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Adult vs. adolescent rats differ in biobehavioral responses to chronic nicotine administration

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Abstract

More than 90% of smokers begin smoking during adolescence, suggesting that nicotine's actions may differ in adults vs. adolescents in ways that render adolescents vulnerable to smoking initiation. This experiment tested the hypothesis that nicotine's biobehavioral actions differ in adult and adolescent rats. Forty-two male (21 adolescents, 21 adults) and 41 female (21 adolescents, 20 adults) Sprague–Dawley rats were administered saline or 12 mg/kg/day nicotine via osmotic minipump for 21 days. Body weight, feeding, and locomotion (horizontal activity, vertical activity, center time) were measured before, during, and after saline or nicotine administration. Nicotine's effects depended on age and sex. Nicotine reduced body weight and feeding of adult males and females, and of adolescent males, but not of adolescent females. In addition, adolescent males were more sensitive than adults or adolescent females to nicotine's activity-enhancing effects. In cessation, nicotine-exposed adolescent males continued to exhibit greater activity than saline-exposed animals. Results indicate that nicotine's biobehavioral actions differ depending on age and sex.

Keywords: Sprague–Dawley rats; Males and females; Feeding; Vertical activity; Adult rats; Nicotine; Body weight; Center time; Adolescent rats; Locomotion; Horizontal activity

1. Introduction

More than 90% of adult smokers initiated tobacco use before age 20 (Dappen et al., 1996; Chassin et al., 1996; U.S. Department of Health and Human Services [USDHHS], 1989), indicating that understanding why adolescents initiate and maintain tobacco use is the key to prevention. Despite extensive prevention efforts, rates of cigarette smoking among American adolescents have been resistant to change, with 20% of high school seniors smoking daily (Johnston et al., 1992) and with 3000 American children a day beginning to smoke (USDHHS, 1994). Studies of adolescent tobacco use have focused on psychosocial factors that influence initiation, maintenance, and cessation. It is possible that an important reason for initiation and maintenance of tobacco use by adolescents is one that has not been thoroughly considered or evaluated: differences between adults and adolescents in nicotine's biobehavioral actions.

Adolescents, like adults, report smoking for reasons related to nicotine's behavioral and biological actions, including: feelings of pleasure or reward; control of body weight and feeding; and modulation of arousal and mood states (Frank et al., 1991; Klesges et al., 1997; Stanton et al., 1993; Sarason et al., 1992; Tuakli et al., 1990; Byrne et al., 1995; Friedman et al., 1985; Botvin and McAlister, 1981). It is not known, however, whether adolescents differ from adults in behavioral sensitivity to nicotine in ways that make young people particularly vulnerable to smoking initiation and maintenance. Such studies are difficult to perform in young people, in part because of ethical concerns (i.e., 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 studies of nicotine effects have not been reported to date in adolescent rats.

There is some evidence that adolescent drug use vulnerability is neurobiologically based in age-associated developmental changes in the brain (Spear, 2000). In particular,

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adolescent rodents appear to be less sensitive than adults to the activity-stimulating effects of dopaminergic agonists such as amphetamine and cocaine (Lanier and Isaacson, 1977; Spear and Brick, 1979; Bolanos et al., 1998; Snyder et al., 1998; Laviola et al., 1999). Evidence that adolescence may be associated with differential behavioral sensitivity to some drugs may be relevant to human adolescent smoking initiation. The purposes of this experiment were to: (1) compare nicotine's chronic effects in adolescent vs. adult rats; (2) compare nicotine's cessation effects in adolescent vs. adult rats; and (3) determine whether there are gender differences in nicotine's effects during nicotine administration and/or cessation in adolescent rats. The dependent measures were body weight, feeding, and locomotion. These measures were selected because they are indices of nicotine's actions and substantial research literature is available in adult rats for comparison. Locomotion is a particularly useful behavior because different aspects of locomotion (e.g., horizontal activity, vertical activity, time spent in the center of an open field) have been interpreted to reflect different physiological or emotional states (i.e., arousal and dopaminergic stimulation, exploration, and possibly fearfulness or anxiety, respectively) (e.g., Ader and Conklin, 1963; Archer, 1973; Walsh and Cummins, 1976; Consroe et al., 1982; Sanberg et al., 1983; Nichols and Schreur, 1987; Young and Johnson, 1991; Crawley et al., 1997; Zocchi et al., 1998; Faraday et al., 1999b). Increased time spent in the center of an open field vs. in the margin, for example, may be an index of anxiolysis.

Nicotine was administered chronically via osmotic minipump (12 mg/kg/day). This route of drug administration avoids the stress of repeated injections. This relatively high dosage was used because: (1) it produces behavioral effects in adult rats that parallel behavioral changes in adult human smokers (e.g., Grunberg, 1982; Winders and Grunberg, 1989); (2) it produces clear and near-maximal behavioral effects without harm in adult rats (e.g., Grunberg and Bowen, 1985; Acri et al., 1991, 1995; Faraday et al., 1998, 1999a,b); and (3) if adolescents are less sensitive than adults to dopaminergic agonists, then a lower dosage might be ineffective.

Nicotine's chronic effects may be relevant to understand behaviors of heavy smokers who are likely to maintain nicotinic cholinergic receptors in a chronically desensitized state as a result of frequent and intensive nicotine self-administration (Benwell et al., 1995). In addition, many smokers maintain a significant concentration of nicotine in plasma throughout much of the day and some nicotine replacement therapies (e.g., nicotine patch) provide continuous nicotine administration (Benowitz et al., 1990; Russell, 1990). Use of this dosage via this route of administration, however, may limit extrapolation of findings to relatively heavy smokers.

Reports of nicotine's chronic effects vary depending on nicotine dosage, rat sex, and rat strain (i.e., Faraday et al., 1999b). In adult Sprague–Dawleys, the 12-mg/kg/day dosage decreases body weight and feeding, with greater effects generally reported in females (i.e., Grunberg, 1982; Grunberg and Bowen, 1985; Grunberg et al., 1984, 1986, 1988; Bowen et al., 1986). This dosage increased horizontal and vertical activity after 8 days of chronic infusion in adult Sprague–Dawley males (Grunberg and Bowen, 1985). In contrast, the same dosage in female Sprague–Dawleys did not reliably alter activity (Bowen et al., 1986). Females as well as males were included in the present experiment to determine whether sex differences in sensitivity to nicotine existed among adolescents.

Changes in body weight, feeding, and locomotion also have been used to quantify withdrawal from chronic nicotine exposure. For Sprague–Dawley males, body weight remains suppressed (i.e., nicotine-exposed animals do not return to control animal body weights) and feeding does not change; for Sprague–Dawley females, body weight returns to control level and feeding increases (i.e., Grunberg, 1982; Grunberg and Bowen, 1985; Grunberg et al., 1984, 1986, 1988; Bowen et al., 1986). With regard to locomotion, in Sprague–Dawley males, cessation decreased activity (Grunberg and Bowen, 1985). In Sprague–Dawley females, no changes in activity occurred in cessation (Bowen et al., 1986).

