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Alteration of Avoidance and Ingestive Behavior After Destruction of Central Catecholamine Pathways with 6-Hydroxydopamine

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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(5) 639–649, 1974. – Alterations of shuttle-box avoidance acquisition, ingestive behavior, and catecholamine content in 4 different parts of brain were determined following bilateral infusion of 6-hydroxydopamine into the ventral tegmental area containing A-10 dopamine cell bodies, the tegmental segment of the ascending norepinephrine pathways, the globus pallidum, or the caudate-putamen. The maximum antagonism of active avoidance acquisition occurred following placement of 6-hydroxydopamine into the ventral tegmental and caudate areas. No effect on either avoidance or ingestive behavioral measures occurred after infusion of 6-hydroxydopamine into the norepinephrine pathways. Factor analysis of behavioral and biochemical data suggested that only striatal dopamine content bore a high relationship to avoidance behavior, while ingestive behavioral measures were highly related to both striatal and limbic dopamine content. Results suggest a functional–anatomical differentiation of dopamine pathways in brain.

6-Hydroxydopamine Norepinephrine Dopamine Active avoidance Ingestive behavior

THE hypothesis that central catecholamine neural systems are of functional significance for active avoidance behavior was suggested by the observation that depression of avoidance responding produced by reserpine could be reversed by dihydroxyphenylalanine (L-DOPA) [23]. This proposal has been supported by the observation that α-methyltyrosine, a specific depletor of catecholamines [25], also disrupted active avoidance behavior [21]. Recent work with 6-hydroxydopamine, a neurotoxic agent producing destruction of central catecholamine-containing neurons when injected into brain [5,8], has also provided evidence that active avoidance behavior is dependent on intact catecholamine-containing neural systems [13]. Using methods to selectively destroy noradrenergic or dopaminergic neurons, Cooper *et al.* [11] found that depletion of dopamine in brain produced a deficit in shuttle-box avoidance behavior whereas destruction of noradrenergic fibers did not interfere with the acquisition or performance of the active avoidance response.

In the present studies, the role of the central catecholamine neural systems in active avoidance behavior was explored further by administering 6-hydroxydopamine directly into specific catecholaminergic pathways in brain tissue [26,27]. This approach was employed to delineate the pathways responsible for avoidance responding deficits observed after intracisternal 6-hydroxydopamine treatment and to define the specific area(s) of brain important to active avoidance behavior. In addition to the alterations of shuttle-box avoidance acquisition produced by placing 6-hydroxydopamine into the ascending noradrenergic and dopaminergic pathways, this paper will also describe certain changes in ingestive behavior which were noted in the course of these experiments.

METHOD

Animals

Male Sprague-Dawley (Holtzman) rats, weighing 250–350 g at the time of 6-hydroxydopamine treatment were used in this study. All animals were individually caged and maintained on a 12 hour light–dark cycle (light; 6 a.m. to 6 p.m.) with free access to food and water.

Treatment Groups

Different groups of rats were prepared with bilateral microinjections of 6-hydroxydopamine aimed at various structures containing the ascending catecholamine neural pathways. The 6-hydroxydopamine was dissolved in isotonic saline containing 0.5 percent ascorbic acid for infusion into brain tissue. Prior to treatment each rat was

anesthetized with ether and a 30 g stainless steel cannula through which injections were made was stereotaxically aimed at the specified site. When the cannula was in place, 6-hydroxydopamine (2 μg base/μl) or the ascorbic-acid-saline vehicle was injected into the brain with an infusion pump (Sage Instrument Co., White Plains, N. Y.) at a rate of 1 μl/min over a 4 min period. Thus, during the 4 min infusion period, at total dose of 8 μg of 6-hydroxydopamine was delivered in a total volume of 4 μl [16,26].

Injection sites were located with the aid of Ungerstedt's [26] mapping of the central catecholamine pathways. The following coordinates, with reference to the atlas of zero of König and Klippel [19], were derived from examination of histological material in rats weighing 275–325 g and were used for injection of 6-hydroxydopamine into the following structures: the ventral tegmental area of Tsai containing A-10 dopamine cell bodies and dopamine axons arising from A-8 and substantia nigra (A + 3.0, L ± 1.5, V − 1.8); the posterior portion of the globus pallidum just lateral to the internal capsule which contains the nigro-striatal DA tract (A + 8.0, L ± 3.0, V −1.5); the dorsal caudate nucleus (A + 10.0, L ± 3.0, V −0.0); and the ventral caudate nucleus (A + 10.0, L ± 3.0, V −2.0). For some animals, injections were placed both in the ventral and dorsal region of the caudate nucleus described by the coordinates specified above. Other animals referred to as site controls received infusions of the saline-ascorbic acid vehicle at each set of coordinates corresponding to the 6-hydroxydopamine treatment groups described above. A group of intact, unoperated controls was included with each set of experimental animals prepared.

Behavioral Procedures

Weight change after surgery was used as an indication of the acute interruption of ingestive behavior which can be produced by 6-hydroxydopamine [7,27]. Each animal was weighed at the time of surgery and 3 days after receiving the various 6-hydroxydopamine or vehicle injections. Animals which lost over 20 g were given 5 to 10 ml of a warm Meritene solution (Doyle Pharmaceutical Co., Minneapolis, Minn.) by oral intubation and in cases of more severe weight loss, treated rats were also given a 5 ml daily injection (i.p.) of a warm protein hydrolysate solution, USP. Treatment was continued until animals were capable of maintaining body weight on a diet of rat chow and water for at least 10 days. Following recovery from the acute effects of 6-hydroxydopamine or after a minimum of 20 days after treatment for groups not displaying an acute alteration of ingestive behavior, consumption of a 5% sucrose solution substituted for water was measured as an index of the chronic alteration of ingestive behavior reported to accompany intracisternal injection of 6-hydroxydopamine [7]. Water consumption over a 24 hr period was determined by weighing the drinking bottles at the beginning and end of a test period.

