Pharmacology Biochemistry and Behavior, Vol. 61, No. 3, pp. 281–289, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(98)00094-X

Nicotine Administration Impairs Sensory Gating in Long–Evans Rats

MARTHA M. FARADAY, MATTHEW A. RAHMAN, PETER M. SCHEUFELE AND NEIL E. GRUNBERG

Uniformed Services University of the Health Sciences, Bethesda, MD

Received 9 January 1998; Revised 18 March 1998; Accepted 25 March 1998

FARADAY, M. M., M. A. RAHMAN, P. M. SCHEUFELE AND N. E. GRUNBERG. *Nicotine administration impairs sensory gating in Long–Evans rats.* PHARMACOL BIOCHEM BEHAV **61**(3) 281–289, 1998.—In rats, effects of nicotine administration on sensory gating as indexed by prepulse inhibition (PPI) of the acoustic startle reflex (ASR) are unclear. We have found that nicotine administration enhances ASR and PPI in Sprague–Dawley rats, but other investigators, using Long–Evans rats, have reported no effects or enhancement of PPI only. Numerous methodological differences exist among studies in addition to subject strain, however, making it unclear whether inconsistent behavioral responses are the result of different experimental procedures or indicate a true strain difference. To investigate the role of strain in nicotine's effects on ASR and PPI, 192 male and female Long–Evans rats were administered 12 mg/kg/day nicotine via osmotic minipump for 14 days using identical methodologies employed in studies with Sprague–Dawley subjects. Effects of grouped vs. individual housing on these responses also were examined. Nicotine administration impaired ASR and PPI in Long–Evans subjects. These effects occurred in female rats regardless of housing condition, and interacted with housing in male rats. Results indicate that sex and housing are important variables in nicotine's effects. Results suggest that subject strain may be an important variable in nicotine's effects on sensory gating, and that responses of Sprague–Dawley vs. Long–Evans rats may represent a true strain difference. © 1998 Elsevier Science Inc.

Nicotine Acoustic startle reflex Prepulse inhibition—Long–Evans rats Strain differences Housing conditions

ABOUT one-quarter of the U.S. population smokes cigarettes despite the well-established deleterious health effects. Several studies have suggested that biological factors contribute significantly to smoking behavior. For example, twin studies indicate a mean heritability estimate of 53% for tobacco use (34). In addition, genetic factors contribute to smoking initiation, age of onset, and number of cigarettes smoked per day (20,29,31). Genotype may contribute to individual variability in smoking behavior via differential effects of nicotine, the primary active and addictive pharmacologic agent in tobacco. The specific effects the individual experiences may influence the likelihood of initiation, maintenance, and cessation of tobacco use. For example, some smokers report that smoking cigarettes enhances attentional processes (32,45,54, 55). Laboratory studies also suggest that nicotine may enhance the attentional processes of some individuals on simple tasks (32,49,55). Exploration of genotypic differences in response to nicotine in an animal model may illuminate individual differences in human smoking behavior.

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 (16) that has been reported to be sensitive to changes in attentional processes in humans (6,7). When the startling sound is preceded by a nonstartling stimulus (a prepulse), the amplitude of the startle response is reduced (9,21). 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 (51), and possibly attention (1–5,25,42).

The ASR-PPI paradigm has been widely used to index the effects of dopaminergic agonists such as apomorphine (17,53), *d*-amphetamine (19,35,52), and cocaine (30,51). In addition, the effects of nicotine administration and cessation have been studied using this procedure (1–5,15,33,42,43). The ASR-PPI and nicotine literature, however, is contradictory. Nicotine

Requests for reprints should be addressed to Martha M. Faraday, Department of Medical and Clinical Psychology, Uniformed Services University of the Health Sciences (USUHS), 4301 Jones Bridge Road, Bethesda, MD 20814.

administration has been reported to enhance startle amplitude and enhance prepulse inhibition (1–5,42) and also to have no effect on startle but enhance prepulse inhibition (15). Nicotine cessation has been reported to have no effect on startle and reduce prepulse inhibition (1,4), and also to enhance startle (33,43).

These inconsistent results might be explained by the strain of subjects used or methodological differences among studies. Experiments reporting enhancement of ASR and PPI during nicotine administration used albino rats of the Sprague–Dawley strain (1–5,42). Studies reporting no effect on startle and reduced PPI during nicotine cessation (1,4) also used Sprague–Dawley subjects. In investigations reporting no effects of nicotine on startle and enhancement of PPI only during nicotine administration (15), a nonalbino strain—Long–Evans hooded rats—was used. Long–Evans subjects also were used in studies reporting enhancement of startle in nicotine cessation only (33,43).

Important differences among studies in addition to subject genotype also exist that prevent the attribution of differing results to strain. Two of the experiments reviewed above used female Sprague–Dawley rats as subjects (5,42), whereas the remaining studies used male subjects. Some investigators used a chronic nicotine administration paradigm via an implanted minipump (1–4,33,43). Others used acute nicotine injections (5,15,42). The only studies that examined female rats also used acute nicotine injections (5,42).

Further, the time of ASR and PPI testing during the circadian cycle varied across studies. Some investigators tested responses during the active portion of the cycle (dark portion) (1,2,4,5), whereas others tested responses during the resting portion of the cycle (light portion) (3,15). Some studies do not report time of testing (33,42,43). Time of testing is relevant because startle amplitudes are more stable (18) and are greater during the dark cycle (13). Specifically, startle amplitudes increase by up to 100% during the dark portion of the daily cycle over mean startle values measured during the light portion (13). Because PPI is calculated, analyzed, and reported as a portion or percentage of startle amplitude, time of testing may affect findings with regard to this measure because it affects the responses from which PPI is derived.

