Psychopharmacologia (Berl.) 30, 227—240 (1973)

The Use of *d*-Amphetamine Pellet Implantation as a Model for *d*-Amphetamine Tolerance in the Mouse*

R. J. Hitzemann**, H. H. Loh**, F. B. Craves, and E. F. Domino***
Wayne State University, School of Pharmacy, Detroit

Received September 18, 1972

Abstract. The use of *d*-amphetamine pellet implantation as a method for producing rapid central drug tolerance was investigated. Mice were implanted with *d*-amphetamine pellets containing 2 mg of drug and were challenged 24 h later, a time when no detectable drug was present, with various doses of *d*-amphetamine i.p. Implantation was found to potentiate the stereotyped activity and produced tolerance to the exploratory activity induced by *d*-amphetamine. Daily pellet implantation for 3 days was not found to produce tolerance to the stereotyped activities. Animals administered a single pellet showed no difference in the brain disposition or metabolism of a subsequent dose of ^{3}H-*d*-amphetamine. Twenty-four hour pellet implantation markedly increased the rate of conversion of ^{3}H-tyrosine to ^{3}H-dopamine (330%) and ^{3}H-norepinephrine (61%) in the subcortex. However, this effect was reversed by the administration of 10 mg/kg of *d*-amphetamine.

Key words: *d*-Amphetamine — Pellet — Implantation — Tolerance — Locomotor — Activity — Exploratory Activity — Catecholamines.

Although there are a number of reports concerning tolerance to the anorexic effect of *d*-amphetamine (Harrison *et al.*, 1953; Brodie *et al.*, 1969, 1970; Kasman and Unna, 1967), much less is known of tolerance mechanisms to the drug's central stimulant effect. Kalant (1966) has reported case histories where apparent tolerance developed to the stimulant action of *d*-amphetamine. However, this phenomena has not been shown to occur in laboratory animals. Recently, it was demonstrated that repeated injections of *d*-amphetamine to mice produced habituation, i.e., decreased responsiveness but not tolerance (a shift in the dose-response curve) to certain psychomotor behavior facilitated by *d*-amphetamine (Hitzemann and Loh, 1972). Somewhat similar results in attempting to produce tolerance to amphetamine's stimulant effects have been described by Lewander (1971).

The present experiments were undertaken to evaluate a new approach to produce tolerance to the centrally mediated locomotor behaviors

* This work represents a portion of the dissertation submitted to the College of Pharmacy, Wayne State University, by R. J. Hitzemann in partial fulfillment of the requirements for the degree of Master of Science.

** Present address: Department of Pharmacology, University of California Medical Center, San Francisco, California 94122, U.S.A.

*** Michigan Neuropsychopharmacology Research Program Lafayette Clinic, University of Michigan, Ann Arbor, Michigan, U.S.A.

increased by *d*-amphetamine. The technique tested involves the s.c. implantation of a small pellet containing *d*-amphetamine. At appropriate times after implantation, the effects of challenging doses of *d*-amphetamine on psychomotor behavior were evaluated. In addition, the possibility that the observed effects of pellet implantation on behavior were related to differences in drug disposition or in brain catecholamine metabolism was investigated.

Materials and Methods

Materials. *d*-Amphetamine pellets were kindly prepared by Dr. Robert Gibson, University of California School of Pharmacy, San Francisco, California. The formulation of the pellet was 148 mg microcrystalline cellulose, 2.0 mg *d*-amphetamine · HCl calculated as base, 0.75 mg fused silicone dioxide, and 0.75 mg calcium stearate. Placebo pellets were of similar composition but lacked *d*-amphetamine. ^{3}H-*d*-amphetamine (general label, specific activity 1 Ci/mmol) was obtained from New England Nuclear Corp., Boston, Mass. ^{3}H-tyrosine (2′,3′-side chain label, specific activity 11.4 Ci/mmol) was obtained from Amersham-Searle Corp., Chicago, Ill. *d*-Amphetamine and p-hydroxyamphetamine were a gift of Smith, Kline and French Laboratories, Philadelphia, Pa. Norephedrine and p-hydroxynorephedrine were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin. All other reagents were obtained from standard suppliers.

Experimental. Male CF_1 mice (Carworth Farms, Portage, Michigan) weighing 20—22 g were used one week after acclimatization. Lighting conditions were maintained exactly as they were with the supplier, i.e., lights on at 7 A.M. and ligths off at 7 P.M. Room temperature was maintained at 72° F. Mice to be implanted with pellets were placed in individual cages several hour before and maintained in isolated conditions throughout the experimental period. The pellets were implanted s.c. by first pinching the skin on the back of the mouse, cutting a small hole with scissors at the base of the fingers and then inserting the pellet. This innocuous procedure can be done easily in restrained animals after sufficient surgical skill has been acquired. No general anesthesia is necessary. Throughout the entire period food and water were provided *ad libitum*. All control animals were implanted with a placebo pellet.

Twenty-four hour after pellet implantation, animals were administered i.p. various doses of *d*-amphetamine sulfate (calculated as base) dissolved in distilled water. Motor activity was then assessed by one of the methods described below. In some experiments, 0.5 h after the injection of *d*-amphetamine, animals were administered 1 μCi/g body weight of ^{3}H-tyrosine. Animals were sacrificed 15 and 30 min later by decapitation and the brains removed. The cortex and cerebellum were dissected away and the remainder of the brain, the diencephalon-midbrain-brain stem (DMB), was frozen at −40° C until analysis, which was performed within 48 h. The DMB tissue obtained from two animals was pooled for each determination of endogenous and ^{3}H-tyrosine and catecholamines. In other experiments, the effects of pellet implantation *per se* on motor activity and brain amines were determined. For catecholamine and amphetamine determinations, animals were sacrificed every 2 h after pellet implantation, brains removed, and the whole brain was frozen until analysis. The pellet was carefully removed from the carcass to determine residual pellet amphetamine levels.