After determination of sucrose consumption, rats were tested for acquisition of a shuttle-box avoidance response using a modified automated shuttle-box (LeHigh Valley, Inc.) which has been described previously [11,13]. A single 100 trial session was used to examine acquisition. One minute after a rat was placed in the shuttle-box, the session began with the lighting of a lamp (conditioned stimulus). The light remained on until the animal crossed to the opposite compartment, thereby avoiding shock, or for a maximum interval of 10 sec. If the animal did not cross to the other compartment during the 10 second interval, it received a continuous electric shock of 0.8 mA which was terminated when the rat escaped to the opposite compartment, or at the end of 5 sec. Each avoidance response, escape response, or failure to escape was followed by a 30 sec interval before initiation of the next trial. Performance during each successive 25 trial period was recorded for 100 trials and used to assess acquisition.

Shuttle-box avoidance performance was determined for animals displaying acquisition of the avoidance response. Each rat was tested for 25 trials a day over a 5-day period and then pretreated with 40 mg/kg L-α-methyltyrosine (i.p.) 4 hours before a 25 trial session on the 6th day [11,12]. A session 24 hr after drug treatment was used to determine if performance had returned to pretreatment levels.

Drugs

6-Hydroxydopamine was purchased from Regis Chemical Co. (Chicago, Ill.) and prepared for infusion into brain tissue as previously described. L-α-methyltyrosine was a gift of Merck Sharp & Dohme (West Point, Pa.) and was prepared for injection (i.p.) at a concentration of 10 mg/ml by suspending the drug in distilled water.

Catecholamine Determination

At the completion of the behavioral experiments, rats were killed by cervical fracture. The brain was removed from each animal and immediately dissected into 4 parts which shall be referred to as hypothalamus, striatum, septal-forebrain, and dorsal cortex plus hippocampus. Prior to dissection, each brain was placed cortex down on an ice-cold petri dish so that the ventral surface was exposed. The first cut was made in the sagittal plane, beginning just caudal to the mammillary bodies and terminating just anterior to the superior colliculi. The brain stem region and cerebellum were removed. When appropriate, the brain stem was kept for histological examination. A second sagittal cut was then made at the level of the optic chiasm through to the cortex in a plane perpendicular to the petri dish. After the striatal tissue was excised, the remaining septal and cortical tissue anterior to this cut constituted the septal-forebrain region. The hippocampus and the overlying cortex were then peeled away from the tissue block caudal to the cut at the level of the optic chiasm and this tissue constituted the hippocampus plus cortex region. The small amount of remaining striatal tissue was excised from this block of tissue and added to that removed from the anterior section to constitute the striatum. The hypothalamus was dissected using the anterior commissure as a guide to separate the hypothalamic tissue from the overlying thalamus, and the cortical margins were then trimmed away. Thus the hypothalamus was bounded rostrally by the optic chiasm, caudally by the cortical margins and medial border of the internal capsule. Weights ± S.E.M. (N = 116) for the brain parts were 0.111 ± 0.001 mg for hypothalamus, 0.136 ± 0.001 mg for striatum, 0.428 ± 0.003 mg for septal-forebrain, and 0.548 ± 0.004 mg for hippocampus plus cortex.

After dissection, each part was homogenized in 10 ml of ice-cold 0.4N perchloric acid and the homogenate kept frozen at −20° C until analyzed within the next 24 to 48 hr. After the homogenate was thawed and centrifuged, an aliquot of the supernatant was stirred with alumina and

transferred to a column as previously described [8,9]. Catecholamines were eluted from the alumina with 0.2N acetic acid and norepinephrine and dopamine in the eluate were analyzed spectrofluormetrically [3, 4, 15].

Histology

Representative animals from the various 6-hydroxydopamine treated groups and vehicle control groups were used to evaluate histological changes produced by the injection of 6-hydroxydopamine. After each brain was embedded in paraffin, 16 μ sections were taken from the area containing the cannula tip and were stained according to the method of Klüver and Barrera [18] for microscopic examination of the injection site.

Statistical Analysis

Differences between the 6-hydroxydopamine treated groups, vehicle controls, and intact animals with regard to catecholamine concentrations in various parts of brain, weight change, sucrose consumption, and active avoidance acquisition were initially evaluated with an one-way analysis of variance. Differences between treatment groups were examined using the Duncan's Range test. Dunnett's t test was used when it was of interest to compare all treatment groups to control. Effects on active avoidance acquisition were evaluated using repeated measures two way analysis of variance. Stepwise discriminant analysis [1] was used to determine the extent to which animals could be placed within the appropriate 6-hydroxydopamine injection groups on the basis of the homogeneity of their biochemical and behavioral values. Possible interrelationships between biochemical and behavioral measures were examined using factor analysis [1].

RESULTS

Histological and Biochemical Effects of 6-Hydroxydopamine Injections

The typical localization of the injection sites used in these experiments, based on examination of histological material from representative animals, are shown in Fig. 1 in drawings from the atlas of König and Klippel [19]. A diagrammatic representation of the ascending noradrenergic and dopaminergic neural systems, based on the work of Ungerstedt [26], is also included to show the approximate relationship of the injection sites with respect to these pathways (Fig. 1).

