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Pimozide Prevents the Response-Reinstating Effects of Water Reinforcement in Rats

AARON ETTENBERG AND JON C. HORVITZ

Behavioral Pharmacology Laboratory, Department of Psychology
University of California, Santa Barbara, CA 93106

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ETTENBERG, A. AND J. C. HORVITZ. *Pimozide prevents the response-reinstating effects of water reinforcement in rats.* PHARMACOL BIOCHEM BEHAV **37**(3) 465–469, 1990.—Thirsty animals were trained to traverse a straight runway once each day for a reward consisting of 100 licks from a water-filled drinking tube. Once running speeds had stabilized, single daily extinction trials were initiated during which no water reinforcement was provided in the goal box. Extinction trials continued until running had slowed to levels approximately half of that observed during reinforced trials. A single treatment trial was then conducted in which some animals found water in the goal box and others continued to find an empty water bottle. Those subjects that were reinforced on treatment day subsequently demonstrated a reinstatement of their operant running response on the very next trial (i.e., 24 hr later). However, pretreatment with 1.0 mg/kg (but not 0.5 mg/kg) of the dopamine antagonist drug, pimozide, attenuated this response-reinstating effect of water-reinforcement. This action of pimozide was not likely a consequence of some residual sedative or motor incapacitation since a) the test day was conducted 24 hr after the treatment day by which time the pharmacological actions of the drug had greatly subsided; b) a Motor Control group administered pimozide *after* the reinforced trial exhibited normal response-reinstatement 24 hr later on Test Day; and c) on treatment day, pimozide did not reliably attenuate running times, latency to initiate drinking, nor the rate of licking behavior. Together, these data suggest that dopamine receptor antagonism can produce an attenuation in the reinforcing efficacy of water.

Neuroleptics Water reinforcement Reward Pimozide Operant behavior Dopamine

DOPAMINE antagonist drugs produce dose-dependent attenuations in the performance of water-reinforced operant responses and, at higher doses, in the unconditioned consumption of water (8, 13–15, 17–20). While many researchers have proposed that such effects are best accounted for by drug-induced alterations in reward processes [e.g., see review (24)], others attribute the neuroleptic effects predominantly to an interference with extrapyramidal motor function. Ljungberg (18), for example, has observed that scopolamine reverses haloperidol-attenuated lever-pressing for water but not haloperidol's disruption of unconditioned drinking. The author concludes that "blockade of operant responding by low doses of neuroleptics is probably related to the extrapyramidal side-effects of neuroleptics seen in the clinic, as both phenomena can be counteracted by anticholinergics" (p. 205). Indeed, it has been demonstrated that neuroleptic drugs do produce a slowing in motoric ability that contributes to the reduction in operant response rates observed during drug trials (10,11). Since this "slowing" in operant behavior gets progressively stronger as the session progresses (6) this action might explain the "extinction-like" response patterns often exhibited by neuroleptic-treated subjects during reinforced trials (24). While such data do not preclude the possibility that neuroleptics disrupt reward processes, they do exemplify the interpretive complexities inherent in attempts to dissociate reward and performance factors when the dependent measures are obtained from animals drugged at the time of testing (4).

To circumvent some of these interpretative difficulties, we have recently described a behavioral test paradigm in which the putative reward-attenuating effects of neuroleptics were assessed independent of any performance-debilitating action of the drugs. This was accomplished by separating the drug treatment and the behavioral test phases of the experiment in a manner that permitted data collection to occur well after the direct pharmacological effects of the drug had subsided (16). Animals were trained to traverse a straight runway for food reward during single daily trials. Once the operant was established, reinforcement was removed and animals experienced single daily extinction trials until running speeds had substantially slowed. A single reinforced trial in the midst of this extinction phase was sufficient to reinstate runway responding on the very next trial (i.e., 24 hr later). In this situation, therefore, the effects of reinforcement presentation on one day produced a change in operant behavior measured during the next day's trial. When subjects were pretreated with haloperidol, the response-reinstating effect of food reinforcement was dose-dependently prevented. Even though these animals were no longer drugged at the time of testing, they performed equivalently

to animals that had received no food reinforcement the previous day (16). We concluded from these data that, in a response-reinstatement test paradigm, dopamine receptor antagonism disrupts the reinforcing actions of food. It was of interest, therefore, to determine if this behavioral methodology could be applied to assess whether or not neuroleptic challenge might similarly interfere with the response-reinstating action of water-reinforcement. The present experiment was devised to test this possibility.

