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Effects of Nicotine and Stress on Startle Amplitude and Sensory Gating Depend on Rat Strain and Sex

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FARADAY, M. M., V. A. O'DONOGHUE AND N. E. GRUNBERG. *Effects of nicotine and of stress on startle amplitude and sensory gating depend on rat strain and sex.* PHARMACOL BIOCHEM BEHAV **62**(2) 273–284, 1999.—We recently reported that 14 days of nicotine administration (12 mg/kg/day) reduced acoustic startle reflex amplitude and impaired prepulse inhibition (PPI) of startle in male and female Long–Evans rats (24). These findings contrasted with reports of nicotine-induced enhancement of startle and PPI in Sprague–Dawley (a different strain) male rats (2–4). The present experiment administered 0, 6, or 12 mg/kg/day nicotine via osmotic minipump for 14 days to 120 Sprague–Dawley rats (male and female) and to 120 Long–Evans rats (male and female) and examined ASR and PPI. Half of the subjects also were stressed by immobilization once each day to examine nicotine–stress interactions. Nicotine enhanced ASR and PPI responses of Sprague–Dawley rats but impaired these responses in Long–Evans rats, regardless of sex. Effects of stress were complex and depended on strain, sex, and drug dose. These findings indicate that effects of nicotine on measures of reactivity (ASR) and sensory gating (PPI) depend on genotype and that nicotine–stress interactions depend on genotype, sex, and nicotine dosage.

Nicotine Immobilization ASR PPI Sprague–Dawley Long–Evans Strain differences Sex differences Males Females

THE effects of nicotine in the human smoker are complex. Some of these effects, such as relief from stress, are reported robustly across individuals (71,72,80,83). These reports are paradoxical, given that nicotine, a sympathomimetic, increases physiological and biochemical stress responses (80). It is possible that this dissociation between subjective experience and biologic responses—known as Nesbitt's paradox [e.g., (57)]—results from nicotine's effects to normalize behavior under stress (2). Other nicotine effects, such as attentional enhancement, are not uniformly reported (40,68,80,81). The extent to which nicotine's effects are consistent or vary across individuals likely depends on many factors, including psychological, environmental, and biological influences. Several studies have suggested that the smoker's genotype contributes to smoking behavior, with a mean heritability estimate of 53% for tobacco use (43), and genetic factors relevant to smoking initiation, age of onset, and number of cigarettes smoked per day (23,35,39). Consistent self-reports of stress-relief from cigarette smoking across individuals suggest these effects of nicotine may obtain regardless of genotype. Mixed findings with regard to nicotine-induced attentional enhancement suggest that genotype may explain some of the variance in these nicotine effects (40,68,74,80,81).

The role of sex, as a subset of genotype, in attentional and stress-relieving effects of nicotine also may be relevant. Many studies investigating effects of nicotine on attentional processes in humans have examined only men [e.g., (47,74)] or only women [e.g., (42,46)]. In studies that tested both male and female subjects [e.g., (64,82)], analyses by gender are often not reported, and the number of subjects per cell (typically $n = 6$ of each sex) may have been insufficient to reliably distinguish sex differences. In addition, recent studies indicate that men and women differ in baseline sensory-gating and information-processing abilities (75), further complicating interpretation of studies with human females. Further, although several studies have indicated that females are more sensitive

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than are males to some behavioral and biological effects of nicotine (11,30,32–34), female humans and rats are less adept than are males at discriminating nicotine from placebo or adjusting nicotine intake after preloads (60,69). This contrast between behavioral sensitivity and interoceptive insensitivity, therefore, also complicates interpretations of human male vs. female self-reports of nicotine effects.

The extent to which nicotine's attentional effects and interactions with stress may be biologically controlled (i.e., by genotype, including sex) is relevant to optimize prevention of tobacco use and to maximize success of cessation. Examination of genotypic differences, including sex differences, in attentional and stress responses to nicotine in an animal model may illuminate individual differences in human smoking behavior. In addition, if genotype is a powerful variable in nicotine's attentional effects, then the efficacy of nicotine or nicotine analogs and the development of nicotine-related pharmaceuticals as cognition-enhancing agents in clinical populations (e.g., Alzheimer's patients) also may depend on these individual differences.

The acoustic startle response (ASR) and prepulse inhibition (PPI) of the ASR constitute a behavioral paradigm that may index basic cognitive processes, and has been used to evaluate drug effects on these processes. The ASR is an unconditioned behavioral index of reactivity to external acoustic stimuli (19) that has been reported to be sensitive to changes in attentional processes in humans (8,9). When the startling sound is preceded by a nonstartling stimulus (a prepulse), the amplitude of the startle response is reduced (12,27). The inhibition of startle as the result of a prepulse - prepulse inhibition (PPI) - is believed to index central processes related to information processing and sensory gating (76), and possibly attention (1–5,31,63).

The ASR–PPI paradigm has been widely used to index the effects of dopaminergic agonists (20,22,36,48,76–78). In addition, the effects of nicotine administration and cessation have been studied using this procedure (1–5,18,24,41,63,65). We recently reported that chronic nicotine administration (12 mg/kg/day) reduced startle amplitude and prepulse inhibition in male and female Long–Evans rats (24). These results contrast with our findings in Sprague–Dawley males, in which nicotine administration enhanced startle and PPI (2–4), and contrast with findings of other investigators using Long–Evans rats as subjects who also used different methodologies (e.g., testing during the light portion of the circadian cycle, different forms and dosages of nicotine, and different routes of administration) (18,41,65). Our findings are consistent, therefore, with a true strain difference in the ASR and PPI responses of Sprague–Dawley vs. Long–Evans rats in response to nicotine, but because they are based on the data from only one study, do not unequivocally establish it. Several other important questions also remained, including: the effects of chronic nicotine on ASR–PPI responses of Sprague–Dawley females and the responses of Long–Evans males and females to an intermediate dosage of nicotine (6 mg/kg/day).

The present experiment was designed to replicate and extend previous work by examining ASR and PPI responses to nicotine in male and female Sprague–Dawley and Long–Evans rats within the same study. In addition, to begin to investigate whether the two strains had differently shaped or positioned dose–response curves, the experiment included two nicotine dosages. Further, immobilization stress was included to examine stress effects on ASR and PPI responses as well as the stress–-nicotine interaction within and across strains, within and across sexes, and in within-strain, within-sex groups. A further goal of the experiment was to assess possible sex differences within strains apart from drug or stress effects.

Immobilization stress was used because this stressor alters ASR and PPI responses. Specifically, immobilization increased startle and PPI in Sprague–Dawley males (1,2). In addition, immobilization produces reliable peripheral biochemical changes in the form of elevated adrenocorticotropin hormone (ACTH), beta-endorphins, and corticosterone consistent with a stress response [e.g., 1,2,44,66). These biochemical stress responses do not diminish with repeated exposure to the stressor (45), and are similar in males and females (44).

