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Strain Differences in Amphetamine Sensitivity in Mice

I. A Diallel Analysis of Open Field Activity

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Abstract. Experiments are reported which show that 1 mg/kg of *d*-methylamphetamine HCl induced hyperactivity in pigmented strains (C57BR, C57BL/6, and SEC) and hypoactivity or no change in albino strains (BALB/c, A, and AKR) of mice. In F1 hybrids, the B6 genotype was partially dominant over BR and C, and BR over C. In animals back-crossed to C parents widespread distributions with two peaks were obtained in control experiments, and amphetamine induced hyperactivity in 38% of the albino population, and hypoactivity or no significant change in 45% of the pigmented one. This genetic study indicates that genes influencing locomotor activity are independent from those influencing amphetamine sensitivity. From results obtained in back-crosses and C57BL/6-c^{2J} mice, the albino gene does not seem to be involved in the hypoactive effect of amphetamine.

Key words: Amphetamine sensitivity – Inbred strains – Strain difference – Diallellic analysis – Back-crossing – Albino and pigmented strains

In using genetically controlled inbred strains of mice, it has been largely accepted that there are clear strain differences in some behavioral traits (emotionality, learning ability, etc.) (Bovet et al., 1969; Thiessen et al., 1970; Thompson, 1956; Abeelen, 1966), and that the mode of inheritance of such characteristics may be influenced by one or several genes (Thiessen et al., 1970; Oliverio et al., 1973). Recently, many authors have reported that amphetamine as well as scopolamine provoke strain-dependent effects on some behavioral traits in mice, such as open field emotional activity (Abeelen et al., 1971; Oliverio et al., 1973; Moisset and Welch, 1973; Moisset, 1977; Anisman, 1976). However, it is not clear that such a difference is determined by a polygene system (Oliverio et al., 1973) or a single major gene (Moisset, 1977). The present study was aimed at clarifying the mode of inheritance of open field ambulation and strain-dependent responses to amphetamine, using 3×3 diallellic crosses and back-crosses among C57BR, C57BL/6, and BALB/c strains. A/J, AKR, and SEC mice were also studied independently. Furthermore, the strain C57BL/6-c^{2J}, with a mutation at the albino locus, was studied to check the role of the albino gene on ambulation scores or amphetamine sensitivity.

Materials and Methods

Animals. The subjects were naive male and female mice ($N = 702$), (25 – 30 g) from inbred strains C57BL/cd/Orl. (BR), C57BL/6 Orl. (B6), BALB/c Orl. (C), AKR/Orl. (AKR), A/J/Orl. (A) (Centre d'élevage du CNRS à Orléans), and SEC, as well as hybrids and back-crosses produced from matings of all possible combinations among BR, B6, and C parents. Thirteen males and 26 females from each strain were crossed to produce reciprocal F1 progeny (indicated by BRCF1 for C57BR ♀ × BALB/c ♂, CBRF1 for BALB/c♀ × C57BR ♂, B6BRF1 for C57BL/6 ♀ × C57BR ♂, etc.). To obtain the back-cross generation (B1 or B2), F1 mice were mated with each of their progenitor lines (CBRF1 × BR indicates hybrid CBRF1 ♀ × C57BR ♂, etc.).

For mating, one male and two female mice were placed together in a plastic cage and the males were removed when the females were pregnant. The litters were weaned at 21 days and divided according to sex. Animals were maintained in plastic cages in groups of 4 or 5, with free access to food and water, under conditions of a 12-h light-dark cycle (temperature 24° ± 1° C). Tests on behavior started when the mice were 8 – 10 weeks of age.

Apparatus and Procedure. An open field (126 × 126 cm) was used which was without restraining walls and 80 cm off the floor. The surface was divided into 36 squares (21 × 21 cm) and illuminated by a dim white light (10 lux) or, in certain cases, by a dim red light (1 lux). The animal, either nontreated or after having received an IP injection of saline (0.9% NaCl, 0.12 ml), was placed in the center of the surface and allowed to explore it for 1 min. After this habituation, the number of lines crossed during 3 min (ambulation score) was measured. No significant difference was observed between nontreated and saline-injected animals. Between observations, the floor was cleaned with a

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wet sponge and dried. The same animal was then tested again in the open field 30 min after IP injection of *d*-methylamphetamine HCl (1 mg/kg) diluted in 0.9% NaCl solution (0.12 ml). According to preliminary data with several doses of this drug (1, 2, and 4 mg/kg), we have observed the same alterations of locomotor activity and have chosen the dose of 1 mg/kg, i.e., the effective dose for locomotor activity without or with few stereotyped behaviors. All tests started between 9 a.m. and 12 noon. The data were analysed by analysis of variance. An unweighted means formula was used because of the unequal sample size of each cell (Winer, 1962).

