Pharmacology Biochemistry & Behavior, Vol. 33, pp. 667–678. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Genetic Influences on Nicotine Responses

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Received 8 August 1988

MARKS, M. J., J. A. STITZEL AND A. C. COLLINS. *Genetic influences on nicotine responses.* PHARMACOL BIOCHEM BEHAV **33**(3) 667–678, 1989.—Male mice from 19 inbred strains were tested for the effects of nicotine on six responses: respiratory rate, acoustic startle response, Y-maze crosses, Y-maze rears, heart rate and body temperature. Dose-response curves were constructed for each strain on each test in a multitest battery. Results indicated that the responses were strongly influenced by the genotype of the animal. Comparison of the results from the six tests measured in this study and the results previously reported for nicotine-induced seizures in these same strains indicated that the responses could be grouped into two major classes: a set characterized by Y-maze crosses, Y-maze rears and body temperature and a set characterized by seizure sensitivity and seizure latency. Responses observed for respiratory rate and startle response shared characteristics with both of these sets, while nicotine effect on heart rate was fairly unique. The results have identified strains of mice which are differentially sensitive to the effects of nicotine.

Nicotine Pharmacogenetics Respiratory rate Acoustic startle response Locomotor activity Heart rate Body temperature

ADMINISTRATION of nicotine to rodents affects, among other responses, locomotor activity, body temperature, heart rate, respiration, and, at high doses, causes convulsions (1–3, 7–9, 14, 18, 22, 24, 26, 28–30). Not only does nicotine interact with specific receptor sites in the central nervous system and periphery, it also has biphasic effects on its receptors. Langley and Dickinson (13) were the first to note that low doses of nicotine will stimulate autonomic ganglia while higher doses will induce blockade. Nicotine induces a transient stimulation of electrical activity which is followed by a longer lasting blockade (12). This phenomenon is referred to as desensitization of the nicotinic receptor.

Genotype apparently affects the sensitivity of an animal to nicotine. The influence of genotype on the effects of nicotine on locomotion have been most thoroughly studied in both rats (3, 7, 8, 26) and mice (2, 10, 14, 17). Other responses have been less well studied, but genotype also influences nicotine-induced hypothermia (14, 16, 18), bradycardia (18), respiratory stimulation (14,18), acoustic startle response (14,18) and convulsions (22, 23, 26). Genotype also influences the extent of tolerance development with chronic nicotine treatment (15,19). Although these results indicate that genotype influences sensitivity to nicotine, very little is known about the genetic architecture that regulates sensitivity to nicotine, perhaps because most of the studies that have been published, to date, have employed a relatively small number of inbred strains.

Because of the complexity of nicotine response, the ability to measure several different parameters is of value in fully assessing the effects of this drug. To accomplish this purpose, a test battery consisting of six measures (respiratory rate, acoustic startle response, Y-maze crosses, Y-maze rears, heart rate and body temperature) has been developed (18). This battery allows the measurement of these responses in an individual animal with no measureable intertest interactions. The availability of this test battery makes efficient use of experimental subjects possible such that a more extensive analysis of genetic influences on nicotine response is feasible. This paper presents the results of such an analysis.

Nineteen inbred mouse strains have been tested for the effects of nicotine using the test battery. A full dose-response curve has been constructed for each strain to determine whether qualitative or merely quantitative differences in response occur. Since several measurements of nicotine effects were obtained, further analyses of the results obtained in this paper, and those on nicotine-induced seizures (23), have been made to determine if a relationship among the various responses exists. Several of the strains used in the current study are closely related, while others are thought to derive from substantially different populations. This design was chosen to determine the stability of nicotine sensitivity (from similarity of related stocks) and to maximize the likelihood of identifying widely divergent responses (by testing mice of different origins).

METHOD

Animals

Male mice of 19 inbred strains were used in this study. Mice of

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the A/J/Ibg, C57BL/6/J/Ibg, DBA/2J/Ibg, and C3H/2Ibg were bred at the Institute for Behavioral Genetics, University of Colorado, Boulder, CO. These strains have been maintained at the Institute for at least 20 generations. Male mice of the BALB/cByJ strain, originally obtained from the Jackson Laboratories, Bar Harbor, ME, were also bred at the Institute, but have been maintained there for fewer than eight generations. All mice were weaned at 25 days of age and were housed with male littermates. Animals were 60–90 days old when tested. Male mice of the following strains were purchased from Jackson Laboratory, Bar Harbor, ME: AKR/J, BUB/BnJ, CBA/J, C57BL/10J, C57BR/cdJ, C57L/J, C58/J, DBA/1J, LP/J, P/J, RIIIS/J, SJL/J, ST/bJ, and SWR/J. All mice were 4–6 weeks old when they were received and were housed five per cage in our mouse colony until they were 60–90 days old. A 12-hr light/12-hr dark cycle (lights on 7 a.m. to 7 p.m.) was maintained and animals were given free access to food (Wayne Lab Blox) and water.

Nicotine Administration

Nicotine was obtained from Sigma Chemical Co., St. Louis, MO and was redistilled periodically. The drug was dissolved in physiological saline and was administered by intraperitoneal injection. Injection volume was 0.01 ml/g body weight. Drug doses were adjusted to assure that a full range of responses was observed for each strain. To facilitate analysis, saline and 1.0 and 2.0 mg/kg nicotine were administered to mice of every strain.

Testing

All mice were tested using the six component test battery described below. Six to twelve mice were tested at each nicotine dose. Timing and duration of the tests has been established previously (18), and the results obtained using the battery are the same as those determined with each test individually. Details of the conduct of each test follow.

Respiration

Respiratory rate was measured using a Columbus Instruments Respiration Rate Monitor. Prior to injection of nicotine, the mouse was placed in a glass jar (diameter, 10.5 cm; height, 17 cm) the bottom of which was covered with aspen shavings. After 10 min, the mouse was removed and injected. The animal was then returned to the jar and a lid containing a pressure-sensitive transducer was placed on the jar to form a closed system. Monitoring was begun 1 min after injection of the nicotine. Respiratory rate was observed for 1 min during which time five equally spaced recordings were made. Animals were tested 1 min after injection because this is the time at which nicotine maximally stimulates respiratory rate (14).

Startle Response

The response of mice to an acoustic startle was measured using a Columbus Instruments Responder Startle Reflex Monitor. The startle reflex was measured 3 min after injection of nicotine. The mouse to be tested was placed inside a box made of acrylic plastic (length, 14 cm; width, 5 cm; height, 16 cm) and the box was covered with a lid of acrylic plastic. The bottom of the box was the sensor platform. An auditory stimulus (frequency, 6250 Hz; intensity, 120 dB; duration, 50 msec) was presented ten times, with a 10-sec interval between stimuli. Both the response time and amplitude were recorded. The sensor sensitivity was set at 5.00

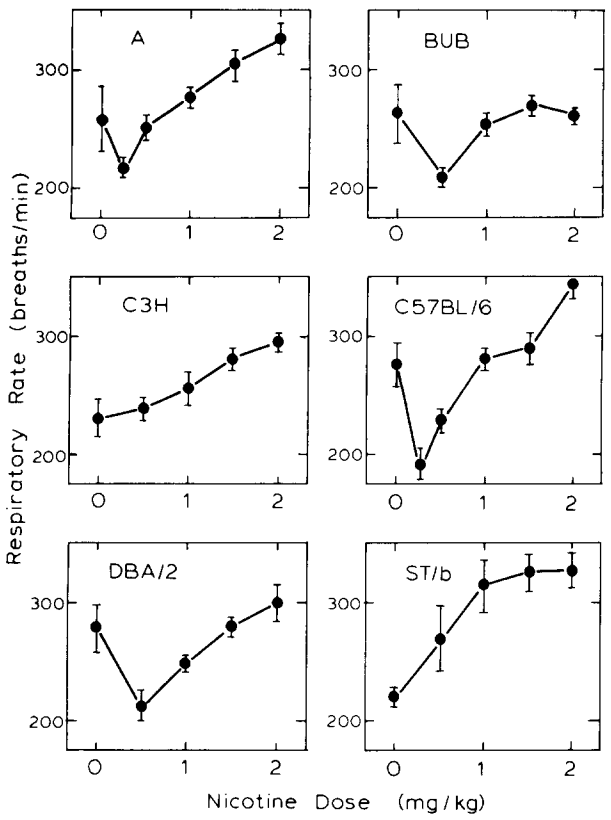

FIG. 1. Nicotine effects on respiratory rate. Respiratory rate was measured for 1 min beginning 1 min after IP administration of nicotine. Data represent mean ± SEM for 6–12 individual mice at each dose.

