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# Chronic Stress Induces Strain-Dependent Sensitization to the Behavioral Effects of Amphetamine in the Mouse

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BADIANI, A., S. CABIB AND S. PUGLISI-ALLEGRA. *Chronic stress induces strain-dependent sensitization to the behavioral effects of amphetamine in the mouse.* PHARMACOL BIOCHEM BEHAV **43**(1) 53–60, 1992. – Following 10 days of daily restraint stress, sensitization developed to the stimulatory effect of amphetamine on locomotion in DBA/2 but not in C57BL/6 mice tested 24 h after the last stressful experience regardless of their being naive or habituated to the test cages. Saline-injected C57BL/6 mice, however, showed an increase of locomotion 24 h after chronic stress treatment. Chronically stressed mice of the two strains did not exhibit any alteration of dopamine and metabolites (3-4-dihydroxyphenylacetic acid, homovanillic acid, and 3-methoxytyramine) levels in the frontal cortex, caudatus putamen, or nucleus accumbens septi, thus ruling out that stress-induced alteration of basal dopamine metabolism affected the behavioral response to amphetamine challenging in DBA/2 mice. Ten daily amphetamine injections (5 mg/kg) did not significantly modify the behavioral response to amphetamine in either strain of mice tested 24 h after the end of the chronic treatment and did not increase locomotion in saline-injected C57BL/6 mice. Finally, chronically stressed hybrids B6D2F1 did not show sensitization to the locomotor effects of amphetamine, suggesting a dominant mode of inheritance in the response to chronic stress of the C57BL/6 strain.

Dopamine metabolism  Locomotion  Dopamine autoreceptors  Genotype

THERE is increasing evidence that repeated exposure to environmental stress is capable of producing sensitization-like changes in brain and behavioral responses to psychostimulants (2,9,12,15,26,27), leading to the hypothesis that stimulant drugs and stress may act upon the same neurobiological substrates.

There are large individual differences in the effects of repeated amphetamine treatment in both humans and laboratory animals [(26) for review]. Moreover, the existence of robust strain differences in the effects of chronic amphetamine treatment suggests that genetic factors influence behavioral sensitization processes (26). Since behavioral sensitization in laboratory animals might represent an animal analog of amphetamine psychosis (26) and may predict vulnerability to amphetamine self-administration (20), it is of considerable interest to study genotype-dependent differences in the susceptibility to stress-induced behavioral sensitization.

A number of studies conducted in this laboratory (6,8,24) have shown that, following chronic stress, C57BL/6 (C57) and DBA/2 (DBA) mice exhibit behavioral and biochemical alterations that suggest genotype-dependent differences in the adaptation of brain dopamine (DA) systems to stress. Moreover, Robinson (26) has shown that DBA mice are significantly more susceptible to behavioral sensitization produced by repeated amphetamine than C57. These results suggest that DBA and C57 are the strains of choice for investigating how genetic factors influence brain organization to modulate susceptibility to sensitizing processes.

## GENERAL METHOD

Naive, male mice of the C57 and DBA strains were obtained from Charles River Labs. (Calco, Como, Italy). They were maintained, six to a standard breeding cage, with food and water ad lib in a 12 L : 12 D cycle (lights on from 0700–1900 h) and tested always during the second half of the light period (between 1400 and 1600 h).

Mice were stressed by restraining them in a snug-fit apparatus (3–8) for 2 h daily for 10 consecutive days and tested or killed 24 h after the last stressful experience. Stress sessions, testing, and sacrifices were performed in different rooms.

Locomotor activity was measured by an automated apparatus consisting of eight toggle-floor boxes (3), each divided into 20 × 10 cm compartments. For each mouse, the number

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of crossings from one compartment to the other was recorded by means of microswitch connected to the tilting floor of the box.

Tests were carried out in sound-attenuated cubicles where a 30-W lamp was the only source of illumination. Cubicle temperature was kept constant.

## EXPERIMENT 1

### METHOD

Mice were stressed and tested as described in the General Method section.

Behavioral tests were performed 24 h after the last stress session. Mice were injected with *d*-amphetamine sulfate dissolved in saline (0.9% NaCl) or with saline alone. All injections were made IP in a volume of 10 ml/kg. Testing sessions started immediately after treatment and lasted 60 min.

Data were analyzed by three-way analysis of variance (ANOVA), the factors being strain (two levels: C57 and DBA), stress (two levels: controls and stressed), and amphetamine (three levels: 0, 0.5, and 1 mg/kg). Individual between-group comparisons were carried out by posthoc test (Duncan's multiple-range test).

