Pharmacology Biochemistry and Behavior, Vol. 1, pp. 23–28. ANKHO International Inc., 1973. Printed in the U.S.A.

Neurochemical Mediation of Reward: A Significant Role for Dopamine?[1]

ARNOLD S. LIPPA, SEYMOUR M. ANTELMAN, ALAN E. FISHER AND DON R. CANFIELD

Psychobiology Program, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

(Received 26 September 1972)

LIPPA, A. S., S. M. ANTELMAN, A. E. FISHER AND D. R. CANFIELD. *Neurochemical mediation of reward: a significant role for dopamine?* PHARMAC. BIOCHEM. BEHAV. 1(1) 23–28, 1973.– Intraventricular injection of 6-hydroxydopamine resulted in only a temporary decrement of self-stimulation. Treatment with the alpha-adrenergic receptor blocking agent, phentolamine, had virtually no effect on self-stimulation in either 6-hydroxydopamine treated or normal rats; whereas haloperidol, a dopamine receptor blocking agent, markedly reduced self-stimulation in normal rats. Treatment with FLA-63, a potent inhibitor of dopamine-beta-hydroxylase, had no effect on self-stimulation but very significantly reduced eating. These results imply that dopamine may be importantly involved in the mediation of positive reinforcement and/or that the role of norepinephrine may be a minor one.

Self-stimulation 6-Hydroxydopamine Norepinephrine Dopamine

MUCH evidence has accumulated in recent years which suggests a major role for one or both of the catecholamines (CA) in mediating positive reinforcement, with norepinephrine (NE) receiving by far the most attention. Many drugs which depress CA levels or interfere with their action also decrease bar pressing for electrical brain stimulation (self-stimulation) [9, 21, 35], while drugs which potentiate the action of CA increase self-stimulation rates [36]. Unfortunately, interpretation of such data often proves difficult. First, most pharmacological manipulations thus far utilized affect both dopamine (DA) and NE, making their individual roles unclear. Achieving a clear distinction between the effects of changes in NE and DA levels must be an ultimate goal of any crucial assessment of the NE reward hypothesis. However, results which seem to implicate NE in the mediation of positive reinforcement are suspect on other grounds as well. For example, disulfiram and diethyldithiocarbamate (DEDTC) have been reported to decrease self-stimulation, presumably due to a decrease in NE levels [35]. Yet, disulfiram has central depressant effects [20] and also results in inflammation of the peritoneum [29] whereas DEDTC affects energy metabolism [34]. In addition, both chelate trace metals [34].

Such generalized side effects make it necessary to seek less confounded methods for depleting brain CA, and 6-hydroxydopamine (6-OHDA) shows promise of becoming a valuable tool in this field of research. When injected into the ventricles of the brain, 6-OHDA depletes most central CA containing nerve terminals and causes a permanent depletion of brain CA [32, 33]. Since toxic or side effects unrelated to CA depletion should be temporary, the effects on self-stimulation actually due to CA depletion can be assessed by a series of post-drug tests. Furthermore, utilization of other drugs in conjunction with selected dosages of 6-OHDA can produce a virtually complete functional inactivation of NE or DA with or without a marked depletion of the other [6, 12]. It seems then, that some rather basic tests of the NE reward hypothesis, and of alternatives to it, can now be made.

In a previous report [2] we stated that 6-OHDA treated animals provided priming stimulation (experimenter delivered stimulation not contingent on bar pressing) returned to normal self-stimulation rates within 3–7 days. In the present report, we provide evidence that priming is not a crucial factor for such early recovery, and that self-stimulation survives a combined drug treatment which should virtually eliminate functional utilization of NE.

EXPERIMENT 1

The purpose of this experiment was to determine the effects of 6-OHDA induced CA depletion on self-stimulation rates. Two different testing regimens were used to determine the necessity of priming stimulation.

METHOD

Animals

Animals were male Sprague-Dawley rats from Marland Farms (250–300 g). All rats were housed in individual cages with continual access to food and water.

