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Self-Injection of Apomorphine in the Rat: Positive Reinforcement by a Dopamine Receptor Stimulant

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BAXTER, B. L., M. I. GLUCKMAN, L. STEIN AND R. A. SCERNI. *Self-injection of apomorphine in the rat: positive reinforcement by a dopamine receptor stimulant.* PHARMAC. BIOCHEM. BEHAV. **2**(3) 387–391, 1974. – Rats with jugular cannulas self-administer the dopamine receptor stimulant apomorphine at doses from 0.125 to 1 mg/kg/injection. Pretreatment with the dopamine receptor blocker pimozide (0.5 or 1 mg/kg) disrupted the self-injection of apomorphine. These data support the idea that the activation of dopamine receptors yields positive reinforcement, although other neurochemical actions of apomorphine have not been ruled out. Dopaminergic mechanisms may also be involved in the self-administration of psychomotor stimulants and narcotics.

Drug-addiction Apomorphine Self-administration Pimozide Self-injection Dopamine Reward Norepinephrine Reinforcement

EVIDENCE from studies of brain self-stimulation has led to the suggestion that central noradrenergic neurons mediate rewarding or positively reinforcing effects on behavior [3, 24, 29, 34]. Self-stimulation data obtained recently have been interpreted to indicate that the activation of dopamine neurons also yields positive reinforcement [9, 15, 16, 21, 31]. As a test of this idea, we attempted to determine whether rats would self-administer a central dopamine receptor stimulant. The self-injection method is generally regarded as a valid technique for assessing the reinforcing properties of a drug [26]. The agent most widely employed in the laboratory for dopamine receptor stimulation is apomorphine [2, 7, 11], a non-narcotic derivative of morphine [27]. Self-administration of apomorphine could therefore be taken as a demonstration of dopamine-mediated reinforcement, provided only that other neurochemical actions of apomorphine may be ruled out.

METHOD

Twenty-one female rats weighing 230–250 g had a cannula implanted into the right external jugular vein. The cannula, fabricated from a combination of silastic and polyethylene tubing [32], was brought around the body subcutaneously to an exit from the back of the neck. At least 14 days were allowed for recovery from surgery. During initial periods on test, all animals lived continuously in individual stainless steel wire mesh cages (18 × 24 × 18 cm); they were removed for 2 hr each morning for cage cleaning and equipment servicing. After these initial 22-hr per day self-injection periods, 4 animals were changed to a 5-hr daily schedule (11:00 a.m. to 4:00 p.m.).

Each test cage contained a standard rodent operant lever. Solutions were delivered to the jugular cannula by a motor driven pump connected via a feed-thru swivel to a saddle which the rats wore while on experiment. Each lever press produced an injection (FR-1) of 0.08 ml during a 2.5-sec infusion period. Food and water were available ad lib.

Apomorphine hydrochloride (Merck) was dissolved in physiological saline which contained 0.02% ascorbic acid. Pimozide was suspended in 1% Tween 80 for intraperitoneal injection. At least 1 week elapsed between administrations of pimozide.

RESULTS

In a previous study [4] 6 rats that had saline available for self-injection during a 10-day acquisition period (22-hr daily tests) never took more than 50 injections on the first day, nor more than 25 injections on any day thereafter (Fig. 1). In the present experiments, a stringent criterion of drug-induced reinforcement was used. Apomorphine was considered reinforcing only in those rats that self-adminis-

