Psychopharmacology 48, 59–67 (1976)

Psycho–
pharmacology

Changes in Brain Norepinephrine Associated with Sensitization to *d*-Amphetamine

PETER H. SHORT and LOUIS SHUSTER*

Department of Biochemistry and Pharmacology, Tufts University School of Medicine, 136 Harrison Ave., Boston, Massachusetts 02111, U.S.A.

Abstract. Pretreatment of $B6AF_1/J$ mice with *d*-amphetamine HCl 10 mg/kg, twice daily for 5 days, produced a 4-fold increase in the running response to a test dose of 5 mg/kg amphetamine. Amphetamine pretreatment decreased whole-brain norepinephrine levels to 50% of control values and whole-brain dopamine to 85%. The test dose of 5 mg/kg amphetamine lowered whole brain norepinephrine levels of control mice from 0.50 µg/g to 0.28 µg/g in 2 h. In amphetamine-pretreated mice, this injection caused an increase in whole-brain norepinephrine levels from 0.22 µg/g to 0.55 µg/g at 30 min, followed by a decrease to 0.22 µg/g at 60 min. No change in whole brain dopamine levels was observed in either group. Amphetamine sensitization and norepinephrine depletion were still evident 25 days after pretreatment. No cross sensitization to morphine or cocaine was observed. Reserpine pretreatment resulted in a 3-fold increase in locomotor activity following injection of *d*-amphetamine, 5 mg/kg. No sensitization or changes in catecholamine levels were observed in amphetamine-treated A/J mice. These results suggest that the sensitization produced by amphetamine pretreatment may be related to the depletion of brain norepinephrine.

Key words: Amphetamine – Sensitization – Catecholamines – Running activity.

INTRODUCTION

Chronic amphetamine treatment has been shown to produce tolerance to the hypothermic (Harrison et al., 1952), anorexigenic (Tormey and Lasagna, 1960), toxic (Lewander, 1968) and cardiovascular (Day and Rand, 1963) effects of the drug. There have also been reports of sensitization to the central stimulant effects of amphetamine (Magos, 1969; Wallach and Gershon, 1971; Ranje and Ungerstedt, 1974). The central stimulant effects of amphetamine are thought to involve the release of loosely bound or newly synthesized catecholamines (Chuieh and Moore, 1975; Carr and Moore, 1970; Carlsson, 1970; Azzaro and Rutledge, 1973).

Amphetamine produces a characteristic pattern of hyperactivity which is thought to be mediated by norepinephrine (Randrup and Munkvad, 1970; Welch and Welch, 1970) or dopamine (van Rossum, 1963; Ernst, 1969). There is also evidence favoring the involvement of both norepinephrine and dopamine in amphetamine-induced locomotor activity (Tseng and Loh, 1974).

The prior implantation of *d*-amphetamine pellets into mice has been shown to enhance the running response to subsequent injections of amphetamine (Hitzemann et al., 1973). The response of rats to amphetamine is increased by amphetamine pretreatment (Segal, 1975a; Rech et al., 1975). Pretreatment with reserpine has also been shown to potentiate the locomotor response of rats to amphetamine (Stolk and Rech, 1968). The purpose of our work has been to investigate the development of *d*-amphetamine sensitization in mice and to examine the relationship of brain catecholamines to this sensitization.

MATERIALS AND METHODS

Animals. Unless otherwise stated, male $B6AF_1/J_1$ mice (Jackson Laboratories, Bar Harbor, Maine) 6–8 weeks old were used. The mice were housed in plastic cages lined with hardwood bedding and fed Purina chow and water ad libitum. The animal room was air-conditioned and maintained at 22°C with a lighting cycle of 12 h light and 12 h darkness.

Locomotor Activity. Motor activity was measured by a modification of the method of Dews (1953). Food and water were removed just

* To whom offprint requests should be sent.

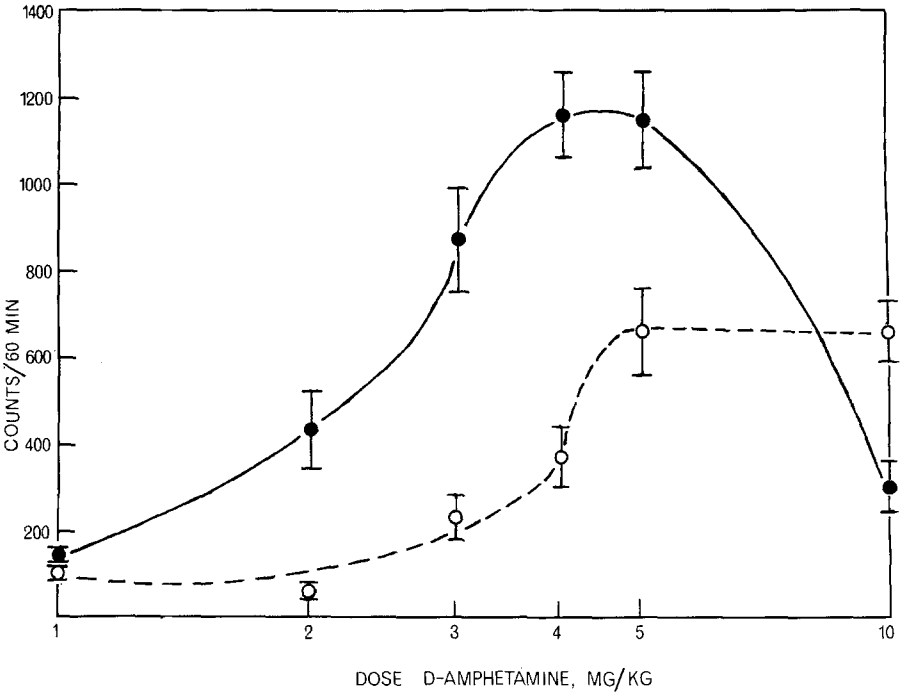

Fig. 1. Running response of saline- and amphetamine-pretreated mice to different doses of *d*-amphetamine HCl. Amphetamine-pretreated mice (●——●) were injected with 10 mg/kg *d*-amphetamine HCl, twice daily for 5 days. Saline-pretreated mice (○----○) received 0.15 M NaCl on the same schedule. All testing was done 70 h after the last pretreatment injection. Each point is the mean running activity obtained from 8 mice ± S.E. for the first 60 min after injection. All values for pretreated mice significantly different from controls ($P \leq 0.01$) except at the dose of 1 mg/kg

