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Strain and sex alter effects of stress and nicotine on feeding, body weight, and HPA axis hormones

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Abstract

Gender and genotype result in differential sensitivity to stress and to nicotine. Male and female Sprague–Dawley and Long–Evans rats exhibit different behavioral responses to immobilization stress and to chronically-administered nicotine, suggesting that these animals may be useful to model human variability in stress and nicotine sensitivity. It is possible that differences in sensitivity of the hypothalamo–pituitary–adrenocortical (HPA) axis might account for these sex and strain differences. This experiment examined corticosterone (CORT) and adrenocorticotropin hormone (ACTH) responses of male and female Sprague–Dawley (n=117) and Long–Evans (n=120) rats administered 0, 6, or 12 mg/kg/day nicotine for 14 days; half of each treatment group was exposed to immobilization stress (20 min/day). Feeding and body weight also were measured. Nicotine increased CORT and ACTH levels of Sprague–Dawley females only. Stress increased CORT and ACTH levels of all groups except for Long–Evans females. Nicotine and stress decreased feeding and body weight with greatest effects in Long–Evans females. CORT, feeding, and body weight were positively correlated among stressed females. These findings suggest that strain differences in HPA axis, body weight, and feeding responses to nicotine and to stress are robust among females but not among males. CORT reactivity and female sex hormones may explain these differences.
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1. Introduction

Individuals differ markedly in physiological and behavioral responses to stress (Cannon, 1898; Broadhurst, 1960; Mason et al., 1968; Petrides et al., 1979; Lupien et al., 1995; McEwen, 1998). Effects of nicotine, the primary active substance in tobacco, also vary across individuals and may depend on whether an individual is experiencing stress (Acri, 1994; Perkins, 1995; Pomerleau and Pomerleau, 1990). Sensitivity to stress is linked to the development of physical and psychological disorders, including substance abuse (McEwen, 1998; Sinha, 2001; Gordon, 2002; Weiss et al., 2001). Sensitivity to nicotine is associated with greater addiction liability and relief from stress is a widely-reported reason for smoking and for cessation relapse (Wills and Shiffman, 1985; USDHHS, 1988; Kassel, 2000; Pomerleau, 1995). Understanding why individuals exhibit differential responses to stress, to nicotine, and to nicotine in combination with stress, therefore, is important to prevent and to treat stress- and tobacco-related health problems in vulnerable individuals.

Gender is one major variable that appears to confer differential sensitivity to stress and to nicotine (Gallucci et al., 1993; Frankenhaeuser et al., 1976; Lerner and Kannel, 1986; Stoney et al., 1998; Verbrugge, 1985; Brown and Grunberg, 1996; Haleem et al., 1988; Taylor et al., 2000; Grunberg and Bowen, 1985; Grunberg et al., 1991). Individuals also vary within-gender in responses to stress and to nicotine, however, indicating that stress and nicotine sensitivity are a function of factors in addition to gender

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(e.g., other genetic or environmental factors; Krantz and Durel, 1983; Lerner and Kannel, 1986; Lupien et al., 1995; Sapolsky, 1983; Henry et al., 1993; Broadhurst, 1960; Sternberg et al., 1992; Acri, 1994; Pomerleau, 1995; Perkins, 1995; Collins et al., 1988).

We have found that male and female Sprague–Dawley and Long–Evans rats exhibit different behavioral responses to immobilization stress and to chronically-administered nicotine, suggesting that these animals may be useful to model human variability in stress and nicotine sensitivity (Faraday, 2002; Faraday et al., 1998, 1999a,b, 2003). In particular, immobilization (20 min/day for 3 weeks): decreased feeding and body weight of males but generally not of females; had no effect on male 15 min activity levels and decreased 15 min activity levels of Sprague–Dawley females but not of Long–Evans females; increased acoustic startle reflex (ASR) responses of Sprague–Dawley males and females but not of Long–Evans males and females; and reduced pre-pulse inhibition (PPI) of Long–Evans females but not of other groups (Faraday, 2002). With regard to nicotine: 6 mg/kg/day increased horizontal activity among Long–Evans but not among Sprague–Dawleys, with greater effects in Long–Evans females; 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; and 6 and 12 mg/kg/day increased ASR amplitude and PPI in Sprague–Dawleys but decreased these responses in Long–Evans (Faraday et al., 1998, 1999a,b, 2003).

One possible explanation for these behavioral sex and strain differences is differential sensitivity of the hypothalamo–pituitary–adrenocortical (HPA) axis to stress and to nicotine. It is well-established that stress increases corticosterone (CORT) levels (Kant et al., 1983, 1987; Raygada et al., 1992). Comparisons of HPA axis activity between Sprague–Dawley and Long–Evans males and females, however, have not been made. CORT also is important in behavioral effects of nicotine. CORT facilitates the development of tolerance to some nicotine actions (e.g., analgesia) and the development of sensitization to other nicotine actions (e.g., locomotion; Pauly et al., 1988, 1990, 1992; Grun et al., 1992; Caggiula et al., 1993, 1998; Shoaib and Shippenberg, 1996). In male rats, nicotine increases CORT and adrenocorticotropin hormone (ACTH) levels when acutely administered (Balfour et al., 1975; Turner, 1975; Cam and Bassett, 1984; Bugajski et al., 1998; Matta et al., 1998). Responses of females and of different rat strains have not been examined thoroughly.

HPA axis activity in the stressed state also may be relevant to nicotine's actions. For example, Sprague–Dawley male rats exposed to a mildly stressful environment that increased CORT levels before nicotine injection exhibited tolerance to nicotine's analgesic actions (Caggiula et al., 1993). When the stressful experience occurred repeatedly, CORT responses habituated in saline-injected animals but not in nicotine-injected animals, suggesting that HPA axis adaptation to stress may not occur in the presence of nicotine (Benwell and Balfour, 1982). Non-habituating HPA activity in the stressed state may promote tolerance and sensitization to specific nicotine actions. If tolerance develops to desired nicotine actions or if the outcome of combined tolerance and sensitization is a reinforcing state, then stressed individuals may maintain and/or increase nicotine self-administration. Nicotine–stress effects on HPA axis activity, however, have not been examined in females or across strains.