2. Methods

2.1. Subjects

Subjects were 42 male (21 adolescents and 21 adults) and 41 female (21 adolescents and 20 adults) Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA). Animals were housed in same-sex, 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 at 50% relative humidity on a 12-h reverse light/dark cycle (lights on at 1900 h). Locomotor activity was measured during the dark (active) phase of the light cycle (between 0900 and 1600 h). At the beginning of the experiment, adult animals were approximately 60 days old (average male weight = 268.7 g, average female weight = 200.1 g) and adolescent animals were approximately 30 days old (average male weight = 86.1 g, average female weight = 84.1 g). The experiment was conducted as a 2 (male or female) $\times$ 2 (0 or 12 mg/kg/day nicotine) $\times$ 2 (adult or adolescent) full factorial design, with about 10 subjects per treatment cell. Adolescence was defined in consultation with the breeder as the period spanning 35 to 60 days (i.e., this period spans presexual maturation into young adulthood; P. Mirley, Charles River Laboratories, personal communication, 2/98). A review by Spear (2000) published after this experiment had been run indicated that the period during which adolescents in this

experiment were exposed to nicotine (beginning at 40 days old) would be considered late adolescence. This experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

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 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. A second side-to-side array of 16 pairs of additional photocells located 10.5 cm above the arena floor measured vertical activity. Time spent in the center vs. time spent in the margin of the arena also was recorded. 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 2 h. Once subjects were placed in the test arenas, the experimenter turned off the lights and left subjects undisturbed during the testing period. Cagemates 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. Drug administration and surgical procedure

Nicotine (12 mg/kg/day; expressed as nicotine base) or physiologic saline was administered via Alzet osmotic minipumps (Model 2002, Alza, Palo Alto, CA). Physiological saline also was used as vehicle for the nicotine solution. Nicotine solution was made from nicotine dihydrochloride.

Subjects were anesthetized using methoxyflurane (Metofane) in a bell jar inside a vented hood. Minipumps were implanted subcutaneously between the shoulder blades according to procedures described in detail elsewhere (e.g., Grunberg, 1982; Acri, 1994). The duration of the surgery, including anesthesia, was approximately 4 min per subject.

2.4. Procedure

The procedure included three phases: predrug phase (baseline phase), during-drug administration phase (during-drug phase), and drug cessation phase (cessation phase). Body weight and food consumption were measured throughout the three phases. Food consumption data are the average amounts eaten per group over the days between measurements: These data, therefore, are cumulative between measurements and amounts vary depending on the measurement interval.

2.4.1. Baseline phase

Subjects were handled once each day for 2 days to minimize any stress that might occur as a result of necessary handling for body weight and locomotion measurements. All subjects ($N = 83$) also 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 1–2 days after the final acclimation period.

2.4.2. Drug administration phase

After the completion of baseline measures, subjects were assigned within sex and age to drug (0 or 12 mg/kg/day nicotine) groups in a manner that assured comparable, initial body weights in same-sex, same-age groups. Minipumps containing the appropriate solutions were implanted on the last baseline day. Drug Day (DD) 1 was designated as the day after implant. Locomotor activity was measured for 2 h on DDs 1, 4, 7, 10, and 14. Body weight and food consumption were measured on DDs 2, 4, 6, 8, 10, 14, 19, and 21.

2.4.3. Cessation phase

Based on minipump fill volume (measured for each pump when minipumps were filled) and flow rate (provided by the manufacturer), it was calculated that drug administration ceased (i.e., that nicotine volume had been exhausted) on DD 22. Cessation Day (CD) 1 was defined, therefore, as the 23rd day after implant.[1] Locomotor activity was measured on CDs 1, 4, 7, 18, and 26. Body weight and food consumption were measured on CDs 1, 4, 7, 11, 14, 18, 21, 25, and 28.

3. Results

3.1. Data analytic strategy

Drug phase and cessation phase body weight, food consumption, and locomotion data initially were analyzed

[1] For logistical reasons involving access to laboratories, we were unable to remove minipumps in this experiment. We are aware that minipump explant is optimal for the assessment of acute withdrawal and have followed this procedure in other experiments. For this reason, we focus data interpretation on the postnicotine administration period in general rather than on the first 24 to 48 h of withdrawal.

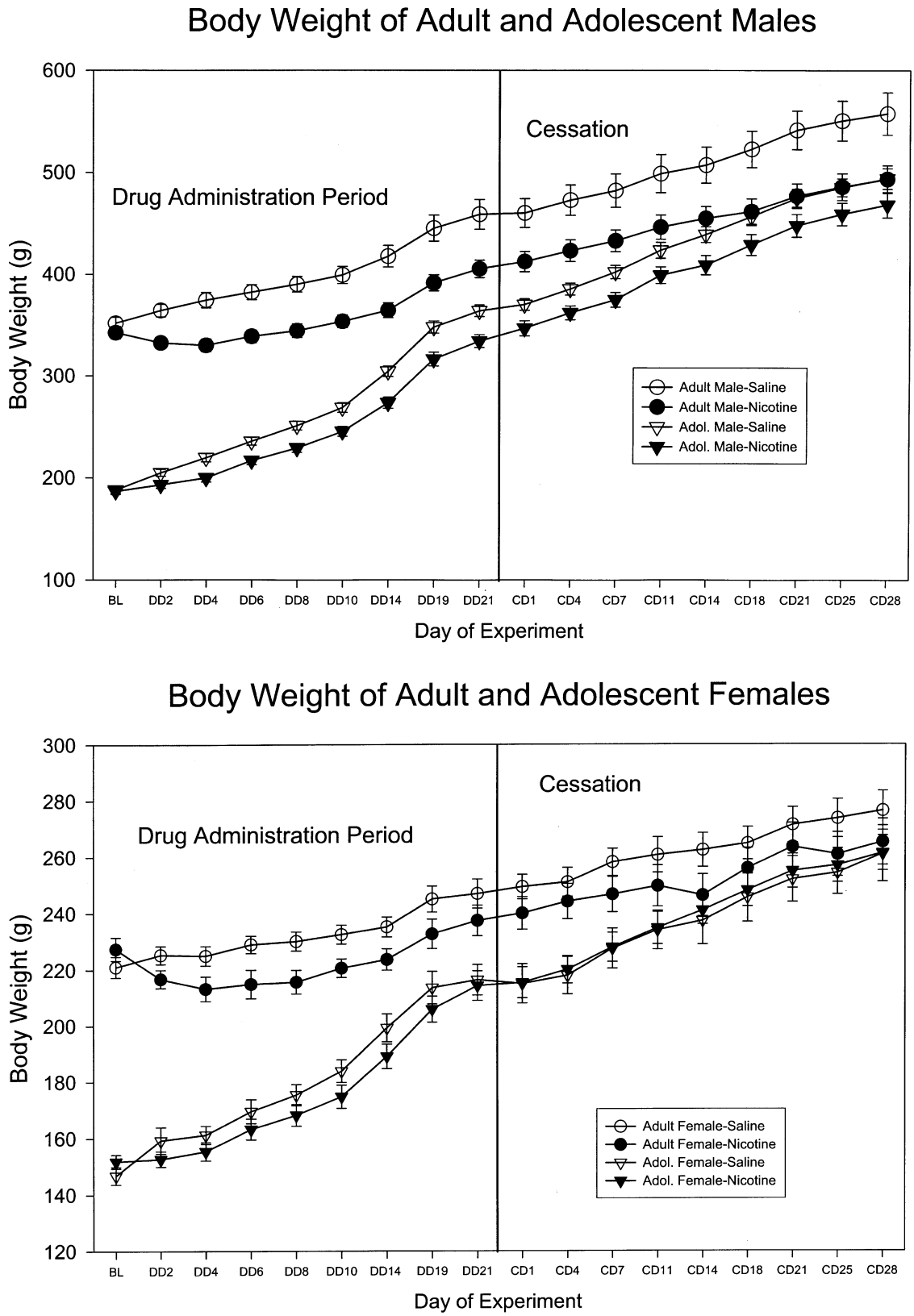

Fig. 1. (a) Body weights (g) (group means ± S.E.M.) for adult and adolescent males during drug administration and cessation. (b) Body weights (g) (group means ± S.E.M.) for adult and adolescent females during drug administration and cessation.

