A GENETIC ANALYSIS OF SPONTANEOUS ALTERNATION IN MICE[1]

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ABSTRACT—A genetic analysis of spontaneous alternation behavior using two 6 × 6 diallel matrices of mice reared under either enriched or standard cage conditions indicated (1) a high degree of directional dominance toward increased alternation rates, (2) no overall effect of early enrichment on alternation, (3) an increase in alternation due to early enrichment only among hybrids with one DBA/1J parent. These results, when examined in conjunction with an earlier genetic study, indicated that the pattern observed in alternation was similar to that for emotional reactivity to experimenter handling, suggesting either pleiotropic gene effects or that the alternation scores obtained were artifacts of genetically influenced fear responses. A second experiment, testing 3 strains under bright and dim illumination supported the hypothesis that fear can suppress alternation in mice, although one strain showed consistently low alternation in both test conditions. The importance of systematic sampling of genotypes, environments, and test parameters is stressed.

BEGINNING with the attempt by Dennis and Sollenberger (1934), a considerable number of reviews and explanations for alternation behavior of animals have appeared (e.g., Solomon, 1948; Dember and Fowler, 1958; Dember, 1961; O'Connell, 1965; Still, 1966a; Lester, 1967). These reviewers have usually taken general theories of curiosity and exploratory behavior and occasionally memory trace or inhibition theories and applied them to specific situations involving *T*-maze alternation. While the various theories can be roughly grouped into adaptation (tedium) theories or exploratory drive (titillation) theories (O'Connell), it is difficult to show unequivocal support for any one class of hypotheses despite the large number of studies in this area.

In contrast to this approach, which attempts to describe alternation behavior in a neurological–psychological framework, Breland and Breland (1966) have emphasized the evolutionary significance of the behavior, suggesting a possible survival value of alternation when level of reward is low after an initial choice of alternatives. This hypothesis has received little attention, perhaps partly because of the difficulty of directly testing it. While no simple direct method to test the survival value of alternation behavior is available, the use of techniques from population genetics does allow one to obtain strong indirect information concerning the adaptability of certain characteristics. It is generally acknowledged (e.g. Robertson, 1955; Falconer, 1960; Breese and Mather, 1960; Mayr, 1963; Bruell, 1964) that the effects of genes on characters connected with fitness will show

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dominance while effects not closely connected with fitness do not. Furthermore the directionality of dominance, if any, indicates the direction of scores contributing to increased fitness. In the present situation, for example, one would expect to find higher alternation frequencies among F_1 hybrids than inbred parent strains. In addition, such a characteristic should be relatively buffered against environmental intervention during early development (Waddington, 1957); hence, one would expect to find relatively small effects of different early rearing environments on alternation. The first experiment described examines genetic and early environmental influences on alternation using a diallel-cross analysis of mice reared in either standard cage or enriched cage conditions for the first six weeks of life. If spontaneous alternation is of some selective value there should be relatively little overall influence of rearing conditions on alternation rate and a large portion of the genetic variation obtained should be due to directional dominance.

EXPERIMENT I—A DIALLEL-CROSS ANALYSIS OF ALTERNATION BEHAVIOR IN MICE

Method

Subjects and Design. A total of 288 highly inbred mice from 6 strains *(A/J, BALB/cJ, RF/J, C3H/HeJ, C57BL/10J. DBA/1J),* and 240 F_1 crosses from the 6 strains were used. Genetic design was a diallel cross method wherein the 6 strains were mated to form all 36 combinations of offspring, 30 of which would be F_1's and the remaining 6 reproducing the original inbred strains. The 30 F_1 combinations consisted of the 2 reciprocal crosses from each of the 15 possible genetic combinations. There was an equal number of males and females in each of the genetic combinations; half of the mice from each combination were reared in standard laboratory cages and the remaining half in larger cages containing a variety of enrichment objects. Summing over environments, sex, and reciprocals, the 15 F_1 combinations were each represented by 16 mice, and the 6 inbred lines were each represented by 48 mice.