Additional methodological differences among studies complicate interpretation. For example, the form of nicotine used varied across studies. Some experiments used nicotine dihydrochloride (1–5,42), some used nicotine tartrate (33), and some experiments employed nicotine ditartrate (15,42). The differences in solubility among nicotine forms also require minipumps of different sizes for delivery of reported dosages in studies using a chronic administration paradigm. Specifically, the 200-μl capacity Alzet Model 2002 was used in studies employing nicotine dihydrochloride, which also were studies using Sprague–Dawley subjects, but the larger, 2-ml Model 2ML2 minipump was used in studies employing nicotine tartrate and nicotine ditartrate—the studies using Long–Evans rats as subjects.

It is not clear, then, to what extent the ASR and PPI differences reported constitute a strain difference and to what extent they are accounted for by methodological dissimilarities. Work on other drugs indicates that strain of subject can affect ASR and PPI responses to drugs. For example, apomorphine has been found to disrupt PPI in rats of the Wistar strain but not in Sprague–Dawley subjects (51), and the same drug had no effect on Wistar subjects' startle but increased Sprague–Dawley subjects' startle (44). With regard to nicotine, in mice, depending on the strain of the subjects, nicotine enhanced, decreased, or had no effect on startle responses (14,23,36,37).

The present experiment was designed to investigate whether reported differences in nicotine's effects on ASR and PPI responses in rats were the result of genotype or of differing experimental procedures. This experiment, therefore, used methods identical to those we have employed in studies using Sprague–Dawley subjects [e.g., (1–4)], but used Long–Evans rats as subjects. Additional variables of sex and differential housing were included to ensure that the study would yield information even if results indicated that differences between Long–Evans and Sprague–Dawley responses to nicotine were due solely to methodological variations.

These particular variables were chosen because there are human sex differences in effects of nicotine (22,28,40) and the responses of female rats to chronic nicotine administration in the ASR-PPI paradigm have not been examined. Therefore, female as well as male Long–Evans rats were used. Second, the effects of differential housing (grouped vs. individual housing) also were investigated. This environmental manipulation was selected because it affects appetitive behaviors (e.g., feeding) relevant to nicotine's effects and other behaviors (e.g., drug self-administration) indicative of altered drug effects (10–12). In addition, many smokers report smoking as well as increased likelihood of relapse from smoking cessation only in social groups or only when bored and alone (47,48). Therefore, the extent to which nicotine's behavioral effects are altered by housing also may be relevant to individual differences in smoking behavior. Therefore, the effects of this environmental manipulation with and without nicotine administration and cessation also were examined by having half of the subjects in each cell live in grouped conditions during the drug administration and cessation phases of the experiment.

METHOD

Subjects

Subjects were 96 male and 96 female Long–Evans rats (Charles River Laboratories, Wilmington, MA). During the baseline phase (predrug and prehousing manipulation) all animals were individually housed 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. The housing room was maintained at 23°C at 50% relative humidity on a 12-h reverse light/dark cycle (lights on at 1900 h). 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., (4,39,50,53)]. At the beginning of the experiment subjects were 51–55 days old. Average male weight was 234 g, and average female weight was 194 g. The experiment was conducted as a 2 (male or female) × 2 (0 or 12 mg/kg/day nicotine) × 2 (individual or grouped housing) × 2 (during nicotine administration or nicotine cessation) 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, Utah). 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 15 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 (38), 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.

Drug Administration and Surgical Procedure

Nicotine (12 mg/kg/day; expressed as nicotine base) or physiologic saline was administered via Alzet osmotic minipumps (Model 2002, Alza 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. This dosage has resulted in significant changes in ASR and PPI responses in other experiments (2–4).

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. Cessation phase subjects also underwent explant of minipumps to insure drug cessation following the same anesthetic and surgical procedures.

Environmental Manipulation

During the baseline phase all subjects were individually housed in standard shoebox cages. Individual housing was maintained during the baseline period to ensure comparability with other studies of nicotine's effects (2–4,15,33,43). At the beginning of the drug administration phase, subjects were assigned to an individual housing or grouped housing condition in a manner that insured comparable body weights between conditions. Grouped housing was established based on empirically determined procedures (10,11) to produce environmental conditions that have been reported to alter behavioral responses of rats. Specifically, 1 day after surgery animals in the individually housed condition were transferred to clean standard shoebox cages. Grouped subjects were placed in same-sex groups of six. For grouped subjects floor space per animal was adjusted based on mean body weights to provide approximately 55% of U.S. Department of Health and Human Services (USDHHS) recommended floor space per animal. Grouped males (mean = 292.8 g) were placed in standard shoebox cages (six subjects per cage). This cage size provided approximately 143.5 cm^2 of floor space per male subject (55% of USDHHS recommended floor space for weight range 300–400 g). Grouped females (mean = 210.4 g) also were placed in standard shoebox cages (six subjects per cage), and the amount of floor space was adjusted using a polypropylene divider bolted to the cage top. The divider was placed so that each female subject had approximately 102.9 cm^2 of floor space (55% of USDHHS recommended floor space for weight range 200–300 g). Individually housed animals had cages changed twice a week. Grouped subjects' cages were changed every other day and were checked twice daily to ensure that subjects had adequate food and water.

Procedure

The procedure included three phases: a predrug, prehousing manipulation phase (baseline phase), a during drug administration and housing manipulation phase (during drug phase), and a drug cessation phase in which drug administration ceased but the housing manipulation continued (cessation phase). Decreased rates of body weight gain are well-established effects of nicotine administration at this dosage in rats in the dynamic growth phase [e.g., (24,54,56)]. Therefore, subjects' body weights were measured every third day throughout the three phases as validation of drug administration.

Baseline phase. Subjects were gentled once each day for 3 days. All subjects (n = 192) then underwent an acclimation exposure to the startle procedure in which they 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 to drug (0 or 12 mg/kg/day nicotine), housing (individual or grouped), and phase (during nicotine administration or nicotine cessation) groups in a manner that assured comparable, initial body weights. This assignment resulted in 16 balanced groups of 12 subjects each (eight groups of males; eight groups of females). Minipumps containing the appropriate solutions were implanted on during drug phase day 1. Twenty-four hours after surgery, subjects were placed in their assigned housing condition (individual or grouped).