Motor Activity. Motor activity or various components of the activity repertoire were measured in two different ways. The first employed a simple open field apparatus (Newton and Levine, 1968; Gupta and Holland, 1972). The open field consisted of a regular cage (15×45 cm) with a grid of 20 squares placed in the bottom. The mice

were removed from the individual cages and placed in the middle of the grid. Motor activity was measured for 90 sec according to two criteria: One count was registered for every square completely crossed via forward locomotion and one count was registered for every rearing movement. Stereotyped behavior was measured during the same interval as the time the animal spent in highly repetitive licking, grooming and short distance (< one grid square) locomotive behaviors. The testing rooms were completely dark except for a 20 watt lamp positioned 2 feet above the open field. Animals tested in the daytime were not exposed to darkness for more than one-half hour before testing. The open field test was scored by a naive observer in a double-blind arrangement.

The second method of measuring motor activity utilized the Pharmacia M/P Fc motility meter. This meter consists of 40 photocells (8×5) covered by a sheet of plexiglass and arranged such that a standard clear plastic laboratory cage exactly fits the photocell design. The photocells were activated by standard laboratory lighting during the day and a 20 watt bulb during the night. One count is registered for every photocell contact broken.

Catecholamine and Tyrosine Determination. The procedures used to isolate endogenous and ^{3}H-DA, NE and tyrosine have been described in detail elsewhere (Hitzemann and Loh, 1972b). After homogenization in 0.4 N $HClO_4$ and centrifugation, the catechol and non-catechol metabolites in the supernatant were separated by batch alumina absorption (Anton and Sayre, 1962). The non-catechol fraction was analyzed for endogenous and ^{3}H-tyrosine as described by Weiner and Rabadjija (1968). Catechols were eluted from the alumina with 0.2 N acetic acid and a portion was used for the determination of endogenous DA and NE (Ansell and Beeson, (1968). To the remainder of the alumina elute was added 100 μg each of DA and NE. ^{3}H-DA and ^{3}H-NE were separated via column chromatography (Taylor and Laverty, 1969).

Amphetamine and Amphetamine Metabolites. Pellet and brain *d*-amphetamine levels were determined by the method of Axelrod (1954). ^{3}H-*d*-amphetamine and ^{3}H-*d*-amphetamine metabolites were determined essentially as described by Thoenen *et al.* (1966).

In experiments involving ^{3}H material, a zero time blank was prepared by adding a known amount of ^{3}H-substance to a brain homogenate and carrying through all procedures. Statistical analysis was performed using the student's *t*-test (two-tailed).

Results

Relationship of Motor Activity, Brain Catecholamine Levels and Amphetamine Levels after d-Amphetamine Pellet Implantation

The time course of changes in amine levels and motor activity after pellet[1] implantation is given in Fig. 1. Animals in these and subsequent experiments were implanted only at 9 A.M. in order to minimize the effects of circadian rhythms in catecholamine levels and motor activity. The data in Fig. 1 illustrate that the maximum depletion of brain DA (54%) and brain NE (71%) occurred 4 and 6 h after pellet implantation, respectively. The levels of both DA and NE returned to nearly normal values within 12 to 14 h, but complete recovery was not observed even by 24 h. Motor activity was increased for the first 2 to 4 h, gradually declined over the next 14 h, and finally a marked depression of activity

1 "Pellet" will refer only to the *d*-amphetamine pellet unless otherwise noted.

