

Feeding Behavior in Rats Is Differentially Affected by Pimozide Treatment Depending on Prior Experience

DIANE C. HOFFMAN AND RICHARD J. BENINGER

Department of Psychology, Queen's University, Kingston, Canada K7L 3N6

Received 11 February 1985

HOFFMAN, D. C. AND R. J. BENINGER *Feeding behavior in rats is differentially affected by pimozide treatment depending on prior experience*. PHARMACOL BIOCHEM BEHAV 24(2) 259-262, 1986 —It has been observed in previous research that rats treated with the dopamine receptor antagonist, pimozide, failed to eat palatable food pellets. Two possible interpretations for this effect were evaluated, namely, a reduction in the primary level of food motivation or drug-induced hypokinesia resulting in a subsequent failure to find the food. The effects of several doses of pimozide (1.0, 2.0 and 4.0 mg/kg IP) on feeding in rats with or without experience eating food pellets in an experimental chamber were tested. Pimozide had little effect on feeding in rats with prior experience whereas the drug produced a dose-dependent impairment in rats without similar experience. In addition, although the initial impairment was severe, the nonexperienced rats became more efficient at locating the food despite pimozide treatment. These results appear to rule out a primary motivational interpretation for the effect of pimozide on feeding; rather, the impairment is likely due to the drug's hypokinesia-producing properties.

Pimozide	Feeding	Experience	Motivation	Hypokinesia	Learning	Rats
----------	---------	------------	------------	-------------	----------	------

SEVERAL lines of evidence suggest a role for the central neurotransmitter, dopamine (DA) in mediating the effects of reinforcement on behavior (for reviews see [2,17]). Operant conditioning studies, for example, revealed an extinction-like intra- or inter-session decline in responding for reinforcing stimuli in rats treated with DA receptor blockers [1, 6, 7, 8, 9, 11, 13, 19].

Conditioned reinforcement studies have recently contributed additional support. Normally, when animals receive pairings of a neutral stimulus and a reinforcing stimulus they later show a preference towards the "neutral" stimulus. Davis and Smith [5] demonstrated that rats treated with the DA receptor antagonist, haloperidol, failed to show a preference for a tone which had previously been paired with intravenous injections of apomorphine, a DA agonist with reinforcing properties. Other investigators, employing food as the reinforcing stimulus, found similar results in rats treated with pimozide during conditioning [3,10]. Generally, these results suggested that the learning processes by which reinforcing stimuli affect behavior involve dopaminergic transmission. Not all conditioned reinforcement studies have produced consistent results, however. Tombaugh and his colleagues [14] did not observe a pimozide-induced disruption of conditioned reinforcement in either a place preference or sign-tracking task.

In the studies using food as the reinforcer, when high doses of pimozide were utilized it was noted that a number of animals failed to eat all of the food pellets [3,10]. These rats were eliminated from the experiments and the failures to eat were attributed to failures of finding food in hypokinetic pimozide-treated animals. This hypothesis appeared tenable

as decreased dopaminergic transmission results in hypokinesia and catalepsy (cf. [2]). Alternatively, it may be that the failures to eat reflected reduced levels of primary food motivation, that is, the animals were simply less hungry. Such an explanation could account for the disrupted conditioning seen in pimozide-treated rats and may provide an alternate interpretation for at least some of the DA literature.

Related to this issue are conflicting reports as to whether pimozide treatment in rats affects response initiation or response maintenance in a free-feeding situation. Tombaugh *et al.* [16] observed a disruption in the initiation, but not maintenance, of food pellet consumption in pimozide-treated rats. It was suggested that the results were consistent with a sensory-motor interpretation of pimozide's effect on feeding. Wise *et al.* [18], on the other hand, observed only small increases in latency to initiate eating while response maintenance was significantly impaired. A best-scores analysis suggested that the pattern of results was attributable to a motivational deficit, that is, an attenuation of the 'rewarding impact of food'.

The purpose of the present research was to test whether the observed failures to eat in pimozide-treated animals are due to a reduced level of primary food motivation or to slowness in finding food, possibly resulting from disrupted motor performance. This was accomplished by testing the effects of pimozide on feeding in rats which either had experience or no experience eating food pellets in an experimental chamber. If pimozide reduces primary food motivational levels, then both groups might be similarly affected regardless of their past experience. However, if pimozide leads to

slowness in finding food as a result of disrupted motor activity, nonexperienced rats would be predicted to be more impaired in finding and eating the food

METHOD

Animals

Sixty-nine male Wistar rats (supplied by Charles River) with free-feeding weights of 250–300 g were individually housed in a temperature controlled environment on a 12 hr light-dark cycle. Animals were placed on a restricted diet one week prior to the start of the experiment. The rats reached approximately 80% of their free-feeding weight by the first session and were maintained at this level throughout the experiment.

