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A Test Battery for Measuring Nicotine Effects in Mice

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MARKS, M. J., E. ROMM, S. M. BEALER AND A. C. COLLINS. *A test battery for measuring nicotine effects in mice.* PHARMACOL BIOCHEM BEHAV **23**(2) 325–330, 1985.—A test battery consisting of measurement of respiration, startle response, Y-maze activity, heart rate, and body temperature has been developed to assess the effects of nicotine on the mouse. Results obtained using the test battery were compared to those obtained with each test individually in four inbred strains of mouse (BALB, C57BL, DBA and C3H). No significant differences between the results obtained using the test battery and those obtained with individual tests were found. The results did demonstrate, however, that the genotype of the mouse strongly influenced the responses in several of the tests.

Nicotine Genetics Locomotor effects Heart rate Respiration rate Body temperature Startle response

THE effects of the drug, nicotine, are complex. Not only does nicotine interact with specific receptor sites in the central nervous system and periphery, it also has biphasic effects on its receptors. These biphasic effects are manifested in a number of ways. Langley and Dickinson [10] were the first to note that low doses of nicotine will stimulate autonomic ganglia while higher doses will induce blockade. At the electrophysiological level, it has long been known that nicotine induces a transient stimulation of electrical activity which is followed by a longer lasting blockade [9]. This phenomenon is referred to as desensitization of the nicotinic receptor. Consequently, the physiological and behavioral responses to nicotine are multiple and varied. Administration of this agent to rodents affects, among other responses, locomotor activity, body temperature, heart rate, respiration, and, at high doses, causes convulsions [1, 2, 3, 5, 6, 8, 11, 13, 15, 16]. Not only are many different responses observed, but the magnitude of these responses is affected by the genotype of the animal. For example, the effects of nicotine on locomotion in rats [3, 5, 6, 15] and mice [2, 8, 11] are dependent on the strain of the animal tested. Other responses are also influenced by strain [11,13]

The responses of rodents chronically treated with nicotine also change. Tolerance to the effects of nicotine on locomotion has been observed in both rats [4, 17, 18] and mice [2, 7, 12] after chronic treatment with the drug. Other responses to nicotine are changed by chronic nicotine treatment, as well [12].

Because of the complexity of nicotine response, the ability to measure several different parameters would be of value in fully assessing the effects of this drug. A further advantage would be obtained by the measurement of several responses in an individual animal. This would be particularly valuable in the study of genetic influences on nicotine response and in the assessment of tolerance to the drug after chronic treatment. The measurement of many responses in a segregating or heterogeneous population of mice would permit the calculation of correlations among the responses and would allow genetic analysis of the relationship among these responses. It may also permit the selection of populations of mice with differing responses to this drug. The measurement of many responses in chronically treated animals would provide a more detailed analysis of the effects of the treatment on the response of the animal than would be attained with a single measurement. It would also provide an economy of effort in the study of tolerance development and enhance the power of analysis of the relationship between the responses of the animal and the biochemical and physiological bases for those responses.

The difficulty encountered in using a battery of tests to measure the responses of an animal arises because the repeated testing of a single animal may alter the responses measured at later times of testing; that is, a carryover effect may exist. The present study was undertaken to determine if carryover effects influence the responses of mice to nicotine in a battery consisting of the following tests: respiration, startle response, Y-maze activity (both crosses and rears), heart rate, and body temperature. These studies were conducted using four inbred strains of mouse (BALB, C57BL, DBA, and C3H) to determine if genotype influences the extent of the carryover and to provide additional information about the genetics of nicotine response in inbred mice.

METHOD

Animals

Female mice of four inbred strains were used in this study. C57BL/61bg, DBA/21bg, and C3H/21bg mice

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TABLE 1
TIMING AND ORDER OF MEASUREMENTS IN THE TEST BATTERY

Time after Injection	Response Measured	Test Duration
1 min	Respiratory Rate (5 Observations)	60 sec
3 min	Startle Response (10 Stimuli)	90 sec
5 min	Y-Maze Activity (Crosses and Rears)	120 sec
9 min	Heart Rate (Rate by QRS complexes)	6 sec
15 min	Body Temperature	10 sec

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. Female 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 five generations. All mice were weaned at 25 days of age and were housed with 1–5 female littermates. Animals were 60–90 days old when tested.