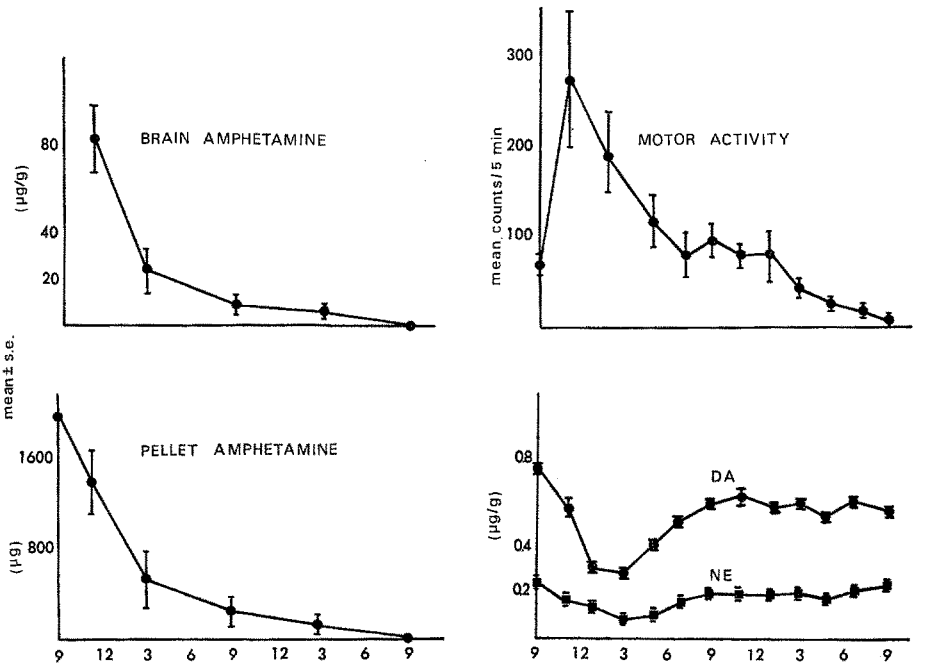

Fig. 1. Relation of brain *d*-amphetamine and catecholamine levels and motor activity as a function of time of pellet implantation. The graph shows the relation of cerebral *d*-amphetamine levels and disappearance of *d*-amphetamine from the pellet to motor activity and brain biogenic amines for 9 A.M. implanted animals. Amine levels are expressed in mean ± S.E. in μg/g except for the pellet which is in μg. Motor activity was measured using the Pharmacia M/P 40 Fc motility meter for a 5 min trial at various times after implantation. Activity data is expressed in mean counts ± S.E./5 min. $N = 10-12$ animals/group

was observed 24 h after implantation. Brain *d*-amphetamine levels rapidly increased with a level of 84 μg/g being measured 2 h after implantation. However, by 24 h after implantation, no *d*-amphetamine was detectable in the brain or in the pellet. Despite the initial high level of *d*-amphetamine, deaths were observed in only 2 to 5% of the animals implanted.

Tolerance to d-Amphetamine

Since at 24 h after pellet implantation, no *d*-amphetamine was detectable in the brain or the pellet, this time was used to assess whether or not tolerance had developed to the effects of a subsequent dose of *d*-amphetamine on activity. Two methods of measuring motor activity were employed. When the activity meter was used, the activity measured was not only exploratory activities but also stereotyped short distance locomotive behaviors located primarily at the ends of the activity arena. The latter behavior has been described in detail by other investigators

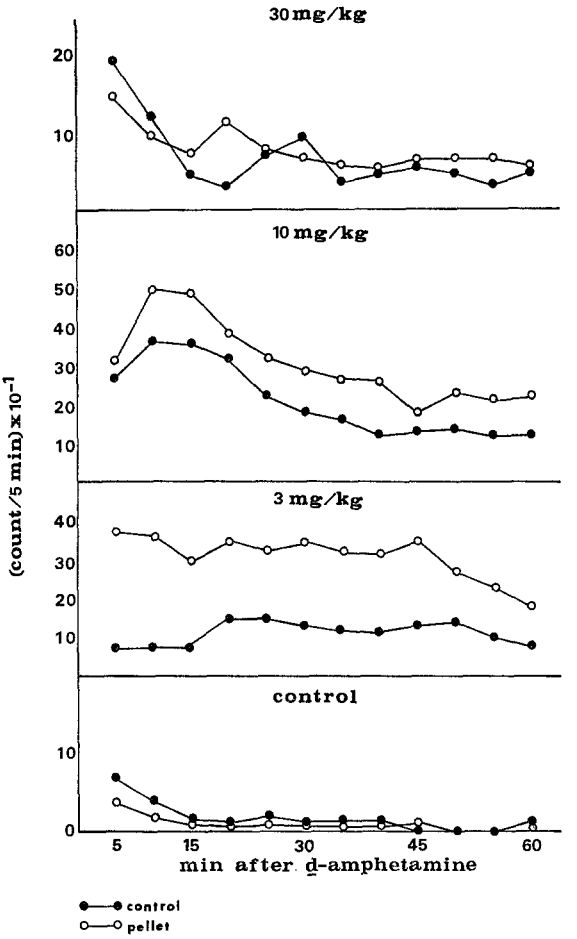

Fig. 2. Effect of *d*-amphetamine on activity in pellet implanted animals (photocell device). Twenty-four hour pellet implanted animals were administered various doses of *d*-amphetamine i.p. and motor activity was measured for 1 h using a Pharmacia M/P 40 Fe motility meter. Data is expressed as (counts/5 min) $\times 10^{-1}$. $N = 8-10$ animals/group

(Lat, 1965; Randrup and Munkvad, 1970). The second measure of activity employed the open field apparatus where the animals were scored for both ambulation and rearing movements.

Fig. 2 illustrates that the 24 h implantation of a single pellet potentiated the effects of challenging doses of 3 and 10 mg/kg but not 30 mg/kg of *d*-amphetamine on activity when the photocell device was used. This potentiation of effect seemed to exhibit an inverse dose-response relationship with 3 mg/kg showing greater potentiation than 10 mg/kg. In some experiments, *d*-amphetamine was administered to

