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Abnormal Open Field Behavior after Anterolateral Hypothalamic Injection of 6-Hydroxydopamine[1]

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YOUNG, R. C., G. N. ERVIN AND G. P. SMITH. *Abnormal open field behavior after anterolateral hypothalamic injection of 6-hydroxydopamine.* PHARMAC. BIOCHEM. BEHAV. 5(5) 565–570, 1976. — To investigate the importance of forebrain catecholamine terminals in open field behavior, rats were microinjected bilaterally in the medial forebrain bundle at the level of the anterolateral hypothalamus with 6-hydroxydopamine (6-OHDA), a specific catecholaminergic neurotoxin. These 6-OHDA microinjections produced extensive loss of forebrain catecholamine terminals; identical vehicle microinjections did not. When 6-OHDA rats were given 8 open field (OF) tests in the first or the fifth postinjection week, they had a longer latency to enter the OF, crossed fewer squares and reared less than normal rats or rats microinjected with vehicle. The abnormal OF behavior of 6-OHDA rats was not a generalized loss of locomotor activity because 6-OHDA rats were normally active in the home cage. The abnormal OF behavior of 6-OHDA rats was also not a result of a generalized lack of reactivity because the OF test elicited an increase of plasma corticosterone in 6-OHDA rats. The possibility that 6-OHDA rats were abnormal in the OF because they were hyperreactive to it was not consistent with the observations that the OF activity of 6-OHDA rats did not change with repetitive testing, 6-OHDA rats did not defecate more than vehicle rats, and 6-OHDA rats did not display freezing behavior. These results suggest, but do not prove, that the abnormal OF behavior of 6-OHDA rats reflects a deficit of exploratory behavior that is correlated with extensive loss of forebrain catecholamine terminals.

Open field test Exploratory behavior 6-Hydroxydopamine Brain catacholamines Corticosterone

CONFRONTED with a novel open field, a rat moves through it, sniffs it, rears in it, and frequently defecates. With repeated exposure to the open field, rats usually defecate less and move as much or more than they did on the first trial [23], but exploratory activity increases or does not change. Although the open field test (OF) was devised to measure emotionality [7], it also measures exploratory behavior, particularly after the first trial [23].

Movement in the OF is temporarily abolished by reserpine [5]. This suggests that brain biogenic amines (catecholamines and serotonin) are necessary for normal OF behavior. Discovery of the selective toxicity of 6-hydroxydopamine (6-OHDA) for catecholamine neurons by Thoenen and Tranzer [20] provided a tool to investigate the relationship of brain catecholamines (norepinephrine and dopamine) to OF behavior without simultaneous alteration of brain serotonin. Intraventricular or intracisternal administration of 6-OHDA produced significant depletion of catecholamines (CA) throughout the brain [22] and decreased OF activity [3, 8, 10, 12, 18]. A more localized destruction of CA neurons (loss of terminals of the nigrostriatal tract and of the forebrain [9]) has been obtained by microinjecting 6-OHDA into the region of the substantia nigra [21]. After substantia nigra microinjec-

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tions rats move less in the OF [2, 13, 21], but such treatment can produce rats that do not move under most conditions [21].

Since OF testing after intracerebral 6-OHDA has been limited to 1 or 2 tests in these experiments, it is not possible to decide if the decreased OF activity is due to inhibition of exploration by hyperemotionality, to a decreased tendency to explore, to a general decrease of locomotor activity, to a general lack of reactivity to the OF, or to a combination of these defects.

To determine the basis of decreased OF behavior after central CA loss, we gave repeated OF tests to rats after they received microinjections of 6-OHDA into the medial forebrain bundle at the level of the anterolateral hypothalamus (AL). At this AL site, 6-OHDA injections produce widespread damage of forebrain CA, but only partial damage of the nigrostriatal tract [6]. After such AL 6-OHDA injections, rats have a transient period of aphagia and adipsia [17], but then appear much more normal than rats that receive 6-OHDA injections into the substantia nigra.

