Pharmacology Biochemistry & Behavior, Vol. 32, pp. 43–47. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Breaking Points on a Progressive Ratio Schedule Reinforced by Intravenous Apomorphine Increase Daily Following 6-Hydroxydopamine Lesions of the Nucleus Accumbens[1]

DAVID C. S. ROBERTS

Department of Psychology, Carleton University, Ottawa, Canada, K1S 5B6

Received 16 March 1988

ROBERTS, D. C. S. *Breaking points on a progressive ratio schedule reinforced by intravenous apomorphine increase daily following 6-hydroxydopamine lesions of the nucleus accumbens.* PHARMACOL BIOCHEM BEHAV 32(1) 43–47, 1989.—It has been shown previously that 6-hydroxydopamine (6-OHDA)-induced lesions of the nucleus accumbens cause extinction of cocaine self-administration behavior, yet fail to affect the rate of apomorphine self-administration on a Fixed Ratio 1 schedule of reinforcement. Since the dopamine (DA) receptors in this nucleus should become supersensitive, and since these receptors are thought to mediate the reinforcing effects of apomorphine, then some change in apomorphine self-administration would be expected. We have therefore reinvestigated the effect of 6-OHDA lesions on apomorphine self-administration using a progressive ratio schedule of reinforcement. Rats were trained to self-administer apomorphine (0.033 mg/inj, IV) on a schedule in which the response requirements increased after each reinforcement. The breaking point was defined as the final ratio completed during each daily session. Sham lesions had no effect on the breaking points, and a steady increase in the breaking point was observed in the 6-OHDA-lesioned animals which may parallel the development of DA receptor supersensitivity.

Apomorphine Self-administration Dopamine 6-Hydroxydopamine Reinforcement

DOPAMINE (DA) receptors appear to be critically involved in the reinforcing properties of psychomotor stimulant drugs. While amphetamine and cocaine also have actions on noradrenaline and serotonin systems, a variety of evidence indicate that they achieve their reinforcing effects through an indirect action on DA terminals. For example, biochemical studies have shown that cocaine analogues block the reuptake of DA from the presynaptic terminal (5,13) and this action correlates strongly with their potency in self-administration experiments (18). Direct DA agonists, such as apomorphine, bromocriptine and piribedil, are self-administered by laboratory animals (1, 6, 26, 28), and DA antagonists, such as haloperidol and pimozide, block psychomotor stimulant self-administration (3,27).

Progress has been made in identifying the anatomical location of the critical DA receptors. Because amphetamine and cocaine are indirect-acting agonists requiring the presynaptic terminal, it has been possible to test whether the DA innervation of particular brain regions is critical to their reinforcing effects. Lesions of the DA terminals in the nucleus accumbens septi (NAS), induced by 6-hydroxydopamine (6-OHDA), were found to disrupt cocaine self-administration behavior (20,22), although 6-OHDA lesions of the dorsal noradrenergic bundle (20), the striatum (24), or frontal cortex (14), were found to have no effect. It appears, therefore, that the mesolimbic DA system is specifically and critically involved in psychomotor stimulant self-administration behavior.

There is one experimental result, however, which is not easily accommodated by this mesolimbic DA hypothesis. Animals sustaining 6-OHDA lesions of the NAS have been tested for apomorphine self-administration and it has been found that the rate of drug intake before and after the lesion is identical. The data pose an interpretational problem because the 6-OHDA lesion should have promoted the development of "supersensitive" DA receptors, and if these receptors are responsible for apomorphine reinforcement then one would have expected a change in the rate of apomorphine self-administration.

It is possible that changes can occur in the reinforcing

[1]Preliminary data from this experiment were presented at the "Conference on Mesolimbic Dopamine" sponsored by the New York Academy of Science, May 4–6, 1987.

efficacy of a drug which are not reflected in an altered rate of drug intake. In the present study, lesion-induced changes were assessed through the use of a progressive ratio (PR) schedule in which breaking point, i.e., the last completed ratio in an escalating series, was used as the dependent variable.