before testing. Mice were run individually in clear plastic cages, 28 cm × 16 cm × 13 cm deep, containing about 1 cm of bedding chips. Each cage was placed between a horizontal light source and photocell of an Autotron model S1AC photoelectric counter (Autotron, Inc., Danville, Ill.). Spontaneous activity was determined for the first four 15-min periods after placing the mice in the cages. The mice were then injected intraperitoneally and activity recorded every 15 min for the next 2 h. Comparative measurements of activity were carried out at the same time of day, usually between 1:00 p.m. and 4:00 p.m.

Determination of Catecholamine Levels. Mice were killed by cervical dislocation. Whole brain concentrations of norepinephrine and dopamine were determined by the method of Ansell and Beeson (1968). The whole brain was homogenized at 4° C in acid-butanol, with ten strokes of a motor-driven glass-teflon homogenizer. Fluorescence was measured with an Aminco-Bowman spectrophotofluorometer.

Measurement of Tyrosine Hydroxylase Activity in vitro. A modification of the assay of Kuczenski and Segal (1974) was used. Whole brains were homogenized in 7.5 ml of ice cold 0.32 M sucrose. 80 µl of the brain homogenate were added to an incubation mixture, which contained in a final volume of 0.5 ml: 120 mM NaCl, 5 mM KCl, 10 mM glucose, 2 mM ascorbic acid, 2 mM disodium EDTA, 50 mM sucrose and 30 mM sodium phosphate, adjusted to give a final pH of 6.6. L-Tyrosine-1-C^{14} (5 µCi, 0.27 µmoles) was then added. The samples were incubated at 37° C for 30 min in glass tubes sealed with serum caps. Filter discs saturated with 75 µl NCS, an organic amine used for trapping CO_2, were suspended inside the tubes by means of a straight pin. After 30 min, the reaction was stopped by injecting 0.5 ml of 10% TCA through the serum caps. The tubes were then incubated at 37° C for an additional 2 h. After incubation, the filter discs were placed in 5 ml of a toluene based scintillation fluid and counted in a Packard scintillation counter.

Drugs and Reagents. Morphine sulfate (Merck and Company, Inc.) cocaine hydrochloride (Merck and Company, Inc.) and *d*-amphetamine hydrochloride (Amend Drug and Chemical Company) were purchased from Gilman Bros., Boston, Mass. Norepinephrine bitartrate was purchased from Nutritional Biochemicals. Dopamine hydrochloride was purchased from the Regis Chemical Company. Certified A.C.S. iso-octane and fluorometric grade 1-butanol were purchased from the Fisher Chemical Company. L-Tyrosine-1-C^{14}, specific activity 55.8 mCi/mM was purchased from New England Nuclear Corp., Boston, Mass. NCS was purchased from Amersham-Searle, Des Plaines, Ill.

Statistical Analysis. Statistical significance of the difference between means was determined by the Mann-Whitney *U* test (Goldstein, 1964).

RESULTS

The Development of Sensitization to d-Amphetamine HCl. Dose response curves for control and amphetamine-pretreated mice (10 mg/kg, twice daily for 5 days) were done in order to demonstrate the effect of amphetamine pretreatment on a subsequent injection of amphetamine (Fig. 1). Amphetamine pretreatment caused a shift in the dose response curve to the left and an increase in the maximal locomotor activity. This result shows that pretreatment with amphetamine enhances the amphetamine-induced running

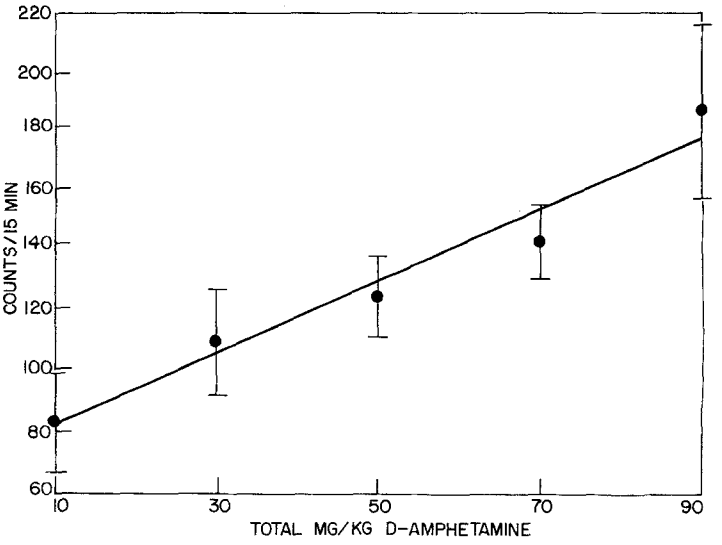

Fig. 2
Running response to daily injections of amphetamine. Mice were injected twice a day for 5 days with 10 mg/kg *d*-amphetamine HCl. Injections were given at approximately 9:00 a.m. and 2:00 p.m. All testing was done following the 2:00 p.m. injection. The running activity during the first 15 min after injection was plotted against the cumulative dose of *d*-amphetamine administered prior to testing. Each point represents the mean value ± S.E. from 10 mice. Total drug dose is directly related to the number of days of administration

response of $B6AF_1/J$ mice. The decreased running response seen with 10 mg/kg *d*-amphetamine in amphetamine-pretreated mice could be a result of sensitization. In this case, the 10 mg/kg test dose could be too high for maximal stimulation of locomotor activity (Randrup and Munkvad, 1972; Hitzemann et al., 1973). Starvation-induced weight losses of approximately 20%, have been shown to enhance the amphetamine-induced running response of rats (Campbell and Fibiger, 1971). Weight loss due to amphetamine pretreatment was never more than 5% at the time of testing in any of the experiments described here, and was not considered a major factor in the response of amphetamine-pretreated mice to amphetamine.

