Effects of d-Amphetamine and Scopolamine on Activity Before and After Shock in Three Mouse Strains'

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Abstract:
In three experiments the following results were obtained: (a) Activity was greater both prior to and following exposure to shock among C57BL/6J mice than in DBA/2J mice, which in turn was greater than that of A/J mice. (b) Scopolamine hydrobromide increased general activity in DBA/2 and A mice, but had either no effect or decreased activity in the C57BL/6 strain. Following exposure to shock, however, the disinhibitory effects of scopolamine were apparent in all three strains. (c) d-amphetamine increased activity in all three strains. Moreover, following a single shock d-amphetamine had excitatory effects among both A and DBA/2 mice such that activity exceeded the level observed with d-amphetamine alone. Following several shock presentations a small but significant excitation was observed in C57BL/6 mice as well. Data were interpreted in terms of disinhibitory and excitatory effects of scopolamine and d-amphetamine, respectively, as well as possible interactions between the catecholaminergic and cholinergic systems. In addition, implications for sources of strain differences in avoidance behavior are discussed.
Keywords: Activity, Behavior genetics, Inbred strain, d-Amphetamine, Scopolamine, Stress

Article:
IT is well known that inbred strains of mice differ widely in the degree of motor activity manifested in various situations [13, 21, 22, 25, 28] , as well as in their rates of learning to avoid electric shock [11, 27, 30, 31] . Furthermore, many studies have been reported which indicate that experimental manipulations often affect avoidance behavior via nonassociative effects on activity or response inhibition [7] . Thus, it is likely that genetic variations in avoidance are due largely to nonassociative effects of shock rather than associative processes [8, 18, 23] .

Previous research has indicated that freezing responses during avoidance training may be disrupted either by interference with the action of acetylcholine using scopolamine [4, 8, 9, 10] or by augmentation of the action of norepinephrine or dopamine using d-amphetamine [8, 14, 20], thereby giving rise to the idea that the balance between cholinergic and catecholamine function largely determines the effective degree of response inhibition or motor activity [15,16] . However, a recent study [5] has shown that the effects of scopolamine and amphetamine are highly dependent on the strain of mouse. In an active avoidance situation performance of A/J is enhanced by both drugs, that of DBA/2J is enhanced only by amphetamine, while no drug effects at all are observable for C57BL/6J. In a simple inhibitory avoidance task performance of all strains is disrupted by scopolamine, and amphetamine affects all but C57BL/6. The results suggest that effects on avoidance are mediated to a large extent by changes in activity or response inhibition. Hence, the present study was conducted to determine whether similar strain-dependent drug effects would occur for simple measures of motor activity independent of an avoidance training contingency.

A number of studies have already reported strain-by-drug interactions on exploratory activities of mice. For example, it has been observed that [26] amphetamine reversed the order of two strains on a factor indicative of freezing. Other results with amphetamine have been inconsistent [12,23] . Scopolamine has been consistently observed to increase the activity of DBA/2 or Balb/c mice and to decrease the activity of C57BL/6 mice [1, 2, 3,

12, 23] . Since these various results are not in agreement with the findings for avoidance learning [5] , the need for further study of drug effects on activity is considerable.

At the outset, one obvious difference between exploratory activity testing and avoidance training is notable, namely the absence or presence of electric shock. It was conceivable that disparate findings of diverse studies might be attributable to different degrees of stress reactions evoked and their consequent modifications of drug effects. Electric shock is known to elicit a brief increase in activity [7,17], followed by a prolonged motor inhibition owing to increased cholinergic activity [4] . It is therefore possible that the effect of a drug on activity of a particular strain depends upon both the normal activity of various neurotransmitters and the degree of stress reaction elicited by the testing situation. Accordingly, the present investigation included measures of general activity (Experiment 1), activity before and after a single noncontingent-inescapable shock (Experiment 2), as well as five inescapable shocks which was more comparable to the conditions encountered early in the usual avoidance training procedures (Experiment 3).

METHOD

Animals

Three independent studies were carried out employing mice of three strains (A/J, DBA/2J and C57BL/6J) procured from the Jackson Laboratory, Bar Harbor, Maine, at seven weeks of age. Mice were housed 5 per cage, separated by sex and strain, and permitted ad lib access to food and water. Mice were tested when they were 70-90 days of age. All testing was carried out during the light phase of a 12 hr on — 12 hr off light cycle. Experiments 1 and 2 each employed 32 male and 32 female mice of each strain, while Experiment 3 employed 12 male and 12 female mice of each strain.