METHOD

Male Wistar rats (Woodlyn Labs., Guelph, Canada) were housed individually in Plexiglas test cages equipped with stimulus lights and lever receptacles. Each rat was implanted, under pentobarbital anesthesia, with a chronically indwelling Silastic jugular cannula. The tubing exited at the mid-scapular level of the back and was connected through a protective spring to a fluid swivel and syringe pump [for complete details see (21)]. Preliminary training consisted of daily 4-hr sessions that began with the positioning of a lever into its receptacle in the housing/test cage. During this initial training, each depression of the lever resulted in an injection of cocaine (FR 1; 0.6 mg/injection) which was signaled by a 20-sec stimulus light. (Responses made during this period were recorded but had no programmed consequence.) Animals that demonstrated a regular response pattern for cocaine were then screened for apomorphine self-administration (0.033 mg/injection). If a regular response pattern was established for apomorphine reinforcement on the FR 1 schedule then a PR schedule was imposed. Animals were tested daily for 5 hr on the PR schedule during which the response requirements to earn an injection escalated throughout the series: the progression was 1, 2, 3, 5, 7, 9, 12, 15, 18, 23, 28, 33, 41, 49, 57, 70, 83, 96, 117, 138, 156, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400. The last ratio completed was defined as the "breaking point."

After one week of baseline training, one group of rats was anesthetized and injected bilaterally with 6-OHDA (8 μg/2 μl, each side, dose expressed as the free base) into the nucleus accumbens according to the following coordinates from stereotaxic zero: AP +11.0 mm, ML ±1.8 mm, DV +2.7 mm, head held in the plane of Paxinos and Watson (15). The control group received vehicle injections alone (2 μl of saline containing 0.1 mg/ml ascorbic acid). Beginning the day after surgery, animals were tested on the PR schedule for apomorphine reinforcement for up to two weeks. Approximately one month after the lesion, the animals were decapitated and the brains were rapidly removed. The caudate nucleus and nucleus accumbens were dissected on ice, frozen in liquid nitrogen, stored at −70°C and subsequently assayed for DA content (12).

RESULTS

The lesion experiment reported here is based on a total of 16 animals: 5 sham-operated and 11 6-OHDA-lesioned animals. These numbers represent rats that demonstrated a stable pattern of apomorphine self-administration on an FR 1, and retained a patent cannula through to postlesion day 10.

It is important to note that the experimental subjects were selected from a larger population. Each was prescreened, initially for cocaine self-administration and then for apomorphine self-administration. Many animals failed to show reliable apomorphine self-administration even though they had previously demonstrated regular cocaine-reinforced responding. Of the 35 animals that had met the cocaine self-administration requirements during the main experiment and during pilot studies, only 22 rats showed a reliable pattern of