In order to examine the development of sensitization to amphetamine, mice were injected twice a day for 5 days with 10 mg/kg *d*-amphetamine. On each day, locomotor activity was measured after the second injection. The amphetamine-induced locomotor activity increased with each day of treatment. The increase in locomotor activity was also directly proportional to the cumulative dose of amphetamine administered (Fig. 2). Similar results have recently been reported in rats (Segal, 1975a). Baseline responses of control mice were either equivalent to or higher than the baseline responses of amphetamine pretreated mice. Under the same test conditions as described in Methods, repeated injections of saline resulted in either no change or a decrease in locomotor activity, as a result of habituation to handling, injections and the test situation. The activity of saline-injected control mice was approximately 40 counts or less in 60 min (Shuster et al., 1975).

Specificity of Amphetamine Sensitization. Mice were pretreated with morphine, cocaine or reserpine and tested with 5 mg/kg *d*-amphetamine 3 days after pretreatment. These drugs were used because in common with amphetamine they are thought to affect catecholaminergic neurons in the CNS. The results of these experiments along with data from saline-pretreated and amphetamine-pretreated controls are summarized in Table 1. Neither morphine nor cocaine enhanced the running response to 5 mg/kg *d*-amphetamine. The slight depression of amphetamine-induced locomotor activity seen with morphine pretreatment is consistent with the findings of Fog (1970), who showed that acute and chronic injections of morphine antagonized amphetamine-induced stereotypic behavior in rats. A single injection of reserpine 5 mg/kg, 3 days before testing, increased significantly the amphetamine-induced running response.

Table 1. Specificity of sensitization to *d*-amphetamine

Pretreatment	*n*	Running response to amphetamine 5 mg/kg Counts/60 min
Saline, twice daily for 5 days	10	661 ± 123
Morphine 25 mg/kg, once daily for 5 days	8	520 ± 90*
Cocaine 20 mg/kg, once daily for 5 days	10	690 ± 88*
Amphetamine 10 mg/kg, twice daily for 5 days	10	1040 ± 66**
Saline, one injection	5	624 ± 196
Reserpine 5 mg/kg, one injection	5	1334 ± 163***

* $P > 0.05$ for the difference from saline controls.
** $P = 0.025$ for the difference from saline controls.
*** $P = 0.01$ for the difference from saline controls.

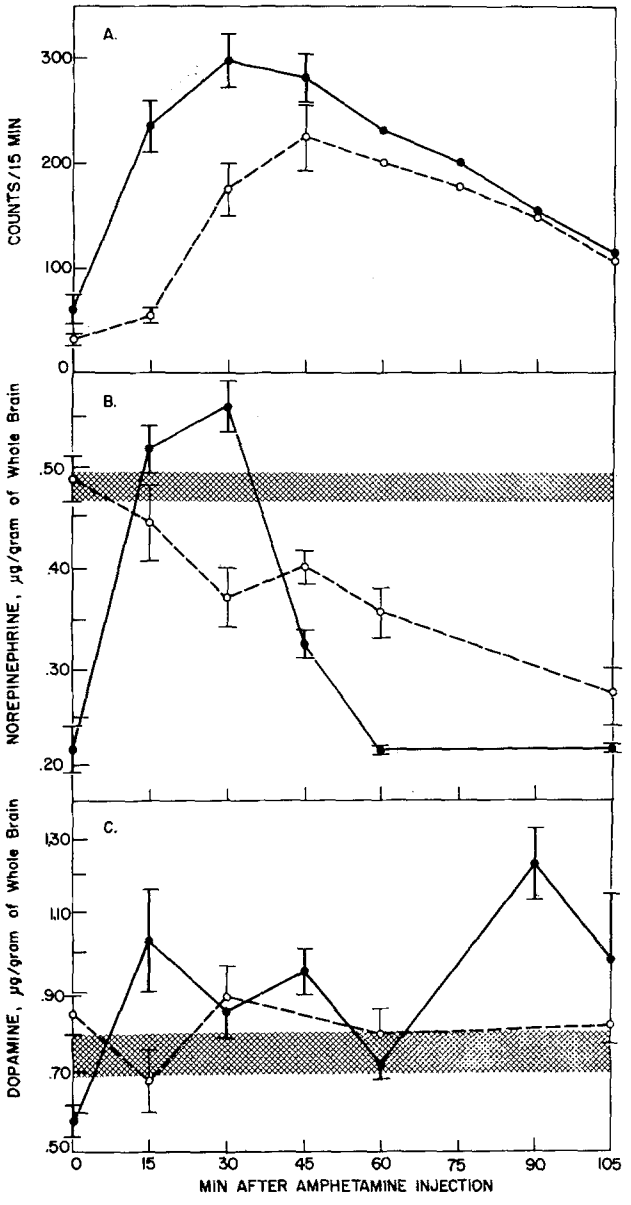

Fig. 3A–C. Running response and catecholamine changes after injection of 5 mg/kg *d*-amphetamine HCl. Thirty mice were pretreated with 10 mg/kg *d*-amphetamine HCl, twice a day for 5 days. Control mice were pretreated with 0.15 M NaCl. All testing was done with 5 mg/kg *d*-amphetamine HCl, 3 days after the last pretreatment injection. (A) Running response of saline (O——O) and amphetamine (●——●) pretreated mice. Where standard errors are shown, $n = 10$, and $P \leq 0.01$ for the difference between sensitized and control groups. Where standard errors are not shown, $n = 6$ and $P > 0.05$. (B) Effect of 5 mg/kg *d*-amphetamine HCl on brain norepinephrine levels. Each point represents the mean of 3 mice ± S.E. The shaded area represents the normal level ± S.E., based on catecholamine determinations in 15 untreated $B6AF_1/J$ mice. (C) Effect of 5 mg/kg *d*-amphetamine HCl on brain dopamine levels. Each point represents the mean of 3 mice ± S.E. The shaded area represents the normal level ± S.E.