Apparatus

The experimental environment consisted of four similar test chambers (23.0×29.0×17.5 cm) constructed of aluminum plate sides with clear Plexiglas tops and doors. Each chamber was located in a ventilated sound-attenuating box and was equipped with a Ralph Gerbrands Co. pellet dispenser (model G5100). Within each chamber, two illuminated 2-watt light bulbs (8.5 cm apart) were situated 11.5 cm above the floor. In the center of the same wall, a feeder cup (2.0×4.0 cm) was positioned at a height of 2.5 cm. Inside each feeder cup was an infrared emitter and detector for measuring the latencies between food presentation and consumption. Data collection was controlled by a Digital Equipment Corporation LSI11/2 computer variably interfaced with a screen or printer.

Procedure

Each of eight different experimental groups was tested according to an experimental design with two phases. In the first phase, four groups of rats (experienced, $n=7$ or 8) received 60-min sessions, one on each of four consecutive days. During each session, one 45 mg food pellet (Bioserv) was delivered 80 times according to a random time 45 sec schedule. The latency between food presentation and consumption was measured by determining the time lapse between pellet delivery and interruption of the infrared beam in the feeder cup by the rat's snout. Another four groups (nonexperienced, $n=7$ or 8) were placed in the experimental box for 60 min on each of the four days but did not receive any food pellets there. These rats were given 80 food pellets in their home cage after each session.

In the second phase, all eight groups were fed 80 food pellets (according to the same schedule) during each of three 60-min sessions and were administered either an IP injection of pimozone (1.0, 2.0 or 4.0 mg/kg) or its vehicle, tartaric acid 4 hr prior to each session. Each dose was given to an experienced group and a nonexperienced group yielding a symmetrical design. Latencies between food presentation and consumption were again measured. Pimozone (Janssen Pharmaceutica) was dissolved in a ratio of 3 parts tartaric acid to 1 part pimozone, by weight, in boiling distilled water and cooled prior to injection. The injection volume for the 1.0 mg/kg dose was 1.0 ml/kg, the volumes for the 2.0 and 4.0 mg/kg doses were 2.0 and 4.0 ml/kg, respectively. For vehicle control injections, tartaric acid was dissolved in distilled water at a concentration of 3.0 mg/ml and was injected at a volume of 1.0 ml/kg. The first drug day occurred im-

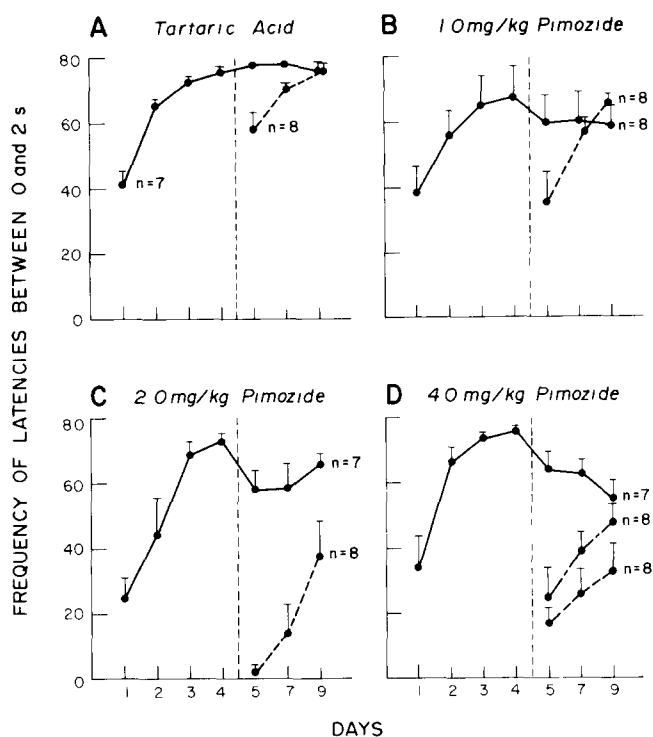


FIG 1 The mean (\pm SEM) number of latencies between pellet presentation and consumption within 0 to 2 sec, panels a to d represent the nonexperienced (— — —) and experienced (—) groups treated with 0, 1.0, 2.0 and 4.0 mg/kg of pimozone, respectively. Also included in panel d is the 4.0 mg/kg control group (— · — · —). Days 1 to 4 are nondrug pre-exposure sessions, days 5, 7 and 9 represent test sessions where all animals received either an IP injection of pimozone or its vehicle tartaric acid.

mediately following the last nondrug day and the remaining drug sessions were separated by 48 hr.

