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Performance Effects with Repeated-Response Measures During Pimozide-Produced Dopamine Receptor Blockade[1]

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ETTENBERG, A., S. A. CINSAVICH AND N. WHITE. *Performance effects with repeated-response measures during pimozide-produced dopamine receptor blockade.* PHARMAC. BIOCHEM. BEHAV. 11(5) 557–561, 1979.—Rats were trained to lever-press for either food reward or brain-stimulation reward on a continuous reinforcement schedule. Following training each animal was extinguished (i.e. tested with the reward omitted) under the influence of pimozide (0.25 mg/Kg or 0.5 mg/Kg). Pimozide produced a dose-dependent reduction in the mean number of responses to extinction made by the rats in each group. In a second experiment, pimozide produced a similar dose-dependent decrease in the performance of a naturally occurring behavior (nose-poking) that had never been associated with reward. These data suggest that dopamine receptor blockade can produce a performance deficit in situations which require repetitive responses, and that this deficit is unrelated to the presence or absence of reward.

Pimozide Food reward Brain-stimulation reward Self-stimulation Extinction Dopamine Performance deficits

ONE approach that has been used to study dopamine's role in the mediation of reward has been to administer a dopamine receptor blocking agent (e.g. pimozide) to rats responding for food reward [17,18] or for brain stimulation reward [5,6]. In these experiments, animals under the influence of pimozide stop responding slowly, in a manner that resembles that of rats responding in the absence of reward (i.e. extinction). On the basis of this resemblance it has been suggested that dopamine blockade produces a condition equivalent to extinction by blocking the central effects of reward.

Other recent evidence, however, has suggested that disruption of dopamine function can also produce severe performance deficits [2, 3, 10, 11, 14]. If it is true that the disruption of dopamine function produces a simple inability or disinclination to perform certain responses, the reward-blocking interpretation of the effects of dopamine-receptor blockade would be vitiated. The major problem in differentiating between these two hypothesized effects of dopamine disruption has been the fact that they lead to nearly identical predictions in experimental paradigms that require animals to make repetitive responses. However, a slight change in the most common of these paradigms—that in which animals simply make repeated responses to obtain a reward—makes the two hypotheses generate different predictions. If dopamine receptor blockade primarily affects responding in these situations by blocking reward, such blockade should have little or no effect on responding during extinction when there is no reward present. Therefore, in rats that have been well trained to perform a response, dopamine receptor blockade should have no effect on patterns of responding during extinction. The performance-deficit hypothesis, on the other hand, predicts that dopamine receptor blockade during extinction will result in decreases in response output of approximately equal relative magnitude to those that occur during rewarded responding. Experiment 1 tested these predictions using food and brain-stimulation rewards.

EXPERIMENT 1

METHOD

Animals

The animals were 36 male albino rats weighing 300–350 g at the start of the experiment. Each rat was individually housed. Eighteen rats were provided with ad lib access to food and water. The remaining 18 rats were maintained at 75% of their free-feeding weight by a restricted food diet.

Procedure

Each of the normally-maintained rats was stereotaxically

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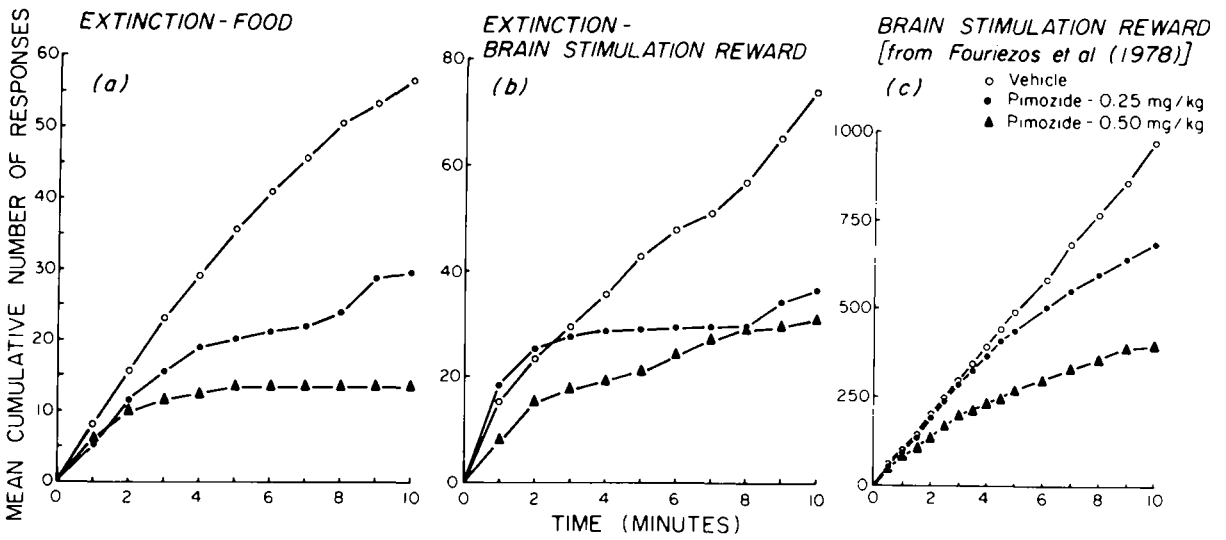