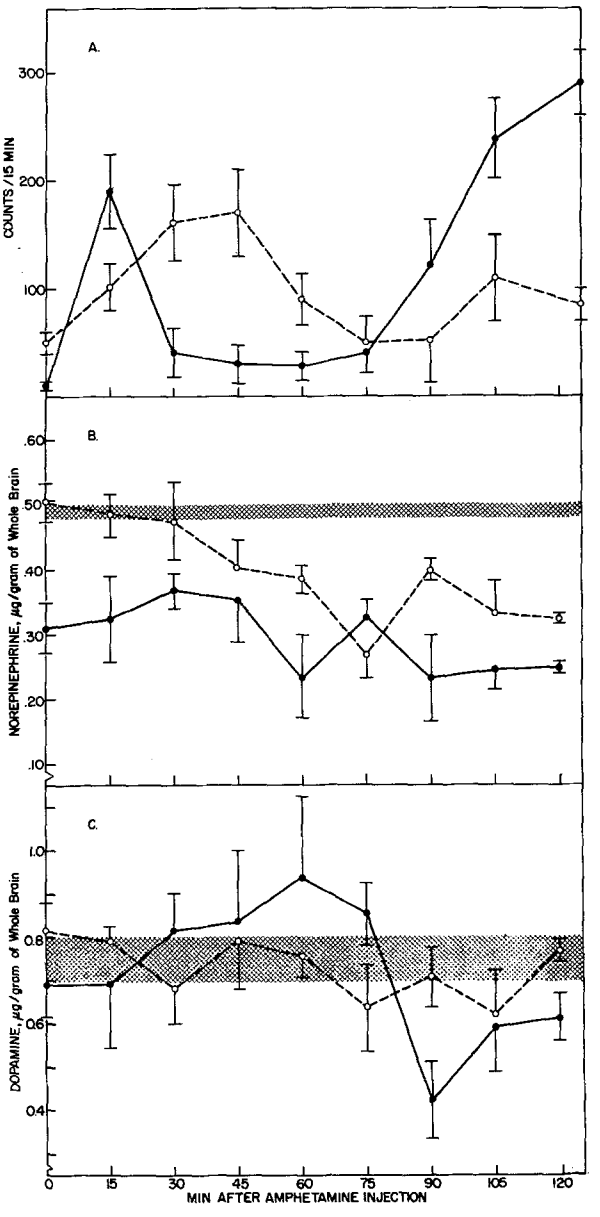

Fig. 4A–C. Running response and catecholamine changes after injection of 10 mg/kg *d*-amphetamine HCl. Amphetamine pretreated mice (●——●) were injected with 10 mg/kg *d*-amphetamine HCl, twice a day for 5 days. Control mice (O——O) were pretreated with 0.15 M NaCl. All testing was done with 10 mg/kg *d*-amphetamine HCl, 3 days after pretreatment. (A) Running response of saline- and amphetamine-pretreated mice. Each point represents the mean of 10 mice ± S.E. (B) Effect of 10 mg/kg *d*-amphetamine HCl on brain norepinephrine levels. Each point represents the mean of 3 mice ± S.E. The shaded area represents the normal level ± S.E., based on catecholamine determinations in 15 untreated $B6AF_1/J$ mice. (C) Effect of 10 mg/kg *d*-amphetamine HCl on brain dopamine levels. Each point represents the mean of 3 mice ± S.E. The shaded area represents the normal level ± S.E.

The Effect of Amphetamine Pretreatment on Amphetamine-Induced Changes in Catecholamine Levels. Mice were injected with *d*-amphetamine 10 mg/kg twice a day for 5 days. Control mice were injected with 0.15 M NaCl on the same schedule. Three days after pretreatment, both groups of mice received *d*-amphetamine 5 mg/kg. Locomotor activity was measured as described in Methods. Catecholamine levels were determined at 15-min intervals. The results are summarized in Figure 3. Both amphetamine-pretreated and control mice exhibited maximal locomotor activity 30–45 min after injection of the amphetamine test dose. The running response of amphetamine-pretreated mice was significantly higher than that of the saline-pretreated controls.

Amphetamine pretreatment produced a 50% depletion of whole-brain norepinephrine levels and a 15% depletion of whole-brain dopamine levels. The 5 mg/kg *d*-amphetamine injection caused whole-brain norepinephrine levels of control mice to decline from 0.50 μg/g to 0.28 μg/g in 2 h. In the amphetamine-pretreated mice, this injection caused whole brain norepinephrine levels to increase from 0.22 μg/g to 0.55 μg/g in 30 min. Norepinephrine levels then declined to 0.22 μg/g by 60 min and remained at this level for the next hour. No significant changes in whole brain dopamine levels were observed in either group.

Locomotor activity and changes in catecholamine levels were also measured in control and amphetamine-pretreated mice following an injection of 10 mg/kg *d*-amphetamine (Fig. 4). Locomotor activity in control mice was maximal at 45 min after injection. The amphetamine-pretreated mice exhibited a biphasic running pattern with peak responses at 15 and at 120 min after injection. The decline in the running activity after the first phase was associated with an increase in stereotypy in the form of rearing, sniffing and licking. As the stereotypy declined, there was a secondary increase in running activity. Similar results have recently been reported with rats (Segal, 1975b). Five mg/kg *d*-amphetamine was more effective than 10 mg/kg *d*-amphetamine in stimulating the running response of amphetamine-pretreated mice. No difference was observed between the two doses in control mice. These results suggest that 10 mg/kg *d*-amphetamine was too high a dose to stimulate optimally the locomotor activity in amphetamine-pretreated mice.

The 10 mg/kg *d*-amphetamine test dose caused no change in norepinephrine levels of amphetamine-pretreated mice. They remained at the depleted level of 0.31 μg/g for the entire testing period. This test dose caused norepinephrine levels of control mice to decline from 0.50 μg/g to 0.30 μg/g in 2 h.

