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Individual Differences in Sensitivity to Nicotine in Mice: Response to Six Generations of Selective Breeding

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SMOLEN, A., M. J. MARKS, J. C. DEFRIES AND N. D. HENDERSON. *Individual differences in sensitivity to nicotine in mice: Response to six generations of selective breeding.* PHARMACOL BIOCHEM BEHAV 49(3) 531–540, 1994.—Four hundred seventeen heterogeneous stock mice were tested for their relative sensitivity to a low dose of nicotine (0.75 mg/kg) using activity in an automated Y-maze and body temperature as response measures. A wide spectrum of individual responsiveness to nicotine, ranging from complete suppression of activity to stimulation above baseline activity, was found. Replicate measures taken 1 week later on the same animals showed the responses to nicotine to be reliable and reproducible. Activity levels and body temperatures following nicotine administration were highly correlated (r = 0.60, df = 415). From analysis of between-litter proportions of variance, the heritability of nicotine-influenced activity was estimated to be 0.12, indicating that selective breeding for differential responsiveness to nicotine would be possible. The 10 most activated and 10 most depressed male and female mice were chosen as breeders for replicate nicotine activated (NA) and nicotine depressed (ND) lines, respectively. The selection criterion was nicotine-induced activity corrected for baseline activity using regression residuals. After six generations of selective breeding a good response to selection was obtained, although the response was better for the ND than for the NA lines. Realized heritability for responsiveness to nicotine calculated from the six selected generations was found to be 0.20, or slightly greater than that estimated from the foundation population. There were no significant differences in response to selection between the replicate NA or ND lines. Nicotine-induced body temperature was measured as a correlated response to selection, and was found to remain highly correlated with nicotine-induced locomotor activity. The response was more robust for the ND lines than it was for the NA lines. In contrast to the large differences between the ND and NA lines in locomotor activity and body temperatures following nicotine administration, mean baseline activities and body temperatures remained nearly identical throughout. This indicates that selection acted specifically on nicotine-induced responses, and not on baseline measurements, as predicted for response to a selection criterion based on regression residuals.

Nicotine Selective breeding Locomotor activity Body temperature Genetics Regression residuals Heritability

NICOTINE is among the most widely used drugs in the world. Approximately 33% of men and 28% of women in the United States smoke cigarettes (35). Cigarette smoking is one of the major causes of xenobiotic-induced morbidity and mortality. Cigarette smoking is the primary cause of lung cancer and emphysema, and is a major factor in development of coronary and major vessel disease (31–33). Its use in pregnant women, one-third of whom smoke, has a number of deleterious effects on the offspring (23,27).

It is likely that much of the pathology associated with cigarette smoking is caused not by nicotine, but by one or more of the other components of cigarette smoke (2,13). However, the driving force for cigarette smoking appears to be the craving for nicotine (34). Nicotine meets all of the criteria usually associated with an addictive substance, including the ability to alter mood and behavior (16,26). Self-administration studies have shown nicotine to be a positive reinforcer in both laboratory animals and humans (14,15). The commonalty of re-

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sponses to nicotine and other addictive substances is indicated by the fact that animals trained to discriminate amphetamine may mistake nicotine for amphetamine (28), and humans with histories of polydrug abuse were unable to discriminate between intravenous nicotine, cocaine, or amphetamine (15).

To understand how individuals differ in their responses to nicotine, we have been investigating genetic influences on nicotine-induced behaviors in mice (3,18–20). Estimates of genetic parameters can be obtained from a variety of designs, but one of the most powerful methods for elucidating the role of genetic factors in drug responses is the derivation of lines genetically altered for the trait of interest (11). The ability to breed for a specific phenotype provides clear evidence of a heritable component for that phenotype, and responses that are found to be correlated with the selected phenotype are presumed to be under similar genetic control. Ultimately, the response of the selected lines to the selection criterion may even exceed the maximum difference originally observed in the foundation population (8,9,21), and this increased responsiveness can be a great advantage when testing hypotheses concerning drug action.

An appropriate foundation population for a selection study should be genetically heterogeneous to ensure that a wide range of individual responses is obtained. The heterogeneous stock (HS) mice (22), produced by intercrossing eight inbred strains, has been used as the foundation population for a number of genetic selection studies, including studies of: acoustic priming-induced seizures (7), hypnotic effect of ethanol (21), hypothermic effects of ethanol (4), differential ethanol-induced locomotor activity (6), severity of the ethanol withdrawal syndrome (5,36), sensitivity to diazepam (12), and sensitivity to opiate antinociception (1). In each case it has been possible to select for both higher and lower responses than those observed in the foundation population.

