Pharmacology Biochemistry & Behavior, Vol. 3, pp. 1097–1106.

Effect of Various 6-Hydroxydopamine Treatments During Development on Growth and Ingestive Behavior

GEORGE R. BREESE, RONALD D. SMITH AND BARRETT R. COOPER

Departments of Psychiatry and Pharmacology, Biological Sciences Research Center
Child Development Institute, University of North Carolina, School of Medicine
Chapel Hill, NC 27514

(Received 19 August 1974)

BREESE, G. R., R. D. SMITH AND B. R. COOPER. *Effect of various 6-hydroxydopamine treatments during development on growth and ingestive behavior.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1097–1106, 1975. – Destruction of catecholamine-containing fibers in brain at 5 days of age with intracisternal injection of 6-hydroxydopamine reduced body growth, intake of a sucrose solution, and acquisition of an active avoidance response. Further characterization of behavioral deficits indicated that treated animals also showed reduced ingestion of saline solution when injected with desoxycorticosterone and a decreased eating response to insulin. In addition, all of these deficits produced by catecholamine depletion with 6-hydroxydopamine were observed in rats in which brain dopamine was preferentially reduced but not in rats having preferential destruction of noradrenergic fibers, suggesting that dopamine depletion accounts for the observed alterations in developing animals. Although animals treated with 6-hydroxydopamine at 14 days showed reduced intake of a sucrose solution, they did not have reduced growth. Since early malnourishment reduced growth, it seems possible that the reduced growth observed after destruction of dopaminergic fibers may be related to an acute reduction of food intake which is perpetuated by persistent deficits in ingestive behavior. Evidence implicating malnourishment in other deficits produced by 6-hydroxydopamine could not be obtained.

6-Hydroxydopamine Ingestive behavior Dopamine Avoidance responding

INTRACISTERNAL injection of 6-hydroxydopamine into neonatal rats prior to seven days of age has been shown to destroy central catecholamine-containing fibers [6,7], to reduce growth and to disrupt a variety of behavioral responses [7, 17, 29]. Since some of these effects have been observed in developing animals in which brain dopamine was preferentially reduced, damage to the dopaminergic pathways has been suggested to be responsible for the growth and behavioral alterations observed following 6-hydroxydopamine treatment [29].

It has also been shown that rats injected with 6-hydroxydopamine at 14 days do not display an alteration in growth [7]. A possible explanation would be that animals had become resistant to the effects of 6-hydroxydopamine. In this regard, Lytle *et al.*, [17] suggested that the time-dependent difference in the action of 6-hydroxydopamine might be related to the level of monoamine oxidase in the developing rat. In the present work, experiments will be described to examine this possibility.

Several studies have indicated that malnourishment of the neonate can affect physical and behavioral development [15, 28, 35]. The observation that an acute aphagia and adipsia can occur in adult rats after treatment with 6-hydroxydopamine [2, 4, 13, 30, 34] suggested that reduced food intake during a critical period of development might contribute to the deficits observed in animals treated with 6-hydroxydopamine when immature. Therefore, one purpose of the present study was to determine if neonatal malnutrition could account for the growth and behavioral deficits characteristic of rats which receive 6-hydroxydopamine at 7 days of age.

While several studies have described chronic deficits in ingestive behavior in adult animals treated with 6-hydroxydopamine [3, 4, 14, 20, 30, 37] similar studies have not been performed in animals treated with 6-hydroxydopamine when immature. In this study, several of the ingestive behaviors found to be altered by 6-hydroxydopamine in adult animals were examined in rats after both catecholamine-containing fibers were destroyed during infancy as well as in developing rats treated to destroy noradrenergic or dopaminergic fibers [29].

METHOD

Animals

Litters of male Sprague-Dawley rats were obtained from Zivic-Miller Laboratories (Pittsburgh, Pa.) 4 days after gestation and were individually housed in clear plastic cages (10 1/2 × 10 1/2 in.) containing wood chip bedding. Male rats were used for all experiments. Mothers and pups were housed under fluorescent lighting conditions (GE F-40/CW bulbs) with 10 hr of light and 14 hr of darkness. Litters

were limited to 10 pups each and were transferred to large wires cages when 14 to 17 days of age. Mothers were not removed from pups until the pups reached 28 days of age to assure that treated animals had sufficiently matured. At 40 days of age, rats were individually housed. Weights were recorded at time of injection and weekly thereafter.

Procedure

Treatments. The 6-hydroxydopamine was administered intracisternally to developing rat pups at various time periods after birth [7,29]. Four types of treatment schedules were used. For the first experiment, 100 μg/brain 6-hydroxydopamine was administered intracisternally on the 5th day after birth. The second procedure involved administering intracisternally 100 or 150 μg of 6-hydroxydopamine on the 14th day after birth. The third procedure was chosen to lower norepinephrine preferentially in the developing rat and consisted of giving intracisternally 15 and 25 μg of 6-hydroxydopamine per brain on the 5th and 7th days after birth, respectively [29]. The last procedure utilized 5-day-old rats which received desmethylimipramine (DMI: 20 mg/kg, IP) 1 hr before the intracisternal injection of 50, 75, 100, or 150 μg of 6-hydroxydopamine. This procedure was used to reduce brain dopamine preferentially [29]. Control rats received vehicle or DMI where appropriate for comparative purposes. Since control animals that received DMI were not found to differ from animals that received only vehicle, their data were combined.

In other experiments, 2 litters of male Sprague-Dawley rats were subjected to a period of neonatal malnutrition. The mothers, and later the pups, were provided a special powdered diet for 1 week containing all synthetic amino acids with the exception of L-phenylalanine and L-tyrosine (Nutritional Biochemical Corp., Cleveland, Ohio). Following this treatment, animals were given a mixture of the special diet and powdered Purina Rat Chow in a 2:1 ratio for an additional 2 weeks, after which time standard laboratory pellets were supplied. Control litters received powdered Purina rat chow during the treatment period.

