Performance of C57BL/6J, DBA/2J, and B6D2F₁ mice in a free-operant avoidance task

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Male mice (*Mus musculus*) 12-16 weeks of age from the C57BL/6J and DBA/2J inbred strains and B6D2F₁ hybrids were trained at one of two shock levels (.5 or 2.0 mA) in a wheel-turn avoidance task during one 60-min session. Following two 10-min blocks during which no shock was given, a free-operant avoidance program was activated that required the animal to turn a wheel .5 revolution at least once every 10 sec in order to avoid a 1-sec footshock. The program was terminated when the mouse reached an acquisition criterion, which was 5 min of successful shock avoidance, or when it completed the 60-min session without reaching criterion. Strain differences in learning were marked and consistent at both shock levels. Most C57BL/6J mice reached criterion, and most DBA/2J mice failed. The performance of B6D2F₁ mice matched that of the C57BL/6J strain.

A variety of paradigms have appeared in the literature related to genetic influences upon the learning phenotype of the inbred house mouse (*Mus musculus*) (see reviews of Wahlsten, 1972a, 1978). Avoidance paradigms have been among the most commonly used to demonstrate strain differences in performance of learning tasks (e.g., Royce & Covington, 1960; Schlesinger & Wimer, 1967), to analyze the genetic components of these differences using diallel analysis (e.g., Collins, 1964; Royce, Yeudall, & Poley, 1971), single-gene Medelian analysis (e.g., Sprott, 1974; Stavnes & Sprott, 1975b), and recombinant inbred strains (e.g., Oliverio, Eleftheriou, & Bailey, 1973), and to correlate physiological and behavioral parameters of learning in inbred strains (e.g., Church & Sprott, 1979). A variety of shock-motivated paradigms have been applied, including passive avoidance, with contingencies involving step-down (e.g., Bovet, Bovet-Nitti, & Oliverio, 1969) responses, and active avoidance, with contingencies involving jump-up (e.g., Stavnes & Sprott, 1975a, 1975b), one-way (e.g., Anisman, 1975), shuttle (e.g., Messeri, Oliverio, & Bovet,

1971; Oliverio, Castellano, & Messeri, 1972), and wheel-turn (e.g., Zerbolio, 1967) responses.

The results of these studies have often shown that particular strains do not perform consistently across tasks (Wahlsten, 1978). For example, studies in one laboratory have consistently reported that DBA/2J mice were superior in performance of shuttle avoidance compared with C57BL/6J mice (Messeri et al., 1972; Oliverio et al., 1972). In contrast, studies at the Jackson Laboratory using a jump-up avoidance paradigm have produced consistent reports that the C57BL/6J strain exhibits superior performance in comparison with the DBA/2J strain (Sprott & Stavnes, 1975b; Stavnes & Sprott, 1975a, 1975b). Mendelian analysis of this genetic variation in jump-up avoidance performance yielded evidence of a single-locus model of inheritance (Stavnes & Sprott, 1975b).

All the aforementioned studies involved active avoidance learning in discrete-trial paradigms. In this situation the contingency to be learned is externally cued. The present investigation compared the C57BL/6J and DBA/2J strains and an F₁ hybrid in a Sidman avoidance task, which involved an internally cued, free-operant avoidance contingency (Sidman, 1953).

EXPERIMENT 1

Method

Subjects. Fifteen male mice (*Mus musculus*) 12-16 weeks old were selected from each of the C57BL/6J and DBA/2J inbred strains and their F₁ hybrid, B6D2F₁ (henceforth referred to as B6, D2, and F₁, respectively). These animals had been obtained from the Animal Resources Division of the Jackson Laboratory at approximately 8 weeks of age. Following their arrival, the mice were housed five to a plastic cage with bedding of wood

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shavings; they were placed in a vivarium maintained at 22°C ± 2°C with a 12-h light/12-h dark cycle. Water and food (Old Guilford 96W) were provided ad lib throughout the experiment.

