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Effects of nicotine and stress on locomotion in Sprague–Dawley and Long–Evans male and female rats

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Abstract

Locomotor activity is widely used to study nicotine effects, including genotypic differences, in rodents. In rats, chronic nicotine's (administered via osmotic minipump) effects on locomotion may differ based on animal strain, with Long–Evans rats more sensitive than Sprague–Dawley rats. Males and females also may differ in sensitivity. No studies, however, have compared males and females of the two strains. In addition, stress relief is a frequently cited reason for smoking, but the behavioral consequences of nicotine–stress interactions have rarely been examined. This experiment evaluated locomotor responses of male and female Sprague–Dawley and Long–Evans rats to 0, 6, or 12 mg/kg/day nicotine administered by minipump. Half of the animals in each drug condition were exposed to 20 min/day of immobilization stress to examine nicotine–stress interactions. Horizontal and vertical activities were measured on Drug Days 4 and 10. Stress effects were minimal and stress did not alter effects of nicotine. Nicotine (6 mg/kg/day) increased horizontal activity among Long–Evans but not among Sprague–Dawleys, with greater effects in Long–Evans females. Nicotine (6 mg/kg/day) increased vertical activity of all groups and 12 mg/kg/day decreased vertical activity of all groups except for Sprague–Dawley males. Results indicate that genotype and sex are relevant to understand nicotine's behavioral actions.

Keywords: Nicotine; Immobilization; Horizontal activity; Vertical activity; Locomotor activity; Sprague–Dawley; Long–Evans; Strain differences; Sex differences; Males; Females

1. Introduction

Locomotor activity is widely used to study nicotine's behavioral actions, especially psychomotor stimulant actions, in rodents (e.g., Rosecrans, 1971, 1972; Collins et al., 1988; Paulus and Geyer, 1991; Malin et al., 1992). Locomotion—especially horizontal activity—also has been used to quantify genetically based differences in nicotine actions in different strains of rats and mice (Stohr et al., 1998; Cabib and Bonaventura, 1997; Murphy et al., 2001; Witkin and Goldberg, 1990; Hatchell and Collins, 1977; Schlatter and Battig, 1979; Battig et al., 1976; Kianmaa et al., 2000). Human genotype influences smoking behavior, including age of initiation and heaviness of smoking (Eaves and Eysenck, 1980; Hannah et al., 1984; Heath and Martin, 1993; Pomerleau, 1995; Rao et al., 2000). Therefore, the sensitivity of measures of locomotor activity to differences in genotype in rats may be useful to understand if variations in genotype alter nicotine's behavioral actions.

In addition, different aspects of locomotion—horizontal and vertical activity—have been interpreted to reflect different behavioral processes. Horizontal activity has been interpreted to reflect general arousal; vertical activity is thought to indicate exploration (Ader and Conklin, 1963; Walsh and Cummins, 1975; Crawley et al., 1997). Nicotine can affect these two behaviors differently depending on route of administration, dosage, and measurement timing (e.g., Stolerman et al., 1973; Jerome and Sanberg, 1987; Qiu et al., 1992). This dissociation may provide a more detailed picture of genetic control of nicotine's behavioral actions.

Few studies have examined nicotine's locomotion effects when administered via minipump. Chronic infusion may provide a useful model that is applicable to the human condition because many smokers maintain a significant

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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). In addition, nicotine's chronic effects are relevant to understand 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). It should be noted, however, that acutely administered nicotine may be a better model to study nicotine actions that occur as a result of bolus nicotine administration to the brain.

Nicotine's chronic effects when administered via minipump vary depending on nicotine dosage, subject sex, and possibly subject strain. Low nicotine dosages in Sprague–Dawley male rats (i.e., up to 4 mg/kg/day) had no effect on horizontal activity (Benwell et al., 1995; Malin et al., 1992) but increased general activity (horizontal and vertical activity summed) (Grunberg and Bowen, 1985). Higher dosages administered to Sprague–Dawley males (i.e., up to 12 mg/kg/day) also increased general activity (horizontal and vertical activity summed) (Grunberg and Bowen, 1985). The same dosages (e.g., 4 to 12 mg/kg/day) in female Sprague–Dawleys did not reliably alter activity (Bowen et al., 1986). In contrast, dosages of 3 and 6 mg/kg/day increased horizontal activity in Long–Evans males for the first 3 days of drug administration (Helton et al., 1993). In addition, 12 mg/kg/day nicotine decreased horizontal and vertical activity in Long–Evans males, and nonsignificantly decreased activity in Long–Evans females (Faraday et al., 1999b).