One study has examined the interaction of chronic nicotine administration and stress on ASR–-PPI responses. In that case, effects of immobilization stress depended on nicotine dose (2). Specifically, administration of 6 mg/kg/day nicotine to stressed male Sprague–Dawley subjects, resulted in nicotine and stress having additive, enhancing effects on ASR and PPI. ASR and PPI responses of stressed males administered 12 mg/kg/day nicotine, however, were indistinguishable from nonstressed saline control responses (2). The fact that high doses of nicotine when combined with stress produced behavioral responses similar to nonstressed, nondrug control subjects is consistent with the report of human smokers that cigarette smoking alleviates stress. Whether chronic nicotine administration and stress interact similarly for females and for subjects of other strains is not known.

METHOD

Subjects

Subjects were 120 Sprague–Dawley (60 male, 60 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 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). Startle and PPI testing were performed during the dark (active) phase of the light cycle (between 0900 and 1600 h) following the procedures of several investigators [e.g., (4,56,75,78)]. Startle amplitudes are greater and more stable at this time (13,21). At the beginning of the experiment, subjects were 49 days old. Average male weight was 228 g, and average female weight was 172 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.

Equipment

Acoustic startle reflex amplitudes and prepulse inhibition were measured in a Coulbourn Instruments Acoustic Response Test System (Coulbourn Instruments, Allentown, PA) consisting of four weight-sensitive platforms inside a sound-attenuated chamber. Platforms were arranged radially around central speakers in the chamber's floor and ceiling. Each subject was placed individually in a 8 × 8 × 16 cm open-air cage that rested on top of the weight-sensitive platform. The open-air cages were small enough to restrict extensive locomotion but large enough to allow the subject to turn around and make other small movements. Subjects' movements in response to stimuli were measured as a voltage change by a

strain gauge inside each platform and were converted to grams of body weight change following analog to digital conversion. Responses were recorded by an interfaced computer as the maximum response occurring within 200 ms of the onset of the startle-eliciting stimulus.

Following placement of subjects in the chamber, a 3-min adaptation period occurred in which no startle stimuli were presented. Startle stimuli consisted of 112 or 122 dB SPL (unweighted scale; re: 0.0002 dynes/cm^2) noise bursts of 20 ms duration sometimes preceded 100 ms by 68 dB 1 kHz pure tones (prepulses). Decibel levels were verified by a Larson-Davis Sound Pressure Machine Model 2800 (Provo, UT). Each stimulus had a 2-ms rise and decay time such that onset and offset were abrupt, a primary criterion for startle. There were six types of stimulus trials, and each trial type was presented eight times. Trial types were presented in random order to avoid order effects and habituation. Intertrial intervals ranged randomly from 10–30 s. Trial types included: 1) 112 dB stimulus, 2) 112 dB stimulus preceded by prepulse, 3) 122 dB stimulus, 4) 122 dB stimulus preceded by prepulse, 5) prepulse only, and 6) no stimulus. The testing period lasted approximately 22 min.

A ventilating fan provided an ambient noise level of 56 dB throughout the testing period to mask effects of noises from outside the sound-attenuating chamber. In addition, although it has been reported that some rats emit ultrasonic vocalizations during startle testing (55), there is no evidence indicating that vocalizations alter startle responses and this paradigm has been used in many published studies of nicotine [e.g., (2–5)]. Nevertheless, the background noise of the ventilating fan also served to minimize the possible influence of ultrasonic vocalizations, should they occur. In addition, subjects were balanced across treatment groups within each testing chamber and session to control for the influence of possible vocalizations. Open-air cages were washed with warm water and dried after each use. Males and females were tested in separate test chambers. Stressed animals were tested separately from nonstressed animals.

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 Corp., Palo Alto, CA). Physiological saline also was used as vehicle for the nicotine solution. Nicotine solution was made from nicotine dihydrochloride. This method of administration avoids the repeated stress of daily injections. These dosages have resulted in significant changes in ASR and PPI responses in other experiments (2–4,24).

Subjects were anesthetized using methoxyflurane (Metofane) and minipumps were implanted subcutaneously (SC) between the shoulder blades according to procedures described in detail elsewhere (2,4). The entire surgical procedure including anesthesia took approximately 4 min per subject.

Stress Manipulation

Animals in the stress condition were restrained in commercially available 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. Restrained animals were checked every 5 min during the stress procedure to ensure the manipulation did not result in pain or undue distress. This restraint procedure has reliably produced elevations in hormones associated with a stress response, including adrenocorticotropin hormone (ACTH) and corticosterone (1,2,44,66).

Procedure

The experiment was conducted in two phases: a baseline phase and a drug administration 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., (30,80,84)]. Therefore, subjects' body weights were measured every other day throughout the drug administration phase as validation of drug administration.

Baseline phase. Subjects were handled once each day for 3 days. All subjects (n = 240) then underwent two acclimation exposures to the startle procedure. In the first acclimation subjects were placed inside the test chamber for 20 min but not exposed to the noise stimuli. In the second acclimation, exposure subjects were placed inside the test chamber and exposed to the noise stimuli. Acclimation was done to minimize the contamination of startle responses by possibly stressful effects of exposure to a novel situation. Three days after the acclimation exposure, ASR and PPI responses of all subjects were measured again. These responses constituted the baseline values.

Drug administration 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 insuring comparable initial body weights. This assignment resulted in 24 balanced groups of 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 administration day 1. On drug day 2, subjects in the stress condition began undergoing 20 min/day of restraint stress. These subjects were stressed every day for the remainder of the experiment.

ASR and PPI were measured for all subjects on drug day 2 (after 24 h of nicotine or saline administration and 1 day of stress manipulation), on drug day 6 (after 5 days of drug administration and stress manipulation), and on drug day 12 (after 11 days of drug administration and stress manipulation). Subjects in the stress condition were stressed approximately 30 min before the ASR–PPI measures. This procedure has resulted in stress-related changes in ASR and PPI (1,2,63). At the end of the experiment, blood and brains were collected for other experiments.

Data Analyses

Three subjects were dropped from analyses: one Sprague–Dawley male (12 mg/kg/day-stress group) and two Sprague–Dawley females (one from the 12 mg/kg/day-no stress group, and one from the 6 mg/kg/day-stress group). In each case, surgical complications resulted in failure of the minipump to deliver drug reliably. This failure was evident because these subjects did not exhibit reduced rates of body weight gain and the site of the minipumps appeared encapsulated.

Body Weight

Body weight data were analyzed by repeated-measures analyses of variance (ANOVAs) to ensure reliable drug delivery. All subjects that received nicotine exhibited a dose–response decrement in rates of body weight gain, with the 12

mg/kg/day nicotine groups exhibiting statistically significant decrements. These findings validated drug administration.