Apparatus. The apparatus was a wheel-turn avoidance chamber constructed of translucent acrylic and stainless steel. The chamber contained two stationary areas, each with a stainless steel grid floor approximately 6.0 x 7.5 cm in area and 10 cm high. Located between these two stationary areas was a movable floor. This floor was formed by a 180-deg arc of the outer surface of a wheel constructed of stainless steel grids; the wheel had a radius of 10 cm, and it connected the two stationary areas. The wheel was mounted on a well lubricated bicycle wheel hub and axle. The grid floor of the wheel was connected to a constant-current shock source and grid scrambler via eight mercury baths, providing electrical contacts with minimal friction. The grid floors of the stationary areas were connected to the same shock source and scrambler. A removable top to the chamber contained housing for a 15-W light that was activated automatically.

The apparatus was placed in a ventilated, light-excluding, sound-attenuating container in a test room. In an adjacent room, there was a rack of standard electromechanical circuitry connected via cables to the apparatus, thus permitting programmed control of the procedure and data recording.

Procedure. Each mouse was tested during one 60-min session of Sidman avoidance. About 30 min prior to its assigned session, an animal was removed from its home cage and placed in a cardboard container for transportation to the testing room. The animal was removed by the tail from the container and placed on top of the wheel of the apparatus. The top of the apparatus was secured in place, the door to the exterior container was closed, and within 5 sec the session was begun.

No shock was available during the first 20 min of the session. During the first 10-min block, the light was off, and the number of revolutions of the wheel was counted as a baseline measurement of spontaneous activity. During the second 20-min block, the overhead light was activated, and the wheel-turn responses were again counted. The change in illumination was designed to reduce the level of spontaneous activity.

After this second block, the Sidman avoidance program was activated with 10-sec shock-shock, response-shock intervals. The overhead light was illuminated for the remainder of the session. Scrambled footshock of 1 sec duration was programmed to occur every 10 sec. The mouse could avoid footshock by turning the wheel at least .5 revolution at least once every 10 sec. When the wheel-turn response was made, the clock reset and another 10-sec interval began, during which another response was required to avoid footshock. Once the shock was activated, the animal could terminate it by making a wheel-turn response. This contingency constituted an escape response. The schedule continued for 40 min or until the acquisition criterion was met, which was a 5-min interval during which no shock was received. When this criterion was reached or when 40 min had elapsed, the shock schedule was terminated and the light was turned off. The mouse was then removed from the apparatus, ear-punched, replaced in the cardboard container, and returned to its home cage about 1 h later.

Six sessions were conducted each day during the light portion of the light cycle. At least two mice from each genotype participated daily. The order of the sessions during the day was randomized across genotypes. The apparatus was cleaned between sessions. The data from two mice from each genotype were lost due to procedural errors or equipment malfunction; therefore, there were 13 mice in each group.

Results

Nearly all B6 mice reached criterion in this task, whereas most D2 mice failed. The performance of F₁ mice matched that of the B6 strain; that is, nearly all reached criterion. The numbers of animals reaching and not reaching criterion from each strain are presented in Table 1. The results of a χ^2 analysis of these data revealed a significant genotype effect [$\chi^2(2) = 16.85$, $p < .001$].

The same pattern of performance across strains was demonstrated in the data on the number of shocks received during the training session. As observed in Table 1, the mean number of shocks that D2 mice received was about three times higher than the number that B6 and F₁ mice received. Because of the substantial variability evident in these data, they were subjected to a square-root transformation before being submitted to one-way analysis of variance (ANOVA). The results revealed a significant genotype effect [$F(2,36) = 8.82$, $p = .0007$]. Individual comparisons were made with the Tukey (a) procedure. D2 mice received significantly more shocks than either B6 or F₁ mice received ($p < .01$), but B6 and F₁ mice did not differ significantly ($p > .05$).

Also shown in Table 1 are data on the mean number of responses emitted by mice of each genotype in the absence of shock in the dark during the first 10 min of the session (first block) and in the light during the second 10 min (second block). B6 mice appeared to emit the highest number of responses during both blocks. These data were subjected to a square-root transformation to reduce variability and then were submitted to one-way ANOVA across each block. The results revealed no significant genotype differences in these measures of spontaneous activity in either block ($p > .05$). It was evident, however, that responses in the light declined in all genotypes to about half the level recorded in the dark.

The mean body weight of each genotype is also presented in Table 1. An ANOVA of these data yielded no significant differences ($F < 1.0$).