The purpose of the present experiment was to examine the HPA axis effects of stress, of nicotine, and of stress in combination with nicotine across sexes and strains. Male and female Sprague–Dawley and Long–Evans rats were exposed to one of three dosages of chronically-administered nicotine (0, 6, or 12 mg/kg/day); half of the animals in each treatment group also were exposed to daily immobilization stress. Feeding and body weight were measured because these indices are sensitive to nicotine and to stress and nicotine's effects on feeding and body weight have not been compared across strains. HPA axis hormones C corticosterone (CORT) and adrenocorticotropin hormone (ACTH) C were measured to examine sensitivity of the axis across sexes and strains. We measured both hormones to examine the possibility that chronic exposure to nicotine and stress might produce a disconnection between these two components of the axis.

The 6 and 12 mg/kg/day nicotine 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., 1999; Faraday et al., 1998, 1999a,b; Malin et al., 1992; Helton et al., 1993). These dosages produce nicotine blood levels of 148 ng/ml and 257 ng/ml respectively (Winders et al., 1998). Chronic nicotine administration via osmotic minipump was used to avoid conditioned release of CORT in response to a nicotine injection (Caggiula et al., 1993, 1998). The minipump eliminates presentation of drug administration cues that can trigger CORT release and also avoids the stress of an injection procedure (Caggiula et al., 1993, 1998). Relatively few studies have examined CORT responses when nicotine was administered chronically via minipump. Investigators generally have reported that administration of relatively low chronic nicotine dosages (1 to 3 mg/kg/day via minipump) does not alter CORT levels in male rats (Fuxe et al., 1990; Singh et al., 2000).

Chronic infusion also may provide a useful model because many smokers maintain a significant concentration of nicotine in plasma throughout much of the day (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).

Immobilization stress was used because it is non-painful 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; Faraday, 2002). Further, CORT responses of Sprague–Dawley males to repeated brief immobilization do not habituate after up to 13 days of daily exposure (Kant et al., 1983; Raygada et al., 1992). To our knowledge, HPA axis responses of Sprague–Dawley females and of Long–Evans rats to repeated brief immobilization have not been examined.

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 stress and nicotine effects. These data also are important for external validity. Sprague–Dawley and Long–Evans animals are widely used to study stress and to study nicotine's reinforcing, behavioral, and neurochemical effects but are rarely compared across dependent variables. If the strains differ systematically in responses to stress and to nicotine, then studies conducted in one strain may not generalize to the other strain and, more importantly, may generalize most accurately to human subgroups rather than to a broad human population.

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 male, 58 female) rats and 120 Long–Evans (60 male, 60 female) rats (Charles River Laboratories, Wilmington, MA). Animals were individually housed throughout the experiment in standard polypropylene shoebox cages (42×20.5×20 cm) on hardwood chip bedding (Pine-Dri). Throughout the study animals had continuous access to rodent chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. 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 animals per treatment group.

2.2. 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; its purity was verified by the laboratory of N. Benowitz.

Subjects were anesthetized by inhalation of methoxyflurane (MetofaneJ) and minipumps were implanted subcutaneously (SC) 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.

2.3. 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 Afingers@ were tightened until subjects were immobilized, but not pinched or in pain. The stress manipulation took place in a room adjacent to the housing room.

2.4. Procedure

The experiment was conducted in two phases: a Baseline Phase and a Drug Administration/Stress Phase.

2.4.1. 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. Body weight was measured throughout this phase.

2.4.2. Drug Administration/Stress Phase

After the completion of the Baseline Phase, animals 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 (6 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 animals in the stress condition began undergoing 20 min/day of restraint stress. These animals were stressed every day for the remainder of the experiment. Food consumption and body weight were measured on Drug Days 1, 3, 5, 11, and 13.

On Drug Day 14 (after 14 days of drug administration and 13 days of stress exposure for stress animals), all

animals were sacrificed by decapitation. Animals in the stress groups underwent immobilization for 20 min and were sacrificed within 5 min of removal from the restrainers. Trunk blood was collected in two 20-ml polystyrene tubes. The tubes from which CORT samples were drawn was allowed to clot at room temperature for 20–25 min. These samples then were spun for 20 min (3000×g at 4 °C) in a refrigerated centrifuge (IEC Centra, Model GP8R, Needham Heights, MA). Serum was pipetted into a set of labeled Eppendorf tubes and frozen in an −80 °C freezer until assay. The second tube contained 20 μl of 15% EDTA; blood in these tube was used to draw samples for ACTH. These tubes were placed on ice immediately after blood collection and then centrifuged as above. The resulting plasma was pipetted into labeled Eppendorfs. Aprotinin (0.56 trypsin inhibitor units per milliliter) was added to each aliquot. Samples were stored at −80 °C until assay. Samples were assayed in duplicate for CORT and ACTH using commercially-available radioimmunoassay kits (ICN Biomedicals).

3. Data analyses

Body weight data were analyzed with repeated-measures analyses of variance (ANOVAs) with a within-subject factor of Day and between-subjects factors of Strain, Drug, and Stress. Because males always weighed more than did females, analyses were conducted separately for males and females. No-stress and stress animals also were examined separately to assess the extent to which nicotine effects depended on stress status. ANOVAs were used to detect differences among groups on specific days. Food consumption data were summed over the drug administration period and analyzed with ANOVAs. Corticosterone (CORT) and ACTH data were analyzed with ANOVAs. For all analyses, Tukey's HSD post hocs were used to determine differences among drug groups. Drug and Stress effects on the dependent measures also were examined by calculating proportions of variance explained (η^2; eta squared) to assess magnitude. For Drug effects, these assessments were made on no-stress animals that received nicotine; for Stress effects, calculations were performed on saline-treated no-stress and stress animals. Pearson's product–moment correlations were used to assess the relationship between CORT, food consumption, and body weight All tests were two-tailed. Results are significant at $p<0.05$ unless otherwise noted.