[1]Supported in part by NSF Predoctoral Traineeship to A. S. L.; by Postdoctoral Fellowship to S. M. A. and by Predoctoral Traineeship to D. R. C., NIH Training Grant MH 1114; and by NIH Grant MH 1951 to A. E. F. We would like to thank Dr. Michael Zigmond for the generous use of his biochemical facilities and Mrs. Claudia Kraft for her help in the preparation of this manuscript.

Surgery

Surgery was performed using sodium Nembutal (50 mg/kg) anesthesia supplemented by atropine sulfate (0.4 mg/kg). Animals were placed in a stereotaxic instrument and a bipolar platinum iridium electrode (0.01 in. dia.) uninsulated at the cross section of the tips was aimed for a site in the posterior hypothalamus (A 4.5; L 1.5; V 8.5) [18]. Twenty-three gauge stainless steel guide cannulae with 30 gauge stainless steel inner plugs were bilaterally implanted into the lateral ventricles (A bregma suture; L 1.5; V 4.25) [18].

Apparatus

The apparatus used for testing self-stimulation was a modified Skinner box with a bar on one wall. Stimulation was administered through a wire lead via a commutator attached to the top of the cage. The stimulation consisted of rectangular pulse pairs of opposite polarity; each pulse was 0.2 msec in duration and the frequency was 100 pulse pairs/sec. Each bar press delivered a 0.3 sec duration pulse train.

Procedure

A large pool of animals were tested for self-stimulation behavior. Ten animals that achieved a rate of 20 reponse per min or higher were selected for this study and tested until self-stimulation rates had stabilized. An animal was considered to have stabilized if its response rate did not vary by more than ± 10% of the mean rate for three days.

Following stabilization of bar-pressing rates, experimental animals were given two 200 μg intraventricular injections (72 hr apart) of 6-OHDA hydrobromide (calculated as free base) in a vehicle of 0.9% saline and 0.1% ascorbic acid. The volume of solution injected was 20 μl. Five control animals were given isovolumetric injections of the vehicle solution. The injection procedure consisted of removing the guide shaft plug and replacing it with a 30 gauge stainless steel cannula connected by PE 20 tubing to a 50 μl Hamilton syringe. The injections were administered over a period of 2 min and all injections were given 1 hr before testing for self-stimulation. The point has been raised [28] that this particular injection schedule might not cause a sufficient depletion of NE, particularly in those nerve fibers involved with positive reinforcement. However, unreported data from rats tested at various times (1–23 hr) before and after 6-OHDA do not differ from that reported here.

Testing was carried out for 30–60 min each day. If an animal did not begin bar-pressing within the first 15 min, priming (experimenter delivered stimulation not contingent on bar-pressing) was given. Two animals (H1 and H2) were not tested on the two days between injections, while 3 animals (H3, H4, and H5) were tested on all days. Once bar-pressing was initiated, no further priming was given and bar-pressing rates were measured for 30 min.

Four 6-OHDA treated animals whose self-stimulation rates had returned to predrug levels were injected intraventricularly with 50 μg of phentolamine mesylate (dose expressed as salt), an alpha adrenergic receptor blocking agent, in a volume of 10 μl distilled water. All injections were given 15 min before the test session and were only given if the criterion of 3 days of stabilized bar-pressing had been reached. Test sessions were conducted daily until self-stimulation rates had returned to preinjection levels. When self-stimulation rates had again stabilized the animals were injected with 100 μg phentolamine mesylate and given an additional series of daily self-stimulation tests.

Biochemistry

One week after animals had completed all testing conditions, they were sacrificed by decapitation and their telencephalons removed for biochemical determination of NE. NE was extracted on alumina and measured following oxidation with an iodine reagent [26]. Fluorescence was measured in an Aminco-Bowman spectro-photofluorometer. Emission spectra were determined for each sample and these were always identical to the spectra obtained from a NE standard (l-arterenol bitartrate, Sigma). Brain NE content was expressed as μg of NE per g of frozen brain tissue. Reported values were not corrected for recovery (87%).

