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The Effects of Pimozide on Drinking Behavior in the Rat: An Investigation Using the Conditioned Taste Aversion Paradigm

KAREN SPIVAK[1] AND ZALMAN AMIT

Center for Studies in Behavioral Neurobiology, Montreal, Quebec, Canada H3G 1M8

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SPIVAK, K. AND Z. AMIT. *The effects of pimozide on drinking behavior in the rat: An investigation using the conditioned taste aversion paradigm.* PHARMACOL BIOCHEM BEHAV 24(6) 1527–1531, 1986.—In an attempt to examine the potential aversive properties of the neuroleptic pimozide, a conventional conditioned taste aversion (CTA) paradigm was employed. Rats were either pretreated with pimozide (1.0 mg/kg) before the presentation of a familiar or novel saccharin-flavored solution or tap water or received injections of pimozide after the presentation of a novel saccharin solution. Following this procedure, rats were given a two bottle choice test under drug-free conditions. All pretreated pimozide groups demonstrated a significant unconditioned reduction in fluid intake relative to the vehicle control group. These pimozide groups having different drinking histories did not differ from one another. Although pimozide did not induce a CTA in rats post-treated with this neuroleptic, overall this group drank significantly less saccharin than the control group. Furthermore, on the two bottle choice test, rats which received contingent exposure to pimozide and saccharin (pre and post conditions), did not demonstrate a preference for the saccharin solution. These results suggest that the reduction in fluid intake observed in the pretreated pimozide groups may be due to some unconditioned aversive state induced by the drug. These data indicate that the mechanisms involved in the reduction of fluid intake induced by pimozide may be unrelated to a manipulation of the reinforcing properties of the appetitive stimulus.

Pimozide Conditioned taste aversion Drinking behavior Unconditioned suppression

IN recent years, neuroleptics have become a major pharmacological tool in studying the role of brain dopamine in mediating a variety of behaviors [1, 3–4, 12] including consummatory responses [5, 8, 11, 13]. In free-feeding situations, the neuroleptic pimozide (a post-synaptic dopamine receptor antagonist) has been reported to reduce both fluid [5–6, 13] and food intake [9,14] in rats. This pimozide-induced suppression has been variously interpreted in terms of a homeostatic deficit [14], reduction in the rewarding properties of appetitive stimuli [13] or interference with motoric responses [5,11]. Recently, it has also been suggested that the pharmacological effects of pimozide may be aversive to animals and that it is this aversive consequence of pimozide exposure which results in subsequent interference with performance [8].

In reviewing the results of previous studies examining neuroleptic-induced effects, it is difficult to independently assess the possible contribution of nonspecific or aversive actions of neuroleptics on appetitive behavior. Therefore, the present study was designed to examine the potential aversive properties of pimozide using a conventional conditioned taste aversion (CTA) paradigm. We hypothesized that the reduction in fluid or food intake observed with pimozide pretreatment may reflect a learned association between the appetitive stimulus and some aversive properties of the drug. In addition, it has been demonstrated that the degree of familiarity with a particular taste is directly related to the magnitude of the CTA [2]. Consequently, to examine the effects of pimozide pretreatment on fluid intake, the present sutdy included groups that received either a familiar or novel flavored solution or tap water on test days. A group of animals was also included which received pimozide treatment following the consumption of a novel saccharin-flavored solution to examine similarities and/or differences in fluid intake between pretreated and post-treated pimozide groups.

METHOD

Subjects

Subjects were 48 male Sprague Dawley rats (Charles River Breeding Farms) weighing 260–330 grams. The animals

[1]Requests for reprints should be addressed to Karen Spivak, Department of Psychology, Concordia University, 1455 de Maisonneuve Blvd W., Rm 1013, Montreal, Quebec, Canada H3G 1M8.