Pimozone had a differential effect on the feeding latencies in the experienced and nonexperienced groups. This observation may have occurred because the nonexperienced rats were unfamiliar with the click of the feeder. That is, pimozone treatment may have resulted in these rats being more startled by the noise, causing them to freeze and perhaps avoid the source. A control group was added to test this possibility. This group (control, $n=8$) received the same training and treatment as the 4.0 mg/kg pimozone nonexperienced group except that the feeder click was presented 80 times according to a random time 45 sec schedule during the first phase of the experiment.

RESULTS

The number of latencies between pellet presentation and consumption that occurred within 2 sec are illustrated in Fig 1. This latency interval was selected because previous studies have shown that after some practice rats locate and consume food pellets usually within 2 sec [3,10]. Panels a to d of Fig 1 represent the nonexperienced and experienced groups treated with 0, 1.0, 2.0 and 4.0 mg/kg of pimozone, respectively. During the four nondrug days, each experienced group showed a similar increase in the number of latencies within 2 sec, thus, they became faster at locating and eating the food pellets across days, reaching an

asymptote in most cases by day 3. This was supported by a two-way analysis of variance (ANOVA) with one repeated measure (day) yielding no significant effects of group or day by group interaction, but a reliable day effect, $F(3,75)=78.59$, $p<0.001$.

Pimozide influenced latencies in both groups, however, the effect was larger as well as dose-dependent in nonexperienced rats (see Fig. 1). Although the nonexperienced pimozide groups showed a lower frequency of 2-sec responding on the first drug day, they all evidenced an acquisition function across days similar to that observed in the vehicle group.

A two-way ANOVA with one repeated measure (day) was conducted across the three drug days. There were significant main effects of group, $F(8,60)=13.32$, $p<0.001$, and day, $F(2,120)=22.60$, $p<0.001$, as well as a significant interaction, $F(16,120)=3.64$, $p<0.001$. Tests of simple main effects were performed yielding significant group effects at each day ($p<0.001$). To determine which groups differed on each day, Newman-Keuls multiple comparisons were carried out.

Within drug day one, the nonexperienced vehicle group differed significantly from each of the nonexperienced pimozide groups. In contrast, the experienced vehicle group did not differ reliably from any of the experienced pimozide groups. This confirms the observation that the same dose of pimozide had a differential effect depending upon the rat's previous experience. Comparing the experienced and nonexperienced groups within each dose, significant differences occurred in every case except within the two vehicle groups. Finally, the high dose control group (also shown in Fig. 1) differed reliably from the high dose experienced group but did not differ significantly from the high dose nonexperienced group, suggesting that the novelty of the click was not contributing to the lower frequency of 2-sec responding in the nonexperienced rats.

On drug day two, the nonexperienced vehicle group differed significantly from all nonexperienced groups except the 1.0 mg/kg pimozide group. Only within the 2.0 mg/kg and 4.0 mg/kg pimozide groups did the experienced and nonexperienced groups differ significantly. No longer did the high dose control group differ with the corresponding experienced group.

There were less significant group differences occurring on day three; this appears to be a function of the increased number of 2-sec latencies observed across all nonexperienced groups. The vehicle group without experience remained significantly different from the corresponding 2.0 and 4.0 mg/kg groups, and only within the 2.0 mg/kg groups did the experienced and nonexperienced groups differ.

To determine if differences existed between nondrug day one latencies of the experienced group and day one latencies of the corresponding nonexperienced group, multiple Student's *t*-tests were performed. Significant differences occurred between the vehicle groups and between the groups receiving 2.0 mg/kg pimozide ($p<0.05$).

DISCUSSION

In prior studies, animals treated with pimozide often did not consume palatable food pellets [3,10]. There was some question concerning the interpretation of this effect and the present experiment addressed the possibility that it may be due to either primary food motivational factors or failures to find the food, possibly as a result of a motor impairment.