Ingestive behavior. When approximately 60 days of age, water consumption was measured for several days. After baseline intake was stable, water was replaced by a 5 percent sucrose solution [4]. Consumption of sucrose was recorded for a 24 hr interval for 3 days.

Some treated animals were permitted a choice of either a saline solution (1 percent) or water [36]. Bottles containing these 2 solutions were positioned on cages so that the metal drinking tubes were 1 in. apart and approximately 1 1/4 in. above the floor of the cage. On each day for 6 days, the amount consumed from each bottle was recorded, and the bottles reversed. This preference procedure was subsequently used to determine if the 6-hydroxydopamine treatments altered sodium appetite increased by DOCA administration [26]. In this latter procedure, each animal was injected with desoxycorticosterone trimethylacetate (DOCA, Percorten; Ciba, Summit, New Jersey) 5.0 mg/kg (salt) for 4 days and then with 10.0 mg/kg daily for 4 days.

Food intake was also measured in some rats after subcutaneous injection of insulin (20 units/kg; Iletin, Lilly, Indianapolis, Indiana) [18,27]. Rats were not deprived of food before the tests. Insulin was injected at approximately 9:00 a.m., and food intake was measured at hourly intervals for 5 hr. The effects of insulin on food consumption were compared with food intake during the same time period on the day preceding the insulin test. The amount consumed during the subsequent 19 hr following insulin was also recorded.

In additional experiments, animals were deprived of food to determine how much water they would drink in the absence of food or how much food they would eat in the absence of water. Animals were deprived of either food or water for 22 1/2 hr and allowed to eat or drink ad lib during the remaining 1 1/2 hr of each day.

Shuttle-box avoidance experiment. In order to examine a response unrelated to ingestive behavior [11] some groups of rats were tested in a shuttle-box avoidance task as previously described [10]. One min after a rat was placed in the shuttle-box, the session was initiated by activating a conditioned stimulus, which consisted of lighting a small lamp over the compartment occupied by the rat as well as initiating a tone from a speaker over the center of the apparatus. The conditioned stimulus continued until the animal crossed to the opposite compartment, thereby avoiding shock, or for a maximal period of 10 sec. If the animal did not cross, the rat received an electric shock of 0.8 mA intensity which terminated when the animal escaped to the opposite compartment or at the end of 5 sec. Each avoidance response, escape response, or failure to escape was followed by an interval of 30 sec until the start of the next trial. Acquisition was determined by recording the number of avoidance responses every 25 trials during a 100 trial session.

Biochemical Determinations. After the various behavioral procedures were completed, animals were killed by cervical fracture and their brains were removed and rinsed in cold water. The cerebellum was removed from all brains. Some brains were left intact while the brains from other animals were dissected into brain parts. The term brain stem as used in the study refers to that area of brain caudal to a cut made at the level of the superior colliculi after removal of the cerebellum. The hypothalamus was dissected rostrally by a cut at the optic chiasm, caudally by a cut at the level of the mammillary bodies, laterally by a cut just medial to the cortical margin, and dorsally by a cut at the level of the anterior commissure. A cut at the level of the optic chiasm permitted dissection of the striatum from the forebrain. The tissue remaining after these dissections was referred to as rest of brain. The whole brain or parts were subsequently frozen on dry ice and kept at -76°C until analyzed. Storage of samples was never longer than 3 days. Determinations of norepinephrine and dopamine were carried out as previously described [5,6]. Values were corrected for a recovery of 99.2 ± 1.7 and 92 ± 3.6 percent, respectively.

Tyrosine hydroxylase was isolated from brain tissue according to the method of Musacchio *et al.* [22]. Enzyme activity was then determined by minor modification of the method of Nagatsu *et al.* [24]. The L-3, 5-H^3 tyrosine (24.7 c/mMole, New England Nuclear Corp.) was purified as described by Mueller *et al.* [21].

Drugs

The 6-hydroxydopamine HBr was purchased from Regis Chemical Company (Chicago, Illinois) and was used without further purification. Desmethylimipramine HCl was kindly supplied by Geigy Laboratories (Ardsley, N.Y.). Pargyline was furnished by Abbott Laboratories (North Chicago, Illinois).

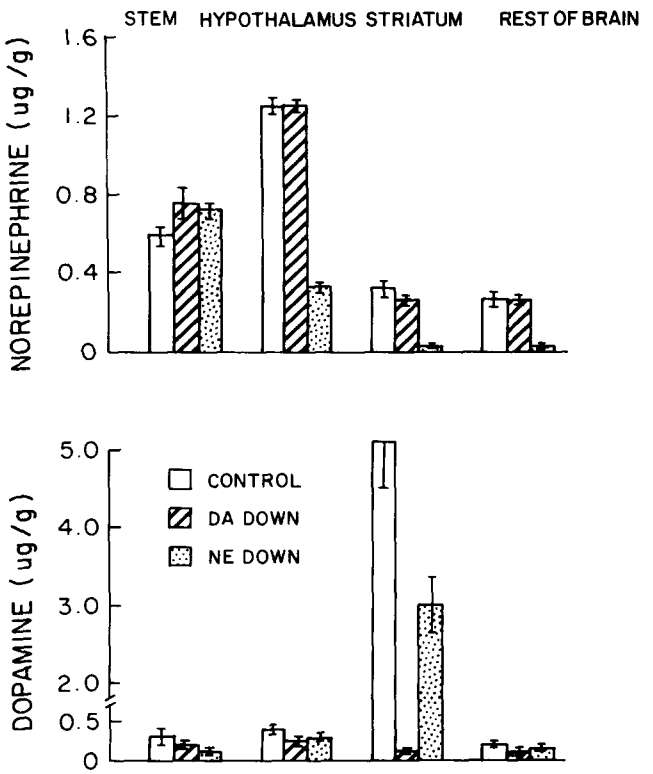

FIG. 1. Effect of 6-hydroxydopamine treatment in developing rats to deplete brain norepinephrine (NE Down) or dopamine (DA Down) preferentially on norepinephrine and dopamine in various brain areas. NE Down rats received 15 and 25 μg of 6-hdyroxydopamine intracisternally when 5 and 7 days of age, respectively. DA Down rats received 100 μg of 6-hydroxydopamine intracisternally one hour after DMI (20 mg/kg, IP) on Day 5. Columns represent the mean ± S.E.M. of from 7 to 12 determinations.