We are in the process of developing lines of mice that are differentially responsive to nicotine (29,30). In this article we report the results of two studies of nicotine sensitivity indices in mice. The first is an analysis of the responses to nicotine in the foundation population of HS mice, which includes sex differences and between- and within-family effects. Two complete test sessions separated by 1 week were used to determine test–retest reliabilities of response measures, and to assess the consistency of family and sex differences across test sessions. The second study describes the response to six generations of selective breeding for differential sensitivity to nicotine.

STUDY 1: RESPONSES TO NICOTINE IN THE FOUNDATION POPULATION FOR SELECTIVE BREEDING

METHOD

Experimental Animals

The animals tested in the foundation population included 204 male and 213 female mice from the first litters born to 38 breeding females from generation 43 of the HS line maintained at the Institute for Behavior Genetics. Mice were born, raised, and tested in the Specific Pathogen-Free facility of the Institute. The colony is maintained in a constant temperature, constant humidity environment with a 12 L : 12 D cycle (lights on 0700–1900 h). Breeding mice were maintained in pairs in plastic cages and were allowed free access to food (Teklad Sterilizable Lab Blox) and water. Cages were checked for litters daily. Litters were weaned at 25 days of age and housed with same-sex litter mates. Most first litters consisted of 9–13 offspring.

All procedures used in this project received prior review and approval by the University of Colorado Animal Care and Use Committee as being consistent with USPHS standards of humane care and treatment of laboratory animals.

Y-Maze Activity

The apparatus is an enclosed symmetrical Y-maze constructed of red Plexiglas (3). The maze consists of three arms that are 26 cm long, 6.1 cm wide, and 10.2 cm high. Each arm of the maze has photoelectric beams at the entrance and midpoint. Crossing of a beam activates a counter that accumulates the number of beams crossed during the 3-min test. The number of beams crossed is recorded as the total locomotor activity score. An additional set of photoelectric beams mounted 5 cm above the floor of the apparatus records the number of rearings.

Body Temperature

Rectal temperature was measured using a Thermalert THS probe (Sensortek, Clifton, NJ). The probe was lubricated with corn oil and inserted 2 cm into the rectum. The probe equilibrates within 5 s and measures temperature to the nearest 0.1 °.

Testing for Sensitivity to Nicotine

Mice were tested for sensitivity to nicotine at 85 ± 15 days of age. On test day 1 mice were injected intraperitoneally (IP) with saline (0.01 ml/g body weight). Five minutes after injection, the mice were placed in a Y-maze and their total locomotor activity and number of rears were measured for 3 min. Fifteen minutes after injection (7 min after completion of the Y-maze test), rectal temperature was measured. Mice were then returned to their home cage. On day 2 the mice were injected with nicotine, 0.75 mg/kg IP in saline (0.01 ml/g body weight), and their activity in the Y-maze and body temperature were measured as on day 1. Because of the many near-zero rearing scores following nicotine administration, only the locomotor activity scores were used in further analysis. All Y-maze testing was conducted between 1000 and 1500 h. The timing of the test and selection of nicotine dose were based on a number of preliminary experiments that have been summarized previously (29). One week later, the complete test was repeated for each mouse.

Data Analysis

For each test session a difference score (saline activity − nicotine activity) and a residual score were calculated. The residual score was calculated by subtracting the observed nicotine activity from the expected nicotine activity (calculated from the regression of nicotine activity on saline activity) (29). Data were analyzed by ANOVA, MANOVA, or Pearson correlation as appropriate using Statistical Package for the Social Sciences (SPSS, Chicago, IL) (24). The data were analyzed separately by sex as well as with sexes combined. Litter effects were assessed by performing ANOVA using litter and sex as main effects.

RESULTS AND DISCUSSION

Means and SDs of activity and body temperature are shown in Table 1 for drug and saline conditions within each test session. Based on studies from our laboratories, we expected that the response of the majority of animals to nicotine would be depression of both locomotor activity and body tem-

TABLE 1

CONTROL AND NICOTINE-INDUCED Y-MAZE ACTIVITY AND BODY TEMPERATURE IN THE FOUNDATION POPULATION

	Test Session 1		Test Session 2	
	Saline	Nicotine	Saline	Nicotine
Y-maze activity (crosses)				
Males	118 (23)	70 (50)	121 (26)	62 (40)
Females	127 (24)	78 (47)	140 (32)	70 (48)
Combined	123 (24)	74 (48)	131 (31)	66 (44)
Range	64–215	0–248	45–227	0–195
Body temperature °C				
Males	38.9 (0.4)	36.8 (1.7)	39.2 (0.5)	37.1 (1.5)
Females	38.7 (0.3)	36.7 (1.5)	38.8 (0.3)	36.8 (1.5)
Combined	38.8 (0.4)	36.8 (1.6)	39.0 (0.4)	36.9 (1.5)
Range	37.5–40.2	32.6–39.9	37.7–40.3	32.2–39.9

Tabulated values are the results of two independent test sessions separated by 1 week for the 204 male and 213 female HS mice comprising the foundation population for the nicotine selection study. Values are means with SD in parentheses.

perature (18–20), but that there would be a small group of animals that would respond with paradoxical stimulation of motor activity (17). As predicted, mean activity levels following administration of nicotine were approximately half of saline-treated baseline scores, and body temperatures following nicotine dropped an average of 2°C. Mean body temperatures following nicotine were below the lowest temperature recorded for any saline-treated animal.