ASR and PPI were measured on during drug phase day 6 (after 5 days of saline or nicotine administration and 4 days of individual or grouped housing) for all subjects (n = 192). ASR and PPI were measured again on during drug phase day 12 (after 11 days of saline or nicotine administration and 10 days of individual or grouped housing) for during phase subjects (n = 96). During phase subjects were sacrificed on during drug phase day 13 and blood and brains were collected and stored for other experiments.

Cessation phase. Cessation phase subjects ($n = 96$) had minipumps explanted on during drug phase day 15. ASR and PPI were measured for these subjects on the third day of nicotine or saline cessation (after 16 days of individual or grouped housing). The different ASR and PPI measurement timing for the during vs. cessation groups was necessary so that groups had the same total number of testing exposures. Cessation phase subjects were sacrificed on drug cessation phase day 5. Blood and brains were collected and stored for other experiments.

Data Analytic Strategy

Body weight. Body weight data were analyzed by repeated-measures analysis of covariance (ANCOVA) with average baseline body weights as covariates. Separate analyses were conducted for during phase males and females and cessation phase males and females, with time as the within-subject factor, and drug and housing condition as between-subjects factors for all analyses. All tests were two-tailed with alpha ≤0.05.

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 (g) of platform displacement on the no-stimulus trials (i.e., the body weight of each subject) from g 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 alone). 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 a prepulse inhibition amount. Percent prepulse (%PPI) was calculated as [(amplitude of trial without prepulse) − (amplitude of trial with prepulse)/amplitude of trial without prepulse] × 100. The product was analyzed as %PPI. These calculations were based on established procedures of several investigators (2,3, 5,51–53).

Day 6 and day 12 amplitude to each stimulus, prepulse inhibition amount, and prepulse inhibition percent were analyzed with analyses of covariance (ANCOVAs) using baseline responses as covariates. The first ANCOVA was done as an overall model with all factors included (sex, drug, housing, and phase). Subsequent separate ANCOVAs were done on males and females, on individually housed and grouped animals, and on during and cessation phase subjects. Additional ANCOVAs were done within sex, housing condition, and phase. All tests were two tailed, with alpha ≤0.05.

RESULTS

Body Weight

Figure 1 presents the body weight data. Nicotine-treated subjects' body weights increased less over time than did saline-treated subjects' body weights regardless of sex or phase [during males, $F(4, 172) = 37.274$, $p < 0.05$, and females, $F(4, 172) = 9.912$, $p < 0.05$; cessation males, $F(5, 210) = 17.488$, $p < 0.05$, and females, $F(5, 210) = 24.782$, $p < 0.05$]. Grouping also decreased body weight gains over time for all subjects [during males, $F(4, 172) = 3.287$, $p < 0.05$, and females, $F(4, 172) = 2.989$, $p < 0.05$; cessation males, $F(5, 210) = 5.499$, $p < 0.05$, and females, $F(5, 215) = 2.220$, $p < 0.05$]. In addition, nicotine reduced body weight gains for during phase females more over time in the grouped condition than in the individually housed condition, $F(4, 172) = 3.555$, $p < 0.05$.

ASR and PPI in During Drug Phase

Startle Amplitude to 112 dB. Table 1 presents these data. Nicotine-treated animals startled less than did saline-treated animals, $F(1, 176) = 4.262$, $p < 0.05$, on day 6. This pattern was evident for females, $F(1, 87) = 7.081$, $p < 0.05$, for individually housed subjects regardless of sex, $F(1, 89) = 5.118$, $p < 0.05$, and for female individually housed subjects, $F(1, 43) = 9.401$, $p < 0.05$. On day 12 these effects were still evident in females but were reversed in males as indicated by a Sex × Drug interaction, $F(1, 85) = 3.688$, $p = 0.058$, with nicotine tending to decrease startle in females, but to increase startle amplitude in males. This Drug × Sex interaction also occurred in grouped subjects' responses, $F(1, 42) = 4.165$, $p < 0.05$. In addition, on day 12 grouped housing increased startle amplitude, $F(1, 85) = 6.599$, $p < 0.05$, especially in female subjects, $F(1, 42) = 4.552$, $p < 0.05$.

Startle amplitude to 122 dB. Table 1 presents these data. Males startled more than did females on day 6, $F(1, 176) = 7.901$, $p < 0.05$. On day 12 nicotine interacted with housing condition such that nicotine tended to decrease startle in individually housed subjects but increase startle in grouped subjects, $F(1, 85) = 3.664$, $p = 0.059$. This interaction also was clear in male responses, $F(1, 42) = 5.173$, $p < 0.05$, and was revealed as a trend for a main effect for drug in individually housed males with nicotine tending to decrease startle, $F(1, 21) = 3.956$, $p = 0.060$.

Amount of PPI to 112 dB with prepulse. Figures 2 and 3 present these data. On day 6 a Drug × Housing interaction revealed that nicotine decreased amount of inhibition in individually housed subjects and slightly increased inhibition in grouped subjects, $F(1, 176) = 4.355$, $p < 0.05$. Among females, nicotine decreased PPI, regardless of housing condition, $F(1, 87) = 9.618$, $p < 0.05$. Nicotine-induced PPI reductions also were evident in individually housed subjects' responses, $F(1, 89) = 8.958$, $p < 0.05$, especially in responses of individually housed females, $F(1, 43) = 8.321$, $p < 0.05$. PPI reductions as a result of nicotine administration also were apparent on day 12 for all subjects, $F(1, 85) = 3.950$, $p < 0.05$,

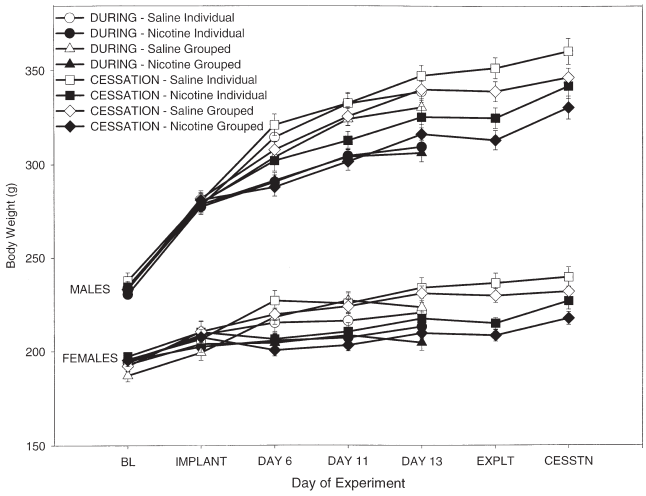

FIG. 1. Body weights of males and females.