RESULTS

Effect of Various 6-Hydroxydopamine Treatments on Body Growth and Brain Catecholamine Content in Whole Brain and Brain Areas

As previously described [7,29], intracisternal administration of 6-hydroxydopamine (100 μg/brain) to 5-day-old rats was found to cause a marked depletion of whole brain catecholamines (see Table 4). In the present study, alterations produced by these treatments was defined in various brain areas. Catecholamines content was reduced in caudate, hypothalamus and rest of brain while content was little affected in brain stem (Fig. 1). Pretreatment of rats with desipramine before administering 6-hydroxydopamine virtually eliminated dopamine content from caudate and other areas of brain examined, while having no significant effect on norepinephrine content. Multiple small doses of 6-hydroxydopamine (15 and 25 μg) reduced norepinephrine in hypothalamus, striatum and rest of brain suggesting that noradrenergic nerve terminals were destroyed by this treatment. However, dopamine was not depleted in cortical tissue after this treatment, providing further support for the view that dopamine terminals are located in cortex [33].

As previously reported [29], a severe reduction of growth accompanied the loss of brain catecholamine-content after 6-hydroxydopamine treatment (Table 2). Since depletion of brain dopamine produced a similar growth deficit while destruction of noradrenergic fibers did not, the deficit observed following 6-hydroxydopamine treatment of the neonate would appear to be dependent upon the destruction of dopaminergic neurons. In order to determine the relationship of growth deficits to the degree of dopamine depletion, several doses of 6-hydroxydopamine were administered to desipramine-treated rats at 5 days of age. In general, the deficits in body growth reflected the depletion of dopamine (Table 1). The 150 μg dose had severe effects on the survival of the animals with only 3 of the 16 animals injected with 6-hydroxydopamine alive 56 days after treatment.

TABLE 1

PREFERENTIAL REDUCTION OF WHOLE BRAIN DOPAMINE WITH INCREASING DOSES OF 6-HYDROXYDOPAMINE (6-OHDA) AFTER DESMETHYLIMIPRAMINE (DMI) PRETREATMENT

Treatment*	Dose (μg per brain)	Pretreatment*	Body Weight (g)	N	Whole Brain Levels (μg/g) Norepinephrine	Dopamine
Control	–	DMI	346 ± 3	10	0.34 ± 0.028	0.52 ± 0.028
6-OHDA	50	DMI	228 ± 23†	11	0.32 ± 0.011	0.13 ± 0.026†
6-OHDA	75	DMI	231 ± 16†	11	0.36 ± 0.018	0.11 ± 0.023†
6-OHDA	100	DMI	165 ± 7†	13	0.30 ± 0.016	0.056 ± 0.010†
6-OHDA	150	DMI	119 ± 33†	3	0.35 ± 0.025	0.062 ± 0.026†

*Desmethylimipramine (DMI, 20 mg/kg, IP) was given 1 hr before the intracisternal injection of 6-OHDA. Animals were treated when 5 days of age and were sacrificed when 56 days of age. N = the number of determinations in each group.

†$p<0.001$ when compared with control

TABLE 2

SHUTTLE BOX AVOIDANCE RESPONSE AND SUCROSE CONSUMPTION IN RATS DEPLETED OF BRAIN NOREPINEPHRINE, DOPAMINE OR BOTH BRAIN CATECHOLAMINES WHEN IMMATURE

Treatment	N	Mean Number of Avoidance Responses	5% Sucrose Intake (ml/100 g body weight)
Control	10	53 ± 10	34 ± 4
6-OHDA – 5 days	10	23 ± 10*	21 ± 2*
NE Down	10	70 ± 8	30 ± 5
DA Down – 5 days	10	20 ± 6*	20 ± 7*

Treatments are described in Fig. 1. Number of avoidance response is during a 100 trial acquisition session.
*$p<0.001$ when compared with control