METHOD

Animals

Male Sprague-Dawley rats (Hormone Assay, Chicago, Illinois), weighing 200–400 g, were housed in individual cages. The room was lighted artificially between 0700 and 1900 hr daily and was maintained at a temperature of 24° C ± 2° C.

Surgery

Rats were anesthetized with chloral hydrate and barbiturate (Equi-thesin, 3 ml/kg, intraperitoneally; Jensen-Salsbery Laboratories, Kansas City, MO). After the scalp and cranium were opened, the tip of a 30 gauge cannula was positioned stereotactically in the anterolateral hypothalamus (AL) (7 mm anterior to the interaural line, 2 mm lateral to the midline, and 8 mm below the dura according to the atlas of deGroot [4]). A 50 μl Hamilton syringe was connected to the cannula by polyethylene tubing (PE 10). Using an automatic dispenser, 4 μl of solution was injected bilaterally over a 30 sec interval. The cannula was left in place for an additional 60 sec and then withdrawn. To produce preferential damage of catecholamine axons and terminals, rats were injected with a solution of 6-hydroxydopamine hydrobromide (Regis Chemical Co., Chicago, Ill.; 6.5 μg of free base/μl) and ascorbic acid 0.4 μg/μl dissolved in cold distilled water immediately prior to use. To control for non-specific damage, rats were injected with a vehicle solution containing ascorbic acid 0.4 μg/μl dissolved in cold distilled water. We have previously demonstrated that such 6-OHDA and vehicle microinjections produce very similar non-specific lesions equal to or less than 300 μm [16]. Such microinjections of 6-OHDA also produce extensive loss of anterolateral hypothalamic and forebrain CA histofluorescence, but vehicle injections produce loss of CA histofluorescence in the region of the cannula tip only [6]. Furthermore, microinjections of 6-OHDA produce a loss of hypothalamic and forebrain catecholamines, but vehicle injections do not [15]. These data support the highly preferential toxicity of our 6-OHDA microinjections for CA axons and terminals. Javoy *et al.* [11] have also reported the ability of obtaining highly specific CA lesions after 6-OHDA microinjections into the substantia nigra or n. caudatoputamen. It is now clear that the relative specificity of the lesion produced by 6-OHDA microinjections varies with injection site and each experiment must provide internal evidence for the degree of specificity claimed under the conditions of that experiment. Under our conditions there is widespread CA loss throughout the entire forebrain and non-specific hypothalamic damage of less than 300 μm in diameter. This is a highly preferential, but not absolutely specific, CA lesion and the results are interpreted accordingly.

Maintenance

Animals had free access to Purina rat chow pellets and tap water. Body weights and food and water intakes were recorded daily for the first two postoperative weeks. Since AL 6-OHDA injections produce short-term aphagia and adipsia [17], body weights of AL 6-OHDA rats were maintained during the first nine postoperative days by two daily gastric intubations at 1300 and 1500 hr of 12 ml of eggnog (13 oz evaporated milk, 125 ml tap water, 250 g dextrose, 1.8 ml Polyvisol, 2 whole eggs, 30 ml Kaopectate). In addition, any animal that did not accept pellets and water by the fourth postoperative day was given wet chocolate chip cookies and 10% sucrose solution until it did.

Apparatus

OF testing was performed in a rectangular area (76 cm × 93 cm) enclosed by brown cardboard walls 60 cm high. The floor was covered with white contact paper and was divided into 8 cm squares by brown lines. Fluorescent ceiling lights 2.6 m above the floor of the OF provided 20 μ Watts/cm ^{2} in the center of the field (40A Opto-Meter, United Detector Technology, Santa Monica, California).

Home cage (HC) activity was determined by housing animals individually in clear bottomless Plexiglas cages (21 cm × 44 cm × 20 cm) mounted on wooden racks with wire mesh (7 mm × 7 mm) floors. Social stimuli were limited by white partitions (20 cm high) between cages. An infrared light source was mounted in the middle of the long axis of each cage 12 mm from the cage floor opposite a photocell detector (Lafayette No. 5811-1, Lafayette Instrument Co., Lafayette, Indiana). Interruption of the light beam triggered a corresponding automatic counter (Lafayette No. 5811) located in an adjacent room.