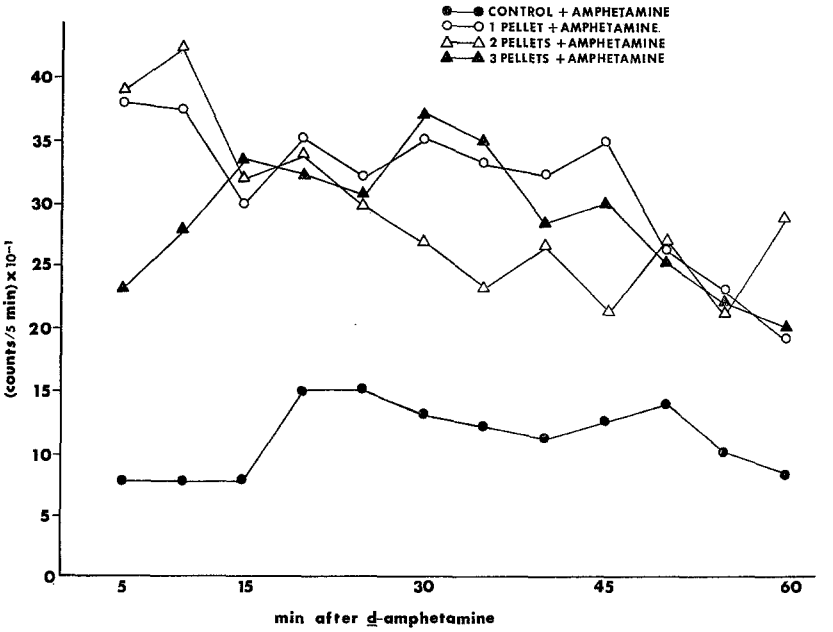

Fig. 3. Effect of multiple pellet implantation on the activity induced by a challenging dose of *d*-amphetamine. Animals were implanted with a single *d*-amphetamine pellet daily for 3 days. Twenty-four hours after each implantation the animals were administered a challenging dose of *d*-amphetamine (3 mg/kg) i.p. Control animals implanted with multiple placebo pellets showed little difference in activity after implantation and the data from this group was pooled. Activity was measured as described in the legend to Fig. 2. $N = 6-8$ animals/group

24 h pellet implanted animals in doses up to 80 mg/kg. While the animals were able to tolerate these high doses fairly well, a second peak of increased motor activity was not observed. Since the *d*-amphetamine disappeared from the pellet fairly rapidly, it was considered possible that only more prolonged *d*-amphetamine levels or repeated pellet implantation would produce tolerance. Fig. 3 demonstrates that when animals were implanted with additional pellets 24 h after the first and so on, tolerance still did not develop to a challenging dose of 3 mg/kg on activity nor was the potentiating effect reversed.

Tolerance was observed when the open field test was employed. Fig. 4 illustrates that the 24 h implantation of a single pellet produced a four-fold tolerance to the effects of subsequent doses of *d*-amphetamine on open field activity. The tolerant animals exhibited a bell-shaped dose-response curve as did the controls with the maximum effective dose for increasing activity being 40 mg/kg. Stereotyped behavior developed more rapidly in the pellet implanted animals with the total time spent

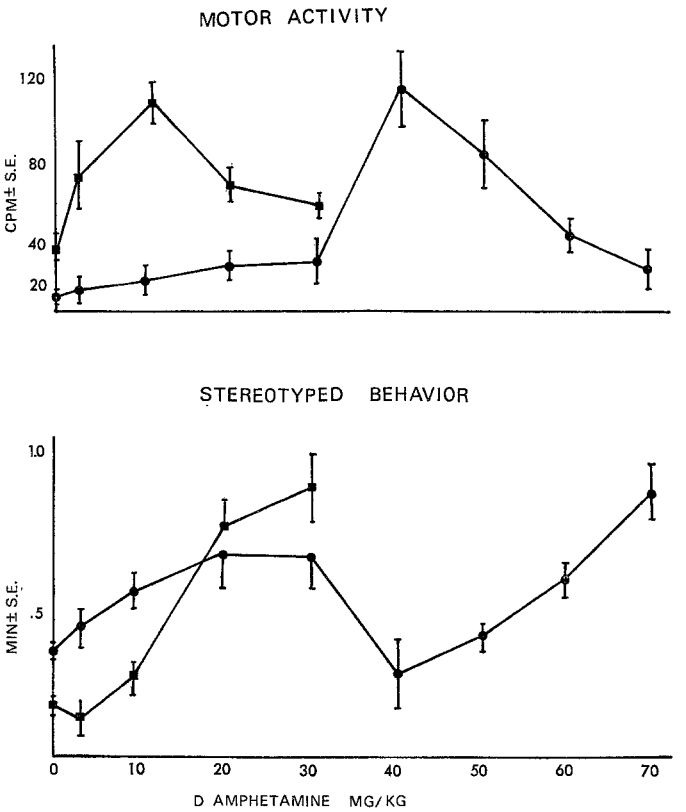

Fig. 4 Effect of *d*-amphetamine on open field activity in pellet implanted animals. Twenty-four hour pellet implanted animals were administered various doses of *d*-amphetamine i.p. One hour later, the animals were given a single 90 sec trial in the open field. The lower graph shows the effect of pellet implantation plus challenging doses of *d*-amphetamine on stereotyped behavior. Stereotyped behavior was scored as the time during the 90 sec trial the animals were engaged in highly repetitive activities. One count was accumulated for every square crossed and/or every rearing movement. Data is expressed as mean counts/min ± S.E. The upper graph shows the effect of this treatment on motor activity as measured in the open field. $N = 10-15$ animals/group

in these activities being significantly greater after challenge doses of 3 and 10 mg/kg of *d*-amphetamine. The maximum time spent in stereotyped behavior was, however, not significantly different between the pellet and control groups. Interestingly, a decrease in stereotyped behavior was observed at those doses of *d*-amphetamine which caused increased open field activity in the pellet implanted animals. Although the doses of *d*-amphetamine used in the open field experiments were fairly large, the mortality rate was less than 5% and overt signs of *d*-amphetamine toxicity were rarely observed.