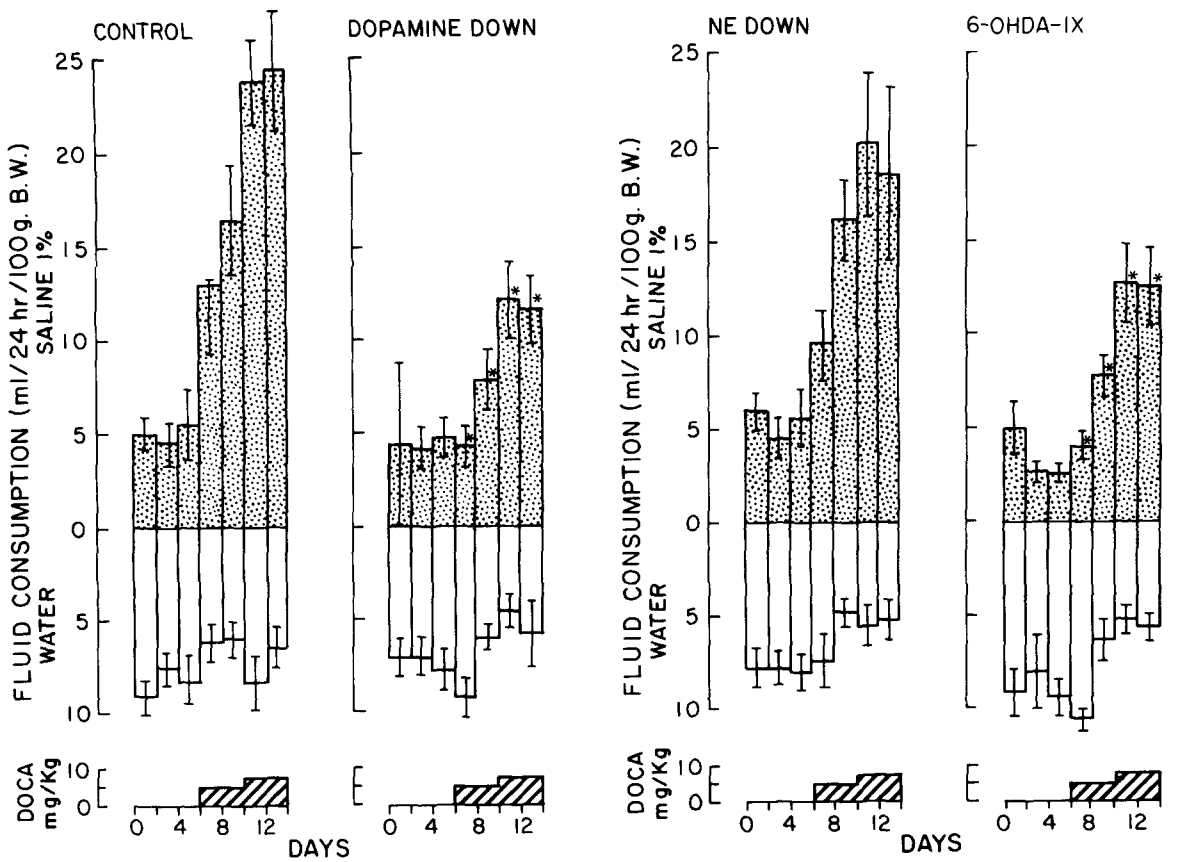

FIG. 2. Effect of desoxycorticosterone acetate (DOCA) on the consumption of water or saline by control rats and rats which received various 6-hydroxydopamine treatments. The striped area below the graph indicates the amount of DOCA administered and the duration of treatment. 6-Hydroxydopamine treatments (DA Down; NE Down; 6-OHDA-IX) are described in Fig. 1 and Method. *$p<0.001$ when compared with saline consumed by control rats.

Effect of Various 6-Hydroxydopamine Treatments During Development on Ingestive Behavior

Several laboratories have suggested that alterations in ingestive behavior produced by lesions of the lateral hypothalamus may be related to an interruption of catecholamine pathways [4, 9, 14, 19, 34, 37], in that such lesions resemble the effects of 6-hydroxydopamine treatment. Previous studies have shown that rats treated with 6-hydroxydopamine as well as developmental rats treated

to reduce brain dopamine had deficits in avoidance responding and ingestion of sucrose [4,29]. Preferential depletion of norepinephrine did not produce these deficits. In order to insure continuity with previous work [29], these deficits were confirmed in animals to be used in the present experiments dealing with ingestive behavior. (Table 2)

Treatment of adults with 6-hydroxydopamine has previously been found to reduce saline preference induced by DOCA [4] (Fig. 2). In developing rats treated with 6-hydroxydopamine to destroy both catecholamine systems, the ingestion of saline was significantly less than the increase observed in control animals. In animals in which brain dopamine had been preferentially reduced, the intake of saline produced by DOCA treatment was similar to the reduction observed when both amines were reduced with 6-hydroxydopamine. Preferential depletion of norepinephrine did not alter the increased saline intake induced by DOCA. This provides evidence that this deficit in 6-hydroxydopamine treated rats is related to a reduction of brain dopamine.

Several workers have reported that the administration of insulin increases food intake [18,27]. In the present study, insulin (20 units/kg, subcutaneously) produced a significant increase in the amount of food consumed during the 5 hr just after the insulin was administered, even though the food intake for the 24 hr period was not altered (Table 3). In confirmation of work in adult rats [4], reduction of both catecholamines prevented the increase in food intake normally observed after insulin treatment. While depletion of brain dopamine (DA Down) prevented insulin-induced eating, the 6-hydroxydopamine treatment to deplete brain norepinephrine did not alter the ability of insulin to increase food intake.

A common ingestive deficit observed after lesions of the lateral hypothalamus is "prandial drinking" [32]. Since there is some controversy whether this occurs in rats treated when adult with 6-hydroxydopamine [4, 14, 19], rats that had been treated with the various 6-hydroxydopamine treatments as infants were tested as to their ability to drink in the absence of food or eat dry food in the absence of water (Table 4). While there were deficits in the total amount of food or water ingested in animals in which both brain amines were reduced and in animals in which brain dopamine was reduced, these changes were not apparent if the intake were corrected for body weight.

Effect of Pargyline Treatment on the Growth of Rats Treated with 6-Hydroxydopamine when 5 or 14 Days of Age

In contrast to the growth reduction produced by 6-hydroxydopamine given prior to 7 days of age, treatment of rats with 6-hydroxydopamine when 14 days of age has been found to cause no reduction in growth rate [7]. This observation was confirmed in the present study (Fig. 3) even though norepinephrine and dopamine were reduced by approximately 80 percent (Table 5). However, the degree of depletion of dopamine in animals treated at 14 days of age was not as pronounced as in rats treated at 5 days of age (Table 5).

Recently, Lytle *et al.* [17], suggested that the depletion produced in the neonate could be related to the development of monoamine oxidase content in brain. Since

TABLE 3

EFFECT OF VARIOUS 6-OHDA TREATMENTS ON INSULIN INDUCED EATING IN DEVELOPMENTAL RATS

Treatment Groups†	Insulin‡ 20 units/kg	N	Food Consumption (g) 5 hr Total	24 hr Total
Control	–	19	7.9 ± 0.7	31.1 ± 2.8
	+		10.2 ± 0.6*	32.5 ± 2.2
NE Down	–	14	6.3 ± 0.6	30.5 ± 1.4
	+		8.3 ± 0.8*	29.9 ± 1.4
DA Down	–	15	4.8 ± 0.9	22.3 ± 1.7
	+		6.1 ± 1.1	24.3 ± 2.9
6-OHDA-lx	–	6	4.9 ± 1.2	25.5 ± 2.6
	+		4.7 ± 1.2	25.5 ± 4.0

†Control and 6-OHDA treatments described in Table 1 and Method.
‡(–) indicates values before insulin: (+) indicates values after the subcutaneous injection of insulin.
*Values significantly greater than the pre-insulin consumption ($p<0.005$).