These findings suggest both strain and sex differences in nicotine's activity effects when administered via minipump. Specifically, it appears that Long–Evans males may be more sensitive than Sprague–Dawley males to the horizontal activity—increasing effects of low nicotine dosages. Higher nicotine dosages may have opposite activity effects in the two strains, with nicotine possibly increasing activity of Sprague–Dawley males and decreasing activity of Long–Evans males. These reports also suggest that females are less sensitive to nicotine's activity effects than are males. More extensive documentation of possible Sprague–Dawley vs. Long–Evans strain differences and of sex differences may be useful to model and to understand human genotypic and gender differences in nicotine's actions. No studies, however, have directly compared the two strains and few studies have examined female responses. These omissions are important because: (1) Sprague–Dawley and Long–Evans animals are widely used to study nicotine's reinforcing, behavioral, and neurochemical effects; and (2) about half of the US cigarette smoking population is female (Centers for Disease Control [CDC], 2000). If the strains differ in responses to nicotine, then studies of nicotine effects conducted in one strain may not generalize to the other strain. In addition, if studies are conducted predominantly in male animals, then it will be difficult to identify the mechanisms that produce human gender differences in nicotine's actions. Based on the activity literature, we hypothesized that Long–Evans would be more sensitive to nicotine's activity-altering effects than would Sprague–Dawleys, with activity increases at low nicotine dosages and activity decreases at high nicotine dosages, and that males would be more sensitive to these effects than would females.

Relief from stress also is a widely reported reason for smoking (Wills and Shiffman, 1985; US Department of Health and Human Services [USDHHS], 1988; Kassel, 2000). It is unclear why nicotine, a sympathomimetic that increases physiological and biochemical stress responses, results in stress reduction (USDHHS, 1988). It is possible that this dissociation between subjective experience and biologic responses—known as Nesbitt's paradox (Schachter, 1973; Parrott, 1998)—results from nicotine's effects to "normalize" behavior under stress—nicotine counteracts stress-induced behavioral alterations, resulting in behaviors in stressed, smoking individuals that are indistinguishable from nonstressed individuals. Few studies have examined the interaction of chronic nicotine administration and stress on behavioral responses. For example, immobilization stress increased acoustic startle reflex (ASR) and prepulse inhibition (PPI) responses of Sprague–Dawley males and 12 mg/kg/day nicotine also increased these responses (Acri, 1994). Administration of 12 mg/kg/day nicotine to animals that also were exposed to immobilization, however, resulted in responses similar to saline no-stress controls (Acri, 1994). Whether nicotine–stress interactions also occur for activity has not been examined.

The present experiment investigated effects of chronic saline or nicotine (6 or 12 mg/kg/day) administration on horizontal and vertical activity in male and female Sprague–Dawley and Long–Evans rats exposed to no stress or to daily immobilization stress. The 6- and 12-mg/kg/day dosages were included to examine dose–response relationships and because these dosages produce clear behavioral effects without harm to the animal (e.g., Grunberg and Bowen, 1985; Acri et al., 1991; Faraday et al., 1998, 1999a,b; Malin et al., 1992; Helton et al., 1993; Benwell et al., 1995). Immobilization stress was used because it is nonpainful and produces reliable peripheral biochemical and behavioral changes consistent with a stress response (e.g., Acri, 1994; Kant et al., 1983, 1987; Raygada et al., 1992). Further, reports indicate that animals do not habituate behaviorally or biologically to daily brief immobilization for up to three weeks (Kant et al., 1987; Faraday, 2002).

2. Methods

The experimental protocol was reviewed and approved by the USUHS Institutional Animal Care and Use Committee. All procedures were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.1. Subjects

Subjects were 117 Sprague–Dawley (59 males, 58 females) rats and 120 Long–Evans (60 males, 60 females) rats (Charles River Laboratories, Wilmington, MA). Animals were individually housed throughout the experiment in standard polypropylene shoebox cages ($42 \times 20.5 \times 20$ cm) on hardwood chip bedding (Pine-Dri). Throughout the study, subjects had continuous access to rodent chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. Housing rooms were maintained at 23 °C at 50% relative humidity on a 12-h reversed light/dark cycle (lights on at 1900 h). Locomotor testing was performed during the dark (active) phase of the light cycle (between 0900 and 1600 h) for face validity (i.e., extrapolating to awake and alert humans). At the beginning of the experiment, subjects were 49 days old. Mean body weights (±S.E.M.) at the beginning of the experiment were: Sprague–Dawley males—224.0 g (1.2 g); Sprague–Dawley females—171.6 g (0.5 g); Long–Evans males—230.9 g (0.9 g); Long–Evans females—172 g (0.7 g). The experiment was conducted as a 2 (Sprague–Dawley or Long–Evans) × 2 (male or female) × 2 (no stress or stress) × 3 (0, 6, or 12 mg/kg/day nicotine) full factorial design with 9 or 10 subjects per treatment group.

