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A Diallel Analysis of Nicotine-Induced Hypothermia[1]

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MARKS, M. J., L. MINER, J. B. BURCH, D. W. FULKER AND A. C. COLLINS. *A diallel analysis of nicotine-induced hypothermia.* PHARMACOL BIOCHEM BEHAV **21**(6) 953–959, 1984.—The hypothermic responses of mice that occur after acute injection of nicotine show genetic influences. The body temperatures of mice of all five strains tested decreased after injection of either 0.75 or 1.5 mg/kg nicotine, but mice of the C3H strain were less affected than were those of the DBA, BALB, or C57BL strains. Mice of the A strain were the most sensitive to nicotine's effects. Genetic effects on nicotine-induced hypothermia were further examined using a five-by-five diallel cross. Additive genetic variance occurred at both nicotine doses. Substantial dominance variance, including directional dominance toward a large hypothermic response induced by injection of a low dose of nicotine (0.75 mg/kg), suggested that an intense response to a low drug dose is adaptive. The directional dominance was absent after treatment with a high dose (1.5 mg/kg) of the drug. Epistatic interactions occurring in crosses involving C57BL mice were pronounced.

Diallel cross Nicotine Hypothermia Genetics Pharmacogenetics

NICOTINE, in the form of tobacco, is one of the most widely used drugs. It has many complex physiological and behavioral effects both in humans and in animals arising in part because of the drug's effects on the autonomic nervous system, at the neuromuscular junction, and in the brain.

Among nicotine's effects in rodents is the induction of hypothermia [3,14]. That the hypothermic response differs among several inbred strains of mice indicates the presence of genetic influences on the drug response. The nicotine-induced hypothermic responses of four inbred strains of mice (BALB, C57BL, DBA, and C3H) recently were compared [14]. Mice of the C3H strain were the least sensitive, those of the BALB strain were the most sensitive, and those of the DBA and C57BL strains were intermediate. In general, hypothermia increased as the nicotine dose increased. Other responses to nicotine are also subject to genetic influences [4, 5, 7, 8, 9, 14, 16].

The presence of strain differences in nicotine-induced hypothermia makes possible the study of the genetic influences on these differences. One method applicable to the study of these differences is the diallel cross. Several previous investigations of drug effects on behavioral and physiological responses in mice have utilized the diallel design [1, 2, 13], but such studies have seldom included complete diallel analyses and have thus failed to obtain all the information possible from this design. The diallel cross described in this paper used five inbred strains that differ in the character of interest, and members of each strain were crossed with members of every other strain to produce all possible combinations. In a five-by-five diallel design, therefore, the 10 F1s and their reciprocal crosses, as well as the five inbred strains themselves, produce a total of 25 groups. Heritability is reflected in the average effects of the crosses; dominance is revealed by the contrast between F1s and the inbred strains; and maternal effects are indicated by differences between the reciprocal crosses [10,15].

In the present study the mice obtained from the diallel cross were tested for the effects of three doses of nicotine (0.0, 0.75, and 1.5 mg/kg) on body temperature, and a diallel analysis of variance was used to analyze the results.

METHOD

Animals

Four of the five inbred strains used in this study (A/Ibg, C57BL/6Ibg, DBA/2Ibg, and C3H/2Ibg) have been maintained in the breeding colony at the Institute for Behavioral Genetics for at least 10 generations. The fifth strain, BALB/cByJ, was obtained from Jackson Laboratory, Bar Harbor, ME.

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Mice to be tested for the nicotine-induced hypothermia were generated from mating pairs representing all possible combinations of the five inbred strains, such that a total of 25 different crosses, 5 of which represented the inbred strains, were obtained. To assure adequate production, 5–6 sets of mating pairs were used for each cross. A 12-hr light/12-hr dark cycle (lights on from 7 a.m.–7 p.m.) was maintained. Mice were permitted free access to food (Wayne Lab Blox) and water. Mating pairs were housed on aspen shavings in 17.5×50×20 cm metal cages and were transferred to 21×62×20 cm metal cages when litters were born. The offspring were weaned when they were 25 days old and were housed with 1–5 like-sexed littermates.