Apparatus and Procedure. At birth a litter was assigned and placed in either a small clear plastic laboratory cage (14 × 20 × 9 cm.) or in a larger cage (55 × 25 × 15 cm.) containing wood and wire mesh ramps, a small maze, and other objects allowing *S*s to receive a wide variety of perceptual and motor experiences throughout early development. Except for weaning at 21 days and a brief test of exploratory behavior and cage cleaning at 28 days animals remained undisturbed in both environments until 6 weeks of age, when they were given a test for spontaneous alternation.

The apparatus for testing alternation consisted of a *T*-maze made out of sheet metal, with corridors 3 cm. wide and 4 cm. high with a 0.6 cm. slot in the cover of the maze to allow for observation of the animal. The starting arm of the maze was 25 cm. long and each place arm 18 cm. from the choice point to the end. The apparatus was designed with swing away walls to allow the animal to be placed in and removed from the maze with minimum disturbance to the animal. The apparatus was placed in a room where the only illumination came from a 25 w.

red lamp located 1.5 m. above the *T*–maze. Both the illumination and the structure of the maze therefore provided minimal visual stimulation for the animals. The testing procedure consisted of placing the animals in the starting arm of the maze and recording the first arm in which the animal completely entered with all 4 feet. The animal was immediately removed and given a second trial using the same procedure and criterion.

RESULTS

Table 1 summarizes tests of significant differences in alternation tendencies between the two environments, inbreds vs. hybrids, and sexes. Regarding main effects, sex differences and environmental differences were nonsignificant, whereas inbreeding significantly reduced alternating. Summed over all genetic combinations the odds are better than two to one that an F_1 hybrid would choose the opposite arm of the *T*–maze on the second trial, whereas among inbreds prediction of trial two based on the first trial was only slightly better than chance. While this superficial analysis supports both the prediction of an inbreeding depression effect and a lack of overall environmental effect on alternation behavior, a more detailed genetic analysis was carried out.

TABLE 1
Percentage of Animals Showing Spontaneous Alternation on the Second Trial

Group Comparison	Number of Animals	Percent Alternating	Chi Square & Significance
Enr. Cgs.	264	61.0	0.07
Std. Cgs.	264	62.5	$p<.90$
Inbreds	288	55.2	9.56
Hybrids	240	68.7	$p<.01$
Males	264	59.0	1.36
Females	264	64.3	$p<.20$

Since additional chi-square tests indicated that sex differences failed to interact significantly with any of the other factors in the experiments, sexes were pooled in further analyses. Preliminary testing of reciprocal differences to determine whether maternal effects were playing any role, were also not significant in both environments ($p > .5$); thus, reciprocal crosses were also pooled before undertaking the biometric analysis. Table 2 indicates the average percentage of animals in each inbred strain and hybrid cross showing alternation behavior. From this table the pattern of overdominance with respect to alternation tendencies can be seen more clearly. Hybrids uniformly show alternation tendencies while only three of the inbred strains showed consistent alternation behavior. Summing over environments, ten of the fifteen crosses show a greater frequency of alternation than either parent strain. A *t*-test between average F_1 performance and the larger parent mean using transformed proportions (probits) indicated that the average F_1 alternation frequency is significantly higher than that of the average larger parent mean in each of the possible crosses ($t = 2.4, p < .02$).

TABLE 2

Percentages of mice reared in standard cages (roman) and enriched cages (italics) alternating on second test trial

Parent	A/J	BALB/ cJ	RF/J	C3H/ HeJ	C57BL/ 10J	DBA/ 1J	Hybrid Av.
A/J	50	88	75	63	75	88	78
	42	*75*	*50*	*50*	*63*	*88*	*65*
BALB		71	75	63	63	50	68
		67	*75*	*63*	*63*	*50*	*65*
RF			50	50	88	50	68
			38	*75*	*88*	*75*	*73*
C3H				71	88	50	63
				58	*63*	*88*	*68*
C57					63	63	75
					71	*100*	*75*
DBA						54	60
						58	*80*

While angular or probit transformations are frequently used to eliminate mean-variance correlations found in proportional data, both of these transformations and the raw percentage scores failed to satisfy the scaling assumption required for biometric analysis of the alternation data using techniques described by Hayman (1954*a,b*). It was found that scaling assumptions could best be met by transforming the percentage data to square roots; thus, the square root of the percentage of animals alternating in each genetic-environment combination was used for the biometric analysis. This transformation depressed the magnitude of dominance variation found, but these estimates were less likely to be inflated by the presence of epistatic interactions than raw scores or other transformations (Jinks, 1955).