FIG. 1. Effects of pimozide on responding in extinction by rats trained to bar press for food reward (a) and for brain stimulation reward (b). The data in (c) are replotted from Fig. 2 in Fouriezos, Hansen and Wise [5], and represent the first ten min of a longer session in which rats bar pressed for brain stimulation reward under the influence of the same doses of pimozide as were used in the present study. Although Wise, Spindler and Legault [17] reported a similar effect of pimozide on animals responding for food reward, their report does not include sufficient data to make a similar comparison for this condition.

implanted with a bipolar stimulating electrode (Plastic Products Company) under 50 mg/Kg sodium pentobarbital anaesthesia. The electrodes were aimed at the lateral hypothalamus. With the tooth-bar of the stereotaxic instrument set at 3.2 mm above the interaural line, the coordinates were: 0.8 mm posterior to bregma; 1.5 mm lateral to midline; 8.6 mm ventral to the skull surface. One week after surgery these animals were trained to lever-press for 0.5 sec trains of 60 Hz sine-wave intracranial stimulation. The training procedure also involved adjusting the current intensity for every animal to a value that produced a steady rate of responding over a 15 min session (current range: 15–35 μA RMS).

The eighteen restricted-diet animals were trained to lever-press for 0.045 g Noyes food pellets on a continuous reinforcement schedule. All rats were run for 15 min every alternate day until their rates of responding had stabilized over three consecutive sessions.

On the test day, six of the brain-stimulation reward animals and six of the food-reward animals received 0.5 mg/Kg of the dopamine receptor blocker pimozide. An additional six animals from each group received 0.25 mg/Kg pimozide. The pimozide was dissolved in a hot aqueous solution of six parts tartaric acid to one part pimozide and was injected intraperitoneally in a volume of 1.0 ml/Kg body weight. The remaining six animals in each group were injected with similar volumes of the tartaric acid vehicle solution. Four hours after the injections each animal was individually placed into the appropriate test chamber (food or brain stimulation). During testing no reinforcement was delivered and the number of responses each rat made was recorded every minute until an extinction criterion of 5 min with no responding was reached.

After the extinction test the rats with electrodes were killed with an overdose of sodium pentobarbital. Their brains were removed and prepared for examination using standard histological techniques.

RESULTS

Figure 1 describes pimozide's effects on responding in extinction by rats trained to bar press for food reward or for brain stimulation reward. Since over 90% of the animals in both groups reached the extinction criterion (5 min with no responses) within 15 min, only data for the first 10 min of the extinction sessions are presented. The figure shows that pimozide produced a clear, dose-dependent reduction in the rates and in the total number of responses emitted by the rats in both groups. Moreover, the magnitude of the drug's effect on responding was small at the beginning of the sessions and became progressively larger as the rats responded, over time. The data from Fouriezos *et al.* [15] in Figure 1c shows that the relative effects of pimozide on responding when brain stimulation is present are similar to the effects of the drug when reward is omitted, as observed in the present study.

For the animals trained to respond for food reward a two-factor analysis of variance, with repeated measures on one factor, was computed on the raw, response-per-minute data (not on the cumulative data shown in Fig. 1). There was a significant effect of drug dose, $F(2,15)=19.6$, $p<0.001$, and of time, $F(9,135)=2.13$, $p<0.001$. A one way analysis of variance showed that the mean numbers of responses made during the first three minutes of the extinction test by the rats in the three groups were not significantly different, $F(2,15)=2.55$. However, there was a significant difference in the mean number of responses made during the final three minutes, $F(2,15)=3.99$, $p<0.02$. During the extinction session the 0.25 mg/Kg dose reduced the total number of responses made in 10 min to 52% of the same value for the vehicle group. The 0.5 mg/Kg dose reduced this figure to 25 percent of the value for the vehicle group. Table 1 shows that the mean total numbers of responses made by the rats in each of the three groups during the final 15 min reward training sessions were approximately equal.