TABLE 4

EFFECT OF FOOD OR WATER DEPRIVATION ON FOOD AND WATER INTAKE IN ANIMALS PREFERENTIALLY DEPLETED OF BRAIN CATECHOLAMINES

Treatment	Body Weight (g)	Food Present for 90 min Food Intake (g)	Water Intake (ml)	No Food for 22 1/2 hr Water Intake (ml)	24 hr Total Water Intake (ml)
			Food Deprivation		
Control	423 ± 10	13.9 ± 0.5	18.2 ± 1.5	21.8 ± 3.4	40.0 ± 4.8
DA Down	256 ± 31*	10.0 ± 0.9	9.2 ± 0.9*	9.8 ± 2.4*	19.0 ± 2.8*
NE Down	385 ± 12	13.0 ± 0.4	18.4 ± 1.4	25.9 ± 3.1	43.8 ± 2.9
6-OHDA-1x	257 ± 14*	12.7 ± 0.3	10.3 ± 2.4*	10.3 ± 2.4*	23.4 ± 2.1*

Treatment	Body Weight (g)	Water Present for 90 min Water Intake (ml)	Food Intake (g)	No Water for 22 1/2 hr Food Intake (g)	24 hr Total Food Intake (g)
			Water Deprivation		
Control	498 ± 39	27.8 ± 1.7	6.5 ± 0.7	24.0 ± 1.8	29.6 ± 2.1
DA Down	276 ± 22*	17.4 ± 1.8*	6.2 ± 0.9	10.7 ± 2.4*	16.9 ± 2.4*
NE Down	530 ± 37	21.9 ± 1.5	6.6 ± 1.1	23.3 ± 2.1	29.9 ± 2.5
6-OHDA-1x	273 ± 30*	18.7 ± 1.9*	4.6 ± 0.8	19.5 ± 1.5	23.9 ± 1.4*

Animals were treated as described in Method and Table 1.

Animals were placed on a 22 1/2 hr food or water deprivation schedule and intake was determined for the period when food and water intake was present for 90 min. Intake was also determined during the deprivation period. Values are from the fifth day of the deprivation schedule.

*Indicates $p<0.001$ when compared with control.

pargyline pretreatment has been shown to enhance depletion of dopamine in adult rats [5,6], pargyline was administered prior to injection of 6-hydroxydopamine to animals 5 or 14 days of age to determine if this treatment would alter the effects of 6-hydroxydopamine on growth and destruction of catecholaminergic fibers. Treatment with pargyline prior to the administration of 6-hydroxydopamine to 5 day old rats caused a small but significant increase in the depletion of both norepinephrine and dopamine. Comparable growth reduction was obtained in pargyline and non-pargyline treated groups (Fig. 3). Administration of pargyline prior to the injection of 6-hydroxydopamine (100 or 150 μg/g) to 14-day-old rats also produced a significant increase in the depletion of both norepinephrine and dopamine (Table 5). However, growth rate was not significantly reduced although there was an increased incidence of death during the first month at the higher dose of 6-hydroxydopamine (150 μg).

Comparison of Animals Treated at 5 or 14 Days with 6-Hydroxydopamine on Sucrose Consumption and Avoidance Responding

Since animals treated at 14 days of age showed no growth deficits, sucrose solution was substituted for water when the animals treated at 14 days reached adulthood in order to determine if animals in either of these treatment groups would display a deficit in the intake of sucrose like that observed in rats treated with 6-hydroxydopamine when adult [4]. As shown in Table 5, rats treated with 6-hydroxydopamine at 5 days of age drank significantly less sucrose solution than did control animals. Rats treated at 14 days with 6-hydroxydopamine also drank significantly less sucrose solution regardless of whether they were pretreated with pargyline.

Since destruction of catecholamine-containing fibers has been shown to block acquisition of the shuttle-box avoidance response, treated animals were also tested in this task permitting examination of a response involving something other than ingestive behavior. In accord with earlier results [29], animals treated when 5 days of age failed to display acquisition of the shuttle-box avoidance response with or without pargyline pretreatment. The avoidance response was also reduced in rats treated with 6-hydroxydopamine in combination with pargyline at 14 days of age. Animals treated with 6-hydroxydopamine alone at 14 days of age showed no deficit in avoidance responding during acquisition of the avoidance task.

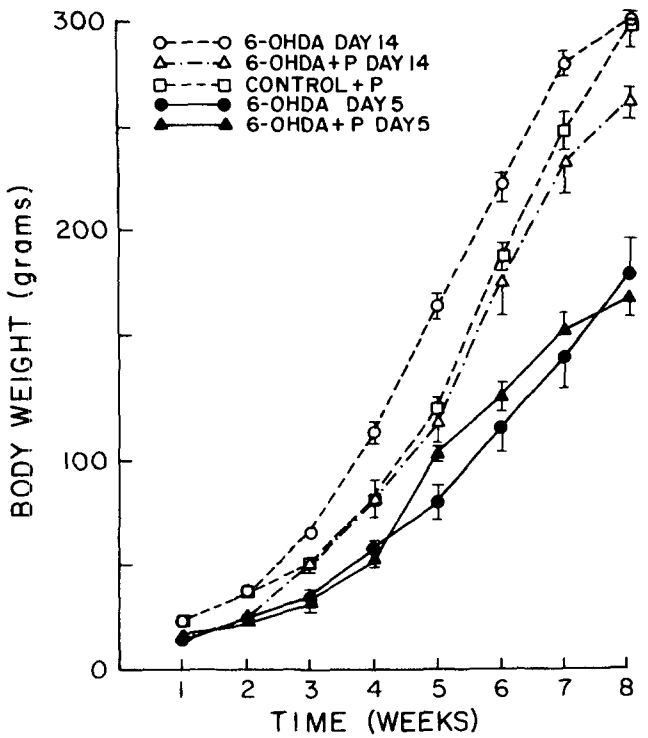

FIG. 3. Effect of pargyline on the effects produced by 6-hydroxydopamine when treated at 7 or 14 days. P indicates the administration of 40 mg/kg of pargyline 30 min before receiving 100 μg of 6-hydroxydopamine (6-OHDA) at 5 or 14 days of age.