Table 1
Genotype Comparisons at .5-mA Shock Level

Parameter	C57BL/6J		B6D2F ₁		DBA/2J	
	Mean	SD	Mean	SD	Mean	SD
Number of Shocks Received	31.9	47.8	35.9	31.4	104.2	58.7
Responses During First Block	140.8	58.7	116.6	66.0	95.6	44.0
Responses During Second Block	59.2	47.8	35.9	31.4	44.2	27.2
Body Weight (g)	28.5	1.6	29.7	2.7	30.1	1.4
Number of Mice Reaching Criterion	11		12		3	
Number of Mice not Reaching Criterion	2		1		10	

Note—Response = ½ wheel revolution.

Discussion

Fuller (1970) tested four strains, including B6 and D2 mice, in shuttle avoidance during a Sidman schedule and observed that D2 mice exhibited performance superior to that of B6 mice; however, this exact comparison was not verified in a statistical test. The results of the present experiment supported past findings that have characterized B6 mice as better performers in active avoidance tasks compared with D2 mice (Sprott & Stavnes, 1974, 1975b; Stavnes & Sprott, 1975b). D2 mice were clearly handicapped in the performance of this free-operant avoidance task. These results contrast with previous findings in other laboratories examining discrete-trial shuttle avoidance that have found D2 mice to be superior performers (Bovet et al., 1969; Messeri et al., 1972; Oliverio et al., 1972) or have found little difference in performance between these two strains (Anisman, 1975; Wahlsten, 1972b).

Although the performance of the parental strains in this experiment was similar to that observed in jump-up active avoidance, the direction of inheritance suggested by the performance of the F_1 mice was opposite to that observed in an earlier experiment (Stavnes & Sprott, 1975b). Dominance was evident in both tasks; however, in the jump-up avoidance task, B6D2 F_1 mice duplicated the inferior performance of the D2 parental strain, whereas in this free-operant avoidance task, the hybrids matched the superior performance of the B6 parental strain.

In studies of shuttle avoidance, there have been reports suggesting a recessive mode of inheritance matching the inferior performance of the B6 parental strain (Messeri et al., 1972; Oliverio et al., 1972). In another study of one-way and shuttle performance, there was the report of dominance and even overdominance in these genetic comparisons (Anisman, 1975). The divergent results of these investigations of active avoidance underscore the contention that genetic influence on learning phenotypes tends toward task specificity rather than toward performance generality (Sprott & Stavnes, 1975a; Wahlsten, 1978).

EXPERIMENT 2

Past experiments using avoidance paradigms have observed a performance interaction between strain and level of shock (Sprott, 1972; Sprott & Stavnes, 1975b; Wahlsten, 1972b). This experiment was designed to determine whether the strain differences observed in Experiment 1 using a relatively low level of shock would be replicated under a higher level of shock.

Method

Subjects. Totals of 15 male mice 12-16 weeks of age were selected from each of the C57BL/6J and DBA/2J strains and their F_1 hybrid, B6D2 F_1 . The origin of the animals and all

conditions of husbandry were the same as described in the preceding experiment.

Apparatus. The same apparatus was used as described above.

Procedure. The exact procedure described in Experiment 1 was duplicated in this experiment, except that the footshock level was raised to 2.0 mA. The data from one B6 animal were lost due to equipment malfunction.

Results

The pattern of performance across genotypes observed in Experiment 1 was duplicated. Table 2 presents the genotype comparisons on several performance parameters. Again few D2 mice were able to reach criterion in this task, whereas most B6 mice did; the performance of F_1 s matched that of the B6 strain. A χ^2 analysis of the numbers of animals reaching and not reaching criterion revealed a significant genotype effect [$\chi^2(2) = 12.48$, $p < .005$]. Compared with the results of Experiment 1, however, it appeared that slightly more animals in the B6 and F_1 genotypes failed to reach criterion when this higher level of shock was used.

With respect to the mean number of shocks received, D2 mice received approximately twice as many as B6 and F_1 mice did, as observed in Table 2. Following a square-root transformation, these data were analyzed in a one-way ANOVA that yielded a significant genotype effect [$F(2,41) = 6.15$, $p = .005$]. The Tukey (a) procedure was used for individual comparisons between groups. The results showed that D2 mice received significantly more shocks compared with B6 mice and with F_1 mice ($p < .01$), but B6 and F_1 mice did not differ significantly ($p > .05$). Compared with data from Experiment 1 on this performance parameter, it appeared that all genotypes received more shocks at this higher level.

