Pharmacology Biochemistry & Behavior, Vol. 27, pp. 67–72. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Some Effects of Pimozide on Nondeprived Rats' Lever Pressing Maintained by a Sucrose Reward in an Anhedonia Paradigm

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Received 24 July 1986

GRAMLING, S. E., S. C. FOWLER AND J. P. TIZZANO. *Some effects of pimozide on nondeprived rats' lever pressing maintained by a sucrose reward in an anhedonia paradigm.* PHARMACOL BIOCHEM BEHAV 27(1) 67–72, 1987.— The present work examined the generalizability of the anhedonia phenomenon (extinction-like responding with repeated neuroleptic treatment) by examining the effects of pimozide (PIM) on nondeprived rats lever pressing for a sucrose solution reward (32%) in an eight day dosing regime. The procedures used replicated the essential features of a previous study (Gramling *et al.* [10]) wherein the effects of PIM on rats licking directly a sucrose solution were assessed. Thirty nondeprived rats were trained to lever press on a CRF schedule for a 32% sucrose solution reward and then assigned to one of five treatment groups (N=6). The treatment conditions included a no-reward group (EXT; vehicle injections), two pimozide (PIM) with reward conditions (either PIM 0.25 mg/kg + RWD or PIM 0.5 mg/kg + RWD), and a vehicle control group (RWD; vehicle injections). These four groups each received their respective injections and operant exposure for eight consecutive days. The fifth group was a home cage (HC) control condition wherein the rats were injected with 0.5 mg/kg PIM each test day but did not receive operant exposure until the fourth test day. The PIM treated rats exhibited a significant curvilinear pattern of responding on the rate measure across eight days of testing, whereas rats in the no-reward condition exhibited a significant downward linear trend across eight days of testing. Within-session analysis revealed that rats in the EXT group responded at significantly higher rates during the first five minutes of testing on the first test day compared to rats in the PIM 0.5 + RWD condition. The results obtained on the rate measure in the present experiment were similar to those obtained when similar procedures were used to test the effects of PIM on rats' licking behavior [10]. The dissimilarity in the response profiles on the rate measure produced by rats treated with PIM relative to rats in a no-reward condition is inconsistent with the anhedonia hypothesis of neuroleptics' effects.

Anhedonia Pimozide Sucrose reward Nondeprived rats Lever press Response duration Neuroleptics

THE "anhedonia" hypothesis of neuroleptic action suggests that the rate reducing effects of neuroleptics in appetitive tasks is due to these drugs' impairment of reward processes [15–17]. The similarity in the across and within session pattern of responding produced by animals treated with neuroleptics and by animals in a no-reward condition has been advanced as critical evidence in support of the anhedonia hypothesis (e.g., [15,17]). More specifically, neuroleptic treatment and no-reward are thought to be functionally similar procedures [17]. The revised anhedonia hypothesis emphasizes that neuroleptics blunt rather than totally block reward efficacy [12,17]. Therefore, the hypothesis predicts that the pattern of responding produced by rats treated with neuroleptics and the pattern of responding

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produced by animals in a no-reward condition should be qualitatively, though not necessarily quantitatively, similar [17].

Gramling *et al.* [10] recently reported that qualitatively different patterns of responding were produced when nondeprived rats licked a sucrose solution and were either tested with the neuroleptic pimozide (PIM) or were exposed to a no-reward condition. Rats in the no-reward condition exhibited a monotonic decrease in lick rate across eight consecutive days of testing whereas rats treated with PIM exhibited an initial decrease in lick rate followed by a trend towards recovery across days 6–8. Moreover, the times between the fastest licks produced by rats in the PIM conditions were lengthened and this effect was not observed in animals in the no-reward condition. These and other data (e.g., home cage controls, tests for transfer) suggested that PIM treatment and no-reward were not qualitatively similar procedures [10].

