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Pharmacology, Biochemistry and Behavior 80 (2005) 567–575

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Adolescent and adult female rats differ in sensitivity to nicotine's activity effects

Brenda M. Elliott*, Martha M. Faraday, Jennifer M. Phillips, Neil E. Grunberg

Department of Medical and Clinical Psychology, Uniformed Services University of the Health Sciences (USUHS), 4301 Jones Bridge Road, Bethesda, MD 20814, USA

Received 20 May 2004; received in revised form 19 October 2004; accepted 17 January 2005

Abstract

More than 90% of cigarette smokers begin smoking during adolescence. This between-subjects repeated-measures experiment examined: (1) nicotine's acute effects on activity in adolescent and adult female Sprague–Dawley rats (Drug Phase I); (2) the effects of age of initial nicotine exposure on activity when nicotine was not administered (Interim Phase); and (3) the effects of age of initial nicotine exposure on later responses to nicotine (Drug Phase II). The experiment consisted of three separate phases. In Drug Phase I, animals were administered either 0 (saline), 0.01, 0.10, 0.50, or 1.0 mg/kg nicotine via subcutaneous injections for 12 days and horizontal activity was measured daily. During the Interim Phase (no drug phase), activity was measured but nicotine was not administered. During Drug Phase II, the same animals were administered the same nicotine dosages as in Drug Phase I for 12 days and activity was measured daily. Drug Phase I revealed dose–response differences between adolescent and adult female rats. In addition, animals initially exposed to nicotine in adolescence exhibited greater sensitivity to nicotine's activity-increasing effects than did females initially exposed to nicotine in adulthood (i.e., Drug Phase II).

Keywords: Nicotine; Adolescence; Locomotor activity; Female rats

1. Introduction

Over 3000 adolescents start smoking every day in the United States (Gilpin et al., 1999; Mowery et al., 2000). The initiation of smoking generally begins in adolescence with greater than 90% of adult smokers reporting first use prior to age 18 (USDHHS, 1994; Chassin et al., 1990; Dappen et al., 1996; Mowery et al., 2000). Individuals who start smoking during adolescence have greater addiction liability and greater difficulty quitting than do individuals who delay nicotine use until adulthood (Chassin et al., 1990; Chen and Millar, 1998; Taioli and Wynder, 1991; Colby et al., 2000; Kendel and Chen, 2000). These facts suggest that adolescence is an important period for the initiation and maintenance of tobacco use. Explanations for why adolescence is important in this context are less clear and likely involve several interrelated factors (i.e., social, environmental, and psychological) (Petraitis et al., 1995; Conrad et al., 1992).

The results of animal studies are consistent with the observation that the initiation of tobacco use during adolescence may be associated with higher smoking rates and greater difficult quitting (Trauth et al., 2000a; Levin et al., 2003). Rats first exposed to nicotine during adolescence self-administer higher levels of nicotine in adulthood compared to rats which begin nicotine self-administration in adulthood (Levin et al., 2003). Other studies with animals suggest that during adolescence, even a brief period of nicotine exposure elicits nAChR upregulation in brain regions associated with nicotine dependence. These changes may make the adolescent brain specifically sensitive to the effects of nicotine (Abreu-Villaca et al., 2003).

Recent animal studies also indicate age differences in nicotine's behavioral effects. For example, recently we reported that chronically-administered nicotine (via osmotic minipump) had greater activity-stimulating effects in adolescent male rats than in adult male rats, and that nicotine

* Corresponding author. Tel.: +1 301 295 9670; fax: +1 301 295 3034.
E-mail address: belliott@usuhs.mil (B.M. Elliott).

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doi:10.1016/j.pbb.2005.01.019

exposure resulted in long-term hyperactivity (in the absence of nicotine) in adolescent males but not in adult males (Faraday et al., 2001). Similar effects were found when nicotine was repeatedly administered via subcutaneous injections to male rats (Faraday et al., 2003). Specifically, initial exposure to nicotine during adolescence, but not during adulthood, resulted in hyperactivity in adulthood in the absence of nicotine. Further, male rats first exposed to nicotine as adolescents exhibited greater sensitivity to nicotine's activity-enhancing effects when nicotine was readministered in adulthood compared to rats that were first exposed to nicotine during adulthood (Faraday et al., 2003). The present experiment extends these findings by examining responses of female adolescent and adult rats to repeated nicotine administration via daily subcutaneous injections.

Sex differences in nicotine's effects have been widely reported (Benowitz and Hatsukami, 1998; Grunberg et al., 1991). Among adolescents, the prevalence of cigarette smoking during adolescence is approximately equal across girls and boys (Wagner, 2000). However, adolescent female smokers generally show less dependence, more severe withdrawal symptoms, and greater difficulty quitting than do their male counterparts (Perkins, 1996). These differences may be explained by gender differences in reasons for smoking or for quitting smoking. It also is possible that these reported gender differences reflect differences between males and females in nicotine sensitivity and the consequences of initial exposure during adolescence.

