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Unilateral Mesolimbicocortical Dopamine Denervation Decreases Locomotion in the Open Field and After Amphetamine[1]

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JESTE, D. V. AND G. P. SMITH. *Unilateral mesolimbicocortical dopamine denervation decreases locomotion in the open field and after amphetamine.* PHARMAC. BIOCHEM. BEHAV. 12(3) 453–457, 1980.—Unilateral denervation of the mesolimbicocortical dopaminergic (DA) system was produced by microinjections of 6-hydroxydopamine (6-OHDA) into the right or left anterolateral hypothalamus after desmethylimipramine pretreatment. Unilateral DA denervation was confirmed by the marked loss of fluorescent fibers in mesolimbic DA terminal fields in brains processed for catecholamine histofluorescence with the glyoxylic acid-paraformaldehyde method. Such unilateral DA denervation significantly decreased the locomotor exploration of an open field and the locomotor response to d-amphetamine (1.5 mg/kg). These results demonstrate that unilateral mesolimbococortical DA denervation is sufficient, and that bilateral denervation of a single mesolimbic terminal field such as the n. accumbens is not necessary, to produce a decrease in the locomotor response to amphetamine or a decrease of locomotion in the open field. The results are consistent with the hypothesis that the mesolimbicocortical DA system energizes behavior, but does not direct it.

d-Amphetamine Open field Locomotion 6-Hydroxydopamine Dopamine
Mesolimbic dopamine system Arousal Exploratory behavior

THE mesolimbicocortical dopaminergic system has been implicated in self-stimulation [4,26], peripheral self-administration of stimulants [26], the locomotor response to low doses of amphetamine [3, 6, 12, 13, 16, 17, 18], the drug induced circling observed after unilateral lesions of the nigrostriatal dopaminergic tract [16] and locomotor and investigatory exploration [7,8]. The major effect of pharmacological blockade, or electrolytic or 6-hydroxydopamine (6-OHDA) lesion of the mesolimbicocortical dopaminergic (DA) system is to decrease the behavioral responses to drug and environmental stimuli without altering their form or causing competing behaviors to appear [6, 10, 11, 13, 17, 18]. This common effect of pharmacological blockade and lesions suggests that a function of the mesolimbicocortical DA system is to increase the frequency of behavioral responses, but not to determine their form. In its simplest form the hypothesis is that the mesolimbicocortical DA system energizes behavior, but does not direct it [4,16].

To test this hypothesis, we measured the responses of rats with unilateral 6-OHDA lesions of the mesolimbicocortical DA system to the novel stimuli of an open field and to a low dose of amphetamine. We reasoned that if the hypothesis is correct, unilaterally lesioned rats should decrease the frequency of their responses to the open field and to amphetamine without changing the form of their responses. If the unilaterally lesioned rats showed new forms of behavior, such as side preference or circling, this would be evidence that was not consistent with the hypothesis. Our results are consistent with the hypothesis.

METHOD

Animals

Male Sprague-Dawley rats (Hormone Assay, Chicago, IL) were housed individually at a temperature of about 24°C and with 12 hr of light (7 a.m. to 7 p.m.). Each animal was handled daily for the week prior to surgery. At the time of surgery rats weighed 200–280 g.

Surgery

To inhibit the uptake of 6-OHDA into noradrenergic axons and terminals at the injection site [18], all animals were pretreated with desmethylimipramine HCl (DMI, 25 mg/kg, IP, a gift of USV Pharmaceutical Corp., Tuckahoe,

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NY) 30–45 min prior to the injection of 6-OHDA or vehicle. The rats were anesthetized with barbiturate and chloral hydrate ("Chloropent," 2 ml/kg, IP, Fort Dodge, IA) and mounted in a stereotaxic apparatus (Baltimore Instrument Co.). Anesthesia was supplemented by inhalation of methoxyflurane ("Metofane," Pitman-Moore, Inc., Washington Crossing, NJ) as needed.