Pimozide had virtually no effect on the ability to find and eat food pellets if the animal had prior practice eating in the experimental chamber, however, the same dose of pimozide produced a dose-dependent impairment in rats without experience eating in the chamber. This differential effect of pimozide on feeding, as well as the observation that nonexperienced rats readily improved at finding and eating the food across days, argues against a primary food motivational interpretation for the failures to eat in pimozide-treated rats. Consistent with this is the previous finding that pimozide treatment in rats did not significantly affect food consumption over a 2 hr period after 24 hr of deprivation [20]. Thus, it does not appear likely that previous research suggesting a role for DA in mediating the effects of reinforcement on behavior could be interpreted in terms of this variable [3,10].

The lower frequency of 2-sec responding in the nonexperienced groups was likely a result of the well-documented hypokinesia-producing effects induced by neuroleptics (cf [2]), possibly leading to longer times to locate the food pellets. This property of the drug may also have caused the small, nonsignificant decline in the number of short latencies in the pimozide-treated rats with experience.

As indicated above, there are inconsistent reports as to whether pimozide treatment in rats affects response initiation or response maintenance in a free-feeding situation [16,18]. Although the present study does not address this issue directly, it does suggest an important variable which may account for the discrepant results: namely, the animal's previous experience with the feeding regimen. In the study by Tombaugh *et al.* [16], the rats had only minimal exposure to the feeding schedule prior to drug treatment whereas in the experiment by Wise *et al.* [18], the rats were habituated to the daily feeding schedule prior to testing (see [17]). Based on the present findings, one might reasonably expect the animals in the Tombaugh *et al.* [16] experiment to show the reported impairment in initiating food consumption. The conflicting reports of pimozide's effect on maintenance of food consumption may be explained with reference to another variable. Wise *et al.* [18] observed a deficit in maintaining feeding in pimozide-treated rats after a large number of pellets were presented during the drug test. Tombaugh *et al.* [16] gave their rats a considerably smaller number of food pellets; therefore, it may not be surprising that a maintenance impairment was not observed.

In the present experiment, there was a learning component reflected by the acquisition curves (see Fig. 1). As days progressed each group became faster at locating and eating the food pellets. Although the nonexperienced pimozide-treated rats (especially in the high dose groups) initially were slower at obtaining the food, they too learned where the food was as days progressed. Similarly, others have reported the acquisition of appetitively-motivated tasks despite pimozide treatment. Rats pre-exposed to light-food pairings under pimozide were not impaired in tracking the light cue in a subsequent drug-free test where no food was presented. Similar results were obtained in a second experiment using a place preference paradigm [14]. These observations were surprising given previous findings suggesting that DA is important in mediating the acquisition of reinforcement-related learning [3, 5, 10].

Recent findings may provide an explanation for these results. Hoffman and Beninger [10], for example, observed that the dose of pimozide necessary to attenuate the establishment of conditioned reinforcement was related to the amount of conditioning. Spyra, Fibiger and Phillips [12]

disrupted the establishment of place preference in rats treated with haloperidol only if the animals were no longer food deprived during the drug-free test. Thus, such variables as the amount of conditioning, dose of pimozide and deprivation level at the time of testing may be important in determining the effects of dopaminergic blockade on the acquisition of appetitive tasks.

Perhaps in the present and other [14] experiments, the simplistic nature of the task may have contributed to pimozide's minimal effect on acquisition. This interpretation was originally suggested by Tombaugh, Szostak and Mills [15] based on Bolles' [4] idea that "if a required response is congruent with an animal's natural food-seeking behavior it will be more readily acquired and more resistant to disruption" ([15], p. 164). Tombaugh *et al.* [15] tested this hypothesis by determining the effects of pimozide on the acquisition and maintenance of two discrimination tasks which varied in response complexity. Using a spatial discrimination task (T-maze), they found that rats pretreated with up to 1.0 mg/kg pimozide showed comparable levels of acquisition and maintenance relative to vehicle controls. Using another discrimination procedure which required bar-press responding, Tombaugh and his colleagues [15] ob-

served that the rats pretreated with 0.25 mg/kg pimozide showed comparable levels of acquisition to the vehicle group, however, significant deficits in response accuracy were evident following day 5. Assuming that the former task is more natural than the latter, these data support the congruency hypothesis.