The purposes of this experiment were to: (1) compare effects of repeated nicotine administration on locomotor behavior in adolescent vs. adult female rats; (2) examine the effects of age of initial nicotine exposure (adolescence vs. adulthood) on locomotion responses when nicotine was not administered; (3) determine whether initial nicotine exposure in adolescence vs. adulthood altered locomotion responses to subsequent nicotine administration; and (4) determine whether nicotine's effects in adolescent and adult females are similar to previous reports of nicotine's effects in adolescent and adult males. Locomotion–horizontal activity–was selected because it is a widely-used behavioral index of nicotine's actions (in particular, the development of tolerance and sensitization), and substantial research literature is available in male and adult rats for comparison.

2. Methods

2.1. Subjects

Subjects were 80 female Sprague–Dawley rats, 40 adolescents and 40 adults (Charles River Laboratories, Wilmington, MA). Animals were housed in same-age groups of two or three in standard polycarbonate shoebox cages (42×20.5×20 cm) on hardwood chip bedding (Pine-Dri). Throughout the study animals had continuous access to rodent chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained at 23 °C and 50% relative humidity on a 12-h reverse light/dark cycle (lights on at 17:00 h). Locomotor activity was measured during the dark (active) phase of the light cycle (between 07:00 and 14:00 h). At the beginning of the experiment, adolescent animals were about 25 days old and adult animals were about 55 days old. Adolescence in rats spans the period of 28 to 42 days old (Spear, 2000). The experiment was conducted as a 2 (adult or adolescent)×5 (saline, 0.01, 0.10, 0.50, or 1.0 mg/kg nicotine) full factorial design, with 8 subjects per treatment cell. This experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee and was conducted in full compliance with the NIH Guide for Care and Use of Laboratory Animals (NIH Pub. 85-23, rev. 1985).

2.2. Equipment

Locomotor activity was measured using an Omnitech Electronics Digiscan infrared photocell system (Test box model RXYZCM [16 TAO]; Omnitech Electronics, Columbus, OH), located in a dedicated room. This room is constructed of cinderblock walls, acoustic tile ceiling, and steel doors so that outside sound is kept to a minimum. Animals were placed singly in a 40×40×30 cm clear Plexiglas arena. A Plexiglas lid with multiple 3.5 cm diameter ventilation holes was placed on top of the arena. A photocell array measured horizontal locomotor activity using 16 pairs of infrared photocells located every 2.5 cm from side-to-side and 16 pairs of infrared photocells located front-to-back in a plane 2 cm above the floor of the arena. Data were automatically gathered and transmitted to a computer via an Omnitech Model DCM-I-BBU analyzer. The apparatus monitored animal activity continuously with data recorded as cumulative activity every 5 min for a total testing period of 1 h. Once subjects were placed in the test arenas, the experimenter turned off the lights and left subjects undisturbed during the testing period. Cage mates always were removed from the cage within 30 s of one another and tested at the same time (in separate chambers) in order to avoid any within-cage order effects. Testing arenas were cleaned with a 50% ethanol solution between subjects.

2.3. Drug administration

Nicotine (0.01, 0.10, 0.50, or 1.0 mg/kg; expressed as nicotine base) or physiologic saline was injected subcutaneously between the shoulder blades. These dosages were selected to span those commonly reported in the research literature and to parallel our previous study with male rats (Faraday et al., 2003). Two different needle gauges were used. During Drug Phase I (see below), 30 gauge needles were used with adolescents and 25 gauge needles were used with adults. The smaller gauge needle was used with adolescents to minimize animal discomfort. During Drug Phase II, when adolescents were adults, 25 gauge needles

were used for all animals. Physiological saline also was used as vehicle for the nicotine solution. Nicotine solutions were made from nicotine dihydrochloride and were pH-adjusted to physiologic saline pH using Na_2PO_4. Nicotine solution was made from nicotine dihydrochloride (MW=235.13) and is expressed as nicotine base. All injection volumes were 1 ml/kg. Injections were given in the procedure rooms in which testing took place. Testing immediately followed the injections.