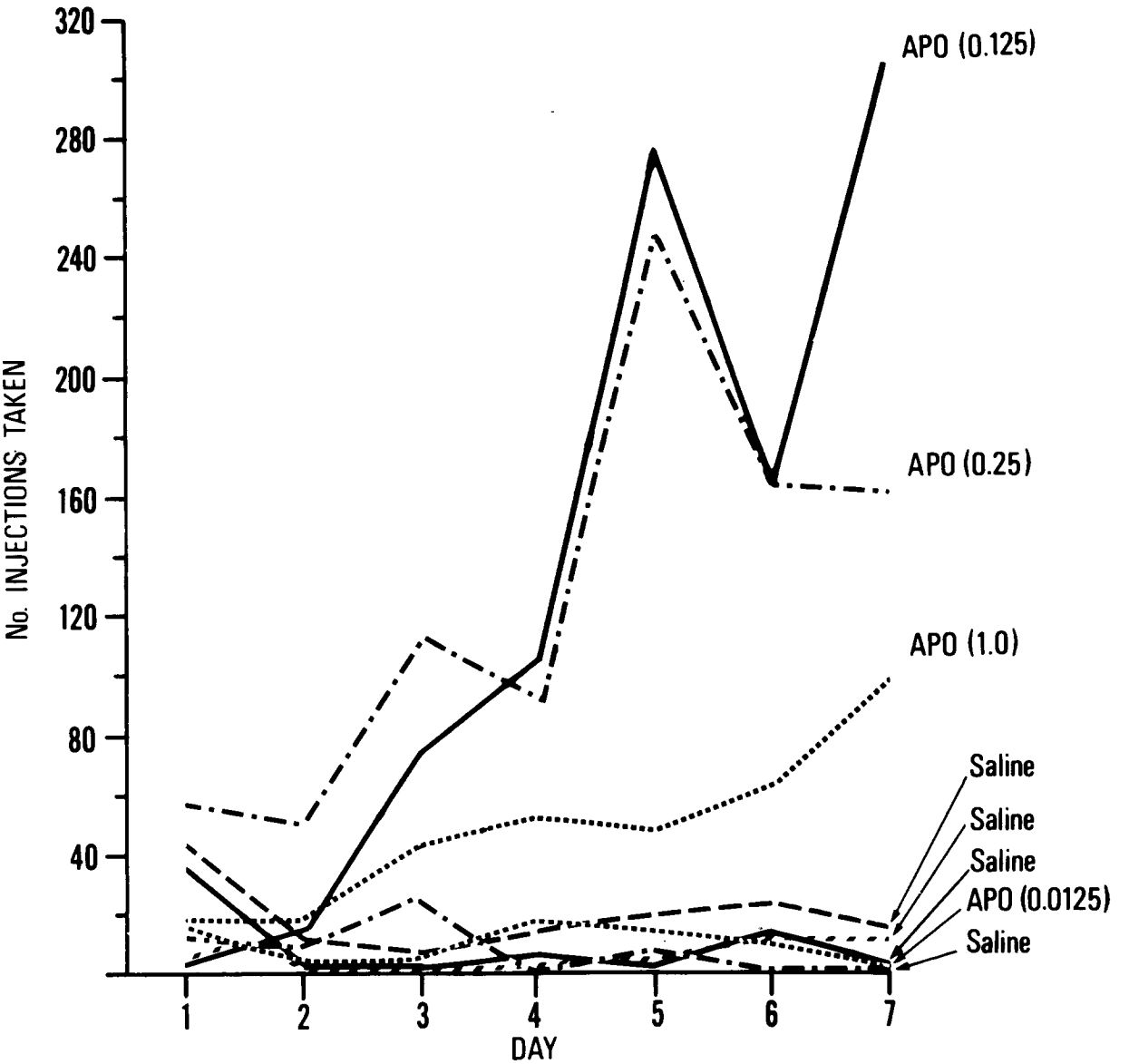

FIG. 1. Sample acquisition curves of apomorphine self-administration in experimentally-naive rats. These curves indicate a reinforcing effect of apomorphine at all doses except 0.0125 mg/kg/injection. (Daily 22-hr tests).

tered the drug at least 75 times per 22-hr test. Data from the first test day were excluded.

Twenty-one rats were given the opportunity to self-administer apomorphine for 10 days in 22-hr daily tests at doses ranging from 0.0125 to 1 mg/kg/injection. Sample acquisition curves during the first week are shown in Fig. 1, and the results for all animals are summarized in Table 1. Using the criterion mentioned above, self-injection was obtained at all doses of apomorphine except 0.0125 mg/kg/injection. At all of the effective doses, sniffing and gnawing were observed. Self-mutilation and weight loss were noted at the highest dose tested (1 mg/kg/injection); in one of these cases, death occurred after 293 self-injections during one day.