Results

Control Group

Table 1 gives means and variances of ambulation scores in the open field and the corresponding sample size for each genotype. Figure 1 is a set of ambulation score histograms.

Inbred Lines. A significant strain difference was observed among six inbred strains [$F(5,374) = 113.7$, $P < 0.01$], but not between sexes [$F(1,374) = 0.01$, NS]. The rank order of activity was BR, B6, AKR, C, SEC, and A. Generally, ambulation scores in pigmented mice were greater than in albino mice. These results were thus identical to those obtained by a number of authors (Thompson, 1956; Abeelen, 1966; Oliverio et al., 1973). However, the activity level of C57BL/6-c^{2J} (albino) was as high as B6 mice, and that of SEC mice (pigmented) was relatively low (Table 1).

Table 1. Genetic study of ambulation scores in the open field (Means, variances, and the corresponding sample size for each genotype) in three mouse strains [C57BR/cd (BR), C57BL/6 (B6), and BALB/c (C)], their hybrids, and back-crosses. Genotypes (16–19) represent the ambulation scores in strains A/J, AKR, C57BL/6-c^{2J} (albino mice), and SEC (pigmented)

	Genotypes	Mean	Variance	*N*
Parents	1. BR	93.0	305.0	100
	2. B6	68.1	270.0	114
	3. C	27.1	161.7	100
Hybrids	4. CBRF1	73.9	305.3	48
	5. BRCF1	68.1	131.2	23
	6. CB6F1	53.2	203.5	35
	7. B6CF1	50.2	93.4	17
	8. B6BRF1	71.5	153.7	21
	9. BRB6F1	73.3	107.6	8
Back-crosses	10. CBRF1 × BR	77.4	332.0	30
	11. CBRF1 × C	49.2	299.6	41
	12. CB6F1 × C	37.1	255.3	27
	13. CB6F1 × B6	57.6	203.4	31
	14. B6BRF1 × BR	78.7	79.0	9
	15. B6BRF1 × B6	72.6	188.6	38
Other strains	16. A/J	5.8	29.2	14
	17. AKR	40.5	228.5	16
	18. C57BL/6-c^{2J}	61.3	244.8	20
	19. SEC	22.0	86.5	10

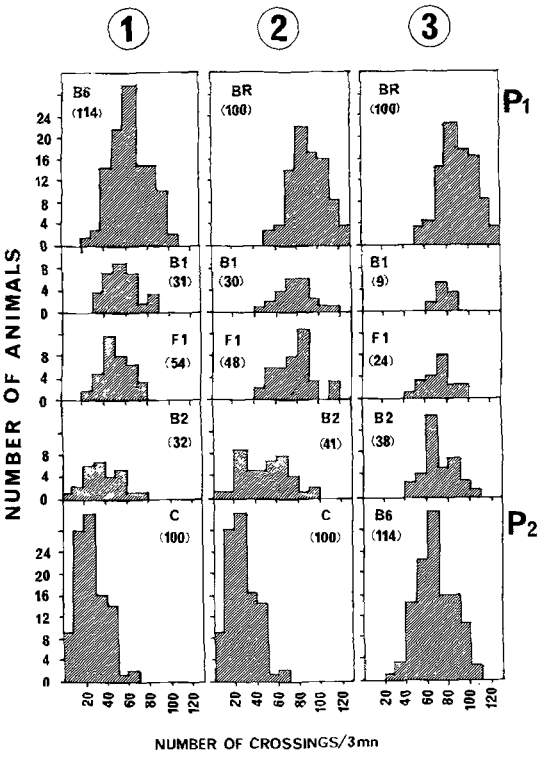

Fig. 1. Histograms of the number of nontreated animals (male and female) as a function of ambulation scores in the open field in parent (BR, B6, and C), reciprocal F1 hybrids (F1), and each of the back-crosses (B1 and B2). The numbers in parentheses indicate the total number of animals tested in each group. Each vertical column represents one set of experiments (Column 1, crossing between B6 and C; Column 2, crossing between BR and C; and Column 3, crossing between BR and B6), with five histograms; Row 1; parent P1 (BR or B6); row 5, parent P2 (B6 or C); row 3, F1 hybrids from P1 × P2; row 2, (B1), offspring from F1 × P1; and row 4 (B2), offspring from F1 × P2. The histograms in parent lines are thus displayed twice to allow easier comparison with the activity of the offspring

The activity of these albino strains (A, AKR, and C) increased under dim red light conditions [between illuminations $F(1,79) = 5.7$, $P < 0.05$; among three strains $F(2,79) = 51.4$, $P < 0.01$] (Table 2), as previously reported (McLearn, 1960; Dixon and DeFries, 1968; Kitahama and Valatx, 1976).