For 6-OHDA lesions, the injection fluid consisted of 6-OHDA hydrobromide, 6.5 μg/μl of free base (Regis Chemical Co., Chicago, IL) and l-ascorbic acid, 0.4 μg/μl dissolved in cold distilled water just before use. A total volume of 3 μl was administered. To control for the nonspecific damage of microinjection, other rats received isovolumetric microinjections of vehicle solution (l-ascorbic acid in cold distilled water, 0.4 μg/μl). Injection solutions were loaded into a Hamilton syringe (50 μl) equipped with an automatic Hamilton dispenser. The Hamilton syringe was connected by polyethylene tubing (PE 10) to a 30 g stainless steel cannula. The cannula was then lowered to the desired coordinates: A7.0, RL2.0 and H8.0 mm down from the dural surface according to the atlas of deGroot [5]; then 3 μl of 6-OHDA or vehicle solution were injected over 60 sec. The cannula was left in place for an additional 30 sec before it was withdrawn. The volume injected was measured by following the movement of a bubble through the tubing during microinjection [23]. In an attempt to detect unilateral asymmetry in mesolimbic DA function that might be analogous to the unilateral asymmetry in striatal function reported by Rothman and Glick [19], four 6-OHDA and 4 vehicle rats were injected in the right anterolateral hypothalamus and four 6-OHDA and 3 vehicle rats were injected in the left anterolateral hypothalamus.

The total dose of 6-OHDA injected at the anterolateral hypothalamic site was 19.5 μg. Such a dose produces large non-specific lesions after microinjections into the substantia nigra [1], but it produces only slight nonspecific damage (largest diameter<300 μm) at this anterolateral hypothalamic site that is not greater than that produced by identical injections of vehicle [7, 20, 22]. Despite equivalent nonspecific damage, anterolateral hypothalamic 6-OHDA injections produce much more extensive loss of catecholamine (CA) histofluorescent fibers as determined by the glyoxylic acid method [7]. The same relationship between specific CA damage and nonspecific damage holds when DMI is given prior to 6-OHDA injections except that the CA damage is predominantly dopaminergic (Fink and Smith, in preparation and reference [8]).

Behavioral Tests

Behavioral testing began 15 days after surgery. Rats were tested first in an open field (OF) and then their locomotor response to d-amphetamine was measured.

Open field test. The test measures the locomotor response to a novel open area. Eight 6-OHDA rats and 7 vehicle rats were observed in the OF for 5-min on 3 successive days. Three successive tests were administered because Whimbey and Denneberg [25] reported that locomotion in the first or second test is related to both emotional and exploratory tendencies, but locomotion in subsequent tests indexes exploration more specifically.

The OF was a 76×93 cm rectangular area enclosed with brown cardboard walls 60 cm high. The floor was covered with white contact paper and divided into 8 cm squares by brown lines. The apparatus was located in a quiet room and was indirectly lighted by a 60 W incandescent bulb. Immediately prior to a test, the OF was wiped with a soap solution and dried. An animal was removed from its home cage, transferred to a wire holding cage for 30 sec and then placed in one corner of the OF with its head toward the corner. During each OF test, the following behavioral measures were recorded by an observer seated in a chair adjacent to the OF:

Locomotion. The number of squares traversed by both front and rear paws.

Rears. The number of vertical extensions of head, body and forelimbs, either free standing or against a wall, excluding those vertical extensions associated with grooming.

Defecation. Presence or absence of defecation.

Amphetamine test. Food and water were removed one hour prior to each test. Rats were placed in activity cages (21×44×20 cm high) made of Plexiglas. The cages had wire mesh floors and were separated by white, wooden walls. These walls prevented visual interaction between rats in adjacent cages during the tests.

After a 40 min adaptation period in the activity cages, rats received intraperitoneal injections of d-amphetamine sulfate (1.5 mg salt/kg) dissolved in 0.5 ml of 0.9% saline or 0.5 ml of 0.9% saline alone. For the next 2 hr, activity was measured by counting the number of interruptions of a photocell beam (Lafayette Instrument Co., Lafayette, ID) mounted 3.2 cm above the floor and halfway along the length of the test cage. In addition, the rat was observed for 60 sec every 10 min and the occurrence of the following behavioral items were noted:

Circling. Complete 360° turn of the animal's body; direction, approximate diameter, and frequency of circling were noted.

