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Pimozide Attenuates Lever Pressing for Water Reinforcement in Rats[1]

GARY J. GERBER, JOHN SING AND ROY A. WISE[2]

Center for Research on Drug Dependence, Department of Psychology
Concordia University, Montreal, Quebec, Canada H3G 1M8

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GERBER, G. J., J. SING AND R. A. WISE. *Pimozide attenuates lever pressing for water reinforcement in rats.* PHARMAC. BIOCHEM. BEHAV. **14**(2) 201–205, 1981.—Rats were trained to lever-press for water on a schedule of continuous reinforcement, then tested every fourth session on five occasions either under conditions of non-reinforcement or following injections of the dopamine receptor blocker pimozide (0.5 or 1.0 mg/kg) or the injection vehicle. The low dose of pimozide did not significantly attenuate responding until the fifth session. The high dose attenuated responding on all occasions, with residual responding decreasing progressively across repeated drug sessions. Responding in the pimozide conditions was never less than that of the non-reinforced control group. Responding in each condition was strongest in the early minutes of a session. After five sessions, rats were switched from the pimozide condition to the non-reinforced condition (or vice-versa) for one additional test day. Decreased responding continued for rats transferred from non-reinforcement to pimozide though not for rats transferred from pimozide to non-reinforcement. These data suggest a general role for brain dopamine in behavior; they reflect the same patterns as have been seen with food reinforcement and with several centrally-acting reinforcers.

Pimozide Dopamine receptor blockade Neuroleptics Water reinforcement Extinction

BLOCKADE of dopamine receptors with neuroleptics such as pimozide has been shown to interfere with the performance of several behaviors maintained by a variety of centrally-acting reinforcers [3–8, 10, 11, 16–18]. While it has been argued that neuroleptic effects on operant behavior might be attributable to some non-specific performance deficit such as a difficulty in initiation of voluntary movement or coordination of sensory-motor acts [1, 2, 4, 10, 11], this possibility can now clearly be ruled out [3, 5–9, 14–18]. It appears that the attenuation of responding observed with brain stimulation reinforcement [5–8, 18] and the increase in responding observed with psychomotor stimulant reinforcement [3, 16, 17] are, at least in major part, the result of decreased efficacy of the reinforcer under neuroleptic treatment. Several facts support this view: (1) pimozide-treated rats usually show normal performance during the initial period of all test sessions under continuous reinforcement; (2) repeated pimozide testing causes progressively less sustained responding in repeated testing, but only if the drug is given in the test sessions and not if it is given in the home cage; (3) performance can be reinstated after response cessation under pimozide through presentation of environmental stimuli which have arousing properties only through past association with reinforcement, though these stimuli, too, lose their efficacy with continued exposure under pimozide. The response deficits are clearly not due to an inability to respond, nor are they due to the development of such an inability as a result of initial responding under drug [3, 5–8, 16–18].

Neuroleptics have been used to challenge only one class of natural reinforcers in paradigms that allow such inferences: food reinforcement [1, 2, 9–11, 13–15]. Inasmuch as the centrally-acting reinforcers studied may all activate the same central mechanism as is synaptically activated by food reward, the generality of speculations regarding dopamine involvement in reward function should be established against tests with a variety of natural reinforcers, and not simply a variety of central reinforcers. The present study examined the effects of pimozide on water-reinforced lever-pressing in thirsty rats, following one of the important paradigms which has been used with food reward [14,15], as a first step toward examining the generality of neuroleptic effects.

METHOD

Animals

Thirty-two experimentally naive Sprague-Dawley rats obtained from Canadian Breeding Farms and Laboratories, St. Constant, Québec, were used. They were housed singly and given free access to food. They were deprived of water for 22 hr prior to each experimental session. The mean weight of the rats was 360 g.

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[2]Send reprint requests to R. A. Wise, Center for Research on Drug Dependence, Department of Psychology, Concordia University, Montreal, Québec, Canada H3G 1M8.