Because self-injection behavior was obtained at 0.125 mg/kg/injection with no apparent toxicity, 4 animals initiated and maintained at this dose were selected for study on a long-term basis. For these tests, which lasted for at least 2 months, self-administration sessions were shortened to 5 hr per day. Within 3 to 4 days on this schedule, a regular pattern of self-injection developed in all cases and a relatively constant amount of apomorphine was taken each day (Table 2). In this Table it will also be noted that daily drug intake was found to be remarkably similar for the different rats. Two rats then were tested at 0.25 mg/kg/injection for 7 days. In both cases, self-injection at this higher dose stabilized at a lower rate than that maintained at the lower dose (Table 2). Examples of the response patterns at both doses are shown for rat 183 in Fig. 2. No evidence of withdrawal symptoms was observed during the 19-hr period between the self-injection sessions. At the beginning of each day the animals appeared normal and

TABLE 1

ACQUISITION OF APOMORPHINE SELF-INJECTION BEHAVIOR (DAILY 22-HR TESTS)

Dose of Apomorphine (mg/kg/injection)	Number of Rats Self-Injecting /Number of Rats Tested
1.0	3/3
0.5	2/2
0.25	4/6
0.125	6/8
0.0125	0/2

were easy to handle.

When saline was substituted for apomorphine, the regular pattern of self-injection was disrupted. During the first hour, the rate of self-administration of saline always exceeded that of apomorphine (e.g., Fig. 2). However, the total number of self-injections taken on the first saline day could be significantly higher, lower, or no different from that obtained on preceding apomorphine days. Saline self-injection then extinguished, with the number of daily self-injections gradually decreasing over a period of one week or more to very low levels.

Pretreatment with 0.5 mg/kg pimozide 4 hr before the start of the session always disrupted the pattern of apomorphine self-administration. Lever presses no longer were regularly spaced and bursts of 2 or more responses often occurred (e.g., Fig. 2). This dose of pimozide had variable effects on the total number of daily self-injections (Table 3). In 2 rats there was a significant decrease in total

TABLE 2

STABILIZED LEVELS OF APOMORPHINE SELF-INJECTION IN 4 RATS

Rat No.	Number of Apomorphine Self-Injections (7 consecutive daily 5-hr tests)	Mean Daily Drug Intake ± S.E.M. (mg/kg)
	0.125 mg/kg/injection	
177	42, 43, 42, 55, 46, 40, 43	5.57 ± 0.24
182	50, 48, 30, 42, 50, 46, 43	5.52 ± 0.33
183	35, 38, 34, 41, 41, 46, 47	5.04 ± 0.23
190	32, 28, 33, 32, 32, 35, 34	4.04 ± 0.12
	0.25 mg/kg/injection	
177	48, 20, 31, 29, 37, 29, 31	8.03 ± 0.81
183	22, 22, 23, 22, 22, 25, 26	5.03 ± 0.24