In C57BL/6-c^{2J} (albino) mice, the activity level increased under dim red light conditions, but it also increased in SEC (pigmented) mice, which showed low ambulation scores (Table 2).

F1 Hybrids (Between BR, B6, and C). No significant difference was observed between each reciprocal crossing in activity distribution or in mean value (Table 1), or between sex. Analysis of variance, assuming a 3 × 3 factorial arrangement, showed a significant difference among female parents [$F(2,354) = 10.37$, $P < 0.01$ for male hybrids; $F(2,102) = 24.0$, $P < 0.01$ for females], and among male parents [$F(2,354) = 21.2$, $P < 0.01$

Table 2. Comparison of the effect of *d*-methylamphetamine (1 mg/kg) on ambulation scores (mean ± SE) in the open field under white illumination (10 lux) and under dim red illumination (1 lux) in three albino strains (BALB/c, A/J, and AKR), C57BL/6-c^{2J}, an albino mutation of C57BL/6, and SEC mice (pigmented)

Strains	*N*	White illumination		Dim red illumination	
		Controls	Treated	Controls	Treated
BALB/c	15	22.6 ± 2.5	14.1 ± 2.7[b]	38.0 ± 6.9[d]	21.0 ± 5.6[b]
A/J	14	5.8 ± 1.5	1.3 ± 0.7[a]	13.9 ± 2.1[c]	4.9 ± 0.9[a]
AKR	16	40.5 ± 3.9	33.9 ± 4.7	51.2 ± 3.6[d]	43.7 ± 4.0
C57Bl/6-c^{2J}	20	61.3 ± 3.6	83.8 ± 6.3[a]	72.0 ± 3.6[d]	76.5 ± 6.7
SEC	10	22.0 ± 3.1	33.1 ± 1.5[a]	32.5 ± 2.3[c]	47.0 ± 4.3[a]

[a] $P < 0.01$; [b] $P < 0.05$; Control group compared with the treated group
[c] $P < 0.01$; [d] $P < 0.05$; Comparison between both control groups under the two different experimental conditions (white and dim red illumination)

Table 3. (A) Distribution of ambulation scores in nontreated albino and pigmented animals resulting from back-crossing with the recessive parents showing the number of offspring resembling BR or B6 or F1 (active and diminant parents), and C (hypoactive parents). (B) Distribution of amphetamine sensitivity in ambulation scores on open field of animals resulting from back-crossing with recessive parents C; the parents C showed decreased locomotor activity after amphetamine

Genotypes and coat colors	A. Spontaneous activity level Phenotypes				B. Amphetamine sensitivity (ambulation scores)			
	BR or B6	F1	C	(Total)	Increase	No change	Decrease	(Total)
CBRF1 × C								
Albino	1	13	8	(22)	7	4	6	(17)
Pigmented	1	10	8	(19)	7	6	1	(14)
Total	2	23	16	(41)	14	10	7	(31)
CB6F1 × C								
Albino	2	5	8	(15)	5	4	6	(15)
Pigmented	1	4	7	(12)	6	7	2	(15)
Total	3	9	15	(27)	11	11	8	(30)

for male hybrids; $F(2,102) = 38.3$, $P < 0.01$ for females].

In B6 × BR crosses activity level was not different from B6 parents, and activity in C × B6F1 as well as C × BRF1 was above the midparent scores: C genotype may therefore be recessive in open field ambulation.

Back-crosses. In animals back-crossed to the active parents (CBRF1 × BR, B6BRF1 × BR, and CB6F1 × B6), ambulation scores did not differ significantly from F1 hybrids. Animals back-crossed to their hypoactive parents (CBRF1 × C, CB6F1 × C, and B6BRF1 × B6) resulted in nonhomogeneous data (Fig. 1). Widespread distribution of individual ambulation scores with two peaks were obtained in each group. In this study, no significant relationship between coat color and activity level was found in CB6F1 × C ($t = 1.3$, NS), or in CBRF1 × C ($t = 1.5$, NS) because of large variability. Table 3A shows the breakdown of coat colors against ambulation scores.