Testing

Mice of either sex were between 60 and 90 days old when they were tested for the effects of nicotine on body temperature. Nicotine was obtained from Sigma Chemical Co. (St. Louis, MO) and was redistilled periodically. The animals were weighed and transferred to a constant temperature room maintained at 23°C. Three doses of nicotine were used: 0.0, 0.75, and 1.5 mg/kg. The injection volume was 0.01 ml/g. Littermates were randomized among treatment groups to minimize possible litter effects. Prior to injection a baseline body temperature was measured by insertion of a probe (Digitec 5910 rectal thermometer; Yellow Springs Instrument Co., Yellow Springs, OH) 2.5 cm into the rectum. Nicotine-containing saline solution was then injected and body temperature was measured at 10-min intervals until it returned to normal. This method allowed two measures of nicotine-induced hypothermia: maximal temperature decrease, and overall drug effect as measured by the area under the time-temperature curve. Similar results were obtained for both measures. The results presented here are for maximal temperature decrease.

Seven males and seven females from each cross were tested at each drug dose. An additional seven mice of each sex from the inbred strains were also tested at each drug dose. Analysis of the results indicated that no significant sex differences occurred so data obtained from both sexes were combined.

Data Analysis

Results were analyzed using the diallel analysis of variance as described by Hayman [10] and applied to the half-diallel design by Jones [12]. Results for each nicotine dose were analyzed independently. Variance-covariance analysis [11,15] was undertaken to test the validity of the additive-dominance model of inheritance assumed in the diallel analysis of variance.

RESULTS

The dose-response curves for nicotine-induced hypothermia in the five inbred strains of mice are shown in Fig. 1. All five strains developed hypothermia after nicotine injection. While the dose-response curves for nicotine-induced temperature decrease observed in four of the inbred strains were linear for the nicotine doses tested, that observed for mice of the A strain was not. The hypothermia developed by mice of the A strain was the same after administration of 1.5 mg/kg nicotine as it was after a dose of 0.75 mg/kg, indicating a floor effect may have occurred. Mice of the C3H strain were less sensitive to nicotine's hypothermic

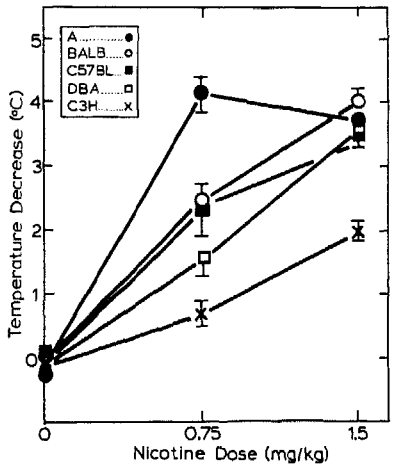

FIG. 1. Effect of nicotine injection on body temperature in five inbred strains of mice. The change in body temperature was measured using 14 males and 14 females of each strain at each of the three doses of nicotine. Results shown are mean±S.E.M.

effect after treatment with either drug dose than were mice of the other four strains. Mice of the A strain developed significantly more hypothermia after treatment with 0.75 mg/kg nicotine than did mice of any of the other strains. However, after a dose of 1.5 mg/kg nicotine, the hypothermic responses of mice of the A, BALB, C57BL, and DBA strains were the same. Saline injection had little effect on body temperature.

The results summarized in Tables 1 and 2 are those observed for all 25 crosses (plus the replicates for the inbreds) after administration of 0.75 mg/kg and 1.5 mg/kg, respectively. Saline injection and subsequent measurement of body temperature tended to increase body temperature slightly, but the changes occurring were small.

The results presented in Table 1 are those for temperature changes observed after administration of 0.75 mg/kg nicotine. A decrease in body temperature occurred for mice of all crosses. The row and column totals are consistent with the results obtained for the inbreds after treatment with 0.75 mg/kg nicotine. Totals from crosses with an A parent were the most negative, while those with a C3H parent were the least negative. Row and column totals for crosses with BALB or C57BL parents were very similar, while those for crosses with DBA parents were slightly less negative. Therefore, the rank orders of row and column totals reflect, in general, the rank order for inbred responses.