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 administrered by intraperitoneal injection. Injection volume was 0.01 ml/g body weight. Four drug doses were used: 0.0, 0.5, 1.0, and 2.0 mg/kg.

Testing

Mice of each of the four strains were divided into six groups. One group was tested using a complete test battery consisting of the five tests described below. Each of the other five groups was tested for the effects of nicotine on only one of the five tests. Ten mice were tested at each nicotine dose in each test group. An outline of the tests used and the timing of the tests are presented in Table 1.

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 with the appropriate dose of nicotine. 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 one min after injection of the nicotine. Respiratory rate was monitored for one min during which time five equally spaced recordings were made. Animals were tested one min after injection because this is the time at which nicotine maximally stimulates respiratory rate [11].

Startle Response

The response of the 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. Those mice which were tested with the battery had previously had their respiration measured. 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 (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). 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 [11]. 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 [11].

Y-Maze Activity

Both locomotor and rearing activity were determined in a symmetrical Y-maze. The maze consists of three arms which are 26 cm long, 6.1 cm wide and 10.2 cm high. Each arm was subdivided into two equal sections. The maze 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 three min. Movements from one section to another were counted, as were the number of rears. Those mice tested as part of the battery had previously been tested for both respiration and startle response. Testing at 5 min after nicotine injection assures near-maximal depression of Y-maze activity [8].

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 the 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. Those mice measured in the test battery had previously been tested for respiration, startle response, and Y-maze-activity.The time of 9 min was chosen from preliminary experiments in which the effect of nicotine was measured as a function of time (results not shown) and found to be maximal or near maximal for 6 to 12 min after injection.

Body Temperature

Body temperature was measured with a Bailey Instru-

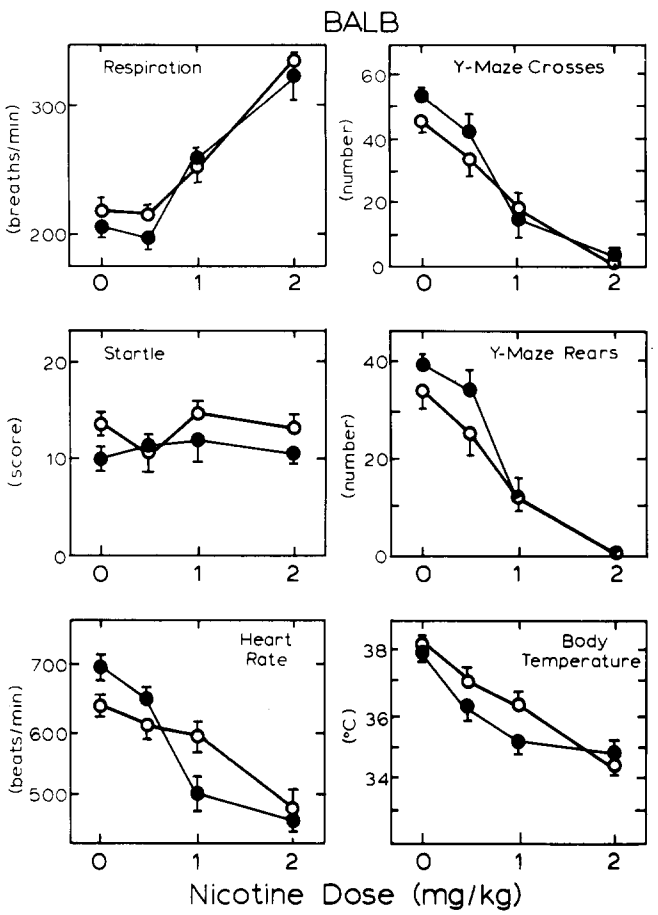

FIG. 1. Nicotine effects on BALB mice. Responses of BALB female mice in each of the tests indicated were measured individually (●) or as part of a test battery (○). Each point represents the mean±SEM of 10 mice.