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 cinder block walls, acoustic tile ceiling, and steel doors so that sound is kept to a minimum. Animals were placed singly in one of sixteen $40 \times 40 \times 30$ cm clear Plexiglas arenas. A Plexiglas lid with multiple 3.5 cm diameter ventilation holes was placed on top of each 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. 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.

2.3. Drug administration and surgical procedure

Nicotine (6 or 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. Nicotine dihydrochloride was made in our laboratory.

Subjects were anesthetized using methoxyflurane (Metofane) and minipumps were implanted subcutaneously between the shoulder blades according to procedures described in detail elsewhere (e.g., Grunberg, 1982; Acri, 1994). The entire surgical procedure including anesthesia took approximately 4 min per subject.

2.4. Stress manipulation

Animals in the stress condition were restrained in finger-like restraining devices (Centrap Cage, Fisher Scientific) 20 min/day beginning the day after surgery. Subjects were placed in the Centrap cage and the restraining "fingers" were tightened until subjects were immobilized, but not pinched or in pain. The stress manipulation took place in a room adjacent to the locomotor testing room. Locomotor testing began within 5 min of removal from the restrainers.

2.5. Procedure

The experiment was conducted in two phases: a baseline phase and a drug administration/stress phase. Decreased rates of body weight gain are well-established effects of nicotine administration at these dosages in rats in the dynamic growth phase (e.g., Grunberg, 1982; Winders and Grunberg, 1989; USDHHS, 1988). Therefore, subjects' body weights were measured every other day throughout the drug administration/stress phase as validation of drug administration.

2.5.1. Baseline phase

During the baseline phase (14 days), animals were acclimated to the facility and were handled every day to minimize any stress that might occur as a result of routine handling for body weight measurement and locomotion testing. During this period, all subjects ($N = 237$) also underwent one acclimation exposure to the locomotion apparatus. Acclimation was done in order to minimize possibly stressful effects of exposure to a novel situation. Four days after the acclimation exposure, locomotion responses of all subjects were measured again. These responses constituted the baseline values.

2.5.2. Drug administration/stress phase

After the completion of baseline measures, subjects were assigned within sex and strain to drug (0, 6, or 12 mg/kg/day nicotine) and stress (no stress or stress) groups in a manner ensuring comparable initial body weights. This assignment resulted in 24 balanced groups of 9–10 subjects per group (six groups each of Sprague–Dawley males, Sprague–Dawley females, Long–Evans males, and

Long–Evans females). Minipumps containing the appropriate solutions were implanted as described in *Drug Administration and Surgical Procedure* on Drug Day 1. On Drug Day 2, subjects in the stress condition began undergoing 20 min/day of restraint stress. These subjects were stressed everyday for the remainder of the experiment. Horizontal and vertical activities were measured for all subjects on Drug Day 4 and on Drug Day 10. These measurement days were selected to evaluate effects of exposure to chronic nicotine administration and repeated daily stress.

3. Data analyses

3.1. Body weight

Body weight data from Drug Day 13 were analyzed with analyses of variance (ANOVAs) to verify effects of nicotine to decrease rates of body weight gain. Tukey's post hoc tests were used to distinguish among drug groups.

3.2. Locomotion

Each animal's responses were summed across the 2-h testing period. Stress effects were assessed statistically by comparing data from no-stress saline animals to data from stress-saline animals using ANOVAs. To determine whether nicotine's actions differed in the presence of stress, data from the 6- and 12-mg/kg/day groups were examined for Time × Stress interactions using repeated-measures ANOVAs. These analyses revealed no consistent effects of stress in saline groups and no Time × Stress interactions in nicotine groups. Therefore, drug groups were collapsed across stress status. ANOVAs were conducted on collapsed data on each drug day to examine effects of nicotine, including strain and sex differences in nicotine effects. Tukey's HSD post hoc tests were used to determine differences among drug groups on specific days. Sex and strain differences also were examined by calculating proportions of variance explained (η^2) for drug effects within same-sex, same-strain groups. Same-sex, same-strain treatment groups did not differ in baseline horizontal