The Gramling *et al.* [10] results were consistent with the idea suggested by others [7] that the anhedonia phenomenon may be response dependent. That is, extinction-like patterns of responding may be obtained with PIM treatment when the response requirements are relatively high (i.e., lever pressing [7]), but not when the response requirements are low (e.g., nose poking [7]). The Gramling *et al.* [10] study differed from traditional tests of the anhedonia hypothesis (e.g., [15]) in a number of other potentially important ways. Specifically, the combined use of nondeprived rats, the use of a natural reinforcer with presumably high hedonic value (i.e., sucrose solutions), the absence of an explicit operant contingency, and an eight day dosing procedure were unique features of the Gramling *et al.* [10] study. The present experiment was designed to replicate these unique features of the Gramling *et al.* [10] lick study using the more traditional (and compared to licking, more kinetically demanding) lever press response.

METHOD

Subjects

Forty-eight male Sprague-Dawley rats (Holtzman Co.) were initially obtained for the present experiment. Thirty rats were actually tested in the treatment phase of the experiment. Some rats (n=11) were excluded because they failed to respond consistently and some rats (n=7) were excluded because their rates greatly deviated from the group mean when matched on the rate variable. The rats were housed in individual home cages and offered food and water ad lib throughout the experiment. The animals' weight averaged 396 g at the beginning of the experiment and increased to an average weight of 488 g by the last day of testing.

Apparatus

Four Gerbrands Co. (Arlington, MA) experimental chambers (model G7410), housed in Gerbrands Co. sound attenuating chambers (model G7211), were used in the present experiment. A 5 cm wide lever protruded 1.5 cm from the center of the front panel of the experimental chamber and was 8 cm above the grid floor, and required 20 g of force for switch closure. When activated, a solenoid operated dipper (model B-LH) presented a 0.1 ml sucrose reinforcer for a period of four sec. The calibration of the levers and up time of the dippers were periodically checked.

Each experimental chamber was serviced by a separate microcomputer (Apple II Plus) which controlled events and recorded the data. The data acquisition software accessed a real time clock which permitted measurement of individual response durations (the amount of time the lever microswitch was held in a closed position) with a resolution of 0.01 sec.

Procedure

The recession which provided access to the dipper was baited with a few drops of the sucrose solution prior to each rat's initial session. Once the animals were licking the solution, the rats were magazine trained on a variable time (VT) 90 sec schedule of reinforcement presentation in 20 min sessions for five consecutive days with the levers removed from the operant chambers. After magazine training, the levers were replaced and the lever press response was manually shaped. Some rats (n=11) were excluded from the present study due to inconsistent responding during the course of training. The rats which reliably responded on a CRF schedule were tested in daily (7 days/week) 35 min sessions for four weeks of baseline data collection.

Following baseline the rats were assigned to treatment conditions after being matched on the rate variable. Three blocks of 10 rats each were formed by excluding those rats (n=7) with the most extreme scores and the rats within each block were randomly assigned to one of five treatment conditions by the method of random block assignment suggested by Cox [5]. Thus, each of the five treatment conditions was comprised of a group of 6 rats, and the mean baseline rate of responding among the five groups was approximately equal.

The five treatment groups included a reward (RWD) condition where rats received vehicle injections and were exposed to normal reward (32% sucrose solution) in the testing situation. A second group served in an extinction (EXT) condition wherein they received vehicle injections and responding resulted in an empty dipper in the test situation (all other cues associated with reinforcement delivery were present except the sucrose solution). Two additional groups (PIM 0.25 + RWD and PIM 0.5 + RWD) received injections of PIM (0.25 mg/kg and 0.5 mg/kg, respectively) and were exposed to normal reward in the testing situation. The final group of rats served as home cage controls (HC). These rats received injections of 0.5 mg/kg PIM on each of the same days as rats in the PIM 0.5 + RWD condition; however, following the first three injections these rats were returned to their home cages and were not tested. On test days 4–8 these rats were injected with 0.5 mg/kg PIM and exposed to the testing situation in the same manner as rats in the PIM 0.5 + RWD group. Thus, the HC group and the PIM 0.5 + RWD group had identical pharmacological histories but different experiential histories. This procedure, which was used by Wise *et al.* [15], serves as a control to insure that the decrease in operant responding observed across test sessions in PIM-treated rats is due to some effect other than a cumulative drug effect carried-over across sessions. All rats in all five conditions were tested for eight consecutive treatment days.

