Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 74 (2003) 917–931
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

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

Martha M. Faraday*, Brenda M. Elliott, 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 28 February 2002; received in revised form 9 August 2002; accepted 7 January 2003

Abstract

More than 90% of cigarette smokers begin smoking during adolescence, suggesting that adolescents may be particularly vulnerable to nicotine's effects. This experiment examined: (1) nicotine's acute effects on locomotion in adolescent and adult male Sprague–Dawley rats (Drug Phase I); (2) the effects of age of initial nicotine exposure on locomotion 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). In Drug Phase I, animals were administered 0, 0.01, 0.10, 0.50, or 1.0 mg/kg nicotine sc for 12 days and horizontal activity was measured daily. During the Interim Phase, activity was measured but nicotine was not administered. During Drug Phase II, 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 adolescents and adults such that adolescents exhibited peak activity at both the 0.50- and 1.0-mg/kg dosages, but adults exhibited peak activity at the 0.50-mg/kg dosage. Initial nicotine exposure in adolescence (0.50 and 1.0 mg/kg), but not in adulthood, resulted in hyperactivity in adulthood in the absence of nicotine (Interim Phase). Reexposure to nicotine when all animals were adults (Drug Phase II) revealed that initial nicotine exposure in adolescence compared to adulthood resulted in dose–response differences in adulthood similar to those in Drug Phase I. In addition, animals initially exposed in adolescence exhibited sensitization to nicotine's activity-increasing effects in adulthood. These findings suggest that there are age differences in nicotine sensitivity that could predispose individuals initially exposed to nicotine in adolescence to long-term smoking.

Keywords: Sprague–Dawley; Nicotine; Horizontal activity; Adult rats; Tolerance; Adolescent rats; Locomotion; Males; Sensitization

1. Introduction

More than 90% of adult smokers initiate tobacco use before age 20 (Dappen et al., 1996; Chassin et al., 1996; U.S. Department of Health and Human Services [USDHHS], 1989). Understanding why adolescents initiate and maintain tobacco use, therefore, is the key to prevention. Prevention efforts have focused on psychosocial factors that influence initiation, maintenance, and cessation. Rates of cigarette smoking among American adolescents have been resistant to change, however, with up to 20% of high school seniors smoking daily and with nearly 3000 American children a day beginning to smoke (Johnston et al., 1992; USDHHS, 1994; Gilpin et al., 1999). It is possible that an important reason for tobacco use by adolescents is one that has not been thoroughly evaluated: differences between adults and adolescents in nicotine's immediate and long-term effects. In particular, exposure to nicotine in adolescence may predispose individuals to become long-term smokers compared with individuals who delay exposure until they reach adulthood.

Experiments comparing the responses of drug-naive adolescent and adult humans to nicotine are difficult to perform because of ethical issues associated with exposing children to an addictive drug. Findings about behavioral effects of nicotine in adult rats have paralleled and predicted findings with adult human tobacco users (i.e., Winders and Grunberg, 1989), but rat models have not been used systematically to examine effects of nicotine in young animals. We recently 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 exposure resulted in long-term hyperactivity (in the absence of nicotine) in adolescent males but not in adult males (Faraday et al., 2001). The present experiment extends these

* Corresponding author. Tel.: +1-301-295-9671; fax: +1-301-295-3034.
E-mail address: Mfaraday@usuhs.mil (M.M. Faraday).

0091-3057/03/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0091-3057(03)00024-8

findings by examining responses of adolescent and adult male rats to repeated-acute nicotine administration.

The purposes of this experiment were to: (1) compare nicotine's repeated-acute locomotion effects in adolescent vs. adult male rats; (2) examine the effects of age of initial nicotine exposure (adolescence vs. adulthood) on locomotion responses when nicotine was not administered; and (3) determine whether initial nicotine exposure in adolescence vs. adulthood altered locomotion responses to subsequent nicotine administration. 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 literature is available in adult rats for comparison.

In adult animals, nicotine produces a dose-dependent biphasic action with an initial decrease of activity and subsequent increase of activity (Clarke and Kumar, 1983a,b; Clarke et al., 1988; Stolerman et al., 1995; see Di Chiara, 2000 for review). Over repeated nicotine exposures, tolerance to nicotine's activity-decreasing actions develops, sensitization to nicotine's activity-increasing effects occurs, and overall activity levels increase. Nicotine's acute actions on activity in adolescents have not been examined.

We also conducted behavioral observations on a separate set of animals that were cage mates to complement the automated locomotion data collection in two ways. First, an animal may exhibit decreased activity because it becomes ataxic, because it is sitting quietly without moving, or because it has fallen asleep. The automated data collection process cannot distinguish among these behaviors. Second, in humans and in rats, adolescence is a period during which social behaviors are learned and practiced. Nicotine alters social behaviors in adult animals (e.g., Scheufele et al., 2000). It is possible that adolescent tobacco use is related to nicotine's property to alter social interactions. Locomotion measurement of individual animals cannot address this question. The behavioral observations, therefore, were included to provide a more detailed picture of nicotine's behavioral actions.

2. Methods

2.1. Subjects

Subjects were 101 male Sprague–Dawley rats, 51 adolescents and 50 adults (Charles River Laboratories, Wilmington, MA). Of the 101 animals, 80 animals (40 adults and 40 adolescents) were administered saline or one of four nicotine dosages and tested in the locomotion apparatus. The remaining 21 animals (10 adults and 11 adolescents) were administered saline or nicotine and returned to their home cage for scoring of behavioral changes (see Behavioral Observations section below). Animals were housed in same-age groups of two or three in standard polycarbonate shoebox cages ($42 \times 20.5 \times 20$ 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 1700 h). Locomotor activity was measured and behavioral observations were made during the dark (active) phase of the light cycle (between 0700 and 1400 h). At the beginning of the experiment, adolescent animals were about 25 days old (average weight = 69.1 g, S.E.M. = 0.9 g) and adult animals were about 55 days old (average weight = 245.0 g, S.E.M. = 1.12 g). 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 eight subjects per treatment cell in the locomotion portion of the experiment and two to three animals per treatment cell in the behavioral observation portion of the experiment. Adolescence was defined as the period spanning 28–42 days (Spear, 2000). 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 $40 \times 40 \times 30$-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 were always 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. Behavioral observations

An additional two or three animals per treatment group (cage mates) received saline or nicotine injections and then were returned to the home cage for behavioral observations. Observations were conducted in ambient light in a procedure room separate from the housing room. Behavioral scoring

was based on a scoring protocol used to quantify adult rat social interactions (Scheufele et al., 2000) with behaviors specific to nicotine effects added (i.e., ataxia). Animals received the same drug dosage daily and were observed daily during Drug Phase I (12 days) and during Drug Phase II (12 days). Observations began immediately after injection. Two trained raters observed animals for 30 min and scored behaviors every 30 s. Behaviors scored were: exploratory (sniff, move, and rear); social (touch, follow, sniff other, groom self, groom other, and wrestle); motor (normal, ataxia, hyperactive, lordosis, and convulsions); and breathing (normal, shallow, rapid, and stopped breathing). Interrater reliability was >.90. It is important to note that these data reflect the effects of nicotine on social behaviors toward other rats exposed to the same nicotine dosage.

2.4. Drug administration

Nicotine (0.01, 0.10, 0.50, or 1.0 mg/kg; expressed as nicotine base) or physiologic saline was administered subcutaneously in the skin between the shoulder blades. These dosages were selected to span those commonly reported in the research literature. The 0.01 dosage is lower than dosages typically used but was selected to ensure that we would not overlook possible age differences in sensitivity to low nicotine dosages. 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 was also 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 dihydrochloride was made in our laboratory; purity was verified by the laboratory of N. Benowitz. All injection volumes were 1 ml/kg. Injections were given in the procedure rooms in which testing took place. Testing immediately followed the injections.