Effect of Pellet Implantation on Drug Disposition and Metabolism

The effects of 24 h pellet implantation on the various activity measures (Figs. 2—4) might be the result of differences in brain drug levels or a shift in the metabolism of *d*-amphetamine from oxidative deamination to p-hydroxylation with the consequent accumulation of the false transmitter p-hydroxynorephedrine in the brain (Smith and Dring, 1970; Dring *et al.*, 1966; Goldstein and Anagnoste, 1965). In order to examine these possibilities, animals were administered 10 mg/kg of ^{3}H-*d*-amphetamine (specific activity 1 μCi/mg) i.p. 24 h after implantation. One hour later, the animals were sacrificed and the brains analyzed for ^{3}H-*d*-amphetamine and for ^{3}H-*d*-amphetamine metabolites. Cerebral ^{3}H-*d*-amphetamine levels were identical in control and pellet mice. No ^{3}H-norephedrine, ^{3}H-p-OH-amphetamine or ^{3}H-p-OH-norephedrine was found in the brains of either the pellet implanted or control groups.

Effect of Pellet Implantation on the Conversion of ^{3}H-Tyrosine to ^{3}H-Catecholamines

Several reports (Sulser *et al.*, 1968; Hanson, 1967; Weissman *et al.*, 1965) have suggested that *d*-amphetamine increases motor activity by potentiating the action of the newly-synthesized brain catecholamines primarily in subcortical structures (Fuxe and Ungerstedt, 1970). Therefore, it was of interest to examine the effects of pellet implantation and pellet implantation plus a challenging dose of *d*-amphetamine on brain catecholamine synthesis. Implanted animals were administered either saline or *d*-amphetamine (10 mg/kg) i.p. 0.5 h prior to the i.v. injection of 1 μCi/g body weight of ^{3}H-tyrosine (specific activity 11.4 Ci/mmol). Animals were sacrificed 15 and 30 min later and the levels of endogenous- and ^{3}H-tyrosine, NE and DA were determined in the diencephalon-midbrain-brain stem (DMB).

The administration of 10 mg/kg of *d*-amphetamine i.p. 24 h after pellet implantation produced a significant decrease in the levels of DA and NE in the DMB (Table 1). In contrast, the levels of DA and NE showed a small but non-significant increase in control animals.

Fig. 5 illustrates the effects of various drug treatments on tyrosine specific activity and the levels of catecholamine radioactivity. In control animals, *d*-amphetamine significantly increased the accumulation of ^{3}H-DA but had no effect on ^{3}H-NE levels. Twenty-four hour pellet implantation alone increased ^{3}H-DA levels at 15 min 40% ($p < 0.05$) and 130% ($p < 0.05$) at 30 min. In this group, ^{3}H-NE levels were significantly increased at 15 min only. These effects of pellet implantation on ^{3}H-catecholamine accumulation were partially reversed by the administration of *d*-amphetamine.

As noted in Fig. 5, tyrosine specific activity was significantly altered by 24 h pellet implantation. A useful calculation for incorporating the

Table 1. Effect of various drug treatments on the levels of endogenous DA, NE and tyrosine in the mouse brain diencephalon-midbrain and brain stem[a]

Group	Tyr	DA	NE
	(μg/g $\pm$ S.E.)		
Control	29.5 $\pm$ 1.0	0.63 $\pm$ 0.07	0.42 $\pm$ 0.03
d-Amphetamine 10 mg/kg	28.0 $\pm$ 1.5	0.75 $\pm$ 0.05	0.51 $\pm$ 0.06
Pellet	25.5 $\pm$ 1.4	0.72 $\pm$ 0.04	0.50 $\pm$ 0.04
Pellet + *d*-amphetamine 10 mg/kg	27.3 $\pm$ 1.1	0.55 $\pm$ 0.05*	0.38 $\pm$ 0.03*

[a] The schedule of drug administration is given in the legend to Fig. 5. Data is taken from 30 min after ^{3}H-tyrosine administration. All data expressed as mean μg/g $\pm$ S.E.

* Significantly different than the pellet control. $p < 0.05$.

Table 2. Effect of various drug treatments on the conversion of ^{3}H-tyrosine to ^{3}H-catecholamines

Group	Time	Conversion index[a]	
		DA	NE
		(mμmol/g/min $\pm$ S.E.)	
Control	15	0.08 $\pm$ 0.01	0.06 $\pm$ 0.01
	30	0.14 $\pm$ 0.01	0.13 $\pm$ 0.02
d-Amphetamine 10 mg/kg	15	0.12 $\pm$ 0.01	0.06 $\pm$ 0.01
	30	0.19 $\pm$ 0.02*	0.11 $\pm$ 0.01
Pellet	15	0.16 $\pm$ 0.03*	0.13 $\pm$ 0.02*
	30	0.61 $\pm$ 0.07*	0.21 $\pm$ 0.02*
Pellet + *d*-Amphetamine 10 mg/kg	15	0.15 $\pm$ 0.03	0.11 $\pm$ 0.02
	30	0.16 $\pm$ 0.03	0.12 $\pm$ 0.02

[a] The schedule of drug and ^{3}H-tyrosine administration is given in the legend to Fig. 5. The details of the calculation of the conversion index are given in the text.