Unlike the previous results, there was evidence of genotypic differences in spontaneous activity, as measured by responses made before shock was activated. These data, shown in Table 2, were submitted to a square-root transformation and then to a one-way ANOVA. A significant genotype effect was evident in the dark [$F(2,41) = 6.34$, $p = .004$]. The results of the Tukey (a) test revealed that during the first block, B6 mice emitted significantly more responses than did D2 mice or F_1 mice ($p < .05$); however, the latter two genotypes did not differ significantly ($p > .05$). During the second block, when the light was activated, there was

Table 2
Genotype Comparisons at 2.0-mA Shock Level

Parameter	C57BL/6J		B6D2 F_1		DBA/2J	
	Mean	SD	Mean	SD	Mean	SD
Number of Shocks Received	69.7	61.1	53.5	45.4	125.6	49.2
Responses During First Block	163.0	54.1	90.0	58.2	93.2	65.1
Responses During Second Block	70.0	56.3	26.3	40.6	48.5	62.9
Body Weight (g)	28.5	2.2	28.9	3.0	29.1	1.7
Number of Mice Reaching Criterion	9		11		2	
Number of Mice not Reaching Criterion	5		4		13	

Note—Response = $\frac{1}{2}$ wheel revolution.

also a significant genotype effect [$F(2,41) = 4.29, p = .02$]. According to the results of the Tukey (a) test, B6 mice responded significantly more only in comparison with F_1 mice ($p < .05$). The comparison between B6 and D2 mice was in the same direction as in the first block, but it was not significant ($p > .05$). The difference between the D2 and F_1 groups was also not significant ($p > .05$).

Table 2 also presents the mean body weights of each genotype. According to the results of one-way ANOVA, there were no significant differences across genotypes ($F < 1.0$).

Discussion

The results of this experiment paralleled those of the preceding one. D2 mice were clearly inferior in performance compared with the superior and similar performances of B6 and F_1 mice. Since the pattern of performance was duplicated at a shock level four times higher, explanations pertaining to motivational factors, such as shock threshold, would seem to be less tenable.

The only observation diverging from the findings in Experiment 1 was evidence of significant genotype differences in baseline activity. In this experiment, B6 mice had a higher level of responding during the two no-shock blocks, compared with the other genotypes. This direction of responding had been observed in Experiment 1 but had not proven statistically significant.

There have been consistent reports that B6 mice exhibit a higher level of general activity compared with D2 mice in a variety of tests (Anisman, 1975; Messeri et al., 1972; Oliverio et al., 1972; Sprott & Eleftheriou, 1974). This behavioral trait would appear to be an advantage to the B6 strain in an active avoidance task; however, past and present findings do not support this assumption (Anisman, 1975; Messeri et al., 1972). There has been little evidence of a correlation between activity and success in active avoidance tasks. Furthermore, in the present results it should be noted that F_1 mice displayed a significantly lower level of responding during the no-shock blocks compared with B6 mice, yet the hybrids matched the superior performance of this parental strain in avoidance responding.

GENERAL DISCUSSION

Consistent with previous reports of performance in jump-up avoidance tasks (Sprott & Stavnes, 1974, 1975a, 1975b), B6 mice were characterized as superior avoiders in comparison with D2 mice in this free-operant task. In contrast to previous observations of F_1 performance in jump-up avoidance, dominance in the F_1 hybrid was in the direction of superior performance. This pattern across genotypes endured across replicate experiments using two levels of shock and, thus, reduced questions concerning motivational factors. In addition, it was difficult to attribute the genotype differences in avoidance learning to differences in baseline activity because B6 mice tended toward greater activity and F_1 mice did not. Whether other nonassociative factors, such as shock reactivity (Anisman, 1975), affected performance remains to be investigated.

The present results demonstrate the utility of the Sidman avoidance paradigm for investigating genetic influences upon the learning phenotypes of mice. With the addition of other control groups, such as yoked-shock controls, the paradigm can be used to assess further other nonassociative factors operating in this task, such as noncontingent response to shock. By lowering the shock level, the task could also be used to assess shock thresholds. The assessment of these performance variables might provide further insight into the genotype-environment interaction observed when different avoidance paradigms are used.

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