(full scale, 10.00). The testing chamber was contained in a sound-insulated box and a low level of white noise (2% of full scale) was present at all times. The responses observed after presentation of the auditory stimulus appear to be of three major types: no response, a modest response (corresponding to a head movement), and an intense response (corresponding to the movement of the head and shoulders or the whole body). At the sensor setting used in these studies, the category of response could be determined by observation of the magnitude of the response recorded by the sensor (no response corresponding to no sensor response, a modest response corresponding to a sensor reading from 1 to 350 and an intense response corresponding to a sensor reading greater than 350). In order for a response to be considered valid, a delay of approximately 25 msec between presentation of the auditory stimulus and the initiation of a startle response was required. By assigning a numerical value of 0, 1, or 2 to these three types of responses, respectively, a single score ranging from 0 to 20 was obtained for each mouse (14). This startle score was then used as a measure of the responsiveness of each mouse. Measurement of the startle response between 3 and 4.5 min after nicotine injection gives maximal response (14).

Y-Maze Activity

Both locomotor and rearing activity were determined in a symmetrical Y-maze. The maze consists of three arms which are

TABLE 1

CONTROL TEST BATTERY RESPONSES OF 19 MOUSE STRAINS

Strain	Respiratory Rate (breaths/min)	Startle Response (score)	Y-Maze Crosses (number)	Y-Maze Rears (number)	Heart Rate (beats/min)	Body Temperature (°C)
A/J/Ibg	266 ± 22	8.5 ± 1.5	28.0 ± 1.8	24.4 ± 2.0	700 ± 16	37.4 ± 0.2
AKR/J	244 ± 18	6.4 ± 1.3	48.4 ± 3.3	26.3 ± 2.2	720 ± 19	37.9 ± 0.2
BALB/cByJ	260 ± 12	3.6 ± 1.2	58.4 ± 5.2	37.2 ± 2.7	614 ± 20	37.3 ± 0.2
BUB/BnJ	263 ± 12	8.3 ± 1.6	63.9 ± 3.5	36.9 ± 5.4	794 ± 16	38.2 ± 0.3
CBA/J	322 ± 21	2.4 ± 0.7	46.9 ± 3.3	26.3 ± 5.4	760 ± 19	37.8 ± 0.2
C3H/2Ibg	231 ± 12	5.6 ± 1.0	38.2 ± 4.2	22.2 ± 2.2	770 ± 13	37.9 ± 0.2
C57BL/6J/Ibg	276 ± 16	4.8 ± 1.2	25.0 ± 4.7	14.1 ± 2.8	594 ± 39	37.8 ± 0.1
C57BL/10J	276 ± 16	7.4 ± 0.8	33.2 ± 3.4	20.0 ± 2.7	696 ± 52	38.0 ± 0.2
C57BR/cdJ	290 ± 19	2.0 ± 0.9	58.8 ± 4.1	38.0 ± 3.5	780 ± 12	37.6 ± 0.1
C57L/J	276 ± 17	0.3 ± 0.3	42.5 ± 3.5	25.0 ± 3.4	735 ± 22	37.4 ± 2.5
C58/J	217 ± 27	6.2 ± 1.0	50.6 ± 14.9	31.8 ± 7.2	740 ± 32	37.7 ± 0.2
DBA/1J	224 ± 15	6.6 ± 0.9	49.4 ± 2.1	31.7 ± 3.5	781 ± 9	38.5 ± 0.2
DBA/2J/Ibg	277 ± 23	5.7 ± 1.1	41.0 ± 9.5	23.0 ± 4.0	737 ± 11	38.2 ± 0.2
LP/J	260 ± 14	4.9 ± 0.9	40.1 ± 10.5	16.6 ± 4.7	787 ± 18	37.6 ± 0.2
P/J	281 ± 17	9.1 ± 2.5	79.3 ± 9.5	36.6 ± 2.8	734 ± 20	37.6 ± 0.1
RIIIS/J	241 ± 29	2.0 ± 1.0	49.9 ± 6.0	34.7 ± 4.0	698 ± 21	37.7 ± 0.4
SJL/J	346 ± 16	2.9 ± 0.6	55.2 ± 2.3	32.4 ± 1.5	706 ± 21	38.4 ± 0.2
ST/bJ	219 ± 17	3.7 ± 0.8	50.8 ± 3.6	22.7 ± 2.7	797 ± 11	38.1 ± 0.2
SWR/J	295 ± 18	4.3 ± 1.1	54.0 ± 6.6	41.4 ± 4.5	788 ± 18	38.5 ± 0.2
ANOVA Results	F=3.02	F=4.02	F=4.75	F=5.39	F=7.48	F=3.21

Responses of mice of each of the 19 inbred strains were measured in the test battery after injection of saline. Values given represent mean ± SEM of 6–12 measurements. All groups for ANOVA had 18 degrees of freedom for between groups and 139 degrees of freedom for within groups. Significance of F for all groups was <0.001.

26 cm long, 6.1 cm side, and 10.2 cm high. Each arm was subdivided into two equal sections. The maze was constructed of black acrylic plastic and was indirectly underlit through a red floor using two 25-cm, 8-watt fluorescent bulbs. The top of the maze was constructed of red translucent acrylic plastic. Testing was begun 5 min after injection of nicotine by placing the mouse in the center of the maze. Testing was conducted for 3 min. Movements from one section to another were counted, as were the number of rears. Testing at 5 min after nicotine injection assures near-maximal depression of Y-maze activity (11).

Heart Rate

Heart rate was measured by placing a mouse in a restrainer to allow the insertion of needle electrodes under the skin. One electrode was placed behind the left foreleg and the other in front of the right hindleg. The electrodes were connected through a preamplifier to a Narco Biosystems E & M Physiograph. Heart rate was measured for 6 sec and the rate was estimated by counting the number of QRS complexes. Heart rate was measured 9 min after injection.

Body Temperature

Body temperature was measured with a Bailey Instruments rectal probe. The probe was lubricated with peanut oil and was inserted 2.5 cm into the rectal cavity. The body temperature was measured 15 min after nicotine injection. This time was chosen to give maximal or nearly maximal drug effect (14).

Data Analysis

Several different analyses were used. All analyses were conducted using the Statistical Package for the Social Sciences adapted for the personal computer (SPSS/PC). Dose-response curves were constructed and least squares linear regression analysis was used to determine the mean effective dose of nicotine. To facilitate comparison of these values the following parameters were defined: for respiratory rate, ED_{260}, the dose required to elevate respiratory rate to 260 breaths/min; for startle response, slope of the dose-response curve was calculated; for Y-maze crosses and Y-maze rears, ED_{50}, the dose required to reduce crosses and rears to 50% of control level; for heart rate, ED_{-100}, the dose required to lower heart rate by 100 beats/min; and for body temperature, $ED_{-2°}$, the dose required to lower body temperature by 2°. The ED values calculated in this manner were used in further analyses described below.