* Significantly different than control, $p < 0.05$.

type of change into the evaluation of drug effects on ^{3}H-catecholamine (CA) accumulation is the conversion index (CI) (Cheney *et al.*, 1971) where

$$\text{CI} = \frac{\text{CA (dpm/g)}}{\text{tyrosine (dpm/mumol)} \times t}$$

and t is the time after ^{3}H-tyrosine administration. The CI values for the various drug treated groups are given in Table 2. The CI was increased 330% for ^{3}H-DA and 61% for ^{3}H-NE in the pellet group at 30 min after ^{3}H-tyrosine administration. *d*-Amphetamine antagonizes the increased CI values observed in the 24 h pellet animals but increases the CI for DA in control animals.

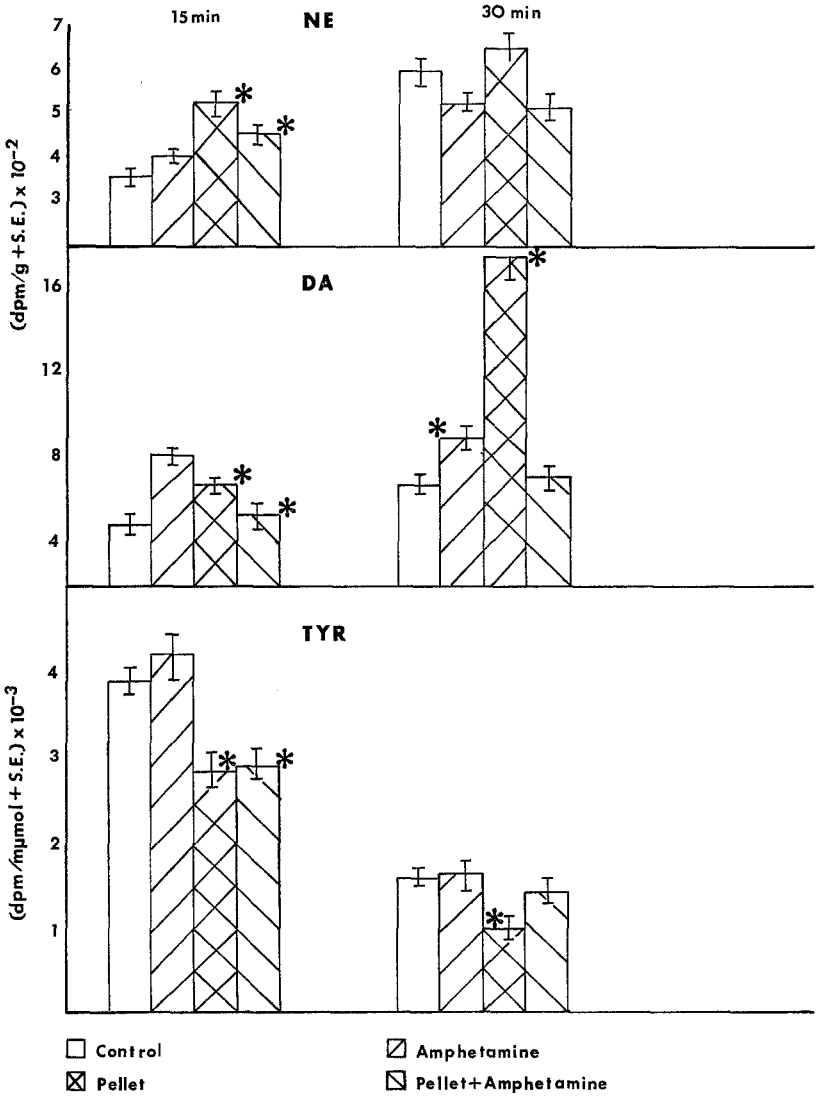

Fig. 5. Effect of pellet implantation plus *d*-amphetamine on DA, NE and Tyr levels in the subcortex. Twenty-four hour pellet implanted animals were administered 10 mg/kg *d*-amphetamine i.p. 0.5 h prior to the injection of 1 μC/g of ^{3}H-tyrosine i.v. Animals were sacrificed 15 and 30 min later and the levels of endogenous- and ^{3}H-Tyr, DA and NE were determined in the diencephalon-midbrain-brain stem. ^{3}H-DA and ^{3}H-NE levels are given as (dpm/g ± S.E.)$\times 10^{-2}$. Tyrosine data is presented as specific activity (dpm/mμmol ± S.E.)$\times 10^{-3}$. * Significantly different than control; $p < 0.05$; $N = 5-6$ determinations/group

Discussion

Of the two methods used to measure activity, only the open field test showed that tolerance had developed after pellet implantation to the psychomotor stimulant effects of *d*-amphetamine. The open field differs from the mechanical measure of activity employed in the present study in that primarily the effect of *d*-amphetamine on exploratory behaviors was measured (Holland *et al.*, 1966). As confirmed in these experiments, others have shown that acute *d*-amphetamine treatment increases the performance of rats subjected to the open field by causing a generalized increase in exploratory locomotor behavior with facilitation of the intermittent exploratory rearing response (Gupta *et al.*, 1971; Gupta and Holland, 1972; Holland *et al.*, 1966). Furthermore, the arrangement of the open field is such that the irrelevant stereotyped locomotor (Lat, 1965; Randrup and Munkvad, 1970) and non-locomotor (Randrup and Munkvad, 1970) behaviors often observed after *d*-amphetamine administration are not scored as part of the ambulation and rearing movements.

Unlike the open field test, the measurement of activity with the photocell device cannot accurately discern between exploratory and stereotyped activities. In some experiments, we attempted to circumvent this problem by reducing the number of photocells (see Methods). However, stereotyped behaviors occurring in the region of even a single medial row of photocells interferred with the accurate measurement of the animals' exploratory activities. The potentiation of activity measured by the counting device when the pellet implanted animals are challenged with *d*-amphetamine is, nonetheless, an interesting phenomenon. These data qualitatively confirm the finding that the dose-response curve for stereotyped behavior is shifted to the left after pellet implantation.

