Pharmacology Biochemistry & Behavior, Vol. 12, pp. 937–941. Printed in the U.S.A.

Increased Self-Administration of d-Amphetamine After Destruction of 5-Hydroxytryptaminergic Neurons[1]

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Received 9 January 1980

LYNESS, W. H., N. M. FRIEDLE AND K. E. MOORE. *Increased self-administration of d-amphetamine after destruction of 5-hydroxytryptaminergic neurons.* PHARMAC. BIOCHEM. BEHAV. 12(6) 937–941, 1980.—Rats will initiate self-administration of d-amphetamine and achieve a stable injection rate within 7–10 days. Animals injected intraventricularly with 5,7-dihydroxytryptamine, which selectively destroys 5-hydroxytryptamine-containing neurons, consistently self-injected larger amounts of d-amphetamine from the first day of training, but the acquisition of a stable rate of drug self-administration was not altered. Bilateral microinjection of 5,7-dihydroxytryptamine into nucleus accumbens failed to alter either the acquisition of d-amphetamine self-administration or the maintenance of a stable rate of injection.

5-Hydroxytryptamine Nucleus accumbens d-Amphetamine Self-administration

THE integrity of dopaminergic nerve terminals in nucleus accumbens has been shown to be of import in both the acquisition and maintenance of self-administration of indirect-acting dopamine (DA) agonists like cocaine [12] and d-amphetamine [7]. Behavioral evidence suggests that 5-hydroxytryptamine (5-HT)-containing neurons may modify some aspects of dopaminergic function in the nucleus accumbens. First, bilateral microinjections of 5-HT into this nucleus can abolish the hyperactivity observed after systemic d-amphetamine administration [11]. Second, destruction of 5-HT neurons within the nucleus accumbens with microinjection of 5,7-dihydroxytryptamine (5,7-DHT) exaggerates the hyperactivity observed after systemic d-amphetamine [2]. Thus, it appears that 5-HT neurons within this nucleus may inhibit DA release. If this is the case then removal of the inhibitory 5-HT neurons should facilitate DA release by an indirect agonist, such as d-amphetamine. As a result the rate of responding for self-injection of d-amphetamine in 5,7-DHT-lesioned rats should decrease since the increased DA being released would act as a stronger reinforcement. It is well known that the rate of self-administration of d-amphetamine decreases as the amount of the drug per injection increases [10]. Unexpectedly, the data presented herein reveals that the self-administration of d-amphetamine is increased in animals pretreated with an injection of 5,7-DHT into the lateral cerebral ventricles. On the other hand, destruction of 5-HT neurons in nucleus accumbens does not influence d-amphetamine self-administration.

METHOD

Male Sprague Dawley rats (250–275 g) were purchased from Spartan Farms, Haslett, MI, pretreated with 25 mg/kg desmethylimipramine (1 hr before 5,7-DHT injections) and anesthetized with 2 ml/kg Equithesin. Rats were placed in a stereotaxic apparatus and, in the case of intracerebroventricular (ICV) injections, 200 μg 5,7-DHT (free base) in 10 μl was injected over a 2 min interval using the coordinates of Pellegrino and Cushman [9]; A 0.0, L+1.5, V−3.2. Bilateral nucleus accumbens 5,7-DHT injections (4 μg/2 μl/2 min) used the coordinates A+2.4, L±1.7, V−7.2.

While anesthetized, rats treated with 5,7-DHT into the lateral ventricle or nucleus accumbens, were surgically implanted with a chronic silastic jugular cannula exiting subcutaneously on the back of the animal as described earlier [16]. Control animals were likewise pretreated with desmethylimipramine, anesthetized and stereotaxically in jected with the appropriate volume of vehicle (0.1 mg/ml ascorbic acid in 0.9% saline). After a 14 day recovery period self-administration studies began.

Animals were placed in self-administration cages (18×20×26 cm) equipped with an operant lever activating a pneumatic device which delivered predetermined volumes of drug or saline as described earlier [7,17]. Initial training sessions were 8 hr (0900–1700 hr) or 16 hr (1700–0900 hr) and consisted of 1 lever press for 1 drug injection (FR-1). Each injection delivered 0.125 mg/kg d-amphetamine sulfate (Sigma Chemical Co.) in sterile saline. Volume of injection was 0.2 ml/kg. Rats were given 3 free injections at the beginning of each of the first 3 training days to initiate self-administration. Both schedules induce stable self-administration rates in comparable time. Furthermore, rats trained on a 16 hr schedule readily continue to self-inject when placed on the 8 hr regimen. The converse is also true.