TABLE 1
GROUP DESIGN

Group	Daily fluid	Test fluid	Drug	Inj. time	Gp. size
Pre-S	Sacc	Sacc	Pimo	pre: 4 hr	n= 7
Pre-W	Water	Water	Pimo	pre: 4 hr	n= 7
Pre-Ts	Water	Sacc	Pimo	pre: 4 hr	n= 7
Pre-V	Water	Sacc	Veh	pre: 4 hr	n= 7
PP	Water	Sacc	Pimo	post: 4 hr	n=10
PV	Water	Sacc	Veh	post: 4 hr	n=10

were individually housed in standard stainless steel cages with free access to food and water prior to the onset of the experiment. The animal colony room was illuminated on a 12 hr day/night schedule.

Drugs

Pimozide (Janssen Pharmaceutica) was dissolved at a concentration of 1.0 mg/ml in a 0.3% (w/v) tartaric acid solution, resulting in a final preparation of pH 3. The tartaric acid vehicle was adjusted to pH 3. An injection volume of 1.0 mg/kg was used for all groups.

Procedure

After 7 days adaptation to laboratory housing conditions, the animals were placed on a 23 hr 40 min water deprivation schedule. Animals were randomly divided into six treatment groups. The treatment groups are outlined in Table 1. For the following 13 consecutive days, tap water was available to rats for a 20 min drinking period in the home cage. One group of animals (Pre-S) received a 0.1% saccharin solution instead of water throughout the experiment. On day 14 (Test day 1) animals were presented with a 0.1% saccharin-flavored solution or tap water. Three groups received intraperitoneal (IP) injections of pimozide 4 hr prior to test fluid presentation and one group received tartaric acid vehicle 4 hr prior to test fluid presentation. Within a minute after termination of the 20 min drinking period, two additional groups received IP injections of either pimozide or tartaric acid vehicle. For 5 days following the first test day, tap water continued to be available for 20 min daily drinking periods. On day 20 (6th day after Test day 1), an identical procedure to that used on Test day 1 was performed. The cycle of test day followed by 5 intervening water days was repeated until 9 test days had been completed. Two days after Test day 9, all animals were given a two bottle choice test with a 0.1% saccharin solution and water for a 20 min drinking period. Animals did not receive drug treatment on this day.

RESULTS

Mean fluid intake on test days for animals pretreated (4 hr prior) with pimozide or vehicle is presented in Fig. 1A. A two-way (4×9) analysis of variance (ANOVA) with repeated measures yielded a significant Group effect, $F(3,36)=25.56$, $p<0.00001$, Days effect, $F(8,192)=2.86$, $p<0.005$, and Group × Days interaction, $F(24,192)=3.0$, $p<0.00001$. Pairwise comparisons using Tukey tests revealed that animals pretreated with pimozide drank significantly less fluid (saccharin and water) than control group Pre-V from Test day 2 onward, $q(4,132)=7.33$; $p<0.05$. These pretreated pimozide groups (Pre-S, Pre-W, and Pre-Ts) having different drinking histories were not significantly different from one another over test days, $q(4,324)=7.33$; $p>0.05$ (see Fig. 1A). Although group Pre-Ts showed a marked decrease in saccharin consumption on Test day 1, Tukey tests revealed that this effect was not significant from the other pretreated groups, $q(4,132)=7.33$; $p>0.05$.

The occurrence of a CTA was determined here by evidence of a significant reduction in saccharin intake of a given experimental group from its own baseline (T1) intake levels. Accordingly, simply a failure to observe an increase in saccharin intake was not considered sufficient evidence to indicate CTA-inducing properties of the conditioning agent.

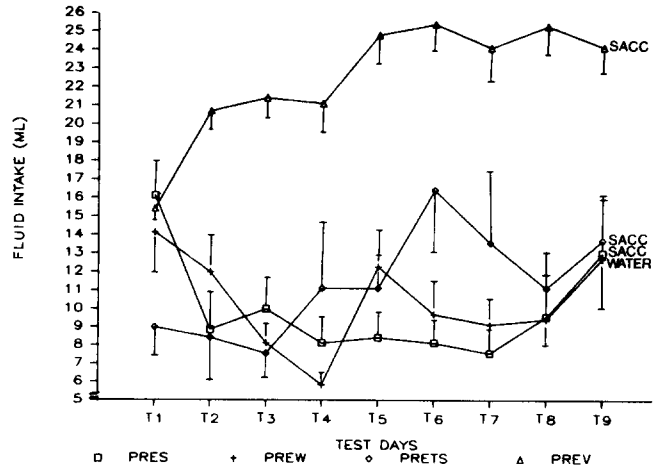

FIG. 1A. Mean fluid intake on test days for groups receiving pimozide (1.0 mg/kg) or vehicle injections 4 hr prior to fluid presentation. Pre-S=pimozide + familiar saccharin solution, Pre-W=pimozide + tap water, Pre-Ts=pimozide + novel saccharin solution, Pre-V=tartaric acid vehicle + novel saccharin solution.