The results presented in Table 2 are those for temperature changes after administration of 1.5 mg/kg nicotine. A decrease in body temperature was seen for mice of all crosses, and the row and column totals are consistent with the responses of the inbreds after a dose of 1.5 mg/kg nicotine. Totals for rows and columns for mice having A, BALB, C57BL, or DBA parents were nearly identical, while the row and column totals for crosses having C3H parents were the least negative.

The effects of nicotine on body temperature that are summarized in Tables 1 and 2 for nicotine doses of 0.75 and 1.5 mg/kg, respectively, were analyzed using a diallel analysis of variance. In general, a diallel cross has the structure of a two-way analysis of variance where rows and col-

TABLE 1

MEAN CHANGES IN BODY TEMPERATURE FOLLOWING INJECTION OF 0.75 mg/kg NICOTINE

Maternal Strain	Paternal Strain A	BALB	C57BL	DBA	C3H	Row Totals
	−4.31 (0.36)					
A		−4.17 (0.33)	−4.35 (0.40)	−2.48 (0.35)	−2.58 (0.28)	−21.88
	−3.99 (0.35)					
		−2.42 (0.43)				
BALB	−3.88 (0.28)		−4.07 (0.53)	−3.23 (0.44)	−2.38 (0.30)	−18.52
		−2.54 (0.41)				
			−2.86 (0.27)			
C57BL	−2.87 (0.25)	−3.79 (0.29)		−3.65 (0.34)	−1.43 (0.27)	−16.84
			−2.27 (0.33)			
				−1.59 (0.53)		
DBA	−3.07 (0.32)	−2.29 (0.39)	−3.93 (0.34)		−2.21 (0.40)	−15.23
				−1.64 (0.40)		
					−0.61 (0.25)	
C3H	−3.07 (0.45)	−1.65 (0.20)	−1.46 (0.38)	−1.56 (0.30)		−9.45
					−1.10 (0.33)	
Column Totals	−21.16	−17.36	−18.94	−14.15	−10.31	

Mean changes in body temperature (±S.E.M.) after injection of 0.75 mg/kg nicotine were determined using 7 males and 7 females per group. All groups showed a decrease in body temperature following nicotine injection.

umns detect additive genetic effects, and rows × columns reveal the non-additive effects of dominance and genic interaction. The analytic method designed by Hayman [10] as applied to the half-diallel design was used for this analysis [12]. This method allows tests for additive genetic variance and for dominance variance, which can be subdivided into tests for directional dominance, non-directional dominance, and apparent dominance due to unequal allelic gene frequency distribution among the parental strains. Since no significant sex differences were found, the analyses were performed with data from both sexes.

This diallel analysis assumes an additive-dominance model. That is, heritability interactions between genes at different loci are the sum of the effects of these genes, and the only non-additive effects arise from dominance relationships between genes at the same loci. The assumption of an additive-dominance model can be tested by variance-covariance analysis [11,15]. If the assumption for a simple additive-dominance model holds, a plot of variance vs. covariance yields a straight line with unit slope. However, if this assumption fails and a straight line is not observed, a more complex model involving epistatic interactions (non-additive interactions among alleles at different loci) must be considered. The regression line obtained using all crosses at the 0.75 mg/kg dose is shown in the main panel of Fig. 2A and that obtained using all crosses at the 1.5 mg/kg dose is shown in the main panel of Fig. 2B. Neither line had unit slope, a result which suggested deviation from the additive-dominance model. The variance-covariance statistics were recalculated omitting crosses with each inbred strain in turn

TABLE 2

MEAN CHANGES IN BODY TEMPERATURE FOLLOWING INJECTION OF 1.5 mg/kg NICOTINE

Maternal Strain	Paternal Strain A	BALB	C57BL	DBA	C3H	Row Totals
A	−3.37 (0.19)	−3.91 (0.27)	−4.35 (0.17)	−3.45 (0.23)	−3.27 (0.24)	−21.85
	−3.50 (0.23)					
BALB	−3.36 (0.27)	−3.79 (0.32)	−3.54 (0.24)	−3.25 (0.31)	−2.83 (0.19)	−20.57
		−3.80 (0.28)				
C57BL	−3.54 (0.29)	−4.07 (0.36)	−3.22 (0.27)	−4.29 (0.55)	−3.15 (0.19)	−21.20
			−2.93 (0.28)			
DBA	−3.80 (0.31)	−3.54 (0.21)	−4.24 (0.28)	−3.06 (0.30)	−2.78 (0.17)	−20.72
				−3.33 (0.27)		
C3H	−3.19 (0.17)	−2.36 (0.22)	−2.62 (0.24)	−2.99 (0.36)	−2.14 (0.26)	−15.62
					−2.12 (0.17)	
Column Totals	−20.76	−21.67	−20.90	−20.37	−16.29	