Effect of Malnutrition During Early Neonatal Development on Brain Chemistry, Ingestive Behavior and Avoidance Behavior When Adult

In view of deficits reported to occur after neonatal malnutrition [35], it was possible that the deficits observed in the 6-hydroxydopamine treated animals were the result of an acute malnutrition produced by 6-hydroxydopamine at a critical time in development. Therefore, immature rats were subjected to a diet specifically deficient in phenylalanine and tyrosine beginning on the 5th postnatal day (see Method). After 3 weeks of this diet, the rats were given laboratory chow ad lib and tested in the various tasks at the appropriate age with animals that received the various 6-hydroxydopamine treatments.

This period of malnutrition severely reduced the growth and body weight of the animals (Table 6). Early reduction of the content of norepinephrine, dopamine as well as a decrease in the content of tyrosine hydroxylase was observed. While body weight had not returned to control levels, content of the brain catecholamines and tyrosine hydroxylase activity were, however, not significantly different from control at the time of behavioral testing (Table 6). Furthermore, deficits in the intake of sucrose solution and avoidance responding were not apparent in the animals exposed to the period of malnutrition during development (Table 6).

DISCUSSION

A major purpose of this study was to examine further various factors related to growth deficits observed in animals treated with 6-hydroxydopamine during develop-

TABLE 5

EFFECT OF 5 OR 14 DAY 6-HYDROXYDOPAMINE TREATMENTS ON BRAIN CATECHOLAMINE CONTENT, SUCROSE INGESTION AND AVOIDANCE RESPONDING

Treatment	N	Whole Brain Content (μg/g) Norepinephrine	Dopamine	5% Sucrose Intake (ml/100 g body weight)	Avoidance Responses (mean ± SEM/100 trials)
Control	20	0.39 ± 0.01	0.57 ± 0.03	53.8 ± 4.0	55 ± 4.0
6-OHDA-5 days (100 μg)	15	0.06 ± 0.008*	0.08 ± 0.01*	32.9 ± 6.3†	21 ± 3.6*
P + 6-OHDA-5 days (100 μg)	10	0.04 ± 0.006*	0.04 ± 0.003*	22.4 ± 5.0*	12 ± 2.2*
6-OHDA-14 days (100 μg)	11	0.07 ± 0.006*	0.13 ± 0.02*	–	–
6-OHDA-14 days (150 μg)	10	0.08 ± 0.008*	0.16 ± 0.03*	29.1 ± 3.2*	63 ± 5.0
P + 6-OHDA-14 days (100 μg)	10	0.03 ± 0.002*	0.07 ± 0.01*	–	–
P + 6-OHDA-14 days (150 μg)	8	0.04 ± 0.006*	0.09 ± 0.02*	27.7 ± 4.3*	37 ± 11.0

Animals were treated on 5 or 14 days of age with 6-hydroxydopamine (6-OHDA). P refers to the administration of 40 mg/kg of pargyline 30 min prior to injection of 6-OHDA. Absence of an asterisk (*) or dagger (†) indicates mean is not significantly different from control.

*$p<0.001$ when compared with control

†$p<0.01$ when compared with control

TABLE 6

EFFECT OF MALNUTRITION DURING EARLY NEONATAL DEVELOPMENT ON GROWTH, BRAIN CATECHOLAMINE CONTENT, TYROSINE HYDROXYLASE ACTIVITY, AND INGESTIVE AND AVOIDANCE BEHAVIORS

Treatment	Age (days)	Body Weight (g)	Whole Brain Content (μg/g)		Tyrosine Hydroxylase Activity ($\mu\mu$M/g/hr)	Sucrose Intake (ml/100 g B.W.)	Avoidance Responses
			Norepinephrine	Dopamine			
Early Effects							
Control	15	38 ± 1.2	0.23 ± 0.01	0.20 ± 0.01	5.3 ± 0.08	–	–
Malnourished	15	19 ± 0.4	0.19 ± 0.01*	0.17 ± 0.01*	4.0 ± 0.02†	–	–
Late Effects							
Control	60	313 ± 8	0.33 ± 0.02	0.67 ± 0.04	29.6 ± 0.9	47 ± 1.3	45 ± 10
Malnourished	60	190 ± 2	0.39 ± 0.05	0.79 ± 0.11	31.6 ± 1.7	46 ± 2.6	37 ± 10

Malnourished animals were fed a diet deficient in L-phenylalanine and L-tyrosine as described in Method.
*$p<0.01$ when compared with control
†$p<0.001$ when compared with control

ment. [7,29]. In agreement with previous findings [6, 7, 17, 29], intracisternal injection of 6-hydroxydopamine into developing animals caused a long-lasting reduction of brain catecholamine content and a decrement of growth if administered at or prior to 7 days of age [7, 17, 29]. Since peripheral administration of 6-hydroxydopamine to newborn rats has not been reported to alter growth [1, 8, 16, 17], this effect appears to result from a disruption of central catecholaminergic fibers.

In contrast to the growth deficit observed in 6-hydroxydopamine treated rats injected at 5 days of age, animals injected intracisternally with 6-hydroxydopamine at 14 days of age did not show reduced growth. Lytle *et al.* [17], who observed that immature rats became increasingly resistant to the effects of 6-hydroxydopamine with age, proposed that the increasing levels of monoamine oxidase in the developing animals may contribute to the apparent loss of potency of 6-hydroxydopamine. This suggestion was based upon observations in adult animals that MAO inhibition enhanced the ability of 6-hydroxydopamine to reduce brain catecholamine content, especially dopamine [5, 6, 13]. While inhibition of monoamine oxidase with pargyline prior to the injection of 6-hydroxydopamine on the 14th day increased the effect of 6-hydroxydopamine on brain catecholamine fibers, it did not significantly alter growth. However, survival rate in animals treated at 14 days with pargyline and 6-hydroxydopamine was not as great as in those rats that received 6-hydroxydopamine without pargyline. Thus, findings would seem to discount the level of monoamine oxidase playing a major role in the explanation of the growth deficit. Rather findings tend to support the view that interruption of central catecholamine function before a specific period of neonatal development results in reduced growth.