Treated Animals

Inbred Lines. BR, B6, and SEC mice, after administration of *d*-methylamphetamine (1 mg/kg), showed increased ambulation in the open field without stereotyped behavior, whereas A, AKR, and C mice showed increased rearing and sniffing patterns.

Under white illumination, BR and B6 mice showed a significant and very homogeneous increase in ambulation scores 30 min after drug injection (141% of baseline, $t = 3.1$, $P < 0.01$ in BR; 145% of baseline, $t = 3.2$, $P < 0.01$ in B6 mice). This increase returned to normal after about 3 h.

Scores of C mice, however, were not homogeneous; an increase in one, a decrease in ten, and no change in four animals were found (Fig. 2, rows 2 and 3). Overall, the mean scores were significantly decreased (48% of baseline, $t = 3.6$, $P < 0.01$) (Table 2), though scores returned to baseline 3 h later. A similar decrease was obtained in A mice ($t = 2.7$, $P < 0.01$); no change in

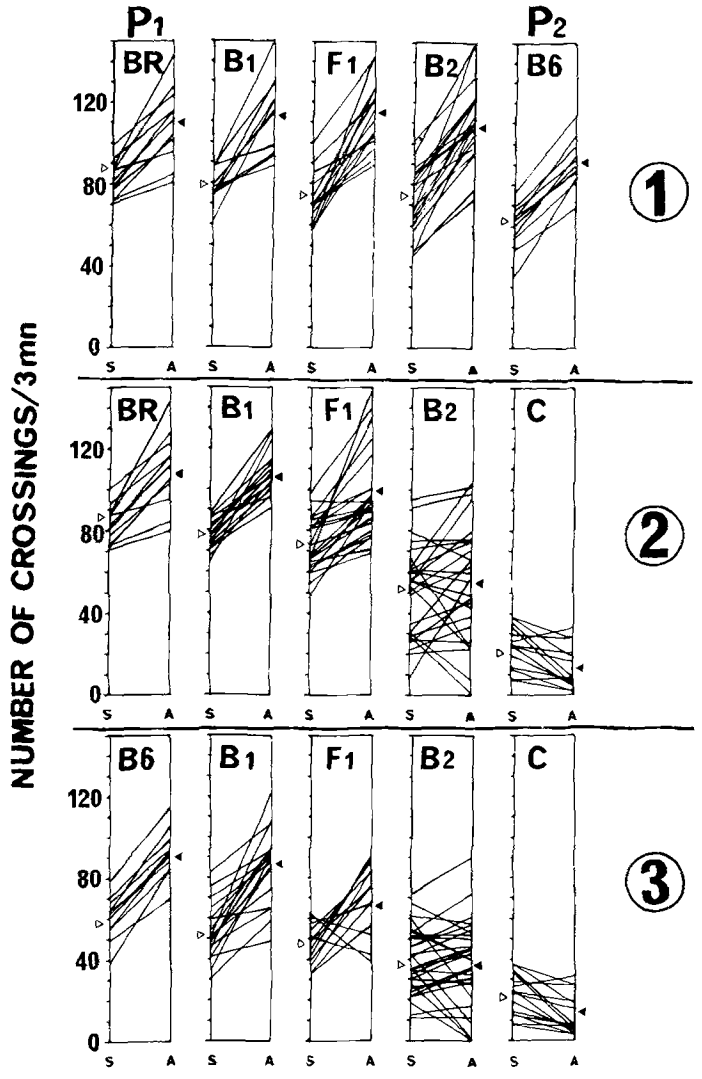

Fig. 2
Effect of *d*-methylamphetamine (1 mg/kg) in individual animals: Mean value of ambulation scores in control animals (S) (▷) and in treated animals (A) (◀). Note heterogeneous modification of activity in CBRF1 × C (row 2, B2) and CB6F1 × C animals (row 3, B2). Rows 1, 2, and 3 correspond with columns 1, 2, and 3 in Fig. 1

AKR. However, in the albino strain C57BL/6-c^{2J}, this drug increased the ambulation scores (Table 2).

Under dim red illumination, this drug induced in the albino strains, a decrease in ambulation scores in C and A mice, and no significant change in AKR and C57BL/6-c^{2J} mice (Table 2).

F1 Hybrids. In F1 hybrids (between BR, B6, and C), B6BRF1 × B6, and animals back-crossed to their active parents (B6BRF1 × BR, CBRF1 × BR, and CB6F1 × B6), all groups showed homogenous increase in ambulation ($t > 3.0$, $P < 0.01$), with the exception of B6CF1 mice, among which some individuals showed a decrease (Fig. 2).