Statistics

Data are expressed as medians and ranges of values. Statistical comparisons were made with the Mann-Whitney U test, 2 tailed, unless specified otherwise.

EXPERIMENT 1

METHOD

Animals

Nine rats received AL injections of 6-OHDA and 8 rats received AL vehicle injections. All 6-OHDA rats had aphagia or anorexia and lost weight during the first postoperative week (mean peak loss = 17.6% of preoperative weight, range: 11–29%). Vehicle injected rats also lost weight (mean peak loss = 8.6%, range: 5–12%). By the 7th postoperative day vehicle rats regained their preoperative

weight (mean loss = 1.4%, range: 0–6%), but 6-OHDA rats did not (mean loss = 15.5%, range: 8–29%).

All 6-OHDA and vehicle rats were tested in the OF on postinjection Days 1, 3, 5, and 7. Four unoperated rats and 2 sham-operated rats (skull holes drilled) were also tested in the OF on four alternate days to define the normal range of behavior in the OF under our conditions.

Open Field Procedure

A rat was removed from its home cage and transferred to a wire holding cage for 30 sec. Then it was placed in one corner of the OF with its head toward the wall. During the next 6 min test period several behavioral measurements were recorded. Then the rat was immediately returned to its home cage. On each test day animals were given two six-minute tests separated by an interval of 55 min. Testing was always performed between 0800 and 1300 hr.

During each OF test, the following behavioral measurements were made:

(1) Latency. The sec elapsed before a rat moved both front and rear paws out of the four squares which defined the starting corner. If a rat did not move from the corner within a 6 min test period, the latency was considered 360 sec.

(2) Corner activity. The number of temporary extensions of both forepaws out of the starting corner (stretches) and the number of times both forepaws were lifted free or against a side wall (rearings). The sum of the incidence of these two behavioral events was the corner activity score.

(3) Exploratory activity in OF. The number of squares traversed by both front and rear paws and the number of rearings (standing free or against a wall) were recorded.

(4) Defecation. Its occurrence was recorded.

Home Cage Activity Procedure

Beginning 3 days prior to injection and for the first postinjection week, all 6-OHDA and vehicle rats were housed in individual photocell cages, except during OF tests. Cumulative photocell counts were recorded daily for each rat and were corrected for counts that occurred within 30 min after OF tests, feeding or weighing.

RESULTS

6-OHDA rats did not respond normally to the OF. They had longer latencies, moved across fewer squares, reared less and defecated less than vehicle or normal rats (Table 1). Although 6-OHDA rats spent most of the test time in the starting corner, they were not as active there as vehicle rats. In those OF tests in which latency was 240 sec or longer, the median incidence of stretches and rears in the corner for 6-OHDA rats (0 in 57 tests) was significantly less than for vehicle rats (8 in 20 tests, $p<0.001$).

In contrast to their poor performance in the OF throughout the first postinjection week, 6-OHDA rats recovered normal activity in the HC by the second postinjection day (Table 2). This was the rate at which vehicle rats recovered (Table 2). Beginning on the third day, some 6-OHDA rats were sporadically hyperactive (see ranges, Table 2).

DISCUSSION

The 6-OHDA rats were less reactive than vehicle rats in all parameters of OF behavior throughout the first post-

TABLE 1

OPEN FIELD PERFORMANCE AFTER 6-OHDA OR VEHICLE

Treatment	Postinjection Day 1	3	5	7
	Squares Traversed			
6-OHDA	0†	0*†	0*†	0*†
	(0)	(0)	(0–159)	(0–232)
Vehicle	62†	113	121†	180
	(0–192)	(0–330)	(0–443)	(37–488)
None	491	514	576	609
	(316–575)	(292–749)	(400–749)	(203–888)
	Latency (sec)			
6-OHDA	720*†	720*†	640†	583*†
	(720)	(720)	(41–720)	(372–720)
Vehicle	366†	218	275	274†
	(33–720)	(15–720)	(2–720)	(80–608)
None	25	9	14	7
	(15–43)	(3–300)	(9–57)	(4–171)
	Rearing			
6-OHDA	0*†	0*†	0*†	0*†
	(0)	(0)	(0–1)	(0–5)
Vehicle	5†	8	19	23†
	(0–18)	(0–51)	(0–69)	(5–54)
None	60	60	64	69
	(33–69)	(28–91)	(38–86)	(53–93)
	Percent Defecating			
6-OHDA	44	0	0	0
Vehicle	38	75	63	100
None	67	67	67	50