[1]This research was supported by USPHS grant MH-13174.

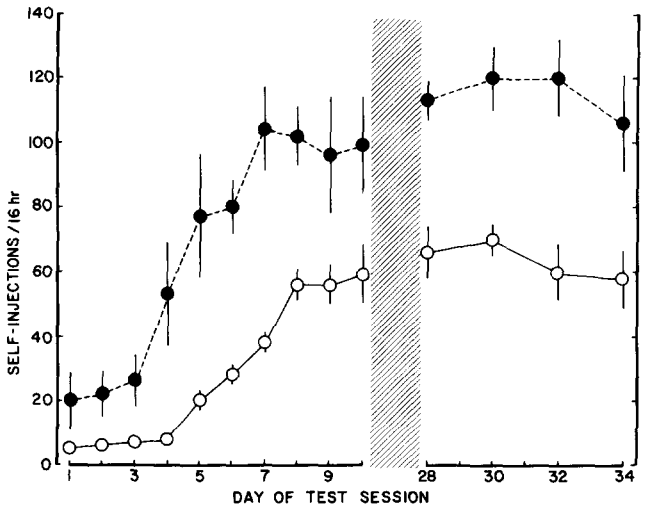

FIG. 1. Effects of ICV 5,7-DHT on self-administration of d-amphetamine. Self-administration studies were started 14 days after ICV injection of 5,7-DHT (●) or vehicle (○). Rats were permitted to self-administer d-amphetamine for 16 hr each day. On Day 28 rats were allowed access to the apparatus only on alternate days. Each value represents the mean and vertical lines ±1 SE as determined from 5 animals in each group.

The total amounts of d-amphetamine self-injected were also similar. Animals on the 16 hr schedule do not consistently self-inject d-amphetamine for the entire test session. Recordings of the sequence of lever presses (Rustrak, Gulton Ind., Manchester, NH) in a 16 hr test session show a near cessation of lever pressing from approximately 0200 hr until 0800 hr in the majority of animals. Periods of self-imposed abstinence have been reported by others [10].

The influence of both ICV 5,7-DHT and microinjection of the neurotoxin into nucleus accumbens was examined using both training regimens. The results were identical.

Biochemical consequences of the ICV 5,7-DHT injections were determined 14 days after termination of self-administration studies. Rats were sacrificed, the brains removed and dissected on ice using the method of Glowinski and Iversen [6]. Tissues were weighed and homogenized in 75% ethanol, centrifuged at 1000×g in a refrigerated centrifuge and the supernatant passed over a 1 cm Bio-Rex 70 weak cation exchange column. The amines (5-HT, norepinephrine (NE) and DA) were eluted with 0.5 N acetic acid [1]. The concentration of 5-HT was determined using the fluorometric method of Curzon and Green [5]. The fluorometric assay described by Chang [3] was used to ascertain the concentrations of DA and NE.

Confirmation of bilateral nucleus accumbens lesions were made by sectioning the brain on a freezing microtome and removing tissue punches of nucleus accumbens and the adjacent striatum. Tissues were homogenized in 0.2 N perchloric acid and DA and NE concentrations determined by radioenzymatic assay [14]. High performance liquid chromatography with electrochemical detection was used to determine 5-HT concentrations in the tissues [8]

RESULTS

Influence of 5-HT Depletion on Acquisition and Maintenance of d-Amphetamine Self-Administration

When d-amphetamine-naive rats which received ICV 5,7-DHT were allowed access to the self-administration apparatus, they self-injected d-amphetamine at a higher rate than controls. The number of lever presses in 5,7-DHT-pretreated animals was significantly ($p<0.05$) greater from Day 1 on (Fig. 1). However, the time required for a stable rate of response to become established (acquisition) appeared to be the same as controls (7–8 days). Therefore, the animals receiving 5,7-DHT did not appear to acquire a stable