2.5. Procedure

The procedure included four phases: a predrug 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 were again 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 within age 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 every day during the dark cycle for 12 days (Drug Days 1–12). Animals were measured in the locomotor apparatus in groups of 16 (16 separate chambers). Each locomotor testing session was counterbalanced by drug dosage and by age. The order of locomotor testing

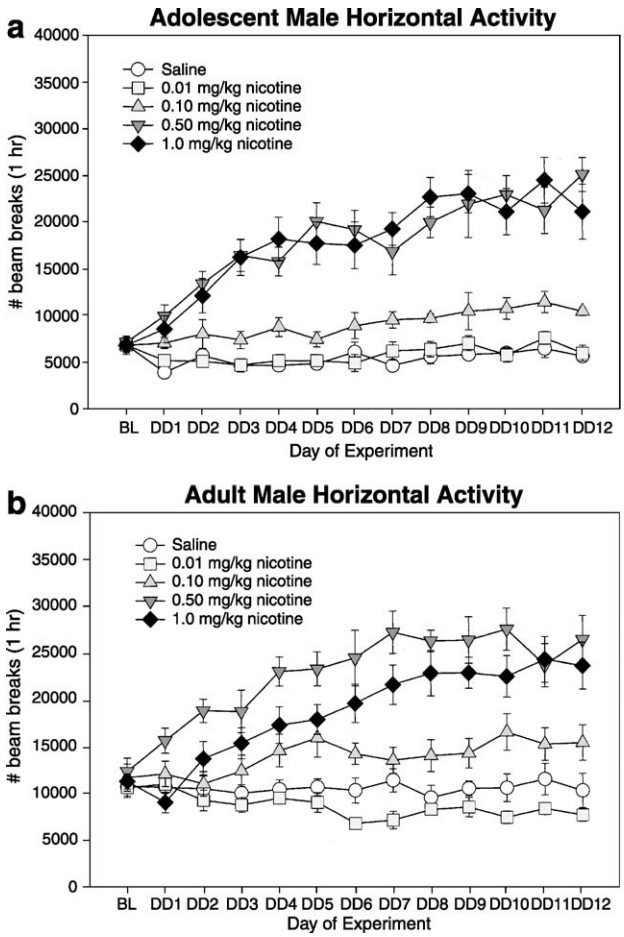

Fig. 1. (a) Horizontal activity (no. of beam breaks) over 1 h (group means ± S.E.M.) for adolescents during Drug Phase I. (b) Horizontal activity in beam breaks over 1 h (group means ± S.E.M.) for adults during Drug Phase I.

was changed every day to control for possible circadian effects. Body weight was measured every day.

Behavioral observation animals also were injected daily for 12 days and behaviors were observed and recorded. Similar counterbalancing procedures were followed for these animals. For each animal (locomotion testing animals and behavioral observation animals), each day's body weight was used to calculate the syringe volume for the next day's injection, ensuring that each animal received a constant drug dosage adjusted daily for increases in body weight. This phase spanned ages 31–42 days for adolescent animals and ages 61–72 days for adult animals.

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

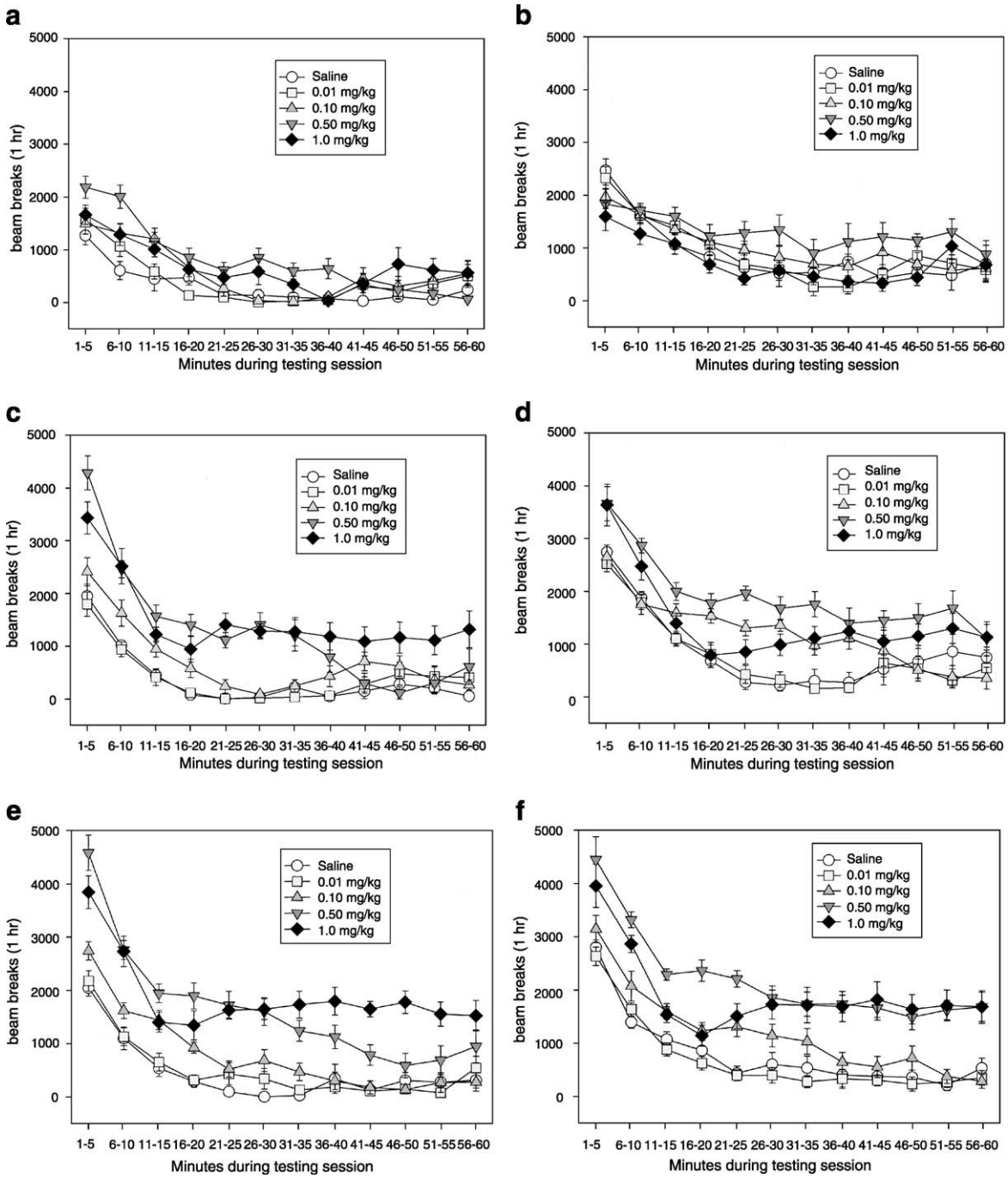

Fig. 2. Horizontal activity (no. of beam breaks) during 5-min periods over 1 h (group means ± S.E.M.) during specific drug days of Drug Phase I. (a) Adolescents—Drug Day 1. (b) Adults—Drug Day 1. (c) Adolescents—Drug Day 4. (d) Adults—Drug Day 4. (e) Adolescents—Drug Day 8. (f) Adults—Drug Day 8. (g) Adolescents—Drug Day 12. (h) Adults—Drug Day 12.

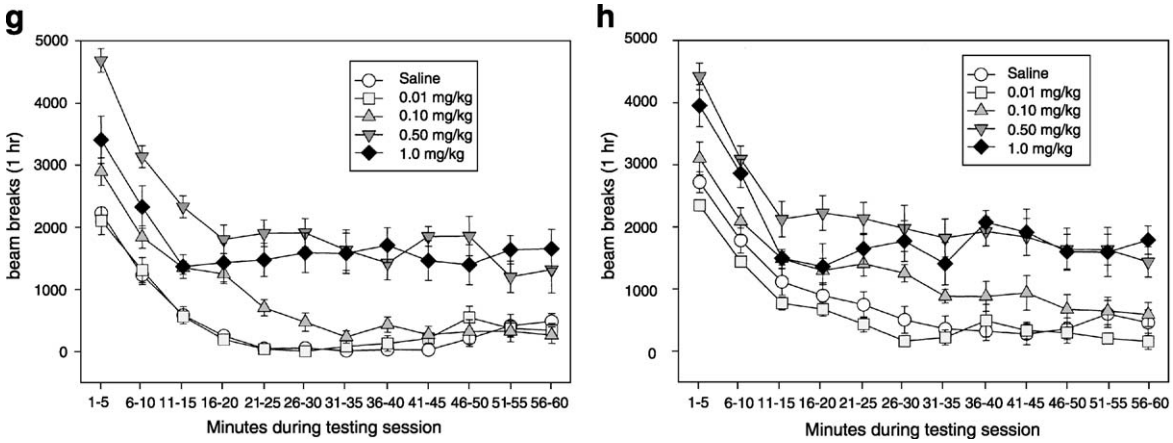

Fig. 2 (*continued*).

were not injected with saline or nicotine and locomotor activity was assessed at four points (Interim Days 3, 6, 10, and 13) to determine whether exposure to nicotine had 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. Behavioral observations were made