During treatment all injections in the above five conditions preceded data collection by four hours. The drug, route of administration (IP), and time since injection were the same as those used by Wise *et al.* [15]. The eight consecutive day dosing regime was the same as that used by Gramling *et al.* [10]. Pimozide (McNeil) was dissolved prior to the start of experimentation in a mixture of tartaric acid and water. The

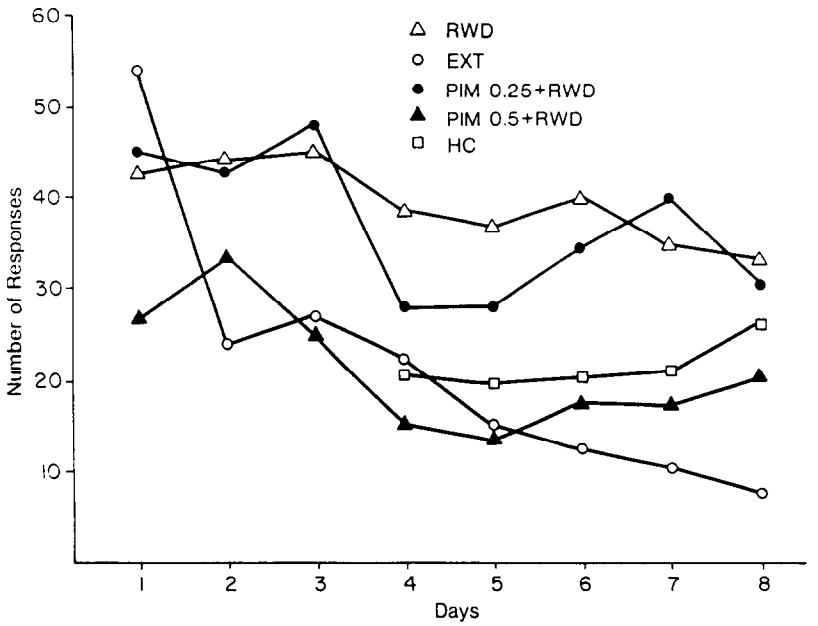

FIG. 1. Mean number of responses in daily 35-min sessions for 5 separate groups of rats. Group RWD (open triangles) received vehicle injections and were exposed to normal reward in the testing situation. Group EXT (open circles) received vehicle injections and were exposed to a no-reward condition in the testing situation. The PIM 0.25 + RWD and PIM 0.5 + RWD groups (darkened circles and darkened triangles, respectively) received pimozide (0.25 and 0.5 mg/kg, respectively) and were exposed to normal reward in the testing situation. The open boxes which are seen on days 4–8 represent the home cage control (HC) rats. These animals received daily injections of pimozide (0.5 mg/kg) but were not exposed to the testing situation until the 4th test day. All of the animals in all the groups received their respective injections daily, four hours prior to testing for eight consecutive days.

vehicle (tartaric acid and water) was used for the injections in the RWD and EXT conditions. The volume per injection was 1.0 ml/kg in all conditions. The doses used in the present study (0.25 and 0.5 mg/kg PIM) were lower than the doses used in other studies (e.g., [10,15]) because pilot data indicated that a dose of 1.0 mg/kg PIM might completely suppress responding.