The 10 mg/kg *d*-amphetamine test dose produced no significant changes in the whole brain dopamine levels of control mice. In the amphetamine-pretreated mice, this injection produced flucutations in whole brain dopamine levels, but there was no obvious correlation with the running pattern. By 90 min after injection, dopamine levels of the amphetamine-pretreated mice were significantly below normal. They stayed below normal for the remainder of the testing period.

Duration of Amphetamine Sensitization and Catecholamine Depletion. Forty mice were pretreated with *d*-amphetamine 10 mg/kg, twice a day for 5 days. At 3, 8, 14, 18 and 25 days after the last pretreatment, 5 mice were injected with *d*-amphetamine 5 mg/kg and their locomotor activity was measured for 30 min. Immediately afterwards, 3 of the mice were killed and whole-brain catecholamine levels were determined. On each day of testing, 3 additional amphetamine-pretreated mice were killed and catecholamine levels measured. These mice were used to determine the duration of norepinephrine depletion following amphetamine pretreatment.

Locomotor activity in response to 5 mg/kg *d*-amphetamine declined from day 3 to day 25 (Fig. 5). However, on day 25, the running response to 5 mg/kg *d*-amphetamine was still 3 times the control value. As a result of amphetamine pretreatment, whole-brain norepinephrine levels were depleted to 55% of normal values. They gradually recovered to 88% of normal values by day 25. In addition, the increase in norepinephrine normally seen 30 min after the administration of 5 mg/kg *d*-amphetamine to amphetamine-pretreated mice gradually disappeared. By day 25, no difference was observed in the whole-brain norepinephrine levels of amphetamine-pretreated mice before or 30 min after an injection of 5 mg/kg *d*-amphetamine. Amphetamine pretreatment caused no significant change in whole-brain dopamine levels. However, as norepinephrine levels began to recover, dopamine levels began to decline, suggesting the operation of some type of feedback mechanism (Costa and Meek, 1974).

Repeated testing of a group of amphetamine-pretreated mice with 5 mg/kg *d*-amphetamine maintained their sensitization for as long as 43 days after pretreatment (Table 2). This table also shows that several different schedules of pretreatment with *d*-amphetamine (5 mg/kg, once daily for 5 days, 10 mg/kg, twice daily for 5 days, or 15 mg/kg, twice daily for 3 days) increased significantly the running response to *d*-amphetamine 5 mg/kg.

Tyrosine Hydroxylase Activity in Control and Pretreated Mice. Tyrosine hydroxylase activity was deter-

Table 2. Duration of sensitization to *d*-amphetamine

Pretreatment schedule	*n*	Days after pretreatment	Running response to 5 mg/kg amphetamine	
			Counts/15 min ± S.E.	Counts/60 min ± S.E.
Saline, twice daily for 5 days	10	3	87 ± 9	672 ± 82
Amphetamine 10 mg/kg, twice daily for 5 days[a]	10	3	373 ± 35	1524 ± 174
		5	300 ± 19	1271 ± 106
		10	327 ± 21	1110 ± 82
		17	337 ± 36	1034 ± 59
Saline, once daily for 5 days	10	3	69 ± 13	699 ± 95
Amphetamine 5 mg/kg, once daily for 5 days[a]	10	3	234 ± 27	1401 ± 182
		7	274 ± 15	1042 ± 100
		29	280 ± 18	988 ± 69
		43	268 ± 25	930 ± 132
Saline, twice daily for 3 days	5	3	96 ± 12	611 ± 86
Amphetamine 15 mg/kg, twice daily for 3 days	5	3	244 ± 33	909 ± 67

The response of all amphetamine-pretreated mice to 5 mg/kg *d*-amphetamine HCl was significantly greater than that of saline-pretreated controls ($P \leq 0.01$).

[a] Following pretreatment these mice were tested repeatedly with 5 mg/kg *d*-amphetamine on the days specified.

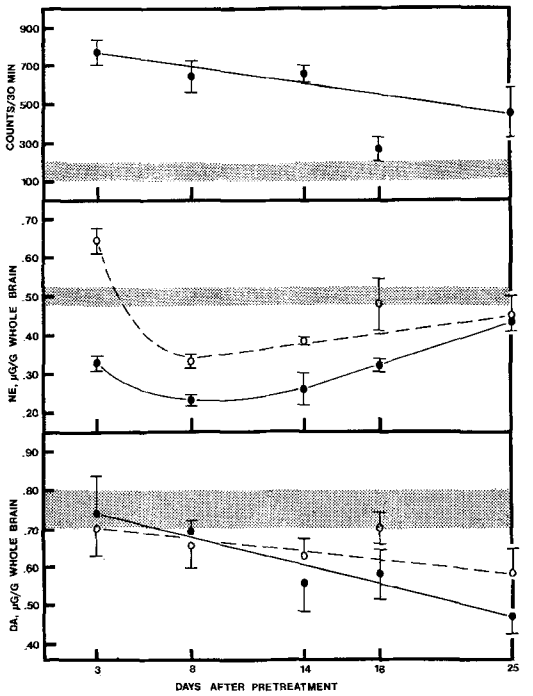

Fig. 5. Duration of amphetamine sensitization and associated catecholamine changes. 40 $B6AF_1/J$ mice were injected with 10 mg/kg *d*-amphetamine HCl, twice a day for 5 days. At 3, 8, 14, 18 and 25 days after pretreatment, the running response to 5 mg/kg *d*-amphetamine HCl and catecholamine levels before (●——●) and 30 min after (○- - - -○) injection of 5 mg/kg *d*-amphetamine HCl were measured. Each point for locomotor activity (counts/30 min) represents the mean value of 5 mice ± S.E. All catecholamine determinations represent the mean values of 3 mice ± S.E. Shaded areas represent the normal levels ± S.E.

mined in brain homogenates from control and amphetamine-pretreated mice, before and 30 min after an injection of 5 mg/kg *d*-amphetamine. There was no significant difference among the four groups of mice examined. This finding suggests that the increase in amphetamine-induced locomotor activity seen following amphetamine pretreatment is not due to an increase in whole brain tyrosine hydroxylase activity as measured in vitro. In another experiment, reserpine, 5 mg/kg, given 2 h before the mice were killed, produced a 60% increase in tyrosine hydroxylase activity (160 ± 8 cpm/mg vs. 103 ± 8 cpm/mg for saline-pretreated controls).