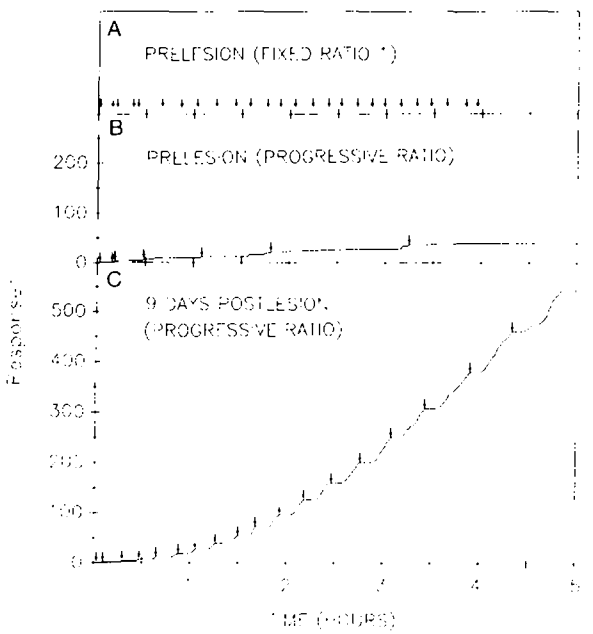

FIG. 1. (A) Event record of a 4-hr apomorphine self-administration session during which each lever response produces a drug injection. Arrows indicate the time of each infusion. (B) Cumulative record of apomorphine self-administration behavior reinforced on a progressive ratio (PR) schedule. The line represents cumulative response summed at one-min intervals. The arrows indicate the time of the apomorphine injections. (C) Cumulative record of apomorphine self-administration on a PR schedule 9 days after 6-OHDA injections into the nucleus accumbens. (Same animal as in Panel B.)

apomorphine intake. Interestingly, three other rats developed a stereotyped behavior pattern which involved the lever. These animals responded on the lever continually at rates of up to 3000 presses/hr and would have overdosed had the pump not been deactivated. Thus, any rat that did not show a postinfusion pause of several minutes was excluded from the study.

As emphasized above, a regular response pattern for apomorphine on an FR 1 schedule was a requisite of every animal in the study. Figure 1 (panel A) shows an example of the regular infusion pattern observed on the 4-hr training session. When the schedule was switched to the PR, the pattern often became inconsistent (Fig. 1, panel B). Prior to the lesion, the animals would self-administer apomorphine on the PR schedule only when the response requirements were relatively low, and usually failed to respond past ratios of 12–15. The temporal pattern of responding was often disrupted by these small response demands.

The SHAM surgery was found to have no significant effect on the self-administration behavior for apomorphine reinforcement on the PR schedule. The control animals continued to extinguish each day after responding to a final ratio of 9–18 (i.e., after 6–9 injections).

The 6-OHDA treatment was found to produce a variable effect on self-administration behavior and on DA content in the nucleus accumbens. DA content ranged from 9–74% of control values. Animals with DA content in the nucleus accumbens greater than 25% of control values were excluded from the initial analysis, leaving 6 animals with average DA

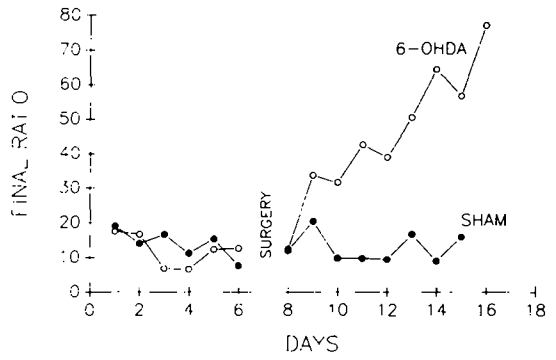

FIG. 2. The effect of 6-OHDA lesions on apomorphine self-administration on a progressive ratio schedule. Each point represents the average final ratio completed during the daily 5-hr session by groups of rats that received either 6-OHDA lesions of the nucleus accumbens (6-OHDA, N=6) or vehicle injections (SHAM, N=5).

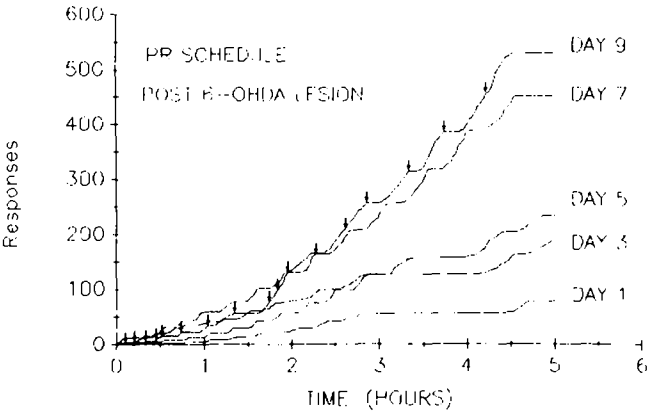

FIG. 3. The effect of 6-OHDA lesions of the nucleus accumbens on the pattern of apomorphine self-administration on a progressive ratio schedule. Each line represents the cumulative record for the specified day following the 6-OHDA lesion. For clarity, the time of the drug infusions (indicated by arrows) are shown only for postlesion day 9.