by separate repeated-measures analyses of variance (ANOVAs) with factors of sex, age, and drug. These global analyses revealed significant interactions of sex, age, or drug with time, or significant sex, age, or drug effects on average over the repeated measurements for each variable during each phase with the exception of center time data from the cessation phase. These analyses served as the justification for overall ANOVAs on each day as post hoc tests to establish the presence of sex, age, and drug effects on specific days. A strict Fisherian strategy was used to limit the number of subsequent tests run. That is, if the overall ANOVA did not reveal significant effects, then no further tests were run on that variable for that day. If the ANOVA revealed significant effects, then further analyses were carried out.

For body weight and food consumption data, the largest significant effect on most days was for sex. Therefore, data for males and females were examined separately, testing for effects of age and drug. If the within-sex analyses revealed significant effects of age or drug, or Age × Drug interactions, then same-sex, same-age groups were examined further for drug effects. If within-sex analyses did not reveal significant effects, then no further analyses were conducted. For locomotion variables, the largest significant effect on most days was for age. Therefore, data for adults and adolescents were examined separately, testing for effects of sex and drug. Same-sex, same-age groups were examined further if this analysis revealed significant effects.

All tests were two-tailed. Results are significant at $P < .05$ unless otherwise noted. Trends (i.e., P values greater than .05) are reported where they are part of an overall pattern of mostly significant effects. There were no differences between same-sex, same-age groups in baseline body weights or food consumption. Adolescent females assigned to the nicotine group exhibited significantly less horizontal activity at baseline than did adolescent females assigned to the saline group. Results for the drug administration period, however, were the same with and without baseline activity as a covariate (i.e., baseline activity was not significantly correlated with activity during drug administration). Analyses are reported without the covariate.

3.1.1. Body weight: drug administration phase

Overall ANOVAs indicated that males were heavier than females, adults were heavier than adolescents, and saline-treated animals were heavier than nicotine-treated animals on every measurement day (see Fig. 1a and b; see Table 1a for F values). Sex × Drug interactions on each day indicated that nicotine effects were greater in males than in females. On DDs 2, 4, 6, and 8, Age × Drug interactions revealed that nicotine effects were greater in adults than in adolescents.

Table 1
Body weight

(a) During nicotine administration

	Significant nicotine effects and interactions on body weight when all animals were considered together			Significant nicotine effects on body weight for same-sex, same-age treatment groups			
	F values (df = 1,75)			F values (df = 1,19)	F values (df = 1,19)	F values (df = 1,18)	F values (df = 1,19)
Drug day	Drug effect	Sex × Drug	Age × Drug	Adult males	Adolescent males	Adult females	Adolescent females
2	24.3	5.7	3.5	14.86	5.76	n.s.	n.s.
4	36.9	12.0	5.2	20.85	11.85	4.41	n.s.
6	35.4	9.1	5.5	20.06	11.39	5.64	n.s.
8	39.5	10.5	4.7	19.74	13.63	7.16	n.s.
10	37.2	10.6	n.s.	18.19	12.22	6.49	n.s.
14	38.8	13.5	n.s.	16.82	17.38	4.90	n.s.
19	27.5	10.5	n.s.	12.85	12.13	n.s.	n.s.
21	19.4	11.1	n.s.	9.92	12.30	n.s.	n.s.

(b) Nicotine cessation

	F values (df = 1,74)			F values (df = 1,18)	F values (df = 1,19)	F values (df = 1,18)	F values (df = 1,19)
Cessation day	Drug effect	Sex × Drug	Age × Drug	Adult males	Adolescent males	Adult females	Adolescent females
1	12.6	7.6	n.s.	7.5	6.5	n.s.	n.s.
4	11.3	8.7	n.s.	7.4	6.8	n.s.	n.s.
7	12.7	7.1	n.s.	6.3	7.9	n.s.	n.s.
11	10.0	5.8	n.s.	5.7	4.8	n.s.	n.s.
14	11.4	6.2	n.s.	6.2	6.2	n.s.	n.s.
18	10.8	8.1	n.s.	7.9	4.3	n.s.	n.s.
21	10.3	8.3	n.s.	8.3	3.7 (P=.07)	n.s.	n.s.
25	11.0	7.0	n.s.	7.6	3.6 (P=.07)	n.s.	n.s.
28	9.2	5.7	n.s.	6.8	n.s.	n.s.	n.s.

(a) F values for significant nicotine effects during nicotine administration when all animals were considered together and when same-sex, same-age treatment groups were considered separately; (b) F values for significant nicotine exposure effects after nicotine cessation when all animals were considered together and when same-sex, same-age treatment groups were considered separately.

Among males, adults were heavier than adolescents and saline-treated animals were heavier than nicotine-treated animals on every measurement day. In addition, Age × Drug Drug interactions on DDs 2, 4, 6, and 8 indicated that body weight decreases in nicotine-treated adult males were larger than those in nicotine-treated adolescent males [$df = (1,38)$; DD 2: $F = 4.5$; DD 4: $F = 4.8$; DD 6: $F = 4.9$; DD 8: $F = 3.9$]. Among females, adults were always heavier than adolescents and nicotine reduced body weight on every day except for DD 21.

Among same-sex, same-age groups, nicotine reduced body weight on every measurement day for adult males and for adolescent males. Among adult females, nicotine reduced body weights on DDs 4, 6, 8, 10, and 14. In contrast, nicotine administration did not significantly reduce body weights of adolescent females on any measurement day.

3.1.2. Drug cessation phase

Overall ANOVAs revealed that on every cessation day, males were heavier than females, adults were heavier than adolescents, and nicotine reduced body weight (see Table 1b). In addition, on each day Sex × Drug interactions revealed that nicotine effects were greater in males than in females.

Among males, on every day, adults were heavier than adolescents and saline-exposed males were heavier than nicotine-exposed males, indicating that during cessation from nicotine body weight remained suppressed for males. For females, only effects of age were evident and occurred on CDs 1, 4, 7, 11, and 14.

Examination of same-sex, same-age groups revealed that nicotine exposure reduced body weight on every measurement day for adult males, and for adolescent males on most days. In contrast, cessation from nicotine did not suppress body weight among adult or adolescent females (i.e., body weight returned almost immediately to saline control levels).

3.1.3. Food consumption: drug administration phase

Overall ANOVAs (see Table 2) indicated that on each day males consumed more than females and saline-treated animals ate more than nicotine-treated animals on DDs 2, 4, 6, 10, and 19 (see Fig. 2a and b). Sex × Drug interactions, with greater nicotine effects in males than in females, were present on DDs 2, 4, 6, and 21. Age × Drug interactions, with nicotine effects greater in adults than in adolescents, were evident on DDs 2, 4, 6, and 19.