TABLE 1

NO DRUG REWARDED BASELINE PERFORMANCE: MEAN NUMBER OF BAR PRESSES PER GROUP (SEM) DURING THE FINAL 15 MIN REWARDED TRAINING SESSION

	Food reward	Brain-stimulation reward
Vehicle	107.3 (8.6)	598.7 (68.5)
Low pimozide	124.5 (10.0)	554.5 (75.6)
High pimozide	120.9 (7.5)	604.8 (48.7)

For the animals trained to respond for brain stimulation reward the results of examining the histological material are shown in Fig. 2. The tips of the electrodes were located in the area of the lateral hypothalamus, dorsolateral to the fornix, at the level of the middle of the ventromedial hypothalamic nucleus. An analysis of variance on the response-per-min data for these rats showed significant effects of drug dose, $F(2,15)=3.7$, $p<0.05$, and of time, $F(9,135)=7.42$, $p<0.01$. A one way analysis of variance showed that the mean numbers of responses made during the first three min of the extinction test by the rats in the three groups were not significantly different, $F(2,15)=0.83$. However, there was a significant difference in the mean number of responses emitted during the last three min, $F(2,15)=4.62$, $p<0.03$. Table 1 shows that the mean numbers of responses made by the rats in each of the three groups during the final 15 min rewarded training session were approximately equal.

In the self-stimulation group the 0.25 mg/Kg dose reduced the total number of responses made in ten min to 49 percent of the same figure for the vehicle group. The 0.5 mg/Kg dose reduced this figure to 42% of the value for the vehicle group. For the data of Fouriezos *et al.* [5], shown in Fig. 1c, the 0.25 mg/Kg dose reduced the total number of responses to 75% of the control value in the first ten min of the session, and the 0.5 mg/Kg dose reduced the total to 36% of the vehicle group's value. A comparison of these effects of pimozide in the three conditions for which data are available (those shown in Fig. 1) shows that there is no consistent difference between the effects of pimozide on responding in the reinforcement and extinction conditions. The major effect of the drug in all the conditions discussed in this experiment seems to have occurred after the rats had been responding for at least a short time. However, the manipulations performed in the present experiment suggest that the presence or absence of reward may not be relevant to the decreased responding observed in this paradigm.

EXPERIMENT 2

An alternative method of studying pimozide's effects on performance would be to determine the drug's influence on a naturally occurring high frequency instrumental response, not previously associated with reinforcement. If the primary result of dopamine receptor blockade is to block reward, then pimozide should have little effect on the operant response levels of such an unreinforced behavior. On the other hand, a pimozide-produced decrease in non-reinforced instrumental responding would suggest that a performance debilitation is produced by dopamine receptor blockade. Experiment 2 describes a test of this nature.