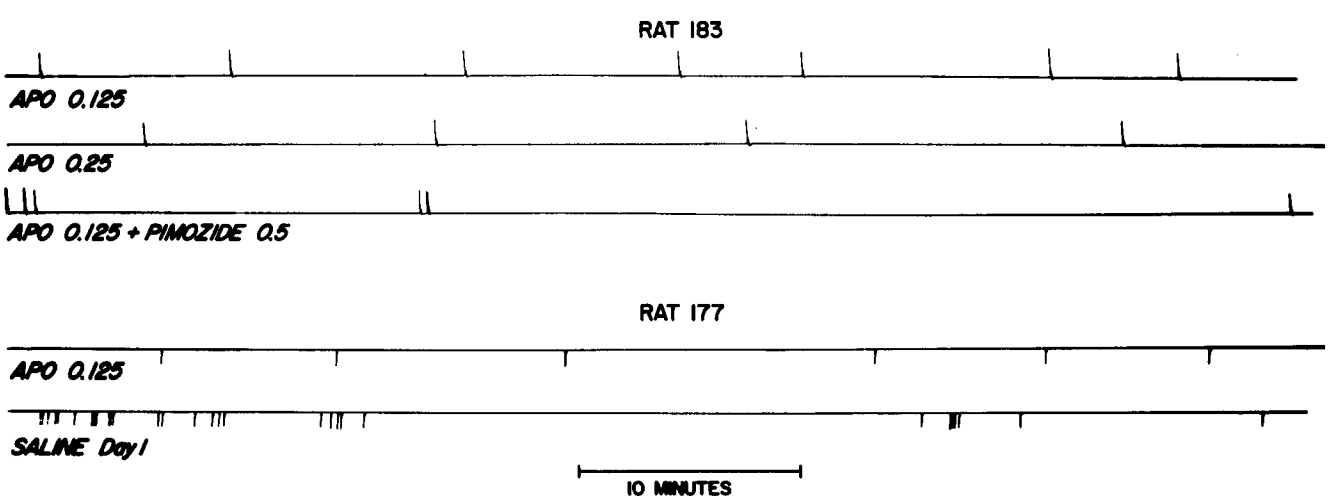

FIG. 2. Strip chart recordings of apomorphine self-injection showing response patterns in the first hour of 5-hr test sessions under the conditions indicated. Each pen deflection indicates an injection. Note especially disruption of regular apomorphine self-administration pattern by pimozide pretreatment (3rd record) and by substitution of saline for apomorphine (5th record).

TABLE 3

EFFECTS OF PIMOZIDE PRETREATMENT ON APOMORPHINE SELF-INJECTION

Rat No.	Self-Injections of Apomorphine (0.125 mg/kg/inj., 5-hr tests)		
	No Treatment (mean of 7 tests prior to pimozide ± S.E.M.)	Pimozide (mg/kg) 0.5	1
177	44.4 ± 1.89	47	18*
182	44.1 ± 2.64	78*	0*
183	40.6 ± 1.96	15*	N.T.
190	32.9 ± 0.99	11†	7*

*Different from No Drug, $p<0.01$ N.T. – Not Tested †$p<0.02$

injections; in one rat there was a significant increase; and in one there was no significant change. At the 1 mg/kg dose of pimozide, the rate of apomorphine self-administration was always significantly reduced (Table 3). In one case at this dose, self-administration behavior was completely abolished. The suppressant effect of 1 mg/kg of pimozide persisted for 2 days in one rat and for 3 days in another.

DISCUSSION

These experiments demonstrate that experimentally naive rats will learn to self-inject apomorphine over a wide range of intravenous doses. Apomorphine causes motor stimulation in the rat [17]. It therefore might be argued that exploratory behavior produces the first few lever presses, and then the stimulant action of apomorphine leads to further accidental self-injections. This idea is contradicted by the following observations: (a) the acquisition of apomorphine self-injection behavior follows a typical learning curve; (b) the intervals between apomorphine self-injections are precisely timed; (c) the day-to-day intake of apomorphine is constant; and (d) high unit doses, which are more stimulating than low doses, produce lower rather than higher rates of self-injection. It therefore seems reasonable to conclude that apomorphine maintains self-injection behavior by a reinforcing action rather than by nonspecific stimulation.

The neurochemical basis of this reinforcing action of apomorphine has important implications for theories of reinforcement. Considerable evidence indicates that apomorphine activates dopamine receptors in the brain [2, 7, 11]. Furthermore, the dopamine antagonist pimozide disrupts the regular pattern of apomorphine self-injection and, at a high dose, suppresses the rate of self-injection. In addition, the precisely spaced pattern of apomorphine self-injection closely resembles the self-injection pattern reported in the rat for amphetamine [22,35], a dopamine releasing agent [6]. All of these results suggest that activation of central dopamine receptors is at least partly responsible for the reinforcing effects of apomorphine and amphetamine. It is intriguing also to speculate that the reinforcing effects of other drugs, particularly morphine and related narcotics, may similarly be dependent on the participation of dopamine systems. Consistent with this interpretation is the finding that the self-administration of morphine is disrupted by haloperidol [13].