TABLE 1
ASR AMPLITUDES, AMOUNT PPI, AND PERCENT PPI (MEANS ± SEM)

		Day 6		Day 12	
		Males	Females	Males	Females
112db					
Startle amplitude (g)	Saline indiv.	60.89 ± 7.39	80.60 ± 8.38	66.98 ± 13.90	67.40 ± 9.77
	Nicotine indiv.	60.10 ± 9.39	57.62 ± 6.04	57.69 ± 10.43	63.55 ± 13.97
	Saline grouped	76.26 ± 14.74	62.83 ± 5.38	68.37 ± 8.60	97.50 ± 11.08
	Nicotine grouped	79.38 ± 11.67	60.40 ± 9.23	86.72 ± 13.89	82.65 ± 15.21
122db					
Startle amplitude (g)	Saline indiv.	125.20 ± 11.28	107.81 ± 14.98	137.41 ± 22.55	100.05 ± 21.94
	Nicotine indiv.	121.20 ± 11.99	92.38 ± 8.41	93.23 ± 14.11	88.78 ± 17.38
	Saline grouped	138.23 ± 20.79	90.95 ± 8.25	124.86 ± 19.11	124.93 ± 26.82
	Nicotine grouped	130.64 ± 19.87	88.84 ± 11.07	152.27 ± 21.05	144.58 ± 22.84
Amount PPI	Saline indiv.	60.69 ± 8.38	39.27 ± 9.32	63.51 ± 14.17	53.85 ± 19.27
	Nicotine indiv.	49.95 ± 8.83	27.67 ± 5.53	34.38 ± 8.05	18.08 ± 6.08
	Saline grouped	45.98 ± 9.56	26.70 ± 6.25	53.54 ± 12.62	51.80 ± 24.89
	Nicotine grouped	47.06 ± 13.99	17.29 ± 5.67	64.73 ± 17.21	61.15 ± 19.40
Percent PPI	Saline indiv.	46.93 ± 5.19	33.39 ± 4.12	43.51 ± 7.28	44.53 ± 6.86
	Nicotine indiv.	36.97 ± 5.24	29.22 ± 4.65	34.43 ± 7.81	26.30 ± 8.53
	Saline grouped	35.99 ± 6.56	25.98 ± 6.29	41.54 ± 7.57	29.24 ± 7.19
	Nicotine grouped	28.05 ± 8.18	18.80 ± 7.90	37.62 ± 9.80	40.08 ± 7.99

for females alone, $F(1, 42) = 4.596$, $p < 0.05$, and for individually housed females, $F(1, 20) = 4.372$, $p < 0.05$.

Amount PPI to 122 dB with prepulse. Table 1 presents these data. On day 6 males exhibited greater PPI amounts than did females, $F(1, 176) = 12.201$, $p < 0.05$, in both individual, $F(1, 89) = 6.603$, $p < 0.05$, and grouped housing, $F(1, 86) = 5.777$, $p < 0.05$. On day 12 a Drug × Housing interaction revealed that nicotine administration decreased individually housed subjects' PPI amounts but increased grouped subjects' PPI, $F(1, 85) = 3.898$, $p = 0.05$. PPI reductions as a result of nicotine administration also were evident for individually housed subjects, $F(1, 42) = 5.421$, $p < 0.05$.

Percent PPI to 112 dB with prepulse. Figures 2 and 3 present these data. Nicotine administration reduced percent prepulse inhibition on day 6, $F(1, 176) = 6.044$, $p < 0.05$, and also interacted with housing condition such that nicotine decreased %PPI in individually housed subjects but not in grouped subjects, $F(1, 176) = 5.437$, $p < 0.05$. Nicotine-induced %PPI reduction was evident in female responses regardless of housing condition, $F(1, 87) = 7.750$, $p < 0.05$, in individually housed subjects regardless of sex, $F(1, 89) = 8.904$, $p < 0.05$, and in individually housed males, $F(1, 45) = 4.550$, $p < 0.05$, and females, $F(1, 43) = 4.175$, $p < 0.05$, with a trend for the same effect in grouped females, $F(1, 43) = 3.745$, $p = 0.065$. In contrast, the interaction was evident in male responses, $F(1, 88) = 5.384$, $p < 0.05$, with nicotine increasing %PPI in grouped males but decreasing %PPI in individually housed males. For grouped subjects nicotine administration increased %PPI in males but decreased %PPI in females, $F(1, 86) = 4.081$, $p < 0.05$. On day 12 nicotine-treated subjects exhibited less %PPI than did saline-treated subjects, $F(1, 85) = 3.845$, $p = 0.05$.

Percent PPI to 122 dB with prepulse. Table 1 presents these data. On day 6 males exhibited greater %PPI than females, $F(1, 176) = 5.380$, $p < 0.05$, especially in the individual housing condition, $F(1, 89) = 4.674$, $p < 0.05$, and individually housed subjects exhibited greater %PPI than grouped subjects, $F(1, 176) = 4.515$, $p < 0.05$.

ASR and PPI in Cessation Phase

Table 2 presents these data. There were no differences between groups in startle amplitude or prepulse amounts to the 112 dB stimulus.