METHOD

Subjects

The subjects were 35 experimentally naive male Sprague Dawley rats (300–325 g) obtained from Charles River Laboratories. Each animal was individually housed in metal wire hanging cages located within a temperature-controlled (23°C) 12-hr light/dark vivarium environment (lights on at 0700 hr). All subjects were provided with ad lib access to food and water in their home cages for two weeks. Access to water was thereafter restricted to a single 15-min period in the home cage each day. These periods occurred at the same time each day approximately one hour after operant testing. It should be noted that animals had free food available in their home cages at all times and in fact continued to gain weight throughout the course of the study. The limited availability of water served to motivate subjects to perform the operant running response for water reinforcement.

Drug/Vehicle

Pimozide (0.5 and 1.0 mg/kg) was dissolved in a warm vehicle solution of 0.002 M lactic acid. Intraperitoneal injections of pimozide or vehicle were administered in a volume of 1.0 ml per kilogram of body weight 4 hours prior to the treatment trial.

Apparatus

All trials were conducted in a straight-arm runway (155 cm long × 15 cm wide × 20 cm high) having a white start box and a black goal box attached at opposite ends. Walls of the runway were constructed of wood while the floor and removable ceiling were composed of wire mesh. A guillotine door separated the start box from the runway. Lifting this door signalled the start of a trial by activating a digital timer which stopped when the animal's presence in the goal box was detected by interruption of an infrared photocell beam located 8 cm inside the goal box entrance. Once inside the goal box, an additional guillotine door was lowered to prevent the animals from reentering the runway at the end of a trial. The operant data for each animal on each trial, therefore, consisted of the time required to leave the start box, traverse the runway, and enter the goal box.

Water reinforcement was delivered through a standard rodent drinking bottle secured to the outside rear wall of the goal box. A small hole was drilled through the goal box wall (along the midline 5 cm from the floor) to provide access to the metal spout (dia. 0.5 cm) of the drinking bottle. This spout was recessed slightly (0.1 cm from the inside surface of the wall) to ensure that measurements were not confounded by paw or whisker contacts with the spout. This was important since a drinkometer was employed to identify licks by completing a circuit between the wire floor of the goal box and the metal drinking spout each time an animal's tongue contacted the spout. This device was in turn wired to an IBM-PC equipped with a customized John Bell Engineering PC Universal I/O board for data collection. Turbo Pascal software (written by Stephen Fowler) directed the A/D converter to sample the output of the drinkometer circuit at a frequency of 256 Hz thereby providing precise information about the number of licks per unit time. In addition, the computer recorded the latency to initiate drinking upon the animal's entry into the goal box.

Procedure

Pretraining. Prior to the start of the data collection phase of the experiment, each animal was individually placed into the goal box (with the runway door closed) where they remained until 2 min of drinking behavior had been recorded. This procedure was repeated on two consecutive days followed by two runway trials in which subjects were placed into the start box with the start door open and allowed to traverse the alley for a reinforcement of 100 licks from the drinking tube. Once removed from the apparatus, subjects were returned to their home cages where, one hour later, their daily 15-min access to water was provided.

Acquisition. Animals received one runway trial each day. On a given day, each animal was placed into the start box and, after 5 sec, the start door was raised, thereby initiating a trial. Once the animal entered the goal box, the timing stopped and the second door was closed to prevent retracing. In the goal box, the subject was allowed 100 licks from the drinking tube after which it was immediately removed from the apparatus and returned to its home cage. For an individual animal, acquisition continued in the manner described until its running time was less than 30 sec on two consecutive trials (on average this required 28 days to accomplish). Once this arbitrary criterion was satisfied, the extinction phase of the experiment was initiated.

Extinction. Extinction trials were conducted once each day in precisely the same manner as that described for acquisition with two exceptions: 1) no water reinforcement was available in the goal box on any trial; and 2) subjects remained in the goal box for 50 sec. Extinction trials continued for a given animal until its running behavior slowed to an arbitrary criterion equal to two times the mean running time of the last two acquisition trials (this required approximately 8 days on average). Once this extinction criterion was satisfied, a single additional extinction trial was conducted to provide a "baseline" condition for subsequent comparisons.

Treatment Trial. Treatment day consisted of a single trial during which subjects experienced one of five different treatment conditions (n = 7/group). One group of rats received a vehicle injection 4 hr prior to a single nonreinforced extinction trial (VEH/NO WATER); other animals received a vehicle injection 4 hr prior to a water-reinforced trial (VEH/WATER); two groups received a single IP injection of either 0.5 or 1.0 mg/kg pimozide prior to a water-reinforced trial ($PIM_{0.5}$/WATER and $PIM_{1.0}$/WATER); a final "motor control" condition consisted of subjects that received the high dose of pimozide 1 hr *after* a water-reinforced trial (W/$PIM_{1.0}$).

Test Day. Twenty-four hours after the Treatment Trial, a single no-drug no-reinforcement (i.e., extinction) trial was conducted with all subjects.

RESULTS

To control for the heterogeneity of variance inherent in response-duration measures, the raw data were converted from running times (X-sec) to their reciprocals, running speeds (1/X sec). Analyses were conducted on the running speed data.