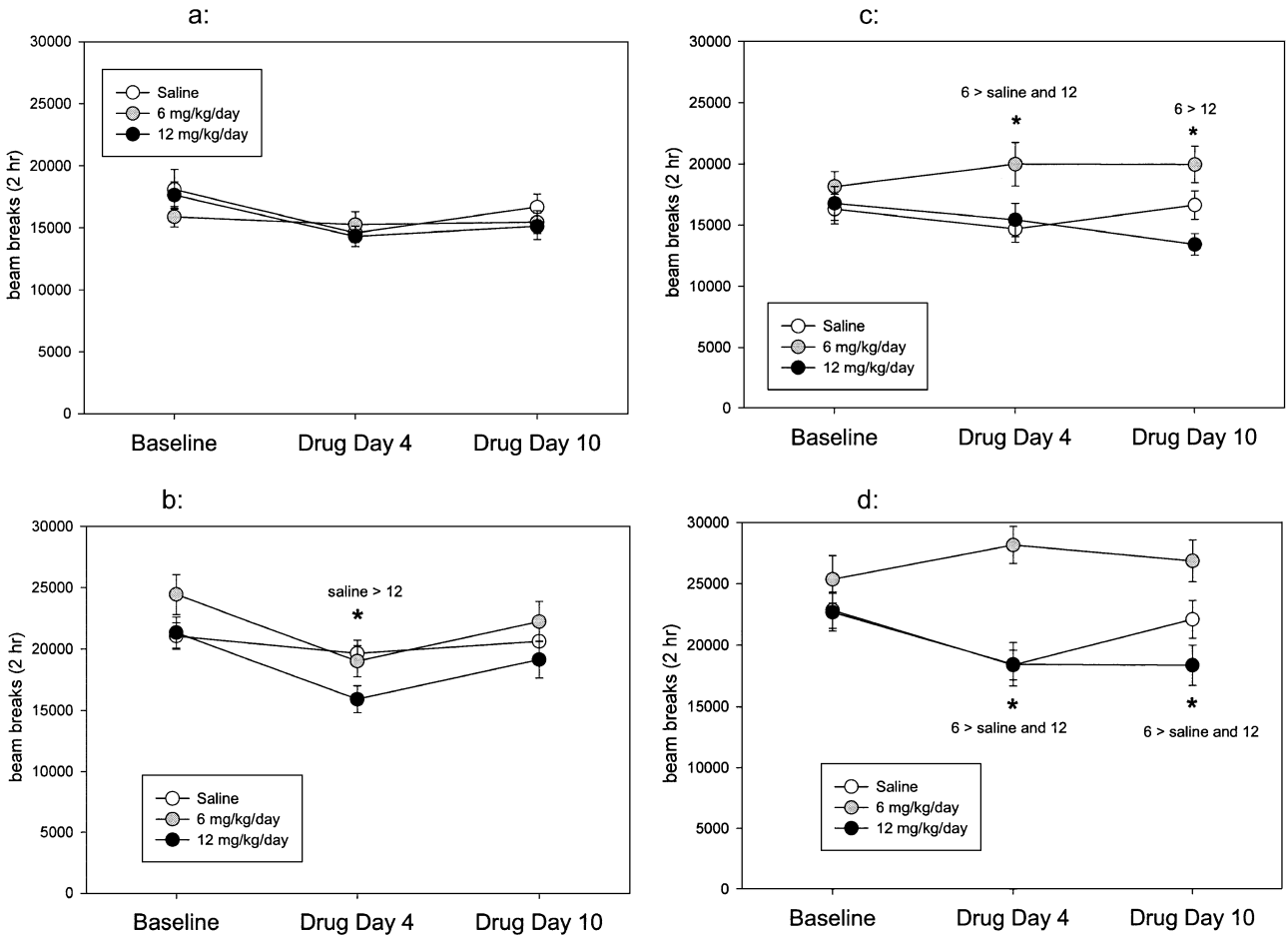

Fig. 1. Horizontal activity (beam breaks over 2 h; mean ± S.E.M.) at baseline, on Drug Day 4, and on Drug Day 10. Notations on graph indicate between-groups differences on specific days ($P < .05$, Tukey's HSD). (a) Sprague–Dawley males, (b) Sprague–Dawley females, (c) Long–Evans males, (d) Long–Evans females (mean ± S.E.M.).

or vertical activity. All tests were two-tailed. Results are significant at $P < .05$ unless otherwise noted.

4. Results

4.1. Body weight

Nicotine administration reduced body weight gain among males [$F(2,107) = 15.3$] and among females [$F(2,106) = 21.6$]. Body weight gain decreases occurred in a dose–response fashion: 12 mg/kg/day>6 mg/kg/day>saline.

4.2. Horizontal activity

See Fig. 1a–d.