Histology

Traditional histology was not performed. However, two procedures were used to obtain evidence that our cannula placements were actually in the lateral ventricle. First, a series of four standard weight control animals were implanted with cannulae at the same stereotaxic coordinates specified for the experimental animals. 6-OHDA was administered twice to the same side of the brain according to the schedule and dosage previously described. Five days later, brains were removed, bisected into right and left halves and separate assays performed on each half brain. The eight samples gave an average depletion of 90% NE and there was no significant difference in depletion between the 6-OHDA injected halves and the contralateral halves.

In addition, the cannula placement of the animals in Experiment 1 and the first group in Experiment 2 was checked by cutting through the brain in the plane of the cannula track. Visual inspection of the exposed track verified that the tip terminated in the lateral ventricle in all cases.

RESULTS

The data in Table 1 indicate a very temporary decrement in self-stimulation rates for 6-OHDA treated animals, with vehicle controls showing no change. It should be noted that our animals recovered in an average of 5 days if primed and seven days if not primed. Since Stein and Wise report data very similar to ours for unprimed animals, but only for the first 6 days following 6-OHDA treatment [27], it would appear that they did not wait long enough to observe the full recovery reported here.

Two other points are of particular interest. First, the two animals not tested on the days between 6-OHDA injections (H1 and H2) failed to resume bar-pressing on the three days following the second 6-OHDA injection unless they were primed (See Table 1). Once primed, however, these animals usually displayed their normal bar-pressing rates. By the seventh postinjection day these two animals had not only recovered or exceeded their predrug response rates but no longer required priming. Second, and more important, the three animals tested throughout the injection regimen demonstrated complete recovery of their predrug response rates without the need for priming. In

TABLE 1

EFFECTS OF 6 OHDA* ON SELF-STIMULATION RATE†

		TEST DAYS										
TREATMENT	ANIMAL	1‡	2	3	4‡	5	6	7	8	9	10	11
200 μg 6 OHDA on Days 1 and 4	H1	92	not	tested	129 (10)	94 (2)	148 (3)	144				
	H2	90	not	tested	53 (100)	104 (80)	117 (10)	115				
	H3	1	31	47	73	118	120	116	110	109	126	119
	H4	52	34	60	63	71	69	79	74	115	114	92
	H5	63	71	94	52	67	72	113	121	150	157	123
Vehicle (0.9% NaCl and 0.1% Ascorbic Acid)	H6	95	94	89	88							
	H7	118	114	113	120							
	H8	111	110	107	111							
	H9	107	113	107	113							
	H10	107	109	91	95							

*Two intraventricular injections of 6 OHDA were given 1 hr prior to start of test. Interval between injections was 72 hr.
†Data presented as percent of mean self-stimulation rate for 3 days prior to first injection.
‡Day of injection.
( )Parentheses refer to the number of primings given at start of session.

addition, the second 6-OHDA injection did not produce even a temporary further decrement in response rates for most animals.

Phentolamine had a very minimal effect in the four 6-OHDA treated animals whose self-stimulation rates had recovered to predrug levels (Table 2). Rate decreases for self-stimulation ranged only from 0–31% with mean decreases of 11% for the 50 μg dose and 13% for the 100 μg dose (Table 2).

TABLE 2

EFFECTS OF PHENTOLAMINE ON SELF-STIMULATION IN 6-HYDROXYDOPAMINE TREATED RATS

Treatment	Self-Stimulation (Percent of Predrug Rate)			
	H2	H3	H4	H5
Vehicle	97	99	79	89
50 μg Phentolamine	84	100	85	89
100 μg Phentolamine	80	69	100	100

Table 3 presents the effects of 6-OHDA on the telencephalic content of NE in the self-stimulating animals. It can be seen that 6-OHDA caused a 90% depletion of telencephalic NE relative to vehicle treated controls. Depletion of telencephalic NE has been found to represent a relatively uniform depletion of NE in all brain regions [15]. Although we were not prepared to do dopamine assays on these animals, similar drug regimens produce a DA depletion of 50–60% [6, 33]. Subsequent DA assays done here confirm such figures.