4. Results

4.1. Body weight

See Fig. 1a–d. Among males, stress exposure [Day×Stress: $F(4, 424)=32.2$ and Stress: $F(1, 106)=9.9$] and nicotine administration [Day×Drug: $F(8, 424)=26.2$ and Drug: $F(2, 106)=9.7$] reduced body weight. The effects of nicotine to reduce body weight also were evident when no-stress males [Day×Drug: $F(8, 216)=19.5$ and Drug: $F(2, 54)=4.8$] and stressed males [Day×Drug: $F(8, 208)=10.0$ and Drug: $F(2, 52)=5.1$] were examined separately. ANOVAs on specific days indicated that nicotine reduced body weight for males on every measurement day (F values ranging from 8.2 to 16.1), with the saline-treated animals weighing more than the 12 mg/kg/day-treated animals (Tukey's HSD), and that stress reduced body weight on Drug Days 5, 11, and 13 (F values ranging from 7.8 to 23.0). These patterns also were present when the strains were examined separately.

There were no clear strain differences among males in terms of stress or nicotine effects. The average magnitude of the stress effect during the drug administration period was similar between the strains (η^2 for saline-treated Sprague–Dawley males=11.7%; η^2 for saline-treated Long–Evans males=9.3%). The average magnitude of the nicotine effect also was similar between the strains (η^2 for no-stress Sprague–Dawley males=20.6; η^2 for no-stress Long–Evans males=21.0%) and the magnitude of these effects was comparable to effects in no-stress animals (stressed Sprague–Dawley males: η^2=22.9%; stressed Long–Evans males: η^2=24.8%). Among females, stress exposure [Day×Stress: $F(4, 424)=2.3$ and Stress: $F(1, 106)=3.6$, $p=0.06$] and nicotine administration [Day×Drug: $F(8, 424)=20.5$ and Drug: $F(2, 106)=14.6$] reduced body weight. The effects of nicotine to reduce body weight were evident when no-stress females [Day×Drug: $F(8, 212)=14.0$ and Drug: $F(2, 53)=13.3$] and stressed females [Day×Drug: $F(8, 212)=7.8$ and Drug: $F(2, 53)=3.4$] were examined separately.

In contrast to males, there were strain differences in the magnitude of stress and nicotine effects to reduce body weight among females. Among saline-treated females, stress reduced body weight markedly among Long–Evans females [Stress×Strain: $F(1, 36)=3.3$] but not among Sprague–Dawley females. The stress effect was significant and large among saline-treated Long–Evans females [$F(1, 18)=10.4$; η^2=36.6%] but not among saline-treated Sprague–Dawley females [nonsignificant F test; η^2= 3.0%]. ANOVAs on each measurement day indicated that among Long–Evans females, stress (F values ranging from 3.4 to 7.5) and nicotine administration (F values ranging from 11.6 to 14.1; Tukey's HSD: saline >6 and 12 mg/kg/day) significantly reduced body weight on every measurement day. Further, significant Stress×Drug interactions (F values ranging from 3.8 to 4.8) on every measurement day indicated that nicotine effects were greatest in no-stress Long–Evans females (Tukey's HSD; no-stress animals: saline >6 and 12 mg/kg/day; stressed animals: saline=6 mg/kg/day; 6>12 mg/kg/day). In contrast, among Sprague–Dawley females only nicotine administration reduced body weight (F values ranging from 6.4 to 8.6; Tukey's HSD: saline >6 and 12 mg/kg/day). The average magnitude of the nicotine effect was greater among no-stress Long–