Figure 1 depicts the mean performance (± S.E.M.) of each group during the extinction baseline and the test day (i.e., the day preceding and following the treatment trial). A two-factor ANOVA computed on these data produced a statistically reliable effect for Group, $F(4,30) = 4.69$, $p<0.01$, for Trials, $F(1,30) = 19.58$,

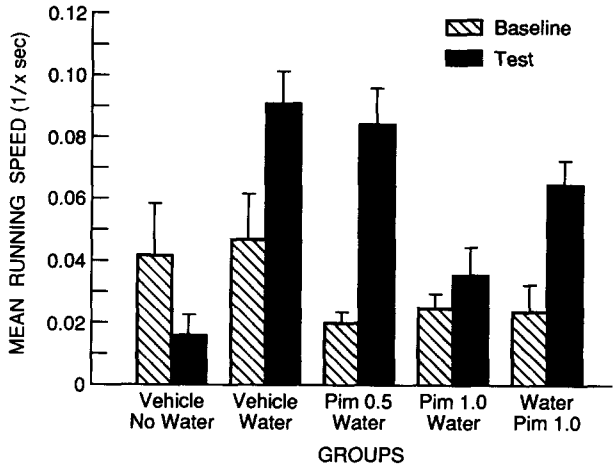

FIG. 1. Mean Baseline (extinction) and Test Day performance for each group (±S.E.M.). Data are expressed as running speeds with higher bars depicting faster operant running.

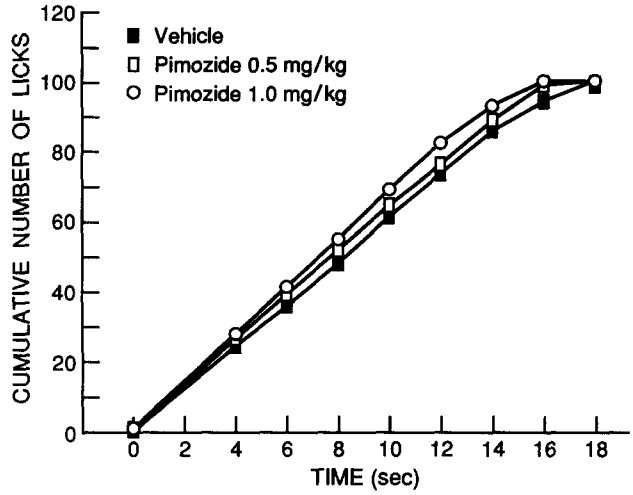

FIG. 2. Mean cumulative records for each group emitting 100 licks for water reinforcement. Pimozide produced no reliable change in the pattern or rate of unconditioned licking behavior.

$p<0.001$, and for the Group × Trials interaction, $F(4,30)=6.86$, $p<0.001$. A one-way ANOVA computed solely on the baseline data produced no statistically reliable effect, $F(4,30)=1.29$, n.s., while a similar analysis computed on the test day data revealed a robust main effect of Group, $F(4,30)=10.68$, $p<0.0001$. These results suggest that the differences revealed in the overall ANOVA are likely attributable to differences in group performance on test day. Analyses of each group's test-day performance compared to its baseline performance revealed that the only groups to demonstrate a reliable change in operant behavior were the VEH/WATER group, $t(6)=2.85$, $p<0.05$, the $PIM_{0.5}$/WATER group, $t(6)=5.91$, $p<0.01$, and the motor control WATER/$PIM_{1.0}$ group, $t(6)=4.62$, $p<0.01$. In each of these three groups, water reinforcement on treatment day was, therefore, sufficient to reliably reinstate operant running on the next trial/day 24 hr later. As one would expect, animals that continued to experience extinction conditions on treatment day (i.e., the VEH/NO WATER group) continued to run slowly on test day, $t(6)=1.39$, n.s. While water-reinforced animals pretreated with the high dose of pimozide (the $PIM_{1.0}$/WATER group) did not exhibit the response-reinstatement observed in the nondrugged or low-pimozide conditions.

Note that the slow running of the 1.0 mg/kg pimozide-water subjects cannot easily be attributed to a residual motor or performance incapacitation since other animals injected *after* the treatment trial (and therefore in closer temporal proximity to the test day; i.e., the WATER/$PIM_{1.0}$ group) were able to perform without impairment on test day. In addition, an analysis of treatment day running speeds revealed no reliable group differences suggesting that even under peak pimozide effects the animals were able to perform a single trial without obvious motoric impairment, $F(4,30)=1.44$, n.s. Pimozide also had no effect on rats' latency to initiate drinking upon entering the goal box, $F(2,18)=1.77$, n.s., nor on the time required to emit 100 licks from the drinking tube once drinking was initiated, $F(2,18)=1.36$, n.s. These final analyses did not include the VEH/NO WATER group since it found an empty water bottle on treatment day, nor the WATER/$PIM_{1.0}$ control group which was undrugged on treatment day and hence redundant with the VEH/WATER group. Figure 2 shows a cumulative record of responses (licks) for the vehicle and pimozide water-reinforced groups. It is clear from both the statistics (above) and Fig. 2 that rate of licking was not impaired by pimozide. Therefore, putative drug-induced differences in reinforcement density cannot account for the changes in runway performance observed on test day 24 hr later.