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Apparatus

Rats were tested in experimental chambers measuring 27×51×38 cm which were equipped with Gerbrands Model 5600 solenoid-operated water dippers located in the center of the shorter side, 4.5 cm above the floor. Plastic levers were 6 cm long and 0.9 cm wide, and were located 8 cm above the chamber floor, 4 cm from the left corner.

Procedure

The water-deprived rats were trained to lever-press on a schedule of continuous reinforcement for five sec access to 0.5 ml of water in the dipper. Lever-pressing was shaped by reinforcing successive approximations and by leaving the rats in the chamber overnight. Following a week of shaping, responding was stabilized for three weeks of daily 15 min sessions until each rat's response rate varied by less than 10% of the mean of the previous five sessions. Rats were assigned to four groups of eight animals each so that mean response rates were equivalent for the groups.

A total of seven test sessions of 45 min duration were run, separated by one day without testing and two days of retraining. On the first five sessions, one group of rats received IP injections of the drug vehicle and responding during the test session was normally reinforced. The next group of rats was tested without their usual water reinforcement; that is, responding resulted in the presentation of the empty dipper. Rats in two more groups received IP injections of 0.5 and 1.0 mg/kg pimozide, respectively, and their responses were normally reinforced with water during the test session. Injections were given four hours prior to testing to allow for peak distribution of pimozide to the brain.

On the sixth test session, all groups received an injection of vehicle to assess any change in response rates due to: (1) motor deficits caused by cumulative drug effects; (2) conditioned taste aversion effects; or (3) differences in training. The seventh test session was run the day immediately following the sixth test. The group that had not been reinforced in test sessions 1 through 5 was given 1.0 mg/kg pimozide and run for a normally reinforced session. Groups that had received pimozide in tests 1 through 5 were given vehicle and were tested without their normal water reinforcer. The purpose of this session was to determine whether decreased responding continued for rats transferred from conditions of non-reinforcement to reinforcement under pimozide, and for rats transferred from reinforcement under pimozide to conditions of non-reinforcement.

Responses were recorded each min for 15 min of the test session, and each 10 min thereafter until the session had run 45 min.

Drugs

Pimozide was prepared in a 0.3% solution of tartaric acid dissolved in distilled water. The tartaric acid solution alone was used for vehicle control injections.

Analysis of Data

Repeated measures analyses of variance were performed on each group's response data for the initial 15 min of the first five sessions (Time×Session). Single sessions were also analyzed using a Group×Dose×Time repeated measures design. Session 1 performance of the 1.0 mg/kg pimozide-treated rats was compared with Session 7 performance of the non-reinforced rats that were given pimozide and tested under normal reinforcement conditions. A Group × Dose × Time analysis for independent groups was used for this comparison. Scheffé tests were used for comparison of group means; the α-level was set at $p<0.10$ as recommended by Scheffé [12].

RESULTS

Normally-reinforced rats, treated with vehicle or 0.5 mg/kg pimozide, began responding vigorously and then showed a gradual decrease in rates within the first test session. Normally-reinforced rats receiving 1.0 mg/kg pimozide and non-reinforced rats responded at rates below those of the first two groups in the initial 15 min of the first test session (Fig. 1A). Response rates differed significantly between groups for the initial 15 min, $F(3,28)=4.39$, $p<0.05$. This was attributable to the difference between the vehicle and the 0.5 mg/kg pimozide groups responding at higher rates than the other two groups ($p<0.05$). The decrease in responding across the session was significant, $F(14,392)=27.72$, $p<0.0001$. A significant Group×Time interaction, $F(14,392)=2.40$, $p<0.0001$, for the first 15 min is the result of the 1.0 mg/kg pimozide and the non-reinforced groups' response rate decreasing faster than that of the other two groups.