ASR and PPI

Each animal's responses were averaged within trial type. Trials during which no stimuli were presented were used to control for normal subject movements on the platform. Amplitudes to each trial type were derived by subtracting grams of platform displacement on the no-stimulus trials (i.e., the body weight of each subject) from grams of platform displacement in response to specific stimuli. The remainder from this calculation represented the amount of platform displacement related to the stimulus (e.g., 112 dB, 112 dB with prepulse, 122 dB, 122 dB with prepulse). Prepulse amounts were calculated by subtracting amplitude to each stimulus with a prepulse from amplitude to the same stimulus without prepulse. The remainder was analyzed as prepulse inhibition amount.

Amplitude and prepulse inhibition amount to each stimulus were analyzed separately at each time point (drug days 2, 6, and 12) with analyses of covariance (ANCOVAs) using baseline responses as covariates. The data analytic goal was to determine the existence of: sex differences within each strain; strain and sex differences in nicotine's effects; strain and sex differences in effects of stress; and strain and sex differences in interactions of nicotine with stress. Therefore, at each time point an overall ANCOVA was done on each dependent variable with all factors included (strain, sex, stress, and drug). The results from this overall analysis are not reported, but were used to guide analyses to answer specific hypotheses. For example, if no overall main effect for drug or strain $\times$ drug interaction was present, then further analyses for drug effects were not pursued. If a strain $\times$ drug interaction was present, then the two strains were analyzed separately for drug effects, and so on. Because of a priori hypotheses that strains and sexes would differ in responses to nicotine and to stress, same strain–same sex groups (collapsed across stress status) also were analyzed separately. To determine the effects of nicotine separate from stress in each same strain–same sex cell, drug effects were examined within the nonstress groups only. To determine the effects of stress separate from effects of nicotine, stress effects were tested in saline cells. Where necessary (e.g., for drug effects) Tukey's HSD post hoc tests were used to determine differences among groups. Further, in each same-strain, same-sex group, contrasts were done between responses of saline-no stress subjects and responses of 12 mg/kg/day-stress subjects to test the hypothesis that high doses of nicotine + stress would result in ASR and PPI responses similar to saline-no stress controls. All tests were two-tailed with alpha < 0.05. All reported results are significant at the $p < 0.05$ level.

Several strategies were employed to minimize the possibility of type I error. First, an initial MANOVA was performed that included startle amplitudes (112 and 122 dB) with and without prepulse from the drug administration phase to determine whether stimulus intensity and stimulus type (i.e., prepulse or no prepulse) significantly affected responses. This analysis indicated that responses were significantly different to each stimulus intensity and stimulus type. Therefore, stimuli were analyzed separately at each time point. Second, all possible main effects and interactions in each model were run, including three-way and four-way interactions, despite the fact that some effects and interactions were not of interest. This tactic ensured that variance partialled to main effects and two-way interactions of interest was not inflated by the presence of a higher order, but untested interactions. Third, despite the number of tests run, the conventional alpha level of 0.05 was judged acceptable for findings from this experiment that replicated other published work from our laboratory using identical methodologies (e.g., main effects for strain, main effects for drug in opposite directions for each strain, and so on). Given that replication of a previous result is a cumulative probability, the type I error level for these findings is the product of the alpha levels from each experiment - actually 0.0025, or lower.

RESULTS

Table 1 presents startle amplitudes to the 112 and 122 dB stimuli on days 2 and 12. Figure 1 a and b presents startle amplitudes on day 6 to the 112 and 122 dB stimuli, respectively. Table 2 presents PPI amounts to both stimuli on days 2 and 12. Figure 2 a and b presents PPI amounts on day 6 to the 112 and 122 dB stimuli, respectively. Because Sprague–Dawley rats startled significantly more and exhibited significantly greater PPI amounts than did Long–Evans rats to the 112 and 122 dB stimuli throughout the experiment, data for the two strains were analyzed separately.

Sex Differences

There were sex differences in startle amplitudes among Long–Evans rats, but not among Sprague–Dawley rats. Specifically, on day 2, Long–Evans males startled more to the 122 dB stimulus than did Long–Evans females, $F(1, 107) = 4.315$. Long–Evans females, however, startled more than did Long–Evans males to the 112dB stimulus on day 6, $F(1, 107) = 5.280$, and on day 12, $F(1, 107) = 3.988$.

With regard to PPI, males had greater PPI than females in both strains. Specifically, Long–Evans males exhibited greater PPI than did Long–Evans females to both stimuli on day 2 [112 dB: $F(1, 107) = 5.258$; 122 dB: $F(1, 107) = 12.716$] and to the 122 dB stimulus on day 6, $F(1, 107) = 6.790$. Among Sprague–Dawleys, males also had greater PPI amounts than females to both stimuli on day 2 [112 dB: $F(1, 104) = 5.387$; 122 dB: $F(1, 104) = 5.505$.

Drug Effects: Startle Amplitudes

Overall, nicotine administration increased amplitudes in Sprague–Dawley rats but decreased amplitudes in Long–Evans rats. Specifically, on days 2 and 6, nicotine administration increased Sprague–Dawley females' startle to both stimuli [day 2 112 dB: $F(2, 51) = 4.015$; 122 dB: $F(2, 51) = 7.585$; day 6 112 dB: $F(2, 51) = 5.198$; 122 dB: $F(2, 51) = 7.681$]. The nicotine-induced startle increase to 122 dB also was present when only nonstressed Sprague–Dawley females were considered [day 2: $F(2, 25) = 6.017$; day 6: $F(2, 25) = 4.142$]. In all cases, post hocs indicated that the 12 mg/kg/day group startled more than did the saline group.

By day 12, drug effects among Sprague–Dawley females had disappeared but significant effects among Sprague–Dawley males were present. Specifically, nicotine increased startle amplitudes of Sprague–Dawley males to both stimuli [112dB: $F(2, 52) = 4.357$; 122 dB: $F(2, 52) = 5.785$]. This nicotine-induced increase to both stimuli also was present when only nonstressed Sprague–Dawley males were considered [112 dB: $F(2, 26) = 6.281$; 122 dB: $F(2, 26) = 4.436$], with the 12 mg/kg/day nicotine group startling more than the saline group in all cases.