TABLE 3

EFFECTS OF 6-HYDROXYDOPAMINE ON BRAIN NOREPINEPHRINE CONTENT

Treatment	Animals	Norepinephrine μg/g	Norepinephrine Percent Control
Vehicle Control	(5)	0.45±0.02	--
2 x 200 μg 6-Hydroxydopamine	H1	0.04	8
	H2	0.05	12
	H3	0.05	12
	H4	0.04	9
	H5	0.06	12

EXPERIMENT 2

The purpose of this experiment was to determine the effects on self-stimulation of several pharmacological agents which would selectively interfere with either NE or DA. Phentolamine was again selected as an agent for blocking the alpha adrenergic actions of NE. FLA-63 (an agent that interferes with the synthesis of NE, but not of DA) was selected because animals treated with this drug do not

exhibit the central nervous system depressant effects associated with drugs like disulfiram and DEDTC [8, 10]. Finally, haloperidol was the drug of choice for selective interference with DA actions, since its blocking effects appear to be specific to DA receptors when low doses are utilized [1, 16, 23].

METHOD

The surgical procedures, apparatus utilized and the self-stimulation training procedures were identical to those described for Experiment 1. Animals meeting the self-stimulation stabilization criteria were divided into two groups. A first group (n=4) received intraventricular injections of haloperidol, phentolamine mesylate or their respective vehicles. Haloperidol (10 μg free base) was injected in a 10 μl vehicle solution which was supplied by the manufacturer (Haldol, McNeil Labs.). Phentolamine (100 μg) was injected in 10 μl distilled water. The animals were injected repeatedly over days but only at points when the criterion of 3 days stabilized responding had been reached. The order of drug administration was: phentolamine vehicle - phentolamine - haloperidol vehicle - haloperidol. All injections were administered 15 min before the testing session.

The second group of animals (n=4) were given intraperitoneal injections of FLA-63 (25 mg/kg), a dopamine-beta-hydroxylase inhibitor, in a hydrochloric acid vehicle solution (pH=5). In addition to monitoring self-stimulation rates, food and water intake were measured 24 hr following injections and compared to the food and water intake for the three days preceding the injections. One week after treatment, these animals were injected with an isovolumetric amount of the vehicle solution and retested for self-stimulation. All intraperitoneal injections were given 7½ hr before the testing session.

If an animal showed a deficit in self-stimulation rate after a drug injection, he was immediately tested for motor impairment [3]. Catalepsy was measured by placing each animal on a table top and recording how many seconds the animal required to appropriately readjust its body posture when a front paw or hind limb was lifted by the experimenter. Each animal was also placed in a vertical position on the front part of its home cage such that the hind quarters were hanging over the top edge of the cage and the front paws were grasping the inside of the cage front. The number of seconds it took each animal to readjust itself to the appropriate horizontal position on the cage floor was recorded as a measure of horizontal stabilization.

RESULTS

As can be seen in Table 4, the attempted selective blockade of NE receptors with phentolamine (100 μg) had minimal effects on self-stimulation rate. However, the attempted blockade of DA receptors with haloperidol caused a 54% reduction (Table 4) in self-stimulation rate on the day of injection. It should be noted that at no time after haloperidol were any motor impairments observed. The animals displayed no catalepsia or impairment of horizontal stabilization. Furthermore, FLA-63 had no effect on self-stimulation rates (Table 4) and water intake, yet reduced food intake by 53%.

TABLE 4

EFFECTS OF PHARMACOLOGICAL AGENTS ON SELF-STIMULATION

Drug Treatment	Animals	Percent of Mean Self-stimulation Rate for 3 Days Prior to Drug
Phentolamine Vehicle	4	94
Phentolamine (100 μg)	4	98
Haloperidol Vehicle	4	97
Haloperidol (10 μg)	4	46*
FLA63 Vehicle	4	96
FLA63 (25 mg/kg)	4	88

*$p<0.01$, t-test.