Despite significant decreases in mean activity and mean body temperature induced by nicotine, it is apparent from a comparison of saline and nicotine SDs, and from the range of scores, that there was considerable variation in responsiveness to nicotine—ranging from complete suppression of activity and 6°C decreases in body temperatures in some animals to activity and body temperatures comparable to the highest saline scores in others. Because activity levels and body temperature are highly correlated following nicotine administration, (r = 0.60, df = 415), animals sensitive to nicotine tended to show marked decreases in both temperature and activity.

Although females showed slightly, but significantly, higher baseline activity in both test sessions, sex differences were not significant following nicotine, nor were there significant sex differences in regression residual scores, discussed below. ANOVAs of each of the possible response measures also confirmed that there was no sex-by-test session interaction, nor were there any significant sex-by-family interactions for any measure. In addition, correlations among dependent variables for both saline and nicotine treatments were very similar for males and females. All of these results indicated that sex was not a significant factor for response to nicotine.

The effects of nicotine on activity and body temperature proved to be highly consistent across test sessions. Repeated-measures ANOVAs examining test session effects failed to yield any significant main effects or interactions with test session for either temperature or activity, whether expressed as raw scores following nicotine or as saline-adjusted scores. These results indicated that a single test session would be sufficient to assess the nicotine response among families and individual mice.

Response to nicotine administration can be assessed in three ways: 1) using raw activity scores and body temperature taken following nicotine administration, ignoring saline baseline measurements; 2) using changes in activity and temperature following nicotine (i.e., nicotine scores minus saline scores); or 3) correcting for saline baseline using an adjusted nicotine activity score based on regression residuals obtained from the regression of drug-induced activity on saline control activity. In the absence of any correlation between saline and nicotine scores, the first option provides the most accurate and easily interpreted criterion measures. This option proved most appropriate for body temperature, as discussed below. In cases where measures taken in the saline and nicotine conditions are correlated, as was the case for Y-maze activity (29), an adjustment for baseline scores using difference scores or regression residuals more accurately reflects drug sensitivity.

Although the use of simple difference scores is common in the pharmacological literature, such scores have two undesirable properties that can be misleading or inefficient. First, the SDs of the two measures must be equal. If they are unequal in the two treatment conditions, the condition with the largest SD will disproportionately influence a simple difference score. Second, if the correlation between the measure taken in the saline and drug conditions is relatively low, a difference score is less reliable than the unadjusted score in the drug condition (29). Both problems are obviated by the use of regression residuals, which adjust drug scores based on the observed regression between saline and drug scores. This proved the method of choice with respect to Y-maze activity. Adjusted nicotine activity scores based on regression residuals were computed for comparison with raw scores. There were no significant sex differences in either regression slopes or intercepts for activity, so a single regression of nicotine activity on saline activity was computed for both sexes combined for test session 1 by the equation: predicted nicotine crosses = 0.45 saline crosses + 19 (eq. 1). For each animal a regression residual was computed from its saline and nicotine activity scores: residual score = (observed nicotine crosses − predicted nicotine crosses). For simplicity of presentation, the population mean (73.9) was then added to the residual score to produce the "saline-adjusted nicotine activity score."

Because the correlation of nicotine-induced activity with saline-induced activity was only moderately large [r = 0.22, df = 415 (29)], adjusting for differences in saline baseline activity resulted in only minor differences in the adjusted and observed nicotine activity scores. The correlation of body temperatures following saline and nicotine injection was lower still (r = 0.15), and adjustments for baseline temperatures were negligible. Unadjusted nicotine-induced body temperatures were therefore used in all further analyses.

Table 2 shows the correlations among the activity and body temperature measures across the two test sessions. These correlations show a high degree of reliability among the measures, particularly: the test-retest reliability (repeatability) coefficients of the saline-adjusted activity measures (AR1-AR2, r = 0.39) and body temperatures (TN1-TN2, r = 0.42); the within-session correlations between adjusted activity and temperature (AR1-TN1, r = 0.67 and AR2-TN2, r = 0.63); and the cross-session correlations between adjusted activity and body temperature (AR1-TN2, r = 0.32 and AR2-TN1, r = 0.29). These latter cross-correlations become 0.79 and 0.72 when adjusted for between-session unreliability. Both the within-session correlations and the adjusted cross-correlations indicate that there is a substantial phenotypic correlation between nicotine-induced decreases in activity and body temperature.