Mean changes in body temperature (±S.E.M.) after injection of 1.5 mg/kg nicotine were determined using 7 males and 7 females per group. All groups showed a decrease in body temperature following nicotine injection.

to determine if crosses involving one of the strains were responsible for the deviation. When all C57BL crosses were omitted, the regression lines improves dramatically (see insets to Fig. 2A and 2B). This result suggests that there is epistasis acting on the response being measured at both nicotine doses and that it is being contributed primarily, if not exclusively, by the C57BL crosses.

Since the additive-dominance model appeared to hold in the absence of C57BL crosses, the analysis was made on the data represented by a four-by-four diallel cross from which all results for crosses with a C57BL parent had been omitted. Analysis of the results obtained after saline injection showed little or no additive or dominance variance, a result consistent with the observation that saline injection had little effect on body temperature. Analysis of the results after a 0.75 mg/kg dose of nicotine indicated that both additive genetic variance (item a) and dominance variance (item b) were evident (see Table 3). In addition, two of the three components of the dominance item (b_1, directional effects, and b_3, residual effects) were significant as well. The dominance item accounted for 5% of the total variance in this analysis. Neither the maternal effects nor the reciprocal effects were significant. Analysis of the results after a 1.5 mg/kg dose of nicotine (Table 3) indicated that the additive genetic variance was significant, but that the dominance variance was not. The smaller magnitude of the additive variance and the disappearance of the dominance variance at this higher dosage level may have arisen from the approach of the hypothermic responses to a floor effect at a temperature decrease of approximately 3.5°.

Because both directional dominance after a dose of 0.75 mg/kg nicotine and epistasis after both drug doses were observed in this study, graphical analyses of the data were made and are presented in Fig. 3A for the 0.75 mg/kg dose and in Fig. 3B for the 1.5 mg/kg dose of nicotine. These figures compare the temperature decreases of the F1s to those of their inbred parents and to the midparent value. At both doses, the decrease in body temperature displayed by the F1s tended to be greater than the midparent value, that is, the F1s were more sensitive to the hypothermic effects of nicotine than predicted by the midparent value. After a dose of 0.75 mg/kg nicotine, 7 of 10 F1s were more affected than predicted by the midparent value. This observation is further supported by the fact that the average temperature decrease of the F1s was 2.95° compared to an average of 2.33° for the inbreds. This difference is not entirely due to directional dominance, however, since all crosses, including those with C57BL parents, have been included in these calculations. Two of these hybrids (BALB × C57BL and DBA × C57BL) displayed a much more intense hypothermic response than did the more sensitive parental line. Since epistasis was apparent with C57BL crosses, the calculations were repeated after removal of all hybrids with a C57BL parent and the

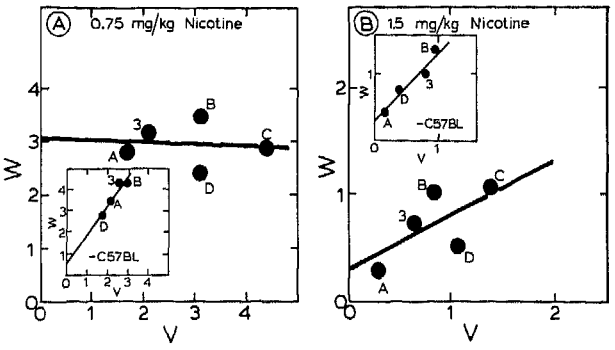

FIG. 2. Variance-covariance diagrams for nicotine-induced hypothermia. The variance of inbred-hybrid responses (W) were graphed as a function of the variances of the inbred responses (V) after administration of either 0.75 mg/kg nicotine (panel A) or 1.5 mg/kg nicotine (panel B). The variance-covariance plots for all crosses are the major figures; the plots obtained by omitting all C57BL crosses are the insets. The equations for the lines are as follows: Nicotine=0.75 mg/kg—W=−0.03V + 3.02, r=−0.09, for all crosses; W=1.35V + 0.51, r=0.92, omitting all C57BL crosses. Nicotine=1.5 mg/kg—W=0.51V + 0.29, r=0.68, for all crosses; W=0.98V + 0.33, r= 0.94, omitting all C57BL crosses.