The effect of injecting 6-hydroxydopamine into the noradrenergic and dopaminergic pathways on catecholamine concentrations in striatum, hypothalamus, septal forebrain, and dorsal cortex plus hippocampus are presented in Table 1. When injected into the ventral tegmental area containing the A-10 dopamine cell bodies and dopamine axons from the substantia nigra (see Fig. 1), content of dopamine as well as norepinephrine was reduced in all brain regions (Table 1). Administration of 6-hydroxydopamine into the ascending norepinephrine pathways (Fig. 1), on the other hand, resulted in a relatively specific depletion of norepinephrine in all parts of brain with little effect on dopamine. Injection of 6-hydroxydopamine into the region of the globus pallidum was found to reduce the content of norepinephrine and dopamine in the striatum, septal-forebrain, and dorsal cortex plus hippocampal regions, while sparing catecholamine containing neurons in the hypothalamus. When 6-hydroxydopamine was injected into both dorsal and ventral areas of the caudate nucleus, striatal dopamine was reduced to approximately the same degree as observed after injection of 6-hydroxydopamine into the ventral tegmental area. A reduction of dopamine content in the septal-forebrain region and differing degrees of norepinephrine depletion in several areas of brain obtained following injection of 6-hydroxydopamine into caudate may be due to some diffusion from the injection site back up the cannula tract.

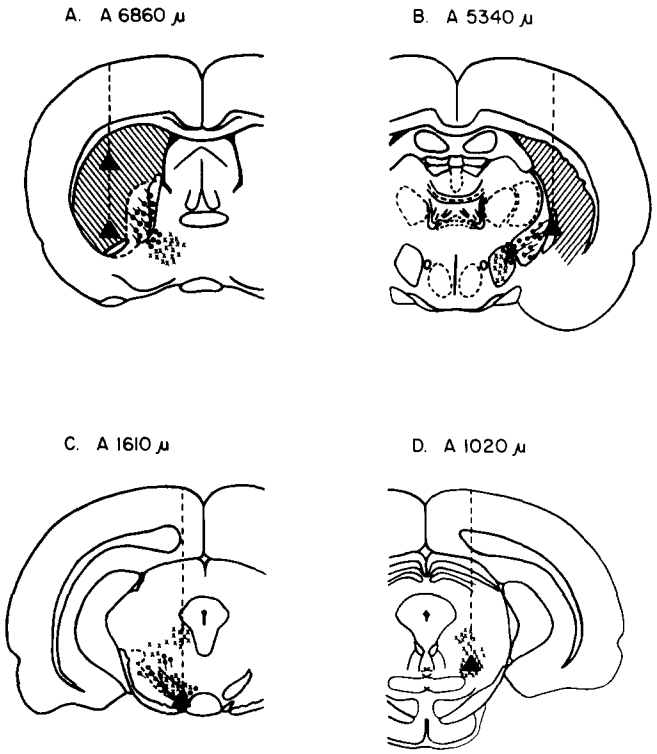

FIG. 1. Schematic representation of the infusion sites in relation to simplified drawings from the atlas of König and Klippel [19]. The approximate location of each injection with reference to Ungerstedt's [26] mapping of the ascending norepinephrine pathways (x) and the dopamine cell bodies and axons (•) are indicated. Shaded areas (///) indicate dopamine terminals in the caudate-putamen. (A) Dorsal and ventral caudate injection sites (B) Globus pallidum injection site (C) Ventral tegmental area of Tsai injection site (D) Norepinephrine bundle injection site in pontine reticular formation. The specific stereotaxic coordinates for these sites using 300 g rats are described in the methods section of the text. Note that the actual coordinates used vary from the atlas coordinates in the anterior-posterior plane since rats used here were twice as large as those used to construct the atlas.

Of the various injection control groups, a small effect on amines was noted in rats with vehicle injections in the ventral tegmental region. This group was found to have slight but significant reduction of norepinephrine in the dorsal cortex and hippocampus and in the septal-forebrain area. Some reduction of dopamine in the hippocampus and cortex region was also obtained. These effects produced by the vehicle injection into the ventral tegmental region may be the result of the cannula tip passing through the ascending norepinephrine containing pathways just dorsal to the

TABLE 1

EFFECTS OF 6-OHDA INJECTIONS ON CATECHOLAMINE CONTENT IN BRAIN PARTS AND ON ACTIVE AVOIDANCE ACQUISITION

	Catecholamine Content (μg/gram)*								
	Striatum		Hypothalamus		Septal-forebrain		Cortex and Hippocampus		
Injection Site	NE	DA	NE	DA	NE	DA	NE	DA	Avoidance Responses During Last 25 Trials of Acquisition
Intact Control (19)	0.33 ±0.01	7.60 ±0.26	1.91 ±0.08	0.34 ±0.07	0.51 ±0.02	0.76 ±0.04	0.40 ±0.01	0.19 ±0.01	18.5 ±2.1
6-OHDA Injection									
Ventral Tegmentum (19)	0.13‡ ±0.03	2.28‡ ±0.53	0.99‡ ±0.13	0.18 ±0.05	0.20‡ ±0.03	0.30‡ ±0.07	0.12‡ ±0.04	0.11 ±0.03	7.0‡ ±2.0
Norepinephrine Pathways (11)	0.05‡ ±0.01	8.21 ±0.53	0.40‡ ±0.06	0.30 ±0.07	0.07‡ ±0.02	0.73 ±0.03	0.06‡ ±0.02	0.28 ±0.06	20.0 ±2.2
Globus Pallidum (8)	0.18‡ ±0.03	3.73‡ ±0.30	1.83 ±0.17	0.42 ±0.03	0.37† ±0.03	0.60 ±0.05	0.21‡ ±0.02	0.07‡ ±0.02	11.1† ±2.5
Dorsal and Ventral Caudate (10)	0.25† ±0.02	2.36‡ ±0.30	1.68 ±0.08	0.39 ±0.02	0.28‡ ±0.02	0.52‡ ±0.05	0.16‡ ±0.03	0.22 ±0.03	5.8‡ ±1.6
Ventral Caudate (8)	0.28 ±0.02	2.89‡ ±0.53	1.85 ±0.17	0.25 ±0.13	0.35‡ ±0.03	0.60 ±0.01	0.25‡ ±0.03	0.15 ±0.02	11.0† ±2.7
Dorsal Caudate	0.29 ±0.02	5.17‡ ±0.38	1.86 ±0.19	0.39 ±0.08	0.35‡ ±0.03	0.70 ±0.01	0.23‡ ±0.02	0.13 ±0.04	17.6 ±3.0
Vehicle Injection									
Ventral Tegmentum (10)	0.31 ±0.53	7.75 ±0.53	2.03 ±0.76	0.26 ±0.11	0.43† ±0.02	0.70 ±0.08	0.33† ±0.01	0.14† ±0.01	19.8 ±2.8
Norepinephrine Pathways (6)	0.30 ±0.03	8.36 ±0.84	2.00 ±0.19	0.29 ±0.18	0.49 ±0.03	0.73 ±0.04	0.39 ±0.01	0.27 ±0.06	21 ±2.0
Globus Pallidum (7)	0.37 ±0.03	7.37 ±0.45	2.04 ±0.13	0.36 ±0.14	0.54 ±0.05	0.81 ±0.07	0.37 ±0.01	0.16 ±0.04	17.6 ±3.3
Dorsal and Ventral Caudate (8)	0.30 ±0.02	7.45 ±0.30	2.12 ±0.15	0.55 ±0.39	0.53 ±0.07	0.73 ±0.01	0.38 ±0.03	0.18 ±0.04	18.5 ±2.6