### RESULTS AND DISCUSSION

Figure 1 shows the effects of chronic stress on amphetamine-induced locomotion. Three-way ANOVA revealed a significant stress main effect, $F(1, 88) = 9.44$, $p < 0.005$, and a significant interaction between the three factors, $F(2, 88) = 3.43$, $p < 0.05$. Individual between-group comparisons showed that chronically stressed DBA mice did not present changes in basal levels of locomotion while an increase of amphetamine-induced locomotion was evident. By contrast, C57 mice were hyperactive following chronic stress while their behavioral response to amphetamine was unchanged.

The present results indicate that, following repeated stressful experiences, the mice of the DBA strain are sensitized to the effects of amphetamine on locomotor activity, while C57 mice are not.

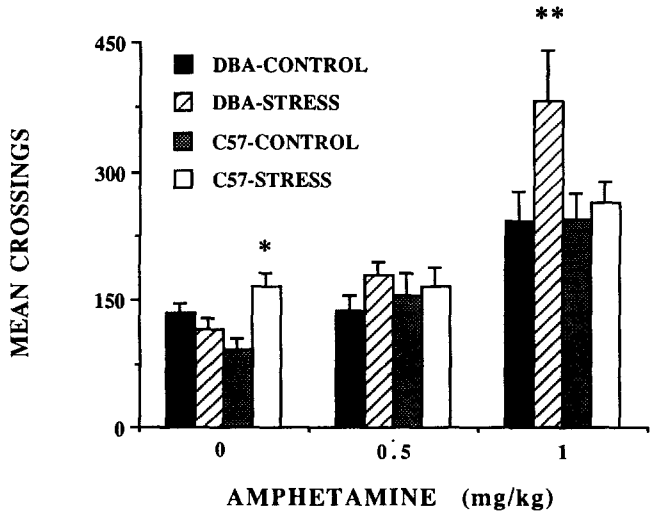


FIG. 1. Effects of amphetamine on locomotor behavior of stressed and unstressed mice of two inbred strains naive to the test cage. Results are expressed as mean (±SE) crossings for the 60-min test. *, **significantly different ($p < 0.5, 0.1$) in comparison with unstressed mice of the same strain receiving the same dose of amphetamine.

## EXPERIMENT 2

It has recently been shown that 120 min of acute restraint have similar effects on DA release in the caudatus putamen (CP) and nucleus accumbens septi (NAS) of DBA and C57 mice (4,7) but induce an increase of homovanillic acid (HVA) in the frontal cortex (FC) of DBA but not C57 mice (4). Moreover, 24 h after the last session of chronic stress C57 do not exhibit any alteration of DA metabolism either in the CP or in the NAS (3). On the basis of these results, it is thus possible that the increased responsiveness to the behavioral effects of amphetamine in chronically stressed DBA mice depends upon the persistence of stress-induced alterations of DA metabolism in one of these brain areas 24 h after the last stressful experience.

The following experiment was designed to evaluate DA metabolism in the three brain areas of DBA and C57 mice 24 h after the end of chronic stress.

### METHOD

Mice were chronically stressed as described and killed 24 h after the last stress session. All animals were killed by decapitation, followed by immediate head freezing. This method was preferred to microwave focusing since this procedure requires animals to be placed in a tube similar to that used for stress; brain DA metabolism alteration in control mice, which may introduce an uncontrollable experimental variable, could not be ruled out. Moreover, it allows concentrations of 3-methoxytyramine (3-MT) to be obtained that are comparable to or lower than those obtained by microwave irradiation (7,8,11, 22,31-33), and to evaluate both increase and decrease of 3-MT concentrations following treatments known to produce these effects (7,8,22,23). After decapitation, the head was plunged directly into liquid nitrogen contained in a thermic box and left for 10 s. The frozen head was then stored at −10°C to allow it to reach a more manageable temperature before removal of the brain. The brain was then fixed vertically on the freeze plate of a freezing microtome. The freeze plate was used as a refrigerating table for punching, with the temperature being maintained at −10°C. Three brain areas were punched: FC, NAS, and CP. The coordinates were measured according to the atlas of Sidman et al. (29) (coronal sections) as follows: FC = two slices from sections 95-140 (2.3-mm tube); NAS = 4 slices from sections 141-210 (1.1-mm tube); CP = 6 slices of 300 m from sections 141-230 (1.5-mm tube). The samples were stored in liquid nitrogen until the day of the analysis.

DA, 3-4-dihydroxyphenylacetic acid (DOPAC), HVA, and 3-MT were determined simultaneously utilizing a reverse-phase high-performance liquid chromatography (HPLC) procedure coupled with electrochemical (EC) detection (3,4,7,8, 22,23). On the day of analysis, frozen samples were weighed and homogenized in 0.1 N $HClO_4$ containing 6 mM Na-metabisulfite and 1mM EDTA. The homogenized samples were centrifuged at 10,000 × *g* for 20 min at 4°C. Aliquots of the supernatant were transferred to the HPLC system. The HPLC system consisted of a Waters 460 EC detector with a glass carbon working electrode and a pump (Waters 510, Waters Assoc., Milford, MA). The potential was set at 800 mV (vs. Ag-Agcl reference electrode). The column, a Bondapak phenyl column (10 μm particle size 300 × 3.1 mm i.d.) was pur-

chased from Waters Assoc. The flow rate was 1.1 ml/min. The mobile phase consisted of 4% methanol in 0.1 M Na-phosphate buffer, pH 3, 0.1 mM $Na_2$ EDTA, and 1-octane sulphonic acid Na salt 0.5 mM (Aldrich, Federal Republic of Germany); 2 mM 3,4-dihydroxyhydrocinnamic acid (Aldrich) was used as internal standard.

