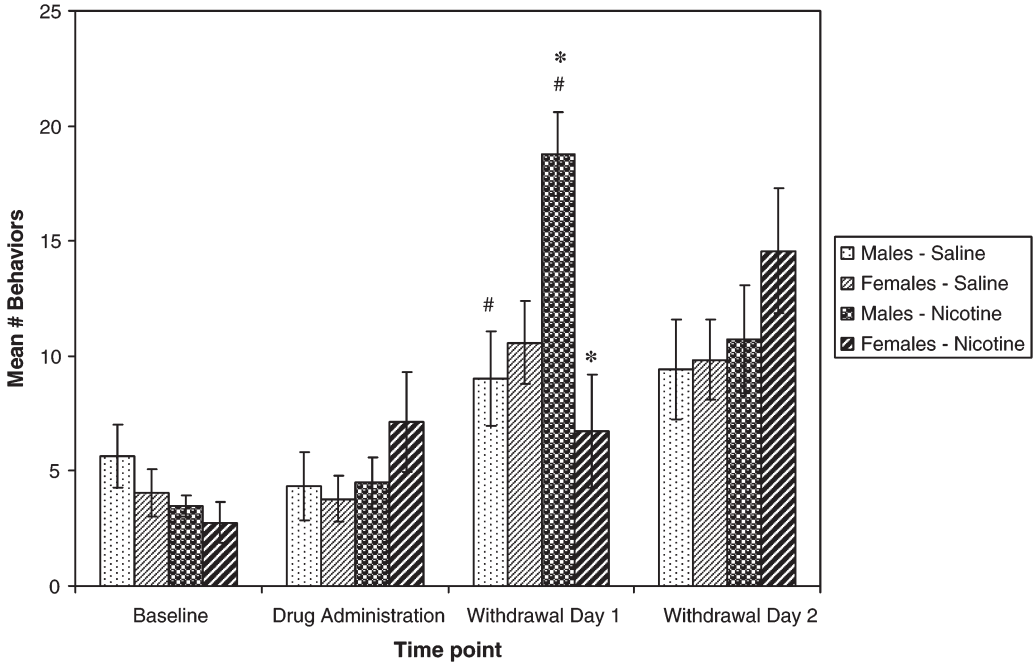

Figure 2. Mean number of total withdrawal symptom behaviors (mean ± *SEM*) observed in all Long Evans rats across four 15-min observation periods. The "#" symbol denotes significance when compared to the same-sex control condition, and the "*" symbol denotes significance when compared to the opposite sex within the same drug condition.

Locomotor Activity in LE Rats

Open-field locomotor activity was similar before saline or nicotine administration. There was a sex difference in Withdrawal Day 1 locomotor activity, $F(1, 44) = 21.032$, $p < .001$, with female rats having more locomotor activity than male rats. There was a sex difference in Baseline center time, $F(1, 45) = 5.985$, $p < .05$, and Withdrawal Day 1 center time, $F(1, 46) = 12.353$, $p < .01$, with females having more center time at both measurements. Male LE rats that had received nicotine were less active than saline cessation animals during the withdrawal period, $F(1, 22) = 17.756$, $p < .001$. Female LE rats displayed more activity during the withdrawal period than at baseline regardless of condition, $F(1, 22 = 30.24$, $p < .001$. Therefore, general activity could not account for differences in withdrawal behaviors. In addition, there were no significant correlations between Withdrawal Day 1 locomotor activity and Withdrawal Day 1 and 2 withdrawal behaviors in LE male and female rats.

General Discussion

The degree to which adolescents display withdrawal behaviors after cessation of tobacco use and the circumstances under which they exhibit withdrawal is an understudied area for several reasons. Previous research has largely focused on youth tobacco prevention and adult tobacco cessation (Backinger, Fagan, Matthews, & Grana, 2003). Additionally, the ethics of conducting invasive, controlled experiments (i.e., randomly manipulating exposure to tobacco or nicotine) to examine tobacco use and withdrawal in adolescents make research in this area difficult. For these reasons, an animal model is a particularly valuable tool to examine nicotine withdrawal in adolescents.