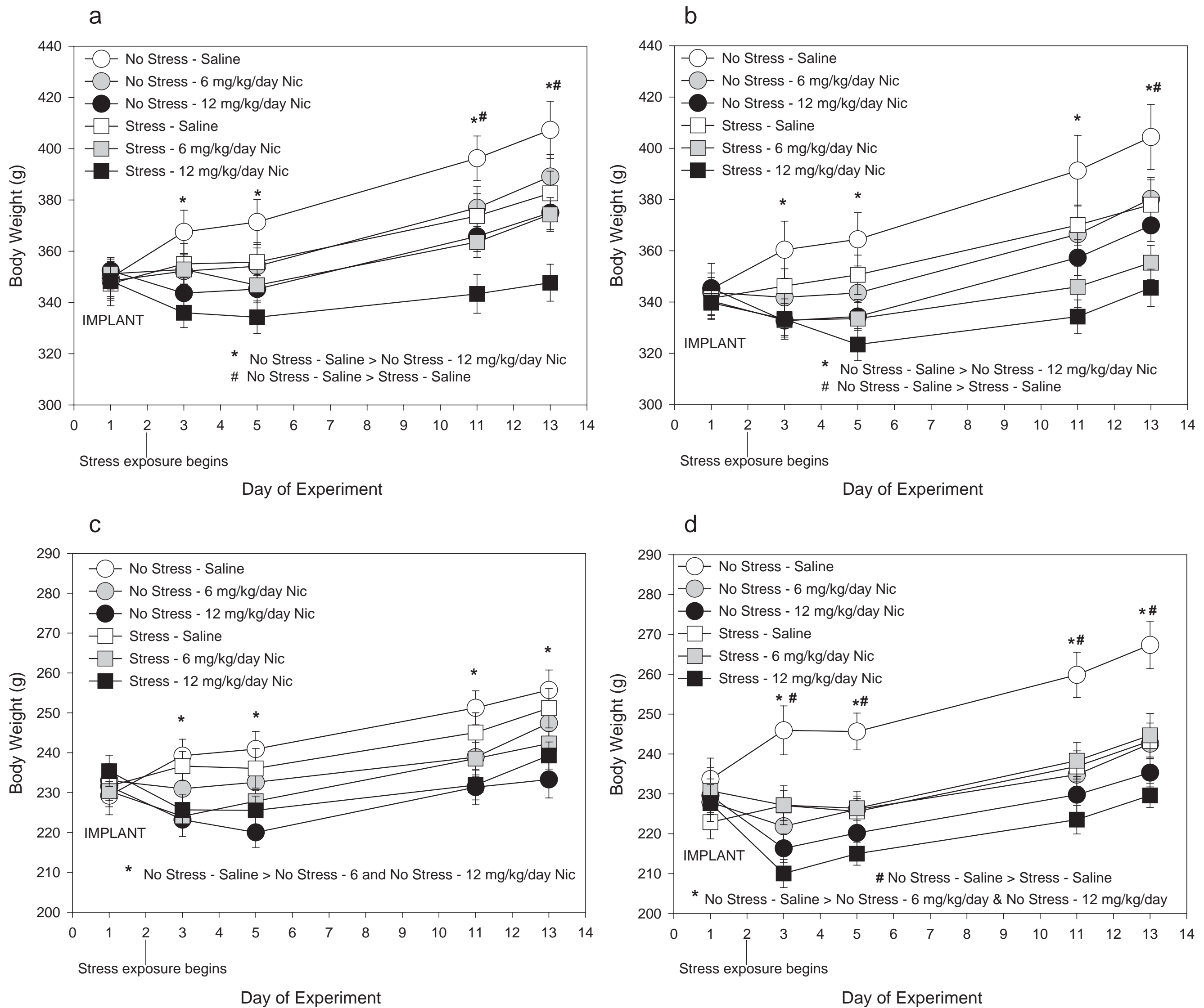

Fig. 1. Body weight in grams (mean±S.E.M.) during the Drug Administration/Stress Phase. (a) Sprague–Dawley males; (b) Long–Evans males; (c) Sprague–Dawley females; (d) Long–Evans females. Detailed data analytic results are presented in the Results section.

Evans females [$F(2, 27)=14.1$; $\eta^2=51.2\%$] compared to no-stress Sprague–Dawley females [$F(2, 26)=5.9$; $\eta^2=31.3\%$). In contrast to males, stress reduced the magnitude of nicotine effects in both strains of females, with eta-squared dropping to 19.1% for stressed Sprague–Dawley females [$F(2, 26)=3.1$; $p=0.06$] and 25.6% for stressed Long–Evans females [$F(2, 27)=4.6$].

4.2. Food consumption

Among males, stress exposure [Stress: $F(1, 107)=27.7$] and nicotine administration [Drug: $F(2, 107)=44.6$] reduced feeding. Nicotine's effects to reduce feeding also were evident when no-stress males [Drug: $F(2, 54)=20.6$] and stressed males [Drug: $F(2, 53)=24.4$] were examined separately. These effects occurred in a dose–response manner, with saline-exposed animals eating significantly more than 12 mg/kg/day nicotine-exposed animals (Tukey's HSD; Fig. 2).

As with the body weight data, there were no clear strain differences among males in terms of stress or nicotine effects. The magnitude of the stress effect was similar among saline-treated Sprague–Dawley males [$F(1, 18)=4.5$, $\eta^2=20.0\%$] and saline-treated Long–Evans males [$F(1, 18)=4.1$; $\eta^2=18.5\%$). The magnitude of the nicotine effect was greater than the stress effect and was similar between no-stress Sprague–Dawley males [$F(2, 27)=9.5$; $\eta^2=41.4\%$) and no-stress Long–Evans males [$F(2, 27)=11.2$; $\eta^2=45.4\%$). In addition, effects of nicotine to reduce feeding in stressed animals were comparable to effects in no-stress animals [stressed Sprague–Dawley males: $F(2, 26)=10.3$; $\eta^2=44.2\%$; stressed Long–Evans males: $F(2, 27)=15.2$; $\eta^2=53.0\%$).

Among females, nicotine administration reduced feeding [Drug: $F(2, 106)=45.6$] and these effects depended in part on

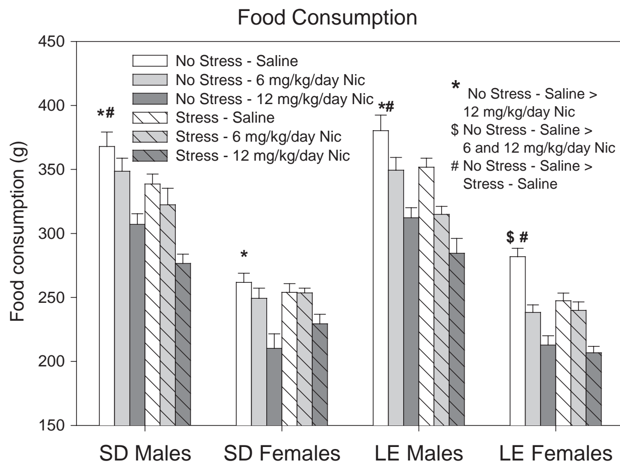

Fig. 2. Food consumption of male and female Sprague–Dawley and Long–Evans rats in grams (mean±S.E.M.) summed over the Drug Administration/Stress Phase. Detailed data analytic results are presented in the Results section.

stress status [Stress×Drug: $F(2, 106)=4.7$] such that differences among drug groups were smaller for stressed animals. Stress also reduced feeding, but only among Long–Evans females [Strain×Stress: $F(1, 106)=5.0$]. Nicotine's effects to reduce feeding were evident among no-stress [$F(2, 53)=30.0$] and among stressed females [$F(2, 53)=17.0$] and within each strain subgroup [no-stress Sprague–Dawleys: $F(2, 26)=9.0$; stressed Sprague–Dawleys: $F(2, 26)=4.9$; no-stress Long–Evans: $F(2, 27)=28.1$; stressed Long–Evans: $F(2, 27)=13.6$]. For Sprague–Dawley no-stress and stressed females and for Long–Evans stressed females, the saline and 6 mg/kg/day groups ate similar amounts and ate more than did the 12 mg/kg/day group (Tukey's HSD). For no-stress Long–Evans females, all groups differed significantly with the saline group eating the most and the 12 mg/kg/day group eating the least.

In contrast to males, there were strain differences in the magnitude of stress and nicotine effects to reduce feeding among females. Stress reduced feeding markedly among saline-treated Long–Evans females [$F(1, 18)=15.3$; $\eta^2=45.9\%$] but not among saline-treated Sprague–Dawley females [nonsignificant F test; $\eta^2=3.5\%$]. Further, the magnitude of the drug effect was much greater among no-stress Long–Evans females [$F(2, 27)=28.1$; $\eta^2=67.5\%$) compared to no-stress Sprague–Dawley females [$F(2, 26)=9.0$; $\eta^2=40.8\%$). Also in contrast to males, stress reduced the magnitude of nicotine effects in both strains of females, with η^2 dropping to 27.4% for stressed Sprague–Dawley females [$F(2, 26)=4.9$] and 50.1% for stressed Long–Evans females [$F(2, 27)=13.6$].