All litters showed mean decreases in both activity and body temperature after receiving nicotine, but the size of the drug effect differed significantly across litters. The results of the ANOVAs for test session 1 are summarized in Table 3. Similar results were obtained with session 2 data. Figure 1 shows the relationship between litter means for body temperature and saline-adjusted activity scores following nicotine administration in test session 1. The activity-temperature correlation using litter means was 0.68, nearly identical to the pooled within-litter correlation of 0.67.

Because a substantial portion of the total variance can be attributed to measurement unreliability, litter effects accounted for only 5.9% and 11.6% of the total variance in activity and temperature scores, respectively. Doubling the between-litter proportions of variance provides an approximate upper estimate of heritability (11). The heritability of nicotine-influenced activity estimated from the foundation population in this way was approximately 0.12. This modest heritability indicates that the response to selection based on saline-adjusted Y-maze activity levels would be moderate, but that the production of selected lines responding differentially to the effects of nicotine would be an attainable goal.

STUDY 2: SELECTION FOR DIFFERENTIAL ACTIVITY LEVELS FOLLOWING NICOTINE ADMINISTRATION

METHOD

The details of the methods employed for production of the nicotine-selected lines have been presented earlier (29) and are briefly summarized here. The selection criterion was nicotine-

TABLE 2

CORRELATIONS AMONG ACTIVITY AND BODY TEMPERATURE MEASURES

	Test Session 1					Test Session 2				
Measure	AS1	AN1	AR1	TS1	TN1	AS2	AN2	AR2	TS2	TN2
Test Session 1										
Y-Maze activity Saline (AS1)	–	0.22	0.00	0.02	−0.02	*0.45*	−0.04	0.10	−0.03	−0.04
Y-Maze activity Nicotine (AN1)		–	0.97	0.03	0.65	0.23	*0.43*	0.39	0.11	0.30
Y-Maze activity Residual (AR1)			–	0.02	0.67	0.13	0.40	*0.39*	0.12	0.32
Temperature Saline (TS1)				–	0.15	0.03	0.09	0.09	*0.56*	0.16
Temperature Nicotine (TN1)					–	0.02	0.28	0.29	0.25	*0.42*
Test Session 2										
Y-Maze activity Saline (AS2)						–	0.25	0.00	−0.03	−0.05
Y-Maze activity Nicotine (AN2)							–	0.97	0.11	0.60
Y-Maze activity Residual (AR2)*								–	0.13	0.63
Temperature Saline (TS2)									–	0.24
Temperature Nicotine (TN2)										–

Correlations greater than 0.10 are significant at the 0.05 level, df = 415. Test-retest reliabilities (repeatability coefficients) are in italics. *Regression residuals for test session 2 are based on the equation: nicotine activity = 0.35 saline activity + 20.

TABLE 3

ANALYSES OF VARIANCE OF SALINE-ADJUSTED Y-MAZE ACTIVITY AND BODY TEMPERATURE FOLLOWING NICOTINE ADMINISTRATION (TEST SESSION 1)

		Adjusted Activity			Body Temperature		
Source of Variance	*df*	MS	*F*	Var (%)	MS	*F*	Var (%)
Litters	37	3526	1.69*	5.9	5.35	2.48*	11.6
Sex	1	6878	3.31	1.0	2.50	1.16	0.1
Litters × sex	36	2149	1.03	0.5	2.52	1.17	2.6
Within groups	342	2081		92.5	2.16		85.7
(Measurement error)				(61.5)			(58.5)

*$p < 0.001$

induced locomotor activity, adjusted for saline (baseline) activity using the regression equation derived from the foundation population (eq. 1). Breeding parents were chosen based on their adjusted nicotine activity scores. Y-maze activity was the selection criterion, rather than body temperature changes following nicotine, to allow direct comparisons with a parallel selection study for differential responses to cocaine being undertaken concurrently (29).

Litters were weaned at 23 ± 2 days of age. Weanlings were housed with like-sex littermates (two–five per cage) until tested at 60 ± 5 days of age. Every animal in every litter was tested over 2 days: a saline baseline measurement on day 1 and nicotine administration on day 2. Measurements made on each day included body weight, Y-maze activity (total number of light beam crossings), and body temperature as described in Study 1 above. The procedure for measuring body temperature was modified slightly, however. For sanitary reasons, mice reared in the Specific Pathogen-Free Laboratory are not touched with hands. They are moved from cage to cage using padded, disinfectant-soaked forceps. However, during the testing procedure, the mice were hand-held for injection and temperature measurement. The mice of the foundation population were noticeably bothered by the procedure. They struggled during injection, and remained highly agitated after being returned to their cage (this is reflected in their body temperatures as described below). In subsequent generations all mice in the selection studies were handled weekly during cage changing. These animals were much more docile during the testing procedure, and baseline temperatures were found to be lower, reflecting an adaptation to handling.