Responses of mice of the 19 strains were compared using Analysis of Variance (ANOVA). Two-way ANOVAs were employed to analyze the effect of strain and dose (doses 0, 1.0, and 2.0 mg/kg, which were used for all strains) for nicotine effects on each test. Analyses were performed both on raw data and data transformed to minimize the effects of baseline differences (for startle response, heart rate, and body temperature the difference between control and test result was analyzed; for Y-maze crosses and Y-maze rears the percentage of control activity was analyzed;

TABLE 2

SUMMARY OF RESPONSIVENESS OF 19 MOUSE STRAINS IN THE TEST BATTERY

Strain	Response					
	Respiratory Rate (ED_{260})	Startle Response (Slope)	Y-Maze Crosses (ED_{50})	Y-Maze Rears (ED_{50})	Heart Rate (ED_{-100})	Body Temperature (ED_{-2})
A/J/Ibg	0.78 ± 0.09	−1.67 ± 0.61	0.80 ± 0.31	0.41 ± 0.21	0.82 ± 0.13	0.55 ± 0.06
AKR/J	1.48 ± 0.09	−0.23 ± 0.46	1.42 ± 0.31	1.26 ± 0.27	1.60 ± 0.25	1.37 ± 0.20
BALB/cByJ	0.67 ± 0.17	+2.04 ± 1.48	1.06 ± 0.06	0.97 ± 0.20	0.95 ± 0.33	0.92 ± 0.17
BUB/BnJ	1.29 ± 0.23	+2.27 ± 1.75	1.89 ± 0.33	1.32 ± 0.36	1.48 ± 0.24	2.53 ± 0.08
CBA/J	0.73 ± 0.31	+2.66 ± 0.94	1.43 ± 0.21	1.41 ± 0.21	1.41 ± 0.19	1.56 ± 0.36
C3H/2Ibg	1.10 ± 0.14	+3.70 ± 0.73	1.78 ± 0.33	1.50 ± 0.10	1.25 ± 0.24	1.32 ± 0.09
C57BL/6J/Ibg	0.95 ± 0.19	−1.77 ± 1.05	0.51 ± 0.18	0.45 ± 0.18	0.90 ± 0.23	0.80 ± 0.16
C57BL/10J	1.14 ± 0.17	+0.73 ± 1.05	0.49 ± 0.21	0.37 ± 0.27	1.12 ± 0.12	0.61 ± 0.21
C57BR/cdJ	0.43 ± 0.24	−0.76 ± 0.22	1.07 ± 0.13	0.92 ± 0.11	1.40 ± 0.20	1.59 ± 0.32
C57L/J	0.97 ± 0.47	−0.10 ± 0.06	1.17 ± 0.27	0.80 ± 0.12	1.36 ± 0.40	1.20 ± 0.11
C58/J	2.66 ± 0.41	+0.49 ± 0.94	1.82 ± 0.08	1.54 ± 0.22	1.28 ± 0.48	2.07 ± 0.06
DBA/1J	1.49 ± 0.11	−0.10 ± 1.32	0.93 ± 0.31	0.94 ± 0.42	0.94 ± 0.24	1.02 ± 0.26
DBA/2J/Ibg	1.25 ± 0.11	−0.80 ± 0.87	0.97 ± 0.31	0.80 ± 0.06	0.94 ± 0.24	0.89 ± 0.19
LP/J	0.75 ± 0.30	−1.18 ± 0.54	1.04 ± 0.34	0.95 ± 0.26	1.79 ± 0.35	1.30 ± 0.15
P/J	0.77 ± 0.23	−0.20 ± 2.30	1.25 ± 0.17	0.96 ± 0.15	1.34 ± 0.23	1.10 ± 0.12
RIIIS/J	0.93 ± 0.43	+1.44 ± 0.41	1.62 ± 0.17	1.46 ± 0.17	1.98 ± 0.79	1.19 ± 0.17
SJL/J	1.00 ± 0.14	+0.11 ± 0.95	1.32 ± 0.24	1.18 ± 0.22	2.03 ± 0.71	1.23 ± 0.09
ST/bJ	0.41 ± 0.04	+4.52 ± 0.94	0.93 ± 0.21	0.64 ± 0.27	0.98 ± 0.18	1.47 ± 0.23
SWR/J	1.19 ± 0.25	−0.28 ± 0.46	1.42 ± 0.49	1.19 ± 0.36	2.19 ± 0.45	1.18 ± 0.20

Dose-response curves for each of the six components of the test battery were analyzed by linear regression and parameters reflecting the sensitivity of mice of each of the 19 inbred strains were calculated to provide a comparison of the relative sensitivity of each of the strains to the effects of nicotine. Since the measurements made for the six tests varied, the following calculations were made: Respiratory rate, ED_{260}, the dose (mg/kg) required to stimulate respiratory rate to 260 breaths/min; startle response, slope of the dose response curve (change in startle score for each 1 mg/kg increase in dose of nicotine); Y-maze crosses and Y-maze rears, ED_{50}, the dose (mg/kg) required to reduce the number of crosses or rears to 50% of control levels; heart rate, ED_{-100}, the dose (mg/kg) required to reduce heart rate by 100 beats/min; and body temperature, $ED_{-2°}$, the dose (mg/kg) required to lower body temperature by 2°. The values given on the table are mean ± SEM calculated from the linear portion of the dose-response curves.

no transformation of respiratory rate data was made). One-way ANOVAs as a function of strain were performed for each dose to determine differences in responsiveness at each nicotine challenge dose.

Intertest comparisons were also made. Correlations among the ED values observed in this study and the ED_{50} for seizure induction and latency to seizure previously determined for these strains (23) were calculated. These parameters were subsequently subjected to factor analysis in an attempt to determine whether underlying similarities among the responses could be found. Factors were extracted by principle components analysis and the factor matrix was subjected to varimax rotation. Other extraction and rotation methods gave substantially similar results. The results obtained from the factor analysis were used as the basis for a cluster analysis to search for similarities among the strains and to group them according to similarity of responsiveness to nicotine. Cluster analysis was based on the Euclidian distance between groups. Scaler differences in measurements were reduced by calculating relative responsiveness of each group by setting the average response to 100. Cluster agglomeration was accomplished by the method of minimum average linkage between groups. Modest variations in cluster formation were obtained when different distance measures of agglomeration methods were employed, but basic cluster patterns were relatively independent of variations in method.

RESULTS

Mice of each of 19 strains were tested for their responsiveness to nicotine using a six-component test battery. Dose-response curves were constructed for each strain. The effects of nicotine on each of the individual tests will be illustrated with six representative strains (A/J/Ibg, BUB/BnJ, C3H/2Ibg, C57BL/6J/Ibg, DBA/2J/Ibg, and ST/bJ). Each result will first be discussed individually and then the results will be integrated with each other and with those observed for nicotine-induced seizure sensitivity measured in these same 19 strains (23).

Respiratory Rate

The effects of nicotine on the respiratory rates of six representative strains are shown in Fig. 1. Two types of dose-response curves were noted: 1) an increase in respiratory rate as dose was increased (e.g., C3H and ST/b) and 2) an apparently biphasic pattern where respiratory rate decreased at the lowest nicotine dose and then increased. The difference between these patterns reflects differences in control respiratory rate; mice showing the biphasic curves have higher initial respiratory rates. Whether these are indeed basal differences or an artifact of response to the test chamber has not been determined. Baseline respiratory rates for all 19 strains are given in Table 1. One-way ANOVA confirms the