Note: Data are median values (except for percent defecating) from 9 6-OHDA, 8 vehicle and 6 control rats. Range of values is in parenthesis. *significantly different from vehicle, $p<0.05$; †significantly different from control, $p< 0.02$.

TABLE 2

HOME CAGE ACTIVITY AFTER HYPOTHALAMIC 6-OHDA OR VEHICLE

Experimental Day	6-OHDA	Vehicle
−2	535 (376–1394)	619 (382–1266)
−1	646 (279–1499)	637 (294–1433)
Microinjection		
+1	237*† (103–517)	369* (82–869)
+2	496 (199–837)	673 (69–1194)
+3	678 (263–2939)	810 (110–1102)
+4	773 (236–3966)	921 (187–1370)
+5	803 (289–1368)	741 (290–1347)
+6	805 (314–2952)	947 (44–1380)
+7	735 (393–5213)	891 (112–1154)

Note: Data are median values from 9 6-OHDA and 8 vehicle rats. Range of values is in parenthesis. *significantly different from day −2, $p<0.05$; †significantly different from Day −1, $p<0.05$.

injection week. The failure of 6-OHDA rats to enter the OF on the first test day could have been an excessive reaction to the OF (freezing). The slight improvement in performance with repetitive testing is consistent with this interpretation. But even in the seventh and eighth OF tests on postinjection Day 7, 6-OHDA rats have very long latencies and moved and reared much less than vehicle rats. Unless 6-OHDA altered the control of defecation, the lack of defecation with repeated testing also supports the view that 6-OHDA rats failed to explore the OF for reasons other than hyperemotionality because defecation in the OF correlates significantly with a variety of emotionality factors [23].

The lack of activity of 6-OHDA rats in the OF contrasted sharply with their normal and sometimes excessive activity in the HC (Table 2). This dissociation demonstrates that the abnormal OF behavior of 6-OHDA rats was not the expression of a general locomotor debilitation.

Although 6-OHDA rats had longer latencies to enter the OF, were less active and reared less in the OF than vehicle rats, vehicle rats were also abnormal on some of these parameters of OF behavior on some test days. Note the erratic behavior of vehicle rats: on Day 3, they were not significantly different from control: on Days 5 and 7, vehicle rats were normal on at least 1 parameter of OF behavior. Presumably the erratic performance of vehicle rats was a result of the non-specific damage of vehicle microinjection. In an attempt to dissociate these presumed non-specific effects of microinjection on OF behavior, we delayed OF testing of 6-OHDA and vehicle rats until 30 days after microinjection.

EXPERIMENT 2

METHOD

Animals

Bilateral microinjections of 6-OHDA (7 rats) or of vehicle (7 rats) were administered as described in the Method section. All 6-OHDA rats had recovered from their initial postoperative aphagia or anorexia and were maintaining body weight on pellets and water at the time of testing.

Open Field Procedure

Two 6 min tests, separated by 55 min (described in Experiment 1), were administered to 7 6-OHDA rats and 7 vehicle rats on postinjection Days 30 through 34. These rats were not the subjects of Experiment 1 and did not have prior OF experience.