4.3. Corticosterone (CORT)

Females had higher CORT levels than did males [$F(1, 213)=92.4$], Sprague–Dawleys had somewhat higher CORT levels than did Long–Evans [$F(1, 213)=7.8$], and stress increased CORT levels [$F(1, 213)=72.4$]. Effects of nicotine on CORT depended on stress status [Drug×Stress: $F(2, 213)=11.3$] with nicotine having little effect on CORT in no-stress animals and decreasing CORT in stressed animals. The findings are most clearly summarized, therefore, by the interactions and by the presence of strain differences among females (see below) but not among males (Fig. 3).

Among males, stress increased CORT [$F(1, 107)=94.6$] and nicotine effects depended on stress status [Drug×Stress: $F(2, 107)=3.5$] with nicotine having little effect in no-stress males and slightly decreasing CORT in stressed males. When no-stress and stressed males were examined separately, there were no significant effects of nicotine. There were no strain differences among males in terms of stress or nicotine effects on CORT. The stress effect was of similar magnitude in saline-treated Sprague–Dawley males [$F(1, 18)=25.4$; $\eta^2=58.5$) and saline-treated Long–Evans males [$F(1, 18)=31.8$; $\eta^2=63.9\%$). The nicotine effects were small and nonsignificant ($\eta^2=1.9\%$ in no-stress Sprague–Dawley males; $\eta^2=13.2\%$ in no-stress Long–Evans males) and were similarly small and nonsignificant in stressed animals ($\eta^2=6.3\%$ in stressed Sprague–Dawley males; $\eta^2=9.6\%$ in stressed Long–Evans males).

Among females, Sprague–Dawleys had somewhat higher CORT levels than did Long–Evans [Strain: $F(1, 106)=5.7$], stress increased CORT [$F(1, 106)=15.9$], and nicotine effects depended on stress status [Drug×Stress: $F(2, 106)=7.8$] with nicotine having little effect in no-stress females and markedly decreasing CORT in stressed females. When no-stress and stressed females were examined separately, among stressed females Sprague–Dawleys had higher CORT levels than did Long–Evans [Strain: $F(1, 53)=9.0$] and nicotine decreased CORT levels [$F(2, 53)=8.8$]. Unlike males, strain differences were evident in

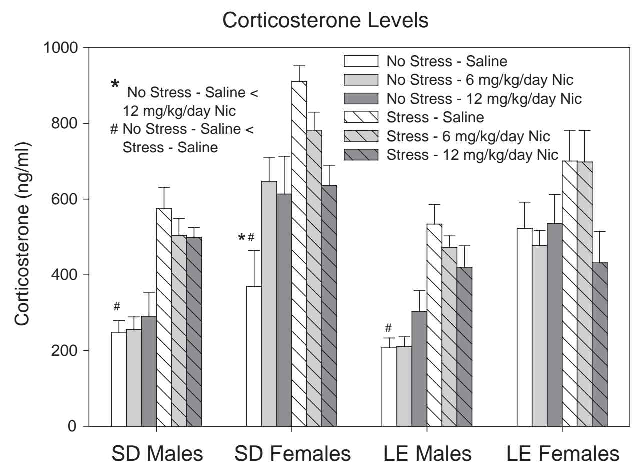

Fig. 3. Corticosterone levels (ng/ml; mean±S.E.M.) of male and female Sprague–Dawley and Long–Evans rats on Day 14 of the Drug Administration/Stress Phase. Detailed data analytic results are presented in the Results section.

terms of the magnitude of stress and nicotine effects on CORT. The stress effect was extremely large in saline-treated Sprague–Dawley females [$F(1, 18)=27.2$; $\eta^2=60.2\%$) compared to saline-treated Long–Evans females [nonsignificant F test; $\eta^2=13.4\%$). The nicotine effect also was much larger in no-stress Sprague–Dawley females [$F(2, 26)=3.2$; $\eta^2=19.5\%$) compared to no-stress Long–Evans females [nonsignificant F test; $\eta^2=1.7\%$). For both strains, the drug effects were larger among stressed animals [stressed Sprague–Dawley females: $F(2, 26)=8.5$; $\eta^2=39.6\%$; stressed Long–Evans females: $F(2, 27)=3.5$; $\eta^2=20.7\%$].

Pearson's product–moment correlations (see Fig. 4a and b) revealed that among stressed females (collapsed across strains and drug dosages) CORT levels were positively associated with body weight on the last day of the experiment ($r=+0.32$) and with food consumption ($r=+0.37$). Correlations were not significant for no-stress males, no-stress females, or stressed males.

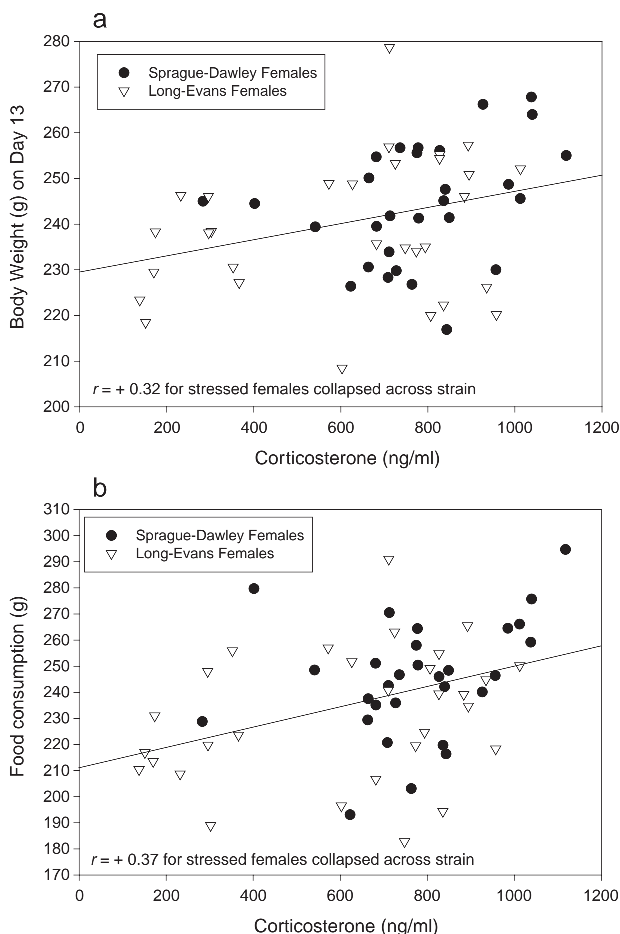

Fig. 4. (a) Relationship between corticosterone levels (ng/ml; mean±S.E.M.) and body weight (g) for stressed females. (b) Relationship between corticosterone levels (ng/ml; mean±S.E.M.) and food consumption (g) summed over the Drug Administration/Stress Phase for stressed females.