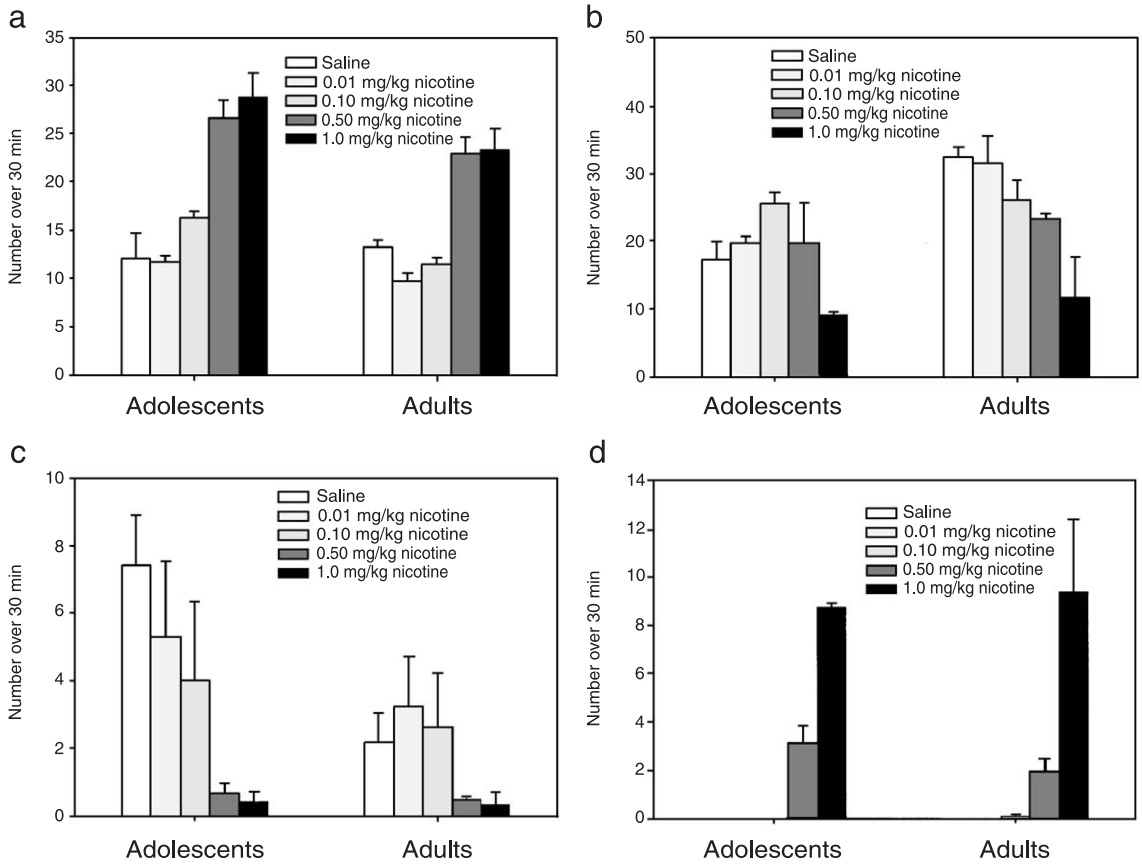

Fig. 3. Number of behaviors over 30 min during behavioral observations conducted during Drug Phase I (group means ± S.E.M.). (a) Move behaviors; (b) Rearing behaviors; (c) Groom other behaviors; (d) Ataxia.

daily on behavioral observation animals. 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. 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 were also examined within each age group. To examine within-session time course effects, within-session data (activity summed over 5-min periods for the 1-h session) were examined with repeated-measures ANOVAs. These analyses yielded within-subjects effects of time and interactions of age and drug with time as well as between-subjects effects of age and drug and Age × Drug interactions. Within-session data were examined from Drug Phase I (Drug Days 1, 4, 8, and 12), from the Interim Phase (average of Interim Phase Days 3 and 6 and of Interim Phase Days 10 and 13), and from Drug Phase II (Drug Days 1, 4, 8, and 12). These data also were examined within each age group. For all analyses, Tukey's post hoc tests were used to determine differences among drug groups. Greater than sign (>) indicates statistically significant rank-order group activity.

Behavioral observation data were averaged over Drug Phase I and Drug Phase II. Data for move, rear, groom other, and ataxia were presented to provide additional information but were not analyzed because of the need to use the dyad or triad as the unit for analysis. Each drug group consisted of two or three animals housed within the same cage; therefore, there was one unit per drug group. These behaviors were selected for presentation because they showed the least variance and therefore were likely to be the most reliable given the small *n* per treatment group.

All tests were two-tailed. Results are significant at $P < .05$, unless otherwise noted. Trends (i.e., *P* values greater than

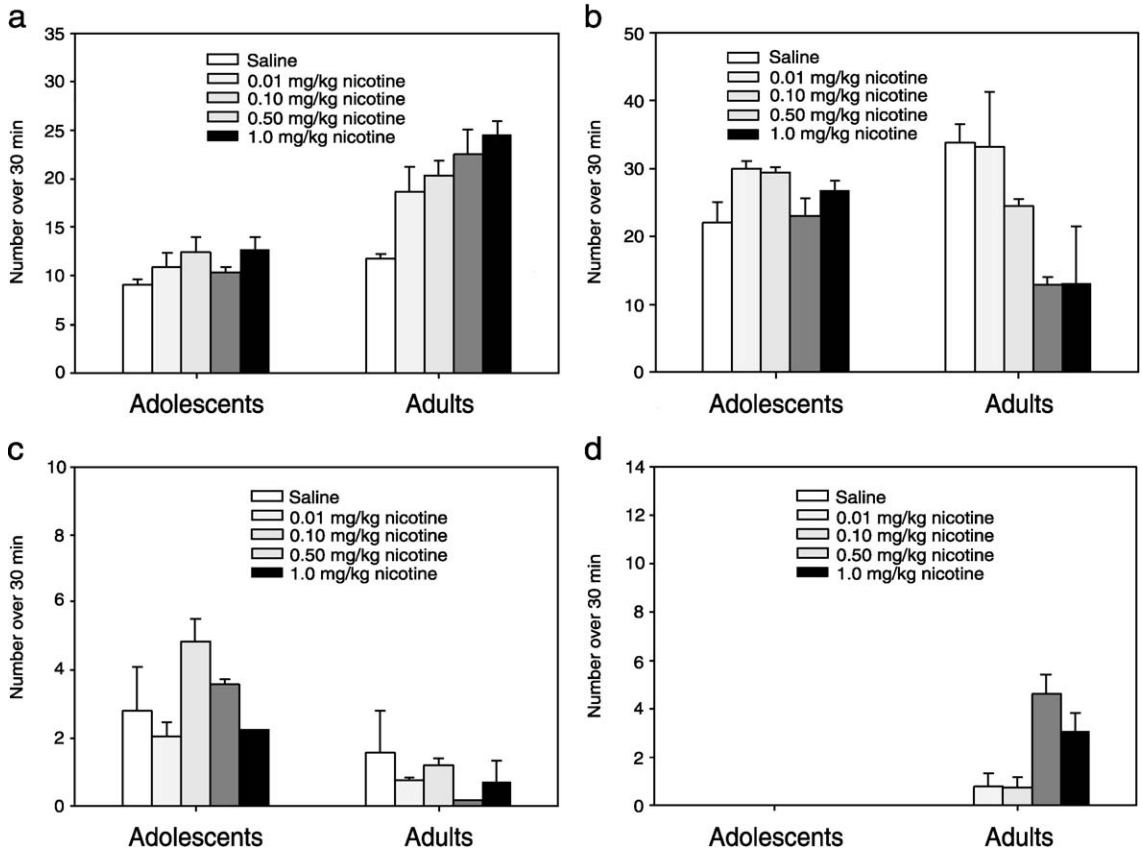

Fig. 4. Number of behaviors over 30 min during behavioral observations conducted during Drug Phase II (group means ± S.E.M.). (a) Move behaviors; (b) Rearing behaviors; (c) Groom other behaviors; (d) Ataxia.

.05) are reported where they are part of an overall pattern of mostly significant effects. There were no differences between same-age groups in baseline body weight and horizontal activity. Animals that were adolescent at the beginning of the experiment are referred to as "adolescents" throughout the results, even though they were adults by Drug Phase II.

3.2. Drug Phase I

3.2.1. Within-phase analyses

Activity levels rose over Drug Days 1 through 12 [day: $F(11,671)=28.7$] and differences among drug groups were greater as Drug Phase I progressed [Day × Drug: $F(44,671)=8.1$] (see Fig. 1a and b). Higher nicotine dosages resulted in greater activity levels [drug: $F(4,61)=50.7$] and adults generally were more active than were adolescents [age: $F(1,61)=17.4$]. Among adolescents, activity increased as Drug Phase I progressed [day: $F(11,319)=18.3$] and differences among drug groups also increased [Day × Drug: $F(44,319)=4.1$]. Nicotine dosage altered activity [drug: $F(4,29)=31.5$] such that the 1.0- and 0.50-mg/kg groups were similarly active and were 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: $F(11,352)=12.3$] and differences among drug groups also increased over time [Day × Drug: $F(44,352)=5.0$]. Nicotine altered activity [drug: $F(4,32)=21.6$] such that the 1.0- and 0.50-mg/kg groups were more active than the saline, 0.01, and 0.10 mg/kg groups but dose–response relationships differed from adolescents in two ways. Among adults, peak activity occurred at the 0.50-mg/kg dosage; 1.0 mg/kg decreased activity levels below this level. This pattern contrasts with adolescents for which 1.0 and 0.50 mg/kg produced similar activity levels. Among adults the 0.10 group was significantly more active than was the 0.01-mg/kg group. This pattern was the result of activity levels in the 0.01-mg/kg group that generally were lower than saline group levels.

3.2.2. Within-session analyses

Every analysis revealed effects of time and effects of age such that adults were more active than were adolescents.