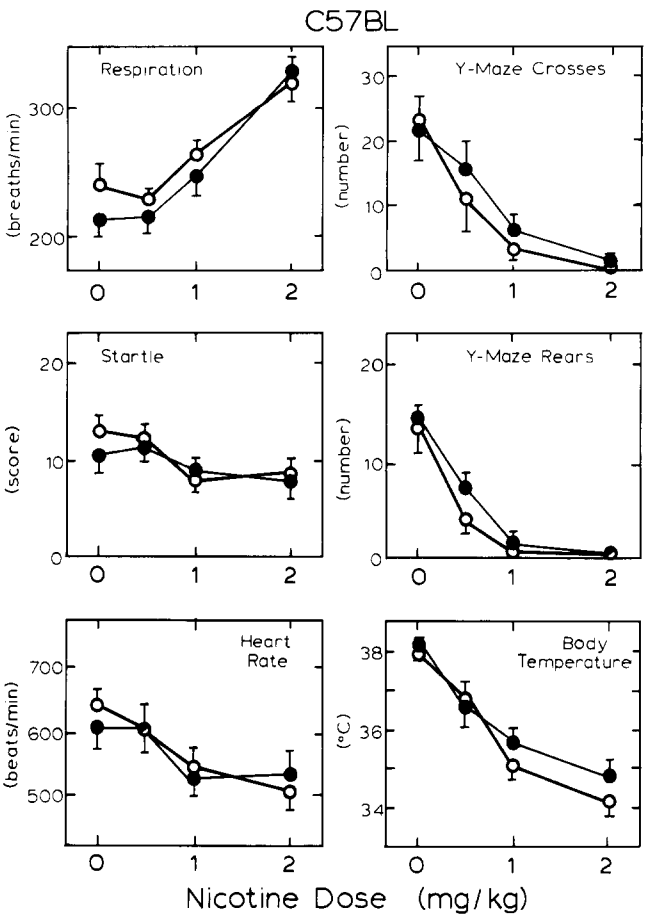

FIG. 2. Nicotine effects on C57BL mice. Responses of C57BL female mice in each of the tests indicated were measured individually (●) or as part of a test battery (○). Each point represents the mean±SEM of 10 mice.

ments 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 [11]. Those mice measured in the test battery had been previously subjected to all of the other tests.

Data Analysis

All data were analyzed using a three-way Analysis of Variance (ANOVA) to determine main effects of strain, dose, and test condition (either individual tests or test battery), as well as interactions among these variables. Untransformed data were analyzed for four of the tests, but since substantial differences in basal (saline-injected) Y-maze activity were found, these data were analyzed using transformed data. The transformation employed (normalizing the scores for the individual test saline activities for each of the four inbred strains) has been previously used to analyze the effects of nicotine on open-field activity in these same four inbreds [9]. For those analyses in which significant effects were observed, the results were subjected to Newman-Keuls' *post hoc* test.

RESULTS

The results for the responses of the four inbred strains, BALB, C57BL, DBA, and C3H, are given in Figs. 1, 2, 3, and 4, respectively. Data from all six tests (respiration, startle response, Y-maze crosses, Y-maze rears, heart rate, and body temperature) are included in each figure, as are the results obtained using individual tests and the test battery. The responses for each test were analyzed separately using three-way ANOVAs (strain×nicotine dose×test condition). For those tests where differences in baseline values were observed, analyses were also carried out on transformed data to eliminate these differences. The results obtained from these analyses will be presented in turn.

A significant effect of nicotine dose, $F(3,265)=106.35$, $p<0.001$, and a significant strain by dose interaction, $F(9,265)=3.34$, $p<0.001$, were found for respiration. No other main effects or interactions were significant. The absence of a significant effect of test condition was to be expected for this measurement, since it was the first response measured in the test battery. The respiratory rate was different at each dose of nicotine, accounting for the main effect of dose. The interaction term arose from the responses of DBA mice. Baseline respiration rate differed among the four in-