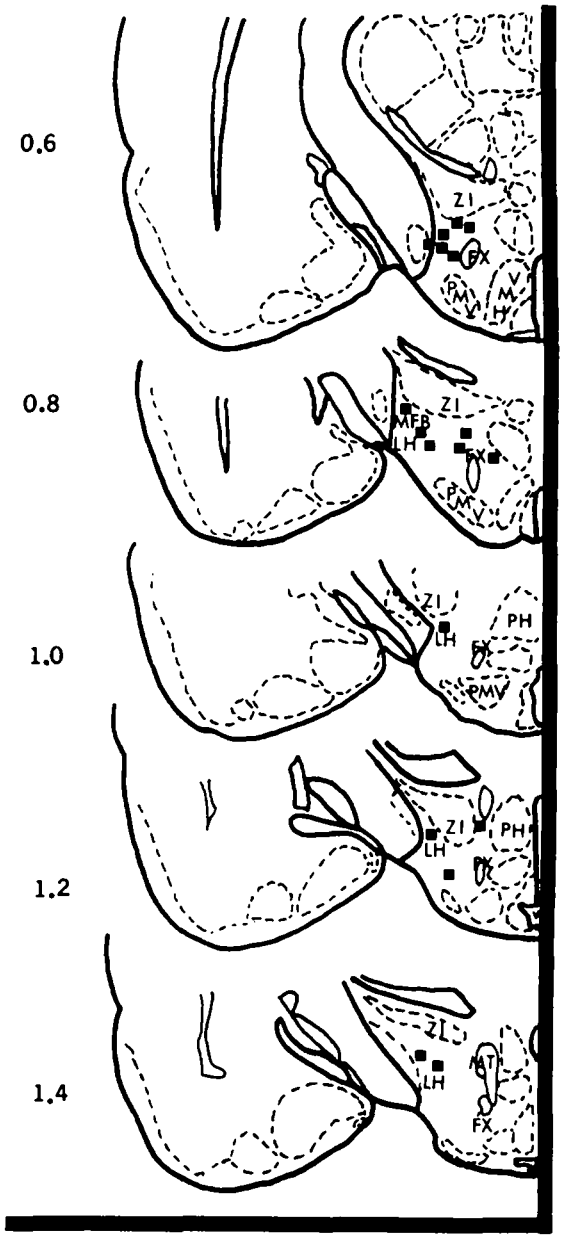

FIG. 2. Electrode placements for the 18 implanted rats used in the experiment. Numbers represent millimeters posterior to bregma. Sections are from Pellegrino and Cushman [9]. Abbreviations: FX, fornix; LH, lateral hypothalamus; MFB, medial forebrain bundle; MT, mamillothalamic tract; PH, posterior hypothalamus; PMV, ventral premamillary nucleus; VMH, ventromedial hypothalamus; ZI, zona incerta.

METHOD

Animals

The animals were 18 male albino rats weighing between 350–375 g at the start of the experiment. All animals were individually housed and provided with ad lib access to food and water.

TABLE 2
MEAN NOSE-POKE LATENCY AND MEAN NUMBER OF NOSE-POKES TO EXTINCTION

Drug treatment (mg/kg)	Response latency mins (SEM)	Number of responses to extinction (SEM)
Vehicle	1.9 (.60)	39.5 (8.48)
0.25 pimozide	2.5 (.07)	27.5 (8.84)
0.50 pimozide	3.0 (.63)	6.7 (2.04)

Procedure

Prior to the test day the animals were allowed seven days to adapt to the laboratory environment. Every animal was handled for several minutes each day during this period. On the test day, six of the rats were injected with 0.50 mg/Kg pimozide, six animals were injected with 0.25 mg/Kg and the final six animals received only the vehicle solution. The drug was prepared and injected in the same manner as in Experiment 1.

Four hr after the injections, the animals were individually placed into the test apparatus. This apparatus consisted of a Plexiglas cubicle (25×18×25 cm) with a metal grid floor located inside a sound-attenuating box. The walls and ceiling of the cubicle were black. A 2 cm-diameter hole was located in the middle of one of the walls 3 cm from the floor of the cubicle. An illuminated Plexiglas disk was suspended inside the hole. When the disk was pushed a microswitch was closed. The latency to the first nosepoke and the total number of nosepokes in the test session were recorded for each animal. A test session was terminated when a rat did not respond during any five min period after making the initial response.

RESULTS

Table 2 shows that increasing doses of pimozide produced slight increases in latency to make the first nosepoke response; however, a one-way analysis of variance showed that there was no significant difference among these means, $F(2,15)=0.70$. This result suggests that, at least at the start of the session, the tendency of the rats to respond was not greatly influenced by any overall effect on activity that may have been produced by the drug. The drug produced a considerably larger effect on the number of responses to the 5 min response cessation criterion. A one-way analysis of variance showed that the dose-dependent reduction in this measure was significant, $F(2,15)=5.36$, $p<0.02$. These data show that the effects of pimozide were similar in this experiment and in Experiment 1. Although there may have been a slight general reduction in the rats' disposition to respond at all, the major effect occurs after the animals begin responding. As no reward was present in the experimental test situation, nor had the rats ever been rewarded in this (or any other) experimental situation, it is not possible to attribute the observed effect of pimozide to a blockade of reward.

DISCUSSION

It has previously been demonstrated that when rats respond for food reward or brain-stimulation reward under the influence of the dopamine-receptor blocker pimozide, a gradual dose-dependent reduction in rate of responding occurs [5, 6, 17, 18]. In Experiment 1 a similar dose-dependent effect was observed in the absence of reward (i.e. during extinction). Moreover, the effect was also observed on the performance of a non-rewarded instrumental behavior in Experiment 2. It is therefore likely that pimozide's action in these situations was independent of reward, since similar effects on responding are produced by the drug whether or not reward is present. This conclusion is at least partly consistent with the results of other studies that suggest an important role of brain dopamine in various motor functions [1, 8, 11, 14, 15].