The analysis of variance of diallel crosses (Hayman, 1954a), modified by replacing reciprocal crosses with animals reared in the two environments, indicated a highly significant directional dominance effect ($F = 10.8$, $df = 1/10$, $p < .01$), some significant residual dominance ($F = 3.8$, $df = 9/10$, $p < .02$), and a significant Line X Environment effect ($F = 5.2$, $df = 5/10$, $p < .02$). The latter interaction was primarily due to the increased frequency of alternation of *DBA* crosses reared in the enriched cages, while other lines showed no significant difference in the two environments. Estimates of genetic variance, using a variance-covariance analysis, indicated that additive and dominance variance for the combined environments was .22 and .48, respectively, or approximately two-thirds of the genetic variation was due to dominance effects. A frequently used measure of dominance is $\sqrt{\text{for 2 Dom.Var./Add.Var.}}$ which ranges from 0 to 1 when there is no dominance to complete dominance detected, and to values greater than 1 when overdominance is present. In the present case this ratio is 2.10, indicating considerable overdominance. Furthermore, the high correlation ($r = .94$) between the proportion of dominant genes possessed by each of the 6 parental lines and the proportion of animals alternating in each of these lines, indicates the unidirectionality of the dominance effect toward greater alternation frequency.

Because of the difference in *DBA* crosses in the two environments the above analysis was also carried out separately for animals reared in each environment. While the magnitude of genetic effects were slightly greater among enriched than normally reared mice (add. var.: $STD = .14$, $ENR = .28$; dom. var.: $STD = .18$, $ENR = .34$), dominance ratios again indicated overdominance in both environments. Analysis of the data also suggested that as few as two gene blocks may be involved in this behavior, although no upper limit can be established.

DISCUSSION

While both the superficial analysis and the more detailed biometric analysis both tended to support the initial hypotheses of directional dominance and relatively little influence of the rearing environment, the more detailed analysis reveals a genotype-environment interaction in which animals from DBA crosses reared in the enriched environment did show an increase in alternation tendency whereas other crosses and inbreds did not.

A possible explanation of these results may be obtained by comparing the results of the current study to one in which four of the six lines currently used were also used in a diallel-cross analysis of reactivity to experimenter manipulation (Henderson, 1967). In that study its was found that inbreds generally reacted to moderate stimulation (as measured by increased open-field emotionality) whereas hybrids did not until noxious shock stimulation was used. The major exception to this involved the hybrid crosses with one *DBA* parent, which reacted more like the inbreds, in terms of reaction to moderate experimenter manipulation.

This similarity in genetic pattern in the fearful response to experimenter handling in the earlier experiment and alternation behavior in the present experiment suggests rather strongly that either the genes responsible for alternation are also responsible for resistance to moderate emotional stress (gene pleiotropy), or that the presence of fear reduces alternation tendency. On the other hand there is only a modest relationship between alternation in the present experiment and exploratory activity of these lines reported elsewhere (Bruell; Henderson). The genetic evidence therefore supports the hypothesis that fear suppresses alternation more strongly than a hypothesis of relationship between exploration and alternation tendencies. If in the present case reduced alternation of inbreds and *DBA* hybrids reared in standard cages is an artifact of their increased fear response to handling, one must conclude that the emergence stress phenomena described by Fuller (1967) is also genetically influenced.

While some evidence already exists that fearfulness may serve to suppress alternation behavior in rats (e.g., Still, 1966b, 1968; Lester), a confirmation of this hypothesis using mice is advisable. Experiment II attempts to provide such evidence using three of the strains which showed significant differences in alternation rates in Experiment I.

EXPERIMENT II—ALTERNATION FREQUENCY IN MICE TESTED IN LIGHT AND DARK CONDITIONS

Since mice are generally light aversive one might expect that *Ss* tested in a brightly lit room would show a greater fear response and thus suppress alternation tendencies more than mice tested in a darkened area, having a minimum of experimenter handling prior to testing. Experiment II attempts to provide two testing situations which fall on either side of the test situation used in Experiment I with respect to the noxious level of the test situation for the subjects.