The present findings reveal significant nicotine withdrawal behaviors in adolescent rats that vary with sex and genetic strain. Locomotor activity results indicate that the increases in withdrawal behavior did not result from a general increase in activity. In fact, reduced locomotor activity during nicotine cessation in SD rats that had received nicotine is consistent with one of the originally noted rat nicotine withdrawal phenomena, reduced locomotor activity (Malin et al., 1992). Adolescent SD male and female rats showed significant effects of nicotine withdrawal when compared to adolescent SD males and females administered saline. The withdrawal effects persisted for 2 days after cessation of nicotine and there were no significant sex differences in nicotine withdrawal. In male SD rats, locomotor activity on Withdrawal Day 1 was significantly inversely correlated with withdrawal behavior, while it was significantly inversely correlated with Withdrawal Day 1 withdrawal behavior in female SD rats. However, because there were no sex differences in withdrawal behavior in SD rats, the correlations do not explain withdrawal behaviors.

In contrast, LE adolescent rats showed sex differences in nicotine withdrawal. Male LE rats administered nicotine displayed withdrawal behaviors 1 day after nicotine that were comparable to the nicotine withdrawal behaviors observed in SD rats. Unlike SD rats, however, there were no significant withdrawal effects in male LE rats on the second day of nicotine administration cessation. Female adolescent LE rats displayed no significant effects of withdrawal on either day after cessation of nicotine administration. Additionally, Withdrawal Day 1 locomotor activity was not correlated with withdrawal behavior on either Withdrawal day in LE male and female rats.

Although direct rat strain comparisons between SD and LE rats were not made in previous research, behavioral signs of nicotine withdrawal have been reported in adult male Wistars (Epping-Jordan et al., 1998; Hildebrand et al., 1997; Watkins et al., 2000), adult male SD rats (Carboni et al., 2000; Hamilton et al., 2009; Watkins et al., 2000), and adult female SD rats (Hamilton et al., 2009). These results are consistent with behavioral signs of nicotine withdrawal in male and female adolescent SD rats and male LE rats in the present research but inconsistent with the absence of withdrawal signs in LE adolescent females.

Studies directly comparing nicotine withdrawal in adult and adolescent rats (O′Dell et al., 2004, 2006) reported that withdrawal was lower in adolescents than adults. While nicotine withdrawal was reduced in adolescents in previous research, the present results indicate that nicotine withdrawal occurs in adolescents and differs by sex and strain. Interestingly, in a direct comparison of nicotine withdrawal in adult and adolescent male Wistar rats using a nicotine receptor antagonist (O′Dell et al., 2004, 2006), it was reported that only adult Wistar males displayed somatic behavioral signs of withdrawal, while adolescent Wistar males did not. The absence of behavioral signs in adolescent Wistar males (O′Dell et al., 2004, 2006) is consistent with the absence of behavioral signs of withdrawal in LE adolescent females but is inconsistent with behavioral signs observed in SD males and females and LE males in the present research. These differences highlight the important influences of sex, strain, and age on nicotine withdrawal. In previous research, we reported sex differences in the effects of environment on nicotine withdrawal in SD adult rats (Hamilton et al., 2009). Males displayed less nicotine withdrawal in a dimly lit environment than in a brightly lit environment, while females displayed similar amounts of withdrawal behavioral signs in both environments. In the present research, all adolescent rats were examined in a dimly lit environment and a sex difference occurred in LE rats. While sex differences emerged in the dimly lit environment in the previous and present experiments, the differences were in the opposite direction, with male adolescent LE rats displaying more behavioral signs of withdrawal than female LE rats. Patterns of nicotine withdrawal differed in our previous and current research by sex and strain in adults and adolescents. The different patterns that emerged underscore the importance of age, sex, and strain differences in rat models of nicotine withdrawal. Research should consider effects of environmental manipulations on nicotine withdrawal in rats of different sex, age, and genetic strains. It may be that the environmental conditions act as additional stressors or that particular environments act as conditioned cues.