2.4. Estrus cycle staging

To determine estrus cycle staging for adolescent and adult female rats, vaginal smears were performed to determine whether rats were in estrus, metestrus, diestrus, or proestrus. Estrus staging was performed after measures of locomotor activity were completed on each day. The tip of a sterile, Dacron tipped swab was inserted into 1 mm of rat's vagina. Cells from the vaginal epithelium were then removed from the vagina and transferred to a labeled glass microscope slide (Emery and Schwabe, 1936; Jerse, 1998). Each slide was fixed using a Hema 3 stain set® and viewed under a light microscope at 40× magnification. Consistent with the criteria described by Hafez (1970), estrus cycle staging were determined using the following criteria: estrus, presence of cornified epithelial cells only; metestrus, presence of 50% cornified epithelial cells and fifty percent leukocytes; diestrus, presence of leukocytes only; and proestrus, presence of round epithelial cells only. A slide was rated as being representative of a particular stage when fifty percent of the cells seen were characteristic of that particular stage. Although estrus cycle varied throughout the study, no Nicotine×Estrus effects were detected. Therefore, this information is not provided in any detail in Results.

2.5. Procedure

The procedure included four phases: a pre-drug phase (Baseline Phase); an initial drug administration phase during which animals were either adolescent or adult and were administered saline or nicotine daily (Drug Phase I); an interim phase during which adolescent animals became adults and no drug was administered (Interim Phase); and a second drug administration phase during which all animals were adult and the same animals were administered saline or nicotine (Drug Phase II).

2.5.1. Baseline phase

Subjects were handled for 2 min each day for 2 days to minimize any stress that might occur as a result of necessary handling for injections and locomotion measurements. All subjects were acclimated to the locomotion apparatus before baseline measurements were obtained by placing them in the apparatus for 1 h on two separate days prior to baseline testing to minimize effects of novelty or possible stress. Baseline testing occurred 1 day after the final acclimation period. Animal body weights were measured during this period for the purpose of balancing experimental groups. The Baseline Phase spanned ages 25–30 days for adolescents and 55–60 days for adults.

2.5.2. Drug phase I (12 days)

After the completion of baseline measures, subjects were assigned to drug groups (saline, 0.01, 0.10, 0.50, or 1.0 mg/kg nicotine) in a manner that assured comparable, initial body weights and horizontal activity levels in same-age groups. Animals were injected and locomotor activity was measured for 1 h everyday during the dark cycle for 12 days (Drug Days 1 through 12). Animals were measured in the locomotor apparatus in groups of 16 (16 separate chambers). To control for order effects and to try and minimize circadian effects, each locomotor testing session consisted of animals representing different drug groups and animals of different ages. Body weight was measured every day and was used to prepare drug dosages for each subject.

2.5.3. Interim phase (17 days)

The purpose of the Interim Phase was to allow adolescent animals to grow into adults. During this Phase, animals were not injected with saline or nicotine and locomotor activity was assessed at four points (Interim Days 5, 7, 10, and 14) to determine whether previous exposure to nicotine altered activity patterns. This Phase spanned ages 43–59 days for adolescent animals and ages 73–89 days for adult animals.

2.5.4. Drug phase II (12 days)

The purpose of Drug Phase II was to determine whether exposure to nicotine during adolescence altered later, adult responses to nicotine. Animals were injected daily for 12 days with saline or nicotine and locomotion was measured for 1 h on each day. The drug administration and locomotor procedures were identical to those followed during Drug Phase I. Animals were administered the same drug dosage as in Drug Phase I. Body weight was measured every day and used to prepare drug dosages for each subject. This Phase spanned ages 60–72 days for previously adolescent animals and ages 90–102 days for adult animals.

3. Results

3.1. Data analytic strategy

Drug Phase I, Interim Phase, and Drug Phase II horizontal activity data were analyzed by separate repeated-measures analyses of variance (ANOVAs) with a within-subjects factor of Day and between-subjects factors of Age and Drug. These analyses yielded within-subjects effects of Day and interactions of Age and Drug with Day, and between-subjects effects of Age and Drug and Age×Drug interactions. Data also were examined within each age group. For all analyses, Tukey's HSD post-hoc tests were

used to determine differences among drug groups (mean across days). Only significant results of these analyses are described.

All tests were two-tailed. Results are significant at $p<0.05$ unless otherwise noted. There were no differences between same-age groups in baseline body weight or horizontal activity. Animals that were adolescent at the beginning of the experiment are referred to as "adolescents" throughout the Results section, even though they were adults by Drug Phase II.

3.2. Drug phase I

Activity levels rose over Drug Days 1 through 12 [Day: $F(11, 726)=32.63$] and differences among drug groups were greater as Drug Phase I progressed [Day×Drug: $F(44, 726)=6.38$]. Higher nicotine dosages resulted in greater activity levels [Drug: $F(4, 66)=35.1$]. Dose response curves for nicotine's effect on locomotor behavior differed between adolescents and adults [Drug×Age: $F(4, 66)=2.47$]. Among adolescents, activity increased as Drug Phase I progressed [Day: $F(11, 385)=19.55$] and differences among drug groups became greater over time [Day×Drug: $F(44,385)=3.49$]. Nicotine dosage altered activity [Drug: $F(4, 35)=29.52$] such that the 0.50 mg/kg and 1.0 mg/kg groups were similarly active. Both the 0.5 mg/kg and the 1.0 mg/kg groups were significantly more active than the saline, 0.01, and 0.10 mg/kg groups during Drug Phase I. Among adults, activity also increased as Drug Phase I progressed [Day: $F(11, 341)=13.81$] and differences among drug groups became greater over time [Day×Drug: $F(44, 341)=3.65$]. Nicotine altered activity [Drug: $F(4, 31)=10.34$] such that only the 0.50 mg/kg group was significantly more active than the saline group, 0.01, and 0.10 mg/kg groups. Among