The biochemical mechanism underlying the pellet-induced shift in the open field dose-response curve for exploratory activity is presently unclear. The increase in activity observed in the pellet implanted animals after challenging doses of 40 to 50 mg/kg of *d*-amphetamine is not sensitive to reserpine and/or α-methyltyrosine pretreatment (unpublished observations) suggesting that *d*-amphetamine is no longer acting indirectly via the brain catecholamines. Perhaps a direct interaction between the drug and noradrenergic and/or dopaminergic receptors should be considered.

Of more pertinent pharmacologic interest, at least in terms of the doses employed, is the mechanism by which pellet implantation potentiates the ability of low doses of *d*-amphetamine to produce stereotyped behavior. Fuxe and Ungerstedt (1970) have presented evidence that exploratory activity is related to an increase in noradrenergic receptor activity in the hypothalamus and limbic system while the stereotyped behaviors can be attributed to an activation of brain dopaminergic

neurons in the neostriatum (Randrup and Munkvad, 1970). From these data, the authors have postulated that the ratio of DA to NE receptor activity will determine the nature of the animal's response to *d*-amphetamine. Using this model, one would conclude that in pellet implanted animals receiving challenging low doses of *d*-amphetamine the ratio of DA to NE receptor activity increases.

To test this hypothesis, the effect of pellet implantation plus challenges of *d*-amphetamine on the cerebral conversion of ^{3}H-tyrosine to ^{3}H-catecholamines and on changes in endogenous catecholamine levels was examined. Costa and Groppetti (1970) and Persson (1969) have shown that acute *d*-amphetamine treatment increases the conversion of a pulse label ^{3}H-tyrosine to ^{3}H-DA but not ^{3}H-NE in the rat brain. The present study confirms that this effect also occurs in the mouse brain. This increased conversion to ^{3}H-DA is thought to represent an increase in brain DA turnover (Costa and Groppetti, 1970). Pellet implantation apparently effected an accumulation of *d*-amphetamine's actions on brain DA. In 24 h implanted animals, the CI for DA was increased 330% while the CI for NE was increased only 61%. If an increase in conversion truly is representative of an increase in turnover and, consequently, an increase in transmitter release, these data would suggest that the ratio of DA/NE receptor activity is increased after pellet implantation. These data may help to explain the increase in stereotyped behavior in pellet implanted animals receiving no additional *d*-amphetamine (see Fig. 4).

In 24 h pellet implanted animals administered 10 mg/kg of *d*-amphetamine, no change from control was observed in the conversion of ^{3}H-tyrosine to ^{3}H-catecholamines. The mechanism by which a single injection of *d*-amphetamine can reverse the increased conversion observed in the pellet controls is thought to be related to the rapid decrease in endogenous catecholamine levels (Table 1). This attenuated mobilization of catecholamines from intraneuronal storage vesicles could reverse the increase in ^{3}H-catecholamine synthesis by increasing the levels of catecholamines in the nerve-ending cytosol which competes with the reduced pteridine co-factor for the oxidized tyrosine hydroxylase (Ikeda *et al.*, 1966; Kopin *et al.*, 1968; Weiner *et al.*, 1972).

These data suggest that in pellet implanted animals receiving a challenge dose of *d*-amphetamine, the ratio of DA to NE synthesis is similar to control and would argue against the hypothesis that the ratio of DA to NE receptor activity is increased in this group. In what manner the increase in the release of both endogenous DA and NE may contribute to the increase in stereotyped over exploratory behaviors remains to be determined.

The present study demonstrates that pellet implantation may be useful in rapidly producing tolerance to at least one *d*-amphetamine

induced behavior, namely exploratory behavior, but is not useful when other behaviors such as stereotyped activity are examined. The importance of using at least more than one method to assess tolerance development was also emphasized. Furthermore, the present study suggests that chronic *d*-amphetamine treatment potentiates the ability of subsequent "pharmacological" doses of the drug to produce stereotyped behavior.

This work was supported in part by USPHS Grants MH-11846 (EFD) and MH-20559 (HHL).

References

Anton, A. H., Sayre, D. F.: A study of factors affecting the aluminium oxide-trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. exp. Ther. **138**, 360—375 (1962).

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).

Axelrod, J.: Studies on sympathetic amines. II. The biotransformation and physiological disposition of *d*-amphetamine, *d*-P-hydroxyamphetamine and *d*-methamphetamine. J. Pharmacol. exp. Ther. **110**, 315—326 (1954).

Brodie, B. B., Cho, A. K., Stefano, F. J. E., Gessa, G. L.: On mechanisms of noradrenaline release by amphetamine and tyramine and tolerance to their effects. In: Advances in biochemical psychopharmacology (E. Costa and P. Greengard, Eds.), pp. 219—238. New York: Raven Press 1969.

Cheney, D. L., Goldstein, A., Algeri, S., Costa, E.: Narcotic tolerance and dependence: Lack of relationship with serotonin turnover in the brain. Science **171**, 1169—1170 (1971).

Costa, E., Groppetti, A.: Biosynthesis and storage of catecholamines in tissues of rats injected with various doses of *d*-amphetamine. In: Amphetamines and related compounds (E. Costa and S. Garattini, Eds.), pp. 231—256. New York: Raven Press 1970.