For females, nicotine reduced food consumption on every measurement day except for DD 21 when nicotine-treated animals ate more than did saline-treated animals. For males, nicotine reduced food consumption on every measurement day except for DD 19: similar to females, on DD 21, nicotine-treated males ate more than did saline-treated males. These drug effect reversals on DD 21 are consistent with minipump volume being exhausted (i.e., nicotine administration was tapering off). In addition, for males, significant Age × Drug interactions were revealed on DDs 2, 4, and 19, with food consumption decreases in nicotine-treated adult males larger than those in nicotine-treated adolescent males.

Examination of same-sex, same-age groups (see Table 2) revealed that nicotine reduced food consumption on every measurement day for adult males except for DD 21 and for adolescent males on DDs 2, 4, and 10. For adult females, nicotine reduced food consumption on DDs 2, 4, 6, and 19. Among adolescent females, nicotine reduced feeding only on DD 4. On DD 21, nicotine increased feeding by adult females, adolescent females, and adolescent males, consistent with the beginning of nicotine cessation.

3.1.4. Drug cessation phase

Overall ANOVAs revealed that males consumed more than females on every cessation day. Adults consumed more than adolescents on CDs 11 and 14. Saline-exposed animals consumed more than nicotine-exposed animals on CDs 11, 14, and 18 [$df = (1,74)$; CD 11: $F = 4.8$; CD 14: $F = 8.0$; CD 18: $F = 4.4$]. In addition, there was a Sex × Drug interaction

Table 2
Food consumption

	During nicotine administration						
	Significant nicotine effects and interactions on food consumption when all animals were considered together			Significant nicotine effects on food consumption for same-sex, same-age treatment groups			
	F values ($df = 1,75$)			F values ($df = 1,19$)	F values ($df = 1,19$)	F values ($df = 1,18$)	F values ($df = 1,19$)
Drug day	Drug effect	Sex × Drug	Age × Drug	Adult males	Adolescent males	Adult females	Adolescent females
2	47.1	5.1	7.7	26.9	8.9	14.2	n.s.
4	116.3	12.5	32.0	85.7	11.5	31.6	4.8
6	37.0	4.3	4.6	14.7	n.s.	52.1	n.s.
10	22.4	n.s.	n.s.	14.0	14.3	n.s.	n.s.
19	5.0	n.s.	7.4	7.4	n.s.	5.5	n.s.
21	n.s.	15.7	n.s.	n.s.	4.7	4.9	11.1

F values for significant nicotine effects during nicotine administration when all animals were considered together and when same-sex, same-age treatment groups were considered separately.

with greater nicotine effects in males than in females on CDs 14 and 21 [$df = (1,74)$; CD 14: $F = 5.7$; CD 21: $F = 5.9$]. Separate analyses for each sex indicated that saline-exposed males ate more than nicotine-exposed males on CDs 11, 14,

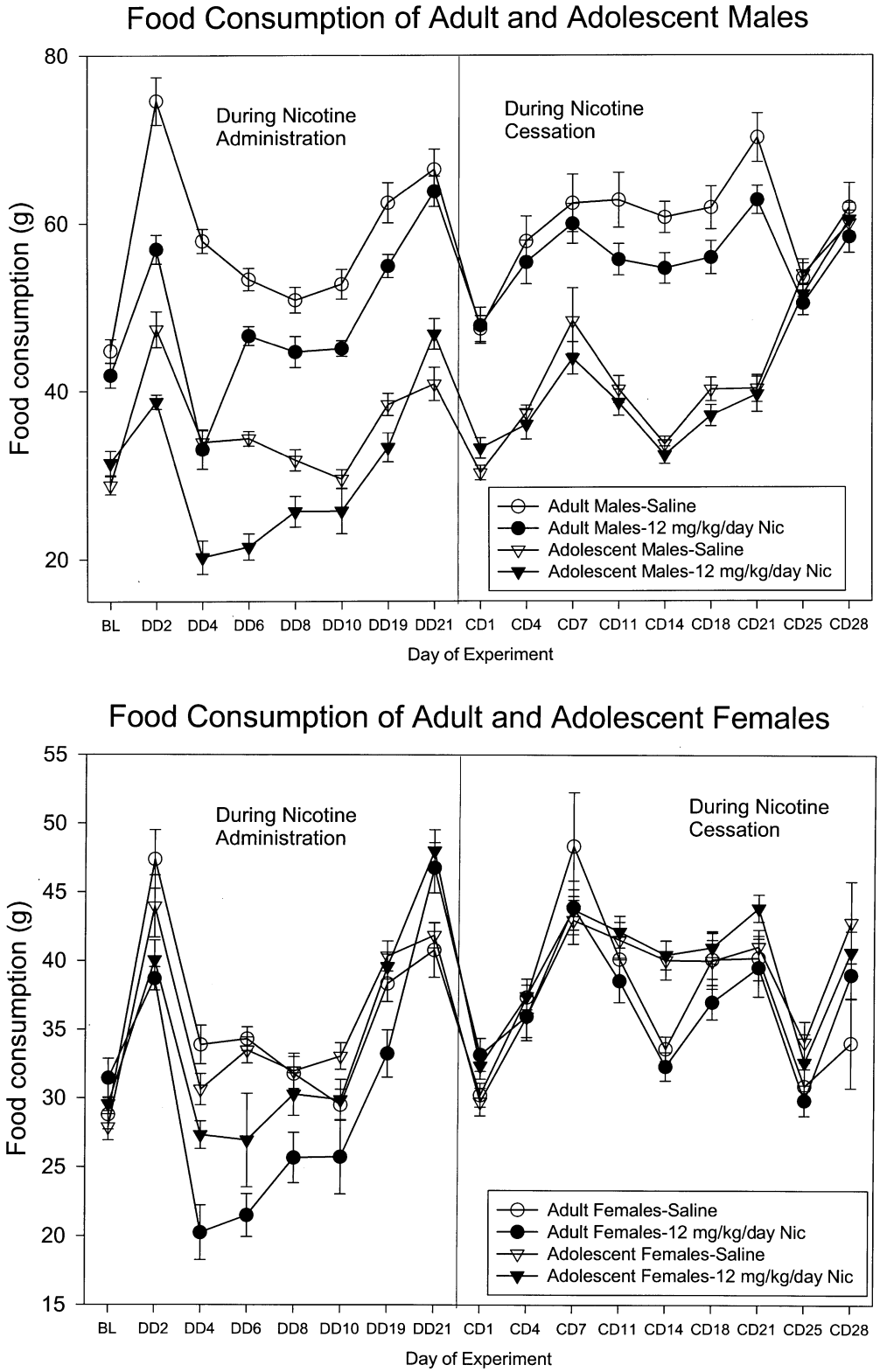

Fig. 2. (a) Food consumption (g) (group means ± S.E.M.) for adult and adolescent males during drug administration and cessation. (b) Food consumption (g) (group means ± sem) for adult and adolescent females during drug administration and cessation.

18, and 21. Saline-exposed females ate less than nicotine-exposed females on CD 1.

Examination of same-sex, same-age groups revealed that nicotine exposure reduced food consumption among adult males on CDs 11, 14, 18, and 21 [$df = (1,18)$; CD 11: $F = 3.54$, $P = .07$; CD 14: $F = 5.5$; CD 18: $F = 3.4$, $P = .08$; CD 21: $F = 4.9$] and among adolescent males on CD 14 [$F(1,18) = 4.4$].