3.2.2.1. Drug Day 1. Nicotine altered activity [Time × Drug: $F(44,770)=1.4$ and drug: $F(4,70)=7.4$] but these effects depended on animal age [Time × Drug × Age: $F(44,770)=1.8$ and Drug × Age: $F(4,70)=2.5$] (see Fig. 2a and b). Among adolescents, the 0.50- and 1.0-mg/kg groups were more active than the other groups for most of the session [Time × Drug: $F(44,385)=2.4$]. On average, 1.0 and 0.50 mg/kg>saline; 0.50>0.01 mg/kg [drug: $F(4,35)=6.6$]. In contrast, among adults on average 0.50 mg/kg>saline and 1.0 mg/kg [drug: $F(4,35)=4.0$].

3.2.2.2. Drug Day 4. Nicotine altered activity during the session [Time × Drug: $F(44,770)=3.6$ and drug: $F(4,70)=31.5$]. These effects depended on animal age [Time × Drug × Age: $F(44,770)=2.8$ and Drug × Age: $F(4,70)=2.5$] (see Fig. 2c and d). Among adolescents, 1.0 mg/kg increased activity throughout the session, 0.50 mg/kg increased activity during the first two thirds of the session, and the other groups were generally similar to saline [Time × Drug: $F(44,385)=4.4$]. On average, 1.0 and 0.50 mg/kg>saline, 0.01, and 0.10 mg/kg [drug: $F(4,35)=20.0$, with post hoc]. In contrast, among adults [Time × Drug: $F(44,385)=2.2$], 0.50 mg/kg increased activity throughout the session and 1.0 mg/kg increased activity only in the middle third of the session. On average, 0.50 mg/kg>all of the other groups; 1.0 mg/kg>saline and 0.01 mg/kg [drug: $F(4,35)=14.3$].

3.2.2.3. Drug Day 8. Nicotine altered activity [Time × Drug: $F(44,770)=4.4$ and drug: $F(4,70)=59.8$]. Among adolescents, 1.0 mg/kg increased activity throughout the session and 0.50 mg/kg increased activity during the first two-thirds of the session [Time × Drug: $F(44,385)=3.7$] (see Fig. 2e and f). On average, 1.0 and 0.50 mg/kg>saline,

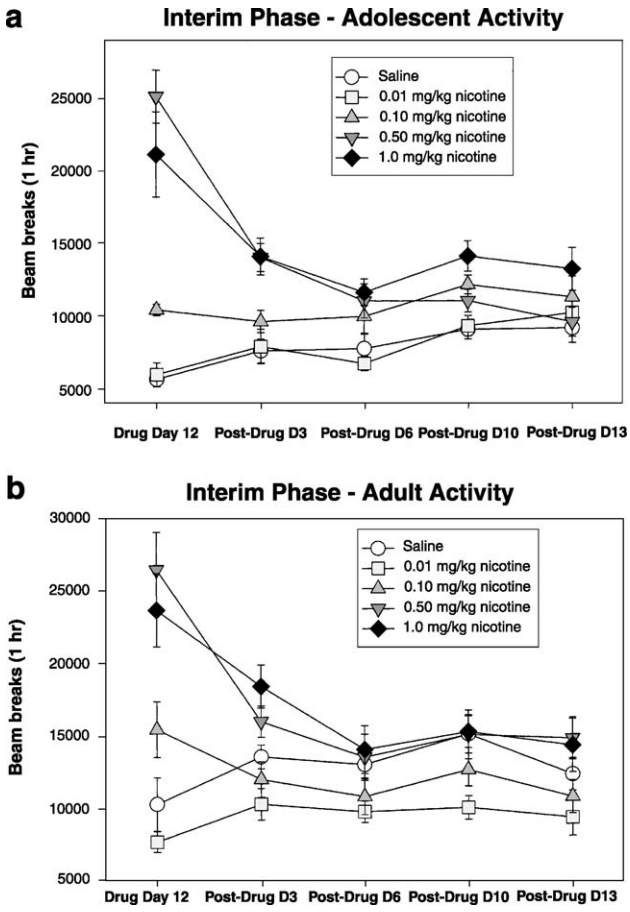

Fig. 5. (a) Horizontal activity in beam breaks over 1 h (group means ± S.E.M.) for adolescents on the last day of Drug Phase I (Drug Day 12) and the four Interim Phase days during which no nicotine was administered. (b) Horizontal activity in beam breaks over 1 h (group means ± S.E.M.) for adults on the last day of Drug Phase I (Drug Day 12) and the four Interim Phase days during which no nicotine was administered.

0.01, and 0.10 mg/kg [drug: $F(4,35)=36.5$]. In contrast, among adults, 0.50 mg/kg increased activity throughout the session and 1.0 mg/kg increased activity during the last two-thirds of the session [Time × Drug: $F(44,385)=2.7$]. On average, 1.0 and 0.50 mg/kg>saline, the 0.01, and 0.10 mg/kg [drug: $F(4,35)=26.3$].

3.2.2.4. Drug Day 12. Nicotine altered activity [Time × Drug: $F(44,770)=6.2$; and drug: $F(4,70)=44.9$]. Among adolescents, 1.0 and 0.50 mg/kg increased activity throughout the session; 0.10 mg/kg increased activity during the first half of the session [Time × Drug: $F(44,385)=4.6$] (see Fig. 2g and h). On average, 1.0 and 0.50 mg/kg>saline, 0.01, and 0.10 mg/kg [drug: $F(4,35)=32.0$]. Among adults, 0.50 mg/kg increased activity throughout the session and 1.0 mg/kg increased activity during the second half of the session [Time × Drug: $F(44,385)=2.7$]. On average, 1.0 and 0.50 mg/kg>saline, 0.01, and 0.10 mg/kg; 0.10>0.01 mg/kg [drug: $F(4,35)=16.9$].

3.2.3. Behavioral observations

In the familiar social context of the home cage in the presence of a cage mate, nicotine altered exploratory, social, and motor behaviors (see Fig. 3a–d). Nicotine's effects on exploratory behaviors (e.g., move, similar to horizontal activity), however, were different from patterns in the locomotion testing apparatus when animals were tested alone. Increasing nicotine dosages increased movement similarly in adolescents and adults in contrast to the different horizontal activity dose–response curves revealed when animals were tested alone (Fig. 3a). Increasing nicotine dosages altered rearing in an inverted U-shaped pattern for adolescents with the 1.0-mg/kg dosage decreasing rearing (Fig. 3b). For adults, increasing nicotine dosages decreased rearing in a dose–response manner. Nicotine also altered social behaviors. For adolescents, nicotine dosages (except for the 0.01 mg/kg dosage) reduced touch behaviors; for adults, the 0.01 dosage increased touch behavior and the other dosages had no effect (data not shown). Nicotine at the 0.50- and 1.0-mg/kg dosages decreased groom other behaviors for adolescents and adults (Fig. 3c). Nicotine reduced wrestling behaviors in a dose–response manner for adolescents and adults (data not shown). The 0.50- and 1.0-mg/kg dosages produced increasing amounts of ataxia in adolescents and adults (Fig. 3d).