Responding in successive test sessions was analyzed separately for each of the four groups. There was no significant change in responding across the five test sessions for the vehicle or 0.5 mg/kg pimozide groups, $F(4,28)=1.25$, and $F(4,28)=2.09$, respectively. Both the non-reinforced and the 1.0 mg/kg pimozide groups showed significant changes across sessions, $F(4,28)=10.25$, $p<0.0001$, and $F(4,28)=11.62$, $p<0.0001$, respectively, for the first 15 min of the session. Significant Session×Time interactions were found in the non-reinforced and the 1.0 mg/kg pimozide groups, $F(56,392)=1.41$, $p<0.04$ and $F(56,392)=1.70$, $p<0.003$, respectively, indicating that responding decreased sooner in successive test sessions. This effect is seen by comparing responding in Sessions 1 and 5 (Figs. 1A and 1B). Responding of the non-reinforced and 1.0 mg/kg pimozide groups diminished more rapidly in Session 5 than in Session 1.

Responding of the 0.5 mg/kg pimozide group was equivalent to that of the vehicle control group in Session 1. In Session 5, responding of the 0.5 mg/kg pimozide group was lower than that of the control group for the first 9 min of the session. The analysis of variance for Session 5 showed a significant group effect, $F(3,28)=39.61$, $p<0.0001$, but the difference between these two groups did not reach statistical significance. The group effect was attributable to the difference between the control group and both the 1.0 mg/kg pimozide and the non-reinforced groups ($p<0.10$). A significant decrease in responding across time, $F(14,392)=29.89$, $p<0.0001$, and a significant Group×Time interaction, $F(42,392)=5.92$, $p<0.0001$, were the result of high initial rates in the control group.

On the sixth test day, the control group and both pimozide-treated groups were injected with vehicle and tested with normal reinforcement. There were no significant differences in responding between the three groups, $F(2,21)=3.15$, which showed similar decreases in responding over time (Group×Time interaction was not significant, $F(28,291)=0.95$).

The test of transfer from non-reinforced, non-drug conditions to pimozide-treated, reinforced conditions, and from