In conclusion, the present results suggest that previous observations of impaired reinforcement-related learning probably do not reflect primary food motivational changes in neuroleptic-treated rats. The unexpected observation of a learning curve in pimozide-treated rats further suggests that the effect of DA blockade on reinforcement-related learning is influenced by the type of response required, this variable can be added to a growing list including neuroleptic dose, amount of conditioning and level of deprivation at the time of testing.

ACKNOWLEDGEMENTS

Pimozide was the generous gift of Janssen Pharmaceutica. This research was supported by grants from the Natural Sciences and Engineering Research Council and the Ontario Ministry of Health to R. J. B.

REFERENCES

- Beninger, R. J. A comparison of the effects of pimozide and nonreinforcement on discriminated operant responding in rats. *Pharmacol Biochem Behav* **16**: 667-669, 1982.
- Beninger, R. J. The role of dopamine in locomotor activity and learning. *Brain Res Rev* **6**: 173-196, 1983.
- Beninger, R. J. and A. G. Phillips. The effect of pimozide on the establishment of conditioned reinforcement. *Psychopharmacology (Berlin)* **68**: 147-153, 1980.
- Bolles, R. C. Reinforcement, expectancy, and learning. *Psychol Rev* **79**: 394-409, 1972.
- Davis, W. M. and S. G. Smith. Catecholaminergic mechanisms of reinforcement. Direct assessment by drug self-administration. *Life Sci* **20**: 483-492, 1977.
- Faustman, W. O. and S. C. Fowler. An examination of methodological refinements, clozapine and fluphenazine in the anhedonia paradigm. *Pharmacol Biochem Behav* **17**: 987-993, 1982.
- Fouriez, G. and R. A. Wise. Pimozide-induced extinction of intracranial self-stimulation. Response patterns rule out motor performance deficits. *Brain Res* **103**: 377-380, 1976.
- Gerber, G. J., J. Sing and R. A. Wise. Pimozide attenuates lever pressing for water in rats. *Pharmacol Biochem Behav* **14**: 201-205, 1981.
- Gray, T. and R. A. Wise. Effects of pimozide on lever pressing behavior maintained on an intermittent reinforcement schedule. *Pharmacol Biochem Behav* **12**: 931-935, 1980.
- Hoffman, D. C. and R. J. Beninger. The effects of pimozide on the establishment of conditioned reinforcement as a function of the amount of conditioning. *Psychopharmacology (Berlin)* in press.
- Mason, S. T., R. J. Beninger, H. C. Fibiger and A. G. Phillips. Pimozide-induced suppression of responding. Evidence against a block of food-reward. *Pharmacol Biochem Behav* **12**: 917-923, 1980.
- Spyraki, C., H. C. Fibiger and A. G. Phillips. Attenuation by haloperidol of place preference conditioning using food reinforcement. *Psychopharmacology (Berlin)* **77**: 379-382, 1982.
- Tombaugh, T. N., H. Anisman and J. Tombaugh. Extinction and dopamine receptor blockade after intermittent reinforcement training. Failure to observe functional equivalence. *Psychopharmacology (Berlin)* **70**: 19-28, 1980.
- Tombaugh, T. N., L. J. Grandmaison and K. A. Zito. Establishment of secondary reinforcement in sign tracking and place preference tests following pimozide treatment. *Pharmacol Biochem Behav* **17**: 665-670, 1982.
- Tombaugh, T. N., C. Szostak and P. Mills. Failure of pimozide to disrupt the acquisition of light-dark and spatial discrimination problems. *Psychopharmacology (Berlin)* **79**: 161-168, 1983.
- 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 (Berlin)* **66**: 219-225, 1979.
- Wise, R. A. Neuroleptics and operant behavior. The anhedonia hypothesis. *Behav Brain Sci* **5**: 39-87, 1982.
- Wise, R. A. and L. M. Colle. Pimozide attenuates free feeding. Best scores analysis reveals a motivational deficit. *Psychopharmacology (Berlin)* **84**: 446-451, 1984.
- Wise, R. A., J. Spindler, H. deWit and G. J. Gerber. Neuroleptic-induced "anhedonia" in rats. Pimozide blocks reward quality of food. *Science* **201**: 262-264, 1978.
- Zis, A. P. and H. C. Fibiger. Neuroleptic-induced deficit in food and water regulation. Similarities to the lateral hypothalamic syndrome. *Psychopharmacology (Berlin)* **43**: 63-68, 1975.