Data were analyzed by two-way ANOVA, factors being stress (two levels: control, stress) and strain (two levels: DBA and C57).

### RESULTS AND DISCUSSION

The effects of chronic stress on basal DA metabolism are presented in Table 1. These results indicate major strain-dependent differences in the basal levels of DA and its metabolites in the three brain areas examined while no alteration of DA metabolism in chronically stressed animals of both strains killed 24 h after the last stressful experience was evident.

These results rule out the possibility that different alterations of basal DA metabolism account for the different responses to amphetamine in mice of the DBA and C57 strains following chronic stress.

## EXPERIMENT 3

The previous results indicate that, following repeated stressful experiences, mice of the DBA strain are sensitized to the effects of amphetamine on locomotor activity while C57 mice are not. It should be noted that C57 mice are sensitized to the behavioral effects of amphetamine following repeated injection with this psychostimulant (26). The discrepancy between the present results and those obtained with chronic amphetamine treatment appears to suggest that not all the mechanisms implicated in the development of stress- and amphetamine-induced behavioral sensitization are similar. However, a number of factors related to the different experimental procedure used might account for the discrepancy between the effects of repeated stress (present result) and of chronic amphetamine (26) on amphetamine-induced locomotion in C57 mice. Among these, the most relevant appear to be the time interval between the end of the chronic treatment and the behavioral test and previous habituation to the testing environment (26).

In the following experiments, we compared the effect of chronic stress and chronic amphetamine treatments on the behavioral response to amphetamine in mice tested 24 h after the end of the chronic treatments in an apparatus to which they were previously habituated.

### METHOD

Four groups of mice ($n$ = 7-8) for each strain were used for these experiments: control, chronic stress, chronic saline, and chronic amphetamine. Control and chronic stress groups were treated as previously described. Mice of the other groups received an injection of 5 mg/kg *d*-amphetamine sulfate dissolved in saline (0.9% NaCl) or of saline alone (SC) daily for 10 consecutive days.

Twenty-four hours after the last stress session or the last injection of amphetamine or saline, all mice were left 1 h inside the cages of the testing apparatus described above, then removed and left undisturbed for the following hour. Mice coming from the different pretreatments were then injected with 0.5 mg/kg *d*-amphetamine sulfate dissolved in saline (0.9% NaCl) or with saline alone (IP) and tested immediately in the same cages for the following 60 min.

Data were analyzed by three-way ANOVA for independent factors: pretreatment (four levels: control, stress, saline, and amphetamine), strain (two levels: C57 and DBA), and treatment (two levels: saline and amphetamine) and by four-way ANOVA factors being: repeated measure (10, 20, 30, 40, 50, and 60 min of tests), pretreatment (two levels: control and stress or saline and amphetamine), strain (two levels: C57 and DBA), and treatment (two levels: saline and amphetamine). Individual between-group comparisons were carried out by posthoc test (Duncan's multiple-range test and Student's *t*-test).

### RESULTS AND DISCUSSION

In Fig. 2 are presented the results as mean crossings for each group over the 60-min test. Three-way ANOVA revealed

TABLE 1

DA METABOLISM IN CP, NAS, AND FC OF UNSTRESSED MICE (CONTROLS) AND MICE STRESSED FOR 10 CONSECUTIVE DAYS AND KILLED 24 h AFTER THE LAST STRESSFUL EXPERIENCE (STRESSED)

| | Controls | | | | Stressed | | | |
|---|---|---|---|---|---|---|---|---|
| | DA | DOPAC | HVA | 3-MT | DA | DOPAC | HVA | 3-MT |
| CP | | | | | | | | |
| C57BL/6 | 14,129 ± 253 | 1,276 ± 37 | 1,871 ± 35 | 195 ± 8.9 | 14,212 ± 267 | 1,321 ± 39 | 1,793 ± 41 | 158 ± 18 |
| DBA/2 | 12,521 ± 196 | 1,123 ± 27 | 1,459 ± 63 | 158 ± 18 | 12,403 ± 201 | 1,139 ± 30 | 1,403 ± 58 | 149 ± 13 |
| NAS | | | | | | | | |
| C57BL/6 | 11,692 ± 359 | 1,031 ± 98 | 1,469 ± 73 | 74 ± 4.2 | 11,407 ± 311 | 1,058 ± 87 | 1,493 ± 78 | 71 ± 5.6 |
| DBA/2 | 12,216 ± 189 | 1,060 ± 17 | 1,011 ± 37 | 70 ± 2.7 | 12,173 ± 371 | 1,081 ± 38 | 1,051 ± 46 | 73 ± 3.4 |
| FC | | | | | | | | |
| C57BL/6 | 251 ± 5.8 | 40 ± 1.4 | 136 ± 7.4 | | 237 ± 5.0 | 42 ± 1.1 | 151 ± 3.1 | |
| DBA/2 | 128 ± 5.2 | 36 ± 1.9 | 107 ± 11 | | 124 ± 4.4 | 37 ± 2.2 | 110 ± 6.9 | |