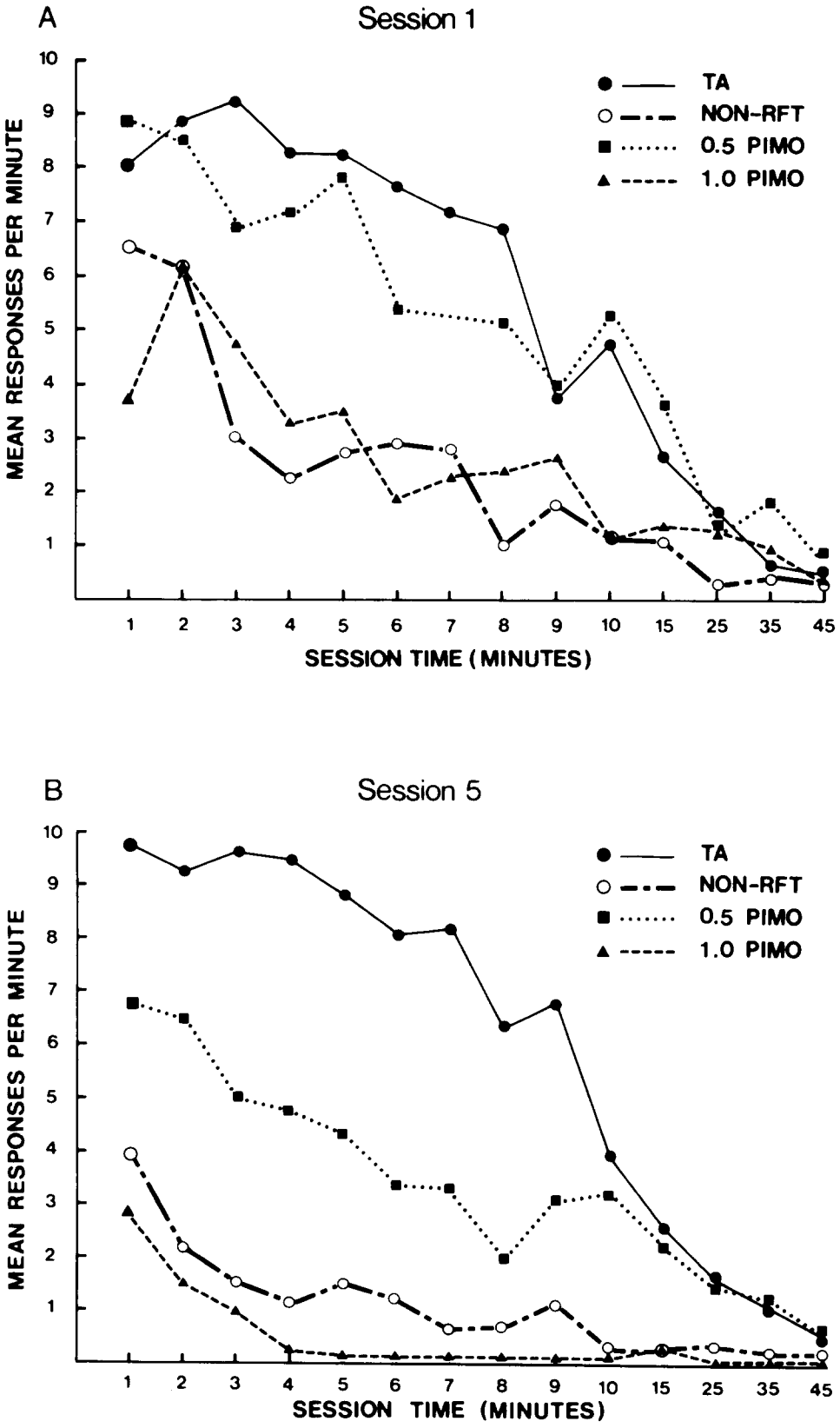

FIG. 1. Mean response rates across sessions for normally-reinforced groups treated with tartaric acid (TA), 0.5 mg/kg, or 1.0 mg/kg pimozide, and for the non-reinforced group. Test day 1 is shown in panel A, and test day 5 is shown in panel B.

pimozide-treated reinforced conditions to non-reinforced, non-drug conditions was performed in Session 7. A comparison of the Session 7 performance of rats that had received five sessions of non-reinforcement and were tested with 1.0 mg pimozide with the Session 1 performance of rats that received normal reinforcement and 1.0 mg/kg pimozide showed that experience with non-reinforcement significantly reduced the performance, $F(1,14)=5.42$, $p<0.05$ for the first 15 min of the session. There was no transfer between pimozide testing experience and non-reinforced testing. The two groups that received pimozide for five test sessions, and were tested without water reinforcement on Session 7, responded more than did the non-reinforced group in Session 1.

DISCUSSION

Repeated experience with pimozide progressively reduced lever-pressing in rats responding for water on a continuous reinforcement schedule. The amount of reduction depended on a history of receiving pimozide while responding for water and on the dose of pimozide administered. Several facts argue against the possibility that these results are due to response-debilitating effects of pimozide. First, the responding of the low dose group was not abnormally low on the first few days of testing; in this group responding was lower than that of normally reinforced, non-drugged animals only on the final day of testing. However, this difference was not statistically significant. At this dose of pimozide the animals are clearly capable of normal responding, but did not show such responding on the fifth day of testing. It is very unlikely that this simply reflects a progressive accumulation of unmetabolized pimozide, since analogous data in a food reinforcement paradigm followed the same pattern when pimozide was given in the testing situation, but not when pimozide was given in the home cage [14]; pimozide given in the home cage, which should accumulate equally, does not cause the response attenuation that is seen when pimozide is given under lever-pressing conditions.

Responding in the high dose condition was attenuated even on the first day of testing. Again, it is unlikely that this represents a consequence of performance incapacitation. Even in the high dose condition, responding was never less than that seen in non-reinforced control animals, and, as in the low dose condition, responding on the fifth day did not reflect the performance capacity demonstrated on the first day. The high dose of pimozide in the present study was the same dose that fails to cause performance difficulties in food-reinforced testing on the same schedule [14].