Method

Subjects and Apparatus. Subjects were 150 mice with equal numbers from the *A/J, C57BL/*10, and *DBA/1J* strains. *Ss* were born in the laboratory and reared in 33 cm. × 15 cm. × 13 cm. plastic mouse cages until adulthood, when they were placed in larger pen cages until 11 to 14 weeks of age, when testing began. The apparatus consisted of a *T*–maze of identical construction and height and width dimensions of that used in Experiment I but the longer starting runway (40 cm.), and longer choice arms (27 cm.).

Experimental Design and Procedure. A 3 × 2 factorial analysis was used with Strains and Test Conditions as main factors. Twenty five animals, with approximately half of each sex, were used in each cell of the design. In the dark, or low noxious test situation, the *T*–maze was placed directly in the animal colony room during the dark lighting cycle when the room is illuminated only by a series of 7½ w. red ceiling lamps. *Ss* were removed from their normal living cages and tested immediately using care to minimize the disturbances to *Ss* prior to and during the test. In the light test, or high noxious test situation, the *T*–maze was set up in a well lighted room and animals carried from the darkened colony room to the test room in temporary holding cages. Time of day of testing was the same for both test conditions. In both cases *Ss* were placed in the starting end of the *T*–maze and the choice of arms recorded. This procedure was repeated for four consecutive test trials. *Ss* were scored as to whether they alternated on the second test trial and were also scored from 0 to 3 based on the number of alternations made during the four trials.

RESULTS AND DISCUSSION

Table 3 indicates the percentage of animals alternating on the second test trial and the mean percentage of alternation for each strain in the two test conditions. It can be seen that alternation frequency was lower in all strains on both measures in the lighted test condition although initial alternation rate was only marginally significant ($\chi^2 = 2.76$, $df = 1$, $p < .10$), while an analysis of variance on four trial alternation performance was highly significant ($F = 7.1$, $df = 1/145$, $p < .01$). On both measures strain differences were detected with *A/J* showing significantly less alternation than *C57* and *DBA* mice (initial alternation frequencies $\chi^2 = 4.28$, $df = 1$, $p < .05$; means four trial percentages $F = 10.0$, $df = 2/145$, $p < .01$). No interaction between strain and test situation was found.

TABLE 3

Percent animals alternating on second test trial (roman) and mean percent of alternation for each group during all four test trials (italics)

	Strain		
Test Cond.	A/J	C57BL/10J	DBA/1J
Dark	56	76	68
	47	*74*	*59*
Light	40	60	56
	29	*56*	*53*

The above findings lend support to the hypothesis that alternation behavior is depressed in a more fearful or noxious test situation and confirms the earlier finding of the existence of genetic influences on alternation behavior. The present experiment also suggests that the low rate of alternation among *A/J* mice is generally consistent across tests whereas relatively low alternation in Experiment I of *DBA* inbreds was influenced in some way by the test situation.

CONCLUSION

While Experiment I tended to support the hypothesis that alternation behavior does have some selective advantage for mice, subsequent analysis and a comparison with already available data from similar strains suggested that the effects found may be largely due to genetic differences in fearfulness during testing. Since evidence is already accumulating that fearfulness does play a role in suppressing alternation behavior it may be said that the current study provides only collaborative evidence and no new information concerning the alternation phenomena. It should be pointed out however that this relatively parsimonious finding was obtained only through a systematic sampling of genotypes, early rearing environments, and test conditions. An inspection of Table 2 suggests that if a number of different experimenters had worked with various smaller subsets of lines, crosses, or environmental conditions, a variety of apparently conflicting results would have emerged, thus adding to both the volume of literature and confusion in this area.

It is precisely this elimination of potential blind alleys plus the efficiency of using earlier, directly comparable genetic data, that lends support to Henderson's earlier arguments (1967, 1968, 1969) that selected genetic material and a broader sampling of test and treatment conditions should be incorporated whenever possible in behavioral experiments with lower animals.

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