Data are expressed as mean (±) SE ng/g wet weight. Statistical analysis performed with two-way ANOVA revealed a significant main effect of the factor strain for DA, $F(1, 42) = 176.7$, $p < 0.0001$, DOPAC, $F(1, 42) = 48.36$, $p < 0.0001$, HVA, $F(1, 42) = 35.44$, $p < 0.0001$, and 3-MT, $F(1, 42) = 30.41$, $p < 0.0001$, concentrations in the CP. A significant strain main effect was also evident for HVA concentrations in the NAS, $F(1, 42) = 124.6$, $p < 0.0001$. Finally a significant strain main effect was found for DA, $F(1, 36) = 520.5$, $p < 0.0001$, DOPAC, $F(1, 36) = 7.42$, $p < 0.01$, and HVA, $F(1, 36) = 21.1$, $p < 0.001$, concentrations in the FC. The statistical analysis did not reveal either an effect of the factor stress or a significant interaction between factors in any of the areas analyzed.

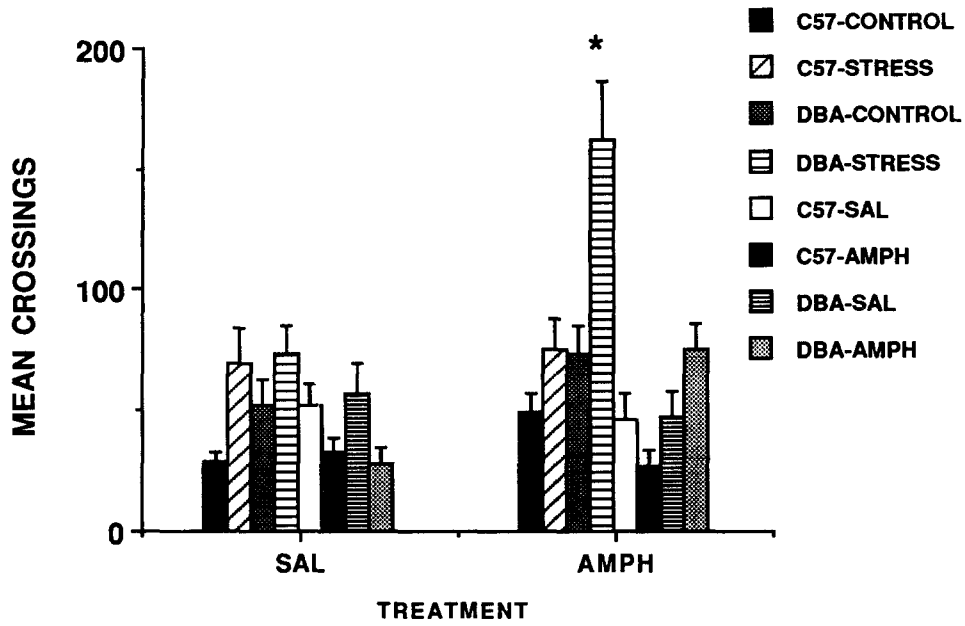


FIG. 2. Effects of repeated restraint stress or repeated amphetamine on amphetamine-induced (0.5 mg/kg) locomotion in mice habituated to the test cage. Results are expressed as mean (± SE) crossing for the 60-min test. *significantly different ($p < 0.01$) in comparison with all other groups.

significant main effects of pretreatment, $F(3, 100) = 14.76$, $p < 0.0001$, strain, $F(1, 100) = 17.64$, $p < 0.001$, and treatment, $F(1, 100) = 6.03$, $p < 0.05$. Significant interactions were found for pretreatment × strain, $F(3, 100) = 2.73$, $p < 0.05$, pretreatment × treatment, $F(3, 100) = 5.75$, $p < 0.005$, and for the three factors, $F(3, 100) = 3.95$, $p < 0.05$. Individual between-group comparisons revealed that stressed DBA mice treated with amphetamine had significantly higher locomotion than all the other groups. These results, showing that repeated stress experiences increase the behavioral response to amphetamine in mice of the DBA but not of the C57 strain are in accordance with those previously presented and indicate that habituation to the testing apparatus does not change the strain-dependent difference in the response to amphetamine following chronic stress.