RESULTS

Average Lever Press Rate

Across-session. The RWD, EXT, 0.5 PIM + RWD, and 0.25 PIM + RWD rate data (in Fig. 1) were entered into a split-plot factorial analysis of variance (SPF-ANOVA). A significant between groups effect was obtained, $F(3,20)=5.01$, $p<0.01$, indicating that the four groups differed in their overall amount of responding. The across days repeated measures effect was also significant, $F(7,140)=9.71$, $p<0.01$, as was the group by days interaction, $F(21,140)=2.30$, $p<0.01$. Thus, the pattern of across-day changes in responding depended on group membership.

Tests for simple main effects revealed significant trends across the eight days for both the PIM 0.5 + RWD and the EXT conditions, $F(7,140)=2.94$, $p<0.01$, and, $F(7,140)=10.71$, $p<0.01$, respectively. The PIM 0.25 + RWD condition approached, but did not reach statistical significance in the across session test for simple main effects, $F(7,140)=1.98$, $p<0.1$. Visual inspection of the data in Fig. 1 suggested that the rats in the EXT condition exhibited a monotonic decrease in response rate across the eight test days, whereas the two drug conditions exhibited rate decreases only through the first five days of testing followed by a trend towards recovery (rate increases) across the final three days of testing. Specifically, the EXT condition would seem to be best described as a linear function whereas both of the drug conditions seem to be better described by a curvilinear function. Tests for trends using the method of orthogonal polynomials described by Bruning and Kintz [1] were used to verify these visual impressions. When tests for trends for the EXT data only were calculated, the linear component of the across session repeated measures effect was significant, $F(1,5)=29.33$, $p<0.001$, and accounted for 76.11 percent of the variance. Tests for other trend components in the EXT data were not significant. Conversely, tests for trends in the drug data revealed that only the quadratic trend components yielded significance, PIM 0.5 + RWD, $F(1,5)=9.4$, $p<0.05$ (32.46 percent of the variance accounted for); PIM 0.25 + RWD, $F(1,5)=9.04$, $p<0.05$ (9.7 percent of the variance accounted for). These statistical analyses indicate that (1) the patterns of responding produced by animals in the drug condition differed from the pattern produced by animals in the EXT condition across the eight days of testing and (2) the kind of pattern produced by the EXT animals was largely linear whereas the kind of patterns produced by the drug animals were largely curvilinear in form.

The rate data from the home cage control (HC) group's first day of operant exposure but fourth day of PIM 0.5