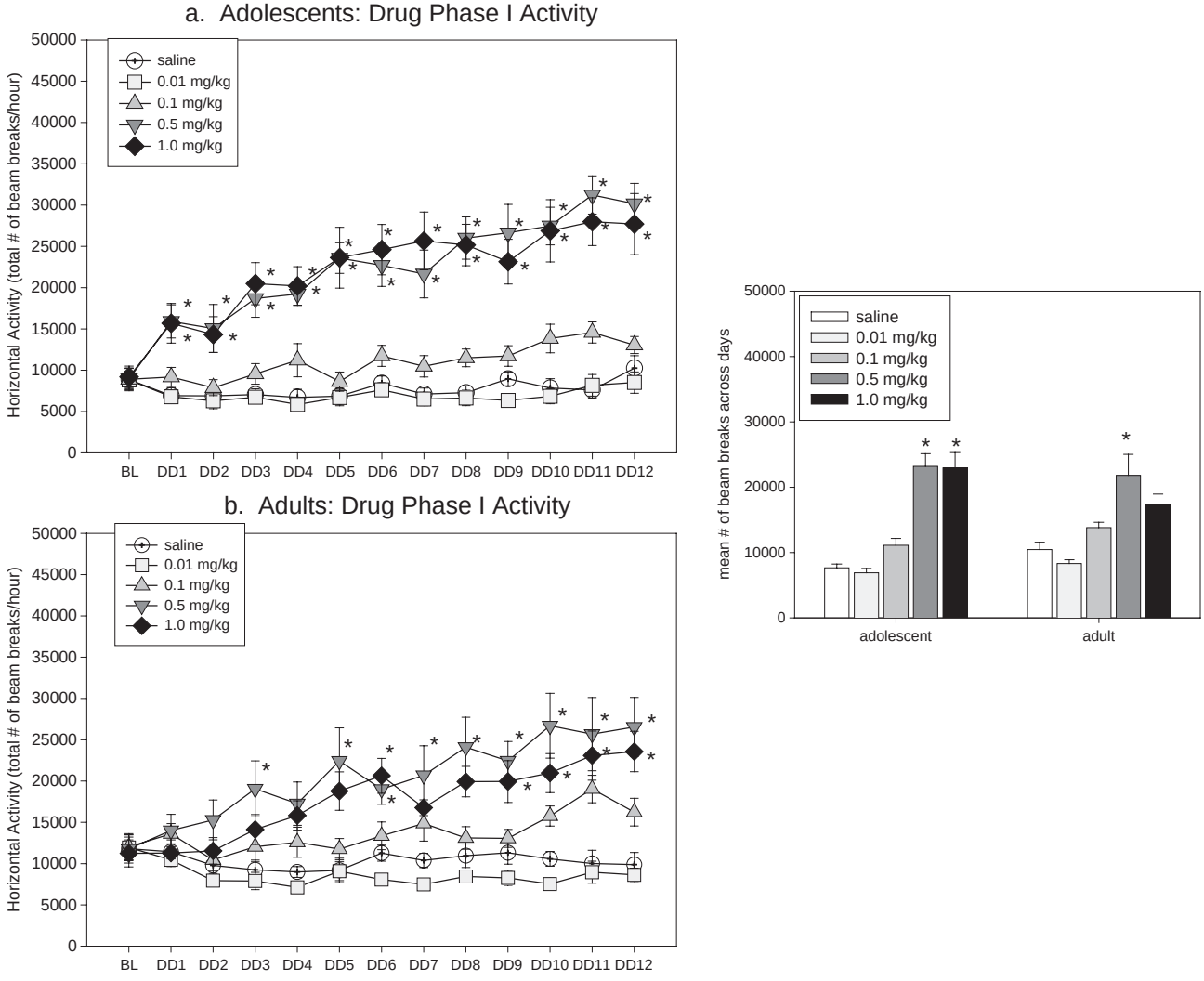

Fig. 1. (a) Horizontal activity (# beam breaks) over 1 h (group means±SEM) for adolescent females during Drug Phase I (BL=Baseline Phase; DD=Drug day). Asterisks indicate significant differences from saline. (b) Horizontal activity in beam breaks over 1 h (group means±SEM) for adult females during Drug Phase I. Asterisks indicate significant differences from saline. *Inset graph*. The inset graph in (a) and (b) represents mean horizontal activity (# of beam breaks) averaged over the 12 drug days (group means±SEM) for adolescent and adult females during Drug Phase I. Asterisks indicate significant differences from saline.