C57 mice tested with amphetamine 24 h after the last injection of a chronic amphetamine treatment do not show an increase but actually a decrease of behavioral response to the psychostimulant. This result could suggest that a longer interval is needed between the last session of chronic treatment (either stress or amphetamine) and the testing session to observe a sensitization in this strain of mice. However, it should be noted that only a slight increase of amphetamine-induced locomotion, significantly lower than that observed 24 h after the last stressful experience, is produced in DBA mice 24 h after the last injection of the chronic amphetamine pretreatment. Moreover, while chronically stressed C57 mice exhibit a slight nonsignificant increase in amphetamine-induced locomotion, a slight nonsignificant reduction of this response is evident in amphetamine-pretreated mice of this strain. These data, together with those showing that chronic amphetamine reduces locomotion in saline-injected mice of both strains while chronic stress increases it, suggest that the two pretreatments do not produce identical behavioral effects.

In Figs. 3A and 3B, the same results are presented as mean crossings for the six time blocks (10 min each) of the total test duration. The effects of chronic stress on locomotor activity are shown in Fig. 3A. ANOVA revealed significant main effects of repeated measure, $F(5, 240) = 80.15$, $p < 0.001$, pretreatment, $F(1, 240) = 16.35$, $p < 0.001$, treatment, $F(1, 240) = 11.26$, $p < 0.005$, and strain, $F(1, 240) = 14.97$, $p < 0.001$. Significant interactions were found for pretreatment × treatment × strain, $F(1, 240) = 4.91$, $p < 0.05$, and for repeated measure × pretreatment, $F(5, 240) = 2.28$, $p < 0.05$. The absence of a significant global interaction did not allow individual between-groups comparisons to be carried out. These results further confirm strain-dependent differences in the effects of repeated stress on amphetamine-induced locomotion and indicate that this difference is dependent upon an increase of amphetamine-induced locomotion in chronically stressed DBA mice over the entire test session.

As far as the effects of chronic amphetamine are concerned (Fig. 3B), ANOVA revealed only significant main effects of strain, $F(1, 280) = 20.14$, $p < 0.001$, and repeated measure, $F(5, 280) = 64.53$, $p < 0.001$. A significant interaction for pretreatment × treatment × strain, $F(1, 280) = 13.52$, $p < 0.001$, and a significant global interaction, $F(5, 280) = 3.28$, $p < 0.01$, were also found. Individual between-groups comparisons revealed that amphetamine-pretreated DBA mice showed a significant depression of saline-induced locomotion and a significant increase of amphetamine-induced locomotion in the final part of the test in comparison with saline-pretreated groups. These results indicate that although 24-h intervals are not sufficient for a full sensitization to develop to chronic amphetamine an increase of the behavioral effects of the psychostimulant was already evident in the second part of the testing session in mice of the DBA strain.