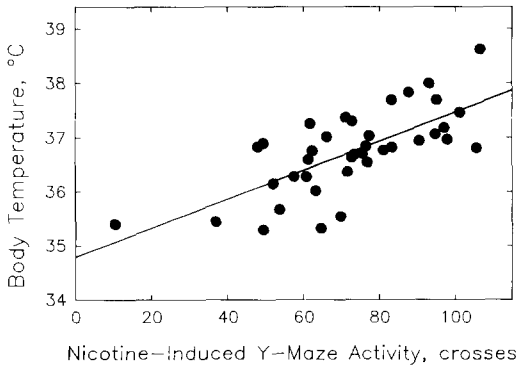

FIG. 1. Relationship between nicotine-induced body temperature and saline-adjusted Y-maze activity for each of the 38 HS families comprising the foundation population. Plotted values are the mean responses of all of the offspring, male and female, in each family (N = 3–15 per family).

The selected lines were named for their nicotine-induced locomotor responses. The duplicate "high" lines, which had greater locomotor activity following nicotine administration than in their saline baseline condition, were termed "nicotine activated" (NA1 and NA2). The duplicate "low" lines, which had lower locomotor activity following nicotine administration than their saline condition, were termed "nicotine depressed" (ND1 and ND2). The unselected control lines were termed control 1 and control 2 (C1 and C2).

Ten males and 10 females were chosen at random from the founding population to form one of the control lines (C1). A second control line, C2, was established 3 months later, in conjunction with the establishment of a set of cocaine-sensitive lines (29). The ND and NA lines were formed from the mice remaining after this control line was established. The 20 males and 20 females with adjusted activity levels most depressed by nicotine were mated at random. These were designated as parental stock 1, to produce selected generation 1 of the ND lines. Half the pairings were randomly designated ND1 and the remaining half ND2. The two NA lines were constructed in an analogous fashion from the 20 males and 20 females with activities most stimulated by nicotine (highest saline-adjusted Y-maze scores). No more than two same-sex mice from each of the 38 HS families were included in a single line. Following the initial assignment to one of the six lines, all matings involved families within a designated line.

Each line was then maintained by within-family selection, in which one male and one female were chosen from each family. This ensured that each family would be represented in each subsequent generation and it also minimized inbreeding (11). The study thus involved two forms of selection. The first selected generation was produced by mass selection, whereas generations 2–6 were subjected to within-family selection.

Animals were selected for mating to produce the next selected generation on the basis of their saline-adjusted nicotine activity scores. For the ND1 and ND2 lines, for example, the male and female in each litter with the greatest nicotine-induced depression of Y-maze activity were chosen for mating to produce the next generation. The selected males and females from 10 different litters were then mated at random to form the 10 mating pairs for the next generation. The male and female from each litter that were most activated by nico-

tine were chosen as breeders for the NA1 and NA2 lines. The control lines were tested in the same manner as the selected lines. One male and one female were chosen at random from each litter without regard to their activity scores and were mated randomly to form the next generation of control animals. The C2 line was tested with nicotine only in generations 4 and 6.

Mean scores of each selected line in each generation were based on an average sample size of 82.8 mice (range 61–112). The C1 line generation means were based on an average N of 79.6 mice (range 69–91).

RESULTS AND DISCUSSION

Table 4 summarizes the mean baseline activity activity scores, nicotine-induced activity scores, and body temperatures of the parents chosen from the foundation population to form the four selected lines and the control line, C1. Saline baseline scores ranged from 122 to 136 crossings and remained at this level for all six lines for all six generations. The relatively small group effects of adjusting nicotine activity scores for saline activity levels is evident in Table 4, with respect to both line means and SDs. Relative to the total foundation population, the founding parents of the ND lines were approximately 1.4 SDs below the population mean in activity, whereas the founding parents of the NA lines were approximately 1.8 SDs above the population mean in activity following nicotine administration. The ND founding parents also showed substantially more depressed body temperatures following nicotine than the founding population, with a mean 1.2 SDs below the population mean. Conversely, the mean nicotine-induced body temperature of the NA lines was approximately 1 SD above that of the founding population.

The response to selection can be seen in Table 5, which shows activity and temperature means for all offspring of the founding parents. Activity means of all four generation 1 selected lines shifted in the direction of selection, although the ND2 line decrease was not significant. A comparison of ND and NA lines indicated a highly significant difference in nicotine-induced activity levels, $F(1, 392) = 34.4, p < 0.001$. Relative to the control line, the ND and NA lines showed a relatively symmetrical response to selection in the high and low directions.