levels of 14.5% of control. Dopamine levels were as follows (mean±S.E.M.): Control=2.45±0.2 μg/g; 6-OHDA=0.35±0.05 μg/g. Animals that sustained substantial depletions of DA showed a dramatic increase in breaking point. An example of apomorphine self-administration on the PR schedule is shown in Fig. 1 (panel C). In contrast to their behavior prior to the lesion, animals in the 6-OHDA group developed a regular pattern of apomorphine intake and would respond to high ratios in order to earn the drug. Following the lesion there was a steady increase each day in the final ratio completed.

Figure 2 shows the breaking points before and after surgery for the two groups. Note that the breaking points for the SHAM group did not change throughout the experiment, whereas the 6-OHDA group displayed a steady increase. Repeated measures ANOVA revealed a significant difference between the 6-OHDA group and the SHAM-operated animals, $F(1,9)=5.91$, $p<0.05$. The "Day" factor, $F(14,126)=5.98$, $p<0.001$, and the "Day × Group" interaction, $F(14,126)=6.23$, $p<0.01$, were also statistically significant; a finding that is accounted for by the daily increase in breaking point displayed by the 6-OHDA group. The group of 6-OHDA-treated animals with less severe DA depletions (N=5) showed an intermediate and variable effect.

The PR scale was designed to ensure that the ratios required to earn an injection in the third hour would be quite high guaranteeing that a breaking point would be established assuming normal rates of self-administration. In theory, responding should have extinguished by the beginning of the fifth hour and the breaking point could be defined as the final ratio. However, by the ninth day postlesion, many animals continued to respond through the five hours of the test, largely due to their slow pace. For whatever reason, they were incapable of obtaining more than 17 or 18 injections during the 5 hours, which resulted in a "ceiling effect" at a final ratio of 83 or 96. The experiment was therefore truncated at the ninth day postlesion, rather than alter the ratio scale or increase the duration of the session.

Figure 3 shows an example of the development of the increase in breaking point over days. On the first day after surgery, the pattern of responding was similar to prelesion days with the animal failing to respond relatively early. The pattern after surgery becomes more regular, with the animal continuing to respond through to the end of the 5-hr session.

DISCUSSION

The major finding of the present study was that 6-OHDA lesions dramatically changed the self-administration behavior on the PR schedule for apomorphine reinforcement. The data show a daily increase in breaking points for the 6-OHDA group. Since the experiment was truncated at postlesion day 9 due to the "ceiling" effect imposed by the 5-hr session (see the Results section), the real limit of this effect is unknown. Pilot animals tested on a steeply escalating PR have been found to respond on ratios as high as 300 after several weeks which suggests that the augmentation continues well past the increase reported here. The data indicate that 6-OHDA lesions of the nucleus accumbens increase the reinforcing efficacy of apomorphine. Whether the effect is due to an upward or leftward shift in the dose/response curve cannot be determined from the present data. It should be noted that the dose tested is at the lower end of the self-administration curve. Apomorphine at the dose of 0.1 mg/kg is only moderately effective as a reinforcing stimulus in control animals, as evidenced by the fact that the drug would not maintain responding on an FR 1 schedule in 13 of 35 rats that had been trained previously to self-administer cocaine. This appears to be consistent with the data of Baxter *et al.* (1) who report that a third to a quarter of the group of rats tested in this dose range failed to acquire apomorphine self-administration behavior. Both Baxter *et al.* (1) and Yokel and Wise (28) report much higher proportions acquire a stable response pattern at higher doses.

A second indication that the dose of apomorphine tested is a relatively weak reinforcing stimulus comes from the present demonstration that those nonlesioned rats which did respond regularly on an FR 1 schedule, displayed only modest breaking points when tested on the PR schedule. The final ratio completed by nonlesioned animals was seldom above 18. This can be compared to breaking points often exceeding 100 for various doses of cocaine (19,23) and heroin

(unpublished). Whether higher doses of apomorphine will maintain responding at higher ratios in control animals remains to be tested.