Stereotypy. Rapid and repetitive movements such as sniffing or bobbing of the head.

Locomotion. Forward movement of the animal in the cage.

At least 96 hr elapsed between amphetamine and saline tests. All rats were tested with amphetamine first and then with saline.

Statistical Procedures

Data were analyzed by nonparametric statistical methods [21]. The Mann-Whitney U test, two-tailed, was used to compare 2 independent groups (e.g., 6-OHDA and vehicle group) on any given measure. Friedman's Two-Way Analysis of Variance was used to compare repeated measures in the same group.

Histochemical and Neuropathological Procedures

Brains from 1 vehicle and four 6-OHDA injected rats were processed for CA histofluorescence with the glyoxylic acid method of Lindvall and Björklund [14] as adapted for cryostat sections by Bloom and Battenberg [2]. Animals were pretreated with nialamide (300 mg/kg IP, 1–3 hr before sacrifice), anesthetized, immersed in an ice bath (−3 to 0°C) and perfused transcardially with 400 ml of cold 2% glyoxylic acid monohydrate and 0.5% paraformaldehyde in phosphate buffered mammalian Ringer's (pH 7.4). The brain was rapidly dissected from the cranium, the desired region cut into 2 or 4 mm blocks, frozen onto cryostat chunks on dry ice and sectioned on the cryostat at 16–24 μm at −12°C. Sections were thawed onto chilled glass slides, immersed in glyoxylic acid (2% in phosphate buffered Ringer's) for 11 min, dried for 3–7 min under a warm air stream (45°C) and heated at 100°C for 3–10 min in a closed glass container. Sections were mounted in paraffin oil, stored at room temperature and examined by fluorescence microscopy using a

Zeiss fluorescence microscope equipped with a 200 W HBO lamp, a Schott BG 12 primary lamp filter, and Zeiss 41 and 47 barrier filters. The extent of CA fluorescence loss was determined by comparing the CA fluorescence of the vehicle injected or 6-OHDA injected side with the uninjected side. The severity of denervation was rated on a + and 3+ scale. When denervation is rated 3+ under our conditions there are few or no fluorescent fibers visible [6,7] and tyrosine hydroxylase activity is equal to or less than 12% of control activity [7].

RESULTS

Open Field Performance

There was no significant difference between the open field performance of rats injected with 6-OHDA in the right anterolateral hypothalamus and of rats injected with 6-OHDA in the left anterolateral hypothalamus. Therefore, the results were combined for analysis.

The 6-OHDA rats moved across significantly fewer squares on all test days than vehicle rats (Fig. 1). The 6-OHDA rats also reared significantly less than vehicle rats on the third test day. The percentage of rats that defecated in the OF was smaller in the 6-OHDA group than in the vehicle group on all three test days, but this difference was not significant (Table 1).

TABLE 1
REARING AND DEFECATION IN OPEN FIELD

	6-OHDA (n=8)	Vehicle (n=7)
	Rearing	
Day 1	10 (1–18)	11 (6–27)
Day 2	9 (0–20)	13 (4–20)
Day 3	7* (0–14)	14 (11–21)
	Percent defecating	
Day 1	25	43
Day 2	25	71
Day 3	13	71

Numbers are median values with their respective ranges in parentheses. *p=0.004, 6-OHDA vs vehicle.