Genetic Differences in Sensitization to Amphetamine. The parental strains of the B_6AF_1/J hybrids used in these experiments were also tested for sensitization to amphetamine. C57B1/6J and A/J male mice were injected twice a day for 5 days with 10 mg/kg *d*-amphetamine. Three days after pretreatment, the running response to 5 mg/kg *d*-amphetamine was measured and compared with the response of saline-pretreated mice of the same strains. The A/J mice showed only a slight difference ($P = 0.05$) in running response (counts/30 min) between control (252 ± 42) and amphetamine pretreatment (366 ± 41). The C57B1/6J mice pretreated with amphetamine exhibited a 2.5-fold increase ($P = 0.01$) in locomotor activity from 183 ± 107 for controls to 519 ± 46 counts/30 min for the amphetamine-pretreated group.

Table 3. *d*-Amphetamine sensitization and catecholamine changes in A/J and B6AF$_1$/J mice

Strain	Pretreatment	Running response to amphetamine 5 mg/kg[a]	Catecholamine levels[b]			
			Control (μg/g whole brain)		30 min after 5 mg/kg amphetamine (μg/g whole brain)	
		Counts/30 min	NE	DA	NE	DA
A/J	Saline	252 ± 42	0.42 ± 0.03	0.63 ± 0.04	0.41 ± 0.03	0.67 ± 0.06
	Amphetamine	366 ± 41 ($P = 0.05$)	0.46 ± 0.05	0.69 ± 0.05	0.45 ± 0.05	0.63 ± 0.03
B6AF$_1$/J	Saline	145 ± 44	0.51 ± 0.03	0.82 ± 0.06	0.48 ± 0.05	0.69 ± 0.09
	Amphetamine	773 ± 68 ($P < 0.005$)	0.33 ± 0.02	0.74 ± 0.10	0.64 ± 0.03[c]	0.70 ± 0.07

[a] $n = 5$.
[b] $n = 3$.
[c] Significantly different from amphetamine-pretreated controls ($P = 0.01$).

Whole-brain catecholamines of control and amphetamine-pretreated A/J mice were measured before, and 30 min after, the injection of 5 mg/kg *d*-amphetamine. These data were compared with those obtained from similarly-treated B6AF$_1$/J mice (Table 3). In A/J mice, where no sensitization was observed, amphetamine pretreatment did not produce norepinephrine depletion. The 5 mg/kg *d*-amphetamine test dose also had no effect on norepinephrine levels of A/J mice. In B6AF$_1$/J mice, where there was sensitization, amphetamine pretreatment produced depletion of norepinephrine. Also, the test dose caused an increase in norepinephrine levels from 0.33 μg/g to 0.64 μg/g at 30 min after injection. These results suggest that the development of sensitization to amphetamine is correlated with the depletion of brain norepinephrine.

Running Response and Catecholamine Levels after Amphetamine in Reserpine-Pretreated Mice. Mice were given 1 injection of reserpine, 5 mg/kg. Three days later, they were injected with 5 mg/kg *d*-amphetamine. Locomotor activity was measured for the first 30 min following injection. Immediately afterwards, 3 mice were killed and catecholamine levels determined. The single injection of reserpine caused a 2.5-fold increase in the running response to 5 mg/kg *d*-amphetamine, based on a comparison with naive mice. The single injection of reserpine 5 mg/kg also resulted in a 70% depletion of norepinephrine and a 55% depletion of dopamine. The 5 mg/kg *d*-amphetamine injection caused a 3.5-fold increase in norepinephrine (from 0.126 ± 0.023 μg/g to 0.426 ± 0.067 μg/g) and a 5.5-fold increase in dopamine (from 0.34 ± 0.09 μg/g to 1.89 ± 0.41 μg/g).

DISCUSSION

The results presented here show that it is possible to produce long-lasting sensitization to the amphetamine-induced running response in mice. Sensitization to the amphetamine-induced running response was found to last for as long as 25 days after the final pretreatment injection. Similar results have recently been reported with rats (Segal, 1975a; Rech et al., 1975). No cross-sensitization to morphine or cocaine was observed in our mice, suggesting that the mechanism of sensitization to amphetamine is different from that for either morphine or cocaine (Shuster et al., 1975; Shuster and Yu, manuscript in preparation). Villarreal et al. (1973) have also concluded that the mechanism by which morphine increases motor activity is different from that for amphetamine.

Administration of amphetamine to mice in doses of 5 mg/kg or higher has been shown to deplete brain norepinephrine (Axlerod, 1970). We have found that when mice are injected repeatedly with *d*-amphetamine HCl, in doses of 5 mg/kg or higher, sensitization develops to the amphetamine-induced running response. The development of sensitization to amphetamine was correlated with a depletion of brain norepinephrine. In addition, 30 min after administration of a 5 mg/kg test dose of *d*-amphetamine to sensitized mice, norepinephrine levels rose from 50% below normal to 10% above normal (0.22 μg/g to 0.55 μg/g). Sensitization to amphetamine and the associated catecholamine changes were also obtained following depletion of norepinephrine levels with reserpine. This observation is consistent with previous findings that reserpine pretreatment enhanced the

amphetamine induced running response in rats (Stolk and Rech, 1968) and in mice (Smith, 1963).

At 25 days after the last pretreatment injection, norepinephrine levels had returned to 88% of normal. With the recovery of norepinephrine levels, there was a decline in the amphetamine-induced running response and a gradual loss of the increase in norepinephrine following the injection of 5 mg/kg *d*-amphetamine. Under these conditions, there was little change in dopamine levels. The decrease in dopamine levels that was observed as norepinephrine levels returned to normal may reflect feedback regulation of catecholamine synthesis by norepinephrine (Costa and Meek, 1974). These results also suggest that the role of dopamine cannot be ignored in the response of amphetamine-pretreated mice to amphetamine (Tseng and Loh, 1974; Segal, 1975a).