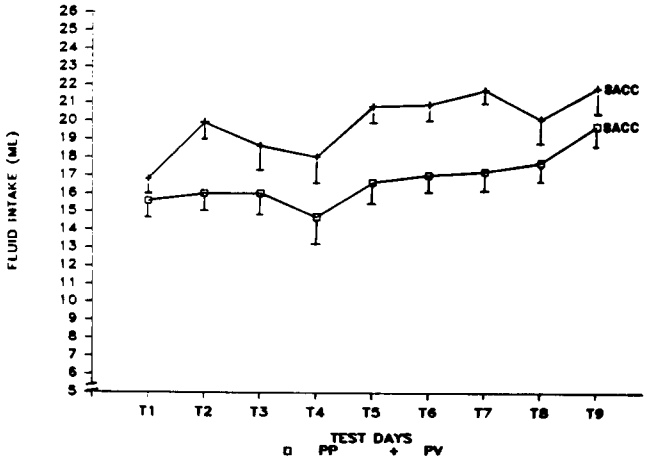

FIG. 1B. Mean saccharin intake on test days for groups receiving pimozide (1.0 mg/kg) or vehicle injections immediately after saccharin presentation. PP=post-treatment pimozide, PV=post-treatment tartaric acid.

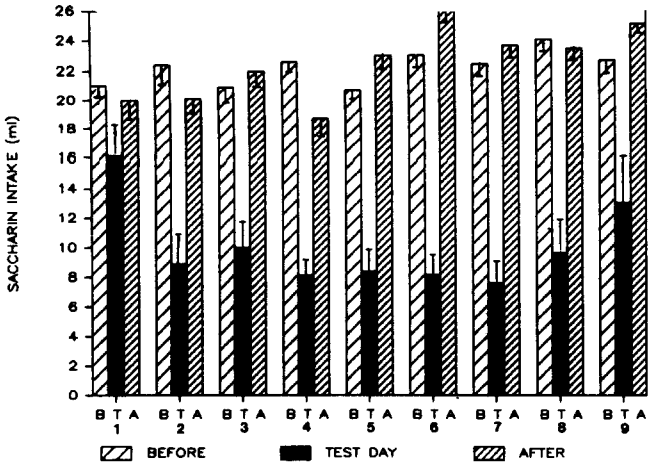

FIG. 2A. Mean saccharin intake for 'pretreated pimozide + familiar saccharin' (Pre-S) group before test day (B) on test day (T) and after test day (A).

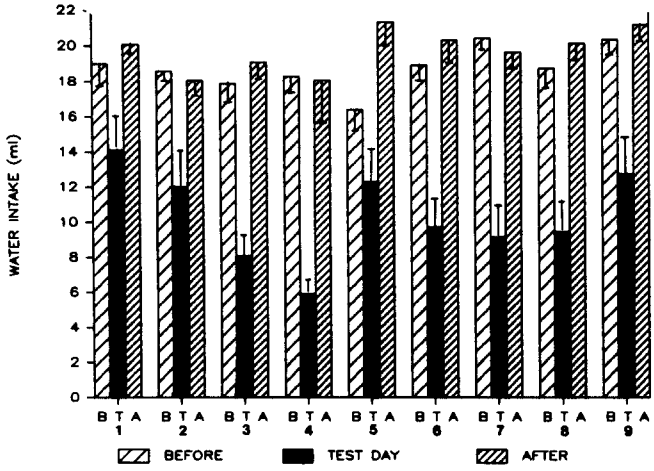

FIG. 2B. Mean water intake for 'pretreated pimozide + tap water' (Pre-W) group before test day (B) on test day (T) and after test day (A).

