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Destruction of Dopaminergic Nerve Terminals in Nucleus Accumbens: Effect on d-Amphetamine Self-Administration[1]

W. H. LYNESS, N. M. FRIEDLE AND K. E. MOORE

Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824

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LYNESS, W. H., N. M. FRIEDLE AND K. E. MOORE. *Destruction of dopaminergic nerve terminals in nucleus accumbens: Effect on d-amphetamine self-administration.* PHARMAC. BIOCHEM. BEHAV. **11**(5) 553–556, 1979.—Control rats initiate self-administration of d-amphetamine and achieve stable injection rates within 7–10 days. Rats in which dopamine nerve terminals in nucleus accumbens were destroyed by bilateral microinjections of 6-hydroxydopamine (6-OHDA) did not initiate self-administration of d-amphetamine when tested for as long as 19 days. In rats previously trained to self-administer d-amphetamine, 6-OHDA injections into nucleus accumbens abolished d-amphetamine self-administration. These results suggest that dopaminergic nerve terminals in nucleus accumbens are necessary for both the acquisition and maintenance of d-amphetamine self-administration.

Nucleus accumbens d-Amphetamine Dopaminergic nerve terminals

RATS can be trained to lever press for intravenous self-injections of d-amphetamine [4,12]. A number of pharmacological and surgical manipulations suggest that dopaminergic neurons play a predominant role in mediating this behavior (see review, [11]). For example, dopamine (DA) agonists, apomorphine and piribedil, attenuate the lever pressing response to d-amphetamine [14], and pretreatment with haloperidol, a DA antagonist, blocks the rewarding effects of amphetamine in lever pressing rats and leads to an initial increase in the rate of self-administration followed by extinction [1]. Similarly, both pimozide and (+) butaclamol (DA antagonists), initially increase the rate of self-injection; an effect comparable to replacement of d-amphetamine with saline [13].

The interest of this laboratory has been to identify dopaminergic neuronal systems participating in amphetamine self-administration. Earlier works suggest that the nucleus accumbens participates in the hyperactivity syndrome produced by d-amphetamine. Injections of the drug directly into nucleus accumbens produce hyperactivity [5]. Furthermore, microinjection of DA (10 μg) into nucleus accumbens produces an increase in motor activity of nialamide-pretreated rats comparable to 1.0 mg/kg d-amphetamine [5,6]. Local administration of 5-hydroxytryptamine or norepinephrine, on the contrary, does not increase motor activity but decreases it [6]. Moreover, bilateral destruction of DA containing neurons within the nucleus accumbens with 6-hydroxydopamine (6-OHDA) abolishes the hyperactivity seen after systemic d-amphetamine [2].

Recent evidence also suggests that DA terminals in nucleus accumbens are of some importance in the self-administration of cocaine, which, like amphetamine, is an indirect-acting DA agonist. Depletion of DA in the nucleus accumbens with 6-OHDA reduces the self-administration of cocaine in lever pressing rats, but does not affect self-administration of apomorphine, a direct acting DA agonist [7].

METHOD

Male Sprague-Dawley rats (250–300 g) were purchased from Spartan Farms, Haslett, MI, anesthetized with 3.0 ml/kg Equithesin and surgically implanted with a chronic silastic jugular catheter exiting subcutaneously on the back of the animal as described earlier [9]. After a 5-day recovery period, self-administration training began.

Animals were placed in self-administration cages (18×20×26 cm) equipped with 2 levers, one activating a pneumatic device which delivered predetermined volumes of drug or saline [9,10]. The other lever was a dummy apparatus. The number of presses on the dummy lever were not recorded in these experiments. The operant lever (Micro Switch, Freeport, IL [BRS/LVE 121-03]) is situated at the rear of the cage 3 cm above the cage floor and 4 cm from the left wall. The dummy lever is on the right rear of the cage symmetrically placed. Levers are separated by 5.5 cm from each other and shielded against accidental presses as described earlier [10]. Initial daily training sessions was 16 hr (1700–0900 hr) regimens 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. The volume of

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injection was 0.2 ml/kg. Rats were given 3 free presses of d-amphetamine on each of the first 3 training days to help initiate self-injection. Upon stabilization of the number of drug injections in each animal (ca. 7–10 days) rats were switched to an 8 hr schedule (0900–1700 hr) and again, time allowed for a stable baseline response to be established, i.e., less than 20% variation in the number of self-injections over a 3-day period. Each animal, however, maintains its own characteristic amount of drug injected. For example, the mean number of self-injections per test session ranged from 49 in one rat to 152 in another. During all test sessions animals were allowed free access to food and water.