The term "progressive ratio" has been used to describe a variety of schedules employed in self-administration experiments involving primates (7–9, 11) and dogs (16,17). Usually the term implies a *fixed ratio* schedule that is increased from one day to the next day (i.e., not within a session). Thus "breaking points" are defined as the ratio in effect on the day on which responding falls below some set level. It, therefore, takes many days to establish one breaking point. In the present study, the schedule requirement increased with each reinforcement, as originally suggested by Hodos (10) for food reinforcement and adapted by Bedford *et al.* (2) for self-administration studies. This procedure yields a daily assessment of the breaking point. The ability to assess the breaking point on a regular basis is critical when evaluating changes that might rapidly occur after a lesion.

It should be reemphasized that the rate of apomorphine self-administration on a continuous reinforcement schedule (FR 1) is not changed by the 6-OHDA lesions to the nucleus accumbens (20). However, the present data clearly show that the 6-OHDA-treated animals display greater breaking points following the lesion. These data force the conclusion that the treatment produced marked changes in the reinforcement efficacy of the injection and suggest that this change is due to the development of supersensitivity of the DA receptor within the nucleus accumbens (25). Since the rate of intake on an FR 1 schedule is not changed, these data imply that important motivational influences on drug intake may be overlooked if rate of self-administration is used as the only dependent variable.

The finding that changes in reinforcing efficacy can take place in the absence of any change in rate of self-administration has widespread implications for the interpretation of experiments in which a treatment has failed to affect rate of self-administration. For example, the conclusion has been drawn that neither noradrenergic nor opiate mechanisms are involved directly in stimulant reinforcement based on the failure of noradrenergic lesion (20) or opiate antagonists (4) to affect the rate of cocaine self-administration. It now appears that such conclusions should not be drawn based solely on results showing no change in rate of drug intake.

ACKNOWLEDGEMENTS

This work was supported by a Natural Sciences and Engineering Research Council of Canada grant to D.C.S.R. The excellent technical assistance of G. Vickers is gratefully acknowledged.