adults the 0.50 mg/kg dosage group had the highest activity overall and was the only group that differed significantly from saline. This pattern contrasts with adolescents for which both the 0.50 and 1.0 had higher activity levels than all other groups and suggests that dose response relationships differ for adult and adolescent rats. In addition, among adolescent female rats there was an increase in activity for the 0.5 mg/kg group of 223% compared to saline, and an increase in the activity for the 1.0 mg/kg group of 206% compared to saline. In contrast, among adult female rats there was an increase in activity for the 0.5 mg/kg group of 110% compared to saline, and an increase in the 1.0 mg/kg group of 84% compared to saline (see Fig. 1a and b).

3.3. Interim phase

Over the Interim Phase, activity levels changed [Day: $F(3, 210)=3.46$]. Overall levels of activity did not appear to be significantly affected by previous drug exposure (see Fig. 2a and b).

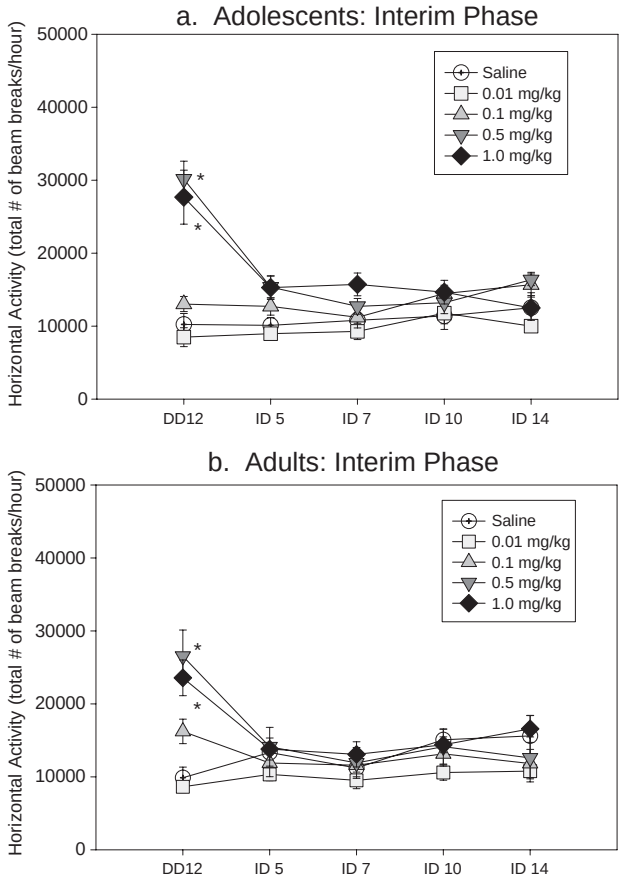

Fig. 2. (a) Horizontal activity in beam breaks over 1 h (group means±SEM) for adolescent females on the last day of Drug Phase I (Drug Day 12) and the four Interim Phase days during which no nicotine was administered. (DD=Drug day; ID=Interim Day Phase Day). Asterisks indicate significant differences from saline. (b) Horizontal activity in beam breaks over 1 h (group means±SEM) for adult females on the last day of Drug Phase I (Drug Day 12) and the four Interim Phase days during which no nicotine was administered. Asterisks indicate significant differences from saline.

3.4. Drug phase II

Activity levels increased as the phase progressed [Day; $F(11, 770)=3.75$] and drug effects became larger as the phase progressed [Day×Drug; $F(44, 770)=2.86$]. Animals receiving nicotine generally had the highest activity levels [Drug; $F(4, 70)=34.95$]. Among animals that had been exposed to nicotine during adolescence, activity levels increased over the phase [Day; $F(11, 385)=3.39$] and activity increases at the 0.50 mg/kg and 1.0 mg/kg dosages became larger over time [Day×Drug; $F(44, 385)=1.73$]. On average, activity in the 0.10, 0.50, and 1.0 mg/kg groups (animals first exposed to nicotine during adolescence) was significantly greater than was activity for the 0.01 mg/kg and saline groups [Drug; $F(4, 35)=31.71$]. Among animals first exposed to nicotine as adults activity levels also increased during phase II, and these effects differed depending on Drug [Day×Drug; $F(11, 385)=1.7$]. On average, activity for the 0.50 mg/kg and the 1.0 mg/kg groups (animals first exposed to nicotine during adulthood) was greater than was the activity level for the saline and 0.01 mg/kg groups. Also, the activity for the 0.10 mg/kg was greater than the activity for the 0.01 mg/kg group [Drug; $F(4, 35)=9.25$]. In addition, for animals first exposed to nicotine as adolescents, there was an increase in activity for the 0.1 mg/kg group of 114% compared to saline, an increase in activity for the 0.5 mg/kg grou p of 242% compared to saline, and an increase in activity for the 1.0 mg/kg group of 161% compared to saline. In contrast, among animals first exposed to nicotine as adults, there was an increase in activity for the 0.1 mg/kg group of 57% compared to saline, an increase in activity for the 0.5 mg/kg group of 104% compared to saline, and an increase for the 1.0 mg/kg group of 87% compared to saline (see Fig. 3a and b).