*Values represent the mean ± S.E.M. for either catecholamine content in brain parts (μg/gram) or avoidance responses made during the final 25 trial period of acquisition, as indicated. Injection sites are shown in Fig. 1, and the brain parts are described in Methods. Numbers in parenthesis indicate the number of rats in each group.

†$p<0.05$ when compared with intact control using Dunnett's t-test.

‡$p<0.01$ when compared with intact control using Dunnett's t-test.

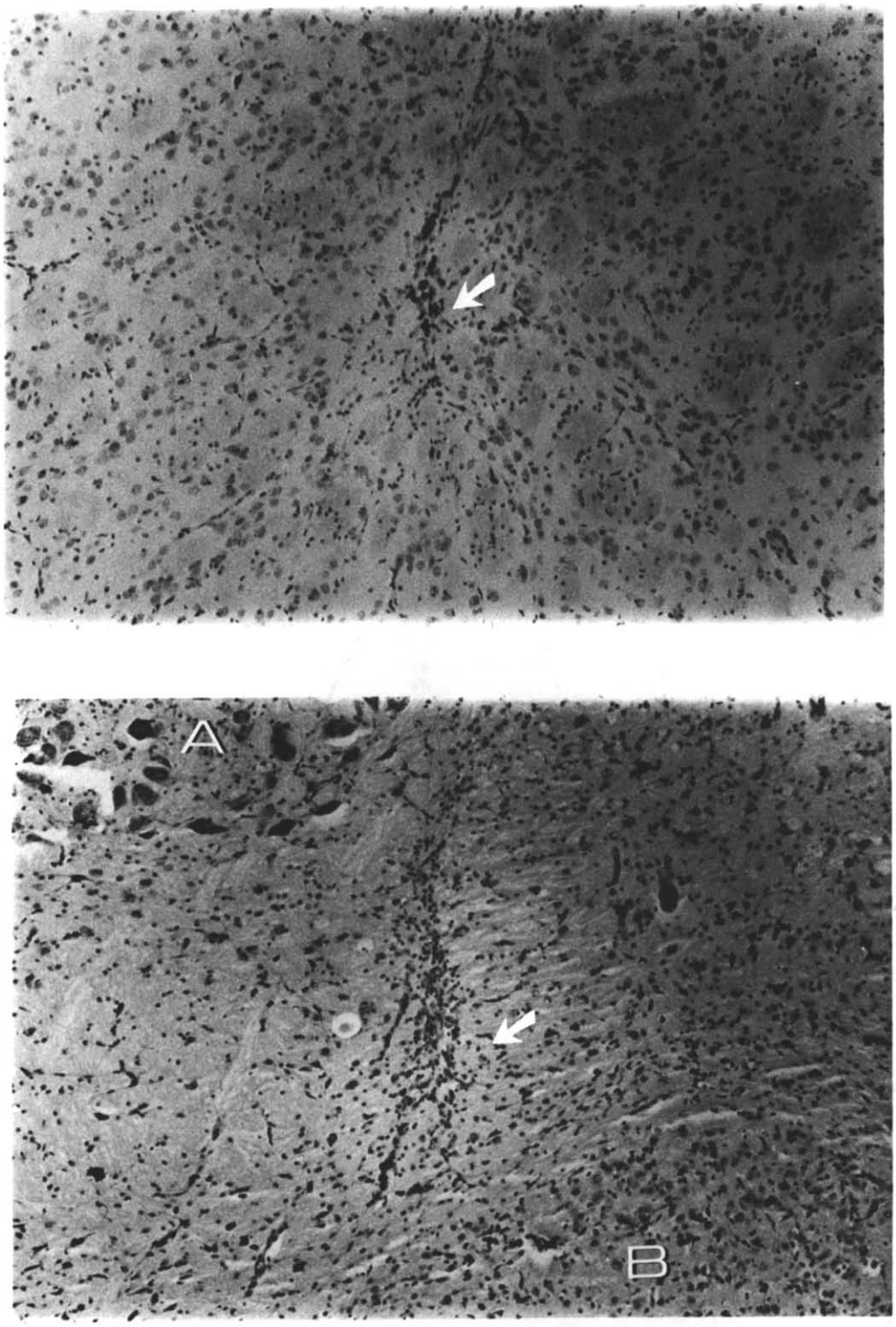

FIG. 2. Photomicrographs of representative brain sections from 6-hydroxydopamine treated rats showing cannula tips (←) in either the caudate (top) or the ventral tegmental area (bottom). Sections were stained for both cells and fibers using the method of Klüver and Barrera [18]. Intact neurons in the red nucleus (A) and interpeduncular nucleus (B) adjacent to the cannula tip in the ventral tegmental area (bottom) suggest that lesioning of non-catecholaminergic neurons did not occur in areas proximal to the 6-hydroxydopamine injection site.

injection site and penetrating the A-10 dopamine cell bodies.