DA nerve terminals in nucleus accumbens were destroyed by acute bilateral microinjections of 6-OHDA (8 μg free base in 2 μl). Rats were pretreated with 25 mg/kg desipramine 1 hr before injection and were anesthetized with 2.0 ml/kg Equithesin. Control animals were likewise pretreated with desipramine and Equithesin and then injected with vehicle (0.1 mg/ml ascrobic acid in 0.9% NaCl). Stereotaxic coordinates used were those of Pellegrino and Cushman [3]: A 3.4, L ± 1.7, V - 7.2. Injections were made through 30 gauge stainless steel cannulae via a motor driven 10 μl Hamilton syringe at a rate of 1 μl/min. All animals were allowed 14 days for recovery before self-administration studies were initiated or resumed.

To ascertain the extent of the 6-OHDA lesions in nucleus accumbens and surrounding tissue, rats were given bilateral injections of either vehicle or 6-OHDA and sacrificed 14 days later. Brains were immediately frozen on dry ice and later sectioned on a freezing microtome. Tissue punches from nucleus accumbens and adjacent striatum were homogenized in 0.2 N perchloric acid and the concentration of DA determined by radioenzymatic assay [8].

RESULTS

Acquisition of d-Amphetamine Self-Administration

When naive rats, which had received bilateral injections of vehicle into nucleus accumbens, were placed in the self-administration apparatus and given access to 0.125 mg/kg d-amphetamine (FR-1 schedule), a stable rate of self-administration of the drug was achieved within 7–10 days (Fig. 1). These rats usually maintained a constant hourly injection rate frequented by pauses of 10–15 min duration as has been reported by others [4]. Naive rats pretreated with 6-OHDA in the nucleus accumbens did not acquire self-administration behavior even when allowed access to the drug for up to 19 days. The number of self-injections were comparable to those seen in untrained rats receiving non-rewarding saline injections which will not initiate lever pressing. Furthermore, when non-cannulated rats were placed in the cages for one week they made an average of 2–3 presses/session, with a maximum of 6. This low level of self-injection activity probably results from accidental contact with the lever.

Maintenance of d-Amphetamine Self-Administration

When test sessions were abruptly stopped for 14 days, resumption of the test schedule in trained rats resulted in an immediate reinitiation of lever pressing behavior (Fig. 2). The number of d-amphetamine self-injections were comparable to those seen before the 14 day rest period. Bilateral injections of 6-OHDA into nucleus accumbens followed by a 14 day rest period resulted in cessation of d-amphetamine self-administration.

When d-amphetamine in the syringe is replaced with saline, rats trained to self-administer d-amphetamine temporarily increase their rate of responding prior to extinction