4. Discussion

This experiment examined the effects of repeated nicotine administration (saline, 0.01, 0.10, 0.50, or 1.0 mg/kg daily) on locomotor activity of adolescent and adult female rats. The experiment had four purposes: (1) to determine whether age differences in nicotine's acute effects existed for females (Drug Phase I); (2) to examine the effects of age of initial nicotine exposure (adolescence vs. adulthood) on locomotion activity when nicotine was not administered (Interim Phase); (3) to determine whether age of initial nicotine exposure altered responses to later nicotine administration (Drug Phase II); and (4) to determine whether nicotine's effects in adolescent and adult female rats are similar to previous reports of nicotine's effects in adolescent and adult male rats.

4.1. Drug phase I

Adolescents as well as adults exhibited sensitization to nicotine's activity-increasing actions over the 12 days of

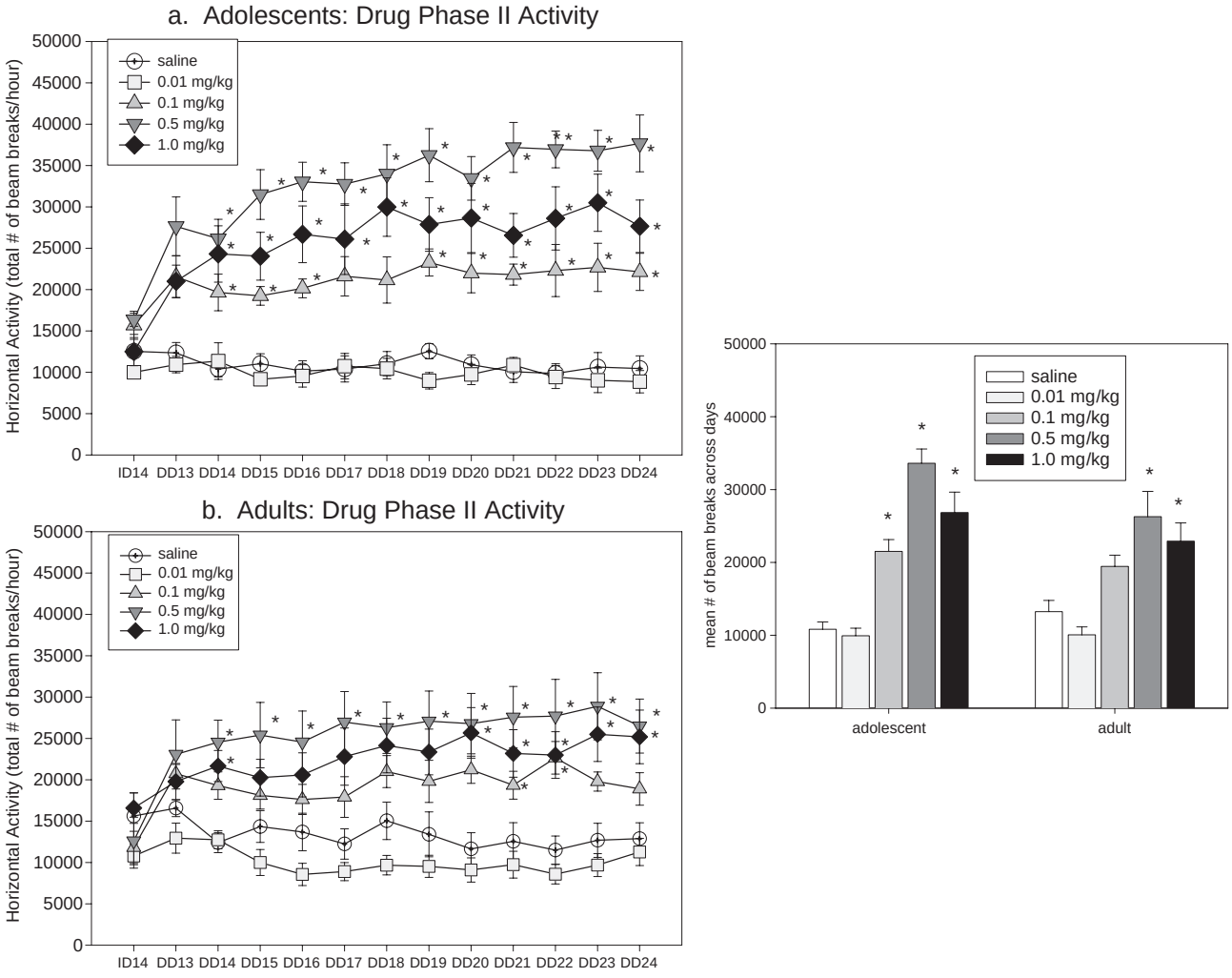

Fig. 3. (a) Horizontal activity (# beam breaks) during 5 min periods over 1 h (group means±SEM) for adolescent females on the last Interim day (ID 14) and during Drug Phase II. Asterisks indicate significant differences from saline. Asterisks indicate significant differences from saline. (b) Horizontal activity (# beam breaks) during 5 min periods over 1 h (group means±SEM) for adult females on the last Interim day (ID 14) and during Drug Phase II. Asterisks indicate significant differences from saline. *Inset graph*. The inset graph in (a) and (b) represents mean horizontal activity (# of beam breaks) averaged over the 12 drug days (group means±SEM) for adolescent and adult females during Drug Phase II. Asterisks indicate significant differences from saline.

drug administration as evidenced by the increasing activity levels of the 0.50 and 1.0 mg/kg groups during Drug Phase I. These activity patterns replicate data reported by other investigators (e.g., Clarke and Kumar, 1983a,b; Clarke et al., 1988; Ksir et al., 1987; Stolerman et al., 1995) and are similar to the patterns we previously reported in males (Faraday et al., 2003).

Nicotine's effects on activity differed in adolescent and adult female rats. Among adolescents animals receiving the 0.50 mg/kg and 1.0 mg/kg nicotine dosages had the highest activity levels overall, differing significantly from all other groups, including saline. Further, animals receiving 0.5 mg/kg and 1.0 mg/kg nicotine had significantly higher activity levels than the saline group on each drug day (mean increases above saline were 223% and 206%, respectively). Among adults, animals receiving the 0.50 mg/kg dosage of nicotine had the highest activity overall (mean increase above saline was 110%) and differed significantly from the saline, 0.01, and 0.1 mg/kg groups. The 0.5 mg/kg group was significantly more active than the saline group on drug days 