TABLE 3

DIALLEL ANALYSIS OF HYPOTHERMIC RESPONSE OF MICE AFTER NICOTINE INJECTION OMITTING ALL C57BL CROSSES

		Nicotine Dose			
		0.75 mg/kg		1.5 mg/kg	
Item	*df*	MS	F	MS	F
a. Additive Variance	3	5.60	46.63†	1.36	20.94†
b. Dominance Variance	6	0.39	3.24**	0.092	1.41
b_1. Directional	1	1.11	9.25**	0.050	0.77
b_2. Unequal	3	0.013	0.11	0.146	2.25
b_3. Residual	2	0.41	3.38*	0.030	0.46
c. Maternal Effects	4	0.12	1.03	0.025	0.41
d. Reciprocal Effects	6	0.11	0.88	0.048	0.74
Error	262	0.12		0.065	
Heritability					
Narrow Sense			0.30		0.19
Broad Sense			0.35		0.20

Summaries of the analyses of variance of nicotine-induced hypothermia are provided for results obtained from the four-by-four diallel cross from which all C57BL crosses have been omitted. Each cell was composed of 7 males and 7 females. Replicate cells for the inbred mice were included. The degrees of freedom (*df*) for each item in the analysis are indicated. Both the mean square (MS) and the F-value are listed for each item at each nicotine dose. The major items in the analysis are: (a) additive genetic variance, sum of the average effects of the genes in a genotype; (b) dominance variance, difference between the additive genotypic value and the actual genotypic value; (c) maternal effects, effects dependent on the strain of the mother; (d) reciprocal effects, differences between reciprocal crosses not ascribable to (c). In addition, the dominance variance (b) has been subdivided into three items: (b_1) directional dominance, deviation from additive value toward the response of one of the parents; (b_2) unequal gene frequencies, an indication that some of the parents are carrying more dominant genes than are other parents; and (b_3) non-directional dominance (residual effects), dominance variance unique to each F1. Narrow-sense heritability is the fraction of the phenotypic variance resulting only from additive genetic factors; broad-sense heritabililty is the fraction of phenotypic variance arising from all sources of genetic variance.

*$p<0.05$.

**$p<0.01$.

†$p<0.001$.

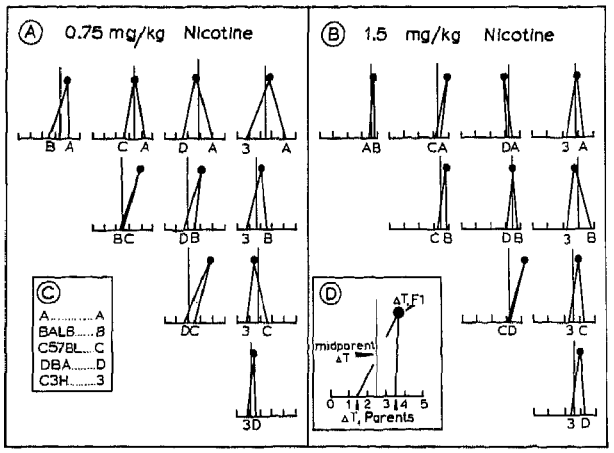

FIG. 3. Comparison of hypothermic responses of F1 hybrids to the parental inbred strains. Panels A and B show the relative hypothermic responses of the F1 hybrids after injection of 0.75 mg/kg or 1.5 mg/kg nicotine, respectively. The diagram in panel D is provided as a reference for interpretation of the diagrams of the actual results shown in panels A and B. The mean hypothermic response for the F1 hybrid (ΔT, F1) is represented by the point (●) at the apex of each triangle. The response of the F1 hybrid is compared to the hypothermic responses exhibited by the parental strains (ΔT parents), which are the values at the base of the triangles and are identified by the single letter codes explained in panel C. The vertical line in each diagram is the average temperature response shown by the two parents (midparent ΔT). A tilt of the triangle to the right indicates that the response of the F1 hybrid was more intense than that predicted by the midparent value, whereas a tilt to the left indicates a less intense response.