Percent PPI to 112 dB w/prepulse. A Sex × Housing interaction revealed that individually housed males exhibited more

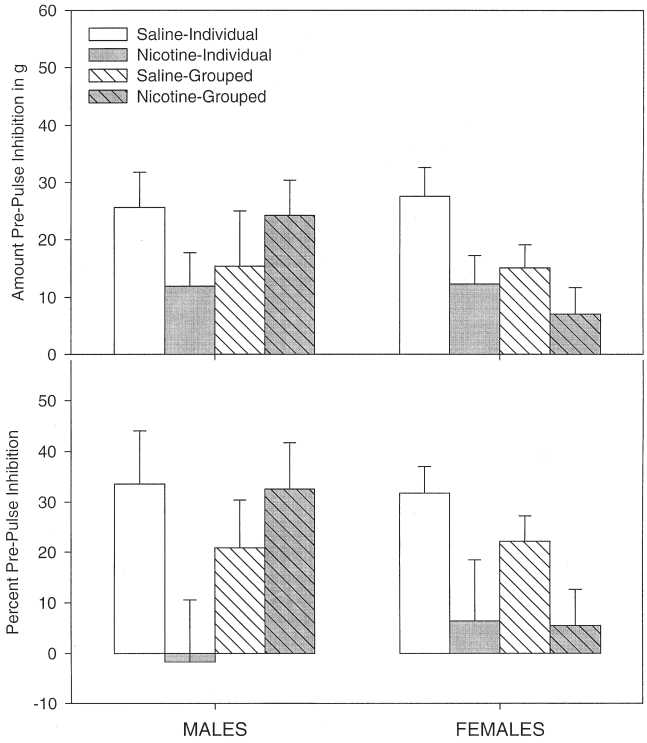

FIG. 2. Day 6 prepulse inhibition to 112 dB.

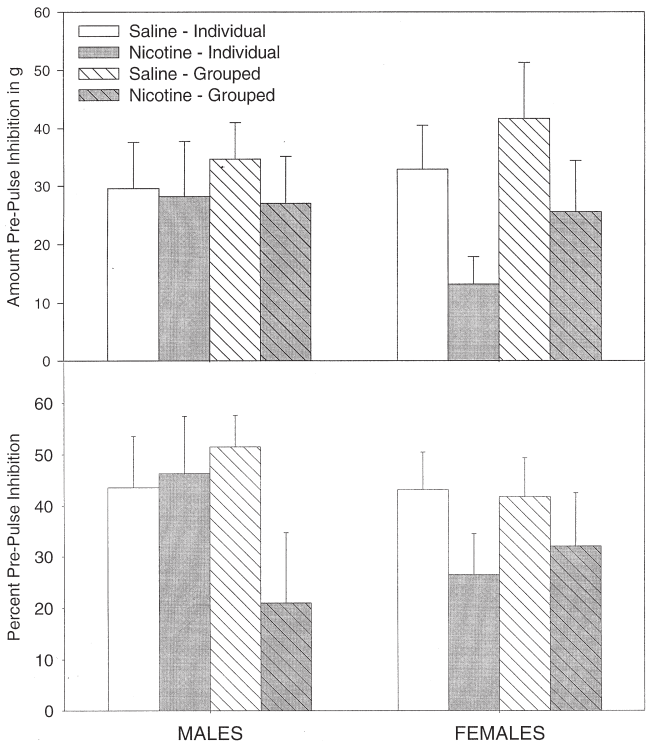

FIG. 3. Day 12 prepulse inhibition to 112 dB.

%PPI than did grouped males, but grouped females exhibited greater %PPI than did individually housed females, $F(1, 87) = 6.101$, $p < 0.05$. The effects of grouping to decrease %PPI in males, $F(1, 21) = 4.169$, $p = 0.054$, but increase %PPI in females, $F(1, 43) = 4.224$, $p < 0.05$, also were clear as main effects when the sexes were analyzed separately.

Startle amplitude to 122 dB. Males startled more than did females, $F(1, 87) = 5.264$, $p < 0.05$.

Amount PPI to 122 dB w/prepulse. Males exhibited greater PPI than did females, $F(1, 87) = 7.642$, $p < 0.05$, especially among individually housed subjects, $F(1, 43) = 5.117$, $p < 0.05$.

DISCUSSION

The purpose of the present experiment was to examine nicotine's effects on attention in Long–Evans rats as operationalized in the acoustic startle response (ASR) and prepulse inhibition (PPI) of the ASR. In previous studies with Sprague–Dawley subjects, 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. Results from studies using Long–Evans male rats as subjects vary. Two studies reported no effects of nicotine administration on startle (33,43). One study reported enhanced PPI as a result of nicotine administration (15). Multiple methodological differences among studies (e.g., route of administration, form of nicotine used, time of testing during the circadian cycle) precluded interpretation of behavioral differences as simply the product of subject strain.

Whether strain differences in nicotine's effects on attention existed, therefore, was not clear. In addition, because the effects of chronic nicotine administration on ASR and PPI responses of female rats had not been explored, the present experiment examined responses of female as well as male Long–Evans rats. Further, environmental conditions such as grouped housing can alter effects of drugs and may be relevant to individual differences in smoking behavior. Therefore, this experiment assessed the effects of this environmental manipulation and its interaction with nicotine administration and cessation on ASR and PPI responses of Long–Evans males and females.

Strain Differences

The most striking finding of this experiment was that nicotine administration decreased startle amplitude and impaired PPI in Long–Evans subjects. These findings contrasted with previously reported findings in Sprague–Dawley subjects conducted with identical methodologies indicating that nicotine enhanced startle and PPI (1–5). In addition, these findings contrasted with data from Long–Evans subjects obtained during the light portion of the circadian cycle when nicotine was administered acutely (15) and with reports that chronic nicotine administration had no effect on startle in Long–Evans subjects (33,43) . These findings suggest, therefore, that when similar methodologies are employed across strains, a true strain difference in nicotine's attentional effects between Sprague–Dawley and Long–Evans subjects may exist. These findings also indicate that differences in experimental procedures may obscure important strain differences in drug responses.