3–12. The activity of the 1.0 mg/kg group did not differ significantly from the saline group when activity levels were collapsed across drug days (mean increase above saline was 84%). When examined separately by drug day, the 1.0 mg/kg group had higher activity levels than the saline group on DD 6 and DD 9–12 only. These results indicate that the dose–response curves for nicotine's effect on locomotor behavior differ between female adolescent rats and adults female rats. Although a direct comparison of female and males rat cannot be made in this study, the pattern of results is similar to our previously reported findings in male rats.

4.2. Interim phase

Age differences in the effects of nicotine exposure on locomotor activity were less evident during the Interim Phase–the phase during which no nicotine was administered–than it had been during the dosing phase. During the four Interim Phase Days the activity levels of female adolescent and adult rats previously exposed to nicotine did not differ significantly from the activity levels of animals previously exposed to saline. The Interim Phase findings differ from previously reported findings for males (Faraday et al., 2003). That is, male rats that receive nicotine in adolescence show residual activity after nicotine administration is discontinued. Female rats do not. These findings suggest that adolescent and adult females may be less sensitive than are adolescent and adult males to the hyperactivity effects that occur in the absence of nicotine.

The apparent insensitivity of females to the hyperactivity effects of nicotine that follow nicotine cessation is consistent with previous behavioral and biological reports. Previously we reported that the persistent hyperactivity effects following nicotine exposure via minipump occurs for adolescent males, but not for adolescent females (Faraday et al., 2001). Following nicotine treatment in adolescence, male adolescent rats exhibit nicotinic upregulation in the midbrain, cerebral cortex, and hippocampus that persists a month after termination of drug. Effects for female rats are less persistent or do not appear until much later (Trauth et al., 1999, 2001). Trauth et al., (2000a,b), for example, recently reported that females show decreased locomotor activity following nicotine cessation and suppressed cholinergic tone. The absence of persistent hyperactivity in females is consistent with Trauth's findings and adds further support to previous reports that early-nicotine exposure affects adolescent males and females differently. More specifically, it may be that adolescent males' behavioral responses to nicotine continue after cessation of nicotine administration whereas female responses to nicotine occur only in the presence of nicotine. Alternatively, the responses of females after cessation of nicotine may require other behavioral measures to detect.