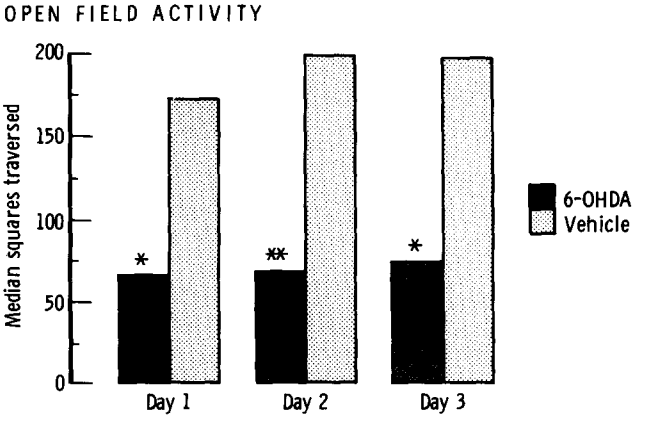

FIG. 1. Data are from eight 6-OHDA and 7 vehicle rats tested in the open field for 5 min on 3 successive days. *p<0.05, **p<0.02, Mann-Whitney U test.

Response to Amphetamine

Since there was no significant difference in response to amphetamine related to the side of 6-OHDA injections, the results were combined for analysis. 6-OHDA rats had significantly less photocell counts after amphetamine than vehicle rats (Fig. 2). The decreased locomotion was not the result of inhibition of locomotion by competing movements because 6-OHDA rats did not show circling or stereotypies after amphetamine.

Although 6-OHDA rats ran less than vehicle rats after amphetamine, both 6-OHDA and vehicle rats had significantly more photocell counts after amphetamine than after saline (Table 2; p<0.02 for both groups, Friedman's Two-Way Analysis of Variance). The similarity of response to saline (Table 2) and of the pre-injection activity (Fig. 2) supports the significance of the difference in response to amphetamine (Fig. 2).

Histochemical Neuropathology

All four 6-OHDA rats (2 lesioned on the right side and 2 lesioned on the left side) had severe (3+) denervation of n. accumbens, olfactory tubercle and anteromedioventral striatum. There was also moderate (2+) to severe (3+) loss of CA terminals in the lateral septum and dorsal bed nucleus of the stria terminalis in all 4 cases, and in the diagonal band and ventral bed nucleus of the stria terminalis in 2 of the 4 cases. One rat had slight (1+) loss of DA fluorescence in the lateral part of the anterior striatum and another rat had moderate (2+) loss of DA fluorescence more posteriorly in the caudate. Their responses to the OF and amphetamine were apparently not different from the responses of the other 2 rats that did not have this additional striatal damage.

The vehicle injected rat had no apparent loss of CA terminals except at the injection site. The nonspecific damage at the injection site appeared equal after vehicle and 6-OHDA injections and the greatest diameter was not more than 300 μm. Thus, the relative specificity of the 6-OHDA injections in this experiment was very similar or identical to what we have observed in previous experiments over the last 5 years [7, 20, 22].

The DMI pretreatment apparently protected noradrenergic (NA) fibers from 6-OHDA damage under our conditions because fibers with the characteristic morphology of NA fibers were observed very close to the injection site and in the parietal cortex in equal density on both sides. This also confirms our previous experience (Fink and Smith in preparation and reference [8]).

The pattern of histofluorescent neuropathology in these four 6-OHDA rats appears to be the same as we observed in a more extensive analysis of material after identical unilateral and bilateral 6-OHDA lesions in a recent study of exploratory behavior (Fink and Smith, in preparation and reference [8]). In that study, we also used the vibratome

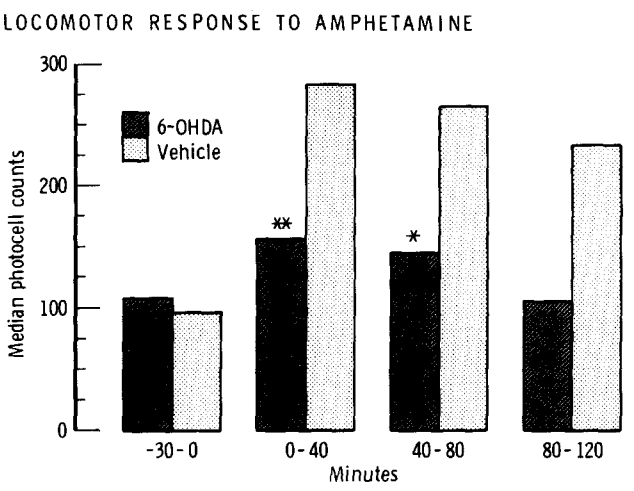

FIG. 2. Data are from seven 6-OHDA and 6 vehicle rats. $*p<0.05$, $**p<0.02$, Mann-Whitney U test.