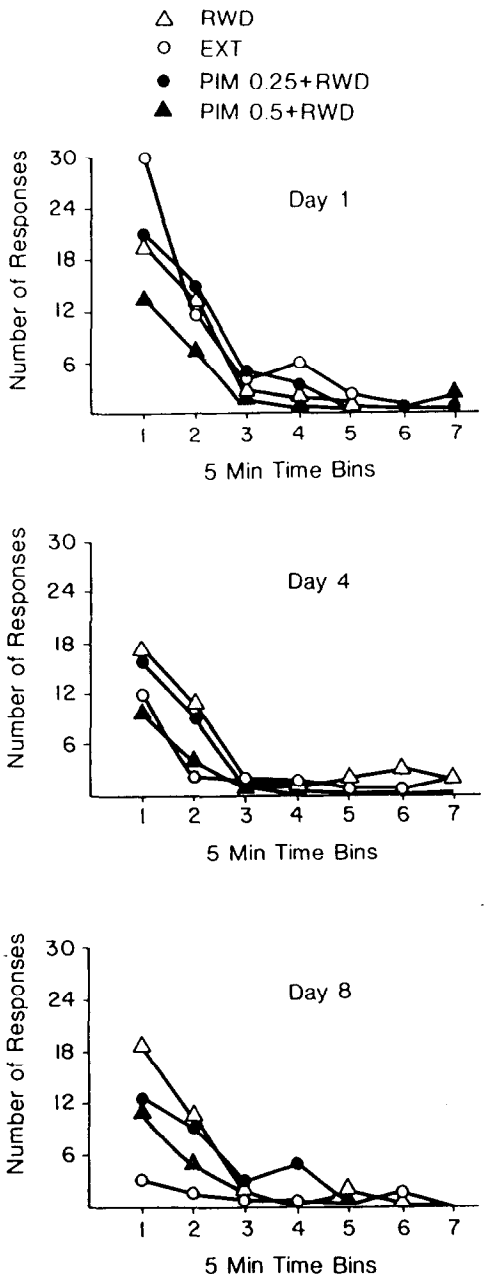

FIG. 2. Average number of responses in 5 minute time bins for the first four groups of rats described in Fig. 1 on days 1, 4, and 8.

mg/kg were compared with the fourth test day data of the PIM 0.5 + RWD condition with a t-test for independent groups. The difference between these two groups was not significant, $t(10)=1.4, p>0.1$. Additionally, an SPF-ANOVA was performed on the rate data of days 4–8 for these two groups of rats and, again, significant group differences were not obtained, $F(1,10)=0.69$, $p>0.2$. In other words, the difference between the HC animals and the drug plus operant exposure condition observed by Wise *et al.* [15] was not replicated with these procedures. A final SPF-ANOVA was performed comparing the rate data of the HC animals on days 4–8 with the rate data of the PIM 0.5 + RWD on days 1–5. The only significant finding was a days by group interaction, $F(4,40)=3.37$, $p<0.05$, indicating that the pattern of responding produced by animals in these two conditions differed according to group membership.

First day comparisons and within-session analysis. The rate of responding produced by animals in the eight consecutive day treatment procedure (Fig. 1) were further analyzed to determine if the apparent differences in patterns of responding produced by PIM and EXT procedures on the first day of testing were genuine. Visual inspection of Figs. 1 and 2 suggested that the animals in the EXT condition exhibited relatively high rates of responding on the first test day (day 1 in Fig. 1) as well as relatively high rates of responding in the early part of their first exposure to extinction procedures (time bin 1 of top axes in Fig. 2). To verify these impressions the test sessions were divided into seven, 5 minute time bins, and rate of lever pressing was calculated for each bin on days 1, 4, and 8 (Fig. 2; days 4 and 8 are presented for comparison purposes).

An SPF-ANOVA on the time bin data on day 1 for the four groups depicted in Fig. 2 (top set of axes) revealed significant group differences, $F(3,20)=3.28$, $p<0.05$, in overall amount of responding (the group differences in overall amount of responding on day 1 are illustrated in Fig. 1, day 1). Newman-Keuls multiple comaprisons test indicated that the group effect was due to significant differences between the PIM 0.5 + RWD condition and the EXT condition. Tests for simple main effects from this analysis (tests for group differences at each time bin; top set of axes in Fig. 2) indicated that significant group differences were obtained only in the first time bin, $F(3,140)=8.74$, $p<0.001$. Moreover, Newman-Keuls post-hoc comparison of these first bin group differences indicated that the group effect was due to significant differences between the PIM 0.5 + RWD condition and the EXT condition. The first day comparison and within-session analysis confirm the visual impression that EXT and PIM conditions produced opposite effects on measures of overall amount of responding on the first test day (day 1 in Fig. 1) and within-session in the first five minutes of testing (top set of axes in Fig. 2). Since the anhedonia hypothesis maintains that the pattern of responding produced by animals in these two conditions should be similar to each other, testing for significant differences between these two groups in this manner would seem to be a legitimate (and conservative) way to test this hypothesis.