RESULTS

When tested in the OF on postinjection Days 30 through 34, 6-OHDA rats had significantly longer latencies, moved across fewer squares and reared less than vehicle rats (Table 3). There was a tendency for 6-OHDA rats to defecate more than vehicle rats during these OF tests, but OF activity did not increase when 6-OHDA rats defecated less than vehicle rats (Day 34, Table 3). Furthermore, repetitive OF testing did not elicit better OF performance. These results do not support hyperemotionality as the explanation for the

TABLE 3

OPEN FIELD PERFORMANCE 30-34 DAYS AFTER ANTEROLATERAL HYPOTHALAMIC 6-OHDA OR VEHICLE

Treatment	Postinjection Day 30	31	32	33	34
			Squares Traversed		
6-OHDA	0* (0–199)	0* (0–187)	0* (0–59)	0* (0–59)	0* (0–4)
Vehicle	510 (321–674)	481 (246–796)	357 (204–638)	477 (332–593)	409 (278–624)
			Latency (sec)		
6-OHDA	720* (28–720)	720* (32–720)	720* (425–720)	720* (55–720)	720* (373–720)
Vehicle	47 (10–120)	5 (3–67)	9 (2–39)	13 (7–38)	23 (15–34)
			Rearing		
6-OHDA	0* (0–17)	0* (0–11)	0* (0–10)	0* (0–6)	0* (0–12)
Vehicle	60 (49–77)	40 (13–77)	36 (22–54)	56 (26–86)	40 (26–81)
			Percent Defecating		
6-OHDA	29	29	43	43	14
Vehicle	14	14	14	0	29

Note: Data are median values (except for percent defecating) from 7 6-OHDA and 7 vehicle rats. Range of values is in parenthesis.
*significantly different from vehicle, $p<0.05$.

decreased activity in the OF. Since vehicle rats responded to the OF like normal rats (compare Control data, Table 1), the abnormal behavior of 6-OHDA rats in these OF tests can be correlated with the loss of CA after 6-OHDA because the apparently equivalent non-specific neuropathological effects of vehicle microinjection did not produce any abnormal OF behavior.

DISCUSSION

Experiments 1 and 2 demonstrated major deficits in OF behavior that did not improve with repetitive daily testing. This poor OF performance of 6-OHDA rats was not correlated with a general loss of motor activity (Table 2), a non-specific postoperative effect (Table 3) or with excessive defecation (see percent defecating, Tables 1 and 3).

It is possible that the abnormal OF behavior of 6-OHDA rats reflects a general diminished reactivity of 6-OHDA rats to the stimuli of the OF. To test this possibility, we measured the plasma corticosterone response of 6-OHDA rats to the OF. In normal rats, plasma corticosterone increases during an OF test [1], but the increase of plasma corticosterone does not depend on locomotion in the OF [19]. This dissociation between the neuroendocrine response and the behavioral responses to the OF permits us to use the neuroendocrine response to the OF as an independent measure of reactivity of 6-OHDA rats to OF stimuli. It should be noted that the CA innervation of the median eminence appears intact in the 6-OHDA rats [6]. If plasma corticosterone increased in 6-OHDA rats that had deficits in the OF, that would demonstrate that 6-OHDA rats reacted to the OF test neuroendocrinologically. This result would not be consistent with the suggestion that the OF deficit of 6-OHDA rats reflects a diminished reactivity of all response systems to the OF.

EXPERIMENT 3

METHOD

Animals

The animals were from Experiment 2 plus 3 6-OHDA and 4 vehicle rats of similar weight.

Procedure

On postinjection Day 35, 7 6-OHDA and 6 vehicle rats from Experiment 2 were given a single 6 min OF test at 1000 hr. At the end of the test, the rats were decapitated. Samples of trunk blood were heparinized, centrifuged, separated and frozen immediately.

Three 6-OHDA rats and 4 vehicle rats were removed quickly from their HC on postinjection Day 35 at 1000 hr and decapitated to obtain plasma samples. Concentration of corticosterone in all the plasma samples was determined by competitive protein-binding radioimmunoassay [14] through the courtesy of Dr. Peter Stokes of this Department.

RESULTS

Plasma corticosterone in 6-OHDA rats was significantly higher after an OF test than in the HC (Table 4). This is evidence that 6-OHDA rats react to OF testing despite failing to move normally in the OF (Table 4). Median plasma corticosterone of 6-OHDA rats in the HC was smaller than that of vehicle rats, but the difference was not significant for this small number of animals.