Examination of the histological material obtained from representative animals indicated that there was no evidence of nonspecific lesioning associated with the injection of 6-hydroxydopamine. Representative photomicrographs of sections from animals treated with 6-hydroxydopamine can be seen in Fig. 2, which shows the area at the termination of a cannula tract situated either in the region of the A-10 dopamine cell bodies medial to the substantia nigra or in the caudate nucleus.

Since it was not possible to verify histologically the location of the injection sites in most animals whose brains were used for catecholamine determinations, a stepwise discriminant analysis was used as an evaluation of the consistency of the effects of 6-hydroxydopamine. This analysis, by comparing 16 biochemical and behavioral variables available for each rat with the profile of the means and variances of the different 6-hydroxydopamine or control treatments, allowed a post hoc assignment of rats to the various groups. Table 2 shows the results of the analysis which indicate that there was considerable homogeneity of the effects of the 6-hydroxydopamine infusions within the treatment groups.

Effect of 6-Hydroxydopamine Injections on Active Avoidance Acquisition

In accord with previous results which implicated dopamine neural systems in the maintenance of avoidance responding, [10,11] analysis of the data revealed that injection of 6-hydroxydopamine into various brain regions associated with the nigrostriatal dopamine pathway produced deficits in the acquisition of the shuttle-box avoidance response (Fig. 3).

Duncan's range test after appropriate analysis of variance revealed that administration of 6-hydroxydopamine into the ventral tegmental area (A-10) caused a significant reduction of performance during the last two 25 trial periods of acquisition ($p<0.05$). Even though this latter procedure reduced dopamine as well as norepinephrine in several brain regions, results of avoidance acquisition tests using rats with 6-hydroxydopamine placed in the dorsal and ventral ascending noradrenergic pathways argued against this deficit in avoidance responding arising from a disruption of noradrenergic pathways (Table 1). Performance of this latter group did not differ from control during any 25 trial period of acquisition (Fig. 3), in spite of the fact that norepinephrine was reduced by 85% in most brain areas (Table 1).

TABLE 2

STEPWISE DISCRIMINANT ANALYSIS

Treatment Groups	N†	Treatment Group Assignment* CONT	VT	NE.P	GP	D&VC	VC	DC
Combined Controls (CONT)	50	**50**						
Ventral Tegmentum (VT)	19	2	**14**			2	1	
Norepinephrine Pathways (NE.P)	11			**11**				
Globus Pallidum (GP)	8		1		**7**			
Dorsal and Ventral Caudate (D&VC)	10					**9**	1	
Ventral Caudate (VC)	8						**8**	
Dorsal Caudate (DC)	8							**8**

*Values represent the number of rats assigned to the various 6-hydroxydopamine or control treatment groups by a stepwise discriminant analysis. This analysis based on the sixteen biochemical and behavioral measurements available for each rat, determined how closely the animal matched the mean and variance of these measures for each group in making an "assignment".

†N refers to the total number of animals in each group, and can be compared with the values in bold face in the table which represent rats which were designated members of that group by the analysis. Values not in bold face in the same row represent those rats which had biochemical and behavior values more closely matching those produced by the other treatments.

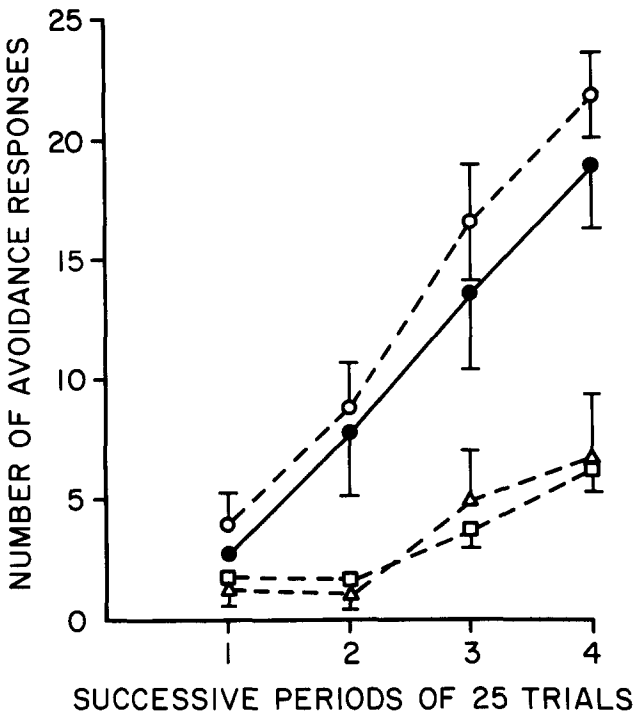

FIG. 3. Effects of 6-hydroxydopamine injection into various brain regions on acquisition of a shuttle-box avoidance response. Treatment groups consisted of intact controls (●——●), and rats receiving infusions of 6-hydroxydopamine either into the ascending norepinephrine pathways (○– – –○), the ventral tegmentum (△– –△), or into both dorsal and ventral caudate (□– –□). Details of these treatments are described in Methods and location of the injection sites is shown in Fig. 1. Repeated measures analysis of variance revealed a significant groups X trial block interaction, $F(30,221) = 1.96$, $p<0.01$). Subsequent one way analyses showed significant effects related to injection group at $p<0.05$ for trial block 2, $F(10,104) = 2.11$ and $p<0.01$ for trial blocks 3 and 4. $F(10,104) = 3.24$ and 4.36, respectively, for both tests. See text for further statistical comparisons.