DISCUSSION

With an eight day dosing procedure both the present experiment and the Gramling *et al.* [10] study failed to observe extinction-like patterns of responding in PIM treated rats. The essential procedural difference between the present study and the Gramling *et al.* [10] study was the change in the response requirements (lever press vs. lick). The similarity in the patterns of responding produced by PIM treated animals in both of these experiments suggests that the failure to observe anhedonia was due to some factor other than the type of response employed. These two experiments were similar to each other, but different from other tests of the anhedonia hypothesis, in their use of nondeprived animals and a natural reinforcer of high hedonic value (i.e., sucrose), thereby keeping motivational influences to a minimum and at the same time emphasizing the hedonic value of the reward in maintaining responding. These two studies were also unique in their use of an eight consecutive day dosing regime. Minimizing motivational factors may have attenuated

PIM's extinction-like effects and may partially account for the qualitatively different patterns of responding between PIM treated rats and rats exposed to extinction procedures. However, since the qualitative differences were most apparent across the last four days of testing the extended dosing regime may be required to detect such differences. Curvilinear patterns of responding might be observed under other motivational conditions if an eight consecutive day, rather than the typically used four intermittent test days, were used.

One might argue that the curvilinear patterns of responding observed in the PIM treated rats are the result of slight supersensitivity effects that may have developed by days 6–8 [13]. However, numerous reports suggest that even with daily dosing, supersensitivity takes appreciably longer to develop (e.g., [21]) and even then is detected only after the neuroleptic is discontinued [13]. In the present experiment the functional dose that the animals were exposed to increased across daily injections since the half-life for PIM probably exceeds twenty-four hours [12]. Therefore, even if supersensitivity were occurring, it would seem reasonable to assume that there was sufficient PIM to maintain dopamine receptor blockade at nearly constant levels. These data may be better interpreted in terms of behavioral tolerance (cf., [3]). For instance, these data seem congruent with the reinforcement density hypothesis of behavioral tolerance [14]. This hypothesis predicts that when the effect of a drug is to decrease the density of reinforcement below predrug levels, tolerance to the drug effect will occur [14]. In the present experiment, perhaps the trend towards recovery on the rate measure reflects adaptation to a subtle motor deficit analogous to the adaptation observed in humans to neuroleptics' early onset extrapyramidal motor side affects. Given the numerous reports in the animal literature which emphasize neuroleptics' effects on motor processes (e.g., [4, 6–8]) it seems plausible that the recovery exhibited by the PIM treated rats on the rate measure reflects an adaptation to PIM's motor and/or sedative effects.

Under the conditions used here, eight consecutive days of PIM treatment produced clear behavioral effects but failed to produce patterns of responding similar to animals in the no-reward condition. Rather than exhibiting across-session declines in rate, PIM treated animals displayed a trend towards recovery on the rate measure across the last three days of testing. The largely curvilinear across-session pattern of responding produced by the PIM treated animals was in sharp contrast to the largely linear across-session decrease in rate exhibited by rats in the EXT condition. Moreover, first day comparisons revealed significant differences in the overall amount and pattern of responding produced by animals in the EXT condition relative to animals in the PIM 0.5 + RWD condition. A within-session analysis of the first test day revealed similar differences in the pattern of responding produced by these two groups. Namely, animals in the EXT condition exhibited relatively high rates of responding when first exposed to no-reward conditions whereas animals treated with PIM exhibited a relative rate decreases during the same time period. Taken together, these findings seem to compromise the generality of the anhedonia explanation of neuroleptics' behavioral effects. These data suggest that the anhedonia phenomenon may be dependent on the motivational state of the animal and/or the type of reinforcer employed. Alternatively, the criteria outlined by the anhedonia hypothesis to test for the putative reward-reducing effects of neuroleptics may be inadequate. Perhaps, as suggested by others [9], neuroleptic treatment should be compared with reduced-reward rather than no-reward conditions.

ACKNOWLEDGEMENTS

This study was conducted by the first author under the supervision of the second author in partial fulfillment of the requirements for the Ph.D. degree at the University of Mississippi. We thank Dr. Robert Cleary, Department of Pharmaceutics, School of Pharmacy, University of Mississippi for preparation of the pimozide solution. The pimozide was a gift from McNeil Inc. and is gratefully acknowledged.

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