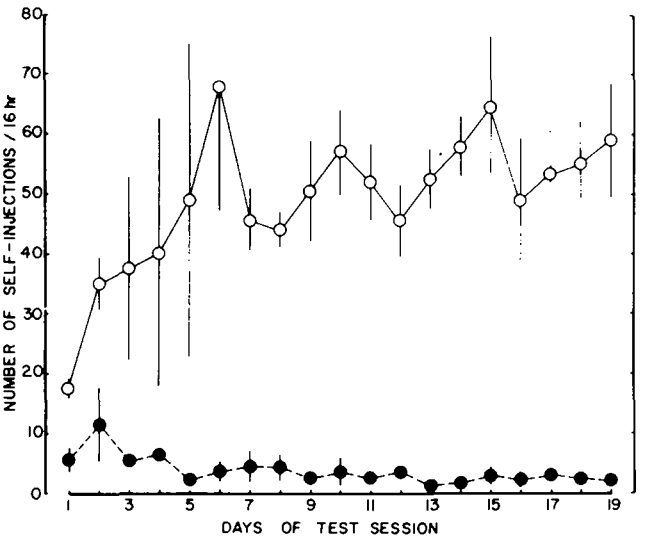

FIG. 1. Effect of 6-OHDA-induced lesions of DA nerve terminals in nucleus accumbens on the acquisition of d-amphetamine self-administration. Self-administration studies were started 14 days after the bilateral injection of vehicle (○) or 6-OHDA (●) into the nucleus accumbens of rats (4 rats in each group). Each symbol represents the mean and vertical lines ± 1 SEM of the number of self-injections of d-amphetamine (0.125 mg/kg/injection; FR-1) made during 19 consecutive daily 16 hr sessions. Where no vertical line is shown the SEM is less than the radius of the symbol.

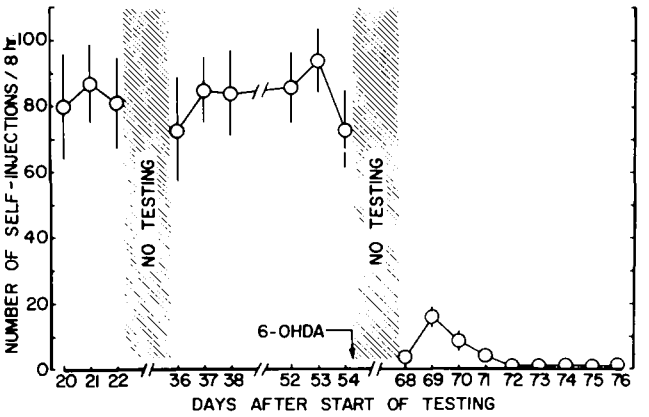

FIG. 2. Effects of 14 day abstinence and the injection of 6-OHDA into nucleus accumbens on self-administration of d-amphetamine. Rats were permitted to self-administer d-amphetamine (0.125 mg/kg/injection; FR-1) for 16 hr each day for 10 days and then switched to a 8 hr daily schedule from Day 11 onward. A 14-day period of no access to the self-administration cages was imposed from Day 23–35. 6-OHDA was injected on Day 55 and self-administration tests were resumed on Day 68. Each symbol represents the mean and vertical lines ± 1 SEM as determined from 5 animals.

[13]. In the present experiment the responses of trained rats without 6-OHDA lesions (Group 1, Table 1) increased by 400–600% on the day of saline replacement. When untrained rats, which were given 6-OHDA into nucleus accumbens, were allowed access to the self-administration apparatus they did not learn to lever press for the drug and, as expected, when saline replaced d-amphetamine no increased responding was observed (Group 2, Table 1). In rats previously trained to self-administer d-amphetamine and then lesioned with 6-OHDA in nucleus accumbens, saline replacement also failed to increase lever pressing (Group 3, Table 1).

Dopamine Concentration in Nucleus Accumbens and Striatum

Bilateral microinjections of 6-OHDA into nucleus accumbens reduced the DA concentration in this nucleus to approximately 5% of control (Table 2). The effect of the 6-OHDA was essentially restricted to this nucleus since the DA concentration in the adjacent striatum was not altered significantly (89% of control).

TABLE 1

SUBSTITUTION OF SALINE FOR d-AMPHETAMINE ON THE NUMBER OF SELF-ADMINISTRATION RESPONSES

Animal Groups (N)	Number of Self-injections 1) d-Amphetamine	2) Saline
Group 1-vehicle+training (6)	65.6 ± 8.7	249.7 ± 23.7
Group 2-6-OHDA+training (5)	4.5 ± 2.6	4.3 ± 2.6
Group 3-training–6-OHDA (4)	1.2 ± 0.9	2.8 ± 1.2

Values represent the mean ± SEM of the number of self-injections during a test period for: (1) d-amphetamine on the day prior to the saline substitution, and for (2) saline on the following day when saline was substituted for d-amphetamine. Animals that received microinjections of vehicle (Group 1) or 6-OHDA (Group 2) into nucleus accumbens prior to daily training sessions are the same animals as those depicted in Fig. 1; saline substitution was made on Day 21. Animals that were trained to self-administer d-amphetamine prior to receiving an injection of 6-OHDA into nucleus accumbens (Group 3) are the same animals as those depicted in Fig. 2; saline substitution was made on Day 77.