Evidence supporting a possible role for the nigrostriatal pathway was provided by the finding that the placement of 6-hydroxydopamine into both dorsal and ventral regions of the caudate produced results similar to those obtained after injection of 6-hydroxydopamine into ventral tegmentum medial to the substantia nigra. Performance after these two treatments was significantly less than all other groups during the last two 25 trial periods ($p<0.05$; see Fig. 3). Rats which received 6-hydroxydopamine into the globus pallidum or into the ventral portion of the caudate nucleus had significantly fewer avoidance responses only during the last 25 trial period of acquisition (Table 1; not shown in Fig. 3).

Enhanced Sensitivity to Depressant Effects of α-Methyltyrosine

Previous studies have demonstrated that 6-hydroxydopamine treated rats, if capable of performing a behavioral task, show increased sensitivity to α-methyltyrosine [10, 11, 12, 22]. Therefore, following the tests for acquisition of the active avoidance response, those rats which made a total of 10 or more avoidance responses during the last 25 trial period of 5 days of performance testing were given 40 mg/kg of L-α-methyltyrosine. As shown in Table 3, an enhanced sensitivity to the depressant effects of α-methyltyrosine was observed in those animals that received 6-hydroxydopamine in the region of the ventral tegmentum or the ventral caudate. Although the 40 mg/kg dose of α-methyltyrosine decreased performance of rats with injections into the globus pallidum, they were not significantly different from controls. The animals selected from the intact control group and the animals selected from the vehicle injection control groups were not affected by this α-methyltyrosine treatment. Performance of six rats selected from the group with selective destruction of norepinephrine pathways was also not affected by the α-methyltyrosine treatment. Since shuttle-box avoidance performance of those rats which had dopamine as well as norepinephrine reduced (Table 3) was depressed by the 40 mg/kg dose of α-methyltyrosine, while performance of rats with large depletions of just norepinephrine was not, these findings are consistent with previous reports indicating that the enhanced sensitivity to α-methyltyrosine is related to dopamine remaining in brain after 6-hydroxydopamine treatment [10,11]. The view that dopamine is of functional significance to avoidance responding is further supported by the observation that rats which displayed acquisition of the avoidance response did not have as great a reduction of dopamine content in various brain areas as those rats which did not display acquisition of the avoidance response (Table 1 vs 3).

Effects of 6-Hydroxydopamine Injections on Ingestive Behavior

Although the initial emphasis of this work was to be concerned with active avoidance, weight records kept after surgery provided a means to infer acute changes in ingestive behavior after 6-hydroxydopamine treatment. Consistent with the proposal that damage to the nigro-striatal dopamine system produces an acute aphagia and adipsia [27], rats in which 6-hydroxydopamine was placed into the ventral tegmentum lost weight, while rats injected with 6-hydroxydopamine into the ascending dorsal and ventral noradrenergic pathways gained weight during the same 3 day observation period (Table 4). Rats with 6-hydroxydopamine injections in the region of the globus pallidum also lost weight, whereas no loss of weight was produced by injections of 6-hydroxydopamine into the caudate nucleus. This latter finding was especially surprising, since striatal dopamine was reduced by this latter treatment to the same extent as treatment with 6-hydroxydopamine at the level of the A-10 dopaminergic cell bodies in the ventral tegmentum (Table 2). Further complicating the relationship of striatal dopamine depletion to acute loss of weight after 6-hydroxydopamine treatment was the finding that bilateral injection of 6-hydroxydopamine into the globus pallidum produced a somewhat greater weight loss than observed after injection into the ventral tegmentum, while having significantly less effect on striatal dopamine than did the ventral tegmentum treatment with 6-hydroxydopamine ($p<0.01$).

Since previous work [7] has demonstrated that rats with severe depletion of dopamine in brain produced by intracisternally injected 6-hydroxydopamine fail to increase fluid intake to the same degree as control rats when a 5% sucrose solution is substituted for water, this measure was used to assess chronic effects of these treatments on ingestive behavior. Those animals receiving injections of 6-

TABLE 3

ACTIVE AVOIDANCE PERFORMANCE AFTER 40 MG/KG L-α-METHYLTYROSINE AND CATECHOLAMINE CONTENT IN BRAIN PARTS*

			Catecholamine Content (mg/g)							
	Avoidance Responses		Striatum		Hypothalamus		Septal-forebrain		Cortex and Hippocampus	
Treatment Groups	Pre-drug	L-αMT	NE	DA	NE	DA	NE	DA	NE	DA
Combined Controls (20)	22.7 ±0.6	22.2 ±0.8	0.32 ±0.02	7.71 ±0.30	2.02 ±0.08	0.36 ±0.07	0.50 ±0.02	0.75 ±0.04	0.37 ±0.01	0.19 ±0.01
Ventral Tegmentum† (5)	16.4 ±1.3	4.0‡ ±1.7	0.19 ±0.05	3.19 ±1.0	1.16 ±0.21	0.20 ±0.13	0.25 ±0.06	0.16 ±0.05	0.21 0.06	0.07 ±0.01
Globus Pallidum† (3)	22.3 ±2.1	15.0 ±7.1	0.20 ±0.05	4.00 ±0.3	2.03 ±0.26	0.14 ±0.07	0.24 ±0.10	0.33 0.07	0.26 0.01	0.08 ±0.03
NE Pathways (6)	24.0 ±0.7	23 ±1.0	0.05 ±0.01	8.36 ±1.0	0.36 ±0.09	0.75 ±0.05	0.05 0.02	0.76 0.02	0.05 ±0.02	0.22 ±0.05
Ventral Caudate† (6)	22.0 ±2.0	8.1‡ ±3.0	0.27 ±0.02	3.50 ±0.2	1.98 ±0.38	0.34 ±0.17	0.33 ±0.02	0.22 0.08	0.24 ±0.02	0.09 ±0.03
Dorsal Caudate (6)	19.8 ±3.1	17.4 ±3.4	0.29 ±0.03	5.02 ±0.5	1.80 ±0.25	0.35 0.12	0.34 ±0.02	0.32 0.06	0.21 ±0.02	0.07 ±0.02

*Values represent the mean ± S.E.M. for either shuttle-box avoidance performance prior to (Pre-drug) and 4 hours after a 40 mg/kg dose of L-α-methyltyrosine (i.p.) or the catecholamine content in brain parts. Catecholamine content was determined at least two weeks after α-MT. Animals are a part of the groups shown in Table 1. Numbers in parentheses represent the number tested.