TABLE 2

SUBJECTS DEMONSTRATING SACCHARIN PREFERENCE (EXPRESSED AS GREATER THAN 50% OF TOTAL FLUID INTAKE) USING THE TWO BOTTLE CHOICE TEST FOR ALL TREATMENT GROUPS

	Groups					
	Pre-S	Pre-W	Pre-Ts	Pre-V	PP	PV
No. of Ss >50%	7	1	4	6	6	5
N per group	7	7	7	7	10	10
Prop. Pref %	100	14	57	86	60	50

Mean saccharin intake for animals treated with pimozide or vehicle following saccharin presentation is presented in Fig. 1B. A two-way ANOVA with repeated measures yielded a significant Group effect, $F(1,18)=10.86$, $p<0.004$, and Days effect, $F(8,114)=4.31$, $p<0.001$. The Group × Days interaction was not significant, $F(8,144)=0.60$, $p>0.05$. Pairwise comparisons using Tukey tests revealed that there were no significant differences in saccharin intake on T1 between groups PP and PV, $q(2,152)=3.29$, $p>0.05$. Although group PP did not demonstrate a significant reduction in saccharin intake on T2 compared to its baseline measure (T1), $q(9,144)=1,18$, $p>0.05$, overall, they drank significantly less saccharin than the control group PV. To examine whether these groups represented two distinct drinking populations, a comparison of water intake on the day preceding the first test day revealed that the mean water consumption was identical for groups PP and PV (mean=17.6±1.1, mean=17.6±0.91, respectively). Figures 2A and 2B represent mean saccharin intake and water intake on the days before and after test days for groups Pre-Ts and Pre-W, respectively.

There were no differences in saccharin or water consumption before or after each test day. However, there was a marked reduction in saccharin or water intake on test days. As shown in Figs. 2A and 2B, there is a tendency for test fluid intake to decrease over the first four test days and then maintain at that level over the next four days. There is some recovery by Test day 9.

Saccharin consumption during the two bottle choice test is shown in Table 2. Saccharin preference was designated to those subjects whose saccharin intake was more that 50% of total fluid intake. A chi square distribution using arcsine transformations [7] revealed significant differences among the proportions of saccharin intake in the 6 treatment groups, $\chi^2(95)=16.8$, $p<0.05$. Multiple comparisons based on the arcsine transformation [7] indicated that groups Pre-S and Pre-V preferred saccharin in comparison to groups Pre-Ts, PP and PV, using the lower and upper limits of 95% simultaneous confidence intervals. Group Pre-W demonstrated the least saccharin preference in comparison to all the other groups.

DISCUSSION

Results of the present experiment confirmed previous findings that pimozide pretreatment reduced fluid intake in rats [5–6, 13–14]. All groups pretreated with pimozide showed a marked reduction in fluid intake in comparison to the vehicle control group. Based on the data collected in this

study, the results suggest that this suppression may not reflect a reduction in the rewarding property of the appetitive stimulus [13]. All pretreated pimozide groups showed a strong suppression in fluid intake whether these groups received a familiar or novel saccharin solution or tap water. Xenakis and Sclafani [13] reported that rats pretreated with pimozide showed a stronger suppression of a saccharin-glucose solution than plain water. They suggested that the flavored solution was more rewarding than water and that these rats would therefore be more influenced by pimozide's effects. The discrepency between the present study and that of Xenakis and Sclafani [13] may be due to procedural differences. In the present study, animals received the same drug treatment regimen for 9 test days, whereas rats in the other experiment received varying doses of pimozide and one test trial per dose. It may be that repeated experience with pimozide results in a maximal suppression effect independent of type of fluid presented. However even if repeated pimozide treatment produced maximal suppression effects, significant differences between water and saccharin intake should have been observed on the first test day on the basis of the hypothesis suggested by Xenakis and Sclafani [13]. In the present study no differences in amount of fluid suppression was observed between the three pretreated pimozide groups on the first day and across test days.