C57BL inbreds. After omission of these crosses, 5 of 6 F1s still showed directionality toward greater drug effect, a pattern consistent with the significant b_1 item calculated using the Hayman analysis. This directional dominance was further supported by the fact that the average temperature decrease of the 6 remaining F1s was 2.76° compared to an average of 2.28° for the four remaining inbreds. However, after a dose of 1.5 mg/kg nicotine no directionality was found in the absence of C57BL crosses. Only 2 of 6 F1s displayed greater nicotine-induced hypothermia than that predicted from the midparent value and the average temperature decrease for the F1s was only slightly greater (3.24°) than that of the four inbreds (3.13°). All four F1s with a C57Bl parent, however, were sensitive to nicotine and two of these crosses (C57BL × A and C57BL × DBA) were more profoundly affected than the more sensitive parent. When all crosses were included, the F1s had an average temperature decrease of 3.44° compared to an average of 3.13° for the five inbreds. It appears that any nonadditive hypothermic response observed at the higher dose arose from epistasis rather than from dominance, a finding completely consistent with the Hayman analysis.

The variance-covariance plots constructed in the absence of C57BL crosses can be used to estimate which of the four remaining inbreds carry the greater numbers of dominant or recessive genes. The points representing those inbreds carrying more dominant genes lie closer to the origin than do those carrying fewer dominant genes. The rank order of the inbreds changed slightly at the two nicotine doses. After a dose of 0.75 mg/kg, DBA mice appeared to carry more dominant genes than did the other three strains, followed by A mice and then by C3H and BALB mice, which appear to carry the fewest dominant genes. After the 1.5 mg/kg dose, the rank order became: A>DBA>C3H>BALB.

DISCUSSION

This study represents one of the first diallel analyses for a drug effect. Several previous studies that used a diallel design [1, 2, 13] did not apply diallel analyses of variance to the results. The existence of a strong genetic component in nicotine-induced hypothermia found in the present study is consistent with the results obtained in strain comparisons for nicotine-induced hypothermia in mice [14]. The pattern of inheritance is complex, indicating that many genes are involved. Additional genetic analyses will be required to describe this pattern more completely.

The results of the diallel analysis differed as a function of dose. In the analysis that omitted all C57BL crosses (which conformed to the additive-dominance model), considerable additive genetic variance was found for the hypothermic response after administration of either 0.75 mg/kg or 1.5 mg/kg of nicotine (see Table 3). This effect was more evident at the lower dose, however, probably because larger variation in hypothermic response occurred at this dose. The smaller range of responses and the approach of these responses to a floor effect may be responsible for the decrease in the additive genetic variance. The significant dominance effects found after a dose of 0.75 mg/kg were not evident at the 1.5 mg/kg dose, and the genetic variance at the higher dose was strictly additive in nature—i.e., the response of the F1 hybrids corresponded closely to the average of the two parental responses (see Fig. 3B, ignoring the C57BL crosses). Of particular interest is the significant directional dominance seen after a dose of 0.75 mg/kg nicotine. The direction is toward a more intense hypothermic response, i.e., the F1 hybrids tend to resemble their more sensitive parent.

The presence of a dominance component in a study such as this is often interpreted from an evolutionary viewpoint. Heterosis has been defined as the average superiority of the F1 animals over the average performance of the parental strains [6]. Therefore, it would be expected that the presence of dominance for a more severe hypothermic reaction to nicotine should bear some relationship to fitness in mice. This at first seems counterintuitive, since a large temperature drop is more likely to cause death. However, it is likely that temperature drop is confounded with rate of temperature loss, i.e., animals that suffer larger temperature decreases are also affected more quickly. If this is indeed the situation, it would indicate that a mouse would be more fit if it were rapidly and markedly affected by nicotine. Given that nicotine is lethal to mice in high doses, it would make sense in fitness terms that a rapid response to nicotine may serve to prevent ingestion of a lethal dose and thus would be highly adaptive. That the directional dominance disappears at the higher drug dose (in the absence of C57BL crosses), at which an intense response may be fatal, is consistent with this notion. It remains to be determined whether this pattern of response is specific for nicotine or if dominance for a rapid and intense response to a potentially toxic substance is a generalized phenomenon.