†The number of rats tested in these groups represents the total number which displayed acquisition and performance. Animals in other groups were selected at random from the total number treated.

‡$p<0.01$ when compared with Intact Control, Dunnett's *t*-test.

TABLE 4

EFFECTS OF DIFFERENT 6-HYDROXYDOPAMINE TREATMENTS ON POSTOPERATIVE WEIGHT CHANGE AND SUCROSE CONSUMPTION

		24 Hour Fluid Intake (ml)	
Treatment Group*	Weight Change 3 Days After Treatment (gm)	Water	Sucrose (5%)
Combined Controls	10 ±1.2	36 ±2	143 ±10
Ventral Tegmentum	−20.0† ±6	34 ±2	92† ±10
Norepinephrine Pathways	8.0 ±2.	40 ±3	136 ±9
Globus Pallidum	−27.3† ±6.	34 ±2	54† ±5
Dorsal & Ventral Caudate	6.3 ±2	31 ±3	144 ±11
Ventral Caudate	5.3 ±4	37 ±1	149 ±11
Dorsal Caudate	11.0 ±2.	39 ±2	148 ±14

*Treatment groups are described in Methods and Figure 1.

†$p<0.01$, when compared with control, Dunnett's *t*-test.

hydroxydopamine in the ventral tegmentum and globus pallidum drank significantly less sucrose in 24 hours than did the vehicle controls, intact controls, and rats receiving injections of 6-hydroxydopamine in the caudate or in the norepinephrine-containing pathways (Table 4). Like weight loss, sucrose consumption did not appear to bear a strict correlation to striatal dopamine content.

Factor Analysis

In order to gain insight into the interrelationships of the biogenic amine alterations and the behavioral changes produced by injection of 6-hydroxydopamine, data from the intact control group and 6-hydroxydopamine treatment groups were subjected to factor analysis [1]. Since the site controls were similar to intact controls with regard to almost every measure, data from site control groups were not included in the analysis which is summarized in Table 5.

The factor analysis revealed that there were 4 independent factors which together accounted for 0.41 of the total variance in the intercorrelation matrix. Factor 1 accounted for 35.5 percent of this variance, Factor 2 accounted for 32.2 percent, Factor 3 accounted for 21.0 percent, and Factor 4 accounted for the remaining 11.3 percent of the variance.

Table 5 shows that the first factor was composed primarily of the active avoidance measures, and hence was designated an active avoidance factor. Caudate dopamine was the only amine which had a loading on the first factor greater than the arbitrary cut off of 0.333. The high relationship of norepinephrine content in the various brain parts with Factor 2, suggested that this factor was primarily a norepinephrine factor. Weight change 3 days after surgery also had a weak loading on this factor. The third factor appeared to be related to dopamine content in the striatum, in the septal-forebrain, and in the hippocampal-cortical dissections, as well as to weight change and sucrose consumption. Because of its relationship to these latter measures, it was arbitrarily labeled an ingestive behavior factor. The fourth factor appeared to be related to water and sucrose consumption. It did not bear a relationship to any of the biogenic amines above the criterion of 0.333, and may be related to the general condition of the rat at the time of testing.

TABLE 5

FACTOR ANALYSIS OF BIOCHEMICAL AND BEHAVIORAL EFFECTS OF 6-HYDROXYDOPAMINE*

Variables†	Active Avoidance 1	Norepinephrine 2	Ingestive Behavior 3	4
Striat NE		0.88		
Striat DA	0.53		0.62	
Hypo NE		0.89		
Hypo DA				
S-F NE		0.89		
S-F DA			0.68	
Ctx. & H. NE		0.84		
Ctx. & H. DA			0.61	
Weight Chng.		0.34	0.70	
Water Intake				0.84
Sucrose Intake			0.49	0.59
Avoids 1–25	0.78			
Avoids 26–50	0.88			
Avoids 51–75	0.87			
Avoids 76–100	0.76			
Avoids Total	0.93			

*Values represent factor coefficients (or "loadings") which indicate the magnitude of relationship between the sixteen biochemical and behavioral measures taken in this study with each of four orthogonal factors derived by factor analysis [1]. Only those factor loadings greater than 0.33 were included in the table. Factors were assigned arbitrary names as suggested by the magnitude and pattern of the relationship of the factor with the dependent variables.

†Abbreviations for the variables are as follows: NE, norepinephrine; DA, dopamine; striat, striatum; hypo, hypothalamus; S-F, septal forebrain; Ctx. & H, cortex and hippocampus; weight chng., weight change; avoids, avoidance responses. Numbers following "avoids" indicates a particular 25 trial period during the 100 trial acquisition session (see Fig. 3 and Methods).