GENERAL DISCUSSION

The main findings of Experiment 1 were (a) that animals chronically depleted of NE with 6-OHDA recovered predrug self-stimulation rates in 7 days or less, and (b) that priming was not essential for this recovery. Although these data suggest strongly that temporary deficits (and recovery) of self-stimulation after 6-OHDA are related to factors other than NE depletion, the possible role of receptor supersensitivity should still be considered. However, even though increases in sensitivity to NE attributable to postsynaptic receptor changes are well documented, such effects (unlike the increased responsiveness to NE attributable to interference with presynaptic reuptake of NE) typically take weeks to develop [25, 30]. In fact, Stein and Wise have cited a study in which receptor supersensitivity is not evident until the twenty-third day after NE depletion [28]. Since our animals are fully recovered by Day 7, it seems unlikely that such recovery can be attributed to receptor supersensitivity. Nevertheless, we attempted to control for this possibility by the use of phentolamine, an adrenergic receptor blocking agent. The use of both 6-OHDA and phentolamine should virtually eliminate functional utilization of NE. Nevertheless, phentolamine had no effect on self-stimulation rates in recovered 6-OHDA treated animals, even though equivalent doses of phentolamine in intact animals decrease locomotor activity and block the excitatory effects of NE and amphetamine [17].

Since the biochemical assay showed a 90% depletion of NE in the 6-OHDA treated animals utilized in these studies, our data strongly suggest that mediation of positive reinforcement is not critically dependent upon it. Thus, temporary decrements in self-stimulation after 6-OHDA treatment most probably relate to factors other than a selective interference with a positive reinforcement system.

In this regard, Laverty and Taylor [19] report that sedation, lethargy and piloerection are common symptoms during the first few days after a 200 μg injection of

6-OHDA. Conditioned avoidance responding and runway activity were also temporarily depressed and the time course of recovery was similar to that reported here for recovery of self-stimulation.

If such data do put the NE theory of reward in jeopardy, are any reasonable alternatives suggested by the available data? Although it is doubtful that any single transmitter will be found to be crucially and solely related to the mediation of positive reinforcement, some compelling evidence suggests that dopamine may play a very significant role.

First, our finding that a second injection of 200 μg of 6-OHDA had no marked effect on self-stimulation (See Table 1) is of interest in light of the Breese and Taylor [6] report that the administration of a second 200 μg dose of 6-OHDA further depletes brain NE, but has no further depleting effect on brain DA. This suggests the hypothesis that the depression of self-stimulation observed after the initial 6-OHDA injection may be related more directly to DA depletion than to NE depletion. The transient nature of the effect might relate to a combination of minimal depletion of dopamine and toxic effects of 6-OHDA.

A direct test of the dopamine hypothesis was made in Experiment 2. The data indicate that although intraventricular administration of 100 μg of the adrenergic blocker phentolamine had no significant effect on self-stimulation in normal animals, intraventricular administration of 10 μg of haloperidol (a selective DA receptor blocker at low doses) reduced self-stimulation rates by more than 50%. This decrease does not seem to be due to motor impairment since the animals appeared normal on all tests of motor function which were used. Furthermore, FLA-63, a potent dopamine-beta-hydroxylase inhibitor, had virtually no effect on self-stimulation rates at a dosage level which has been shown to deplete NE 70% but leaves DA levels unchanged [10]. Although we do not as yet have our own biochemical assay data for FLA-63 treated animals, we do have indirect evidence of an effective depletion of NE. The marked decrease in 24-hr food intake is highly suggestive of a major NE depletion since NE has been strongly implicated as the neurotransmitter most directly related to feeding [13].

The importance of DA in relation to the self-stimulation phenomenon is implied by several other lines of evidence: (1) Breese *et al.* [5] report that self-stimulation deficits lasting 35 days can be obtained by a single injection of pargyline prior to 6-OHDA, and then cite this as support for the NE reward hypothesis. However, pargyline has been shown to potentiate the depleting effect of 6-OHDA on brain DA without any further depletion of brain NE [6]. This would seem to implicate DA rather than NE in the mediation of self-stimulation. (2) Tricyclic antidepressants which selectively block reuptake of NE [7] have no effect on self-stimulation [4]. On the other hand, cocaine, which blocks reuptake of dopamine as well as NE [24] facilitates self-stimulation [4]. (3) Crow [11] has reported that the majority of mesencephalic sites from which he could elicit self-stimulation correspond to areas A9 and A10 of Dahlstrom and Fuxe, which consist of dense groups of dopamine containing cell bodies.