Animals Back-crossed to Their Less Active Parents. (CBRF1 × C and CB6F1 × C) mice showed no significant change in mean ambulation scores. In fact, in the CBRF1 × C group (Fig. 2, row 2, B2), 14 mice showed an increase, 7 a decrease, and 10 mice no change (Table 3B). Similarly, in the CB6F1 × C group (Fig. 2, row 3, B2), 11 animals showed increase, 8 a decrease, and 11 no change (Table 3B).

Discussion

Results obtained in control animals, B6 and C strains, and their crossings were identical to those of DeFries and Hegman (1970) and Oliverio et al. (1973) in spite of different techniques. Furthermore, BR mice were used instead of B6 because comparison between strains which widely differ in activity levels as well as in sensitivity to drugs may more effectively clarify the mode of inheritance. In fact, BR mice were three-times more active than C mice, and the BR genotype was dominant over C genotypes with respect to inheritance of ambulation score and wide range of distribution; in CBRF1 × C mice two peaks were obtained. These

results may confirm the hypothesis (DeFries and Hegman, 1970) that open field ambulation may be determined by a single major gene and some minor polygenes at many loci.

A strain difference was evident in the sensitivity to methylamphetamine (1 mg/kg) which induced hyperactivity in BR, B6, and SEC mice, and hypoactivity or no change in albino mice (A, C, and AKR). This confirms data previously reported by Oliverio et al. (1973) for C and B6 mice, and by Anisman et al. (1975) for A mice, using *d*-amphetamine (0.5–2 mg/kg). This hypoactive effect of amphetamine was recessive, as shown by results obtained in reciprocal hybrids F1 (BR × C as well as B6 × C) and in animals back-crossed to C mice, in which amphetamine-induced hypoactivity was shown in 25 of 36 of the population resembling each progenitor strain. These results could be attributable to a recessive gene linked to the albino gene "c". However, these effects are not completely linked to a "c" allele because not all albino mice showed hypoactivity; 38% of albino animals exhibited hyperactivity. In the same manner, 45% of pigmented mice showed hypoactivity. Thus, amphetamine sensitivity was not linked to the level of activity as might have been thought from consideration of the parent strain alone. Environmental factors may be important in amphetamine-induced hypoactivity, because results in B6CF1 mice, nourished by a B6 female, were not homogeneous. Moisset (1977), also reported hypoactivity induced by *d*-amphetamine (5 mg/kg) in B6CF1 mice.

On the other hand, the hypoactive effect was observed only in the open field situation: C mice showed hyperactivity in their home cage (Moisset and Welch, 1973). This drug induced a wakeful state for 3–5 h in C, as well as in C57 (BR and B6) mice after the injection (Kitahama and Valatx, 1979). With respect to amphetamine sensitivity in open field activity, disinhibition of emotionality may be the mode of action in pigmented parent strains. In albino mice, however, amphetamine may accentuate the emotional state, possibly due to photophobia mediated by the visual system (DeFries et al., 1966; McLearn, 1960). But increased activity under dim red light in albino strains (C, A, and AKR) was also inhibited, suggesting that the amphetamine effect may be independent of photophobia.

Amphetamine-induced hyperactivity may be mediated by catecholaminergic systems; dopamine (DA) (Carlsson, 1970) and/or noradrenaline (NA) (Kety, 1972; Korf et al., 1968; Javoy et al., 1968). However, there are very few biochemical data concerning the strains we have used. Some functional differences between strains have been shown: NA turnover is higher in DBA and SEC than in B6 mice (Kempf et al., 1974; Eleftheriou, 1971). The mean level of tyrosine hydroxylase activity in the brain stem (locus coeruleus) is higher in B6 than in C mice (Natali et al., 1979). These differences may be related to a variation in the number of NA neurons (Touret et al., in progress) or DA neurons (Ross et al., 1976). Another possible explanation involves strain differences in NA receptor sensitivity (Segal et al., 1975). Thus, the genetic support shown by our results could be a structural variation of the central nervous system.

In summary, it is possible that several genes may play a role in determining the sensitivity to amphetamine in the open field situation. Similar conclusions were reached by Oliverio et al. (1973) and Jori and Rutczinsky (1978). In our experiments, however, the sensitivity to amphetamine does not seem to be necessarily linked to the albino gene, but perhaps to some genes located on the same chromosome. The present work underlines the importance of the study of not only pure strains, but also hybrids, back-crosses, and mutant strains to understand the action mechanisms of amphetamine or other drugs.

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