3.2. Locomotion

3.2.1. Horizontal activity: drug administration phase

Overall ANOVAs revealed that females were more active than males on DDs 1, 7, 10, and 14 (see Fig. 3a and b). Adults were more active than adolescents on DDs 1, 4, 7, and 10. Nicotine-treated animals were more active than saline-treated animals on DDs 1, 7, 10, and 14 (see Table 3a).

Among adults, females were more active than males on DDs 7 and 10. Nicotine-treated adults were more active than saline-treated adults on DD 14 [$F(1,36) = 5.8$]. Among adolescents, females were more active than males on DDs 10 and 14. Nicotine-treated adolescents were more active than saline-treated adolescents on DDs 1, 7, 10, and 14 [$df = (1,38)$; DD 1: $F = 13.9$; DD 7: $F = 13.7$; DD 10: $F = 4.3$; DD 14: $F = 6.1$].

Among same-sex, same-age groups (see Table 3a), nicotine increased activity on DDs 1, 7, 10, and 14 for adolescent males and on DDs 1 and 7 for adolescent females.

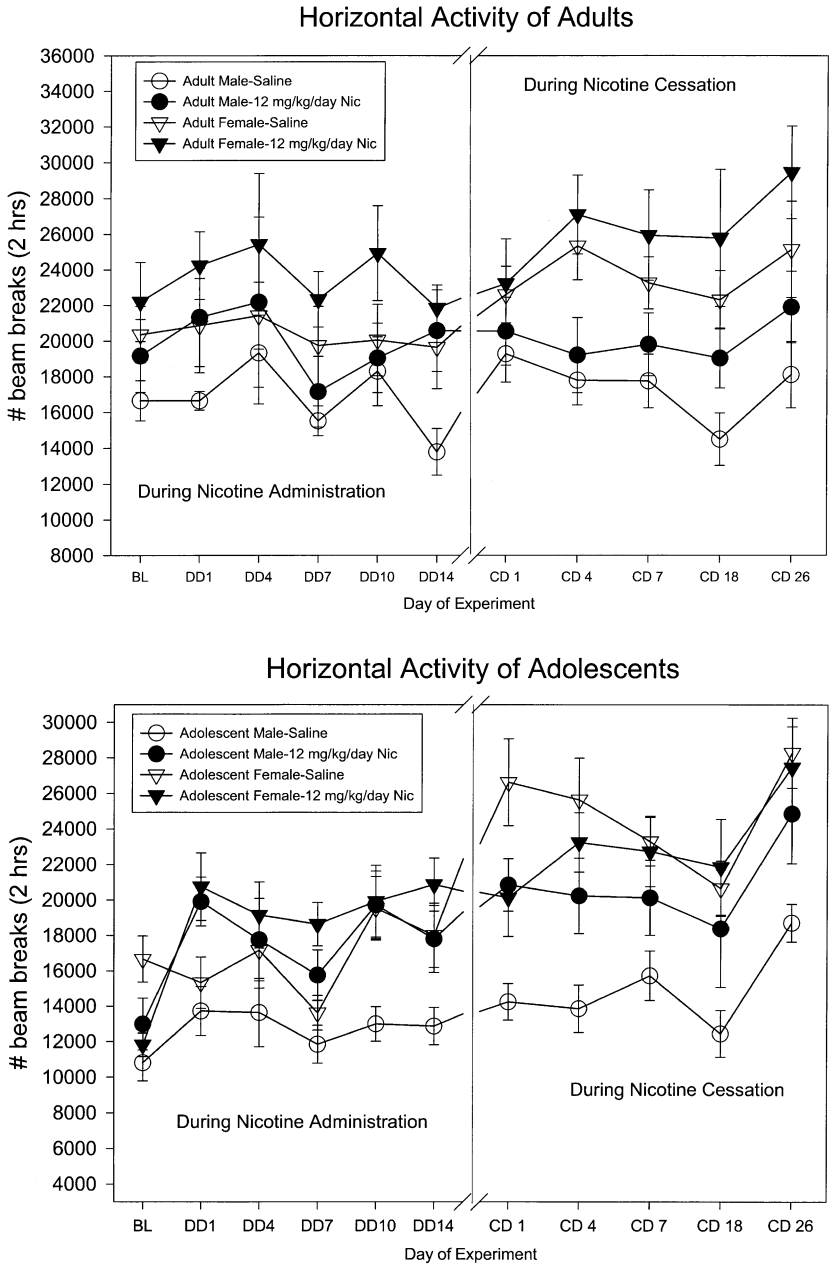

Fig. 3. (a) Horizontal activity over 2 h (number of beam breaks; group means ± S.E.M.) for adult males and females during drug administration and cessation. (b) Horizontal activity over 2 h (number of beam breaks; group means ± S.E.M.) for adolescent males and females during drug administration and cessation.

Table 3
Locomotion

	(a) During nicotine administration: horizontal activity				
	Significant nicotine effects: all animals considered together	Significant nicotine effects for same-sex, same-age treatment groups			
	F values (*df* = 1,75)	*F* values (*df* = 1,19)	*F* values (*df* = 1,19)	*F* values (*df* = 1,18)	*F* values (*df* = 1,19)
Drug day	Drug effect	Adult males	Adolescent males	Adult females	Adolescent females
1	14.2	n.s.	10.0	n.s.	5.0
4	n.s.	n.s.	n.s.	n.s.	n.s.
7	9.9	n.s.	4.8	n.s.	9.6.
10	5.4	n.s.	10.4	n.s.	n.s.
14	11.8	6.6	5.4	n.s.	n.s.
	(b) Center time				
	Significant nicotine effects: all animals considered together	Significant nicotine effects for same-sex, same-age treatment groups			
Drug day	Drug effect	Adult males	Adolescent males	Adult females	Adolescent females
1	8.8	n.s.	9.3	n.s.	n.s.
4	n.s.	n.s.	n.s.	n.s.	n.s.
7	4.9	n.s.	n.s.	4.2	6.6
10	3.1 (*P* = .08)	n.s.	5.6	4.5	n.s.
14	11.1	n.s.	n.s.	4.6	n.s
	(c) Nicotine cessation: horizontal activity				
	F values (*df* = 1,73)	*F* values (*df* = 1,18)	*F* values (*df* = 1,19)	*F* values (*df* = 1,18)	*F* values (*df* = 1,19)
Cessation day	Drug effect	Adult males	Adolescent males	Adult females	Adolescent females
1	n.s.	n.s.	13.9	n.s.	n.s.
4	n.s.	n.s.	6.7	n.s.	n.s.
7	n.s.	n.s.	3.0 (*P* = .09)	n.s.	n.s.
18	5.3	4.2	3.2 (*P* = .09)	n.s.	n.s.
26	4.7	n.s.	4.9	n.s.	n.s.

(a) Horizontal activity: *F* values for significant nicotine effects during nicotine administration when all animals were considered together and when same-sex, same-age treatment groups were considered separately; (b) Center time: *F* values for significant nicotine effects during nicotine administration when all animals were considered together and when same-sex, same-age treatment groups were considered separately; (c) Horizontal activity: *F* values for significant nicotine exposure effects after nicotine cessation when all animals were considered together and when same-sex, same-age treatment groups were considered separately.

For adult males, nicotine increased activity on DD 14. Nicotine did not significantly increase activity of adult females on any measurement day.