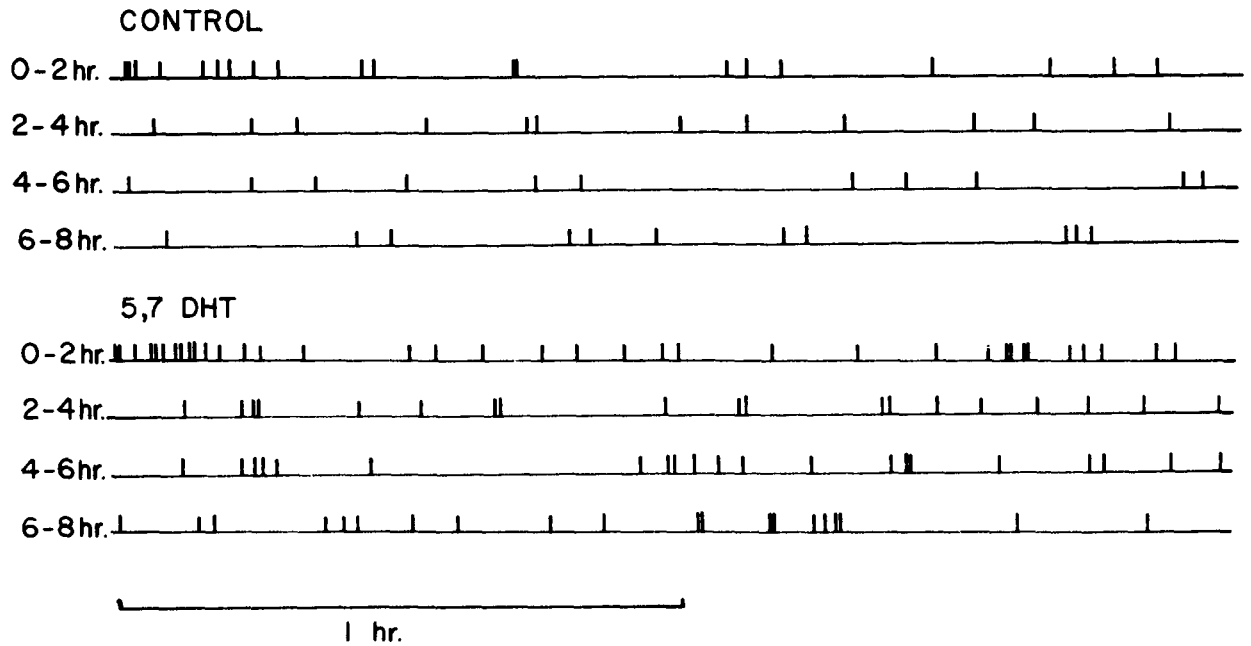

FIG. 2. Event recordings of d-amphetamine self-injection in individual rats. Animals began self-administration of 0.125 mg/kg/injection on the left side of the panel and continued for 8 hrs. Pretreatments consisted of ICV vehicle (control) or ICV 5,7-DHT. Recordings were made on Day 30 of training. Each vertical line represents 1 self-injection.

TABLE 1
REGIONAL ANALYSIS OF BRAIN AMINES AFTER INTRAVENTRICULAR 5,7-DHT

	DA (μg/g)		5-HT (μg/g)		NE (μg/g)	
Area	Vehicle	5,7-DHT	Vehicle	5,7-DHT	Vehicle	5,7-DHT
Hypothalamus	—	—	1.09 ± 0.30	0.44 ± 0.10*	1.70 ± 0.14	1.78 ± 0.22
Hippocampus	—	—	0.34 ± 0.02	0.08 ± 0.02*	—	—
Striatum	7.12 ± 0.37	6.89 ± 0.41	0.59 ± 0.05	0.06 ± 0.05*	—	—
Cortex	—	—	0.24 ± 0.01	0.04 ± 0.01*	0.29 ± 0.02	0.32 ± 0.06

Rats were sacrificed 14 days after termination of self-administration studies or 48 days after the ICV injection of 5,7-DHT. Values represent the mean ± SE of 7 animals.

*Values in 5,7-DHT-pretreated rats which were significantly different from vehicle-treated rats ($p<0.01$; Student's t-test).

rate of self-administration faster, although the amounts of drug administered were markedly greater. Once stable response rates were established (maintenance) the 5,7-DHT pretreated animals still self-injected larger amounts of d-amphetamine. Identical results were obtained in rats subjected to the 8 hr regimen, i.e., the 5,7-DHT treated rats self-administered significantly more d-amphetamine from Day 1 of training on (data not shown).