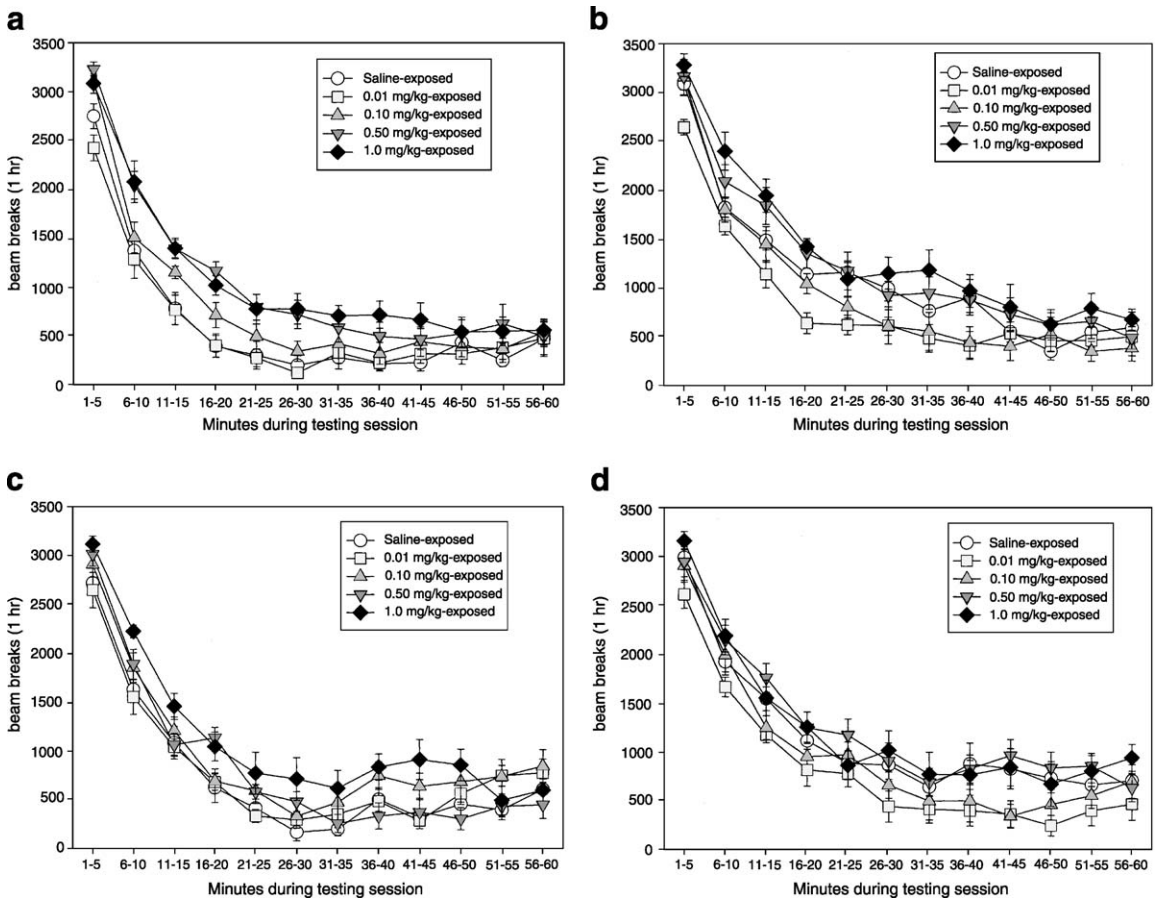

Fig. 6. Horizontal activity (no. of beam breaks) during 5-min periods over 1 h (group means ± S.E.M.) during Week 1 and Week 2 of the Interim Phase during which no nicotine was administered. (a) Adolescents—Week 1. (b) Adults—Week 1. (c) Adolescents—Week 2. (d) Adults—Week 2.

3.3. Interim Phase

3.3.1. Within-phase analyses

Over the Interim Phase, activity levels changed [day: $F(3,210) = 9.5$], and these changes depended on past drug exposure [Day × Drug Exposure: $F(12,210) = 2.5$ and drug exposure: $F(4,70) = 9.5$], age [Day × Age: $F(3,210) = 2.3$], and both factors [Day × Drug Exposure × Age: $F(12,210) = 1.7$, $P = .06$] (see Fig. 5a and b). Among adolescents, animals previously exposed to 1.0 and 0.50 mg/kg exhibited greater activity than did saline-exposed animals [Day × Drug Exposure: $F(12,105) = 3.2$ and drug exposure: $F(4,35) = 7.0$]. In contrast, among adults, the 1.0- and 0.50-mg/kg-exposed groups exhibited activity levels similar to the saline-exposed group but greater than the 0.01-mg/kg-exposed group [drug: $F(4,35) = 4.9$]. Adults were more active than were adolescents [age: $F(1,70) = 19.8$].

3.3.2. Within-session analyses

Within-session data were averaged from Week 1 (Interim Days 3 and 6) and from Week 2 (Interim Days 10 and 13). Every analysis revealed effects of time.

3.3.2.1. Week 1. Activity depended on previous drug exposure [Time × Drug Exposure: $F(44,770) = 2.1$ and drug exposure: $F(4,70) = 11.9$] as well as on age [Time × Age: $F(11,770) = 4.5$ and age: $F(1,70) = 24.5$] (see Fig. 6a and b). Among adolescents, previous exposure to 1.0 and 0.50 mg/kg resulted in activity levels greater than the saline group for most of the session and, on average, throughout the session [Time × Drug: $F(44,385) = 1.4$ and drug: $F(4,35) = 9.4$]. Among adults, previous exposure to 1.0, 0.50, and 0.10 mg/kg resulted in higher average activity levels than the 0.01-mg/kg-exposed group [drug exposure: $F(4,35) = 4.9$].

3.3.2.2. Week 2. Activity depended on previous drug exposure [drug exposure: $F(4,70) = 4.8$], age [Time × Age: $F(11,770) = 3.0$ and age: $F(1,70) = 9.6$], and on both factors [Drug Exposure × Age: $F(4,70) = 2.5$] (see Fig. 6c and d). Among adolescents, the 1.0-mg/kg-exposed group was more active during most of the session than the other groups and, on average, was more active than the saline and 0.01-mg/kg-exposed groups [Time × Drug: $F(44,385) = 1.5$ and drug: $F(4,35) = 4.3$]. Among adults, on average, the 1.0- and 0.50-mg/kg-exposed groups were more active than the 0.01-mg/kg-exposed group but not the saline group [drug: $F(4,35) = 3.3$].

3.3.3. Drug Phase II

Because of equipment failure, data from two animals in each drug group, except for the adult 0.01 mg/kg group, were not recorded on Drug Days 9 and 10. Therefore, within-phase analyses were performed on animals for which all data had been obtained. Because data were collected for all animals on Drug Days 1, 4, 8, and 12, within-session analyses were performed on data from all animals.

3.3.4. Within-phase analyses

Activity levels increased as the phase progressed [day: $F(11,572) = 4.8$], and drug effects became larger as the phase progressed [Day × Drug: $F(44,572) = 2.4$]. Nicotine generally increased activity levels [drug: $F(4,52) = 50.5$] (see Fig. 7a and b). Among "adolescents" (now adults), activity levels increased over the phase [day: $F(11,275) = 3.3$] and activity increases at the 1.0 and 0.50 mg/kg dosages became larger over time [Day × Drug: $F(44,275) = 2.6$]. On average, 1.0, 0.50, and 0.10 mg/kg>saline and 0.01 mg/kg [drug: $F(4,25) = 30.4$]. Among adults, activity levels also increased during the phase [day: $F(11,297) = 2.2$]. On average, 0.50 mg/kg>all of the other groups, 1.0 mg/kg>saline and 0.01 mg/kg, and 0.10>0.01 mg/kg [drug; $F(4,27) = 21.4$]. Visual inspection suggested that the Day × Drug interaction was more pronounced among adolescents than adults. Therefore, estimates of variance explained (η^2) were calculated. The Day × Drug interaction accounted for nearly three times more variance in adolescents (29.2%) than in adults (10.5%).

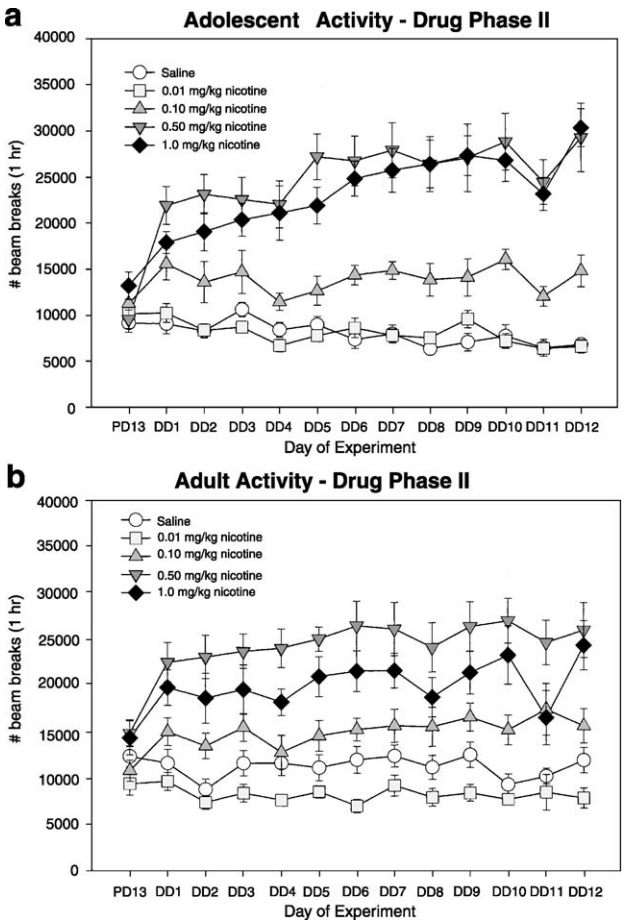

Fig. 7. (a) Horizontal activity (no. beam breaks) over 1 h (group means ± S.E.M.) for adolescents on the last Interim Phase Day (PD13) and during Drug Phase II. (b) Horizontal activity in beam breaks over 1 h (group means ± S.E.M.) for adults on the last Interim Phase Day (PD13) and during Drug Phase II.

3.3.5. Within-session analyses

Every analysis revealed effects of time.

3.3.5.1. Drug Day 1. Nicotine altered activity levels [Time × Drug: $F(44,770) = 1.9$ and drug: $F(4,70) = 23.1$] (see Fig. 8a and b). Among "adolescents," 0.50 mg/kg increased activity throughout the session and 1.0 mg/kg increased activity during the last two-thirds of the session [Time × Drug: $F(44,385) = 1.4$, $P = .06$]. On average, 1.0 and 0.50 mg/kg>saline and 0.01 mg/kg; 0.50>0.10 mg/kg [drug: $F(4,35) = 13.0$]. Among adults, 0.50 mg/kg increased activity throughout the session and 1.0 mg/kg dosage increased activity during the second half of the session [Time × Drug: $F(44,385) = 1.5$]. On average, 1.0 and 0.50 mg/kg>saline and 0.01 mg/kg; 0.10>0.01 mg/kg [drug: $F(4,35) = 10.8$].