Whereas the genetic influence on nicotine-induced hypothermia consists of both additive and dominance components, it also appears that epistatic gene interactions are present and are contributed primarily by the C57BL strain. The epistasis is most pronounced in the C57BL × DBA hy-

brids. The C57BL and DBA strains are very similar in their hypothermic responses (see Fig. 1 and [14]). Other studies have compared these strains and found that they are also very similar in their rates of metabolism of nicotine [9], in their susceptibility to nicotine-induced seizure [17], and in their brain nicotinic receptors [14]. Yet, despite these similarities, their F1 progeny are much more sensitive to nicotine-induced hypothermia than is either parent strain. This finding certainly merits further investigation of the differences in the biochemical mechanisms of nicotine's actions in the DBA × C57BL crosses that are not apparent in the inbreds themselves.

REFERENCES

1. Anisman, H. Effects of scopolamine and d-amphetamine on the one-way shuttle and inhibitory avoidance: A diallel analysis in mice. *Pharmacol Biochem Behav* **3:** 1037–1042, 1975.
2. Anisman, H. Effects of scopolamine and d-amphetamine on locomotor activity before and after shock: A diallel analysis in mice. *Psychopharmacologia* **48:** 165–173, 1976.
3. Ankier, S. J., R. T. Brittain and D. Jack. Investigation of central cholinergic mechanisms in the conscious mouse. *Br J Pharmacol* **42:** 127–136, 1971.
4. Baer, D. S., G. E. McClearn and J. R. Wilson. Effects of chronic administration of tobacco smoke to mice: Behavioral and metabolic measures. *Psychopharmacology (Berlin)* **67:** 131–137, 1980.
5. Bättig, K., P. Driscoll, J. Schlatter and H. J. Uster. Effects of nicotine on the exploratory locomotion patterns of female Roman High- and Low-Avoidance rats. *Pharmacol Biochem Behav* **4:** 435–439, 1976.
6. Falconer, D. D. *Introduction to Quantitative Genetics*. New York: Ronald Press, 1960.
7. Garg, M. The effects of some central nervous system stimulant and depressant drugs on rearing activity in rats. *Psychopharmacologia* **14:** 150–156, 1969.
8. Garg, M. Variations in effects of nicotine in four strains of rats. *Psychopharmacologia* **14:** 432–438, 1969.
9. Hatchell, P. C. and A. C. Collins. The influence of genotype and sex on behavioral sensitivity to nicotine. *Psychopharmacology (Berlin)* **71:** 45–49, 1980.
10. Hayman, B. I. The analysis of variance of diallel tables. *Biometrics* **10:** 235–244, 1954.
11. Jinks, J. L. The analysis of continuous variation in a diallel cross of "Nicotiana rustica." *Genetics* **39:** 767–788, 1954.
12. Jones, R. M. Analysis of variance of the half-diallel table. *Heridity (Edinburgh)* **20:** 117–121, 1965.
13. Kitahana, K. and J. L. Valatx. Strain differences in amphetamine sensitivity in mice. I. A diallel analysis of open field activity. *Psychopharmacology (Berlin)* **66:** 189–194, 1979.
14. Marks, M. J., J. B. Burch and A. C. Collins. Genetics of nicotine response in four inbred strains of mice. *J Pharmacol Exp Ther* **226:** 291–302, 1983.
15. Mather, K. and J. L. Jinks. *Introduction to Biometrical Genetics*. London: Chapman and Hall, 1977.
16. Schlatter, J. and K. Bättig. Differential effects of nicotine and amphetamine on locomotor activity and maze exploration in two rat lines. *Psychopharmacology (Berlin)* **64:** 155–161, 1979.
17. Tepper, J. M., J. R. Wilson and K. Schlesinger. Relation between nicotine-induced convulsive behavior and blood and brain levels of nicotine as a function of sex and age in two inbred strains of mice. *Pharmacol Biochem Behav* **10:** 349–353, 1979.