Apparatus

The testing apparatus was a 30 × 30 × 30 cm clear Plexiglas open field with a grid floor consisting of 0.23 cm stainless steel rods spaced 0.83 cm apart. The floor beneath the grid was divided into 7.5 × 7.5 cm squares, thus demarcating the open field into 16 areas of equal size. Footshock of 300 μA could be delivered through the grid floor from a high voltage-high resistance source. The grid floor was wired to a diode bridge, connecting every fourth bar in common to reduce the probability of a mouse finding two bars of the same polarity.

Procedure

Mice of each strain received intraperitoneal injection of either scopolamine hydrobromide (1, 3, 5 or 10 mg/kg) or d-amphetamine sulfate (1,3,5 or 10 mg/kg) in a 0.5 mg/ml solution or saline (2 ml/kg). Ten minutes following injection mice were individually placed in the open field and activity (defined in terms of square crossings) was recorded for a 15 min period. A square crossing consisted of the animal placing all four legs into a particular square.

The procedure of the second experiment was the same as that of Experiment 1, except that activity was recorded for 10 min, after which a single 2 sec shock (300 microamperes) was delivered. Following shock, activity was again recorded for a 10 min period, exclusive of the 10 sec period immediately following shock. In the third experiment mice received IP injection of either scopolamine (1 mg/kg), d-amphetamine (10 mg/kg) or saline (5 ml/kg). These dosages were selected on the basis of Experiment 2. Ten minutes after injection animals were placed in the apparatus. Activity was recorded for 3 min, after which a 2 sec, 300 μA shock was delivered. Ten seconds after the shock activity was again recorded for a 3 min period, after which animals were again shocked. This procedure was repeated until animals received 5 shocks.

RESULTS AND DISCUSSION

The mean number of squares entered in 15 min (Experiment 1) is shown in Fig. 1 for each Strain and Drug treatment combination. Raw scores were transformed with $\sqrt{X+1}$ to reduce heterogeneity of variance. Analysis of variance of the transformed scores revealed a significant Strain × Drug interaction, $F(16,189) = 2.5$, $p<0.01$. Subsequent Newman-Keuls multiple comparisons revealed that under saline the C57BL/6 strain was substantially more active than the other two strains, while A and DBA/2 strains did not differ significantly.

Amphetamine increased activity of A and DBA/2 mice at 5 and 10 mg/kg dramatically, but it did not significantly alter the activity of C57BL/6 mice at any dose. The only indication of a decline in activity produced by amphetamine was for A mice under 1 mg/kg; only one animal in this group reached the level of any A mouse under saline. Scopolamine increased activity of A and DBA/2 mice significantly only at the 1 mg/kg dose. C57BL/6 mice showed a significant decline in activity only under the high 10 mg/kg dose; no motoric impairment was evident at this dose. Lower doses of scopolamine did affect changes in activity during a session for C57BL/6 mice, however. Under saline the C57BL/6 mice averaged 88.6 square crossings in the first 3 min period and 54.0 crossing in the last 3 min period. Under 1 mg/kg scopolamine this decline in activity was eliminated; mean crossings for the first and last periods were 81.3 and 79.9, respectively. A similar pattern held for C57BL/6 mice under other scopolamine doses. Under 10 mg/kg scopolamine the activity of C57BL/6 mice actually increased from 33.6 to 42.9 square crossings between the first and last periods.