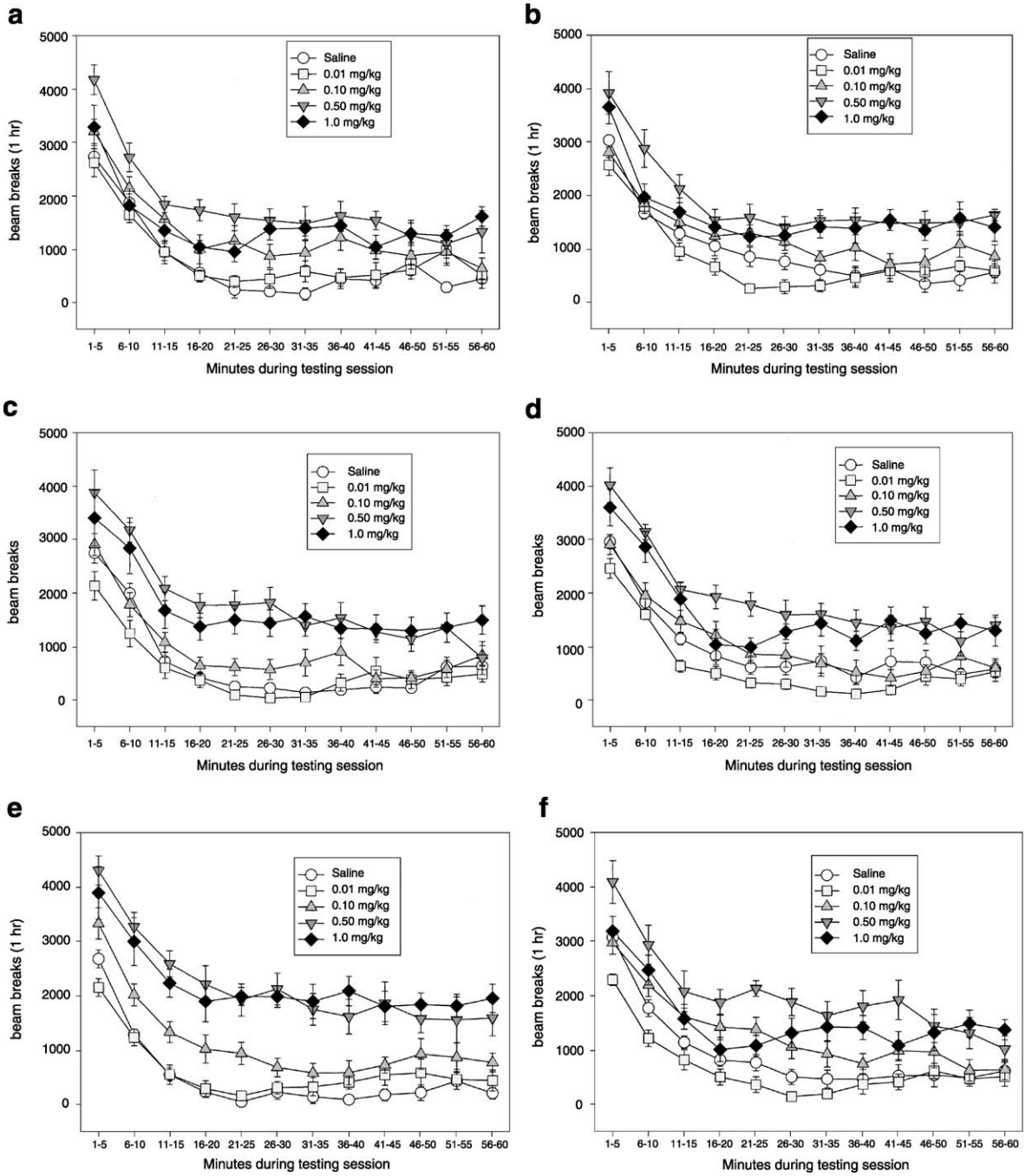

Fig. 8. Horizontal activity (no. beam breaks) during 5-min periods over 1 h (group means ± S.E.M.) during specific drug days of Drug Phase II. (a) Adolescents—Drug Day 1. (b) Adults—Drug Day 1. (c) Adolescents—Drug Day 4. (d) Adults—Drug Day 4. (e) Adolescents—Drug Day 8. (f) Adults—Drug Day 8. (g) Adolescents—Drug Day 12. (h) Adults—Drug Day 12.

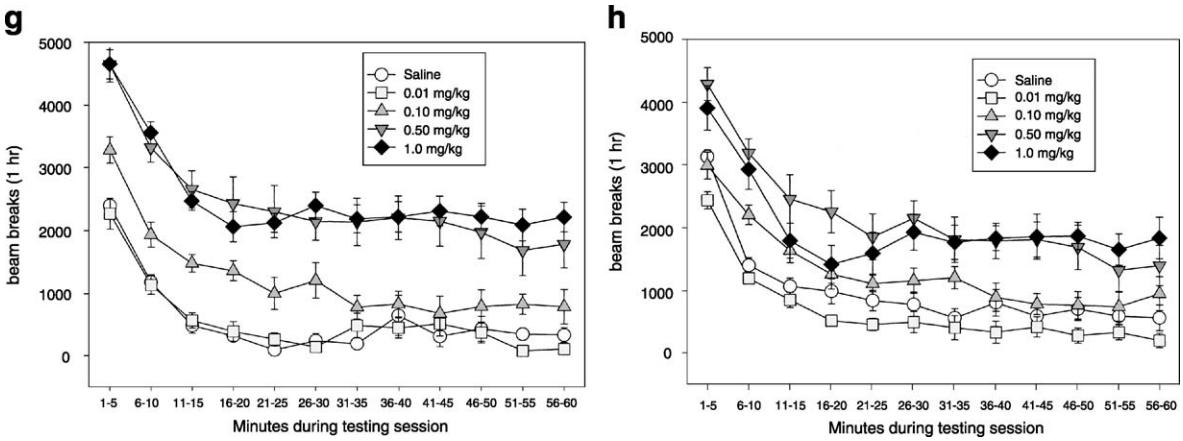

Fig. 8 (*continued*).

3.3.5.2. Drug Day 4. Nicotine altered activity levels [Time × Drug: $F(44,770) = 2.1$ and drug: $F(4,70) = 29.0$] (see Fig. 8c and d). Among "adolescents," 1.0 and 0.50 mg/kg increased activity throughout the session [Time × Drug: $F(44,385) = 1.9$]. On average, 1.0 and 0.50 mg/kg>saline, 0.01, and 0.10 mg/kg [drug: $F(4,35) = 13.6$]. Among adults, 0.50 mg/kg increased activity throughout the session and 1.0 mg/kg increased activity during the second half of the session. On average, 1.0 and 0.50 mg/kg>saline, 0.01, and 0.10 mg/kg [drug: $F(4,35) = 15.6$].

3.3.5.3. Drug Day 8. Nicotine altered activity [Time × Drug: $F(44,770) = 2.3$ and drug: $F(4,70) = 33.1$] and these effects depended on animal age [Age × Drug: $F(4,70) = 2.9$] (see Fig. 8e and f). Among "adolescents," on average 1.0, 0.50, and 0.10 mg/kg>saline and 0.01 mg/kg [drug: $F(4,35) = 24.8$]. Among adults, 0.50 mg/kg increased activity throughout the session and 1.0 mg/kg increased activity during the second half of the session [Time × Drug: $F(44,385) = 2.0$]. On average, 1.0 and 0.50 mg/kg>saline and 0.01 mg/kg; 0.10 mg/kg>saline [drug: $F(4,35) = 10.9$].

3.3.5.4. Drug Day 12. Nicotine altered activity [Time × Drug: $F(44,770) = 2.1$ and drug: $F(4,70) = 42.5$] and these effects depended on animal age [Age × Drug: $F(4,70) = 2.1$, $P = .08$] (see Fig. 8g and h). Among "adolescents," on average 1.0, 0.50, and 0.10 mg/kg>saline and 0.01 mg/kg groups [drug: $F(4,35) = 30.9$]. Among adults, 1.0 and 0.50 mg/kg increased activity throughout most of the session [Time × Drug: $F(44,395) = 1.4$]. On average, 1.0 and 0.50 mg/kg>saline, 0.01, and 0.10 mg/kg [drug: $F(4,35) = 13.8$].

3.3.6. Behavioral observations

In contrast to marked nicotine effects on home cage behaviors during Drug Phase I in adolescents and adults, during Drug Phase II nicotine effects in "adolescents" largely disappeared (see Fig. 4a–d). For adolescents, drug dosage did not systematically alter move, rear, or groom other behaviors. In addition, there were no ataxic behaviors at any drug dosage. For adults, however, most drug effects were similar to effects during Drug Phase I, with a dose-dependent increase in move behaviors and dose-dependent decreases in rear and groom other behaviors. Ataxia was still present among adults at the higher drug dosages, although reduced from Drug Phase I levels.

4. Discussion

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

4.1. Drug Phase I

Adolescents as well as adults exhibited tolerance to nicotine's activity-decreasing actions and sensitization to nicotine's activity-increasing actions over the 12 days of drug administration as evidenced by the increasing activity levels of the 0.50- and 1.0-mg/kg groups during Drug Phase I (dose–response curves shifted upward over time). 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).