4.3. Drug phase II

During phase II, when nicotine was readministered, age differences in response to nicotine were evident. Animals exposed to nicotine initially as adolescents exhibited significantly greater activity levels compared to saline. Nicotine increased activity above saline at the 0.1, 0.5, and 1.0 mg/kg dosages (increases above saline were 114%, 242%, and 161%, respectively). In animals initially exposed as adults, only the 0.5 and 1.0 mg/kg nicotine dosages increased activity significantly above the saline level (increases above saline were 104% and 87%, respectively). Overall, nicotine's effects to increase activity were greater in animals initially exposed during adolescence compared to animals initially exposed during adulthood. These effects of nicotine are similar to previous reports for adolescent and adult male rats (Faraday et al., 2003). There are, however, differences in Phase II between the results reported here and the findings of Faraday et al.'s (2003) male study: (1) responses to 0.1 mg/kg and 0.5 mg/kg nicotine re-exposure were greater in adolescent females than in adolescent males; (2) the dose–response curve for adolescent females (saline=0.01 mg/kg<0.1 mg/kg<1.0 mg/kg<0. 5 mg/kg) differed from the dose–response curve for adolescent males (saline=0.01 mg/kg<0.1 mg/kg<0.5 mg/kg=1.0 mg/kg). These findings suggest that the adolescent female may be more sensitive to low nicotine dosages in adulthood when exposed initially in adolescence.

4.4. Summary and implications

These findings indicate that: (1) adolescent and adult female rats exhibit different dose–response effects with acutely-administered nicotine; (2) exposure to nicotine in females (adolescent or adult) does not result in persistent behavioral consequences in adulthood when nicotine is no longer administered; (3) initial exposure to nicotine in adolescence compared to adulthood alters responses to nicotine in adulthood; and (4) there appear to be no sex differences in response to initial exposure in adolescence. This last point is based on a comparison between the current findings and our previous results in adolescent males. Specifically, female adolescent rats responded similarly to male adolescents during initial exposure to nicotine, but exhibited greater sensitivity to lower nicotine dosages upon re-exposure in adulthood when compared to adolescent males. In contrast, adolescent female rats did not exhibit the hyperactive effects in the absence of nicotine as previously reported in adolescent male rats.

Findings from animal studies provide evidence that the nicotine's effects on the adolescent brain are distinctly different than the effects observed in adults. Specifically, nicotine exposure in adolescence results in cell damage, persistent nicotinic receptor upregulation, and increased dopamine turnover during exposure (Trauth et al., 1999, 2000a, 2001). These effects do not occur when nicotine exposure occurs during adulthood, suggesting that the adolescent brain responds differently to nicotine. Whether these differences extend to humans and whether they are associated with proposed differences in addiction liability remains unclear.

The central nervous system undergoes rapid changes during adolescence (Spear, 2000, for review). Exogenous influences (e.g., stress, drugs) that are introduced during this period of rapid growth and development may alter various aspects of brain function, including the response to drugs and these changes may persist into adulthood (see Slotkin, 2002, for review). Previous studies have revealed that rats exposed to nicotine during adolescence show an upregulation of nicotinic receptors that persists up to a month

following cessation (Trauth et al., 1999). Further, nicotine administered (via SC injections) to adolescent rats for 10 days produced an increase in nicotinic acetylcholine receptor gene expression 5 weeks after treatment ended, when the rats were adults (Adriani et al., 2003). Finally, adolescent rats that received twice daily injections of nicotine for seven days beginning on postnatal day 30 exhibited increased nicotinic acetylcholine receptor binding in the midbrain immediately after treatment ended. These effects persisted for at least a month following nicotine cessation (Abreu-Villaca et al., 2003). Further, these changes did not occur for adult rats. Together, these findings suggest that nicotine exposure during adolescents induces long-lasting neurochemical changes in the adolescent brain that persist into adulthood and that differ from the effects of nicotine observed in adults.

The present findings also may explain gender differences in effects of tobacco and successful abstinence from tobacco use. The activity-increasing effects of nicotine did not persist in the Interim Phase (when no nicotine was being administered but after it had been administered) for female rats, but these effects did persist for male rats in our earlier experiments. In addition, female rats appeared more sensitive to nicotine upon re-exposure (Drug Phase II) which suggests less tolerance to the drug than that demonstrated by male rats. The lack of persistence of nicotine's effects on dopaminergic systems and less tolerance to nicotine in the female rats are consistent with reports that female smokers are less dependent than are male smokers (Perkins, 1996). The fact that female rats in the present experiment appeared more sensitive than were male rats in our previous experiments to the re-exposure effects of nicotine suggests that females may exhibit greater nicotine sensitivity than males after a period of nicotine abstinence. If true, then these findings may partially explain why female smokers have greater difficulty giving up tobacco smoking (Perkins, 1996).

Acknowledgements

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences. This work was supported by a grant from the Robert Wood Johnson Foundation Grant (G172DF).

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