TABLE 3

ANOVA RESULTS OF TEST BATTERY DATA

Test	Main Effect of Strain	Main Effect of Dose	Strain by Dose Interaction
		Untransformed Data	
Respiratory Rate	$F(18,402) = 7.43$	$F(2,402) = 41.56$	$F(36,402) = 2.03$
Startle Response	$F(18,402) = 11.13$	$F(2,402) = 4.67$	$F(36,402) = 1.68$
Y-Maze Crosses	$F(18,402) = 8.49$	$F(2,402) = 226.77$	$F(36,404) = 1.82$
Y-Maze Rears	$F(18,402) = 9.32$	$F(2,402) = 313.80$	$F(36,402) = 2.72$
Heart Rate	$F(18,402) = 21.70$	$F(2,402) = 228.12$	$F(36,402) = 1.32$
Body Temperature	$F(18,402) = 15.00$	$F(2,402) = 559.61$	$F(36,402) = 3.38$
		Transformed Data	
Startle Response	$F(18,402) = 4.24$	$F(2,402) = 4.33$	$F(36,402) = 1.72$
Y-Maze Crosses	$F(18,402) = 2.79$	$F(2,402) = 201.22$	$F(36,402) = 1.62$
Y-Maze Rears	$F(18,402) = 2.76$	$F(2,402) = 299.88$	$F(36,402) = 2.06$
Heart Rate	$F(18,402) = 3.55$	$F(2,402) = 210.31$	$F(36,402) = 1.94$
Body Temperature	$F(18,402) = 10.26$	$F(2,402) = 490.87$	$F(36,402) = 3.69$

Test battery results collected after administration of 0 (saline) mg/kg, 1.0 mg/kg, or 2.0 mg/kg nicotine were analyzed by two-way ANOVA (strain by dose). These doses were chosen for analysis because they were administered to mice of each of the 19 inbred strains. The two sets of analyses were conducted because significant control differences in response among the strains occurred (Table 1). Transformations were accomplished as follows: respiratory rate, none made; startle response, heart rate and body temperature, a difference score for each strain was calculated by subtraction of the average control value for each strain from each individual value for that strain, average control score was then zero; Y-maze crosses and rears, a ratio score was calculated by dividing each score by the average number of crosses or rears measured for that strain, average control score for each strain was then 1.0. Transformation eliminated all differences observed for saline-treated mice.

baseline differences among all 19 strains (Table 1).

Dose-response curves for the 19 strains were analyzed to provide a comparison among the mice and the values for ED_{260} (dose required to elevate respiratory rate to 260 breaths/min) are given. These values were calculated from the curves, omitting control respiratory rate. This method of calculation eliminates the biphasic pattern noted for many of the strains. Values for ED_{260} vary among the strains with a low value of 0.41 ± 0.04 mg/kg for ST/b to a high value of 2.66 ± 0.41 mg/kg for C58. Values for all strains are summarized in Table 2.

Since mice of all strains were tested at doses of 0, 1 and 2 mg/kg, results obtained at these doses were also analyzed by ANOVA. This analysis indicated highly significant effects of strain and dose, as well as a significant strain-by-dose interaction (see Table 3). Significant differences among control respiratory rates may have contributed to the interaction term, so respiratory rates observed at the 1 and 2 mg/kg doses were analyzed as a function of strain with a one-way ANOVA. These analyses also indicate substantial differences among the strains occurred [for dose 1.0 mg/kg, $F(18,135) = 4.23$, $p < 0.001$ and for dose 2.0 mg/kg, $F(18,128) = 5.72$, $p < 0.001$].

Acoustic Startle Response

The effects of nicotine on the acoustic startle response of representative strains are shown in Fig. 2. The results obtained with these strains illustrates the three general effects that nicotine can have on the acoustic startle response: an increase (BUB, C3H and ST/b), no significant effect (DBA/2), or a decrease (A, C57BL/6). The pattern of the response is not dependent on control startle response (note A and BUB, as well as C3H and C57BL/6 strains have nearly identical control startle scores, but quite different response to nicotine). Control startle response scores for all strains are given in Table 1; significant differences in the control startle scores were observed.

Acoustic startle response differed from all other responses measured in that stimulation, depression and absence of effect of nicotine on this response were seen. Calculation of an ED_{50} analog is therefore impossible. As an alternative, the effects of nicotine on the slopes of the dose-response curves were calculated and are given in Table 2. These values varied from a slope of -1.77 ± 1.05 for C57BL/6 mice to 4.52 ± 0.94 for ST/b mice, with six strains showing increased responsiveness, four strains showing decreased responsiveness, and the remaining nine strains showing little change in responsiveness.

The results were further analyzed by ANOVA. The two-way ANOVA of untransformed acoustic startle scores [strain by dose (0, 1, 2 mg/kg)] confirms that highly significant strain and dose effects were present as well as a significant strain-by-dose interaction (Table 3). However, these data are confounded by differences in control acoustic startle response. To overcome this difference, the results were also analyzed after data transformation which was achieved by subtracting control startle response for each strain from all responses observed for this strain. This transformation resulted in average control values of zero for all strains. The results of the two-way ANOVA of data transformed in this manner are comparable to those obtained with untransformed data (see Table 3). The major difference between the analyses was

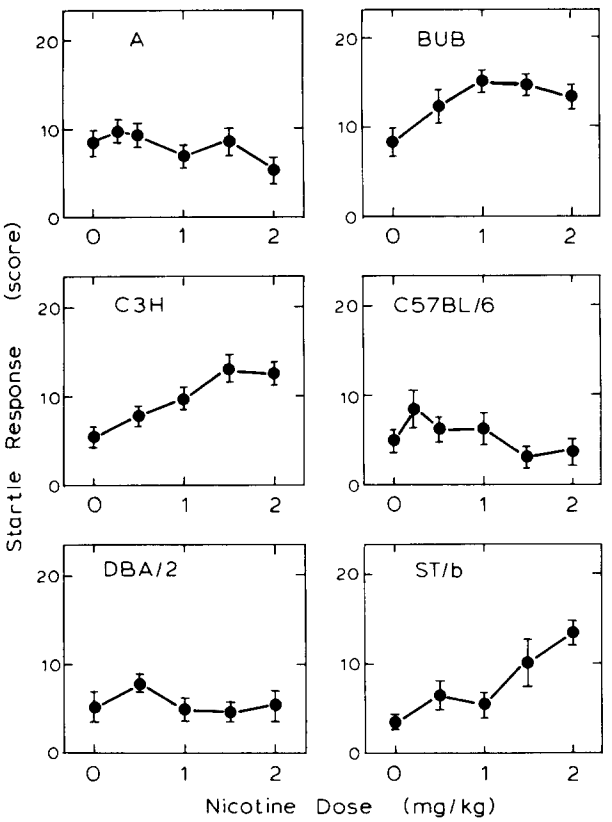

FIG. 2. Nicotine effects on acoustic startle response. Each mouse was exposed to ten acoustic stimuli 10 sec apart beginning 2.5 min after nicotine administration. Results shown represent mean ± SEM for 6–12 individual mice at each nicotine dose.

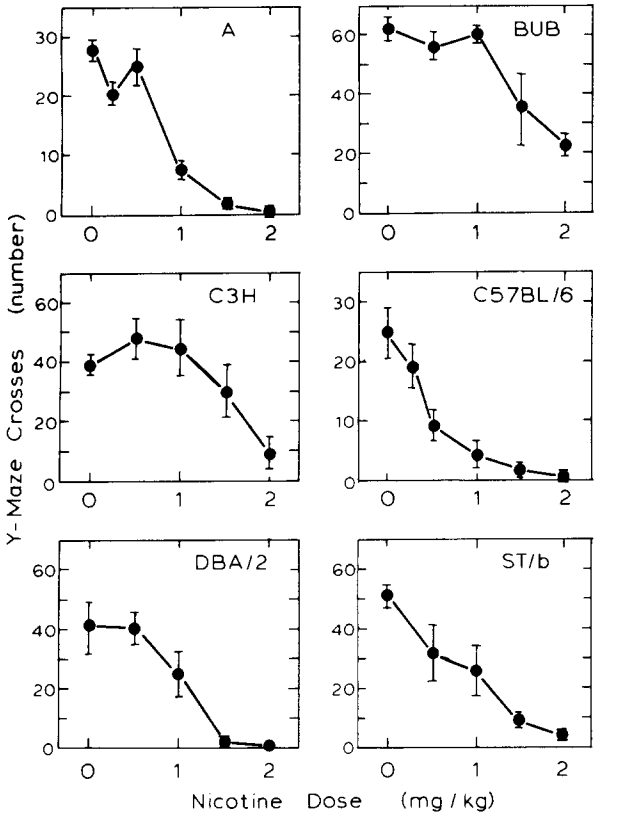

FIG. 3. Nicotine effects on Y-maze crosses. Total line crossings in a symmetrical Y-maze were counted during a 3-min period beginning 5 min after nicotine injection. Results shown are mean ± SEM of 6–12 animals at each data point.

in the magnitude of the strain effect which resulted from removing the influence of baseline differences in response.