The change in procedure for measuring body temperature resulted in a small but significant decrease in body temperature in both the saline and nicotine conditions for generation 1 and subsequent generations. Compared to the foundation population mean of 38.8°C, the first generation control line, C1, had a body temperature of 38.4°C in the saline condition, $t(506) = 3.51$, $p < 0.001$. An even larger temperature decrease was found following nicotine administration [foundation population mean = 36.8°C, C1 mean = 35.7°C, $t(506) = 6.71$, $p < 0.001$]. The procedural change in temperature measurement precluded assessing mass selection effects from generation 0 to generation 1. Comparisons among the selected lines within generation 1 do suggest that selection had been successful, at least for the NA lines. The two ND lines showed significantly lower body temperatures than the NA lines, $F(1, 1392) = 31.7$, $p < 0.001$. In contrast to the results found with Y-maze activity, body temperatures of ND and NA lines did not diverge at equal rates, relative to the control line. The two ND lines did not differ significantly from C1, whereas both NA1 and NA2 had significantly higher body temperatures than C1 following nicotine administration ($p < 0.02$ in both cases).

The significant divergence of ND and NA lines yielded a good response to selection, but this was not large relative to the substantial differences in the founding parent means shown in Table 4. This limited response to selection was expected, based on the heritability estimate of nicotine-induced activity obtained in the foundation population (0.12). Table 6 shows the realized heritabilities for selected generation 1 (mass selection, estimated from the parent and offspring means) and for generations 2–6 (within-family selection). The first and third columns summarize heritabilities based on saline-adjusted activity means without regard to the control line mean. The second and fourth columns summarize heritabilities using means adjusted for changes in the control line, C1, using regression methods (11). For the first generation both analyses yielded heritabilities of approximately 0.20 for both ND and NA lines, reflecting the symmetry of selection response seen in Table 5. The realized heritability of 0.20 was

TABLE 4

Y-MAZE ACTIVITY AND BODY TEMPERATURE FOLLOWING NICOTINE ADMINISTRATION FOR THE FIRST PARENTAL GENERATION

Group	Saline Activity	Nicotine Activity (Observed)	Nicotine Activity (Adjusted)	Nicotine Body Temperature
Foundation population	123 (24)	74 (48)	74 (47)	36.8 (1.6)
Control line 1	122 (23)	81 (50)	82 (46)	36.9 (1.2)
Nicotine depressed	136	8	2	34.6
line 1	(28)	(12)	(14)	(1.6)
Nicotine depressed	127	8	6	35.2
line 2	(21)	(7)	(8)	(1.3)
Nicotine activated	123	166	166	38.6
line 1	(22)	(32)	(26)	(0.7)
Nicotine activated	125	153	152	38.1
line 2	(26)	(21)	(15)	(0.8)

The foundation population ($N = 417$) was the first litter of generation 43 HS mice. All other lines are the 10 males and 10 females selected from the foundation population for breeders for the first selected generation.

TABLE 5

Y-MAZE ACTIVITY AND BODY TEMPERATURE FOLLOWING NICOTINE ADMINISTRATION FOR THE FIRST SELECTED GENERATION

Group	N	Activity (Actual)	Activity (Adjusted)*	Body Temperature
Control line 1	91	77 (57)	70 (52)	35.7 (2.1)
Difference†		0.1	−0.1	−0.7‡
Nicotine depressed line 1	112	54 (50)	50 (49)	35.3 (1.9)
Difference		−0.4‡	−0.5‡	−0.9‡
Nicotine depressed line 2	94	69 (54)	64 (53)	35.8 (2.0)
Difference		−0.1	−0.2	−0.6‡
Nicotine activated line 1	99	97 (57)	91 (57)	36.8 (1.8)
Difference		0.5‡	0.4‡	0.0
Nicotine activated line 2	91	92 (57)	87 (56)	36.5 (2.3)
Difference		0.4‡	0.3‡	−0.2
All offspring	487	77 (57)	72 (56)	36.0 (2.0)
Difference		0.1	0.0	−0.5‡

*Adjusted for saline activity level using regression residuals. †Difference between offspring line (ol) and foundation population (fp) means in phenotypic SD units: $(M_{ol} - M_{fp})/SD_{fp}$. ‡Significant difference between offspring line and foundation population ($p < 0.05$).

somewhat higher than the 0.12 predicted from the foundation population. Interestingly, the realized heritability of 0.20 was nearly identical to the narrow-sense heritability of open field activity following 0.75 mg/kg nicotine (0.18) estimated from a diallel analysis of five inbred mouse strains (19).

Figure 2 shows the progress of selection for nicotine-induced activity (top panel) across all six selected generations. The relatively low between-family effects observed in the foundation population led to the expectation that response to selection would progress more slowly using within-family selection than that obtained for the mass-selected generation 1. Generation 2 showed increased nicotine activity for all lines, including C1, making interpretation of the substantial activity increases of NA1 and NA2 and the return of the ND1 and ND2 lines to near the founding population mean difficult. The C1 activity means then decreased in generations 3 and 4, and were quite comparable in generations 4 and 6 to mean activity levels of C2, the second control line. Data were not collected for control lines in generation 5.