TABLE 1
STARTLE AMPLITUDE (g) (MEANS ± SEM)

		Day 2		Day 12	
112dB		Males	Females	Males	Females
Sprague–Dawley	Saline—no stress	68.69 ± 9.90	40.23 ± 6.04	31.36 ± 8.58	50.95 ± 8.13
	6 mg/kg/day—no stress	61.11 ± 15.62	67.43 ± 14.25	60.78 ± 12.33	73.93 ± 11.48
	12 mg/kg/day—no stress	65.76 ± 13.36	69.49 ± 14.21	92.00 ± 17.55	46.51 ± 5.96
	Saline—stress	86.13 ± 15.48	42.01 ± 5.49	52.90 ± 9.88	60.32 ± 20.14
	6 mg/kg/day—stress	77.90 ± 14.09	45.36 ± 8.99	69.70 ± 16.11	53.83 ± 12.24
	12 mg/kg/day—stress	58.71 ± 21.07	72.39 ± 9.98	65.91 ± 11.90	60.96 ± 14.07
Long–Evans	Saline—no stress	68.94 ± 12.76	54.26 ± 9.63	45.46 ± 10.79	45.89 ± 8.00
	6 mg/kg/day—no stress	37.85 ± 6.77	31.36 ± 3.78	49.00 ± 13.71	66.46 ± 10.96
	12 mg/kg/day—no stress	44.50 ± 9.83	41.81 ± 8.32	31.98 ± 7.78	62.06 ± 8.19
	Saline—stress	48.68 ± 7.23	54.84 ± 13.38	45.26 ± 7.23	53.06 ± 9.12
	6 mg/kg/day—stress	26.70 ± 3.82	29.01 ± 3.69	51.04 ± 13.98	62.64 ± 10.95
	12 mg/kg/day—stress	45.08 ± 22.70	31.08 ± 8.36	54.06 ± 13.26	49.18 ± 10.39
122dB					
Sprague–Dawley	Saline–no stress	180.11 ± 21.46	102.34 ± 14.85	133.28 ± 14.67	109.59 ± 14.90
	6 mg/kg/day—no stress	165.21 ± 25.19	131.31 ± 26.75	194.25 ± 31.24	126.29 ± 22.68
	12 mg/kg/day—no stress	177.86 ± 25.15	219.39 ± 26.84	230.83 ± 30.57	168.92 ± 26.26
	Saline—stress	210.99 ± 32.57	113.95 ± 9.71	144.09 ± 30.03	154.30 ± 33.10
	6 mg/kg/day—stress	237.73 ± 46.78	150.63 ± 37.63	224.56 ± 49.63	149.50 ± 34.17
	12 mg/kg/day—stress	200.46 ± 52.52	169.50 ± 17.38	261.49 ± 47.36	206.38 ± 43.65
Long–Evans	Saline–no stress	111.34 ± 9.52	90.08 ± 6.01	80.20 ± 8.36	93.71 ± 18.74
	6 mg/kg/day—no stress	100.23 ± 18.93	84.34 ± 12.41	122.58 ± 25.87	130.25 ± 20.15
	12 mg/kg/day—no stress	114.73 ± 21.25	111.99 ± 18.92	116.35 ± 19.05	126.60 ± 21.73
	Saline—stress	144.58 ± 16.88	78.20 ± 17.83	114.93 ± 15.44	101.34 ± 16.69
	6 mg/kg/day—stress	87.88 ± 12.74	67.81 ± 10.95	113.46 ± 25.06	105.46 ± 19.99
	12 mg/kg/day—stress	77.04 ± 17.30	69.18 ± 14.07	126.50 ± 23.09	96.55 ± 11.48

With regard to Long–Evans rats, on day 2 nicotine administration decreased amplitudes to the 112 dB stimulus of Long–Evans males, $F(2, 53) = 3.226$, and Long–Evans females, $F(2, 53) = 7.533$. The decrease to 112 dB also was significant when only nonstressed Long–Evans females were considered, $F(2, 26) = 8.102$. In all cases, the 6 mg/kg/day group startled less than did the saline group by post hoc. On day 6, nicotine decreased amplitudes of Long–Evans males to both stimuli [112 dB: $F(2, 53) = 4.012$; 122 dB: $F(2, 53) = 13.217$], with the 6 mg/kg/day group startling less than the saline group to 112 dB, and both 6 mg/kg/day and 12 mg/kg/day groups startling less than the saline group to 122 dB. When only nonstressed Long–Evans males were considered, this pattern remained, $F(2, 26) = 3.695$, with the 6 mg/kg/day nicotine group startling significantly less than the saline group.

Contrasts. Orthogonal contrasts are a standard statistical technique used to compare means of specific subgroups within a larger study to test a priori hypotheses (38). Contrasts were used to compare responses of stressed, high nicotine animals with responses of nonstressed saline controls. Among Long–Evans females, the responses of these two groups were indistinguishable throughout the course of the experiment. For Long–Evans males, the responses of these two groups also were similar, except on day 6 when the stressed 12 mg/kg/day nicotine group startled less to the 122 dB stimulus, $t(54) = 2.13$, than did the no stress-saline group. For Sprague–Dawley males, these two groups also responded similarly, except on day 12 when the stressed 12 mg/kg/day nicotine group startled more to the 122 dB stimulus, $t(53) = 2.51$, than did the no stress-saline group. For Sprague–Dawley females, however, the two groups generally responded differently. Specifically, the stressed 12 mg/kg/day nicotine group startled significantly more than the nonstressed saline group on day 2 to both stimuli [112 dB: $t(52) = 2.24$; 122 dB: $t(52) = 2.07$], on day 6 to both stimuli [112 dB: $t(52) = 2.54$; 122 dB: $t(52) = 3.32$], and on day 12 to 122 dB, $t(52) = 2.28$.

Drug Effects: PPI Amounts

Nicotine generally increased PPI of Sprague–Dawley rats and decreased PPI of Long–Evans rats. Specifically, nicotine increased PPI of Sprague–Dawley females on day 6 to both stimuli [112dB: $F(2, 51) = 3.817$; 122 dB: $F(2, 51) = 5.298$]. Nicotine also increased PPI of Sprague–Dawley males on day 12 to 122 dB, $F(2, 26) = 4.667$. In all cases, the 12 mg/kg/day nicotine group exhibited greater PPI than the saline group by post hoc.

For Long–Evans rats, on day 2 nicotine decreased PPI to both stimuli among Long–Evans females [112 dB: $F(2, 53) = 3.166$; 122 dB: $F(2, 53) = 4.198$], with both the 6 mg/kg/day and the 12 mg/kg/day groups exhibiting less PPI than the saline group in response to 112 dB, and the 12 mg/kg/day group exhibiting less PPI than the saline group in response to 122 dB. On days 2 and 6, nicotine administration decreased PPI to 122 dB in Long–Evans males [day 2: $F(2, 53) = 4.802$; day 6: $F(2, 53) = 4.088$], with the 12 mg/kg/day group exhibiting less PPI than the saline group on day 2, and the 6 mg/kg/day group exhibiting less PPI than the saline group on day 6.