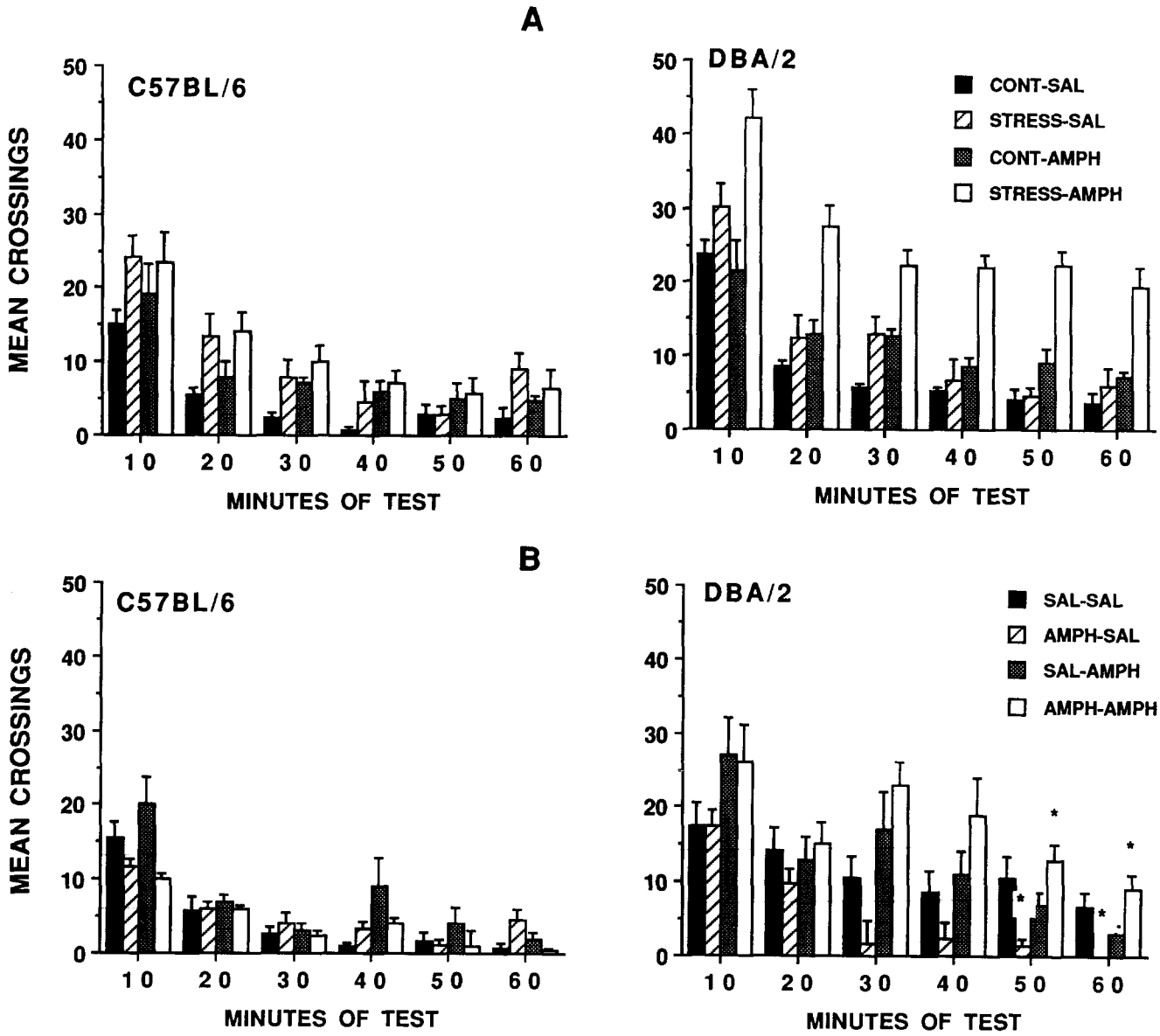


FIG. 3. Effects of repeated restraint stress (A) or repeated amphetamine (B) on amphetamine-induced (0.5 mg/kg) locomotion in mice habituated to the test cage. Results are expressed as mean (±SE) crossings. *significantly different ($p < 0.05$) in comparison with saline-pretreated groups.

## EXPERIMENT 4

We have recently shown that chronically stressed DBA mice are characterized by reduced responsiveness to both behavioral and biochemical effects of low, supposedly presynaptic doses of apomorphine, while C57 mice are hyperresponsive, indicating opposite changes in DA autoreceptor sensitivity in the two strains following chronic stress (8). A genetic analysis of the response to apomorphine in chronically stressed hybrids of the F1 and F2 generations and backcrosses revealed a dominance of C57 response to repeated stress (6). If genotype-dependent alteration of DA autoreceptor sensitivity are involved in the differences of behavioral response to amphetamine following chronic stress, then B6D2F1 hybrids should show the same response as the C57 parental strain.

This experiment was designed to test this hypothesis.

### METHOD

Naive, male B6D2 F1 mice were obtained from Charles River Labs. and housed as described in the General Method section. Mice were stressed and tested in an automated apparatus to which they were habituated following injection of 0.5 mg/kg amphetamine (IP) as previously described 24 h after the last stressful experience.

Data were analyzed by three-way ANOVA, factors being: strain (three levels = C57, DBA, and B6D2F1), pretreatment

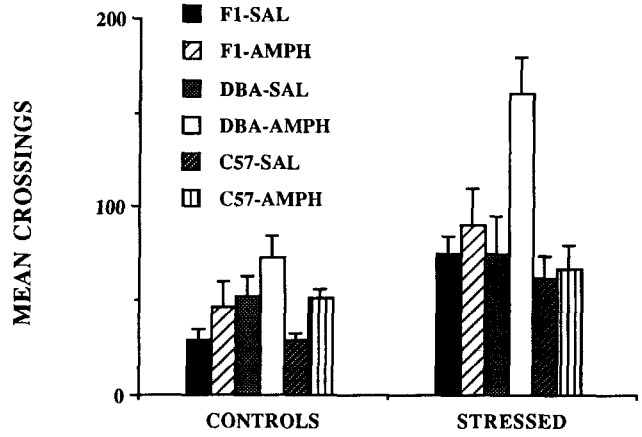


FIG. 4. Effects of repeated restraint stress on amphetamine-induced (0.5 mg/kg) locomotion in mice of the C57 and DBA strain and in their F1 hybrids habituated to the test cage. Results are expressed as mean (±SE) crossing for the 60-min test. Lack of significant global interaction did not allow individual between-groups comparison to be performed. See text for ANOVA results.