4.2.1. Drug Day 4

Nicotine altered activity [$F(2,213) = 14.6$] and a Strain × Drug interaction indicated that these effects occurred mainly among Long–Evans [$F(2,213) = 9.1$]. Females were more active than males [$F(1,213) = 32.4$]. When the strains were examined separately, females were more active than males among Sprague–Dawleys [$F(1,111) = 17.1$] and among Long–Evans [$F(1,114) = 16.8$]. Nicotine (6 mg/kg/day) increased horizontal activity of Long–Evans [$F(2,114) = 16.4$] but not of Sprague–Dawleys. Because of the sex effects, same-sex, same-strain subgroups also were examined. Nicotine had no effect on Sprague–Dawley male activity but 12 mg/kg/day decreased Sprague–Dawley female activity [$F(2,55) = 3.0$ with Tukey's]. Nicotine (6 mg/kg/day) increased activity of Long–Evans males [$F(2,57) = 4.0$ with Tukey's] and of Long–Evans females [$F(2,57) = 13.8$ with Tukey's]. Eta-squared calculations indicated that the drug effect accounted for the following variance percentages: Sprague–Dawley males, 1%; Sprague–Dawley females, 9.8%; Long–Evans males, 12.3%; Long–Evans females, 32.6%.

4.2.2. Drug Day 10

Nicotine altered activity [$F(2,225) = 11.3$] and a Strain × Drug interaction indicated that these effects occurred among