Nicotine's repeated-acute effects differed, however, in adolescent and adult rats. Peak activity levels occurred among adolescents at the 1.0- and 0.50-mg/kg dosages but peak activity for adults occurred at the 0.50-mg/kg dosage. Further, among adults, the 1.0-mg/kg dosage reduced activity

below the 0.50-mg/kg group level and the 0.01-mg/kg dosage tended to reduce activity below the saline level. It is likely that with more animals per group, the difference between the 0.01-mg/kg and the saline groups would have been significant (e.g., greater statistical power was needed). These activity-decreasing effects were not present among adolescents, suggesting that adolescents were less sensitive to these effects.

To our knowledge, no past studies of locomotion have tested a 0.01-mg/kg dosage. This finding for adults, therefore, appears to be new. Previous work has indicated that mecamylamine blocks nicotine's activity-decreasing and activity-stimulating actions, indicating that central nicotinic cholinergic systems are involved in both effects (Stolerman et al., 1995). It has been hypothesized that actions at different nicotinic cholinergic receptor subpopulations might account for the biphasic pattern, but to-date, this question remains unanswered (Di Chiara, 2000). If this hypothesis is accurate, then the data reported here suggest that low nicotine dosages only activate the subpopulation of nicotine receptors responsible for the activity-decreasing action. Low dosages, therefore, could be used to dissociate nicotine's biphasic actions and study the activity-decreasing action. In addition, these data may indicate that one reason for age differences in nicotine's actions is related to differences in receptor subpopulations between adolescent and adult rats.

Within-session data provide a more detailed picture of age differences in nicotine's dose–response effects. On Drug Day 1, the first exposure to nicotine, among adolescents, the 1.0- and 0.50-mg/kg dosages increased activity above the saline level for most of the session. In contrast, among adults, the 1.0-mg/kg dosage decreased activity below the saline level in the early part (first 10 min) of the session and resulted in activity levels similar to saline-treated animals throughout the remainder of the session. The 0.50-mg/kg dosage also decreased activity below the saline level in the early part of the session, but activity then increased for the remainder of the session to levels above the saline group. This pattern, in which adolescents were less sensitive than adults to nicotine's activity-decreasing actions, became more pronounced as Drug Phase I continued.

On Drug Days 4 and 8, among adolescents, the 1.0-mg/kg dosage increased activity throughout the session and the 0.50-mg/kg dosage increased activity for most of the session, with activity becoming similar to saline group levels during the last third of the session. Among adults, however, the 0.50-mg/kg dosage increased activity throughout the session and the 1.0-mg/kg dosage resulted in a drop to saline levels during the early part of the session with a rebound above the saline level during the later part of the session. These data suggest that not only were adolescents less sensitive than adults to nicotine's activity-decreasing actions at the 1.0-mg/kg dosage but that they were somewhat less sensitized to nicotine's activity-increasing actions at the 0.50-mg/kg dosage.

On Drug Day 12, adolescents continued to exhibit less sensitivity to nicotine's activity-decreasing actions at the 1.0-mg/kg dosage but were no longer less sensitive to nicotine's activity-increasing actions at the 0.50-mg/kg dosage. Both dosages produced peak activity during most of the Drug Day 12 session. Adults, however, continued to exhibit sustained peak activity at the 0.50-mg/kg dosage and a drop in activity at the 1.0-mg/kg dosage to the saline level early in the session with a rebound above the saline level later in the session.

The behavioral observation data add important details to this picture. First, these data indicate that decreased activity by adults at the 0.01-mg/kg dosage is unlikely to have occurred because of ataxic motor responses. Observations indicated that ataxia occurred at the 0.50- and 1.0-mg/kg dosages, but not at the 0.01-mg/kg dosage. Second, observation data indicate that nicotine's activity-altering effects may differ qualitatively and quantitatively depending on the testing environment—a nonsocial environment (e.g., the locomotor testing chamber) compared to a familiar social environment (e.g., the home cage in the presence of a cage mate). Other investigators have reported that nicotine's activity-enhancing effects are greater when animals have been acclimated to the testing environment (Stolerman et al., 1995). To our knowledge, the present report is the first that nicotine's activity effects may be sensitive to environmental context.

The observation data also provide information about nicotine's effects on social behaviors. For example, behaviors associated with dominance and possibly aggression—grooming other and wrestling—were decreased by nicotine administration. Importantly, adolescents exhibited higher rates of these behaviors than did adults and nicotine decreased these behaviors more in adolescents than in adults. These findings are consistent with our report that nicotine chronically administered via minipump can reduce aggressive behavior in adult animals when tested in the unfamiliar social environment of the social interaction test (Scheufele et al., 2000). It also is possible, however, that these decreases occurred because nicotine induced behaviors that were incompatible with wrestling and grooming other (e.g., increased activity and increased ataxia). Given the small number of animals tested, these data are preliminary, but may indicate that testing of drug behavioral actions should occur in social as well as nonsocial environments to better understand why humans self-administer reinforcing drugs.

4.2. Interim Phase

Age differences in the consequences of nicotine exposure were evident in activity responses during the Interim Phase, during which no nicotine was administered. When responses were considered across the four Interim Phase days, adolescents that had been exposed to the 0.50- and 1.0-mg/kg dosages exhibited increased horizontal activity compared to saline-exposed animals. Adults exposed to the same dosages exhibited activity levels indistinguishable from saline-exposed animals and greater than the 0.01-mg/kg group.

Similar findings were revealed by the within-session analyses.

Adolescents, therefore, were more sensitive than were adults to the nicotine exposure effect of increased activity when nicotine was not administered. It is possible that the increases for adolescents reflect an associative learning process rather than a pharmacologic effect of prior nicotine exposure. For example, animals may have associated the activity testing chambers with nicotine injections, resulting in increased activity when placed in the chambers even though no injections were given. However, these data replicate our report that nicotine administered chronically during adolescence to males results in hyperactivity in adulthood when nicotine is no longer present (Faraday et al., 2001). In the chronic study, because nicotine was administered via osmotic minipump, no cues for drug administration were present that might trigger activity increases. In addition, Trauth et al. (1999) reported that adolescent nicotine exposure in male rats is associated with up-regulation of nicotinic cholinergic receptors that persists into adulthood. The hyperactivity in the Interim Phase reported here may be the behavioral consequence of this long-term receptor-level change.

4.3. Drug Phase II

When data were considered across the 12 days of Drug Phase II, all animals exhibited continued tolerance to nicotine's activity-decreasing actions and increased sensitization to nicotine's activity-increasing actions (evidenced by rising activity levels over the phase). Tolerance to the activity-decreasing actions despite having not received nicotine for over 2 weeks replicates reports that this type of tolerance can persist for weeks to months without nicotine administration (Stolerman et al., 1973; Clarke and Kumar, 1983a).

Continued age differences in response to nicotine were evident, even though adolescent animals were now adult, in that dose–response curves for each age group continued to differ. In particular, the 0.50- and 1.0-mg/kg dosages continued to produce peak activity among animals that had been exposed to nicotine initially as adolescents but peak activity for animals exposed initially as adults occurred at the 0.50-mg/kg dosage and the 0.01-mg/kg dosage decreased activity. In addition, among animals initially exposed as adolescents, the 0.10-mg/kg dosage also increased activity above the saline level. Overall, nicotine's effects to increase activity at the 0.10-, 0.50-, and 1.0-mg/kg dosages appeared to be greater in animals initially exposed in adolescence compared to animals exposed in adulthood. The variance explained by the Day × Drug interaction among adolescents (29.2%) was nearly triple the variance explained by the same interaction among adults (10.5%).

Within-session patterns on Drug Day 1 of this phase were similar for "adolescents" and adults; the 0.50-mg/kg dosage increased activity throughout the session and the 1.0-mg/kg dosage decreased activity to the saline level with rebound above the saline level later in the session. Age of initial exposure effects emerged by Drug Day 4 and was evident through Drug Day 12. On these days, for adolescents, the 1.0- and 0.50-mg/kg dosages increased activity above the saline level throughout the session and these effects grew larger as the phase progressed. For adults, however, peak activity occurred at the 0.50-mg/kg dosage, with the 1.0-mg/kg dosage decreasing activity to the saline group level early in the session and rebounding above the saline level later in the session. As in Drug Phase I, these patterns suggest that adolescents were less sensitive than adults to nicotine's activity-decreasing actions. In addition, these patterns suggest that initial exposure to nicotine during adolescence resulted in increased sensitivity to nicotine's activity-increasing actions upon nicotine reexposure.

The behavioral observation data from Drug Phase II are strikingly different from Drug Phase I and indicate again the importance of examining drug behavioral effects in different environments. During Drug Phase II, nicotine increased activity robustly in the locomotion chambers in animals first exposed in adolescence, but during the same period nicotine effects in the home cage essentially disappeared for animals initially exposed in adolescence. In the home cage, therefore, adolescents appeared to become tolerant to all of nicotine's actions—the activity-increasing actions as well as the activity-decreasing actions. In contrast, home cage behaviors of adults during Drug Phase II were similar to behaviors during Drug Phase I, suggesting that this tolerance did not occur in animals initially exposed to nicotine as adults.