Contrasts. Contrasts were performed as described above. For Long–Evans females and Sprague–Dawley males, PPI amounts for the saline nonstressed groups and the 12 mg/kg/day nicotine-stressed groups were indistinguishable through-

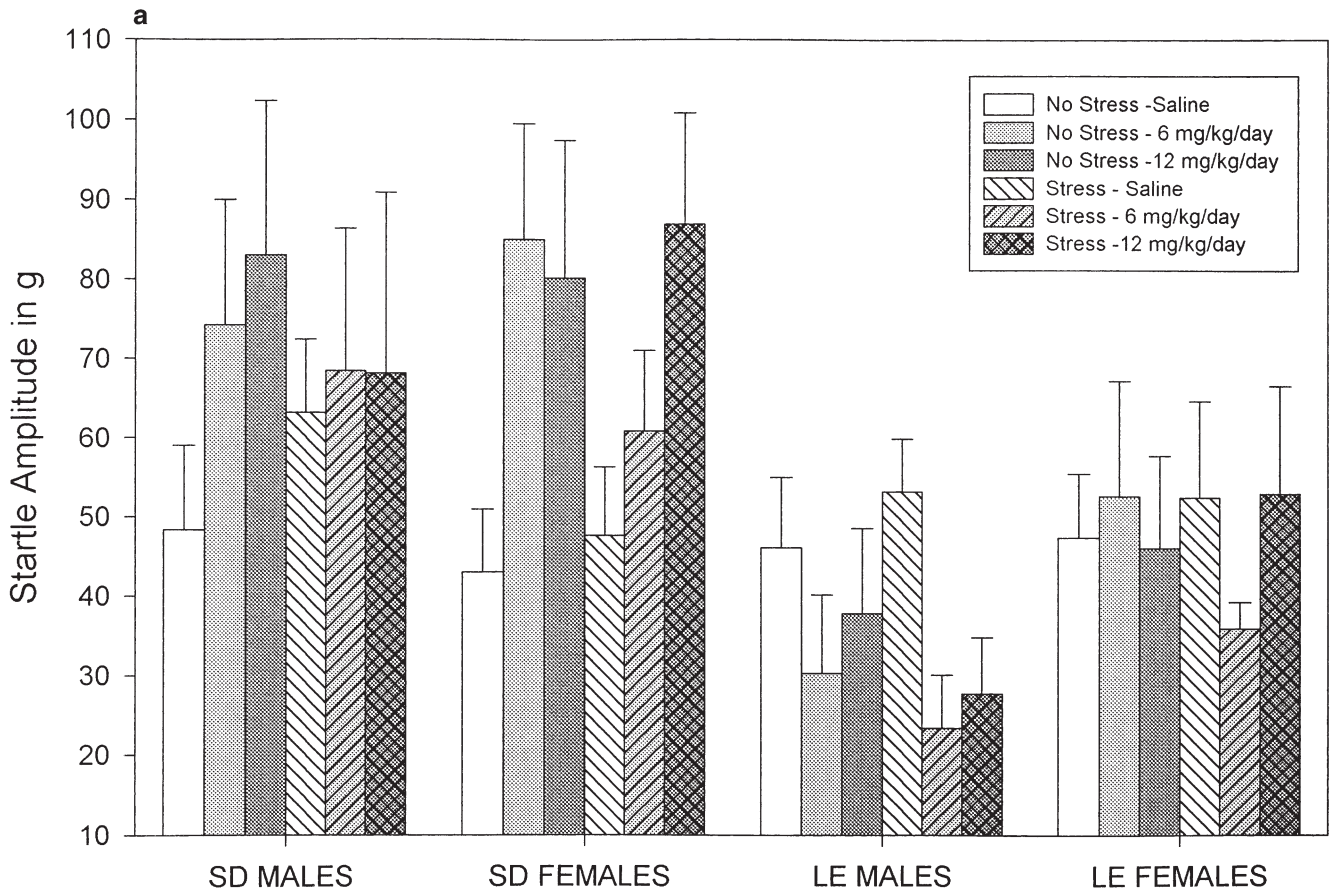

FIG. 1. (a) Day 6 startle amplitude to 112 dB stimulus.

out the experiment. For Long–Evans males, these two groups' responses also were similar, except on day 2 when the stressed 12 mg/kg/day nicotine group exhibited less PPI, $t(54) = 2.59$, than did the no stress-saline group to 122 dB. Among Sprague–Dawley females, the stressed 12 mg/kg/day nicotine group had greater PPI to the 122 dB stimulus than did the no stress-saline group on day 6, $t(52) = 2.36$, and on day 12, $t(52) = 2.83$.

Stress Effects

On day 2, stress decreased Long–Evans female startle to the 122 dB stimulus, $F(1, 53) = 4.938$. Stress increased startle of saline-treated Sprague–Dawley males on day 6 to 122 dB, $F(1, 17) = 6.270$, and on day 12 to 112 dB, $F(1, 17) = 6.392$.

With regard to PPI amounts, on day 2, stress and drug interacted for Long–Evans males in response to 122 dB such that stress increased PPI in the saline and 6 mg/kg/day groups above nonstressed subjects' responses but decreased PPI in the 12 mg/kg/day group below nonstressed subjects responses, $F(2, 106) = 3.624$. On day 6 stress increased PPI of Sprague–Dawley males to 122 dB, $F(1, 52) = 5.288$. This increase remained when only saline-treated Sprague–Dawley males were considered, $F(1, 17) = 5.642$. On day 12 in response to 112 dB stress increased PPI of Long–Evans females, $F(1, 53) = 4.443$, and of saline-treated Sprague–Dawley males, $F(1, 17) = 4.348$. In response to the 122 dB stimulus, stress increased PPI of Sprague–Dawley females, $F(1, 51) = 5.344$.

DISCUSSION

The present experiment extended previous work by examining effects of chronic nicotine with and without stress on reactivity and sensory gating in male and female Sprague–Dawley and Long–Evans rats as operationalized by the acoustic startle response (ASR) and prepulse inhibition (PPI) of the ASR. In previous studies with Sprague–Dawley males, nicotine enhanced ASR and PPI (2–5). This enhancement has been interpreted as analogous to the attentional enhancement demonstrated empirically in certain human subjects and reported by some human smokers when they smoke cigarettes [e.g., (25,81)]. In one study with Long–Evans rats, nicotine reduced startle amplitude and PPI (24). The present study replicated and extended these findings, indicating that robust and consistent strain differences in rats exist in ASR and PPI responses to nicotine. Therefore, these two rat strains may provide models of individual differences in smoking behavior, i.e., of smokers who smoke for nicotine's attentional effects and smokers who do not.