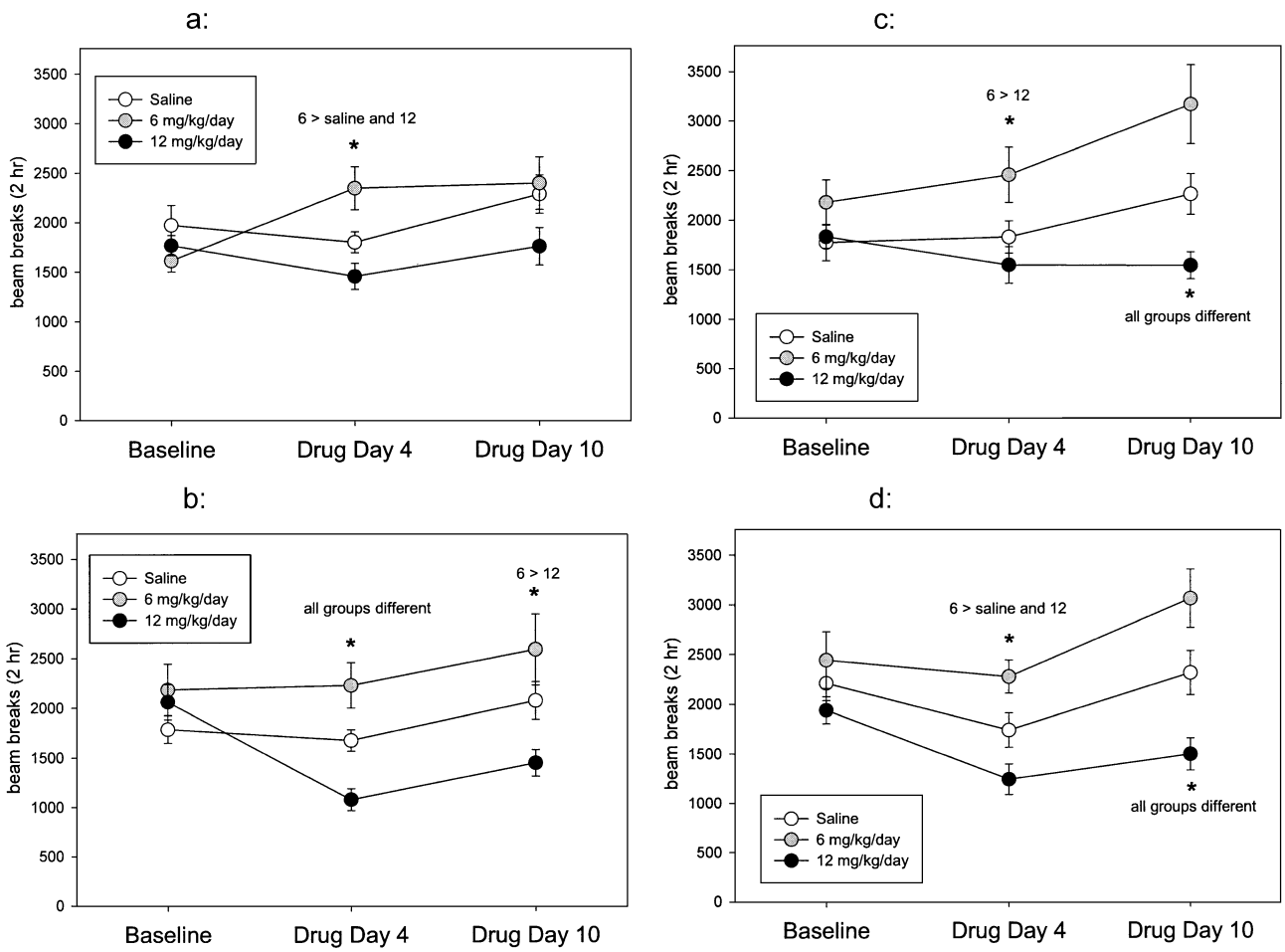

Fig. 2. Vertical activity (beam breaks over 2 h; mean ± S.E.M.) at baseline, on Drug Day 4, and on Drug Day 10. Notations on graph indicate between-groups differences on specific days ($P < .05$, Tukey's HSD). (a) Sprague–Dawley males, (b) Sprague–Dawley females, (c) Long–Evans males, (d) Long–Evans females.

Long–Evans [$F(2,225)=4.6$] but not among Sprague–Dawleys. Females were more active than males [$F(1,225)=45.4$] and this effect was evident among Sprague–Dawleys [$F(1,111)=21.4$] as well as among Long–Evans [$F(1,114)=24.1$]. Nicotine altered activity among Long–Evans [$F(2,114)=13.7$] such that 6 mg/kg/day increased activity and 12 mg/kg/day decreased activity (Tukey's). Among Long–Evans males, the drug effect [$F(2,57)=7.3$] was evident with the 6-mg/kg/day group exhibiting greater activity than the 12-mg/kg/day group (Tukey's). Among Long–Evans females, the 6-mg/kg/day group was more active than the saline and the 12-mg/kg/day groups [$F(2,57)=6.8$]. Eta-squared calculations indicated that the drug effect accounted for the following variance percentages: Sprague–Dawley males, 2.3%; Sprague–Dawley females, 3.5%; Long–Evans males, 20.4%; Long–Evans females, 19.3%.

4.3. Vertical activity

See Fig. 2a–d.

4.3.1. Drug Day 4

Nicotine altered activity [$F(2,225)=31.9$] such that 6 mg/kg/day increased activity and 12 mg/kg/day decreased activity (Tukey's). Females were more active than males [$F(1,225)=3.8$]. Because of the sex difference in activity, males and females were examined separately. Effects of nicotine to alter activity were evident among males [$F(2,113)=11.6$] with 6 mg/kg/day increasing activity above the saline level (Tukey's). Among Sprague–Dawley males, the 6-mg/kg/day group was more active than the saline and 12-mg/kg/day groups [$F(2,56)=7.9$ with Tukey's]. Among Long–Evans males, the 6-mg/kg/day group was more active than the 12-mg/kg/day group [$F(2,57)=4.7$ with Tukey's]. Among females, nicotine also altered activity [$F(2,112)=22.6$] such that 6 mg/kg/day increased activity and 12 mg/kg/day decreased activity (Tukey's). This pattern was clear among Sprague–Dawley females [$F(2,55)=13.1$] with all groups differing significantly (Tukey's) and among Long–Evans females [$F(2,57)=9.8$] with the 6-mg/kg/day group more active than the saline and 12-mg/kg/day groups. Eta-squared calculations indicated that the drug effect accounted for the following variance percentages: Sprague–Dawley males, 22.0%; Sprague–Dawley females, 32.3%; Long–Evans males, 14.1%; Long–Evans females, 25.5%.

4.3.2. Drug Day 10

Nicotine altered activity [$F(2,225)=26.1$] such that 6 mg/kg/day increased activity and 12 mg/kg/day decreased activity and these effects were strongest among Long–Evans [Strain × Drug: $F(2,225)=2.3$]. Among Sprague–Dawleys, 12 mg/kg/day decreased activity [$F(2,111)=7.5$]; this effect was the result of Sprague–Dawley female responses [$F(2,55)=5.4$] with the 6-mg/kg/day group more active than the 12-mg/kg/day group (Tukey's). Among Long–Evans, 6 mg/kg/day increased activity and 12 mg/kg/day decreased activity [$F(2,57)=9.0$ with Tukey's]. These effects were evident among Long–Evans males [$F(2,57)=9.0$] and among Long–Evans females [$F(2,57)=11.3$], with all groups differing significantly. Eta-squared calculations indicated that the drug effect accounted for the following variance percentages: Sprague–Dawley males, 7.8%; Sprague–Dawley females, 16.4%; Long–Evans males, 24.1%; Long–Evans females, 28.5%.