REFERENCES

1. Baxter, B. L.; Gluckman, M. I.; Stein, L.; Scerni, R. A. Self-injection of apomorphine in the rat: Positive reinforcement by a dopamine receptor stimulant. Pharmacol. Biochem. Behav. 2:387–391; 1974.
2. Bedford, J. A.; Bailey, L. P.; Wilson, M. C. Cocaine reinforced progressive ratio schedule performance in the rhesus monkey. Pharmacol. Biochem. Behav. 9:631–638; 1978.
3. deWit, H.; Wise, R. A. Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide, but not with noradrenaline blockers phentolamine and phenoxybenzamine. Can. J. Psychol. 31:195–203; 1977.
4. Ettenberg, A.; Pettit, H. O.; Bloom, F. E.; Koob, G. F. Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. Psychopharmacology (Berlin) 78:204–209; 1982.
5. Farnebo, L. -O. Effect of D-amphetamine on spontaneous and stimulation induced release of catecholamines. Acta Physiol. Scand. [Suppl.] 371:45–52; 1971.
6. Gill, C. A.; Holz, W. C.; Zirkle, C. The role of dopamine in self-administration of psychomotor stimulants. Reported to the NAS-NRC Committtee of Drug Dependence, 1975.
7. Griffiths, R. R.; Bradford, L. D.; Brady, J. V. Progressive ratio and fixed ratio schedules of cocaine-maintained responding in baboons. Psychopharmacology (Berlin) 65:125–136; 1979.
8. Griffiths, R. R.; Brady, J. V.; Snell, J. D. Progressive-ratio performance maintained by drug infusions: comparison of cocaine, diethylproprion, chlorphentermine, and fenfluramine. Psychopharmacology (Berlin) 56:5–13; 1978.
9. Griffiths, R. R.; Findley, J. D.; Brady, J. V.; Gutcher, K.; Robinson, W. W. Comparison of progressive-ratio performance maintained by cocaine, methylphenidate and secobarbitol. Psychopharmacologia 43:81–83; 1975.
10. Hodos, W. Progressive ratio as a measure of reward strength. Science 134:943–944; 1961.
11. Hoffmeister, F. Progressive-ratio performance in the rhesus monkey maintained by opiate infusions. Psychopharmacology (Berlin) 62:181–186; 1979.
12. Jacobowitz, D.; Richardson, J. Method for rapid determination of norepinephrine, dopamine and serotonin in the same brain region. Pharmacol. Biochem. Behav. 8:515–519; 1978.
13. McKenzie, G. M.; Szerb, J. C. The effect of dihydroxyphenylalanine, pheniprazine and dextroamphetamine on the *in vivo* release of dopamine from the caudate nucleus. J. Pharmacol. Exp. Ther. 162:302–308; 1968.
14. Martin-Iverson, M. T.; Szostak, C.; Fibiger, H. C. 6-Hydroxydopamine lesions of the medial prefrontal cortex fail to influence intravenous self-administration of cocaine. Psychopharmacology (Berlin) 88:310–314; 1986.
15. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.
16. Risner, M. E.; Goldberg, S. R. A comparison of nicotine and cocaine self-administration in the dog: Fixed ratio and progressive-ratio schedules of intravenous drug infusion. J. Pharmacol. Exp. Ther. 224:319–326; 1983.
17. Risner, M. E.; Silcox, D. L. Psychomotor self-administration by beagle dogs in a progressive-ratio paradigm. Psychopharmacology (Berlin) 5:25–30; 1981.
18. Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219–1223; 1987.
19. Roberts, D. C. S.; Bennett, S. A. L.; Vickers, G. J. Gender and estrous cycle affect cocaine self-administration on a progressive ratio schedule. Psychopharmacology (Berlin); in press.
20. Roberts, D. C. S.; Corcoran, M. E.; Fibiger, H. C. On the role of ascending catecholamine systems in intravenous self-administration of cocaine. Pharmacol. Biochem. Behav. 6:615–620; 1977.
21. Roberts, D. C. S.; Goeders, N. Drug self-administration: Experimental methods and determinants. In: Boulton, A. A.; Baker, G. B.; Greenshaw, A. J., eds. Neuromethods. Clifton, NJ: Humana Press; in press.
22. Roberts, D. C. S.; Koob, G. F.; Klonoff, P.; Fibiger, H. C. Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol. Biochem. Behav. 17:901–904; 1980.

23. Roberts, D. C. S.; Loh, E. A.; Vickers, G. Self-administration of cocaine on a progressive ratio schedule in rats: Dose response relationship and effect of haloperdiol pretreatment. Psychopharmacology (Berlin); in press.
24. Roberts, D. C. S.; Zito, K. A. Interpretation of lesion effects on stimulant self-administration. In: Bozarth, M. A., ed. Methods of assessing the reinforcing properties of abused drugs. New York: Springer-Verlag; 1987:87–103.
25. Ungerstedt, U. Postsynaptic supersensitivity after 6-hydroxy-dopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol. Scand. [Suppl.] 367:69–95; 1971.
26. Woolverton, W. L.; Goldberg, L. I.; Ginos, J. Z. Intravenous self-administration of dopamine receptor agonists by rhesus monkeys. J. Pharmacol. Exp. Ther. 230:678–683; 1984.
27. Yokel, R. A.; Wise, R. A. Increased lever pressing for amphetamine after pimozide in rats: implications for a dopamine theory of reward. Science 187:547–549; 1975.
28. Yokel, R. A.; Wise, R. A. Amphetamine-type reinforcement by dopaminergic agonists in the rat. Psychopharmacology (Berlin) 58:289–296; 1978.