It is important to note that adolescent animals have been reported to exhibit higher activity levels than adult animals when exposed to novel environments. These effects are especially marked in social environments and in environments that allow exploration such as two-chamber apparatuses and hole-poke boards (see Laviola et al., 1999, for review). In this experiment, the reverse pattern was evident with adult animals consistently exhibiting higher activity levels than adolescents. Greater activity by adults may have occurred because the testing environment was not novel and the locomotion chamber provided limited opportunity for exploration. Before the first drug exposure, animals had been exposed to the locomotor boxes three times—two acclimation exposures and a baseline testing session. During the study, animals were exposed to the apparatus an additional 28 times—12 sessions during Drug Phase I, 4 sessions during the Interim Phase, and 12 sessions during Drug Phase II.

It also is important to note that there were activity differences between the saline groups even when adolescents had become adults, with older animals exhibiting greater activity levels. It is possible that differences between the saline groups continued to reflect age differences in activity levels when animals are repeatedly tested in a familiar environment. For example, by Drug Day 1 of Drug Phase II (see Fig. 7a and b), activity levels of saline-treated "adolescents" were

similar to activity levels of saline-treated adults on Drug Day 1 of Drug Phase I—on these days animals were about the same age (about 60 days old). However, it also is evident that saline-treated "adolescents" tended to decrease activity over Drug Phase II to levels lower than saline-treated adults during Drug Phase I. It is possible that, because adolescents are reported to be more sensitive to novel environments than adults, repeated testing in a familiar environment had the opposite effect and acted to suppress activity in adolescents compared to adults. This is an important point that requires more empirical examination.

4.4. Summary and implications

These findings indicate that: (1) adolescent and adult male rats exhibit different dose–response effects to acutely administered nicotine; (2) exposure to nicotine in adolescence has 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) exposure to nicotine in adolescence results in the development of tolerance to nicotine's effects in certain environments in adulthood. These findings may be relevant to understand the vulnerability of adolescent humans to smoking initiation and maintenance.

For example, when animals initially exposed to nicotine as adolescents were reexposed to nicotine in adulthood, they appeared to exhibit not only less sensitivity to nicotine's activity-decreasing actions but greater sensitivity to nicotine's activity-increasing actions than animals initially exposed as adults (Drug Phase II data). The locomotion-enhancing effects of nicotine occur because nicotine stimulates dopaminergic transmission (Walter and Kuschinski, 1989; O'Neill et al., 1991). These data suggest, therefore, that initial exposure to nicotine in adolescence compared to adulthood results in greater dopaminergic activity when animals are reexposed to nicotine as adults. If these data extrapolate to humans, then exposure to nicotine during adolescence may alter the sensitivity of dopaminergic systems. If these alterations affect motivational systems, such as those implicated in reinforcement, then smoking during adolescence may predispose adolescents to continue to self-administer nicotine in adulthood.

Importantly, exposure to nicotine in adolescence resulted in complete tolerance to all of nicotine's behavioral actions in adulthood in the home cage environment. These findings underscore the role of environment in revealing nicotine's behavioral actions, especially the comparison of behavioral actions in controlled vs. naturalistic environments. If the home cage findings extrapolate to nicotine actions in typical human environments, then these findings may suggest that individuals who begin smoking in adolescence will be likely to smoke more in adulthood to obtain particular effects of nicotine (i.e., because they are tolerant).

Adolescent rodents appear to be less sensitive than adults to the acute activity-stimulating effects of other drug of abuse such as amphetamine and cocaine but can develop greater sensitization to these effects after repeated exposures (Lanier and Isaacson, 1977; Spear and Brick, 1979; Bolanos et al., 1998; Snyder et al.,1998; Laviola et al., 1999). Nicotine's actions in the present experiment were partially consistent with these reports. During Drug Phase I, although dose–response relationships differed between adolescents and adults, the average magnitude of activity increases was similar. Age differences in the magnitude of activity-stimulating effects did not appear until Drug Phase II when animals were reexposed to nicotine. Animals initially exposed as adolescents exhibited greater activity stimulation in response to nicotine than did animals initially exposed as adults.

There are several possible mechanisms that could account for age differences in nicotine's actions. For example, adolescent and adult rats may metabolize nicotine at different rates. Adolescent and adult rats may differ in distribution, density, or affinity of central nicotinic cholinergic receptors (nAChRs), nAChR subpopulations, or in rates of nAChR up-regulation or desensitization in response to nicotine administration. There also may be age differences in consequences of nAChR activation such as in the amount and time-course of dopamine or other neurotransmitter release. All of these questions are relevant to understand why young people smoke and remain to be answered.

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 Robert Wood Johnson Foundation grant 036413.

References

Bolanos CA, Glatt SJ, Jackson D. Subsensitivity to dopaminergic drugs in periadolescent rats: a behavioral and neurochemical analysis. Dev Brain Res 1998;111:25–33.

Chassin L, Presson C, Rose J, Sherman S. The natural history of cigarette smoking from adolescence to adulthood: demographic predictors of continuity and change. Health Psychol 1996;15(6):478–84.

Clarke PBS, Fu DS, Jakubovic A, Fibiger A. Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. J Pharmacol Exp Ther 1988;246:701–8.

Clarke PBS, Kumar R. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br J Pharmacol 1983a;78:329–37.

Clarke PBS, Kumar R. Characterization of the locomotor stimulant action of nicotine in tolerant rats. Br J Pharmacol 1983b;80:587–94.

Dappen A, Schwartz R, O'Donnell R. A survey of adolescent smoking patterns. J Am Board Fam Pract 1996;9(1):7–13.

Di Chiara G. Behavioural pharmacology and neurobiology of nicotine reward and dependence. In: Clementi F, Fornasari D, Gotti C, editors. Handbook of experimental pharmacology, vol. 144. Heidelberg: Springer; 2000. p. 603–750.

Faraday MM, Elliott B, Grunberg NE. Nicotine's biobehavioral actions differ in adult vs. adolescent rats. Pharmacol Biochem Behav 2001; 70:1–15.

Gilpin EA, Choi WS, Berry C, Pierce JP. How many adolescents start smoking each day in the United States? J Adolesc Health 1999; 25(4):248–55.

Johnston LD, O'Malley PM, Bachman JG. National survey results on drug use from the monitoring the future study, 1975–1995. Rockville (MD): U.S. National Institute on Drug Abuse; 1992.

Ksir C, Hakan RL, Kellar KJ. Chronic nicotine and locomotor activity: influences of exposure dose and test dose. Psychopharmacology 1987; 92(1):25–9.

Lanier LP, Isaacson RL. Early developmental changes in the locomotor response to amphetamine and their relation to hippocampal function. Brain Res 1977;126:567–75.

Laviola G, Adriani W, Terranova ML, Gerra G. Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. Neurosci Biobehav Rev 1999;23:993–1010.

O'Neill MF, Dourish CT, Iversen SD. Evidence for an involvement of D1 and D2 dopamine receptors in mediating nicotine-induced hyperactivity in rats. Psychopharmacology 1991;104:343–50.

Scheufele PM, Faraday MM, Grunberg NE. Nicotine administration interacts with housing conditions to alter social and nonsocial behaviors in male and female Long–Evans rats. Nicotine Tob Res 2000;2:169–78.

Snyder KJ, Katovic NM, Spear LP. Longevity of the expression of behavioral sensitization to cocaine in preweanling rats. Pharmacol Biochem Behav 1998;60:909–14.

Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 2000;24:417–63.

Spear LP, Brick J. Cocaine-induced behavior in the developing rat. Behav Neural Biol 1979;26:401–15.

Stolerman IP, Fink R, Jarvik ME. Acute and chronic tolerance to nicotine measured by activity in rats. Psychopharmacology 1973;30:329–42.

Stolerman IP, Garcha HS, Mirza NR. Dissociations between the locomotor stimulant and depressant effects of nicotinic agonists in rats. Psychopharmacology 1995;117:430–7.

Trauth JA, Seidler FJ, McCook EC, Slotkin TA. Adolescent nicotine exposure causes persistent upregulation of nicotinic cholinergic receptors in rat brain regions. Brain Res 1999;851:9–19.

U.S. Department of Health and Human Services. Reducing the health consequences of smoking: 25 years of progress. A report of the Surgeon General. DHHS Pub. No. (CDC)89-8411. Washington (DC): U.S. Government Printing Office; 1989.

U.S. Department of Health and Human Services. Preventing tobacco use among young people: a report of the Surgeon General. Atlanta (GA): U.S. Department of health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. Washington (DC): U.S. Government Printing Office; 1994.

Walter S, Kuschinsky K. Conditioning of nicotine effects on motility and behaviour in rats. Naunyn-Schmiedeberg's Arch Pharmacol 1989; 339(1–2):208–13.

Winders SE, Grunberg NE. Nicotine, tobacco smoke, and body weight: a review of the animal literature. Ann Behav Med 1989;11(4):125–33.