3.2.2. Drug cessation phase

Overall ANOVAs revealed that females were more active than males on every measurement day. Nicotine-exposed animals were more active than saline-exposed animals on CDs 18 and 26 (see Table 3c).

Among adults, females were more active than males on CDs 4, 7, 18, and 26. Among adolescents, females were more active than males on every measurement day. In addition, there were Sex × Drug interactions on CDs 1 [$F(1,38) = 12.4$] and 4 [$F(1,37) = 5.5$] such that nicotine-exposed adolescent males were more active than saline-exposed adolescent males while nicotine-exposed adolescent females were less active than saline-exposed adolescent females.

Examination of same-sex, same-age groups (see Table 3c) revealed that prior nicotine exposure did not significantly alter activity of adult females or adolescent females and increased activity of adult males only on CD 18. In contrast, adolescent males with prior nicotine exposure exhibited increased activity on every measurement day.

3.2.3. Vertical activity: drug administration phase

Overall ANOVAs indicated that adults were significantly more active than adolescents on every measurement day (see Fig. 4a and b). In addition, on DD 10 there was a significant Sex × Age × Drug interaction [$F(1,75) = 3.8$] such that nicotine increased activity of adult females and adolescent males, but not of adult males and adolescent females.

Among adults, vertical activity was not altered by sex or drug. Among adolescents, females were more active than males on DD 7. Nicotine-treated adolescents were more active than saline-treated adolescents on DDs 7 [$F(1,36) = 13.4$] and 10 [$F(1,36) = 4.2$].

Among same-sex, same-age groups, nicotine increased activity on DDs 7 [$F(1,18) = 4.0$, $P = .06$] and

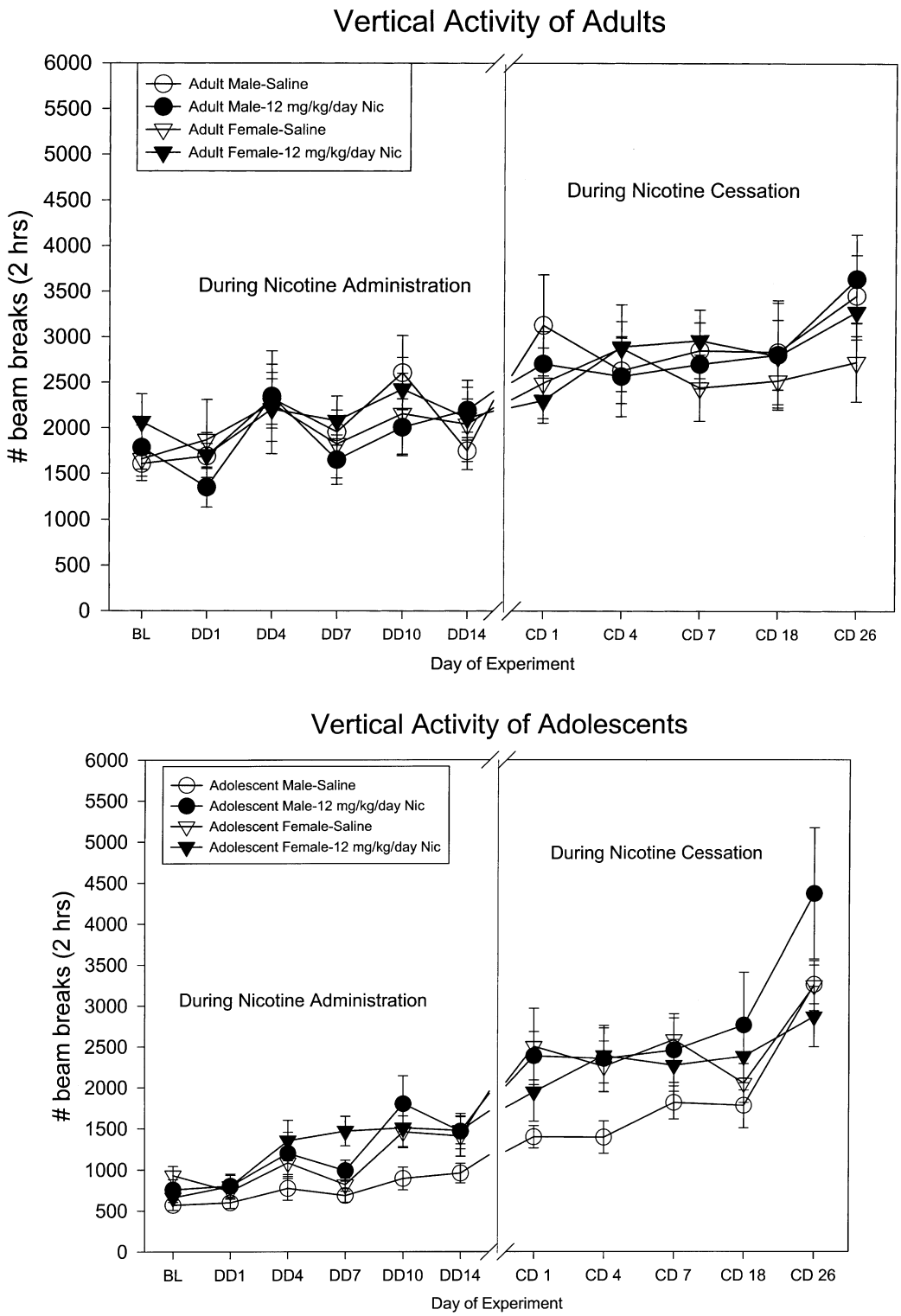

Fig. 4. (a) Vertical activity over 2 h (number of beam breaks; group means ± S.E.M.) for adult males and females during drug administration and cessation. (b) Vertical activity over 2 h (number of beam breaks; group means ± S.E.M.) for adolescent males and females during drug administration and cessation.

10 [$F(1,19) = 6.5$] for adolescent males and on DD 7 [$F(1,18) = 9.5$] for adolescent females. Nicotine did not significantly increase activity of adult males or females on any measurement day.

3.2.4. Drug cessation phase

ANOVAs revealed that adults were more active than adolescents on CDs 1, 4, and 7. Data from adults and adolescents were then analyzed separately for these days.

Table 4
Center time (s) (±S.E.M.) during 2-h measurement periods throughout nicotine administration and cessation