All groups exhibited a decline in responding over time in the first test session. The decline was more rapid for the non-reinforced and the 1.0 mg/kg groups than it was for the vehicle or 0.5 mg/kg groups. The decline in the vehicle control group reflected the effects of satiation. The progressive decrease in responding which is always seen in pimozide-treated animals and in non-reinforced animals (except in the case of intravenous drug reinforcement, where compensatory increases rather than decreases in response rate are seen) cannot simply be a function of satiation, however, since it occurs even when a non-satiating reinforcer such as saccharin is used [15]. Pimozide thus causes a response attenuation which increases progressively both within and across sessions.

These results are interpreted as indicating that reinforced experience under pimozide acts to reduce the response-sustaining value of water, just as it has been argued to reduce the response-sustaining value of food. In this view the water-loaded dipper has for the pimozide-treated animals little more reinforcing value than the empty dipper has for undrugged animals. That such response attenuation is due to a decreased impact of the reinforcer and not due to some progressive impairment of performance capacity which develops within sessions is suggested by the decreased responding across sessions, and by the finding that rats that have ceased to respond in sessions involving brain stimulation reinforcement will reinitiate responding when exposed to a light stimulus which has previously been associated with normal reinforcement in the test situation [8]. Such reinstatement would not be possible if response inadequacy of any form were responsible for the within-session response cessation.

The possibility that progressive decreases in responding might reflect the accumulating acquisition of a conditioned taste aversion (from pimozide-water pairings) can be ruled out by the results of the sixth day of testing. Rats that received vehicle injections after a history of pimozide testing in Sessions 1–5 showed no evidence of a conditioned water aversion; they responded for water as often as did the vehicle control group on the first day of testing. Thus pimozide-water associations do not cause conditioned taste aversions in this paradigm.

There was a significant transfer effect between early non-reinforced testing and subsequent pimozide testing. This effect has been reported in some [14] but not all [13,15] food reinforcement tests, and, though it may not occur under all conditions, it has significant implications when it does occur. The animals that had five non-reinforced test sessions and were then tested under pimozide behaved as if they were undergoing a sixth non-reinforced trial. Prior experience with non-reinforcement resulted in less than half the number of responses that was seen in naive animals. That prior experience with non-reinforcement causes effects in pimozide tests which are similar to the effects of prior experience under pimozide suggests that the non-reinforcing testing and the pimozide testing share some critical attribute. The fact that responding is initiated but not sustained in each situation suggests that this attribute is reduction in the reinforcing impact of the events which usually sustain responding.

There was no positive transfer from initial pimozide testing to subsequent non-reinforcement testing, and this fact is equally important. It clearly indicates, as also argued on other grounds [15], that the pimozide condition and non-reinforcement condition are discriminated by the animal and are thus not totally equivalent. If transfer between the two conditions had been symmetrical, the above interpretation of the positive transfer from non-reinforcement testing to pimozide testing could be viewed with a good deal more confidence. The lack of bi-directionality of transfer does not, however, rule out the interpretation offered above for transfer from non-reinforcement to pimozide conditions. The positive transfer means that some aspects of the two conditions are similar, while lack of transfer from pimozide to non-reinforcement means that other aspects of the two conditions are not. Since responding during extinction can readily be reinstated by environmental stimuli in both natural extinction and pimozide-induced extinction [8], it seems likely that differences in some condition-specific cues to the animal account for the differences in transfer effects. Obvious cues are the drug cue and the cue of the water in the

dipper during pimozide testing. The importance of these cues in transfer effects and the assymetry of transfer effects is the subject of current investigation.

The present experiment demonstrates that pimozide attenuates responding for water just as it attenuates responding for food or brain stimulation reinforcement (including the asymmetrical transfer in both the food and water paradigms). This suggests that whatever the role played by dopamine in reinforcement processes, it is not specific to only one natural reinforcer and the experimental reinforcers capable of activating the same mechanism centrally. Inasmuch as water is only the second natural (peripheral) reinforcer examined in this way, additional reinforcers must be studied. Also, inasmuch as partial reinforcement studies suggest that a more complex analysis (neuroleptics seem to blunt the impact of secondary as well as primary reinforcers: [9, 10, 13]) is needed, additional reinforcement schedules must be studied as well. Whatever the ultimate interpretation of these studies, it is clear that neuroleptics have more interesting and subtle effects than simply producing difficulties in initiating, organizing or sustaining motor or sensory-motor acts.