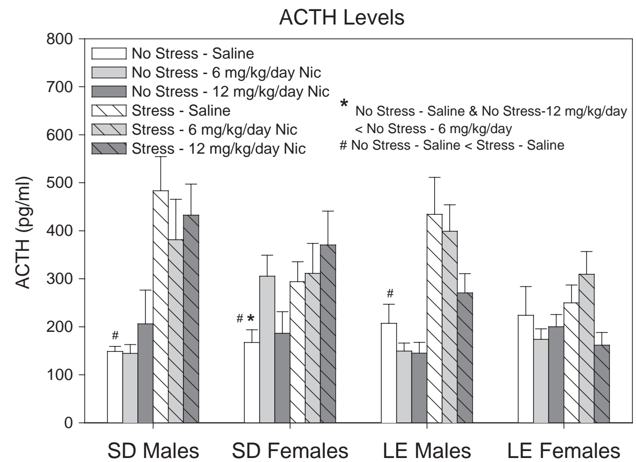

Fig. 5. Adrenocorticotropin levels (pg/ml; mean±S.E.M.) of male and female Sprague–Dawley and Long–Evans rats on Day 14 of the Drug Administration/Stress Phase. Detailed data analytic results are presented in the Results section.

4.4. Adrenocorticotropin hormone (ACTH)

Sprague–Dawleys had somewhat higher ACTH levels than did Long–Evans [$F(1, 209)=4.5$], males had slightly higher ACTH levels than did females [$F(1, 209)=3.5$, $p=0.06$], and stress increased ACTH levels [$F(1, 209)=59.1$]. Effects of stress to increase ACTH were larger in males than in females [Sex×Stress: $F(1, 209)=16.1$]. Nicotine effects on ACTH were complex and depended on strain as well as stress status [$F(2, 209)=3.1$; Fig. 5].

Among males, stress increased ACTH levels [$F(1, 107)=56.0$]. Nicotine administration did not alter ACTH levels and when no-stress and stressed males were examined separately, there were no significant effects of nicotine. There were no clear strain differences among males in terms of stress or nicotine effects on ACTH. The stress effect was somewhat larger among saline-treated Sprague–Dawley males [$F(1, 18)=21.7$; $\eta^2=54.7\%$) compared to saline-treated Long–Evans males [$F(1, 18)=7.8$; $\eta^2=30.1\%$), but the difference was not strong enough to emerge as a strain effect or a Strain×Drug interaction. The nicotine effects were nonsignificant in no-stress animals ($\eta^2=4.7\%$ in no-stress Sprague–Dawley males; $\eta^2=10.2\%$ in no-stress Long–Evans males) and in stressed animals ($\eta^2=0.7\%$ in stressed Sprague–Dawley males; $\eta^2=17.5\%$ in stressed Long–Evans males). Although the effects were larger in Long–Evans males, again the difference was not strong enough to emerge as a strain effect or Strain×Drug interaction.

Among females, Sprague–Dawley animals had higher ACTH levels than did Long–Evans [Strain: $F(1, 105)=4.9$] and stress increased ACTH levels [$F(1, 105)=8.2$]. Unlike males, nicotine effects depended on strain as well as stress status [Strain×Stress×Drug: $F(2, 105)=3.3$]. Among no-stress females, nicotine had no effect on ACTH levels in Long–Evans, but the 6 mg/kg/day dosage increased ACTH

levels in Sprague–Dawleys [Strain×Drug: $F(2, 52)=3.3$]. This drug effect was significant when no-stress Sprague–Dawley females were examined separately [$F(2, 25)=3.9$], with the 6 mg/kg/day group >saline and 12 mg/kg/day groups. Among stressed females, Sprague–Dawleys had higher ACTH levels than did Long–Evans [Strain: $F(1, 53)=4.9$] and, among stressed Long–Evans females, nicotine (12 mg/kg/day) decreased ACTH levels [$F(2, 27)=3.9$; saline=6 mg/kg/day; 6>12 mg/kg/day]. The stress effect among saline-treated Sprague–Dawley females was larger [$F(1, 18)=3.8$, $p=0.06$; $\eta^2=17.6$] than among saline-treated Long–Evans females [nonsignificant F test; $\eta^2=0.07\%$]. The nicotine effect also was larger among no-stress Sprague–Dawley females [$F(2, 25)=3.9$; $\eta^2=23.9\%$] compared to no-stress Long–Evans females [nonsignificant F test; $\eta^2=2.9\%$]. Stress decreased the drug effect magnitude among Sprague–Dawleys [nonsignificant F test; $\eta^2=2.2\%$] and increased it among Long–Evans [$F(2, 27)=3.9$; $\eta^2=22.2\%$].

5. Discussion

This experiment examined effects of chronic nicotine administration (0, 6, or 12 mg/kg/day) with and without daily immobilization stress on body weight, feeding, corticosterone (CORT), and adrenocorticotropin hormone (ACTH) responses of male and female Sprague–Dawley and Long–Evans rats (see Table 1 for summary of results).