Y-Maze Crosses

The effects of nicotine administration on the Y-maze crosses of the six representative strains are shown in Fig. 3. The pattern of response of each strain is a dose-dependent decrease in activity. For some strains, such as C3H, a modest increase in activity after adminstration of low doses of nicotine was suggested but these apparent effects were never found to be significant. The dose required to elicit the decrease in activity varied among the strains. For the strains shown, the dose required for a 50% reduction in the activity (ED_{50}) varied from 0.51 mg/kg for C57BL/6 mice to 1.89 mg/kg for BUB mice.

Control activity varied among the strains and these activities are summarized in Table 1. The most active strain (P) displayed approximately three times the activity of the least active strain (C57BL/6).

The dose-response curves for all 19 strains were analyzed. ED_{50} values were calculated and are shown in Table 2. A large range of ED_{50} values was obtained and the least sensitive strain (BUB, 1.89 mg/kg) required a dose nearly four times that required for the most sensitive strain (C57BL/10, 0.49 mg/kg) to reduce activity to 50% of control.

The results were further analyzed by ANOVA. Analysis of untransformed data confirmed both the strain and dose effects and also revealed significant strain-by-dose interactions (Table 3). This analysis is confounded in part by the large differences in control activity. To correct for these differences, each value for each strain was normalized by dividing by the average control activity of that strain (all average control activities were then 1.0). The transformed data were then subjected to two-way ANOVA (Table 3). The basic pattern of the results was similar to that obtained with untransformed data: significant main effects of strain and dose and a significant strain-by-dose interaction. The major difference between the two analyses was that the magnitude of the strain differences was reduced as a result of the elimination of differences in baseline activity.

Y-Maze Rears

The results shown in Fig. 4 are dose-response curves for the effects of nicotine on Y-maze rears in the six representative strains. Nicotine administration reduced the number of rears observed in the Y-maze for all strains, but the dose required to achieve this reduction varied among them. Similar to the results obtained for Y-maze crosses, a slight stimulation of rearing seemed to occur at low nicotine doses in C3H mice but this apparent effect was not significant. The dose required to reduce Y-maze rears to 50% of control (ED_{50}) varied among these mice with a low value observed for strain A (0.41 mg/kg) and a high value observed for strain C3H (1.50 mg/kg).

Control Y-maze rears varied among the six representative

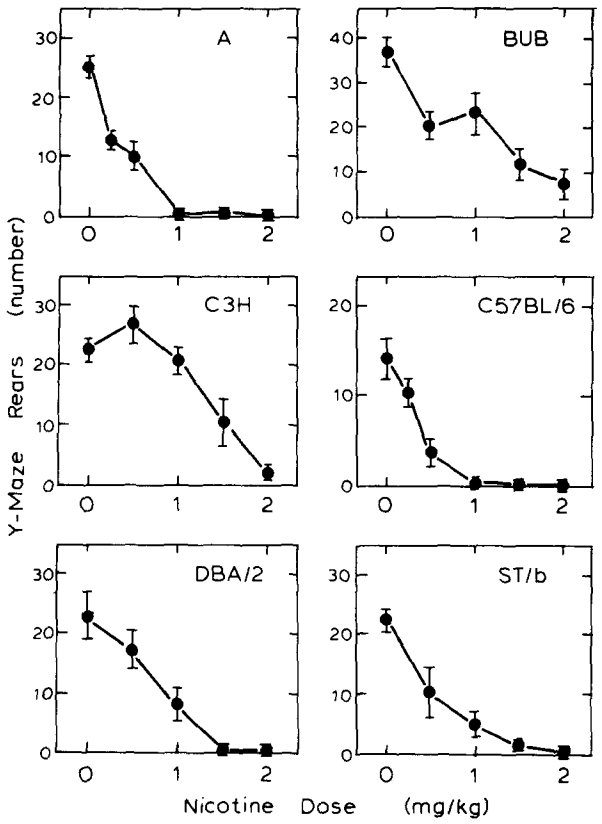

FIG. 4. Nicotine effects on Y-maze rears. Rears occurring in a symmetrical Y-maze were counted during a 3-min test period beginning 5 min after nicotine injection. Results shown are mean ± SEM of 6–12 animals at each data point.

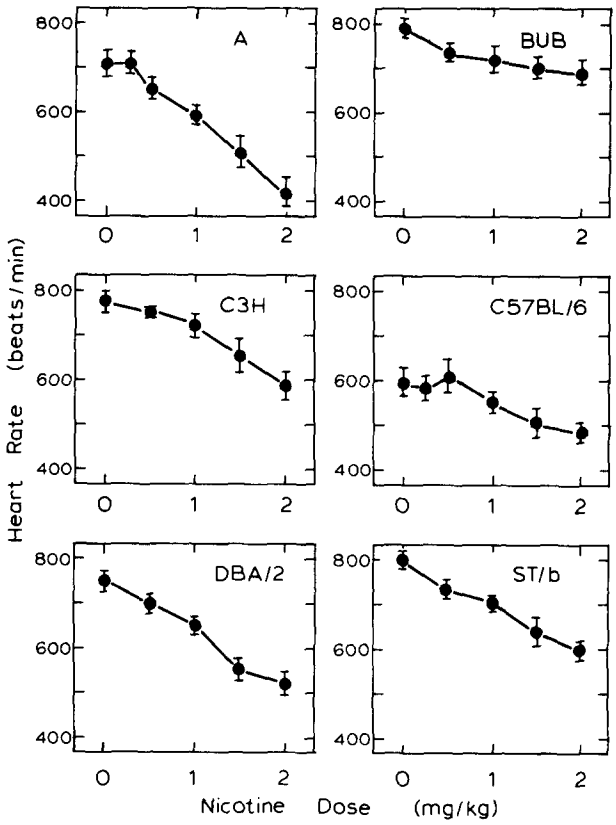

FIG. 5. Nicotine effects on heart rate. Heart rates were measured by counting the number of QRS complexes in a 6-sec period. Heart rate was measured 9 min after nicotine injection. Results represent mean ± SEM of 6–12 individual animals.

strains as indicated in Fig. 4, and control Y-maze rears also differed among all 19 strains as shown in Table 1. Mice of strain SWR had almost three times as many rears as did mice of strain C57BL/6.

The dose-response curves for nicotine effects on Y-maze rears for all 19 strains were analyzed, the ED_{50} values were calculated and these values are summarized in Table 2. A wide range of ED_{50} values was observed from a low of 0.37 mg/kg for strain C57BL/10 to a high of 1.54 mg/kg for strain C58.

The results were further analyzed for effects of strain and dose (0, 1 and 2 mg/kg) by two-way ANOVA. Analysis of the untransformed data confirm both the drug effect and the strain differences as indicated by significant main effects of dose and strain; a significant strain-by-dose interaction was also observed (Table 3). The results of this analysis are confounded by the differences among the strains in control Y-maze rears. To correct for these differences, each value for each strain was normalized by dividing by the average control rearing of that strain (all average control rears were then 1.0). The transformed data were then subjected to two-way ANOVA (Table 3). The basic pattern of the results was similar to that obtained with untransformed data: significant main effects of strain and dose and a significant strain-by-dose interaction. The major difference between the two analyses was that the magnitude of the strain difference was reduced as a result of the elimination of differences in control rearing.