Genetic differences have been reported in the response of different strains of mice to a single injection of amphetamine (Moisset and Welch, 1973; Oliverio et al., 1973). We have also found genetic differences in the ability of mice to become sensitized to amphetamine. In those strains where sensitization to amphetamine was observed, norepinephrine levels were depleted as a result of pretreatment. The norepinephrine levels were also found to increase 30 min after injection of *d*-amphetamine 5 mg/kg. In A/J mice, where no sensitization was observed, there was no change in norepinephrine levels, either following pretreatment or after the 5 mg/kg *d*-amphetamine test dose. We are currently investigating the genetics of the amphetamine response in the F_2 generation obtained by crossing $B6AF_1/J$ mice. We are also examining the amphetamine response of the recombinant-inbred lines of Bailey (1971).

Amphetamine is thought to act by releasing loosely bound or newly synthesized norepinephrine and dopamine (van Rossum, 1963; Chuieh and Moore, 1975; Carr and Moore, 1970). Administration of 5 mg/kg *d*-amphetamine HCl to amphetamine-sensitized mice produced similar changes in the running pattern and whole-brain norepinephrine levels. No obvious correlation between the running pattern and dopamine changes was observed.

No changes in whole brain tyrosine hydroxylase activity were observed following either amphetamine pretreatment or the 5 mg/kg *d*-amphetamine test dose. Similar results have also been reported following chronic amphetamine treatment in rats (Hulme et al., 1974) and acute amphetamine treatment in mice (Smith et al., 1972). Harris et al. (1975) have shown that a single injection of amphetamine can cause increases in tyrosine hydroxylase in several brain areas while causing a decrease in corpus striatum.

With reserpine pretreatment, there was a 60% increase in the activity of whole brain tyrosine hydroxylase. Similar increases have been reported by Mueller et al. (1969). The catecholamine changes following administration of amphetamine to reserpine-pretreated mice were different from those observed with amphetamine-pretreated mice. Both groups of mice showed increases in norepinephrine levels following injection of amphetamine, but increases in dopamine levels were observed only in the reserpine-pretreated mice. Also, reserpine-pretreatment caused marked depletion of both dopamine and norepinephrine, while amphetamine-pretreatment caused depletion of only norepinephrine. These findings suggest that the mechanism of sensitization to amphetamine after reserpine may be different from that involved in amphetamine pretreatment.

The purpose of our work was to examine the development of sensitization to the locomotor effects of amphetamine and to determine whether or not neurochemical correlates exist. For this purpose, the measurement of whole-brain catecholamine levels was sufficient. Recently, the pons-medulla has been shown to be the brain area with the most profound catecholamine changes following amphetamine administration (Cook and Schanberg, 1975). We are planning to examine catecholamine levels and tyrosine hydroxylase activity in discrete brain regions of control and amphetamine-sensitized mice. The possibility of changes in the rate of amphetamine metabolism after amphetamine pretreatment will also be investigated.

The results reported here may have some implications for the study of post-amphetamine depression in man. Following chronic amphetamine use in man, there is a period of lethargy that may last until the next intake of amphetamine (Goodman and Gilman, 1970). Our observations suggest that post-amphetamine depression in man may be related to either the depletion of norepinephrine that is seen immediately following chronic amphetamine administration, or the depletion of dopamine that is observed after norepinephrine levels have returned to normal.

Acknowledgements. We wish to thank Ann Lovejoy and Marsha Hubbard for their excellent technical assistance in some of the experiments described here. This work was supported in part by Grants DA00022 and DA00323 from the National Institutes of Health of the U.S. Public Health Service.

REFERENCES

Ansell, G. B., Beeson, M. F.: A rapid and sensitive procedure for the combined assay of noradrenaline, dopamine and serotonin in a single brain sample. Analyt. Biochem. **23**, 196–206 (1968)

Axlerod, J.: Amphetamine: metabolism, physiological disposition, and its effects on catecholamine storage. In: International

symposium on amphetamines and related compounds, E. Costa and S. Garattini, eds., pp. 207–216. New York: Raven Press 1970

Azzaro, A. J., Rutledge, C. O.: Selectivity of release of norepinephrine dopamine and 5-hydroxytryptamine by amphetamine in various regions of rat brain. Biochem. Pharmacol. **22**, 2801–2813 (1973)

Bailey, D. W.: Recombinant inbred strains. Transplantation **11**, 325–327 (1971)

Campbell, B. A., Fibiger, H. C.: Potentiation of amphetamine-induced arousal by starvation. Nature (Lond.) **233**, 424–425 (1971)

Carlsson, A.: Amphetamine and brain catecholamines. In: International symposium on amphetamines and related compounds, E. Costa and S. Garattini, eds., pp. 289–300. New York: Raven Press 1970

Carr, L. A., Moore, K. E.: Effects of amphetamine on the contents of norepinephrine and its metabolites in the effluent of perfused cerebral ventricles in the cat. Biochem. Pharmacol. **19**, 2361–2374 (1970)

Chuieh, C. C., Moore, K. E.: *d*-Amphetamine-induced release of "newly synthesized" and "stored" dopamine from the caudate nucleus in vivo. J. Pharmacol. exp. Ther. **192**, 642–653 (1975)

Cook, J. D., Schanberg, S. M.: Effect of methamphetamine on norepinephrine metabolism in various regions of brain. J. Pharmacol. exp. Ther. **195**, 87–93 (1975)

Costa, E., Meek, J. L.: Regulation of biosynthesis of catecholamines and serotonin in the CNS. Ann. Rev. Pharmacol. **14**, 491–511 (1974)

Day, M. D., Rand, M. J.: Tachyphylaxis to some sympathomimetic amines in relation to monoamine oxydase. Brit. J. Pharmacol. **21**, 84–96 (1963)

Dews, P. B.: The measurement of the influence of drugs on voluntary activity in mice. Brit. J. Pharmacol. **8**, 46–48 (1953)