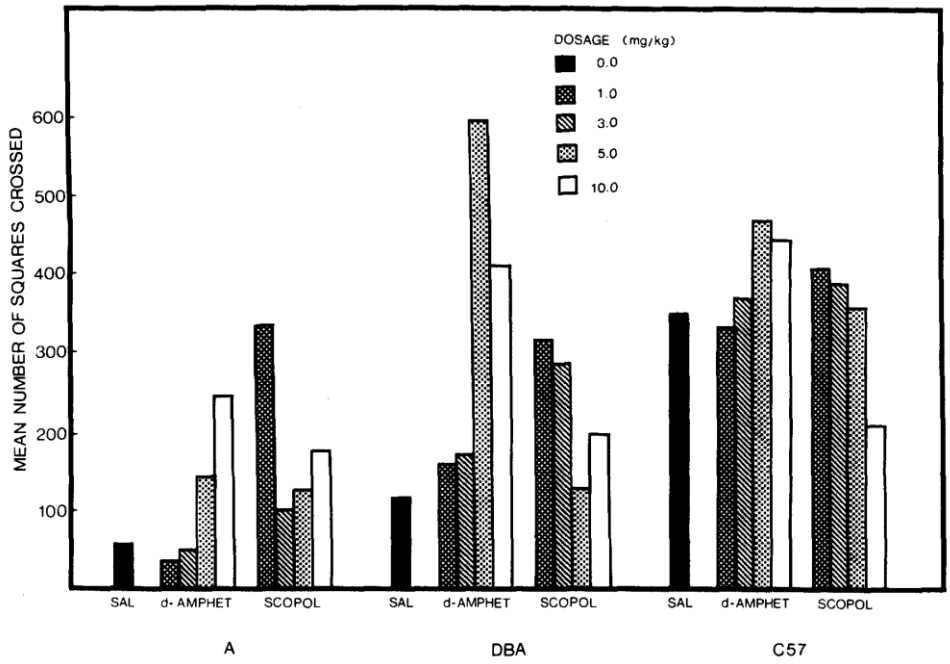

FIG. 1. Mean number of squares crossed as a function of Strain and Drug Treatment.

The results of this experiment are generally consistent with previous studies of strain-dependent drug effects in that strains with normally low to moderate activity levels (A and DBA/2) showed large activity increases in response to both amphetamine and scopolamine. The results for C57BL/6 mice, on the other hand, are not entirely in agreement with other research (e.g. [1, 2, 3]). Decreased activity was seen in C57BL/6 mice only at 10 mg/kg scopolamine in the present study, while others have reported reduced activity of C57BL/6 under either 1.25 mg/kg (1) or 2.0 mg/kg scopolamine [24] . The causes of these different outcomes are unclear; variations in the exploratory tasks employed may be of importance.

Mean square crossings before and after the single, brief foot-shock are portrayed in Fig. 2 for each Strain and Drug treatment combination (Experiment 2). Raw scores were transformed with $\sqrt{X+1}$. Analysis of variance of transformed activity scores yielded a significant Strain × Drug × Period (pre- or postshock) interaction, $F(16,189) = 8.5$, $p<0.01$.

Subsequent Newman-Keuls multiple comparisons revealed that under saline the preshock activity was similar to that observed in Experiment 1; C57BL/6 exceeded A and DBA/2, while DBA/2 was not significantly greater than A. Following the 2 sec electric shock, activity decreased for all three strains. A strain mice showed almost complete cessation of activity for 10 min, while C57BL/6 mice maintained a moderate level of activity. DBA/2 mice were intermediate; additional research in our laboratories has found that DBA/2 mice freeze for 2 to 5 min after a single shock and that their mobility then recovers [32] . Thus, following a single shock A mice show prolonged freezing behavior, DBA/2 mice freeze for a few minutes, and C57BL/6 mice do not freeze at all.

Under amphetamine the pattern of preshock activity was very similar to that observed in Experiment 1. The only disagreement was that a significant but small increase in activity was seen for C57BL/6 under the higher amphetamine doses. After the electric shock, however, a startling pattern of results appeared. Whereas A mice under saline showed intense freezing after shock, under higher amphetamine doses they showed a dramatic increase in activity to a level well above even their preshock activity. Shock-induced hyperactivity was also seen in DBA/2 mice at 10 mg/kg. The major portion of the activity increase occurred soon after shock; during the first 2 min after shock the activity of A and DBA/2 mice at high doses increased by 50 to 100 percent above preshock levels, but within 6 to 10 min after shock activity returned to preshock levels. These rapid changes were temporally correlated with the shock and were not results of drug effects alone. Thus, amphetamine at higher doses appeared to have a genuine excitatory effect when combined with shock; it did not simply disrupt freezing in A and DBA/2 mice. Amphetamine had no significant effect on the postshock activity of C57BL/6 mice; it did not even prevent the usual decline in activity after shock. The only evidence of amphetamine-produced decline in activity was again in A mice at 1 mg/kg prior to shock.