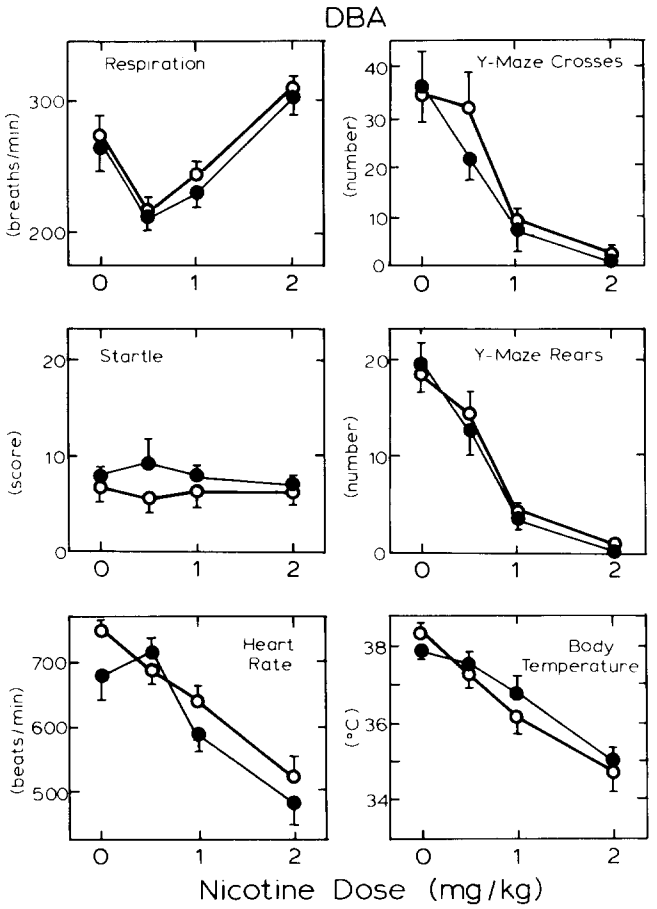

FIG. 3. Nicotine effects on DBA mice. Responses of DBA female mice in each of the tests indicated were measured individually (●) or as part of a test battery (○). Each point represents the mean±SEM of 10 mice.

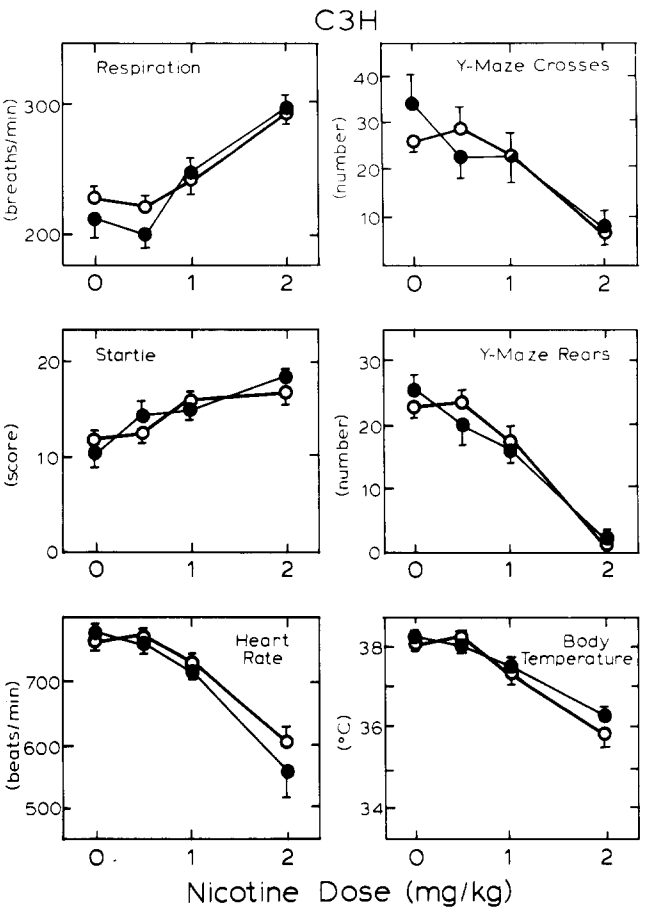

FIG. 4. Nicotine effects on C3H mice. Responses of C3H female mice in each of the tests indicated were measured individually (●) or as part of a test battery (○). Each point represents the mean±SEM of 10 mice.

bred strains, $F(3,65)=4.77$, $p<0.01$. DBA mice had significantly higher control respiratory rates than did any of the other inbreds. It should be noted that DBA mice exhibited more grooming and exploratory behavior in the test apparatus than did mice of the other strains. The difference in respiration rate seen in the DBA strain may be related to this difference. No differences in respiration among the strains was detected after any non-zero dose of nicotine.

While no significant main effect of nicotine dose was found for the startle response, both a main effect of strain, $F(3,269)=40.48$, $p<0.001$, and a strain by dose interaction, $F(9,269)=4.72$, $p<0.001$, were found. Neither the main effect of test condition nor any interaction with test condition was significant. The main effect of strain arose because all four strains differed from each other. The strain by dose interaction term was significant because only C3H mice were affected in this test, displaying a significant increase in startle response after injection of either 1.0 or 2.0 mg/kg of nicotine. Nicotine had no effect on the startle response of the other three strains. These results are confounded because of differences in baseline response, $F(3,65)=3.68$, $p<0.05$. DBA mice had a lesser response than did mice of the other three strains. The results were subsequently analyzed as the differences between control and drug treated groups. Although the effects were less robust, both the main effect of strain, $F(3,269)=8.07$, $p<0.001$, and the strain by dose interaction, $F(9,269)=1.921$, $p=0.0491$, remained significant. All other main effects and interactions were not significant.