5. Discussion

This experiment examined effects of chronic nicotine administration (0, 6, or 12 mg/kg/day) with and without daily immobilization stress on horizontal and vertical activity of male and female Sprague–Dawley and Long–Evans rats. There were strain differences in nicotine's effects on horizontal activity such that effects of nicotine occurred most consistently among Long–Evans, with the largest effects in Long–Evans females. On Drug Day 4, 6 mg/kg/day increased horizontal activity of male and female Long–Evans; on Drug Day 10, 6 mg/kg/day also increased activity above the saline level for both groups but the effect remained significant only for females. Among Sprague–Dawleys, the only effects of nicotine on horizontal activity occurred among females and consisted of 12 mg/kg/day decreasing activity on Drug Day 4. In contrast, there were strain and sex differences in nicotine's effects on vertical activity. Sprague–Dawley males were the least affected, with 6 mg/kg/day increasing vertical activity on Drug Day 4 only. Among Sprague–Dawley females, 6 mg/kg/day increased vertical activity and 12 mg/kg/day decreased vertical activity on Drug Day 4; the same pattern of findings was evident on Drug Day 10 but the difference between the 6-mg/kg/day and saline group was no longer significant. Among Long–Evans males, 6 mg/kg/day increased vertical activity on Drug Day 4; on Drug Day 10, 6 mg/kg/day increased activity and 12 mg/kg/day decreased activity. Responses of Long–Evans females were similar, with 6 mg/kg/day increasing vertical activity on Drug Day 4 and with this dosage increasing activity and 12 mg/kg/day decreasing activity on Drug Day 10.

Effects in Long–Evans males replicate reports that 12 mg/kg/day decreased horizontal and vertical activity (Faraday et al., 1999b). The data reported here do not replicate reports that 6 mg/kg/day nicotine increased horizontal activity in Long–Evans males during the first 3 days of drug administration, but this lack of correspondence may have occurred because activity was not measured until Drug Day 4 in the present experiment. The fact that 12 mg/kg/day also decreased horizontal and vertical activity of Long–Evans females is consistent with the decrease reported in Faraday et al. (1999b) that did not reach statistical significance. The present experiment had a larger sample size per

treatment group once data were collapsed across stress condition, and therefore had greater statistical power.

In contrast, nicotine did not affect horizontal activity of Sprague–Dawley males and only transiently affected Sprague–Dawley female horizontal activity. The lack of nicotine effects in these groups at these dosages is generally consistent with previous reports (Benwell et al., 1995; Malin et al., 1992; Bowen et al., 1986) with two qualifications. First, Grunberg and Bowen (1985) reported that nicotine at similar dosages increased general activity in Sprague–Dawley males, but general activity was the summation of horizontal and vertical activity. Second, Bowen et al. (1986) reported no effects of nicotine on female Sprague–Dawley activity but data were presented collapsed over the entire drug administration period. It is possible that transient effects were not evident when data were averaged across the drug administration period. Further, according to Bowen et al., the mean of the 12-mg/kg/day group was below the saline group mean—the same direction of effect detected on Drug Day 4 in the present experiment. In general, the findings from the present experiment support the hypothesis that Long–Evans are more sensitive than are Sprague–Dawleys to nicotine's horizontal activity effects at a given dosage.

Stress effects on activity were minimal. This minimal effect is consistent with the literature on stress and locomotion (e.g., Faraday, 2002). It is possible that minimal stress effects occurred because 20 min/day of immobilization is a mild stressor. Assessment of effects of a more aversive stressful experience, such as longer immobilization periods or other stressors, might more clearly demonstrate stress–nicotine interactions.

5.1. Summary and implications

Overall, these findings reflect the complexity of nicotine's behavioral actions. In particular, these results suggest that: (1) genotype is relevant to some (i.e., horizontal activity) nicotine actions; and (2) genotype can interact with sex to alter nicotine's behavioral actions (vertical activity).

The most interesting finding in terms of using different rat strains to understand human smoking behavior is that genotype may be relevant to some nicotine actions. We previously have reported that nicotine chronically administered at these dosages enhances ASR and PPI responses in Sprague–Dawleys and impairs these responses in Long–Evans (Faraday et al., 1998, 1999a). Studies also have revealed that Sprague–Dawley and Long–Evans rats differ in nicotine self-administration patterns. Although both strains self-administer nicotine, Sprague–Dawley rats were better able to discriminate nicotine at low dosages in a nose-poke paradigm than were Long–Evans rats (Corrigall and Coen, 1989; Glick et al., 1996; Shoaib et al., 1997). In addition, nicotine self-administration was disrupted by prior nicotine exposure in Long–Evans rats, but not in Sprague–Dawley rats (Shoaib et al., 1997). Further, Long–Evans rats were markedly less sensitive than were Sprague–Dawley rats to the PPI-disrupting effects of various dopaminergic agonists (Swerdlow et al., 2001).