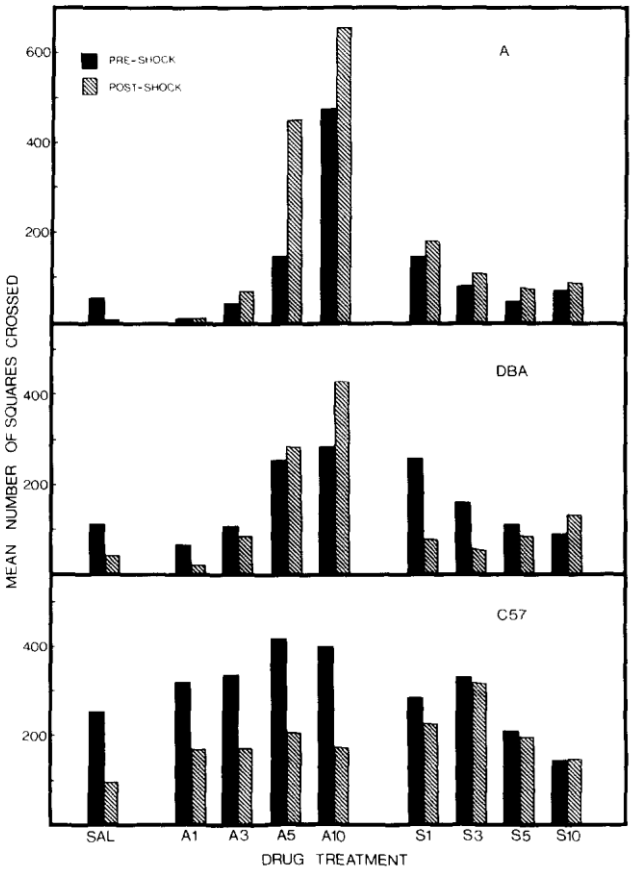

FIG. 2. Mean number of squares crossed prior to and following shock as a function of Strain and Drug Treatment.

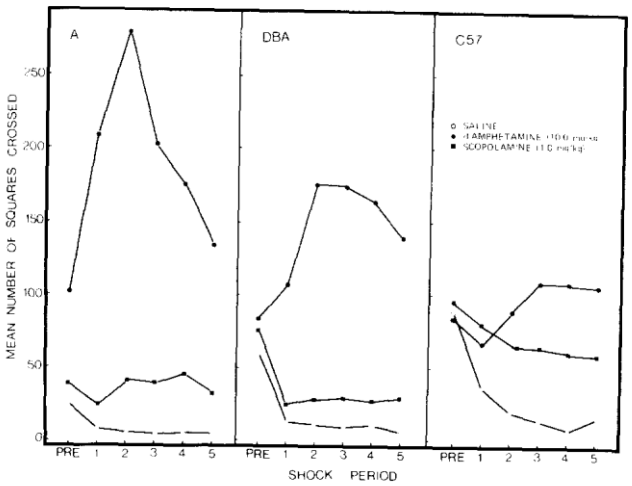

FIG. 3. Mean number of squares crossed prior to shock exposure, and following each of 5 successive shock presentations as a function of Strain and Drug Treatment.

Scopolamine effects on preshock activity were virtually identical to those seen in Experiment 1. The low 1 mg/kg dose increased activity for A and DBA/2 mice, and the high 10 mg/kg dose decreased activity for C57BL/6 mice. Following shock, scopolamine prevented freezing in A mice at all doses, but it did not produce hyperactivity at any dose. Postshock activity increased only at 10 mg/kg for DBA/2, and it was also greater than under saline for C57BL/6 mice at 1, 3 and 5 mg/kg. This latter finding is an important one, because it shows that scopolamine increases activity of C57BL/6 mice only after electric shock.

The mean number of square crossings prior to shock and during the 3 min after each of 5 shocks is given in Fig. 3 for each Strain and Drug treatment combination (Experiment 3). Analysis of variance of transformed ($\sqrt{X+1}$) activity scores revealed a significant Strain × Drug × Sampling period interaction, $F(20,135) = 3.1$, $p<0.01$. Newman-Keuls multiple comparisons revealed that prior to shock saline treated C57BL/6 mice were more active than DBA/2 mice, who in turn were more active than A mice. Freezing was predominant following

all 5 shocks for A mice, while DBA/2 mice showed only slightly higher activity levels than A mice. Activity of C57BL/6 mice declined after the first shock and then declined further with subsequent shocks; after the fourth shock C57BL/6 activity was comparable to that for DBA/2.

Preshock activity changes produced by amphetamine and scopolamine were comparable to those observed in Experiments 1 and 2.

Amphetamine led to hyperactivity following the first shock only for A and DBA/2. With subsequent shocks hyperactivity of A and DBA/2 first increased and then decreased. Surprisingly, activity of C57BL/6 mice increased above preshock levels following the third, fourth and fifth shocks, but the increase was quite moderate compared to effects for A and DBA/2. After 5 shocks, activity of all three strains under amphetamine was far in excess of their activity levels under saline.

Postshock activity under scopolamine showed little change with successive shocks. Freezing was prevented for all three strains, but only for A mice did scopolamine prevent any decline at all in activity following shock. After 5 shocks, all three strains were more active under scopolamine than under saline, but the effects were not nearly as large as those resulting from amphetamine; for DBA/2 the scopolamine effect was not even significant.