	Adults				Adolescents			
	Male		Female		Male		Female	
	Saline	Nicotine	Saline	Nicotine	Saline	Nicotine	Saline	Nicotine
Baseline	701.8 (78.6)	760.9 (121.8)	581.3 (79.8)	917.4 (246.2)	255.5 (40.8)	376.8 (105.2)	359.7 (46.8)	289.8 (47.6)
DD 1	876.4 (83.8)	1329.7 (313.7)	804.0 (201.7)	1123.7 (177.8)	307.9 (33.4)	771.1 (155.1)	480.1 (107.8)	633.5 (132.5)
DD 4	1346.5 (209.9)	1834.9 (353.6)	995.7 (185.0)	1090.2 (182.1)	452.1 (111.8)	676.3 (140.6)	570.9 (135.8)	701.9 (172.8)
DD 7	1139.5 (188.0)	740.9 (173.6)	592.4 (103.6)	1175.0 (264.9)	383.6 (71.9)	624.4 (129.9)	342.2 (46.1)	978.2 (219.6)
DD 10	1215.9 (211.5)	1104.5 (225.6)	805.1 (203.5)	1633.1 (335.2)	600.9 (104.7)	1064.1 (170.2)	932.4 (306.1)	889.3 (199.3)
DD 14	756.5 (138.0)	1370.6 (331.5)	720.2 (127.9)	1231.7 (201.5)	602.5 (99.2)	870.9 (147.9)	641.1 (136.4)	1004.2 (212.1)
CD 1	1770.8 (275.4)	1122.7 (203.4)	1074.1 (342.7)	1267.2 (337.9)	928.2 (218.9)	1316.3 (173.5)	1196.4 (215.6)	898.5 (168.2)
CD 4	1320.2 (147.4)	1169.3 (253.8)	1174.7 (207.7)	1210.3 (287.5)	725.5 (123.7)	1038.8 (213.6)	1176.7 (228.9)	1141.6 (209.0)
CD 7	1281.0 (218.9)	1194.2 (221.6)	1070.5 (183.2)	1365.0 (394.2)	940.5 (154.8)	1012.0 (190.3)	1151.9 (298.9)	858.6 (150.8)
CD 18	1305.0 (331.4)	1230.3 (189.2)	1171.2 (346.0)	1097.3 (256.9)	926.8 (224.5)	1158.2 (328.5)	866.0 (131.7)	1049.2 (204.7)
CD 26	1645.9 (210.9)	1443.0 (170.0)	1298.8 (191.8)	1254.3 (145.2)	1311.0 (176.4)	1884.2 (355.6)	1743.3 (294.5)	1084.5 (160.4)

Among adolescents, there was a Sex × Drug interaction [$F(1,38) = 5.42$] on CD 1 such that nicotine-exposed males were more active than saline-exposed males while nicotine-exposed females were less active than saline-exposed females. On CD 4, nicotine-exposed adolescents tended to be more active than saline-exposed adolescents [$F(1,37) = 2.9$, $P = .09$]. Activity was not altered by sex or drug on CD 7.

Examination of male and female adolescents separately on CDs 1 and 4 revealed that prior nicotine exposure increased male activity on both days [$df = (1,19)$; CD 1: $F = 9.8$; CD 4: $F = 4.9$]. Nicotine exposure did not significantly alter activity of adolescent females.

3.2.5. Center time: drug administration phase

Table 4 presents center time data. Overall ANOVAs revealed that adults spent more time in the center of the field than adolescents on all measurement days. Nicotine-treated animals spent more time in the center than saline-treated animals (see Table 3b) on DDs 1, 7, 10, and 14. Sex interacted with drug on DD 7 such that nicotine increased center time for females but not for males [$F(1,73) = 8.2$].

Among adults, males spent more time in the center than females on DD 4. Nicotine-treated adults spent more time in the center than saline-treated adults on DDs 1 and 14 [$df = (1,37)$; DD 1: $F = 3.5$, $P = .06$; DD 14: $F = 6.8$]. Sex interacted with drug on DDs 7 and 10 such that nicotine increased time in the center for adult females but not for adult males [$df = (1,37)$; DD 7: $F = 6.6$; DD 10: $F = 3.6$, $P = .06$]. Among adolescents, nicotine increased center time on DDs 1, 7, and 14 [$df = (1,38)$; DD 1: $F = 7.2$; DD 7: $F = 9.3$; DD 14: $F = 4.1$].

Examination of same-sex, same-age groups (see Table 3b) revealed that nicotine increased center time for adult females on DDs 7, 10, and 14. Nicotine increased center time of adolescent males on DDs 1 and 10. Nicotine increased center time of adolescent females on DD 7. Nicotine did not increase center time of adult males.

3.2.6. Drug cessation phase

Repeated-measures analysis revealed that center time changed over time. Sex, drug, or age did not reliably alter center time in the cessation phase. Analyses on each day were not performed.

4. Discussion

This experiment examined the effects of chronic nicotine administration for 3 weeks on body weight, feeding, and locomotion responses of male and female adolescent and adult Sprague–Dawley rats. Adolescents and adults differed in responses to nicotine administration and cessation, and these differences depended on the animal's sex.

4.1. Body weight

Nicotine reduced body weights of adults and of adolescent males, but not of adolescent females. Nicotine reduced body weights of adult and adolescent males on every measurement day. Age × Drug interactions among males indicated that these effects were greatest in adult males during the early drug administration period (DDs 2, 4, 6, and 8). Nicotine reduced body weights of adult females during the first 2 weeks of drug administration (DDs 4, 6, 8, 10, and 14), and Sex × Drug interactions indicated that these effects were smaller than those in adult males.

Effects of nicotine to reduce body weight in adult males and females in the present experiment are consistent with previous reports. The present findings that body weight effects of nicotine were greater in adult males than in adult females may appear to contrast with earlier reports of greater effects in females (i.e., Grunberg et al., 1986, 1988). The earlier interpretation that females were more sensitive than males to nicotine's body weight effects was based on within-group comparisons over time. Nicotine-treated female body weights dropped below predrug body

weights during the drug administration period, whereas nicotine-treated male body weights generally did not (i.e., Grunberg et al., 1986, 1988). Within-sex paired *t* tests on body weight data from the present experiment revealed the same pattern of sex differences. Among males, nicotine-treated animals weighed significantly less than they did on the day of implant on DDs 2 [$t=3.4$, $df=9$] and 4 [$t=2.9$, $df=9$]. In contrast, among females, nicotine treatment significantly reduced body weight below implant levels on DDs 2, 4, 6, 8, and 10 [$df=9$ for all comparisons; DD 2: $t=4.7$; DD 4: $t=4.1$; DD 6: $t=3.6$; DD 8: $t=4.8$; DD 10: $t=2.5$]. Therefore, females in the present experiment were more sensitive to nicotine's body weight-reducing effects based on within-group comparisons.

The between-group comparisons in the present experiment revealed greater body weight reductions in adult males than in adult females and also are consistent with past reports. The average difference in body weight between nicotine- and saline-treated animals during the nicotine administration in this experiment was 44 g for adult males and 12 g for adult females. The difference between nicotine- and saline-treated animals' body weights at the 12-mg/kg/day dosage reported previously (when only bland rat chow was available) range from 30 to 50 g in adult males and from 15 to 25 g in adult females (i.e., Grunberg and Bowen, 1985; Grunberg et al., 1984, 1986, 1988; Bowen et al., 1986; Winders and Grunberg, 1990). The present findings that the magnitude of difference was larger in males than in females, therefore, replicates past reports.

Both ways of examining the data—using the within-group change or evaluating the between-groups difference—revealed the consistent finding that adolescents were less sensitive than adults to nicotine's body weight-reducing actions. By the within-groups criteria, adolescents clearly were less sensitive than adults. Neither adolescent males nor females exhibited body weight decreases in response to nicotine that dropped below predrug levels. By the between-groups criteria, adolescents also were less sensitive than were adults, with average body weight differences between nicotine- and saline-treated animals about 23 g for adolescent males (compared to 44 g for adults) and about 7 g for adolescent females (compared to 12 g for adults). Adolescent females were markedly less sensitive than were adolescent males, with significant body weight reductions apparent on every measurement day for adolescent males, but not for females.