Sex Differences

Long–Evans males and females differed in their PPI responses to nicotine administration and to housing conditions in several ways. For females nicotine administration generally impaired PPI, regardless of housing condition. In contrast, for males nicotine's effects always depended on housing condition, with nicotine enhancing PPI in grouped subjects but impairing PPI in individually housed subjects. Nicotine's effects in male and female subjects also followed different time courses as indicated by the statistical detection of drug effects in males or females alone. Specifically, nicotine-induced startle reductions appeared robustly in females on day 6 (startle amplitude to 112 dB) but did not appear in males until day 12 (startle amplitude to 122 dB). Housing effects showed the opposite pattern. Housing condition affected male responses on day 6 (startle amplitude to 122 dB, %PPI to 112 dB), but were not evident in females until day 12 (startle amplitude to 112 and 122 dB) and in cessation (%PPI to 112 dB). Taken together, these results are consistent with female rats having greater sensitivity to nicotine's behavioral effects than males. That is, females were affected more quickly than males by nicotine administration, effects of housing condition on females were minimal compared to effects of nicotine, and effects of housing on females did not appear until day 12. In contrast, males took longer to be affected by nicotine, drug effects always depended on housing, and housing effects appeared on day 6, suggesting that for males the environmental manipulation was a more powerful determinant of responses.

We also have found greater sensitivity among female rats than among male rats to other effects of nicotine, including changes in body weight (24,28), energy intake (8,24,27), and energy expenditure (8,26). It is intriguing that although females appear more behaviorally and biologically sensitive to nicotine, female humans and rats are less adept than males at discriminating nicotine from placebo or adjusting nicotine in-

TABLE 2
ASR AMPLITUDES, AMOUNT PPI, AND PERCENT PPI CESSATION DAY 3 (MEANS ± SEM)

		Males	Females
112dB			
Startle amplitude	Saline indiv.	91.69 ± 12.36	83.72 ± 18.74
	Nicotine indiv.	101.11 ± 23.06	84.07 ± 14.55
	Saline grouped	82.52 ± 18.18	74.22 ± 15.83
	Nicotine grouped	104.33 ± 11.85	87.70 ± 12.74
Amount PPI	Saline indiv.	40.14 ± 8.78	30.15 ± 11.94
	Nicotine indiv.	36.75 ± 7.69	25.52 ± 8.52
	Saline grouped	24.95 ± 10.83	34.09 ± 9.04
	Nicotine grouped	50.03 ± 11.67	43.61 ± 11.08
Percent PPI	Saline indiv.	44.67 ± 6.43	24.18 ± 10.86
	Nicotine indiv.	52.36 ± 13.46	16.38 ± 13.90
	Saline grouped	19.22 ± 12.41	38.15 ± 7.00
	Nicotine grouped	47.05 ± 9.54	45.08 ± 7.37
122dB			
Startle amplitude	Saline indiv.	152.64 ± 21.87	132.91 ± 21.20
	Nicotine indiv.	153.95 ± 24.40	113.71 ± 16.87
	Saline grouped	135.41 ± 20.18	102.30 ± 19.46
	Nicotine grouped	166.35 ± 30.18	89.80 ± 11.09
Amount PPI	Saline indiv.	86.28 ± 15.77	53.86 ± 10.34
	Nicotine indiv.	65.31 ± 9.97	44.57 ± 10.41
	Saline grouped	60.70 ± 15.13	43.80 ± 8.02
	Nicotine grouped	69.78 ± 17.64	22.86 ± 18.47
Percent PPI	Saline indiv.	54.73 ± 4.56	39.71 ± 3.74
	Nicotine indiv.	42.12 ± 6.62	35.79 ± 6.61
	Saline grouped	40.67 ± 10.23	44.70 ± 4.39
	Nicotine grouped	42.75 ± 9.55	21.02 ± 19.68

take after preloads (40,46). The contrast between behavioral sensitivity and interoceptive insensitivity warrants further study.

Apart from effects of nicotine, males and females also responded differently to the two stimuli (112 and 122dB). Males responded in an increasing, linear fashion to acoustic stimuli, with maximal responses occurring to the loudest stimulus. Maximal responses for females, however, depended on environmental conditions as well as on stimulus intensity. This sex difference in Long–Evans subjects contrasts with reports that Sprague–Dawley males and females do not differ in startle or PPI behaviors (50).

Overall, the results replicated other investigators' findings that startle and prepulse inhibition are separately manipulable by drugs (17,19,35,41,51–53). In addition, these results extend this literature by indicating that startle and PPI also are separately affected by housing conditions.

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. To the extent that this is so, the conclusion that genotype, broadly construed to include subject's sex, can alter responses to nicotine and to environmental conditions is supported. Replication of this apparent strain difference by using both strains within the same study, however, is necessary to demonstrate the reliability of this finding.

Future studies, therefore, should include Sprague–Dawley and Long–Evans males and females within the same experiment. Like many drugs nicotine exerts biochemical, physiological, and behavioral effects in an inverted-U-shaped dose–response curve. Opposite behavioral effects as a result of the same drug dosage, therefore, may indicate that one strain is more sensitive to nicotine than the other strain. That is, one strain's inverted U-shaped dose–response curve may be shifted to the left of the other strain's curve. Decreased responses as a result of treatment with 12 mg/kg/day nicotine suggest that Long–Evans subjects might be more sensitive to nicotine's effects than Sprague–Dawley subjects. For example, 12 mg/kg/day nicotine may represent a dosage on the descending limb of the Long–Evans dose–response curve but a point on the ascending limb of the Sprague–Dawley curve. Future studies, therefore, also should examine the effects of additional nicotine dosages to determine dose–response curves for Sprague–Dawley vs. Long–Evans subjects.