Because conditions that result in stress can alter effects of drugs and may interact with genotype and/or sex of subject,

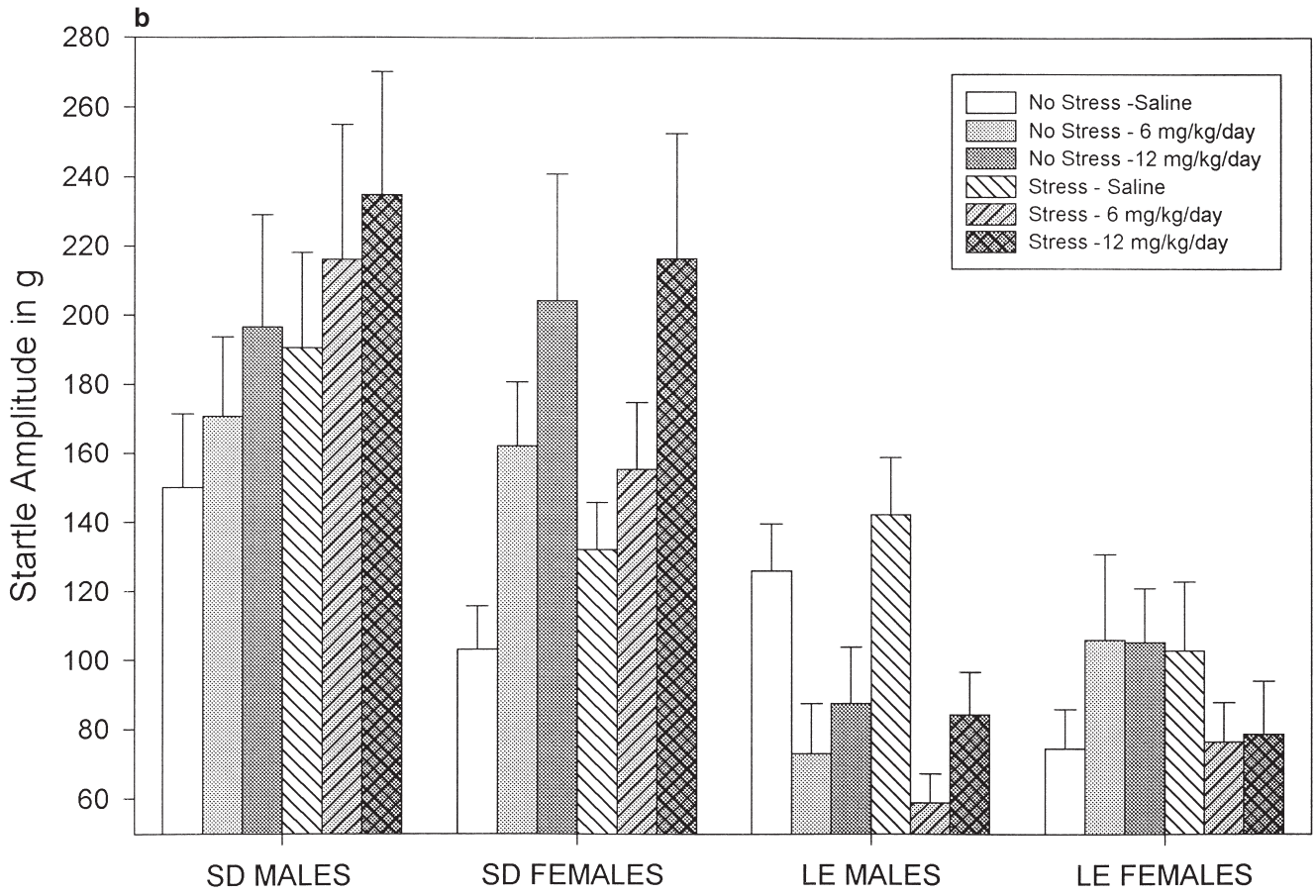

FIG. 1. (b) Day 6 startle amplitude to 122 dB stimulus.

and because smokers commonly report that stress increases smoking and smoking alleviates stress, this experiment also assessed the effects of immobilization stress and its interaction with nicotine administration on ASR and PPI responses. In addition, sex differences in responses separate from drug and stress effects also were investigated. Specific findings with regard to strain, sex, and stress are detailed below.

Strain Differences in Effects of Nicotine

Nicotine generally enhanced startle and prepulse inhibition amounts in Sprague–Dawley subjects but reduced startle and PPI amounts in Long–Evans subjects. For Sprague–Dawley subjects, nicotine's startle and PPI enhancing effects generally occurred in a dose–response pattern with the greatest effects evident at the 12 mg/kg/day nicotine dosage. For Long–Evans subjects, the 6 mg/kg/day and 12 mg/kg/day nicotine dosages generally decreased startle and PPI responses, with the most consistent significant differences occurring at the lower dosage.

One interpretation of these data is that the dose–response curve for Long–Evans subjects was shifted so far to the left of the Sprague–Dawley curve that the 6 mg/kg/day dose produced maximal behavioral suppression. The 12 mg/kg/day dose, then, also would result in behavioral suppression, but because the behavior is reduced to its lowest point by the 6 mg/kg/day dose—a floor effect—no additional suppression would be observed. If this interpretation is correct, then lower doses of nicotine in this paradigm, for example, 1 or 3 mg/kg/day, might produce enhancement of startle and PPI. Alternatively, nicotine's effects on ASR and PPI in Long–Evans subjects may follow a differently shaped dose–response curve than in Sprague–Dawley subjects. Future studies should examine both possibilities.

The two strains also exhibited different temporal responses to nicotine. Drug effects were present in both strains on days 2 and 6 of the drug administration period. By day 12, however, drug effects remained in Sprague–Dawley rats but were largely absent in Long–Evans rats. These data suggest that Long–Evans subjects developed tolerance to nicotine's ASR and PPI effects more quickly than did Sprague–Dawley subjects. Recent work in humans and animals suggests that vulnerability to nicotine dependence is related to high initial sensitivity to nicotine and rapid tolerance development (62). If so, then it is possible that use of these two strains to study nicotine's effects constitutes an animal model of more vulnerable (Long–Evans) and less vulnerable (Sprague–Dawley) smokers.

Sex Differences

Sex differences are relevant in two contexts: as inherent baseline differences in responses, and as differences revealed as a result of a particular manipulation. With regard to inher-

TABLE 2
PREPULSE AMOUNTS (g) (MEAN ± SEM)