Together, these reports suggest that the strains differ in peripheral (e.g., metabolism, drug distribution) or central processes (e.g., receptor and neurotransmitter actions) relevant to nicotine's actions. Whether the strains differ in pharmacokinetic or pharmacodynamic responses to nicotine has not been examined. Several studies indicate, however, that Sprague–Dawleys and Long–Evans differ in central dopaminergic, serotonergic, and noradrenergic system activity (Costa et al., 1982; Horowitz et al., 1997; Park et al., 1990; Swerdlow et al., 2001). The same systems have been implicated in nicotine's actions. Therefore, it is possible that these CNS differences account for strain differences in nicotine's behavioral effects and patterns of self-administration.

Underlying mechanisms for these differences are not clear. Dopamine is the neurotransmitter most strongly linked with the horizontal activity-increasing effects of stimulant drugs and, in the present experiment, nicotine affected horizontal activity in Long–Evans but generally not in Sprague–Dawleys. These findings suggest that Long–Evans might be more sensitive to nicotine's dopamine-releasing actions than Sprague–Dawleys. In addition, PPI impairment in response to nicotine reported in Faraday et al. (1999a) could be the result of nicotine's dopamine-releasing effects. However, Swerdlow et al. (2001) reported that Long–Evans are insensitive to the PPI-impairing effects of dopaminergic agonists, suggesting that some other action of nicotine produced PPI impairments. Increased serotonergic activity also has been associated with PPI impairments (Sipes and Geyer, 1995). It is possible, therefore, that serotonergic differences between Sprague–Dawleys and Long–Evans are most relevant to strain differences in nicotine's actions. Interestingly, depressed behavior in response to stress occurs in Sprague–Dawley females but not in Long–Evans females, and these behaviors in Sprague–Dawleys have been linked to serotonergic dysfunction (Kennett et al., 1986; Haleem et al., 1988; Faraday, 2002).

Greater female than male sensitivity to nicotine has been reported based on nicotine's feeding and body weight effects (e.g., Grunberg et al., 1986, 1991; Bowen et al., 1986). Based on the activity literature, we hypothesized that males would be more sensitive to nicotine's activity-altering effects than would females. In this experiment, sex differences in nicotine effects were evident but were not strong enough to be manifested as Sex × Drug interactions and consisted of females being more sensitive to nicotine's effects than males. For example, values of eta-squared for nicotine effects on horizontal activity were larger in females of each strain—9.8% (Drug Day 4) and 3.5% (Drug Day 10) in Sprague–Dawley females and 32.6% (Drug Day 4) and 19.3% (Drug Day 10) in Long–Evans females—than in males of each strain—1.1% (Drug Day 4) and 2.3% (Drug

Day 10) in Sprague–Dawley males and 12.3% (Drug Day 4) and 20.4% (Drug Day 10) in Long–Evans males. Similarly, eta-squared values for nicotine effects on vertical activity also were larger in females of each strain than in males of each strain (Sprague–Dawley females: 32.3% and 16.4%; Sprague–Dawley males: 22.0% and 7.8%; Long–Evans females: 25.5% and 28.5%; Long–Evans males: 14.1% and 24.1%).

It also is possible that estrus cycling of females may have affected female responses. In this experiment, males and females were housed in the same housing room. Generally, females do not cycle together when exposed to male pheromones. Therefore, several estrus cycle stages should have been represented within each female treatment group on each measurement day and any effects of particular estrus cycle stages should have been spread across treatment groups. Estrus should be measured in future studies to ensure that estrus cycle stages are evenly represented within drug groups on testing days.

It also is important to note that there was some drift in the activity levels of the saline groups over time. These changes may have affected the statistical determination of differences among groups on specific days. More acclimation exposures to the locomotion apparatus may have helped to minimize this drift.

Overall, the results of this experiment indicate that behavioral responses to a specific nicotine dosage differ based on the strain and sex of animal. This information is of potential importance when evaluating the results of studies conducted with different strains and makes clear the need for more extensive strain comparisons. In addition, these strain and sex differences may be useful to develop a fuller understanding of why different individuals manifest different magnitude responses to a given nicotine dosage. These differences could be the result of differences in drug potency (i.e., ED_{50}) as well as of differences in drug efficacy (i.e., maximum effect). A more thorough dose–response analysis is necessary to determine the relevance of these factors. In particular, the possibility that serotonergic systems may be involved in strain differences suggests the potential for using the two strains to study individuals who smoke primarily for affect regulation, especially for nicotine's putative antidepressant effects, compared to individuals who do not. Also, the possible role of serotonergic vs. dopaminergic systems in the effects of nicotine in the different strains may suggest different pharmaceutical approaches to antagonize nicotine's effects in different individuals.

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