Animal models using rats of different strains and sexes may be useful to investigate the role of biologically based individual differences in smoking behavior and in responses to environmental conditions, including differences in vulnerability to nicotine addiction and to situational determinants of smoking. In addition, use of different rat strains may be relevant to development of nicotine analogs intended for clinical use as attention- or cognition-enhancing agents in humans. The findings reported here suggest that effects of substances acting via central nicotinic cholinergic receptors may depend on the subject's genotype, including sex.

ACKNOWLEDGEMENTS

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences. This work was supported by USUHS-DoD grant RO72AR.

REFERENCES

1. Acri, J. B.: Interactions of stress and nicotine on amplitude, prepulse inhibition and habituation of the acoustic startle reflex. Bethesda, MD: Uniformed Services University of the Health Sciences; unpublished doctoral dissertation; 1992.
2. Acri, J. B.: Nicotine modulates effects of stress on acoustic startle reflexes in rats: Dependence on dose, stressor and initial reactivity. Psychopharmacology (Berlin)116:255–265; 1994.
3. Acri, J. B.; Brown, K. J.; Saah, M. I.; Grunberg, N. E.: Strain and age differences in acoustic startle responses and effects of nicotine in rats. Pharmacol. Biochem. Behav. 50:191–198; 1995.
4. Acri, J. B.; Grunberg, N. E.; Morse, D. E.: Effects of nicotine on the acoustic startle reflex amplitude in rats. Psychopharmacology (Berlin) 104:244–248; 1991.
5. Acri, J. B.; Morse, D. E.; Popke, E. J.; Grunberg, N. E.: Nicotine increases sensory gating measured as inhibition of the acoustic startle reflex in rats. Psychopharmacology (Berlin) 114:369–374; 1994.
6. Anthony, B. J.; Graham, F. K.: Evidence for sensory-selective set in young infants. Science 220:742–743; 1983.
7. Anthony, B. J.; Putnam, L. E.: Cardiac and blink reflex concomitants of attentional selectivity: A comparison of adults and young children. Psychophysiology 22:508–516; 1985.
8. Bowen, D. J.; Eury, S. E.; Grunberg, N. E.: Nicotine's effects on female rats' body weight: Caloric intake and physical activity. Pharmacol. Biochem. Behav. 25:1131–1136; 1986.
9. Braff, D.; Stone, C.; Callaway, E.; Geyer, M.; Glick, I.; Bali, L.: Prestimulus effects on human startle reflex in normals and schizophrenics. Psychophysiology 15:339–343; 1978.
10. Brown, K. J.; Grunberg, N. E.: Effects of housing on male and female rats: Crowding stresses males but calms females. Physiol. Behav. 58:1085–1089; 1995.
11. Brown, K. J.; Grunberg, N. E.: Effects of environmental conditions on food consumption in female and male rats. Physiol. Behav. 60:293–297; 1996.
12. Brown, K. J.; Klein, L. C.; Rahman, M. A.; Grunberg, N. E.: Acoustic startle responses as predictors of fentanyl self-administration in rats. Presented at the American Psychological Association, New York, NY; August, 1995.
13. Chabot, C. C.; Taylor, D. H.: Circadian modulation of the rat acoustic startle response. Behav. Neurosci. 106:846–852; 1992.
14. Collins, A. C.; Miner, L. L.; Marks, M. J.: Genetic influences on acute responses to nicotine and nicotine tolerance in the mouse. Pharmacol. Biochem. Behav. 30:269–278; 1988.
15. Curzon, P.; Kim, D. J. B.; Decker, M. W.: Effect of nicotine, lobeline, and mecamylamine on sensory gating in the rat. Pharmacol. Biochem. Behav. 49:877–882; 1994.
16. Davis, M.: The mammalian startle response. In: Eaton, R., ed. Neural mechanisms of startle behavior. New York: Plenum Press; 1984:287–351.
17. Davis, M.: Apomorphine, *d*-amphetamine, strychnine, and yohimbine do not alter prepulse inhibition of the acoustic startle reflex. Psychopharmacology (Berlin) 95:151–156; 1988.
18. Davis, M.; Sollberger, A.: Twenty-four hour periodicity of the startle response in rats. Psychon. Sci. 25:37–39; 1971.
19. Davis, M.; Svensson, T. H.; Aghajanian, G. K.: Effects of *d*- and *l*-amphetamine on habituation and sensitization of the acoustic startle response in rats. Psychopharmacology (Berlin) 43:1–11; 1975.
20. Eaves, L. J.; Eysenck, H. J.: New approaches to the analysis of twin data and their application to smoking behavior. In: Eysenck, H. J., ed. The causes and effects of smoking. London: Maurice Temple Smith; 1980:140–314.
21. Graham, F. K.: The more or less startling effects of weak prestimuli. Psychophysiology 12:238–248; 1975.
22. Gritz, E. R.: Gender and the teenage smoker. In: Ray, B.; Braude, M., eds. Women and drugs: A new era for research. National Institute on Drug Abuse Research Monograph 65. Washington, DC: Department of Health and Human Services; 1986:70–79.
23. Grun, E.; Pauly, J.; Bullock, A.; Collins, A.: Corticosterone reversibly alters brain alpha-bungarotoxin binding and nicotine sensitivity. Pharmacol. Biochem. Behav. 52:629–635; 1995.
24. Grunberg, N. E.: The effects of nicotine and cigarette smoking on food consumption and taste preferences. Addict. Behav. 7:317–331; 1982.
25. Grunberg, N. E.; Acri, J. B.; Popke, E. J.: An animal model to study nicotine's effects on cognition. Presented at the International Symposium on Nicotine, Montreal, Quebec, Canada; 1994.
26. Grunberg, N. E.; Bowen, D. J.: The role of physical activity in nicotine's effects on body weight. Pharmacol. Biochem. Behav. 23:851–854; 1985.
27. Grunberg, N. E.; Bowen, D. J.; Winders, S. E.: Effects of nicotine on body weight and food consumption in female rats. Psychopharmacology (Berlin) 90:101–105; 1986.
28. Grunberg, N. E.; Winders, S. E.; Wewers, M. E.: Gender differences in tobacco use. Health Psychol. 10:143–153; 1991.
29. Hannah, M. C.; Hopper, J. L.; Mathews, J. D.: Twin concordance for a binary trait. II. Nested analysis of ever-smoking and ex-smoking traits and unnested analysis of a committed-smoking trait. Am. J. Hum. Genet. 37:153–165; 1984.
30. Harty, T. P.; Davis, M.: Cocaine effects on acoustic startle and startle elicited electrically from cochlear nucleus. Psychopharmacology (Berlin) 87:396–399; 1985.
31. Heath, A. C.; Martin, N. G.: Genetic models for the natural history of smoking: Evidence for a genetic influence on smoking persistence. Addict. Behav. 18:19–34; 1993.
32. Heishman, S. J.; Taylor, R. C.; Henningfield, J. E.: Nicotine and smoking: A review of effects on human performance. Exp. Clin. Psychopharmacol. 2:345–395; 1994.
33. Helton, D. R.; Modlin, D. L.; Tizzano, J. P.; Rasmussen, K.: Nicotine withdrawal: A behavioral assessment using schedule controlled responding, locomotor activity, and sensorimotor reactivity. Psychopharmacology 113:205–210; 1993.
34. Hughes, J. R.: Genetics of smoking: A brief review. Behav. Ther. 17:335–345; 1986.
35. Mansbach, R. S.; Geyer, M. A.; Braff, D. L.: Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology (Berlin) 94:507–514; 1988.
36. Marks, M. J.; Romm, E.; Gaffney, D. K.; Collins, A. C.: Nicotine-induced tolerance and receptor changes in four mouse strains. J. Pharmacol. Exp. Ther. 237:809–819; 1986.
37. Marks, M. J.; Stitzel, J. A.; Collins, A. C.: Genetic influences on nicotine responses. Pharmacol. Biochem. Behav. 33:667–678; 1989.
38. Miczek, K. A.; Vivian, J. A.; Tornatzky, W.; Farrell, W. J.; Sapperstein, S. B.: Withdrawal from diazepam in rats: Ultrasonic vocalizations and the acoustic startle reflex. J. Psychopharmacol. Abstr. A47; 1992.
39. Morse, D. E.; Davis, H. D.; Popke, E. J.; Brown, K. J.; O'Donoghue, V. A.; Grunberg, N. E.: Effects of ddC and AZT on locomotion and acoustic startle I: Acute effects in female rats. Pharmacol. Biochem. Behav. 56:221–228; 1997.
40. Perkins, K. A.: Sex differences in nicotine versus nonnicotine reinforcement as determinants of tobacco smoking. Exp. Clin. Psychopharmacol. 4:166–177; 1996.
41. Pohorecky, L. A.; Cagan, M.; Brick, J.; Jaffe, L. S.: Startle response in rats: Effects of ethanol. Pharmacol. Biochem. Behav. 4:311–316; 1976.
42. Popke, E. J.; Acri, J. B.; Grunberg, N. E.: Nicotine, stress, and acoustic startle responses of rats. Presented at the American Psychological Association, Los Angeles, CA; 1994.
43. Rasmussen, K.; Czachura, J. F.; Kallman, M. J.; Helton, D. R.: The CCK-B antagonist LY288513 blocks the effects of nicotine withdrawal on auditory startle. Neuroreport 7:1050–1052; 1996.
44. Rigdon, G. C.: Differential effects of apomorphine on prepulse inhibition of the acoustic startle reflex in two rat strains. Psychopharmacology (Berlin) 102:419–421; 1990.
45. Russell, M. A. H.; Peto, J.; Patel, U. A.: The classification of smoking by factorial structure of motives. J. R. Stat. Soc. A: General 137:313–346; 1974.
46. Schechter, M. D.; Rosecrans, J. A.: CNS effect of nicotine as the discriminative stimulus for the rat in a T-maze. Life Sci. 10:821–832; 1971.