Two measures were obtained from the Y-maze: line crossings and rears. Differences in control activities among the strains were evident (baseline crosses, $F(3,64)=11.41$, $p<0.001$, and rears, $F(3,64)=29.23$, $p<0.001$. The actual values for both rears and crosses are given on the figures. These two parameters were analyzed separately for the effects of strain, nicotine dose, and test condition.

The three-way ANOVA of the results of the normalized Y-maze crossings revealed significant main effects both of strain, $F(3,259)=4.29$, $p<0.01$, and nicotine dose, $F(3,259)=90.00$, $p<0.001$. The strain by dose interaction was marginally significant as well, $F(9,259)=1.92$, $p=0.0495$. Neither the main effect of test condition nor any of the other interactions were significant. The main effect of strain arose because the average activity of C3H mice was greater than that of the other three strains. The activities observed after the administration of any dose of nicotine were lower than those of controls, accounting for the main effect of nicotine dose. The modest strain by dose interaction may have arisen from two sources: activity of C3H mice after a dose of 1.0 mg/kg of nicotine was higher than those of the other three strains, and this activity was not significantly lower than

baseline activity for this strain. The analysis of the untransformed data yielded similar results: significant main effects of strain, $F(3,259)=6.36$, $p<0.01$, and dose, $F(3,259)=26.15$, $p<0.001$. However, the strain by dose interaction term was no longer significant, $F(9,259)=1.41$, $p>0.05$. Neither the main effect of test condition nor any interaction was significant.

The pattern emerging from the three-way ANOVA for normalized Y-maze rears was similar to that found for Y-maze crosses: significant main effects of strain, $F(3,259)=10.41$, $p<0.001$, and dose, $F(3,259)=168.6$, $p<0.001$, as well as a significant strain by dose interaction, $F(9,259)=4.00$, $p<0.001$. Neither the main effect of test condition, nor any of the other interactions were significant. As was the case with the Y-maze crosses, the C3H mice displayed significantly more rears than did the other three strains. The number of rears exhibited by BALB mice was greater than that exhibited by C57BL mice, as well.The rearing activity was lower after each dose of nicotine than it was after saline, accounting for the main effect of dose. The strain by dose interaction appeared to arise from the following sources: rearing of C57BL mice was less than that of the other three strains after administration of a dose of 0.5 mg/kg of nicotine, and rearing activity of C3H mice was greater than that of the other three strains after a dose of 1.0 mg/kg of nicotine. The analysis of the untransformed data yielded similar results: significant main effects of strain, $F(3,259)=17.94$, $p<0.001$, dose, $F(3,259)=40.68$, $p<0.001$, and a significant strain by dose interaction, $F(9,259)=3.24$, $p<0.001$. The main effect of test condition and all other interactions remained nonsignificant.

The results of the three-way ANOVA for heart rate revealed significant main effects of strain, $F(3,264)=34.54$, $p<0.001$, dose, $F(3,264)=61.71$, $p<0.001$, and a significant strain by dose interaction, $F(3,264)=1.97$, $p<0.05$. Neither the main effect of test condition nor any of the other interaction terms were significant. The main effect of strain arose because the overall heart rate of C3H mice was greater than that for the other three strains and because the overall rate for DBA mice was greater than that for both BALB and C57BL mice. Part of this effect may have arisen because the control heart rates of C3H and DBA mice were greater than that for BALB and C57BL mice. The overall main effect of dose can be attributed to the significant depression of heart rate observed after the administration of 1.0 or 2.0 mg/kg of nicotine. Again, effects on C3H mice appear to account for the interaction. While the heart rates of three strains were significantly depressed after treatment with 1.0 mg/kg of nicotine, that of the C3H mice was not. Since baseline heart rates differed among the strains, $F(3,64)=12.15$, $p<0.001$; C3H, DBA rates > BALB, C57BL rates, the analysis of the raw data was confounded. Subsequent analysis of the results as change from control indicated that the main effect of dose, $F(3,264)=16.07$, $p<0.001$, was the only significant effect. The main effect of strain and the strain by dose interaction may have been observed because of basal differences among the strains.