The idea that DA might be a neurotransmitter critical for a particular behavior is certainly not a new one. Dopamine has been fully or tentatively implicated in feeding and drinking [31], and in the stimulatory and compulsive behaviors observed after amphetamine [22]. Deficits in DA have been implicated in the symptomology of Parkinsonism [14]. Although our data appear to implicate DA in the mediation of self-stimulation, it now seems precarious to consider either DA or NE as a crucial factor in the mediation of positive reinforcement. Present evidence does suggest that the role played by NE may be a minimal or indirect one, and that other alternatives, including DA (or DA and NE in concert) deserve serious consideration.

REFERENCES

1. Anden, N. E., S. G. Butcher, H. Corrodi, K. Fuxe and U. Ungerstedt. Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. *Eur. J. Pharmac.* **11**: 303–314, 1970.
2. Antelman, S. M., A. S. Lippa and A. E. Fisher. 6-Hydroxydopamine, noradrenergic reward, and schizophrenia. *Science* **175**: 919–920, 1972.
3. Balagura, S., R. H. Wilcox and D. V. Coscina. The effects of diencephalic lesions on food intake and motor activity. *Physiol. Behav.* **4**: 629–633, 1969.
4. Benesova, O. The action of cocaine, atropine and tricyclic anti-depressants on self-stimulation in rats. In: *The Present Status of Psychotropic Drugs. Proc. of the VI Int. Coll. Int. Neuropsychopharmacologium,* edited by A. Cerletti and F. J. Bove. Amsterdam: Excerpta Medica Foundation, 1969. pp. 247–249.
5. Breese, G. R., J. L. Howard and J. P. Leahy. Effect of 6-hydroxydopamine on electrical self-stimulation of the brain. *Br. J. Pharmac.* **43**: 255–257, 1971.
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. Carlsson, A. Recent studies on the mode of action of antidepressant drugs. *Archs Pharmak. exp. Pathol.* **257**: 115–127, 1967.
8. Carlsson, A. Amphetamines and brain catecholamines. In: *Amphetamines and Related Compounds*, edited by E. Costa and S. Garattini. New York: Raven Press, 1970. pp 289–300.
9. Cooper, B. R., W. C. Black and R. M. Paolino. Decreased septal-forebrain and lateral hypothalamic reward after alpha-methyl-p-tyrosine. *Physiol. Behav.* **6**: 425–429, 1971.
10. Corrodi, H. K., K. Fuxe, B. Hamberger and A. Ljungdahl. Studies on central and peripheral noradrenaline neurons using a new dopamine-Beta-hydroxylase inhibitor. *Eur. J. Pharmac.* **12**: 145–155, 1970.
11. Crow, T. J. The relation between electrical self-stimulation sites and catecholamine-containing neurones in the rat mesencephalon. *Experientia.* **27**: 662, 1971.
12. Evetts, K. D. and L. L. Iversen. Effects of protriptyline on depletion of catecholamines induced by 6-hydroxydopamine in the brain of the rat. *J. Pharm. Pharmac.* **22**: 540–543, 1970.
13. Grossman, S. P. Eating or drinking elicited by direct adrenergic or cholinergic stimulation of hypothalamus. *Science* **132**: 301–302, 1960.
14. Hornykiewicz, O. Dopamine and brain function. *Pharmac. Rev.* **18**: 925–964, 1966.