TABLE 2

DOPAMINE CONCENTRATIONS IN NUCLEUS ACCUMBENS AND STRIATUM FOLLOWING MICROINJECTIONS OF 6-OHDA INTO NUCLEUS ACCUMBENS

Treatment (N)	Dopamine (ng/mg protein) Nucleus Accumbens	Striatum
Vehicle (6)	80.7 ± 2.3	113.9 ± 5.1
6-OHDA (5)	3.7 ± 1.7*	101.5 ± 3.3

All rats were pretreated with desipramine (25 μg/kg) 1 hr before either vehicle or 6-OHDA (8 μg/2 μl) were injected into nucleus accumbens. Animals were sacrificed and DA concentration determined 14 days later. Values represent mean ± SEM.

*Significantly different from vehicle treatment ($p<0.01$).

DISCUSSION

The hyperactivity observed after systemic d-amphetamine injections seems, in part, to be related to the action of the drug on dopaminergic neurons in nucleus accumbens [2,5]. It appears that the integrity of DA neurons in this area are also responsible for both the acquisition and maintenance of d-amphetamine self-administration.

DA has been implicated as the neurotransmitter involved in the reward achieved by self-stimulation of d-amphetamine since DA agonists decrease and DA antagonists enhance the self-injection rate [1,13]. Only recently has one specific area of the central nervous system been implicated in self-administration behavior. Lesions of the nucleus accumbens with 6-OHDA sharply reduced the self-injection of cocaine. As would be predicted these same lesions did not alter the self-injection of apomorphine which presumably acts directly on post-synaptic receptors [7].

In the present experiments, 6-OHDA-induced depletion of DA in nucleus accumbens abolished the acquisition of d-amphetamine self-administration. This might be explained by several hypotheses. First, the learning process may have been disrupted by the depletion of DA in nucleus accumbens. This would seem unlikely since we have shown that 6-OHDA lesions of nucleus accumbens did not alter food reinforced operant responding (FR-40; Commissaris, Lyness, Rech and Moore, unpublished observations). Roberts *et al.* [7] also demonstrated that there was no long-lasting disruption of operant responding with this lesion. A second hypothesis is that DA neurons in nucleus accumbens subserve the activation of a reward system. There is much data to support this idea [1, 11, 12, 13, 14]. Removal of dopaminergic nerve terminals would result in the failure of indirect agonists like d-amphetamine to produce a positive (rewarding) stimulus. Therefore, one would expect poor acquisition of self-administration behavior. Finally, a third hypothesis might adequately explain our results, i.e., d-amphetamine has both rewarding (DA receptor stimulation) and aversive properties. On removal of DA neurons innervating nucleus accumbens only the aversive effects of d-amphetamine self-injection would be experienced by the animal. Hence, rats would quickly learn to avoid the self-injection of the drug. Wise *et al.* [12] have demonstrated that under different test conditions d-amphetamine can be both a positive reinforcement or an aversive drug to rats.

Adherence to the third hypothesis would explain why untrained rats administered 6-OHDA never acquire self-administration behavior. The destruction of dopaminergic nerve terminals results in loss of the positive reinforcing properties of d-amphetamine and an unmasking of aversive effects. This hypothesis also explains why previously trained rats administered 6-OHDA into nucleus accumbens never re-acquire their self-injection behavior. If 6-OHDA had only removed the positive reinforcing properties of d-amphetamine, the previously trained rats would be expected to show elevated response rates when given access to the self-administration apparatus. A similar effect occurs when rats are pretreated with the DA antagonist haloperidol [1] or replacement of d-amphetamine with saline [13]. The low response rates observed after 6-OHDA lesions of nucleus accumbens might then be explained by the animals quickly learning that d-amphetamine now causes undesirable effects or a non-rewarding stimulus.

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