It should be kept in mind, however, that both apomorphine and amphetamine also affect noradrenergic transmission. Norepinephrine is released in the brain by amphetamine [18, 28, 33], and central levels of norepinephrine are decreased by high doses of apomorphine [19,20]. Furthermore there is evidence that both dopaminergic and "noradrenergic neurones are involved in the stimulation of motility by apomorphine" ([17] p. 212). In view of the data indicating that norepinephrine is involved in electrical self-stimulation of the brain [24, 29, 34], these considerations suggest that noradrenergic systems also may play an important role in the mediation of apomorphine and amphetamine self-administration. Indeed, if dopamine receptor stimulation causes activation of noradrenergic neurons as suggested by Persson and Waldeck [20] and Maj, *et al.* [17], then it is possible that dopamine-induced reinforcement is mediated via the release of norepinephrine.

Rats tend to self-administer drugs that humans, in some way, find pleasurable [26]. In this regard, the self-injection of apomorphine is surprising. The primary clinical effect of apomorphine is emesis [27]. Apomorphine has been given to patients with Parkinson's disease at relatively low doses with some therapeutic success [5, 8, 10], but no euphoria nor other pleasant effects are reported. Indeed, apomorphine may be used as a punishment in aversive conditioning [12]. The apparent anomaly that the drug may serve both as reward and punishment may depend on the dose, the route of administration, and the susceptibility of the species to the emetic action of apomorphine.

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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. Anden, N., A. Rubenson, K. Fuxe and T. Hökfelt. Evidence for dopamine receptor stimulation by apomorphine. *J. Pharm. Pharmac.* **19:** 627–632, 1967.
3. Arbuthnott, G., K. Fuxe and U. Ungerstedt. Central catecholamine turnover and self-stimulation behavior. *Brain Res.* **27:** 406–413, 1971.
4. Baxter, B. L., M. I. Gluckman and R. A. Scerni. Differential self-injection behavior produced by fenfluramine versus other appetite-inhibiting drugs. *Fedn Proc.* **32:** 754, 1973.
5. Braham, I., I. Sarova-Pinhas and Y. Goldhammer. Apomorphine in Parkinsonism tremor. *Br. med. J.* **3:** 768, 1970.
6. Carlsson, A. Amphetamine and brain catecholamines. In: *Amphetamines and Related Compounds*, edited by E. Costa and S. Garattini. New York: Raven Press, 1970, pp. 289–300.
7. Costall, B. and R. J. Naylor. On the mode of action of apomorphine. *Eur. J. Pharmac.* **21:** 350–361, 1973.