15. Iversen, L. L. and N. J. Uretsky. Biochemical effects ot 6-hydroxydopamine on catecholamine-containing neurones in the rat central nervous system. In: *6-Hydroxydopamine and Catecholamine Neurons,* edited by T. Malmfors and H. Thoenen. Amsterdam: North-Holland Pub. Co., 1971. pp. 171–186.
16. Janssen, P. A. J. The pharmacology of haloperidol. *Int. J. Neuropsychiat.* 3(Supplement 1): 10–18, 1967.
17. Kleinrok, Z. and I. Zebrowska-Lupina. Central action of phentolamine administered intraventricularly in the rat. *Psychopharmacologia* **20:** 348–354, 1971.
18. Konig, J. F. R. and K. A. Klippel. *The Rat Brain.* Baltimore: Wilkins and Wilkins, 1963.
19. Laverty, R. and K. M. Taylor. Effects of intraventricular 2, 4, 5-trihydroxyphenylethylamine (6-hydroxydopamine) on rat behavior and brain catecholamine metabolism. *Br. J. Pharmac.* **40:** 836–846, 1970.
20. Mandell, A. Discussion comment In: *Psychotomimetic Drugs,* edited by D. H. Efron. New York: Raven Press, 1970. p. 145.
21. Poschel, B. P. H. and F. W. Ninteman. Hypothalamic self-stimulation: its suppression by blockade of norepinephrine biosynthesis and its reinstatement by methamphetamine. *Life Sci.* **5:** 11–16, 1966.
22. Randrup, A. and I. Munkvad. Biochemical, anatomical and psychological investigations of stereotyped behavior induced by amphetamines. In: *Amphetamines and Related Compounds,* edited by E. Costa and S. Garattini. New York: Raven Press, 1970. pp. 695–713.
23. Roos, B. F. Changes in homovanillic acid reflecting psychotropic activity of neuroleptic drugs. In: *Neuropsychopharmacology,* edited by H. Brill. Amsterdam: Excerpta Medica Foundation, 1967. pp. 312–313.
24. Ross, R. B. and A. L. Renyi. Inhibition of the uptake of tritiated catecholamines by anti-depressants and related compounds. *Eur. J. Pharmac.* **2:** 181–186, 1967.
25. Sharpless, S. K. Isolated and deafferented neurons: disuse supersensitivity. In: *Basic Mechanisms of the Epilepsies,* edited by H. H. Jasper, A. A. Ward and A. Pope. Boston: Little, Brown, and Co., 1969. pp. 329–348.
26. Shellenberger, M. K. and J. H. Gordon. A rapid simplified assay of norepinephrine, dopamine, and 5-hydroxytryptamine from discrete brain areas. *Anal. Biochem.* **39:** 356–372, 1971.
27. Stein, L. and C. D. Wise. Possible etiology of schizophrenia: progressive damage to the noradrenergic reward system by 6-hydroxydopamine. *Science* **171:** 1032–1036, 1971.
28. Stein, L. and C. D. Wise. 6-Hydroxydopamine, noradrenergic reward and schizophrenia. *Science* **175:** 922–923, 1972.
29. Thornburg, J. E. and K. E. Moore. Stress-related effects of various inhibitors of catecholamine synthesis in the mouse. *Archs int. Pharmacodyn. Ther.* **194:** 158–167, 1971.
30. Trendelenburg, U. Supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.* **15:** 225–276, 1963.
31. Ungerstedt, U. Aphagia and adipsia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta physiol. scand. Supp.* **367:** 95–122, 1971.
32. Ungerstedt, U. Histochemical studies on the effects of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In: *6-Hydroxydopamine and Catecholamine Neurons,* edited by T. Malmfors and H. Thoenen. Amsterdam: North-Holland Pub. Co., 1971, pp. 102–127.
33. Uretsky, N. J. and L. L. Iversen. Effects of 6-hydroxydopamine on catecholamine containing neurons in the rat brain. *J. Neurochem.* **17:** 269–278, 1970.
34. Van der Schoot, J. B. and C. R. Creveling. Substrates and inhibitors of dopamine-beta-hydroxylase. In: *Advances in Drug Research 2,* edited by N. J. Harper and A. B. Simmonds. New York: Academic Press, 1965. pp. 47–88.
35. Wise, C. D. and L. Stein. Facilitation of brain self-stimulation by central administration of norepinephrine. *Science* **163:** 299–301, 1969.
36. Wise, C. D. and L. Stein. Amphetamine: facilitation of behavior by augmented release of norepinephrine from the medial forebrain bundle. In: *Amphetamines and Related Compounds,* edited by E. Costa and S. Garattini. New York: Raven Press, 1970. pp. 463–485.