Rats receiving pimozide treatment following the presentation of a novel-flavored saccharin solution did not demonstrate a CTA. Although group PP overall drank significantly less saccharin than the vehicle control group PV, the more conservative definition of CTA, incorporating an implicit avoidance response (i.e., significant reduction from its own baseline levels) was not observed. An alternative explanation may be that the failure to increase saccharin intake may not reflect a taste aversion but rather maintenance of taste neophobia. In addition, it is possible that a higher dose of pimozide may induce a CTA, since animals receiving a dose of 1.0 g/kg failed to show an increase in saccharin consumption. It follows then, that the reduction in fluid intake observed with pretreated pimozide (1.0 mg/kg) animals does not reflect a CTA.

However, the inclusion of the two bottle choice test provided additional information as to the nature of the suppression observed following pimozide treatment. Animals exposed to saccharin throughout the experiment (group Pre-S) all demonstrated a preference for the saccharin solution, although this same group suppressed saccharin intake on test days. When given a choice however, animals whose exposure to saccharin was contingent with pimozide treatment (pre and post conditions, groups Pre-Ts and PP), did not demonstrate a preference for the saccharin solution. Thus, the two bottle choice test may be a more sensitive measure in reflecting the animal's ability to make an association between some 'aversive' property of pimozide and the novel saccharin flavor. Although a significant reduction in saccharin intake across test days was not evident for the post-treated pimozide group (PP) (see Fig. 1B), the overall suppression may be indicative of this lack of preference for saccharin. Of particular interest is that the post-treated tartaric acid control group (PV), who did not show a suppression of saccharin intake across test days also demonstrated less preference for the saccharin solution in the two bottle choice test. These results suggest that tartaric acid may have some 'aversive' properties as well, which may only be expressed when a more sensitive measure of saccharin intake is employed. This effect however, was not observed for animals pretreated with tartaric acid (group Pre-V). This discrepancy can be explained by the fact that the time interval between tartaric acid administration and saccharin presentation was different for these groups. In the pretreatment condition, tartaric acid was administered 4 hr prior to saccharin presentation. Consequently, the 'aversive' effects of tartaric acid may have subsided before the period of saccharin drinking began.

The results of the present study indicate that alterations in fluid intake may be influenced by a direct drug-fluid consumption interaction. This notion is supported by the finding that animals receiving the familiar saccharin solution or tap water on test days always returned to baseline levels on the day after pimozide treatment. No conditioned suppression effect was evident. Of interest, as shown in Figs. 2A and 2B, is the recovery to baseline levels by Test day 9 for the pretreated pimozide groups (Pre-S and Pre-W) which may possibly be due to some adaptation or tolerance to pimozide's effects.

Furthermore, the results suggest that the suppresssion of fluid intake observed in pimozide treated rats may be due to mechanisms that are unrelated to the mechanisms underlying the reinforcing properties of the fluid. More specifically, animals that received contingent exposure to pimozide (pre and post conditions) and saccharin did not demonstrate a preference for saccharin on the two bottle choice test, suggesting that the suppression may be due to some unconditioned aversive state induced by pimozide [8,10]. Recently, Gramling, Fowler and Collins [5] reported that rats pretreated with pimozide demonstrated a slower licking response for a sucrose solution than non-drugged animals. These authors suggested that this impairment may be due to pimozide's influence on motor performance of the licking response. Thus, the reduction of fluid intake observed in the present study may be a consequence of pimozide's effects on licking response.

The present study suggests that pimozide's influence on drinking behavior is directly related to an unconditioned drug-fluid consumption interaction and not related to a reduction in the rewarding properties of the appetitive stimulus. In addition, results of the two bottle choice test suggest that pimozide may possess aversive properties that may interact with consummatory behavior, resulting in the suppression of fluid intake. Moreover, subtle aversive effects of tartaric acid can be demonstrated when the more sensitive two bottle choice test is employed.

A pimozide-induced homeostatic deficit [14] or a performance deficit [5, 9–10] are also plausible explanations for the reduction in fluid intake because both of these hypotheses suggest drug-induced unconditioned effects. More systematic investigations are necessary to elucidate the mechanisms involved in this consummatory deficit.

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