8. Cotzias, G. E., P. S. Papavasiliou, C. Fehling, B. Kaufman and I. Mena. Similarities between neurologic effects of *L*-DOPA and of apomorphine. *New Engl. J. Med.* **282:** 31–33, 1970.
9. Crow, T. J. A map of the rat mesencephalon for electrical self-stimulation. *Brain Res.* **36:** 265–273, 1972.
10. Duby, S. E., G. C. Cotzias, P. S. Papavasiliou and W. H. Lawrence. Injected apomorphine and orally administered levodopa in Parkinsonism. *Archs Neurol. Psychiat.* **27:** 474–480, 1972.
11. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacologia* **10:** 316–323, 1967.
12. Garcia, J., F. R. Ervin and R. A. Koelling. Learning with long delay of reinforcement. *Psychon. Sci.* **5:** 121, 1966.
13. Hanson, H. M. and C. A. Cimini-Venema. Effects of haloperidol on self-administration of morphine in rats. *Fedn Proc.* **31:** 503, 1972.
14. Janssen, P. A. J., G. J. E. Niemegeers, K. H. I. Scheelikens, A. Dresse, F. H. Lenaerts, A. Pinchard, W. K. A. Schaper, K. M. Van Newten and F. J. Verbruggen. Pimozide, a chemically novel, highly potent and orally long-acting neuroleptic drug. I. The comparative pharmacology of pimozide, haloperidol, and chlorpromazine. *Arzneimittel-Forsch.* **18:** 261–279, 1968.
15. Liebman, J. M. and L. L. Butcher. Effects on self-stimulation behavior of drugs influencing dopaminergic neurotransmission mechanisms. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **277:** 305–318, 1973.
16. 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:** 23–28, 1973.
17. Maj, J., M. Grabowska and L. Gajda. Effect of apomorphine on motility in rats. *Eur. J. Pharmac.* **17:** 208-214, 1972.
18. Moore, K. E., L. A. Carr and J. A. Dominic. Functional significance of amphetamine-induced release of brain catecholamines. In: *Amphetamines and Related Compounds,* edited by E. Costa and S. Garattini. New York: Raven Press, 1970, pp. 371–384.
19. Nybäck, H., J. Schubert and G. Sedvall. Effect of apomorphine and pimozide on synthesis and turnover of labelled catecholamines in mouse brain. *J. Pharm. Pharmac.* **22:** 622–624, 1970.
20. Persson, T. and B. Waldeck. Further studies on the possible interaction between dopamine and noradrenaline containing neurons in the brain. *Eur. J. Pharmac.* **11:** 315–320, 1970.
21. Phillips, A. G. and H. C. Fibiger. Dopaminergic and noradrenergic substrates of positive reinforcement: differential effects of *d*- and *l*-amphetamine. *Science* **179:** 575–577, 1973.
22. Pickens, R. and W. C. Harris. Self-administration of *d*-amphetamine by rats. *Psychopharmacologia* **12:** 158–163, 1968.
23. Pickens, R. and T. Thompson. Cocaine-reinforced behavior in rats: effects of reinforcement magnitude and fixed-ratio size. *J. Pharmac. exp. Ther.* **161:** 122–129, 1968.
24. Ritter, S. and L. Stein. Self-stimulation of the noradrenergic cell group (A6) in the locus coeruleus of rats. *J. comp. physiol. Psychol.* **85:** 443–452, 1973.
25. Rotrosen, J., M. B. Wallach, B. Angrist and S. Gershon. Antagonism of apomorphine-induced stereotypy and emesis in dogs by thioridazine, haloperidol and pimozide. *Psychopharmacologia* **26:** 185–194, 1972.
26. Schuster, C. R. and T. Thompson. Self administration and behavioral dependence on drugs. *Ann. Rev. Pharmac.* **9:** 483–502, 1969.
27. Sollman, T. A Manual of Pharmacology. Philadelphia: Saunders, 1948, p. 491.
28. Stein, L. Self-stimulation of the brain and the central stimulant action of amphetamine. *Fedn Proc.* **23:** 836–850, 1964.
29. Stein, L. Chemistry of reward and punishment. In: *Psychopharmacology, A Review of Progress: 1957–1967,* edited by D. H. Efron. Washington: U. S. Government Printing Office, 1968, pp. 105-123.
30. Thornberg, J. E. and K. E. Moore. The relative importance of dopaminergic and noradrenergic neuronal systems for the stimulation of locomotor activity induced by amphetamine and other drugs. *Neuropharmacology* **12:** 853–866, 1973.
31. Wauquier, A. and C. J. E. Niemegeers. Intracranial self-stimulation in rats as a function of various stimulus parameters. II. Influence of haloperidol, pimozide and pipamperone on medial forebrain bundle stimulation with monopolar electrodes. *Psychopharmacologia* **27:** 191–202, 1972.
32. Weeks, J. R. and J. D. Davis. Chronic intravenous cannulas for rats. *J. appl. Physiol.* **19:** 540–541, 1964.
33. 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.
34. Wise, C. D., B. D. Berger and L. Stein. Evidence of α-noradrenergic reward receptors and serotonergic punishment receptors in the rat brain. *Biol. Psychiat.* **6:** 3–21, 1973.
35. Yokel, R. A. and R. Pickens. Self-administration of optical isomers of amphetamine and methylamphetamine by rats. *J. Pharmac. exp. Ther.* **187:** 27–33, 1973.