(two levels = control and stress), and treatment (two levels = saline and amphetamine). Furthermore, three-way ANOVA for repeated measures (10, 20, 30, 40, 50, and 60 min of test) was also performed on crossings for time blocks of the test session, factors being: pretreatment (two levels = control and stress) and treatment (two levels = saline and amphetamine).

## RESULTS AND DISCUSSION

In Fig. 4, the effects of chronic stress on locomotor activity in B6D2F1 (F1), DBA, and C57 mice are compared. ANOVA revealed significant main effects of stress, $F(1, 79) = 28.11$, $p < 0.001$, amphetamine, $F(1, 79) = 11.9$, $p < 0.001$, and strain, $F(2, 79) = 8.54$, $p < 0.001$. Lack of significant interactions between factors did not allow individual between-groups comparisons to be performed.

The results of the effects of chronic stress on crossings for time blocks of the test session in B6D2F1 hybrids are shown in Fig. 5. Three-way ANOVA for repeated measures revealed a significant main effect of stress, $F(1, 140) = 12.8$, $p < 0.005$, and of repeated measure, $F(5, 140) = 26.86$, $p < 0.001$. A significant interaction between repeated measure and amphetamine was also found, $F(5, 140) = 4.33$, $p < 0.005$. Lack of significant global interaction did not allow individual between-groups comparisons to be carried out.

Chronically stressed B6D2F1 hybrids show the large increase in saline-induced locomotion characteristic of their C57 parental strain. Moreover, the slight increase in amphetamine-induced locomotion in stressed B6D2F1 mice in comparison with controls appears to be due to the increase of basal activity since the increase of locomotion induced by 0.5 mg/kg amphetamine is 60% of saline in control and 20% in stressed mice. These results indicate that following chronic stress B6D2F1 hybrids present the same behavioral profile as the C57 strain, characterized by increased spontaneous (drug-free) locomotion and reduced sensitivity to the stimulatory effect of amphetamine.

## GENERAL DISCUSSION

Repeated stress experiences increase behavioral responsiveness to amphetamine in DBA mice tested 24 h after the last stress session. This effect of stress is evident regardless of whether mice are tested in a novel environment or in an environment to which they are habituated. On the other hand, repeated stress experiences increase spontaneous (drug-free) but not amphetamine-induced locomotion in C57 mice, indicating that strain-dependent alterations are involved in the behavioral effects of amphetamine following chronic stress.

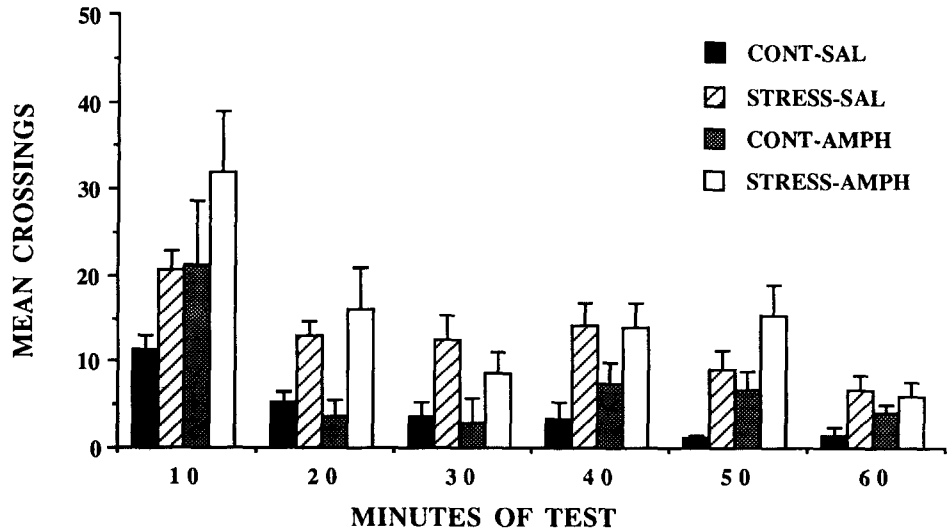


FIG. 5. Effects of repeated restraint stress on amphetamine-induced (0.5 mg/kg) locomotion in mice of the B6D2F1 hybrids habituated to the test cage. Results are expressed as mean (±SE) crossings for the 60-min test. Lack of significant global interaction did not allow individual between-groups comparison to be performed. See text for ANOVA results.

Although major strain-dependent differences were found in the basal levels of DA and its metabolites in the FC, CP, and NAS in chronically stressed animals of both strains sacrificed 24 h after the last stressful experience, no alteration of DA metabolism was evident in comparison with unstressed mice. These results rule out the possibility that different alterations of basal DA metabolism account for the different responses to amphetamine in mice of the DBA and C57 strains following chronic stress. It is worth noting that in FC DBA mice present higher DOPAC/DA and HVA/DA ratios than C57 mice. Previous reports showed an inverse relationship between DA activity in FC and NAS (14) that is not evident in this case since metabolite/ratios in the NAS are similar in both strains. This may depend upon some regulating factor, such as pre- or postsynaptic receptors in mesoaccumbens neurons.