The middle panel of Fig. 2 shows the progress of selection relative to control line C1 by plotting the difference between each selected line and C1 in each generation. Because no data was available for C1 in generation 5, this mean was estimated by averaging the C1 means from generations 4 and 6. Although using differences between selected lines and control lines tends to overweight control line fluctuations, a clear pattern of divergence emerges for the replicate ND and NA lines through generation 3, with little divergence thereafter. The bottom panel of Fig. 2 shows the divergence of the high- and low-selected lines (i.e., NA1–ND1 and NA2–ND2) across generations. The selection plateau related to the lack of response of the NA lines from generation 4 on is clearly evident. Generations 2–6 yielded heritabilities of approximately 0.50 (0.4 adjusted) for the ND lines. Heritabilities for the NA lines did

TABLE 6

HERITABILITY ESTIMATES OF SALINE-ADJUSTED Y-MAZE ACTIVITY FOLLOWING NICOTINE ADMINISTRATION

	Mass Selection (Gen. 1) Heritability Estimated From		Within-Family Selection (Gen. 2-6) Heritabilty Estimated From	
Line	Actual Line (Means)	Control Line (Adjusted)	Actual Line (Means)	Control Line (Adjusted)
ND1	0.29 ± 0.11	0.35 ± 0.10	0.50 ± 0.17	0.36 ± 0.04
ND2	0.15 ± 0.12	0.12 ± 0.12	0.52 ± 0.15	0.41 ± 0.08
ND average	0.22 ± 0.07	0.23 ± 0.11	0.51 ± 0.11	0.39 ± 0.04
NA1	0.18 ± 0.09	0.21 ± 0.09	−0.10 ± 0.13	−0.06 ± 0.14
NA2	0.17 ± 0.11	0.18 ± 0.10	−0.11 ± 0.16	−0.08 ± 0.19
NA average	0.18 ± 0.01	0.20 ± 0.02	−0.10 ± 0.10	−0.07 ± 0.12

Tabled values are means + SEM. Mass selection SEMs are based on SEMs of mean differences in foundation population and selected lines. Within-family SEMs based on regression slope SEMs.

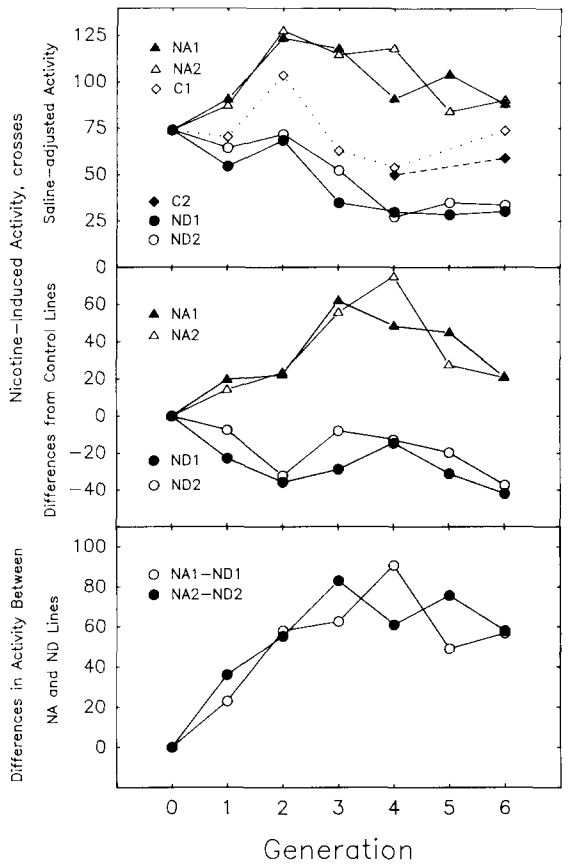

FIG. 2. Progress toward selection from foundation population through generation 6. The top panel shows the saline-adjusted means for all groups; middle panel shows deviations of the ND and NA lines from C1 control line each generation; bottom panel shows differences between ND and NA replicate lines.

not differ significantly from zero, indicating that selection had not progressed in that direction over the last three generations of within-family selection. The locomotor response to nicotine administration has also been shown to have a significant dominance component (19). Because dominance variance is not responsive to selective breeding techniques (11), some limitations in response to selection would be expected.