Heart Rate

Dose-response curves for the effects of nicotine on the heart rates of mice of the six representative strains are shown in Fig. 5. A dose-dependent decrease in the heart rates occurred after nicotine administration for all strains tested. Mice of the various strains were differentially sensitive to the effects of nicotine. The ED_{-100} values (nicotine dose required to decrease the heart rates by 100 beats/min) of the six representative strains varied from 0.82 mg/kg for strain A to 1.48 mg/kg for strain BUB.

Control differences in heart rate among the 19 inbred strains were observed. These values are summarized in Table 1. Variability was less pronounced than that observed for the four tests discussed above: C57BL/6 had the lowest heart rate (594) and ST/b had the highest heart rate (797). Only four strains had average control heart rates of less than 700 beats/min.

Dose-response curves for nicotine effects on heart rates of all 19 strains were analyzed and the ED_{-100} values calculated. These values are summarized in Table 2. A range of values was observed (lowest ED_{-100} was 0.82 mg/kg for strain A and the highest was 2.19 mg/kg for strain SWR), but the 2.7-fold difference in values was not quite as wide as that observed for the other tests.

The results were further analyzed for strain and dose (0, 1 and 2 mg/kg) effects by ANOVA. The results of this analysis confirmed the strain differences observed in ED_{-100} value: significant main effects of strain and dose were observed. The strain-by-dose

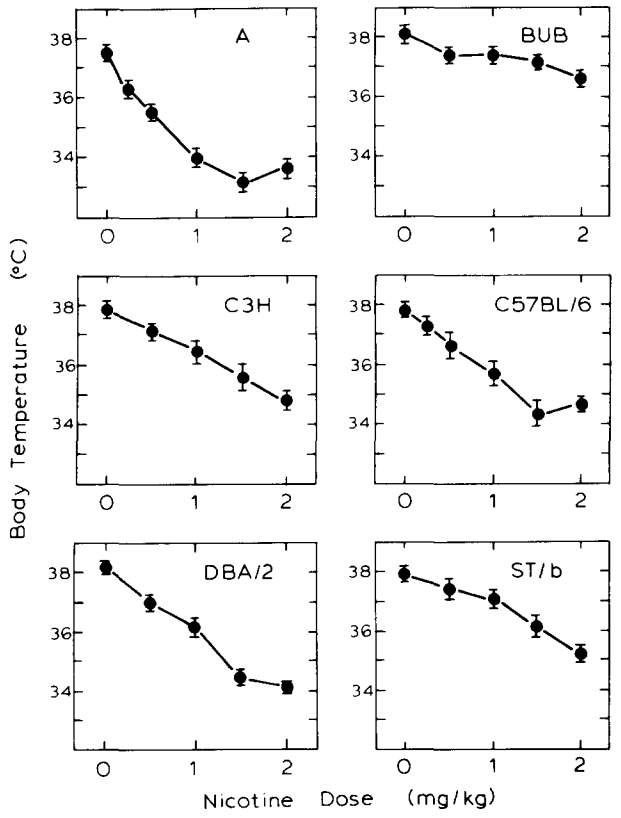

FIG. 6. Nicotine effects on body temperature. Rectal body temperature was measured 15 min after the IP administration of nicotine. Results shown are mean ± SEM of 6–12 mice.

interaction term was not significant (Table 3). A two-way ANOVA was also run on transformed data generated by subtracting average control heart rates from each individual value to yield a difference score (control values were set to zero). This analysis also indicated significant main effects of strain and dose, as well as a significant strain-by-dose interaction. The main effect of strain was less pronounced than that obtained for the nontransformed data (Table 3).

Body Temperature

The effects of nicotine on the body temperatures of the six representative strains are shown in Fig. 6. Nicotine administration lowered the body temperature of all strains tested, but the dose required to achieve the same extent of hypothermia varied among the strains. The $ED_{-2°}$ (dose required to lower body temperature by 2°) varied considerably among the six representative strains with the $ED_{-2°}$ for strain A being 0.55 mg/kg, while that for strain BUB was 2.53 mg/kg.

Control body temperatures varied modestly among the 19 strains (Table 1). Mice of the BALB strain had the lowest body temperature (37.3°) while mice of the DBA/1 strain had the highest (38.5°). Variation in control body temperature was the least extreme of all of the measures. The differences observed were significant, however.

Nicotine affected the body temperature of all 19 strains in the same way as it did that of the six representative strains: drug treatment resulted in a dose-dependent decrease in body temperature. Dose-response curves were analyzed and the $ED_{-2°}$ values calculated from these curves are summarized in Table 2. The high and low $ED_{-2°}$ values are those represented by strain A and strain BUB.

Analysis of the results by two-way ANOVA for effects of strain and dose (0, 1, and 2 mg/kg) confirmed that large differences existed among the strains. A highly significant main effect of both strain and dose was detected as was a significant strain-by-dose interaction (Table 3). Analysis of transformed data which was achieved by subtracting the average control temperature for each strain from each individual value (therefore setting starting temperatures at zero) also revealed significant main effects of strain and dose and a significant strain-by-dose interaction. The main effect of strain was smaller in the analysis of the transformed data, but the effect of data transformation was less marked for this measure, perhaps reflecting the narrower range of control body temperatures compared to the range of the other measures.

Factor Analysis of Nicotine Effects

Measurement of several responses in a number of inbred strains allows the data to be subjected to further analyses. The results on nicotine effects on respiratory rate, acoustic startle response, Y-maze crosses, Y-maze rears, heart rate and body temperature presented above, and of seizure sensitivity after an IP injection of nicotine or latency to seizure after IV infusion of nicotine (23) were compared. The first analysis undertaken was the construction of scattergrams and the calculation of intertest correlations relating the various measures. Several measures of nicotine effects on the 19 strains can be subjected to this analysis including, for example, response after a given dose of nicotine. In order to incorporate all of the observations into the analyses, the measures chosen as indicators of overall nicotine sensitivity were the various ED values for five test battery results and seizure sensitivity, the slope of the dose-response curve for startle response, and latency to seizure after IV nicotine infusion. The scattergrams for these variables are presented in Fig. 7. Correlations among the variables differ widely. For example, Y-maze crosses and Y-maze rears are highly correlated ($r = .93$), but respiratory rate and heart rate are not correlated ($r = .04$).

Inasmuch as many significant correlations among the variables were observed, the likelihood that the underlying physiological basis regulating response in one test shares properties in common with the physiological basis regulating the response in highly correlated test exists. Subsequently, the results were subjected to factor analysis in an attempt to determine whether a simplified relationship among the variables may be suggested. In general, a factor analysis assumes that relationships among several variables occurs because these variables are all influenced by relatively few underlying common variables, called factors. The goal of the analysis is to describe a set of experimental observations with as few underlying factors as possible. The resulting simplification of a complex set of observations may be useful in categorizing the observations and understanding the possible relationships among them. Since several methods of factor extraction (principle components, maximum likelihood and generalized least squares) provided very similar results, only the results obtained from the principle components analysis are presented here. This extraction method yielded two factors: the first with an eigen value of 3.33 accounted for 41.6% of the variance and the second with an eigen value of 2.26 accounted for an additional 28.2% of the variance. The two factors together accounted for 69.8% of the variance among the variables measuring nicotine responsiveness. The graph in the lower section of Fig. 7 displays the factor loadings for the eight measures of nicotine responsiveness. Three of the responses measured, Y-maze crosses, Y-maze rears, and body temperature,