		Day 2		Day 12	
112dB		Males	Females	Males	Females
Sprague–Dawley	Saline—no stress	43.26 ± 6.39	22.85 ± 4.61	22.44 ± 6.50	30.31 ± 7.17
	6 mg/kg/day—no stress	27.60 ± 11.19	29.61 ± 9.44	41.04 ± 9.85	45.03 ± 8.21
	12 mg/kg/day—no stress	40.79 ± 14.36	16.36 ± 9.66	59.20 ± 14.08	26.61 ± 6.38
	Saline—stress	49.94 ± 11.51	18.31 ± 4.21	36.95 ± 6.36	30.60 ± 10.29
	6 mg/kg/day—stress	39.59 ± 13.89	18.38 ± 8.39	49.98 ± 13.06	32.38 ± 8.96
	12 mg/kg/day—stress	20.97 ± 10.41	26.43 ± 10.75	19.76 ± 14.51	23.91 ± 11.85
Long–Evans	Saline—no stress	36.50 ± 10.06	21.75 ± 7.23	13.11 ± 8.60	14.10 ± 4.60
	6 mg/kg/day—no stress	14.40 ± 2.96	10.25 ± 3.57	17.64 ± 5.44	0.65 ± 4.48
	12 mg/kg/day—no stress	17.43 ± 6.30	9.75 ± 3.89	10.78 ± 6.31	5.21 ± 6.92
	Saline—stress	29.46 ± 6.74	22.31 ± 9.72	26.88 ± 9.13	25.70 ± 7.72
	6 mg/kg/day—stress	17.33 ± 2.13	6.59 ± 3.68	19.76 ± 3.92	27.60 ± 7.64
	12 mg/kg/day—stress	30.16 ± 16.06	9.20 ± 4.33	26.40 ± 7.82	13.30 ± 7.00
122dB					
Sprague–Dawley	Saline—no stress	86.45 ± 18.88	45.30 ± 9.14	60.63 ± 13.56	50.90 ± 7.05
	6 mg/kg/day—no stress	53.44 ± 20.43	54.44 ± 16.61	100.06 ± 22.32	49.21 ± 11.73
	12 mg/kg/day—no stress	83.08 ± 17.74	77.03 ± 13.49	102.19 ± 15.88	68.74 ± 15.03
	Saline—stress	98.10 ± 16.55	34.15 ± 8.05	70.19 ± 15.59	77.46 ± 20.60
	6 mg/kg/day—stress	126.31 ± 32.17	61.69 ± 28.19	106.60 ± 28.00	70.19 ± 17.88
	12 mg/kg/day—stress	64.81 ± 25.51	43.79 ± 14.65	93.06 ± 18.30	115.33 ± 21.33
Long–Evans	Saline—no stress	46.28 ± 9.64	13.54 ± 12.16	26.00 ± 5.09	14.93 ± 8.15
	6 mg/kg/day—no stress	24.45 ± 10.71	18.98 ± 4.89	48.91 ± 8.11	42.91 ± 9.24
	12 mg/kg/day—no stress	40.56 ± 11.00	35.03 ± 10.20	54.75 ± 11.50	39.56 ± 13.29
	Saline—stress	65.83 ± 13.59	−11.40 ± 11.03	39.64 ± 9.93	35.06 ± 8.48
	6 mg/kg/day—stress	37.61 ± 11.05	23.14 ± 7.80	26.86 ± 10.43	23.00 ± 8.62
	12 mg/kg/day—stress	6.08 ± 9.26	13.86 ± 7.04	54.36 ± 16.24	24.71 ± 8.79

ent differences in responses, the two strains diverged in the manifestation of sex differences. Among Long–Evans, there were sex differences in startle amplitudes (that depended on the stimulus loudness) as well as in PPI. Among Sprague–Dawleys, there were sex differences only in PPI. In both strains the PPI sex differences consisted of males having greater PPI than females.

Sex differences in nicotine's effects were most clearly revealed in the time course of Sprague–Dawley responses. Sprague–Dawley females' startle responses were increased by nicotine administration on days 2 and 6, and PPI amounts were increased by nicotine on day 6 - the first half of the experiment. In Sprague–Dawley males, however, nicotine effects consisted of startle and PPI increases to the 122 dB stimulus on day 12 - the second half of the experiment. The more rapid onset of drug effects in Sprague–Dawley females vs. males supports the hypothesis of greater female sensitivity to nicotine (11,30,32–34). In contrast, Long–Evans males and females both exhibited decreased startle and PPI as a result of nicotine administration on day 2, and the effects persisted for males on day 6. Therefore, sex differences in effects of nicotine on attention also appear to depend on strain.

Stress

It is striking that the nonpainful physical stressor of immobilization, reported to result in similar biochemical stress responses in males and females, resulted in different behavioral responses across sex and genotype. Responses to stress and to stress + nicotine also depended on day of experiment, stimulus type, and nicotine dosage. Long–Evans females and Sprague–Dawley males were most consistently affected by stress. Stress decreased Long–Evans female startle on day 2 (122 dB) and increased PPI on day 12 (112 dB). In contrast, stress increased startle for Sprague–Dawley males on days 6 (122 dB) and 12 (112 dB), and increased PPI on days 6 (122 dB) and 12 (112 dB). Findings for Sprague–Dawley males are consistent with previous reports (2), as are the relative lack of stress effects in Sprague–Dawley females (63). Findings for Long–Evans females and the lack of stress effects for Long–Evans males, to our knowledge, are new reports.

With regard to nicotine and stress interactions, contrasts between saline-no stress groups and 12 mg/kg/day nicotine-stress groups within strain and sex indicated that ASR and PPI responses of Sprague–Dawley males, Long–Evans males, and Long–Evans females in these groups were for the most part statistically indistinguishable. That is, high dosages of nicotine when combined with stress produced ASR and PPI responses similar to saline, nonstress controls. These results are consistent with past reports in Sprague–Dawley males (4). These results also are consistent with smokers' reports that smoking alleviates stress, despite the fact that nicotine administration elevates physiological and biochemical stress indices. For Sprague–Dawley females, however, nicotine administration and stress together resulted in startle and PPI responses greater than responses of saline-treated, nonstress subjects at every time point. The behavioral responses of Sprague–Dawley females, therefore, indicate that stress and nicotine ex-