The principal finding from this experiment is that drug effects become much larger with repeated shocks. They are also more consistent across strains after 5 shocks; amphetamine has excitatory properties in all three strains, and likewise scopolamine appears to be disinhibitory in all cases. The strain differences, then, are in magnitude of effects, not in their directions. Amphetamine (10 mg/kg) has much larger effects on A and DBA/2 than on C57BL/6 mice, while scopolamine (1 mg/kg) has largest effects on A, smaller effects on C57BL/6 and minimal effects on DBA/2.

When these results of repeated shocks are compared to drug effects on the three strains in avoidance learning [5] , agreement is good except for C57BL/6 mice. Active avoidance performance of C57BL/6 mice is not affected by either drug, in spite of clear drug effects on activity.

GENERAL DISCUSSION

Summarizing, the results of the present investigation revealed that (a) activity both prior to and following shock is greater in C57BL/6 mice than in DBA/2 mice, which in turn, is greater than that of A mice. (b) Scopolamine increases general activity in A and DBA/2 mice, but has either no effect or decreases activity in the C57BL/6 strain. (c) Following shock the disinhibitory properties of scopolamine become apparent in all three strains. (d) Unlike scopolamine, d-amphetamine at high dosages uniformly increases activity in all three strains. (e) Finally, following a single shock d-amphetamine has an excitatory effect upon both A and DBA/2 mice, which enhances activity beyond their baseline levels. Following several shock presentations a small but significant excitation is also observed in C57BL/6 mice.

Turning first to the effects of d-amphetamine on postshock activity, the large augmentation for A and DBA/2 may possibly be attributed to the interaction between a shock-induced noradrenergic reaction, e.g., increased norepinephrine turnover [19] , and the increased noradrenergic and dopaminergic action elicited by the drug treatment. An alternative explanation is that another neurochemical system, possibly a serotonergic one, modifies the response to certain stimulus events. That is, the drug-induced adrenergic action may amplify the responsiveness to stimuli, and consequently shock may produce hyperactivity [29]. The initial response to shock following d-amphetamine treatment corresponds well with the behavior seen in active avoidance tasks [5]. Specifically, in both the A and DBA/2 strains in which a hyperactive response to shock in drugged animals is noted, d-amphetamine enhances active avoidance behavior, whereas in the C57BL/6, where shock does not elicit the large hyperactive response, the drug treatment produces negligible effects on active avoidance behavior. Yet, when the data for repeated shocks are examined, some degree of hyperactivity is noted in C57BL/6 mice. These data suggest that the most important determinant of avoidance behavior is either the

initial activity changes produced by shock (for A and DBA/2) or perhaps potent cognitive factors which limit avoidance behavior even when response inhibitory tendencies are reduced (for C57BL/6). It seems that, although both associative and nonassociative factors determine avoidance response rate, the relative importance of these factors are strain specific [5].

With respect to the differential effects of scopolamine on the activity of the three strains, it is tempting to argue that the normally high level of activity in C57BL/6 mice precludes the possibility of activity being further increased through administration of scopolamine. This position is not a tenable one in that administration of d-amphetamine increases activity in C57BL/6 mice slightly. Moreover, it cannot be argued that C57BL/6 is not susceptive to the effects of scopolamine, since shock-induced response inhibition is reduced following the drug treatment. Thus, although scopolamine may have disinhibitory properties in all strains, the effectiveness of the drug in modulating performance in more complex learning situations may be masked or suppressed by more potent cognitive factors, as appears to be the case with d-amphetamine [5].

Finally, it is abundantly apparent that the response biases elicited by scopolamine and d-amphetamine are different. Whereas the former treatment results in disinhibition, the latter has an excitatory effect. Thus in some instances the effects of d-amphetamine may be more effective in altering behavior than scopolamine, e.g., active avoidance performance [5]. Yet, because the action of d-amphetamine is not of a disinhibitory nature, it may be possible to induce response inhibition in d-amphetamine treated animals more readily than in scopolamine injected animals. For example, it has been observed that in the C57BL/6 strain scopolamine successfully disrupts response inhibition tendencies, whereas amphetamine does not [5]. The question arises then as to whether a balance in fact does exist between the adrenergic and cholinergic systems. In those situations where either drug alone potentiates a given behavior pattern, the drugs act as if they were synergistic. For example, in a shock motivated situation where nonassociative freezing behavior disrupts avoidance, either drug may enhance avoidance (see for example, [6,81). However, where the action necessary to alter a particular behavior pattern is excitation of ordinary behavior, rather than disinhibition of suppressed behavior, e.g., in some cases dealing with response initiation and response perseveration rather than simple disinhibition, d-amphetamine may affect behavior whereas scopolamine may not (see [5]). Finally, in habituation situations it is likely that a disinhibitory agent may have a more potent effect in altering behavior than will an excitatory agent [16] .

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