During cessation from nicotine, body weight for nicotine-exposed adolescent and adult males remained suppressed below saline-exposed animal body weight. Body weights of nicotine-exposed adult females became statistically indistinguishable from saline-exposed animals because of almost immediate weight gain. In contrast, the body weights of adolescent females were unaffected by previous nicotine exposure. These results in adults replicate past reports (Grunberg, 1982; Grunberg and Bowen, 1985; Grunberg et al., 1984, 1986, 1988; Bowen et al., 1986). The results in adolescents are new findings.

4.2. Food consumption

Nicotine reduced food consumption of adult males and females, and of adolescent males, but generally not of adolescent females. Effects were most consistent for adult males (i.e., were present on all but one measurement day). The results in adult females are consistent with past reports (i.e., Grunberg et al., 1986; Bowen et al., 1986). Feeding reductions in adult males contrast, however, with reports of no effects in males (i.e., Grunberg, 1982; Grunberg and Bowen, 1985; Grunberg et al., 1984; Winders and Grunberg, 1990). Examination of these reports revealed an important difference between the subjects in the present experiment and those in these other studies: subject age. In studies that reported no effects of nicotine on feeding in males, animals were 3 to 4 months old at the beginning of the experiment. In the present experiment, adult males were 2 months old. A previous study conducted with young adult males (2 months old) also reported that nicotine at this dosage reduced feeding (Faraday, 1998). Sensitivity to nicotine's feeding actions may change with age. These data also suggest that there are sex differences in this developmental pattern such that the pattern is reversed in females. Specifically, young females were insensitive to nicotine's feeding effects but nicotine reduces feeding in young adult and mature adult females.

In cessation, feeding of nicotine-exposed animals became indistinguishable from saline-exposed animals except for adult males. Among adult males, nicotine-exposed animals ate less than did saline-exposed animals on CDs 11, 14, 18, and 21. Findings in adult females replicate past reports (Grunberg et al., 1986; Bowen et al., 1986). Findings in adult males are in contrast to studies that have reported no feeding effects in cessation but, as with the nicotine effects to reduce adult male feeding reported in the present experiment, this difference may be a consequence of subject age. The finding that feeding in adolescents that had been exposed to nicotine quickly rebounded to saline control levels is new.

4.3. Locomotion

Nicotine increased adolescent male horizontal and vertical activity on every measurement day, with statistically significant increases for horizontal activity on DDs 1, 7, 10, and 14, and for vertical activity on DDs 7 and 10. In contrast, although nicotine increased adolescent female horizontal activity significantly on DDs 1 and 7 and vertical activity on DD 7, a consistent pattern of increased activity for nicotine-treated animals was less clear.

Among adults, nicotine increased adult male horizontal activity only on DD 14; adult female horizontal activity was

not affected by nicotine administration. Vertical activity of adults also was unaffected by nicotine. Findings in adults that chronic nicotine at this dosage increases male horizontal activity late in nicotine administration but that females are unaffected replicates previous reports (Grunberg and Bowen, 1985; Bowen et al., 1986).

With regard to center time, where increased center time may reflect anxiolytic drug actions, nicotine increased time spent in the center of the open field on DDs 7, 10, and 14 for adult females, on Days 1 and 10 for adolescent males, and on Day 7 for adolescent females. Adult male center time was unaffected. The increases for adult females and adolescent males were part of an overall pattern in which these groups of nicotine-treated animals spent more time in the center than saline-treated animals on every measurement day. This finding in adult females is consistent with other reports that nicotine at this dosage is anxiolytic in female rats (Faraday et al., 1999b). Effects varied for adolescent females.

In cessation, only adolescent males were consistently affected by prior exposure to nicotine, with nicotine-exposed animals exhibiting greater horizontal activity than saline-exposed males on every measurement day. Nicotine-exposed adolescent males also exhibited increased vertical activity on CDs 1 and 4. These statistically significant increases were part of an overall pattern in which nicotine-exposed adolescent males were more vertically active than saline-exposed males on every day. Cessation did not alter center time for any treatment group.

5. Summary and implications

These findings reveal important sex and age differences in nicotine's chronic actions. Nicotine's effects to reduce body weight and feeding were greater in adults than in adolescents and, among adolescents, males were sensitive to these nicotine effects but females were not. In contrast, adolescent males were more sensitive than were adults or adolescent females to nicotine's activity-enhancing effects. In addition, adolescent males as well as adult females exhibited increased center time in response to nicotine administration, suggesting that nicotine was anxiolytic for these groups.

In cessation, adult and adolescent male body weight remained suppressed below control animals and adult male feeding also remained suppressed until late in cessation. There were no consistent effects of cessation on adult and adolescent female body weight or feeding. In cessation, only adolescent male activity was affected, with nicotine-exposed animals exhibiting greater activity than saline-exposed animals at every measurement point. Importantly, during this period, animals that had been adolescent at the beginning of the experiment were now fully adult. This finding suggests that exposure in adolescence for males to nicotine results in permanent hyperactivity that extends into adulthood. Interestingly, Trauth et al. (1999) recently reported that adolescent nicotine exposure in male rats is associated with up-regulation of nicotinic cholinergic receptors that persists into adulthood. The hyperactivity in cessation reported here may be the behavioral consequence of this long-term receptor-level change.

The present findings reveal new information that adolescent and adult rats differ in biobehavioral effects of nicotine. Use of this dosage via osmotic minipump may limit extrapolation of findings to relatively heavy smokers, but if these findings do generalize to humans, then they may be relevant to why adolescents initiate and maintain tobacco self-administration. Simply put, the adolescent does not appear to be a "mini-adult." That is, nicotine's actions in the adolescent animal appear to differ quantitatively and qualitatively from those in the adult animal.

There are several possible explanations for these behavioral differences, all of which remain to be evaluated. 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), or in rates of nAChR up-regulation or desensitization in response to nicotine administration. Further, there may be age differences in consequences of nAChR activation such as in the amount and time-course of dopamine or other neurotransmitter release. For example, the activity increases by nicotine-treated adolescent males may indicate that adolescent males are more dopaminergically stimulated and, perhaps, more rewarded by nicotine than adolescent females or adults (i.e., Zocchi et al., 1998). Acute injection studies are necessary, however, to examine this hypothesis.

The activity effects of nicotine contrast with reports that adolescent rodents are less sensitive than adults to the activity-stimulating effects of other dopaminergic agonists such as amphetamine and cocaine (Lanier and Isaacson, 1977; Spear and Brick, 1979; Bolanos et al., 1998; Snyder et al., 1998; Laviola et al., 1999). This difference may be a result of route of administration (acute vs. chronic) or reflect special properties of nicotine.

Surprisingly, adolescent females were minimally affected by the 12-mg/kg/day nicotine dosage in the present experiment in terms of activity, body weight, and feeding. If these findings generalize to human adolescent females, then these data may suggest that although girls believe that smoking will reduce body weight and control appetite, these effects do not occur until adulthood is reached. This lack of effect in adolescent female rodents may be a useful addition to prevention literatures: Young women may be less likely to try cigarettes if they know that body weight and appetite suppression will not occur.

These results also may be relevant to broader questions. These questions include whether adolescent exposure to other drugs or to stressors result in altered behaviors and appetitive drives in adulthood, especially alterations that are associated with problematic behaviors and poor outcomes.

For example, Klein (2001) has reported that nicotine exposure in adolescence for male rats alters opioid self-administration in adulthood. In addition, these findings indicate that gender may be a powerful determinant of specific vulnerabilities associated with particular developmental stages.

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