5.1. Effects of nicotine

Nicotine reduced body weight and feeding. These effects were similar in Sprague–Dawley and Long–Evans males and accounted for about 20% of body weight variance and about 40% of feeding variance in each strain. In contrast to effects in males, nicotine effects in females depended on strain of animal. Body weight effects were larger in Long–Evans females, accounting for about 50% of variance, than in Sprague–Dawley females (about 30% of variance). A similar pattern was evident for feeding effects of nicotine. Effects of nicotine to reduce feeding in Sprague–Dawley females (about 40% of variance) were similar to those in males, but were larger in Long–Evans females (about 68% of variance). These findings are consistent with the existing literature in that nicotine reduced body weight in males and females and effects generally were larger in females than in males (Grunberg and Bowen, 1985; Grunberg et al., 1986; Bowen et al., 1986; Winders and Grunberg, 1989; Faraday et al., 2001). These findings extend this literature by indicating that Long–Evans females are markedly more sensitive to nicotine effects on feeding and body weight than are Sprague–Dawley animals or Long–Evans males.

In contrast, Sprague–Dawley females were markedly more sensitive to nicotine effects on CORT and ACTH than were Sprague–Dawley males or Long–Evans males and females. Nicotine effects accounted for nonsignificant proportions of variance in these subgroups (ranging from 1.7 to 13.2%) but accounted significantly for 19.5% of CORT variance and 23.9% of ACTH variance among Sprague–Dawley females. Specifically, 6 and 12 mg/kg/day nicotine increased CORT levels and 6 mg/kg/day increased ACTH levels among Sprague–Dawley females.

The lack of findings for males and for Long–Evans females is consistent with the existing literature. Although nicotine increases CORT and ACTH robustly when administered acutely (e.g., Bugajski et al., 1998; Matta et al., 1998), studies that have administered nicotine via minipump have reported no effect on CORT and ACTH at dosages up to 3 mg/kg/day (Fuxe et al., 1990; Singh et al., 2000). Findings from the present experiment suggest that chronically-administered dosages up to 12 mg/kg/day also do not increase HPA axis hormones in males and in some strains of females. The fact that chronically-administered nicotine increased CORT and ACTH levels in Sprague–Dawley females in the present experiment is a new finding and may indicate that the Sprague–Dawley female HPA axis is more sensitive to nicotine than is the HPA axis of other groups. One study compared male and female Sprague–Dawley responses to acute nicotine administration and reported that females exhibited greater CORT and ACTH responses than did males (Rhodes et al., 2001). The finding reported here is consistent with this report.

5.2. Effects of stress

Stress also reduced body weight and feeding. Among males, the effects of stress were similar in Sprague–Dawleys and Long–Evans and accounted for about 10% of body weight variance and about 20% of feeding variance in each

Table 1
Effect sizes (η^2; proportion of variance explained)

	Body weight		Food consumption		Corticosterone		ACTH	
	Stress effect	Nicotine effect	Stress effect	Nicotine effect	Stress effect	Nicotine effect	Stress effect	Nicotine effect
SD males	11.7	20.6	20.0	41.4	58.5	1.9	54.7	4.7
LE males	9.3	21.0	18.5	45.4	63.9	13.2	30.1	10.2
SD females	3.0	31.3	3.5	40.8	60.2	19.5	17.6	23.9
LE females	36.6	51.2	45.9	67.5	13.4	1.7	0.07	2.9

strain. In contrast to effects in males, stress effects in females depended on strain of animal. Stress markedly reduced body weight of Long–Evans females C accounting for about 37% of variance C but not of Sprague–Dawley females (3% of variance). A similar pattern was evident for stress effects on feeding. Effects in Long–Evans females (46% of variance) were large; effects in Sprague–Dawley females were minimal (3.5% of variance).

These findings are partially consistent with our previous report that 20 min/day immobilization decreased feeding and body weight more in males than in females and that these effects were more consistent in Long–Evans males based on the number of days for which significant stress effects were obtained (Faraday, 2002). Calculation of effect sizes on these previously reported data were performed for purposes of comparison to the data obtained in the present experiment. These calculations indicated that effects of stress on body weight in males were similar in magnitude to those reported here and were similar between the strains (from Faraday, 2002: Sprague–Dawley males—5.5% of variance; Long–Evans males—11.4% of variance). Effects of stress on feeding in males also were similar in each strain (from Faraday, 2002: Sprague–Dawley males—6.2%; Long–Evans males—6.4%) but smaller than those reported in the present experiment. In females, stress effects on body weight differed based on strain with greater effects in Long–Evans females (10.4% of variance) than in Sprague–Dawley females (2.0% of variance; Faraday, 2002). These effects are in the same direction as in the present experiment but the effect size in Long–Evans females in the previous study was smaller. Effects of stress on feeding in females accounted for less than 1% of variance in each strain in contrast to findings from the present experiment in which effects were substantial in Long–Evans females.

Differences between the two studies may be the result of animal age. Animals in Faraday (2002) were 60 days old at the beginning of the experiment because the goal was to study effects of stress that began in early adulthood. In the present study, animals were 49 days old at the beginning of the experiment. This age was selected because we were interested in effects of nicotine and stress exposure that began in late adolescence and continued into early adulthood (e.g., the period spanning ages 50 to 75 days; Spear, 2000). It is possible that younger animals, especially Long–Evans females, are more sensitive to stress effects on feeding and body weight than are older animals. We have previously reported that sensitivity to nicotine's body weight effects depends on age (Faraday et al., 2001).

With regard to CORT and ACTH responses, immobilization stress significantly increased these responses in all groups except for Long–Evans females. Stressed Sprague–Dawley females exhibited the highest CORT responses but the magnitude of the stress effect was similar among Sprague–Dawley males, Sprague–Dawley females, and Long–Evans males (ranging from 58.5 to 63.9% of variance). In contrast to relatively similar effect sizes for stress effects on CORT, effect size magnitudes for stress effects on ACTH differed among subgroups: Sprague–Dawley males C 54.7% of variance; Sprague–Dawley females C 17.5% of variance; and, Long–Evans males C 30.1% of variance. Findings replicate reports that CORT responses of Sprague–Dawley males to brief immobilization do not habituate after up to 13 days of daily exposure (Kant et al., 1983; Raygada et al., 1992). Findings extend the literature by indicating that CORT responses of Sprague–Dawley females and Long–Evans males also do not habituate after up to 13 days of immobilization exposure. Findings for Long–Evans females also are new, but more difficult to interpret. It is possible that Long–Evans females initially exhibited CORT and ACTH increases to immobilization but habituated after 13 exposures. It also is possible that Long–Evans females are hormonally insensitive to brief immobilization stress. These possibilities remain to be examined.