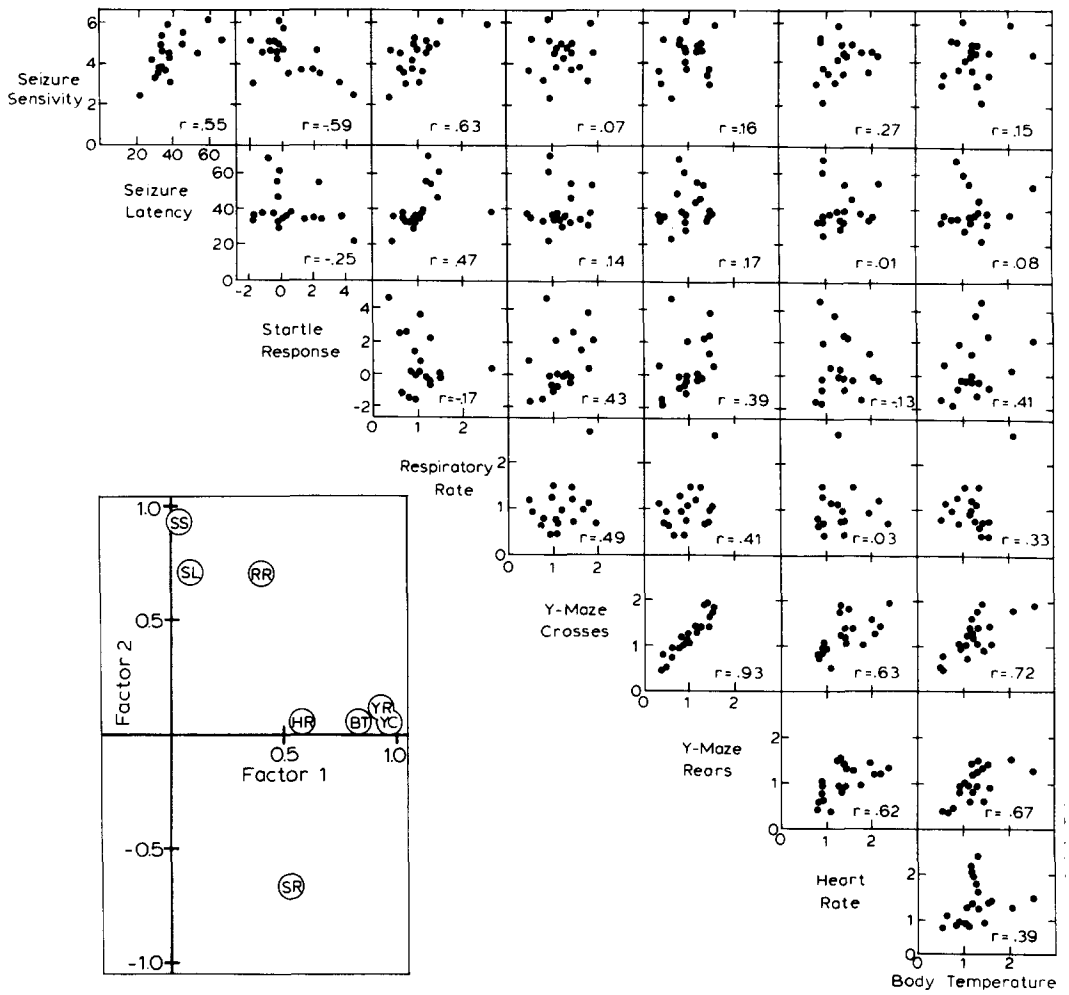

FIG. 7. Intertest correlations and factor analysis. Potential interrelationships among the test battery results and seizure measures (23) were assessed by correlational analysis followed by factor analysis. The following measures of nicotine effects were plotted: for Y-maze crosses, Y-maze rears and seizure sensitivity the ED_{50} value in mg/kg; for seizure latency, the time to clonic seizure after intravenous infusion of 2 mg/kg/min nicotine; for respiratory rate the ED_{260} in mg/kg; for acoustic startle response, the slope of the dose-response curve (change in startle score/mg/kg); for heart rate, ED_{-200}; and for body temperature, ED_{-2°. Each point represents the values measured for an individual strain for the paired tests. Correlation coefficients are given in each panel. The panel in the lower left of the figure depicts the factor loadings determined after a principle components factor analysis followed by varimax rotation of the resulting factors. Two factors were extracted and the loadings calculated for each response measured are plotted. The abbreviations used are: SS, seizure sensitivity; SL, seizure latency; RR, respiratory rate; SR, startle response; YC, Y-maze crossings; YR, Y-maze rears; HR, heart rate; and BT, body temperature.

load heavily and almost exclusively on factor 1, while the two seizure measures load heavily and almost exclusively on factor 2. Although heart rate loads exclusively on factor 1, the loading is much less than those for the other measures. The remaining two measures, respiratory rate and startle response, shared loadings with both factors. The loading patterns of these two responses differ, however. High ED_{260} values for respiratory rate are more likely related to high values for both the seizure group responses and the four-test responses, while enhanced startle response is related to high ED values for the four test factor and to low ED_{50} for seizure sensitivity and short seizure latencies.

The two-factor model explains at least 53% of the variance for all tests except heart rate, for which this model explains only 32% of the variance. Forcing a third factor into the model now accounts for 82.1% of the variance but this factor seems to be unique to heart rate (95% of variance for this variable now explained) but did not markedly improve the fit of any of the other tests.

Overall Differences in Strain Sensitivity to Nicotine

The results generated from the factor analysis grouped the responses of the inbred mice in such a way that a general pattern of sensitivity can be examined using the two factors as the grouping variables. For the first group of responses, the ED values for Y-maze crosses, Y-maze rears, and body temperature were averaged. Since the units of measure for the second group of responses, seizure sensitivity and seizure latency, differ these measures were first normalized by dividing by the average ED_{50} for seizure sensitivity and the average latency to seizure for seizure latency. Since nicotine effects on heart rate were poorly explained

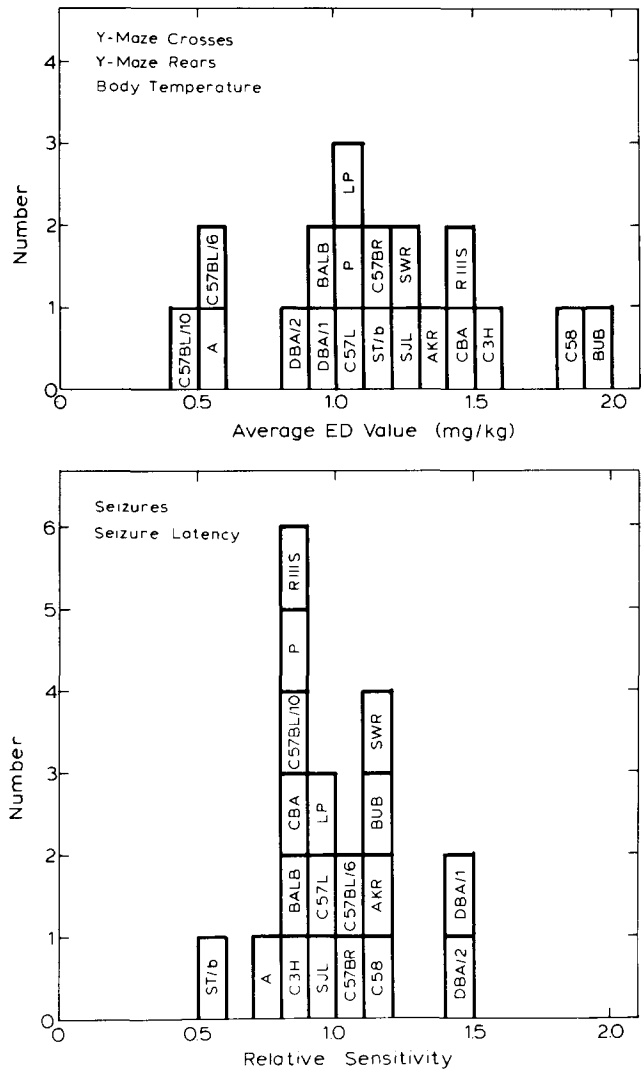

FIG. 8. Overall sensitivity of inbred strains. Distribution of sensitivity of the 19 strains as measured by the two major responses groups extracted by factor analysis were calculated. In the upper panel the ED values for three tests (Y-maze crosses and rears and body temperature) were averaged and in the lower panel measures of responsiveness in the seizure tests were combined (for seizure sensitivity average ED_{50} was defined as 1.0, and for seizure latency average time to seizure was defined as 1.0).

by the two-factor model and since respiratory rate and startle response were best explained by a combination of the two factors, these responses were not included in the strain grouping. The results in Fig. 8 are histograms for the responsiveness of the strains for the two major nicotine response types. These histograms should be regarded as a general overall demonstration of the strain sensitivity since the values presented are composites of several responses.