Changes in generation means do not fully represent the success of bidirectional selection for activity changes following the administration of nicotine. An examination of the frequency distributions of activity scores pooled across generations 4-6 revealed a substantial "floor effect" in the ND lines. Nearly half of these mice had fewer than 10 photocell crosses, although about 25% of this group continued to have activity levels at or above the median of the control lines, whereas approximately 75% of the NA mice exceeded the control line median activity after nicotine. In contrast to the large differences in nicotine-induced Y-maze activity between the ND and NA lines, the mean baseline Y-maze activity scores were 123.7 for the pooled ND lines and 122.5 for pooled NA lines. Selection, therefore, acted specifically on nicotine-induced changes in activity and (body temperature, see below) and not on baseline measurements themselves, as predicted for a selection criterion based on regression residuals.

CORRELATED RESPONSE TO SELECTION: BODY TEMPERATURE

The phenotypic correlations between Y-maze activity and body temperature responses to nicotine within the selection generations were relatively consistent across ND and NA lines. The pooled correlation across generations was an identical 0.62 for ND and NA lines and 0.78 for the C1 line. For the selected lines, the activity-temperature correlation was 0.74 for pooled generations 1-2 and significantly lower [$r = 0.57$, $t(1980) = 6.87$, $p < .001$] for pooled generations 3-6, possibly because there was less genetic variance within lines in the later generations.

Following mass selection from the foundation population, generation 1 NA lines both had significantly higher body temperatures than the C1 line ($p < 0.01$ in both cases), whereas neither ND line differed significantly from C1. In subsequent generations, however, the ND lines had significantly lower body temperatures following nicotine than did C1, although continued divergence from C1 was small. The NA lines did not continue to diverge from C1 after the initial generation following mass selection.

Figure 3 shows the relationship between Y-maze activity and body temperature following nicotine administration for the four selected and two control lines, pooled across the highly similar generations 4-6. The ND and NA lines differed by approximately 1 phenotypic SD on both measures following three generations of selection, suggesting a high additive genetic correlation between activity and temperature responses to nicotine. In contrast to the large differences between the ND and NA lines in body temperatures following nicotine administration, mean body temperatures taken in the saline condition were identical (38.5°C). As was seen with locomotor activity, selection acted specifically on nicotine-induced changes in body temperature and not on baseline measurements, as predicted for a correlated response to a selection criterion based on regression residuals.

GENERAL DISCUSSION

Previous studies from our laboratories have shown a strong genetic component to behavioral and pharmacological responses to nicotine in mice (3,17-20) and suggested that a selection experiment for differential responsiveness to nicotine should be possible. The results of testing 38 families of HS mice for nicotine-induced locomotor activity revealed a possi-

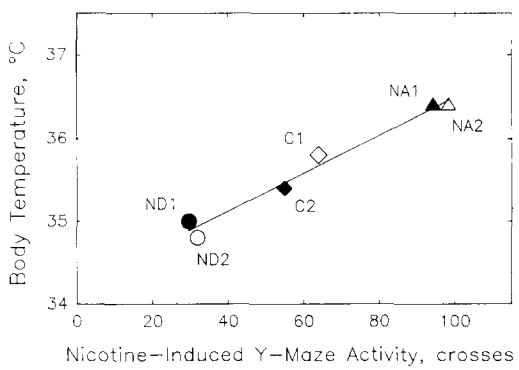

FIG. 3. Mean saline-adjusted activity scores and body temperatures following nicotine for pooled generations 4-6.

ble genetic component to the response (Table 3), with an estimated heritability of 0.12. Because other selection studies have been carried out on phenotypes with heritabilities of this magnitude (5,21,36), this indicated that selective breeding would be possible.

The choice of a phenotype for selective breeding is always a difficult choice, but in virtually every successful selection experiment the selection criterion has been shown to be simple, repeatable, and reliable. For this study we chose drug-induced locomotor activity as the criterion. Locomotor activity has been shown to be a highly reliable and reproducible measure (20), and our data on the foundation population for this study (Table 2) confirmed this finding. Locomotor activity has also been shown to be associated with drug-seeking activity in rodents (10,25). One objective for developing lines of mice differentially sensitive to nicotine is to use them to investigate hypotheses concerning the reinforcement properties of nicotine.

After the first generation of mass selection, selection continued using a within-family design. This design was clearly successful for the ND lines, which showed continued response to selection. For the NA lines, however, it was less successful because there was no further response to selection in generations 4–6. This was not entirely unexpected because the locomotor stimulation by nicotine is really quite a rare phenotype. We expected that selection in this direction would be considerably more difficult than would be selection for locomotor depression. We found that about half the families in the NA lines had no animals actually activated by nicotine. Theory suggests that eventually we will be successful in producing more activated lines, provided there is additive genetic variance for the trait, but progress may be slower than desired. The results of this study suggest that mass selection would be considerably more efficient than within-family selection for this particular phenotype. One reason to use a within-family design is to protect against inbreeding. However, a mass selection design can also be protected from inbreeding provided that a large number of families are used, that one litter does not provide a majority of the breeding stock for the next generation, and that interbreeding of close relatives is avoided.

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