Ernst, A. M.: The role of biogenic amines in the extrapyramidal system. Acta physiol. pharmacol. neer. **15**, 141–154 (1969)

Fog, R.: Behavioral effects in rats of morphine and amphetamine and of a combination of the two drugs. Psychopharmacologia (Berl.) **16**, 305–312 (1970)

Goodman, L. S., Gilman, A.: The phyrmacological basis of therapeutics, Fourth Edition, pp. 293–296. New York: Macmillan 1970

Goldstein, S.: Biostatistics, p. 55. New York: Macmillan 1964

Harris, J. E., Baldessarini, R. J., Roth, R. H.: Amphetamine-induced inhibition of tyrosine hydroxylation in homogenates of rat corpus striatum. Neuropharmacology **14**, 457–471 (1975)

Harrison, J. W. E., Ambrus, C., Ambrus, J. L.: Tolerance of rats toward amphetamine and methamphetamine. J. Amer. pharm. Ass. **41**, 539–541 (1952)

Hitzemann, R. J., Loh, H. H., Craves, F. B., Domino, E. F.: The use of *d*-amphetamine pellet implantation as a model for *d*-amphetamine tolerance in the mouse. Psychopharmacologia (Berl.) **30**, 227–240 (1973)

Hulme, E. C., Hill, R., North, M., Kibby, M. R.: Effect of chronic administration of drugs which modify neurotransmitter reuptake, storage and turnover on levels of tyrosine and tryptophan hydroxylase in rat brain. Biochem. Pharmacol. **23**, 1393–1404 (1974)

Kuczenski, R., Segal, D. S.: Intrasynaptosomal conversion of tyrosine to dopamine as an index of brain catecholamine biosynthetic capacity. J. Neurochem. **22**, 1039–1044 (1974)

Lewander, T.: Urinary excretion and tissue levels of catecholamines during chronic amphetamine intoxication. Psychopharmacologia (Berl.) **13**, 394–407 (1968)

Magos, L.: Persistence of the effects of amphetamine on stereotyped activity in rats. Europ. J. Pharmacol. **6**, 200–201 (1969)

Moisset, B., Welch, B. H.: Effects of *d*-amphetamine upon open field behavior in two inbred strains of mice. Experientia (Basel) **29**, 625–626 (1973)

Mueller, R. A., Thoenen, H., Axlerod, J.: Increase in tyrosine hydroxylase activity after reserpine administration. J. Pharmacol. exp. Ther. **169**, 74–79 (1969)

Oliverio, A., Eleftheriou, B. E., Bailey, D. W.: Exploratory activity: Genetic analysis of its modification by scopolamine and amphetamine. Physiol. and Behav. **10**, 893–899 (1973)

Randrup, A., Munkvad, I.: Correlation between specific effects of amphetamines on the brain and on behavior. Ch. 2. In: Current concepts on amphetamine abuse, E. H. Ellinwood and S. Cohen, eds. Rockville, Maryland: N.I.H. 1972

Ranje, C., Ungerstedt, U.: Chronic amphetamine treatment: vast individual differences in performing a learned response. Europ. J. Pharmacol. **29**, 307–311 (1974)

Rech, R. H., Tilson, H. A., Marquis, W. J.: Adaptive changes in behavior after repeated administration of various psychomotor drugs. Advanc. Biochem. Psychopharmacol. **13**, 263–286 (1975)

Segal, D. S.: Behavioral and neurochemical correlates of repeated *d*-amphetamine administration. Advanc. Biochem. Psyhopharmacol. **13**, 247–262 (1975a)

Segal, D. S.: Behavioral characterization of *d*- and *l*-amphetamine: Neurochemical implications. Science **190**, 475–477 (1975b)

Shuster, L., Webster, G. W., Yu, G.: Increased running response to morphine in morphine-pretreated mice. J. Pharmacol. exp. Ther. **192**, 64–72 (1975)

Smith, C. B.: Enhancement by reserpine and α-methyl dopa of the effects of *d*-amphetamine upon locomotor activity of mice. J. Pharmacol. exp. Ther. **142**, 343–350 (1963)

Smith, C. B., Sheldon, M. I., Bednarczyk, J. H., Villarreal, J. E.: Morphine-induced increases in the incorporation of ^{14}C-tyrosine into ^{14}C-dopamine and ^{14}C-norepinephrine in the mouse brain: Antagonism by naloxone and tolerance. J. Pharmacol. exp. Ther. **180**, 547–557 (1972)

Stolk, J. M., Rech, R. H.: Enhanced stimulant effects of *d*-amphetamine in rats treated chronically with reserpine. J. Pharmacol. exp. Ther. **163**, 75–83 (1968)

Tormey, J., Lasagna, L.: Relation of thyroid function to acute and chronic effects of amphetamine in the rat. J. Pharmacol. exp. Ther. **128**, 201–207 (1960)

Tseng, L. F., Loh, H. H.: Significance of dopamine receptor activity in dl-p-methoxyamphetamine and d-amphetamine induced locomotor activity. J. Pharmacol. exp. Ther. **189**, 717–724 (1974)

van Rossum, J. M.: The significance of brain dopamine for psychomotor stimulation action. Biochem. Pharmacol. **12**, Suppl. 210 (1963)

Villarreal, J. E., Guzmann, M., Smith, C. B.: A comparison of the effects of d-amphetamine and morphine upon the locomotor activity of mice treated with drugs which alter brain catecholamine content. J. Pharmacol. exp. Ther. **187**, 1–7 (1973)

Wallach, M. B., Gerson, S.: Sensitization to amphetamines. Psychopharm. Bull. **7**, 30–31 (1971)

Welch, B. L., Welch, A. S.: Chronic social stimulation and tolerance to amphetamine: Interacting effects of amphetamine and natural nervous stimulation upon brain amines and behavior. Ch. 12. In: Current concepts on amphetamine abuse, E. H. Ellinwood and S. Cohen, eds. Rockville, Maryland: N.I.H. 1972

Received November 18, 1975 / February 27, 1976