Normally, trained animals allowed to the self-administer d-amphetamine maintain a body weight of approximately 80% of that of their litter mates not exposed to the drug. Animals pretreated with 5,7-DHT self-administering d-amphetamine maintain body weights approximately 70% of those of comparably injected rats not given access to d-amphetamine.

On Days 10–26 of the self-administration study (shaded area, Fig. 1), the injection rates of the 5,7-DHT treated rats began to decline toward control values. On changing the self-administration testing to alternate days (Day 28–34), the 5-HT depleted animals again resumed their increased rate of d-amphetamine injection. The body weight of rats in both groups, but especially those of the 5-HT depleted animals, increased as a result. This may indicate that 5,7-DHT treated rats were becoming debilitated by the higher daily intake of d-amphetamine.

There have been conflicting reports that 5-HT depletion either enhances or reduces the stereotypic behavior mediated by high doses of d-amphetamine [4, 13, 18, 19]. An examination of event recordings from both controls and rats treated with ICV 5,7-DHT revealed that the increased self-injection rate in the latter group was not due to stereotypic response patterns (i.e., rapid succession of lever presses) but rather to a steady increase in the frequency of self-injection. Although Fig. 2 illustrates the responses of only one animal from each group, no examples of stereotypic responding were found in any animals.

Examination of the biochemical data (Table 1) revealed that ICV 5,7-DHT was specific in depleting 5-HT with regional DA and NE concentrations remaining unchanged.

Effects of 5-HT Depletion on the Mortality of Rats Trained to Self-Administer d-Amphetamine

Attempts to facilitate self-administration of d-amphetamine using a higher dose of drug per injection (0.25 mg/kg) resulted in an increase in mortality of 5,7-DHT pretreated rats (5 deaths out of 8 rats tested). All deaths occurred within the first 4 days of training. The times of death were not determined so it is uncertain whether death was the result of stereotypic lever pressing. No vehicle-injected rats self-injected lethal amounts of d-amphetamine.

When the drug concentration was decreased to 0.125 mg/kg/injection, mortality was decreased but not absent in the 5,7-DHT treated rats. Three out of 12 5,7-DHT-treated animals self-administered lethal amounts of d-amphetamine on Days 2, 7, and 9 of training. Again, none of the vehicle-injected rats self-administered fatal amounts of d-amphetamine.

Influence of Microinjection of 5,7-DHT into Nucleus Accumbens on the Acquisition and Maintenance of d-Amphetamine Self-Administration

Bilateral microinjections of 5,7-DHT into nucleus accumbens reduced the 5-HT content of this nucleus to 16% of vehicle-injected controls and did not affect DA or NE concentrations (Table 2). Both lesioned and control rats acquired d-amphetamine self-administration at identical rates and maintained equivalent numbers of self-injections per test session (Figure 3). These results suggest that the 5-HT influence, whose absence enhances d-amphetamine self-administration, lies not in nucleus accumbens but elsewhere in the central nervous system.

DISCUSSION

While the participation of DA and NE neurons in the self-administration of DA agonists has been extensively studied, examination of the influence of 5-HT neurons on this behavior are lacking. Available behavioral evidence would suggest an inhibitory role of 5-HT on DA release [2, 4, 11]. Removal of this inhibitory influence, e.g., after ICV 5,7-DHT, might be expected to reduce the rate of self-administration of d-amphetamine by virtue of the facilitation of DA release. However, this was not the case. Paradoxically, animals pretreated with ICV 5,7-DHT and given access to 0.25 mg/kg d-amphetamine per injection exhibited a propensity towards self-injecting lethal amounts of the drug. When the dose was reduced to 0.125 mg/kg/injection this overdose tendency was reduced but the animals consistently self-injected greater quantities of drug than controls. The frequency of self-injection in the 5,7-DHT-pretreated rats was increased over controls but recordings made of the test

TABLE 2

EFFECTS OF BILATERAL MICROINJECTION OF 5,7-DHT INTO NUCLEUS ACCUMBENS ON LOCAL AMINE CONCENTRATIONS

	5-HT (ng/mg protein)		DA (ng/mg protein)		NE (ng/mg protein)	
Area	Vehicle	5,7-DHT	Vehicle	5,7-DHT	Vehicle	5,7-DHT
Nucleus Accumbens	11.5 ± 0.5	1.9 ± 0.3*	84.1 ± 6.0	86.1 ± 4.3	10.2 ± 0.5	10.7 ± 0.6
Striatum	7.8 ± 0.8	7.2 ± 1.0	121.0 ± 11.0	104.0 ± 9.2	8.1 ± 0.6	7.8 ± 0.4

Rats were sacrificed 14 days after termination of self-administration studies or 31 days after injection of 5,7-DHT. Values represent the mean ± SE of 7 animals.