It was also observed that a chronic alteration in several ingestive behaviors accompanied the reduced growth produced by 6-hydroxydopamine. The animals that received the various 6-hydroxydopamine treatments in the present study were found to be comparable to other animals that have been studied in that the reduction of dopamine reduced acquisition of the avoidance response and reduced the increased fluid consumption in response to a sucrose solution [29]. In an extension of this earlier work related to ingestive behavior, the animals treated during development to reduce brain dopamine were found to display a reduced preference for saline when injected with DOCA and an absence of enhanced food consumption in response to insulin administration. Unlike animals lesioned in the lateral hypothalamus [32] food or water deprivation of animals receiving various 6-hydroxydopamine treatments during development did not result in prandial drinking as would be expected if all deficits associated with the lateral hypothalamic syndrome were related to disruption of catecholamine-containing fibers [25,34]. This finding seems to be in accord with other findings in which ingestive behavior has been examined in rats treated with 6-hydroxydopamine when adult [4, 30, 37]. Thus, rats treated during development with 6-hydroxydopamine have persistent deficits in ingestive behavior which are not compensated for by developmental processes. However, chronic disturbance of ingestive behavior alone does not appear to account for the growth retardation, because animals treated at 14 days of age also displayed a deficit in sucrose consumption but showed no alteration in growth rate. However, some caution must be exercised in this interpretation as we are presently uncertain what mechanism is responsible for the failure of animals treated with 6-hydroxydopamine to respond to sucrose by increasing fluid intake.

In order to examine the possibility that an acute period of malnutrition resulting from treatment with 6-hydroxydopamine might account for the growth deficit in rats injected at 5 days of age, mothers were given a diet deficient in L-phenylalanine and L-tyrosine. This diet produced malnutrition in infants sufficient not only to

reduce growth while the animals were on the diet but also an observable effect on catecholamine-containing fibers during the course of the malnourishment (Table 6). Even though animals remained smaller, growth rate increased considerably and catecholamine content returned to control when offered normal diet. The fact that malnourished rats had normal intake of a sucrose solution provides some evidence that ingestive behavior was not chronically altered as observed after treatment with 6-hydroxydopamine. From these data and results obtained concerning chronic deficits following the various 6-hydroxydopamine treatments in developing rats, it could be concluded that an acute alteration in food intake contributes to the growth deficit and that persistent deficits in ingestive behavior may perpetuate the reduced growth. Furthermore, this view concerning the actions of 6-hydroxydopamine would seem consistent with the finding that under-nutrition in weanling rats produced many of the symptoms of the lateral hypothalamic syndrome [31].

Finally, it is worth noting that several laboratories have recently reported data concerned with behavioral or pharmacological mechanisms in which they have utilized rats treated during development with 6-hydroxydopamine [12, 24, 29]. The present work indicates that intracisternal administration of 6-hydroxydopamine to immature rats treated with desipramine is a successful method by which to reduce dopamine specifically without affecting the content of norepinephrine. Thus, this treatment provides an additional method by which to examine the role of dopamine-containing fibers in brain in a variety of physiological, behavioral and pharmacological responses.

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent technical assistance of Marcine Kinkead, Susan Hollister, Edna Edwards, and Joseph Farmer. This project was supported by U.S.P.H.S. Grants MH-16522 and HD-03110. BRC was a postdoctoral fellow in the Neurobiology Program (MH-11107). G.R.B. is a U.S.P.H.S. Career Development Awardee (MH-00013).