TABLE 2

MEDIAN PHOTOCELL ACTIVITY AFTER SALINE INJECTION

	Time (min)		
	0–40	40–80	80–120
6-OHDA	69	24	3
Vehicle	73	17	6

The data are from 6 6-OHDA and 5 vehicle rats.

technique to demonstrate severe (3+) denervation of the mesocortical dopaminergic terminal fields in frontal, anterior cingulate, piriform and suprarhinal cortices. We presume these mesocortical fields were also denervated in the rats in the present study because the 6-OHDA injections were identical.

All the neuropathology in this study was ipsilateral to the injection site. This is consistent with the lack of crossing of significant numbers of mesolimbicocortical DA fibers [15]. The absence of fluorescent axons and terminals raises the possibility of methodological artifact. We evaluated that possibility in two ways. First, the 6-OHDA injected side was compared to the contralateral side in the same section. The loss of catecholamine fluorescence ipsilateral, but not contralateral, to the 6-OHDA injection proved that the loss of fluorescence ipsilateral to the 6-OHDA injection was not an artifact of the histofluorescent technique. Second, the 6-OHDA injected rats were compared to the vehicle injected rat. The mesolimbic fluorescence on both sides of the vehicle injected brain appeared equal and was not different from the contralateral side of 6-OHDA injected brains, but was significantly different from the side of the brain ipsilateral to the 6-OHDA injection. From these two comparisons, we conclude that the loss of fluorescent fibers ipsilateral to the 6-OHDA injection reflected the anterograde degeneration of DA fibers and was not a methodological artifact.

DISCUSSION

The major result of these experiments is that unilateral denervation of the mesolimbicocortical dopaminergic terminal fields decreased locomotor exploration in an OF and the locomotor response to amphetamine. The decrease was more than 50% in both cases. These decreases occurred despite the fact that mesolimbicocortical denervated rats were as active in a photocell cage as intact rats before amphetamine (Fig. 2, Table 2).

The decrease in OF activity after unilateral denervation was equivalent to the decrease observed after bilateral denervation (Fink and Smith, in preparation and reference [8]). The response to amphetamine, however, was significantly larger after unilateral denervation than after bilateral denervation because bilateral denervation abolishes the locomotor response to this dose of amphetamine (Fink and Smith, in preparation).

The significant reduction of the locomotor response to amphetamine by mesolimbicocortical DA denervation is consistent with previous evidence that demonstrated the importance of this DA system for that response (see Introduction). Our results demonstrate a new aspect of this neuropharmacological relationship. Unilateral denervation is sufficient and bilateral denervation of a single terminal field such as the n. accumbens is not necessary for significant impairment of the locomotor response to amphetamine. This conclusion also holds for the much less extensively studied neurobehavioral relationship between locomotor exploration in the OF and the mesolimbicocortical DA system (Fink and Smith, in preparation and reference [8]).

The fact that our test conditions were relatively insensitive for detecting circling may explain why we failed to observe spontaneous circling or circling after amphetamine [9] in the lesioned rats. This could also explain the apparent lack of difference between right unilateral denervation and left unilateral mesolimbicocortical denervation that might have been expected from the work of Glick *et al.* [9]. This point requires further investigation.

In summary, unilateral mesolimbicocortical dopaminergic denervation markedly reduced locomotor exploration of an OF and the locomotor response to amphetamine. These results are consistent with the hypothesis that the mesolimbicocortical DA system energizes behavior, but does not direct it [4, 10, 11, 16, 24].

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