5.3. Stress×nicotine interactions

Stress altered effects of nicotine in females but not in males. For males, nicotine reduced feeding and body weight in a dose-related manner and stressed animals were shifted downward relative to no-stress animals C a straightforward main effect. With regard to CORT and ACTH in males, nicotine did not reliably alter responses and stress shifted responses of all drug groups upward C another main effect.

In females, however, effects of nicotine were affected by stress status and to some extent by strain. With regard to body weight, stress reduced the magnitude of the nicotine effect in both strains. This reduction can be seen in Fig. 1c and d as closer clustering of stressed treatment groups compared to no-stress treatment groups. The strains differed in dose–response relationships. In no-stress and stressed Sprague–Dawley females, nicotine's dose–response relationships were similar, with saline>6 mg/kg/day>12 mg/kg/day. The same dose–response relationship is evident in no-stress Long–Evans females. In stressed Long–Evans females, however, saline=6 mg/kg/day>12 mg/kg/day. Stress also reduced the magnitude of nicotine effects on food consumption in both strains of females but the effect was much weaker and there were no clear strain differences.

With regard to HPA axis hormones, among Long–Evans females CORT levels of stressed animals administered 6 mg/kg/day were greater than CORT levels of no-stress animals administered 6 mg/kg/day but the two 12 mg/kg/day groups exhibited similar responses. A similar pattern was evident for ACTH levels, suggesting that the 6 mg/kg/day dosage in combination with stress had additive effects. Nicotine action on ACTH also differed for no-stress and stressed Sprague–Dawley females, with the 6 mg/kg/day

groups exhibiting similar responses but the stressed 12 mg/kg/day group exhibiting greater ACTH responses than the no-stress 12 mg/kg/day group. These data suggest that, in contrast to Long–Evans females, a higher dosage was necessary in Sprague–Dawley females to produce additive stress–nicotine effects.

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. In support of this point, variances of female and male responses across the dependent variables were similar, suggesting that female responses were not shifting markedly based on estrus stage. In addition, although feeding and CORT responses can be modified by estrus stage, these effects are extremely small compared to the effects of stress and of nicotine on these responses. For example, estrus stage has no effect on cardiovascular responses to mild stressors (Sharp et al., 2002), on nicotine-induced hyperlocomotion (Kuo et al., 1999), on food intake during chronic nicotine administration via osmotic minipump (Blaha et al., 1998), or on nicotine self-administration (Donny et al., 2000). Further, Conrad et al. (2004) concluded that female rats exhibit larger CORT responses during the proestrus phase than during the estrus phase, but the magnitude of these differences is small compared to the size of the stress effects on CORT in the present study.

5.4. Summary and implications

These results suggest that: (1) strain differences in body weight, feeding, and HPA axis responses to nicotine and to stress are robust among females but not among males; (2) among females, feeding and body weight responses to nicotine and to stress can dissociate from HPA axis responses to nicotine and to stress; (3) among females, stress alters nicotine's effects on these responses; and (4) sensitivity to nicotine and sensitivity to stress are not global phenomena that occur across all variables C these effects depend on the variables considered (e.g., feeding and body weight vs. HPA axis hormones).

The most interesting finding is that strain differences in sensitivity were more pronounced among females than among males. This pattern suggests that there are underlying genotypic differences between the strains and that female sex hormones (i.e., estradiol, progesterone) may amplify these differences. Although Sprague–Dawley and Long–Evans rats are used as all-purpose rat strains to address a variety of research questions, the extent to which they differ in central neurochemistry has been addressed by only a few studies, all of which have used male animals.

For example, Long–Evans rats were markedly less sensitive than were Sprague–Dawley rats to the pre-pulse inhibition-disrupting effects of various dopaminergic agonists (Swerdlow et al., 2001). Several studies also suggest that the strains 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). In addition, the strains differ in nicotine self-administration patterns, with Sprague–Dawley rats better able to discriminate nicotine at lower dosages than Long–Evans rats (Corrigall and Coen, 1989; Glick et al., 1996; Shoaib et al., 1997). Taken together, these findings suggest that the two strains differ across systems that have been implicated in effects of stress as well as in effects of nicotine.

No studies, however, have compared neurochemical or self-administration responses of females. It is noteworthy that estradiol modulates activity of dopaminergic, serotonergic, and noradrenergic systems as well as the actions of corticosterone, suggesting that estradiol could be relevant to understand strain differences in females (Galea et al., 2001; Gold and Chrousos, 1999; Joffe and Cohen, 1998; Magiakou et al., 1997; Morissette and Di Paolo, 1993; Parada et al., 1991; Wong et al., 2000). It also is possible that the strains differ in peripheral processes (e.g., metabolism, drug distribution) relevant to nicotine's actions, but these questions have not been addressed.

Findings from the present experiment also raise the question of the health consequences of stress and drug sensitivity revealed as changes in hormonal responses and appetitive behaviors. For example, it is possible that the robust CORT responses of Sprague–Dawley females to immobilization blunted or prevented effects of immobilization to reduce feeding and body weight and that the blunted CORT responses of Long–Evans females to immobilization resulted in greater stress-induced decreases in feeding and body weight. If animals had been provided with a choice of foods rather than standard rat chow, it is possible that stressed Sprague–Dawley females would have exhibited increased feeding and possibly weight gain and the stress-induced decreases of Long–Evans females would have been smaller.

If these findings extrapolate to humans, then they suggest that women who exhibit greater HPA axis activation in response to stress also may increase feeding in response to stress as compared to women who exhibit less cortisol reactivity. In fact, women who were highly reactive to a laboratory stressor in terms of cortisol responses have been reported to consume more calories when stressed than low cortisol reactors (Epel et al., 2001). It also is relevant that Sprague–Dawley females, but not Long–Evans females, exhibit decreases in locomotion when exposed to chronic, mild stress that have been interpreted as behavioral evidence of a depression-like state (Faraday, 2002; Kennett et al., 1986; Haleem et al., 1988). Taken together, these data suggest that HPA reactivity in females

may be associated with physical as well as psychological health problems.

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