*Values in 5,7-DHT pretreated rats which were significantly different from vehicle treated rats ($p<0.01$; Student's t-test).

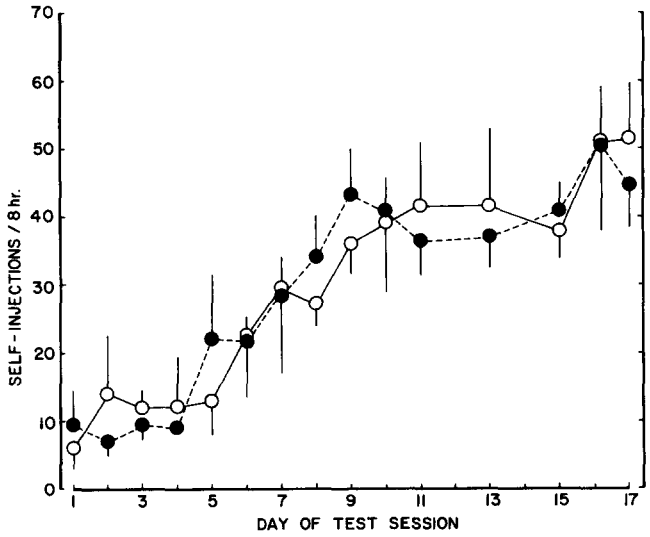

FIG. 3. Effects of 5,7-DHT-induced lesions of 5-HT nerve terminals in nucleus accumbens on the acquisition of d-amphetamine self-administration. Self-administration studies were started 14 days after the bilateral injection of vehicle (○, N=3) or 5,7-DHT (●, N=4) into nucleus accumbens. Rats were permitted to self-administer d-amphetamine for 8 hr each day. Each value represents the mean and vertical lines ±1 SE.

sessions revealed a well-regulated sequence of lever pressing with stereotypic lever pressing the exception rather than the rule. Preliminary experiments employing pretreatment with 1 mg/kg metergoline, a 5-HT antagonist, have also yielded an increase in d-amphetamine self-injection in trained animals. However, event recordings of these test sessions indicate frequent bursts of stereotypic lever pressing which might indicate that metergoline also influences other, possibly catecholaminergic, neuronal systems.

It is uncertain whether the d-amphetamine dose per injection would influence the acquisition of a stable rate of self-administration. However, the number of self-injections during the maintenance phase of training can be predictably and inversely increased by reducing the amount of drug per injection. For example, reducing the concentration of drug per injection by one-half will roughly double the number of self-injections [10]. It would be tempting to speculate that ICV 5,7-DHT pretreatment produced a decreased reinforcing effect of d-amphetamine; hence, an increased rate of lever pressing.

A second possibility is that 5-HT neurons may be involved in an aversive or negative reinforcing system. This hypothesis was suggested earlier when it was found that 6-hydroxydopamine injection into nucleus accumbens abolished self-administration in trained animals. The classic pattern of extinction expected was absent and inconsistent with the concept that DA neurons in the accumbens were essential for only the reinforcing properites of d-amphetamine. The removal of these neurons should have immediately led to an increase in lever pressing followed by extinction; events which were not observed [7]. Wise *et al.* [15] have demonstrated that d-amphetamine can exhibit both positive and negative reinforcing properties. In control animals self-administration would, therefore, be regulated by a balance of the rewarding and aversive properties of the drug. If 5-HT neurons subserve negative reinforcing (aversive) properties of d-amphetamine, the removal of these neurons could conceivably alter the frequency of d-amphetamine self-injection. In any case, the locus of 5-HT influence does not appear to be located in nucleus accumbens since specific lesions of this area failed to influence either the acquisition or maintenance of d-amphetamine self-administration.

ACKNOWLEDGEMENT

The authors acknowledge the help of Dr. J. R. Weeks, The Upjohn Co., Kalamazoo, MI in helping set up the self-administration apparatus.

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