TABLE 4

ADRENOCORTICAL RESPONSE TO THE OPEN FIELD AFTER ANTEROLATERAL HYPOTHALAMIC 6-OHDA OR VEHICLE

Treatment	Plasma Corticosterone (μg/100 ml) Home Cage	Open Field
6-OHDA	1.5 (1.5–3.2)	11.2* (7.4–17.3)
Vehicle	4.7 (0.9–11.4)	18.2* (11.7–30.9)

Note: Data are median values from 3 6-OHDA and 4 vehicle rats in the home cage and from 7 6-OHDA and 6 vehicle rats in the open field. Range of values is in parenthesis. Open field values larger than respective home cage values.

*$p<0.025$, one-tailed. Plasma corticosterone of 6-OHDA rats in Home Cage and Open Field were not significantly different from the values of vehicle rats.

GENERAL DISCUSSION

After bilateral AL 6-OHDA microinjections, rats did not move normally in an OF (Tables 1 and 3). The poor OF performance is not a manifestation of generalized loss of locomotor activity because 6-OHDA rats are normally active or hyperactive in their HC during the period of OF testing (Table 2). The poor OF performance is also not a total lack of reactivity to OF testing because stimuli of the OF elicited an increase of plasma corticosterone in 6-OHDA rats (Table 4). Since the OF elicits emotional and exploratory behavior, it is possible that 6-OHDA rats are hyperreactive to the OF and this inhibits exploratory behavior. We reject this explanation for the following reasons: (1) Repeated testing should diminish hyperreactivity [23], but the performance of 6-OHDA rats did not improve with repetitive OF tests (Tables 1 and 3); (2) Hyperemotional rats frequently assume a tense, crouching posture (freezing), but 6-OHDA rats did not display such behavior in the OF; (3) Hyperemotional rats tend to have exaggerated adrenocortical responses to many stimuli, but 6-OHDA rats did not (Table 4); and (4) Hyperemotional rats defecate more in the OF, but 6-OHDA rats did not (Tables 1 and 3). We consider the lack of data to support the possibility of hyperemotionality and the marked failure to explore the OF as complementary evidence for interpreting the poor OF performance as a failure of exploratory behavior. This interpretation is tentative and it requires further testing in other situations.

The defect of OF behavior is probably the result of the forebrain CA damage produced by 6-OHDA because vehicle microinjections which did not disrupt OF behavior (Table 3) produce the same non-specific damage at the microinjection site in the AL hypothalamus [16] without depleting brain catecholamines [15] or reproducing the pattern of histofluorescent CA loss which occurs after 6-OHDA microinjections [6].

Preliminary analysis of the loss of CA histofluorescence after AL 6-OHDA reveals severe loss of CA afferent terminals in parietal, frontal and piriform cortex, hippo-

campus, AL hypothalamus, anteromedial part of the n. caudatoputamen, nucleus accumbens, dorsal part of the bed nucleus of the stria terminalis, lateral septal nucleus and the olfactory tubercle [6]. The CA histofluorescence of the posterior part of caudate, the posterior hypothalamus, the thalamus and brainstem is intact. (These data will be presented in detail elsewhere, Fink and Smith, in preparation). This pattern of histofluorescent loss after AL 6-OHDA suggests, but does not prove, that CA denervation of limbic forebrain or neocortex or a combination of these sites is critical for the deficits in the OF. Further experiments are necessary to identify the critical sites(s) of CA denervation and the specific transmitter (norepinephrine and/or dopamine) involved.

Since there is some non-specific damage after 6-OHDA microinjections, it is possible that the abnormal OF behavior observed in 6-OHDA rats is correlated with the noncatecholamine damage of 6-OHDA microinjections. The normal OF behavior on Days 30 through 34 after vehicle microinjections that produce similar non-specific damage is not consistent with such a suggestion. We recognize, however, that we have not excluded this possibility in these experiments.

In summary, we consider these experiments to be tentative evidence for the hypothesis that forebrain CA terminals are necessary for normal OF behavior in the rat. Other kinds of experiments will be necessary to prove this hypothesis.

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