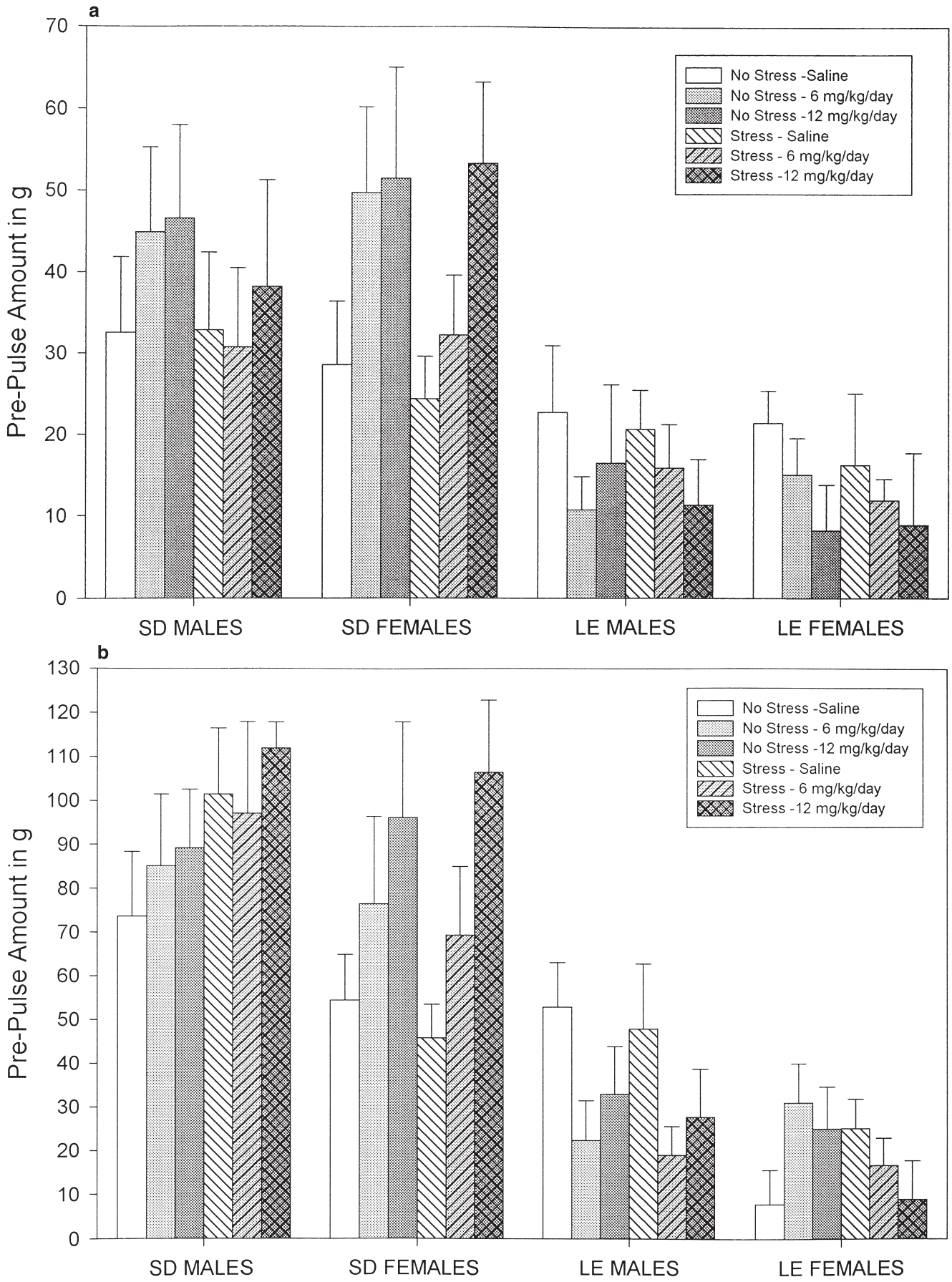

FIG. 2. (a) Day 6 prepulse inhibition amount to 112 dB. (b) Day 6 prepulse inhibition amount to 122 dB.

erted additive effects. This finding is especially striking, given that stress alone did not consistently alter responses of Sprague–Dawley females.

Different behavioral responses by rats of different genotypes, of each sex, and exposed to different environmental conditions may mirror human individual differences in reported effects of smoking and of stress. To the extent that this is so, the conclusion that genotype, broadly construed to include subjects' sex, can alter responses to nicotine and to stress is supported. This conclusion suggests several lines of future work with regard to possible mechanisms for these differences. Because studies examining peripheral metabolism in rodents as mechanisms for behavioral differences in response to chronic nicotine administration have not found metabolic differences in rats (79) or in mice (37,50), different behaviors in response to nicotine administration and different time courses of tolerance development are likely to be the result of changes in central tissue sensitivity rather than changes in peripheral nicotine metabolism.

Strain differences in responses, then, may occur as a result of differences in number, affinity, or distribution of central nicotinic cholinergic receptors, differences in up- or downregulation processes, or as a result of some combination of these factors. In the rodent brain at least two classes of nicotinic cholinergic receptors (nAChRs) exist. The ^{3}H-nicotine probe labels high-affinity, $\alpha4\beta2$-type neuronal receptors (7,10,49, 61,67). A second group of nAChRs, the $\alpha7$-type receptor, is labeled by the snake toxin α-^{125}I-bungarotoxin (14,49,54), and has recently been isolated from the human cortex (59). In mice, binding affinity did not differ among strains that exhibited behavioral differences in response to nicotine for either receptor type, but significant differences across strain were found in receptor numbers, especially in midbrain, hindbrain, hippocampus, hypothalamus, and colliculus (15,51–53). Mouse strains with the greatest behavioral sensitivity to nicotine also had the greatest numbers of nicotinic cholinergic receptors. To our knowledge, comparable studies have not been done across outbred rat strains, but it is possible that similar processes underlie Sprague–Dawley vs. Long–Evans behavioral differences in response to nicotine administration.

Importantly, both receptor subtypes have been implicated in nicotine's effects on cognitive processes. Specifically, in mice the $\alpha4\beta2$ nAChR has been demonstrated to mediate nicotine's effects on startle behavior (28,51). In addition, development of a knockout mouse strain in which the $\alpha4\beta2$ nAChR is not expressed has indicated that nicotine administration in these animals fails to improve passive avoidance performance, a memory task (61). Although the complete molecular mechanism for nicotine's effects on sensory gating (i.e., PPI) is not known, recent work indicates that the $\alpha7$ nAChR presynaptically modulates release of glutamate and GABA, two neurotransmitters implicated in PPI regulation (6). It is possible, therefore, that changes in the $\alpha7$ nAChR system are the mechanism for nicotine's effects on PPI. In addition, the $\alpha7$ nAChR is sensitive to the stress hormone corticosterone, whereas the $\alpha4\beta2$ nAChR is not (28,29,58). This subsystem, then, also may mediate the effects of stress and the effects of nicotine and stress together on PPI.

Future studies, therefore, should examine distribution, affinity, number, and functionality of the $\alpha4\beta2$ nAChR and $\alpha7$ nAChR receptor systems in male and female Sprague–Dawley and Long–Evans subjects in response to nicotine administration, stress, and nicotine + stress manipulations. Delineation of possible receptor level differences may reveal the mechanisms of the observed strain and sex differences in ASR and PPI responses, and also may be relevant to human issues at several levels. First, it is possible that determination of the neurobiological underpinnings of ASR and PPI behavioral differences may illuminate individual differences in susceptibility to nicotine self-administration, addiction, and abuse. It is noteworthy that both of the rat strains used in this experiment will self-administer nicotine (16,17,26,70,73), indicating that rewarding effects of nicotine obtain across strains. The fact that nicotine alters ASR and PPI responses differentially in each strain, however, may provide a useful animal model of smokers who smoke for nicotine's attentional effects in addition to its rewarding effects, and smokers who smoke for other reasons. This information also may enhance the tailoring of smoking cessation therapies to individual needs for maximal success. In addition, individual differences in effects of nicotine that depend on sex and genotype may be important in the use of nicotine and the development of nicotine analogs for therapeutic use, i.e., these two rat strains might be used to provide phase I drug evaluation of potential cognition-enhancing agents related to nicotine.

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