Interestingly, Faraday (2002) reported sex and strain differences in response to stress among male and female SD and LE rats. Adult male and female SD rats and adult male LE rats displayed more vulnerability to stress than did female LE rats. If abstinence from nicotine is a stressor in rats, then the results of the present experiment and Faraday (2002) are consistent; male SD, female SD, and male LE adolescent rats displayed nicotine withdrawal, but female LE adolescent rats did not. However, because hormonal stress responses were not measured in the present experiment, conclusions about stress after nicotine cessation cannot be made.

Although hypothalamic-pituitary-adrenal (HPA) axis responses were not measured in the present research, center time was collected during locomotor activity measurements. The center time parameter provides an index of anxiety, with more center time indicating less anxiety. In the present research, females had more center time than males, with SD females having more center time than SD males at the BL measurement, and LE females having more center time than LE males at both measurements. Therefore, during nicotine withdrawal, LE females were less anxious than LE males, which is consistent with the report of less stress reactivity in LE females (Faraday, 2002).

Potential Limitations

A limitation of the present research is that adult comparison groups were not included. For this reason, direct comparisons of the relative sensitivity of adolescents and adults to nicotine withdrawal cannot be made. In addition, rates of nicotine metabolism were not examined in the present research but may have contributed to observed differences in nicotine withdrawal. Male and female rats metabolize nicotine differently, with female rats having a reduced rate of nicotine metabolism and a larger volume of distribution of nicotine when compared to male rats (Kyerematen et al., 1988). Adolescent rats have an increased rate of nicotine metabolism compared to adult rats (Trauth, Seidler, & Slotkin, 2000). Additionally, possible strain differences in metabolism also could have contributed to strain differences in withdrawal. Rats were observed during the optimal time period for observing nicotine withdrawal in adult male SD rats, as reported by Malin et al. (1992). The reported differences in metabolism and distribution raise the possibility that the optimal time period for observing nicotine withdrawal in females and adolescents may differ from the optimal time for observing withdrawal behaviors in adult males.

Summary and Implications

The current research reveals that individual differences, specifically genetics and sex, are relevant when considering nicotine withdrawal in adolescent rats. If the present results extrapolate to humans, then they suggest that genetic and sex differences merit increased attention to understand tobacco use among adolescents. Optimal pharmacological and nonpharmacological approaches may differ to help boys and girls with different genetic backgrounds successfully abstain from tobacco use.

It seems that "one size" does not "fit all" with regard to understanding and perhaps treating nicotine withdrawal in adolescents. These findings suggest that prevention and cessation treatment approaches must account for individual differences. The current findings are consistent with a focus on pharmacogenomics to prevent and treat tobacco use in youth.

Future Directions

In the present research, adolescent LE females showed fewer behavioral signs of nicotine withdrawal suggesting less sensitivity to the withdrawal effects of nicotine. Future research should focus on why this strain and sex is relatively insensitive to nicotine withdrawal. Future research should examine whether there are differences in nicotine receptors, nicotine metabolism, or physiological responses to nicotine, such as HPA axis reactivity. Faraday (2002) reported sex and strain differences in HPA axis response to stress among male and female SD and LE rats, with less stress reactivity in female LE rats. If the absence of nicotine in nicotine-addicted individuals is stressful, then less stress reactivity in female LE rats may explain why they showed less nicotine withdrawal. In fact, during withdrawal, female LE rats were less anxious than male LE rats. Future research on hormonal stress responses during nicotine withdrawal in LE and SD rats is needed. In addition, in light of recent findings regarding sex differences in nicotine withdrawal in adult male and female SD rats observed in different environments (Hamilton et al., 2009), research also should examine effects of the environment on nicotine withdrawal in adolescents.

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Declaration of Interests

None declared.

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