The final response measured was body temperature, which showed main effects of both strain, $F(3,264)=11.88$, $p<0.001$, and dose, $F(3,264)=62.42$, $p<0.001$. No differences amont the strains in baseline body temperatures were noted, $F(3,63)=1.44$, $p>0.05$. Neither the main effect of test condition nor any of the interactions were significant. The strain difference apparently arose because the body temperature of C3H mice was significantly greater than that of the other three strains and that of DBA mice was greater than that of the C57BL and BALB mice. No differences in control body temperature were evident, however. The main effect of dose arose because the body temperatures measured after injection of 1.0 or 2.0 mg/kg of nicotine were less than those after saline injection. Mice of the C3H strain were less affected than those of other strains: the body temperatures of C3H mice were greater than those of BALB and C57BL mice after treatment with 1.0 mg/kg of nicotine and were greater than those of the other three strains after treatment with 2.0 mg/kg nicotine.

DISCUSSION

Two main types of observations have been made in this study: genetic differences in nicotine effects in mice have been confirmed, and measurement of these effects as part of a test battery yielded results identical to those obtained when each component of the test battery was measured separately. The second result could not have been predicted *a priori*, since intertest interactions may very well have influenced responses measured at later times in the battery. For example, the stress induced in a mouse by the measurement of its startle response may have been predicted to have an effect on its Y-maze activity. A similar effect may also have been predicted for the measurement of heart rate (in which needle electrodes have been inserted into the animal) prior to the measurement of body temperature. The absence of any main effect of test condition (individual measurement vs. test battery measurement) for any of the tests as well as the absence of any interaction between test condition and the other main effects strongly suggests that the results obtained as part of the test battery were identical to those obtained when only a single measurement was made.

The differences among mouse strains in response to nicotine varied from test to test. No significant strain differences were observed for nicotine effects on respiration, a result consistent with that reported previously for these four strains 1 min after drug injection [11]. For the other tests, the genotype of the mouse was an important factor influencing response to nicotine. For several of the tests (Y-maze activity and rears, heart rate. and body temperature) mice of the C3H strain were, in general, less sensitive than those of the other strains. This resistance to the effects of nicotine is consistent with previous observations on Y-maze activity [8] and other locomotor tests [2,11], as well as on body temperature [11]. The genetic influences on heart rate, which have been reported here for the first time, are basically similar to those for the other tests: higher doses of nicotine were required to depress the heart rate of C3H mice than were required to depress the heart rates of mice of the other three strains. However, the statistical analyses of the heart rate data are influenced by the baseline differences among the strains. When the results were analyzed in terms of change from control, no strain differences in nicotine effects were detected. In view of the variability in heart rate within and among the strains, measurement of this response might be best achieved by using each animal as its own control.

While responses of C3H mice in the tests discussed above were qualitatively similar to those of the other three strains, those responses measured for startle were different than those of the other strains. Nicotine had no significant effect on the responses of three of the strains, but increased the magnitude of the startle response of C3H mice in a dose-dependent manner. This fundamental difference in effect of

nicotine on the startle response has been noted previously [11].

The strain differences in response to nicotine observed in this study are not likely to be due to differences in the metabolism or distribution of nicotine, Several studies have assessed nicotine metabolism in these mouse strains [2, 7, 14]. These studies have failed to detect any significant differences in the rate of nicotine elimination or distribution into brain. These findings, coupled with our observation that the relative rank order of the strains to nicotine varies from test to test, suggest that pharmacodynamic rather than pharmacokinetic differences underlie the strain differences in responses to nicotine.

It would appear from these studies that the test battery will be a valuable tool to investigate the responses of mice to nicotine for several reasons. Nicotine had significant effects on each test in the battery (with the exception of startle response in strains other than C3H). This should allow the use of the battery for a number of studies including the assessment of the development of tolerance to nicotine after chronic drug treatment. A smaller test battery has previously been used for this purpose [12]. The differences in responses among the inbred mice and the identity of these responses as measured with the test battery and individual tests should make the use of the test battery valuable in further assessing the genetics of nicotine response both in segregating and heterogeneous populations and should provide a powerful multifactorial method to investigate the inheritance of nicotine effects and the relationship of these responses to underlying biochemical and physiological parameters.

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