DISCUSSION

Over the past few years, several lines of evidence have accumulated to suggest an important role for monoamine neural systems in brain for both active avoidance responding and ingestive behavior. With regard to avoidance responding, the observation that L-DOPA could reverse the depression of motor activity and active avoidance responding produced by reserpine [23] focused attention on the importance of catecholamines in brain for conditioned avoidance behavior. Later work, in which a dopamine-β-hydroxylase inhibitor and L-DOPA were given to reserpinized animals, led to the proposal that norepinephrine plays a significant role in the effective performance of conditioned avoidance responding, but dopamine must be present in order to maintain this function [2].

More recent examination of the relative contribution of norepinephrine and dopamine to active avoidance behavior using 6-hydroxydopamine has supported certain aspects of this latter view. Large, selective depletions of dopamine in brain after intracisternal injection of 6-hydroxydopamine have been found to block shuttle-box avoidance acquisition and performance while selective destruction of noradrenergic neurons failed to produce these deficits [10,11].

The importance of intact dopaminergic systems for active avoidance behavior is also indicated by results from the present experiments. Interruption of the nigro-striatal tract at the level of the substantia nigra, globus pallidum, or the caudate nucleus produced varying deficits in the acquisition of the active avoidance response. The greatest deficit occurred when 6-hydroxydopamine was placed into the region of the ventral tegmentum or into the dorsal and ventral caudate nucleus. Injection of 6-hydroxydopamine at both of these sites produced depletion of striatal dopamine (Table 1), the only tissue amine variable found to have a relationship to shuttle-box avoidance responding when data were subjected to factor analysis (Table 5). Even though the injections having an effect on dopamine in brain also reduced norepinephrine, the large specific reduction of this amine produced by injections of 6-hydroxydopamine into the ascending norepinephrine pathways did not significantly alter acquisition of the shuttle-box avoidance response reducing the likelihood that the blockade was related to a disruption of noradrenergic fibers. Thus, it can be conluded that a dopamine pathway arising from the substantia nigra coursing through the ventral tegmentum and terminating in the caudate region is of functional importance to active avoidance behavior.

In these experiments, however, not all animals receiving 6-hydroxydopamine injections into sites which typically antagonized avoidance acquisition showed deficits characteristic of dopamine depletion. Since earlier studies with intracisternally administered 6-hydroxydopamine indicated that animals which had no chronic behavioral deficits after treatment were more sensitive to the depressant actions of α-methyltyrosine, rats in this study which showed acquisition and performance after 6-hydroxydopamine were treated with this catecholamine synthesis inhibitor. In confirmation of the previous work using intracisternal injection of 6-hydroxydopamine to deplete catecholamine content [11,14], only those animals with dopamine reduced by 6-hydroxydopamine in the various parts of brain displayed an enhanced sensitivity to α-methyltyrosine treatment (Table 3), presumably due to the additional depletions produced by this drug [14]. Rats in which noradrenergic pathways were destroyed resembled control animals in that they both displayed no behavioral depression after α-methyltyrosine. It was also found that animals which acquired the avoidance response from the groups injected in the ventral tegmentum and ventral caudate typically had dopamine values in brain parts that were greater than the means of their respective treatment groups (Table 3). Thus, these results can be interpreted as providing further support for an involvement of dopamine in active avoidance behavior.

In addition to the role of the nigro-striatal dopamine system in active avoidance behavior, this pathway has also been proposed to be of importance to the regulation of certain aspects of ingestive behavior [27]. Indeed, an acute period of aphagia and adipsia results when 6-hydroxydopamine is administered into the dopaminergic cell bodies at the level of the substantia nigra or after the relatively selective depletion of whole brain dopamine following the intracisternal injection of 6-hydroxydopamine in desipramine treated rats [7,27]. After recovery from these acute effects, a chronic deficit in the ability of 6-hydroxydopamine-treated rats to increase consumption of a 5% sucrose solution substituted for water has been observed [7,24]. In the present study, weight loss during a 3-day period after injection, and consumption of a sucrose solution substituted for water were examined. Rats receiving 6-hydroxydopamine injections into the ventral tegmentum or globus pallidum were the only groups which lost weight after treatment, indicating an acute interruption of eating and drinking. Furthermore, both groups also drank significantly less sucrose than the other groups after recovery from the acute effects. Rats with selective destruction of ascending norepinephrine pathways did not show either of these deficits. In this regard, the present results support the view that a dopaminergic pathway originating in the region of the substantia nigra is implicated in ingestive behavior [27]. However, the fact that destruction of catecholaminergic terminals in the caudate did not produce these deficits suggests that dopaminergic terminals other than those affected by injection of 6-hydroxydopamine into this structure are important for maintaining normal ingestive function. Factor analysis also supported the possibility of separate dopamine systems being involved in maintenance of active avoidance and ingestive behavior. In addition to striatal dopamine, dopamine in septal-forebrain and hippocampal-cortical regions bore a relationship to measures of sucrose consumption and weight change. While localization of terminals implicated in ingestive behavior is not clear at this time and requires further exploration, it seems reasonable to suggest that the present results indicate the existence of functionally distinct dopamine pathways in brain.

No clear role for norepinephrine in either active avoidance responding or ingestive behavior was indicated by these experiments. Although factor analysis suggested a weak relationship between weight loss after treatment and norepinephrine content, rats with large specific depletion of this amine gained weight like controls (Table 4). It seems quite possible, therefore, that this weak relationship obtained by factor analysis associating weight loss with reduced norepinephrine may actually be due to the coincidental depletion of norepinephrine produced by injection of 6-hydroxydopamine into the ventral tegmentum and globus pallidum (Table 1).

In summary, these experiments support the role for dopamine neural systems in brain in maintaining active avoidance and ingestive behavior [7, 11, 27]. In addition,

they suggest that these two functions may be mediated by different dopamine pathways. Confirmation of this possibility and further work on possible functional-anatomical differences in the catecholamine systems in animals may eventually be useful for the evaluation of theories relating dopamine to such diverse human mental disorders as schizophrenia [20] and Parkinson's disease [17].

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