REFERENCES

1. Ahlenius, S. and J. Engel. Behavioral effects of haloperidol after tyrosine hydroxylase inhibition. *Eur. J. Pharmac.* **15:** 187–192, 1971.
2. Ahlenius, S. An analysis of behavioral effects produced by drug-induced changes of dopaminergic transmission in the brain. *Scand. J. Psychol.* **20:** 59–64, 1979.
3. deWit, H. and R. A. Wise. Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide but not with the noradrenergic blockers phentolamine or phenoxybenzamine. *Can. J. Psychol.* **31:** 195–203, 1976.
4. Fibiger, H. C., D. A. Carter and A. G. Phillips. Decreased intracranial self-stimulation after neuroleptics or 6-hydroxydopamine: Evidence for mediation by motor deficits rather than by reduced reward. *Psychopharmacology* **47:** 21–27, 1976.
5. Fouriezos, G. and R. A. Wise. Pimozide-induced extinction of intracranial self-stimulation: Response patterns rule out motor or performance deficits. *Brain Res.* **103:** 377–380, 1976.
6. Fouriezos, G., P. A. Hansson and R. A. Wise. Decreased intracranial self-stimulation after neuroleptics: Mediation by reduced reward, not performance debilitation. *J. comp. physiol. Psychol.* **92:** 659–669, 1978.
7. Franklin, K. B. J. Catecholamines and self-stimulation: reward and performance deficits dissociated. *Pharmac. Biochem. Behav.* **9:** 813–820, 1978.
8. Franklin, K. B. J. and S. N. McCoy. Pimozide-induced extinction in rats: Stimulus control of responding rules out motor deficit. *Pharmac. Biochem. Behav.* **11:** 71–75, 1979.
9. Gray, T. and R. A. Wise. Effects of pimozide on lever-pressing behavior maintained on an intermittent reinforcement schedule. *Pharmac. Biochem. Behav.* **12:** 931–935, 1980.
10. Phillips, A. G. and H. C. Fibiger. Decreased resistance to extinction after haloperidol: Implications for the role of dopamine in reinforcement. *Pharmac. Biochem. Behav.* **10:** 751–760, 1979.
11. Rolls, E. T., B. J. Rolls, P. H. Kelly, S. G. Shaw, R. K. Wood and R. Dale. The relative attenuation of self-stimulation, eating and drinking produced by dopamine-receptor blockade. *Psychopharmacology* **38:** 219–230, 1974.
12. Scheffé, H. *The Analysis of Variance*. New York: Wiley, 1959.
13. Tombaugh, T. N., J. Tombaugh and H. Anisman. Effects of dopamine receptor blockade on alimentary behaviors: Home cage food consumption, magazine training, operant acquisition and performance. *Psychopharmacology* **66:** 219–225, 1979.
14. Wise, R. A., J. Spindler, H. deWit and G. J. Gerber. Neuroleptic-induced 'anhedonia' in rats: Pimozide blocks the reward quality of food. *Science* **201:** 262–264, 1978.
15. Wise, R. A., J. Spindler and L. Legault. Major attenuation of food reward with performance-sparing doses of pimozide in the rat. *Can. J. Psychol.* **32:** 77–85, 1978.
16. Yokel, R. A. and R. A. Wise. Increased lever-pressing for amphetamine after pimozide in rats: Implications for a dopamine theory of reward. *Science* **187:** 547–549, 1975.
17. Yokel, R. A. and R. A. Wise. Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats. *Psychopharmacology* **48:** 311–318, 1976.
18. Zarevics, P. and P. Setler. Simultaneous rate-independent and rate-dependent assessment of intracranial self-stimulation: Evidence for the direct involvement of dopamine in brain reinforcement mechanisms. *Brain Res.* **169:** 499–512, 1979.