47. Shiffman, S.: Relapse following smoking cessation: A situational analysis. J. Consult. Clin. Psychol. 50:71–86; 1982.
48. Shiffman, S.: Coping with temptations to smoke. In: Shiffman, S.; Wills, T. A., eds. Coping and substance use. New York: Academic Press; 1985:223–240.
49. Spilich, G. J.; June, L.; Renner, J.: Cigarette smoking and cognitive performance. Br. J. Addict. 87:1313–1326; 1992.
50. Swerdlow, N. R.; Auerbach, P.; Monroe, S.; Hartston, H.; Geyer, M.; Braff, D.: Men are more inhibited than women by weak prepulses. Biol. Psychiatry 34:253–260; 1993.
51. Swerdlow, N. R.; Caine, S. B.; Braff, D. L.; Geyer, M. A.: The neural substrates of sensorimotor gating of the startle reflex: A review of recent findings and their implications. J. Psychopharmacol. 6:176–190; 1992.
52. Swerdlow, N. R.; Mansbach, R. S.; Geyer, M. A.; Pulvirenti, L.; Koob, G. F.; Braff, D. L.: Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. Psychopharmacology (Berlin)100:413–416; 1990.
53. Swerdlow, N. R.; Vaccarino, F. J.; Amalric, M.; Koob, G. F.: Neural substrates for the motor-activating properties of psychostimulants: A review of recent findings. Pharmacol. Biochem. Behav. 25:233–248; 1986.
54. U.S. Department of Health and Human Services: The health consequences of smoking: Nicotine addiction, a report of the Surgeon General. DHHS Pub. No. (CDC)88-8406. Washington, DC: U.S. Government Printing Office; 1988.
55. Wesnes, K.; Warburton, D. M.: Smoking, nicotine and human performance. Pharmacol. Ther. 21:189–208; 1983.
56. Winders, S. E.; Grunberg, N. E.: Nicotine, tobacco smoke, and body weight: A review of the animal literature. Ann. Behav. Med. 11:125–133; 1989.