Dring, L. G., Smith, R. L., Williams, R. T.: The fate of amphetamine in man and other mammals. J. Pharm. Pharmacol. **18**, 402—405 (1966).

Fuxe, K., Ungerstedt, U.: Histochemical, biochemical and functional studies on central monoamine neurons after acute and chronic amphetamine administration. In: Amphetamines and related compounds (E. Costa and S. Garattini, Eds.), pp. 257—288. New York: Raven Press 1970.

Goldstein, M., Anagnoste, B.: The conversion *in vivo* of *d*-amphetamine (+)p-hydroxynorephedrine. Biochim biophys. Acta (Amst.) **107**, 166 (1965).

Gupta, B. D., Dandiya, P. C., Gupta, M. L., Gabba, M. K.: An examination of the effect of central nervous system stimulant and antidepressant drug on open-field performance in rats. Europ. J. Pharmacol. **13**, 341—346 (1971).

Gupta, B. C., Holland, H. C.: Emotion as a determinant of the effects of drugs and their combination on different components of behavior in rats. Neuropharmacology **11**, 31—38 (1972).

Hanson, L. C. F.: Evidence that the central action of (+)-amphetamine is mediated via catecholamines. Psychopharmacologia (Berl.) **10**, 289—296 (1967).

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

Hitzemann, R., Loh, H.: Tolerance to amphetamine-induced psychomotor stimulation in mice. Proc. West. Pharmacol. Soc. **15**, 110—113 (1972).

Hitzemann, R., Loh, H.: Effect of p-hydroxynorephedrine on brain catecholamine synthesis. J. Pharmacol. (1972).

Holland, H. C., Gupta, B. D., Weldon, E.: A note on rearing and on environmental constraint. Activ. nerv. sup. (Praha) **8**, 140—144 (1966).

Hollinger, M.: Effect of reserpine, α-methyl-p-tyrosine, p-chlorophenyl-alanine and pargyline on levorphanol-induced running activity in mice. Arch. int. Pharmacodyn. **179**, 419—424 (1969).

Ikeda, M., Fahien, L. A., Udenfriend, S.: A kinetic study of bovine adrenal tyrosine hydroxylase. J. biol. Chem. **241**, 4452—4456 (1966).

Kalant, O. J.: The amphetamines. University of Toronto Press, Toronto 1966.

Kasman, M. E., Unna, K. R.: Effects of the chronic administration of amphetamine and other stimulants on behavior. Clin. Pharmacol. Ther. **9**, 240—254 (1967).

Kopin, I. J., Breese, G. R., Krauss, K. R., Weise, V. K.: Selective release of newly synthesized norepinephrine from the cat spleen during sympathetic nerve stimulation. J. Pharmacol. exp. Ther. **161**, 271—278 (1968).

Lat, J.: The spontaneous exploratory reactions as a tool for psychopharmacologic studies. Proc. 2nd Intern. Pharmacol. Meeting (Czechoslovak Medical Press, Praha), pp. 47—66 (1965).

Lewander, T.: A mechanism for the development of tolerance to amphetamine in rats. Psychopharmacologia (Berl.) **21**, 17—31 (1971).

Newton, G., Levine, S.: Early experience and behavior, p. 152. Springfield, Ill.: Ch. C. Thomas 1968.

Persson, T.: Drug induced changes in ^{3}H-catecholamine accumulation after ^{3}H-tyrosine. Acta pharmacol. (Kbh.) **28**, 378—390 (1970).

Randrup, A., Munkvad, I.: Biochemical, anatomical and psychological investigations of stereotyped behavior induced by amphetamines. In: Amphetamines and related compounds (E. Costa and S. Garattini, Eds.), pp. 695—715. New York: Raven Press 1970.

Smith, R. L., Dring, R. G.: Patterns of metabolism of β-phenylisopropylamines in man and other species. In: Amphetamines and related compounds (E. Costa and S. Garattini, Eds.), pp. 121—140. New York: Raven Press 1970.

Sulser, F., Owens, M. L., Norvich, M. R., Dingell, J. V.: The relative role of storage and synthesis of brain norepinephrine in the psychomotor stimulation evoked by amphetamine or by desipramine and tetrabenazine. Psychopharmacologia (Berl.) **12**, 322—332 (1968).

Taylor, K. M., Laverty, R.: The metabolism of tritiated dopamine in regions of the rat brain *in vivo*. I. The separation of catecholamines and their metabolites. J. Neurochem. **16**, 1361—1366 (1969).

Thoenen, H., Hurlimann, K. F., Gey, K. F., Haefly, W.: Liberation of p-hydroxynorephedrine from cat spleen by sympathetic nerve stimulation after pretreatment with amphetamine. Life Sci. **5**, 1715—1722 (1966).

Weiner, N., Rabadjija, M.: The effect of nerve stimulation on the synthesis and metabolism of norepinephrine in the isolated guinea-pig hypogastric nerve—vas deferens preparation. J. Pharmacol. exp. Ther. **160**, 61—71 (1968).

Weissman, A., Koe, B. K., Tenen, S. S.: Antiamphetamine effects following inhibition of tyrosine hydroxylase. J. Pharmacol. exp. Ther. **151**, 329—352 (1966).

Horare H. Loh, Ph.D.
Department of Pharmacology
School of Medicine
University of California
Medical Center
San Francisco, California 94122, U.S.A.