REFERENCES

1. Angeletti, P. Chemical symapthectomy in newborn animals. *Neuropharmacology* **10:** 55–59, 1971.
2. Breese, G. R. Chemical and immunochemical lesions by specific neurotoxic substances and antisera. In: *Handbook of Psychopharmacology*, edited by L. L. Iversen, S. D. Iversen, and S. H. Snyder. Plenum Publishing Co., pp 137–189, 1975.
3. Breese, G. R., B. R. Cooper and R. D. Smith. Biochemical and behavioral alterations following 6-hydroxydopamine administration into brain. In: *Frontiers in Catecholamine Research*, edited by E. Usdin and S. Snyder. Pergamon Press, N. Y., 1973, pp. 701–706.
4. Breese, G. R., R. D. Smith, B. R. Cooper and L. D. Grant. Alterations in consummatory behavior following intracisternal injection of 6-hydroxydopamine. *Pharmac. Biochem. Behav.* **1:** 319–328, 1973.
5. Breese, G. R. and T. D. Traylor. Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence for selective degeneration of catecholamine neurons. *J. Pharmac. Exp. Ther.* **174:** 413–420, 1970.
6. Breese, G. R. and T. D. Traylor. Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. *Br. J. Pharmac.* **42:** 88–99, 1971.
7. Breese, G. R. and T. D. Traylor. Developmental characteristics of brain catecholamines and tyrosine hydroxylase in the rat: Effects of 6-hydroxydopamine. *Br. J. Pharmac.* **44:** 210–222, 1972.
8. Clark, D. W. J., R. Laverty and E. L. Phelan. Long-lasting peripheral and central effects of 6-hydroxydopamine in rats. *Br. J. Pharmac.* **44:** 233–243, 1972.
9. Cooper, B. R. and G. R. Breese. Relationship of dopamine neural systems to the behavioral alterations produced by 6-hydroxydopamine administration into brain. In: *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes*, edited by E. Usdin. Raven Press, N. Y., 1974, pp. 353–368.
10. Cooper, B. R., G. R. Breese, L. D. Grant and J. L. Howard. Effects of 6-hydroxydopamine treatments on active avoidance responding: Evidence for involvement of brain dopamine. *J. Pharmac. exp. Ther.* **185:** 358–370, 1973.
11. Cooper, B. R., J. L. Howard, L. D. Grant, R. D. Smith and G. R. Breese. Alteration of avoidance and ingestive behavior after destruction of central catecholamine pathways with 6-hydroxydopamine. *Pharmac. Biochem. Behav.* **2:** 369–649, 1974.
12. Creese, I. and S. Iversen. Blockage of amphetamine induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-hydroxydopamine. *Brain Res.* **55:** 369–382, 1973.
13. Fibiger, H. C., B. Lonsbury, H. P. Cooper and L. D. Lytle. Early behavioral effects of intraventricular administration of 6-hydroxydopamine in rat. *Nature* **236:** 209–211, 1972.
14. Fibiger, H. C., A. P. Zis and E. G. McGeer. Feeding and drinking deficits after 6-hydroxydopamine administration in the rat: Similarities to the lateral hypothalamic syndrome. *Brain Res.* **55:** 135–148, 1973.
15. Guthrie, H. A. and M. L. Brown. Effect of severe undernutrition in early life on growth, brain size and composition in adult rats. *J. nutr.* **94:** 419–426, 1968.
16. Lew, G. and W. Quay. Noradrenaline content of hypothalamus and adrenal gland increased by postnatal administration of 6-hydroxydopamine. *Res. communs chem. path. Pharmac.* **2:** 807–812, 1971.
17. Lytle, L. D., W. J. Shoemaker, K. Cottman and R. J. Wurtman. Long-term effects of postnatal 6-hydroxydopamine treatment on tissue catecholamine levels. *J. Pharmac. exp. Ther.* **183:** 56–64, 1972.
18. Mackay, E. M., J. W. Callaway and R. H. Barnes. Hyperalimentation in normal animals produced by protamine insulin. *J. Nutr.* **20:** 59–66, 1940.
19. Marshall, J. F., J. S. Richardson, and P. Teitelbaum. Nigrostriatal bundle damage and the lateral hypothalamic syndrome. *J. comp. physiol. Psychol.* **87:** 808–830, 1974.
20. Marshall, J. F. and P. Teitelbaum. A comparison of the eating response to hypothermic and glucoprivic challenges after nigral 6-hydroxydopamine and lateral hypothalamic electrolytic lesions in rats. *Brain Res.* **55:** 229–233, 1973.
21. Mueller, R. A., H. Thoenen and J. Axelrod. Increase in tyrosine hydroxylase activity after reserpine administration. *J. Pharmac. exp. Ther.* **169:** 74–79, 1969.
22. Musacchio, J. M., L. Julou, S. S. Kety and J. Glowinski. Increase in rat brain tyrosine hydroxylase activity produced by electroconvulsive shock. *Proc. natn. Acad. Sci. U.S.A.* **63:** 1117–1119, 1969.
23. Nagatsu, T., M. Levitt and S. Udenfriend. A rapid and simple radioassay for tyrosine hydroxylase activity. *Analyt Biochem.* **9:** 122–126, 1964.
24. Nyakas, C., A. M. L. Van Delft, J. Kaplanski and P. G. Smelik. Exploratory activity and conditioned avoidance acquisition after early postnatal 6-hydroxydopamine administration. *J. Neural Trans.* **34:** 253–266, 1973.

25. Oltmans, G. A. and J. A. Harvey. LH syndrome and brain catecholamine levels after lesions of nigrostriatal bundle. *Physiol. Behav.* **8:** 69–78, 1972.
26. Rice, K. E. and C. P. Richter. Increased sodium chloride and water intake of normal rats treated with desoxycorticosterone acetate. *Endocrinology* **33:** 106–115, 1943.
27. Richter, C. P. Increased dextrose appetite of normal rats treated with insulin. *Am. J. Physiol.* **135:** 781–787, 1941.
28. Smart, J. L. and J. Dobbing. Vulnerability of developing brain. II. Effects of early nutritional deprivation on reflex ontogeny and development of behavior in the rat. *Brain Res.* **28:** 85–95, 1971.
29. Smith, R. D., B. R. Cooper and G. R. Breese. Growth and behavioral changes in developing rats treated intracisternally with 6-hydroxydopamine: Evidence for involvement of brain dopamine. *J. Pharmac. exp. Ther.* **185:** 609–619, 1973.
30. Stricker, E. M. and M. J. Zigmond. Effects on homeostasis of intraventricular injection of 6-hydroxydopamine in rats. *J. comp. physiol. Psychol.* **86:** 973–994, 1974.
31. Teitelbaum, P., M.-F. Cheng and P. Rozin. Development of feeding parallels its recovery after lateral hypothalamic damage. *J. Physiol. Psychol.* **67:** 430–441, 1969.
32. Teitelbaum, P. and A. N. Epstein. The lateral hypothalamic syndrome: Recovery of feeding and drinking after lateral hypothalamic lesions. *Psychol. Rev.* **69:** 74–90, 1962.
33. Thierry, A. M., G. Blanc, A. Sobel, L. Stinus and J. Glowinski. Dopaminergic terminals in the rat cortex. *Science* **182:** 499–501, 1973.
34. Ungerstedt, U. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigra striatal dopamine system. *Acta physiol. scand.* (Suppl.) **367:** 95–122, 1971.
35. Winick, M. and J. Coombs. Nutrition, environment, and behavioral development. *A. Rev. Med.* **23:** 149–160, 1972.
36. Wolf, G. Effect of dorsolateral hypothalamic lesions on sodium appetite elicited by desoxycorticosterone and by acute hyponatremia. *J. comp. physiol. Psychol.* **58:** 396–402, 1964.
37. Zigmond, M. J. and E. M. Stricker. Deficits in feeding behavior after intraventricular injection of 6-hydroxydopamine in rats. *Science* **177:** 1211–1214, 1972.