DISCUSSION

Animals that experienced a single water-reinforced trial during extinction reinstate their operant running on the very next trial conducted 24 hr later. Pimozide pretreatment (1.0 mg/kg) produced a reliable attenuation of this response-facilitory action of water reinforcement. These results are, therefore, comparable to those we have previously reported with haloperidol using the same behavioral paradigm with food-reinforcement (16). Since pimozide is a potent and highly selective dopamine receptor antagonist [e.g., (1)], the present data are consistent with the view that central dopaminergic elements are involved in mediating the behavioral effects of water reinforcement (5, 13, 21, 24).

Two novel procedural aspects of the present study strengthen this conclusion in the face of potential alternative "motoric" or performance explanations of neuroleptic action. First, animals were tested on a single trial per day. Since neuroleptic drugs do not dramatically affect operant behaviors at the very outset of the test session (24), we were able to successfully employ doses that others have found to produce profound behavioral impairments in tests requiring continued or sustained responding on the part of the subject [e.g., (6, 8, 9, 12, 13, 22, 23)]. Second, the effect of the neuroleptic on putative reinforcement function was tested 24-hr postinjection at a time when the direct pharmacological actions of the drug had greatly subsided. These two procedural aspects make it highly unlikely that the present data can easily be accounted for by some form of general sedative or performance incapacitation hypothesis. In fact, two pieces of evidence would appear to rule out a motoric explanation of the results: 1) the motor control group (WATER/$PIM_{1.0}$) which was injected after the treatment trial, demonstrated normal response reinstatement on test day, hence drug administration alone was insufficient to account for the slow running observed in the $PIM_{1.0}$/WATER group on test day; and 2) even on treatment day there were no reliable alterations in running speed among drugged and nondrugged groups.

Alternatively, one might account for the present results by a hypothesized change in the motivational state of the drug-treated animals. Pimozide has, for example, been reported to produce an attenuation in unconditioned drinking (3, 14, 15, 17, 25). Therefore, it may be that the observed drug-induced reduction in the response-reinstating effect of water-reinforcement stems not from an attenuation in reward per se but rather an attenuation in "thirst." While this remains a possibility, we observed in the present study no evidence of a drug-induced shift in water motivation. Rather, pimozide animals were just as fast as undrugged animals in traversing the alley, in their initiation of drinking (once they entered the goal box) and in the rate at which they obtained their 100 licks of water reinforcement.

One might ask why pimozide-treated animals licked normally for water if the drug had acted to disrupt water reinforcement. Two possible explanations might account for this. The first possibility is that pimozide attenuates the rewarding effects of water, however, since the animals were allowed only 100 licks of water on the treatment trial (less than 20 sec of drinking), this effect was not yet manifested in the drinking behavior of the animals. Neuroleptic-induced disruptions in drinking behavior are, in fact, seen when animals are allowed to drink for longer periods of time [e.g., (3, 17, 25)]. We chose 100 licks as the reinforcer in the present experiment precisely because pilot data showed that pimozide did *not* disrupt the first 100 licks, thus ensuring that test day reductions in running behavior could not be attributed to differences in treatment day patterns of reinforcer delivery.

Another possibility is that the reinforcement-attenuating actions of pimozide (manifested in test day running behavior) were not due to a blunting of the reward/incentive properties of water per se, but rather to a disruption in the process by which the water-reinforcement produced changes in subsequent operant running. For example, one might presume that the stimuli associated with the primary reinforcer (i.e., the start box, alleyway, and goal box) reacquire conditioned incentive properties on treatment day as a result of their reassociation with the water reinforcer [e.g., see (2)]. Placing the animal in the runway on the next trial (24 hr later) therefore exposes it to these "reactivated" incentive stimuli which in turn motivate the animal to reinstate operant running. In this view, dopamine antagonism may leave the primary incentive properties of the water intact, while interfering with the process by which the alley stimuli regain conditioned incentive value.

The finding that pimozide prevented the reinstatement of the operant running response, but did not impair drinking behavior is consistent with demonstrations that operant behaviors are more vulnerable to neuroleptic challenge than are consummatory behaviors (14, 17, 19). However, while such results have been presumed to reflect a motor impairment, this interpretation cannot account for the present results, since the operant running response was assessed at a time when the direct pharmacological (and thus motoric) effects of the drug had subsided.

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