The histograms in Fig. 8 suggest several distinct subgroups of strains with different sensitivities to nicotine for both of the major response types. The grouping of the strains was subsequently tested using cluster analysis. The cluster analysis is designed to combine into groups those strains displaying the most similarity in ED values and thereby identify those strains responding similary to nicotine. For this analysis the ED values for the individual tests were employed so that the cluster analysis of the activity/body temperature group was based on three measures per strain and that for the seizure group on two measures.

Cluster analysis of the ED_{50} values for the members of the three-test factor indicated that four groups of strains could be classified: 1) a nicotine sensitive group (C57BL/10, C57BL/6, and A; average ED value = 0.47 mg/kg); 2) a modestly nicotine-sensitive group (DBA/2, DBA/1, BALB, C57L, P, LP, St/b and C57BR; average ED value 0.90 mg/kg); 3) a modestly nicotine-resistant group (SJL, SWR, AKR, CBA, RIIIS, and C3H; average ED value = 1.21 mg/kg); and 4) a nicotine-resistant group (C58 and BUB; average ED value = 1.60 mg/kg). Since the correlation between seizure sensitivity and seizure latency was not as robust as that among Y-maze crosses, Y-maze rears, and body temperature, the cluster analysis of these data did not produce as clear a pattern. Nevertheless, cluster analysis seemed to combine the strains into five groups: 1) very seizure sensitive (ST/b alone, relative sensitivity = 0.57); 2) seizure resistant (DBA/1 and DBA/2; relative seizure sensitivity = 1.45); 3) relatively seizure-sensitive by both measures (A, C3H, BALB, CBA, C57BL/10, and RIIIS; relative seizure sensitivity = 0.84); 4) slightly seizure resistant, but with relatively short seizure latencies (P, SJL, C57L, LP, C57BR, C57BL/6, and C58; relative sensitivity = 1.13, relative latency = 0.88); and 5) slightly seizure resistant with relatively long seizure latencies (AKR, BUB and SWR; relative sensitivity = 1.07, relative latency = 1.28).

DISCUSSION

The results presented in this paper confirm and extend the observations that genetic factors influence the responsiveness of mice to the effects of nicotine. The 19 strains tested displayed a wide range of sensitivity to the effects of nicotine on each of the six responses measured. The advantages gained by examination of this large number of inbred strains include the ability to apply factor and cluster analyses in an attempt to group the various responses and search for commonalities among them. In this regard, the results presented here have been modestly successful. Factor analysis suggests that two major variables contribute substantially to the expression of nicotine sensitivity for all responses measured except, perhaps, heart rate. Body temperature, Y-maze crosses and Y-maze rears appear to be primarily affected by one of the variables, while the seizure measures are primarily influenced by the second variable. Respiratory rate and startle response are influenced by both variables. The responses measured are not identical (except perhaps Y-maze crosses and Y-maze rears), however, since a substantial fraction of the inter-strain variability remains to be explained. This result suggests that each response has certain unique aspects, reflecting perhaps the operation of slightly different physiological parameters. But the results of this analysis suggest that at least part of the expression of the major subgroups of responses may be controlled by a common mechanism.

A similar grouping of responses has been suggested by examination of the effects of the nicotinic antagonist, mecamylamine, on nicotine effects on test battery responses and seizure sensitivity (5). In that study, the ED_{50} values for mecamylamine blockade of nicotine effects on DBA/2 and C3H mice comprised two major groups: 1) responses blocked at low doses of mecamylamine (ED_{50} about 0.08 mg/kg for blockade of seizures and enhanced startle) and 2) responses blocked at high doses of mecamylamine (ED_{50} about 1 mg/kg for blockade of nicotine effects on Y-maze crosses, Y-maze rears, heart rate, body temperature and respiration). This grouping is similar to that obtained by factor analysis reported in this study. The responses influenced by both factors (respiratory rate and startle response) were included in different groups by mecamylamine analysis.

The three responses grouped together by the factor analysis (Y-maze crosses, Y-maze rears, and body temperature) also respond in a similar fashion in DBA mice treated chronically with nicotine both as a function of infusion dose (15) and time (20). Tolerance to the effects of nicotine on Y-maze crosses and rears and body temperature was lost 8–12 days after cessation of nicotine treatment (20), but tolerance to seizures induced by IV infusion disappeared 5 days after withdrawal (22). The two-fold difference in rate of return to normal sensitivity suggests that the activity and body temperature measures are controlled by a different process than is seizure latency. In addition, some tolerance to nicotine-induced bradycardia was still present 20 days after withdrawal (20), suggesting that this measure differs from those discussed above. The subdivision of the responses suggested by the withdrawal studies is very similar to that suggested by the factor analysis of the test results presented in the present paper.

The classification of the nicotine effects into two major groups suggests that two different neurochemical mechanisms may be activated by nicotine administration. One possibility is that two receptor systems control nicotine responsiveness. In fact, two different nicotinic binding sites have been identified in rodent brain: one assayed by high affinity nicotine or acetylcholine binding and a second assayed with the binding of the snake neurotoxin, α-bungarotoxin (4, 21, 27). Both sites have pharmacological profiles consistent with those expected for nicotinic receptors. These putative receptors have markedly different properties, are differentially distributed throughout the brain, can be separated from one another, and have different molecular structure. While simple differences in the receptors activated may explain part of the classification of the complex responses measured in whole animals, it is unlikely that an absolute relationship between genetic diversity in receptor levels and in vivo responsiveness will be found. The following paper addresses this point through the measurement of nicotine and α-bungarotoxin binding sites in eight brain regions of each of the 19 strains tested in the present study.

Several pairs of relatively closely related strains have been tested (DBA/1 and DBA/2, C57BL/6 and C57BL/10, C3H and CBA, C57BR and C57L) and in general the responses of mice of these pairs are similar for both major response types, suggesting that responses to nicotine are relatively stable over time. This result may indicate that the underlying neurochemical mechanisms controlling nicotine response are conserved. In contrast to the similarity in nicotine responsiveness between closely related members of the C57 family, members of the larger C57/C58 family (C57/BL/6, C57BL/10, C57BR, C57L and C58) differed markedly in their sensitivity to nicotine. Note that C57BL/6 and C57BL/10 are among the most sensitive strains, while C58 is among the most resistant. Whether this large difference occurred when these strains were established or has arisen from genetic drift is unknown. The similarity observed between closely related substrains argues against drift.

Testing of strains thought to be unrelated to others (AKR, BUB, RIIIS, SJL, SWR and ST/b) has been useful in defining some extremes of responsiveness. For example, ST/b mice appear to be uniquely seizure sensitive while BUB mice are markedly restant to nicotine effects on activity and body temperature.

The results presented in the current study when grouped with those determined previously for the seizure-sensitivity of these same strains (23) define inbred mouse strains that differ markedly in their responses to nicotine. The identification of such widely different strains provides a basis on which to choose strains for further genetic analysis and for the study of genetic influences on both acute and chronic response to nicotine. The use of appropriate strains will substantially improve the power of the genetic analysis of nicotine's effects.

ACKNOWLEDGEMENTS

This work was supported by grant DA03194 from the National Institute on Drug Abuse. A. C. Collins is supported, in part, by a Research Scientist Development Award (DA00116).

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