Repeated amphetamine injections do not produce sensitization to a test dose of amphetamine in mice of both strains tested 24 h after the last injection although a tendency toward an increase of amphetamine-induced locomotion if observable in DBA mice repeatedly injected with the psychostimulant. Since it has been shown that 7 days after repeated amphetamine pretreatment DBA and, to a lesser extent, C57 mice show increased behavioral responsiveness to amphetamine (26), it appears that a long interval from chronic amphetamine treatment should elapse for behavioral sensitization to develop. This conclusion is supported by results obtained in rats showing that a withdrawal period of at least 1 week is needed to observe a fully developed sensitization to amphetamine challenge (19). On the other hand, since in the same experimental paradigms chronically stressed DBA mice show a full-blown sensitization to the behavioral effects of amphetamine it may be suggested that repeated stress and repeated amphetamine pretreatment do not produce identical effects in this strain of mice.

Moreover, both DBA and C57 mice strains show a reduced locomotion in drug-free conditions 24 h after the last injection of amphetamine, indicating a state of withdrawal-induced behavioral depression (19). By contrast, an increase in spontaneous locomotion was observed 24 h after the last stressful experience in C57 mice and in a previous study we have shown that this effect appears to develop as an adaptation process to hypolocomotion produced by acute stress exposure (3). These results, which indicate that repeated stress and amphetamine pretreatments produce opposite alterations in spontaneous behavior in this strain of mice, further support the view that the two pretreatments do not induce similar effects. In this regard, it is worth noting that while amphetamine, as well as other psychostimulants, has been shown to increase DA transmission in the striatum and in the mesolimbic area (10,28) 30–120 min of restraint stress actually inhibit DA release in the CP and NAS of both DBA and C57 mice (4,7,23). Although the involvement of increased DA transmission in the development of behavioral sensitization is still being debated, the opposite effect of stress and psychostimulants at this level may well indicate the existence of some major differences in their central effects.

Evidence exists that disruption of some presynaptic DA processes can interfere with the development of amphetamine sensitization (25,30). Although conflicting results have been reported concerning the involvement of subsensitive DA autoreceptors in behavioral sensitization to amphetamine (13, 16,21,30), it is interesting to note that DA autoreceptor hyposensitivity is the one documented change in brain DA functioning produced by chronic stress (1,17). Hyposensitive DA autoreceptors would not be in contrast with the stress-induced reduction of the DA release in the terminal fields since axonal and dendritic release of DA appear to be inversely related (18).

We have recently shown that chronically stressed DBA mice are characterized by reduced responsiveness to the behavioral effects of low, presynaptic doses of apomorphine, while C57 mice are hyperresponsive (6,8). Moreover, these alterations in the behavioral response to apomorphine parallel opposite alterations of the effects of the direct DA agonist on DA metabolism in the mesolimbic area (8). Taken together, these results indicate that, following chronic stress, the two strains of mice are characterized by opposite changes in DA autoreceptor sensitivity in a brain area that has a major role in the control of amphetamine-induced hyperactivity.

Hyposensitivity of presynaptic DA receptors may well explain the increase of amphetamine-induced locomotor activity observed in chronically stressed DBA mice. On the other hand, chronically stressed C57 mice show an increase in saline-induced locomotion following chronic stress that is not accompanied by a parallel increase in amphetamine-induced locomotion. Moreover, the relative increase of behavior induced by the highest dose of amphetamine in comparison with saline-injected groups is 167% in unstressed and 70% in stressed mice. Taken together, these results suggest that following chronic stress C57 mice are hyporesponsive to the stimulating effects of amphetamine on locomotion, a condition that might depend upon stress-induced increase in autoreceptor functioning.

This hypothesis is supported by the results obtained in chronically stressed B6D2F1 hybrids. In fact, in a previous study we demonstrated through a genetic analysis that the alteration of DA autoreceptor sensitivity characteristic of C57 mice is inherited through a dominant mode of inheritance (6). Thus, the observation that, in the present experiments, chronically stressed B6D2F1 hybrids present the same behavioral response to amphetamine as their C57 parental strain strongly supports the involvement of changes in autoreceptor sensitivity in the strain-dependent sensitization to the effects of amphetamine on locomotion. However, further experiments involving genetic analysis of the effects of amphetamine on DA metabolism in chronically stressed mice are needed to confirm the present results.

In conclusion, the present results show that following repeated stress experiences DBA mice show increased behavioral responsiveness to amphetamine while in C57 mice an actual decrease of responsiveness may be envisaged. The altered responsiveness does not appear to be due to stress-induced changes of basal DA metabolism. Finally, they suggest that strain-dependent alterations of DA autoreceptors are implicated in the effects of stress on amphetamine-induced behavior.

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