A simple performance debilitation hypothesis might, therefore, provide a satisfactory explanation for the effects of dopamine blockade on responding except for the fact that in the present study, as well as in tests in which reinforcement is present (see Fig. 1), the drugged rats begin the session by responding at approximately the same rates as normal control rats, and slow down only later in the session. Therefore, the problem of interpretation posed by the data of the present experiments is to suggest an hypothesis which takes this gradual decline in performance into account.

One possibility suggested by the fact that dopamine blockade is equally effective in the presence and in the absence of primary reinforcers, is that pimozide may act to reduce the influence of secondary reinforcers on behavior. If secondary reinforcers are responsible for prolonging formerly reinforced behaviors during extinction, blocking the central mediation of these conditioned stimuli should produce an earlier cessation of responding. However, at the beginning of the session the drugged rats in the present study responded at the same rates as the control animals. Moreover, Franklin and McCoy [7] trained rats to respond to an explicit secondary reinforcer, and also found that the initial responses to the conditioned stimulus were identical under pimozide and control conditions. Differences between the two conditions appeared only later in the session. These data show that the secondary reinforcement hypothesis has the same weakness as the performance deficit hypothesis. The fact that differences in the behavior of animals treated with pimozide appear only after they have spent some time responding means that a deficit in the effectiveness of secondary reinforcers is a valid hypothesis only if it is assumed that, under the influence of pimozide, the strength of conditioned stimuli is weakened more rapidly than normal by unreinforced responding.

Another obvious explanation of our data centers around the notion of response-produced fatigue. It is possible that pimozide, perhaps because of some peripheral action, simply causes rats to get tired after a relatively small amount of responding [4]. Although the important factors contributing to this proposed effect of the drug are not clearly specified in this form of the response-produced fatigue hypothesis, it nevertheless predicts a behavior change which is operation-

ally indistinguishable from that predicted by the reward-blockade hypothesis.

A second version of the fatigue hypothesis focuses on dopamine receptors in the brain a certain fraction of which are blocked by any given dose of pimozide. The relatively small number of unblocked receptors remaining functional after a pimozide injection may be subject to abnormally high levels of activation during test sessions, and may undergo adaptation processes that are not reflected in behavior when the normal complement of receptors is functional. If the hypothesis that these receptors mediate the effect of reinforcement on behavior is correct, the receptor fatigue notion predicts the behavior deficit that is observed during reinforced responding (Fig. 1c). However, this hypothesis does not predict the behavior deficit observed in the present study, which occurred during unreinforced responding.

It seems clear that the weight of the evidence in the data discussed points to some form of an interaction of the effects of pimozide with response mechanisms. In fact, experimental paradigms using repeated response measures seem ideal for demonstrating deficits that occur partially as a consequence of responding itself. To evaluate the suggestion that dopamine receptor blockade may also produce a reinforcement deficit, an experimental measure that is not influenced by response-produced performance deficits must be used. White and Major [16] reported an experiment in which pimozide-treated rats and control animals were given one trial per day on a water-tube finding task. They found a significant acquisition deficit in pimozide-treated animals whose behavior was positively reinforced by the presence of water, but no effect of pimozide on animals whose behavior was negatively reinforced by the absence of water. In a control condition, animals given pimozide for the first time after they had learned the task showed no significant change in their performance. These data, which were obtained in an experimental paradigm that eliminated the influence of response-produced performance deficits, support the hypothesis that dopamine receptors mediate the effects of positive reinforcement on behavior. Another experimental paradigm which appears to distinguish between the effects of functional disruption of dopamine on performance and reward is the underwater swim maze. When rats are required to negotiate a Y-maze while completely submerged, their latencies to reach air at the end of one of the arms of the maze are greatly increased (a motor deficit) by treatment with spiroperidol or by 6-hydroxydopamine lesions of the DA neurons of substantia nigra [11]. However, the animals do eventually reach the goal box and so can be tested on various learning tasks in the maze. Both of the above treatments also produced an inability to learn a brightness discrimination [12,13], which may have resulted from a reinforcement deficit.

In summary, there appear to be two reasonably well documented consequences of dopamine receptor blockade in rats. The present data clearly suggest a response-produced performance deficit, and other data in the literature suggest that pimozide weakens the effect of positive reinforcers on behavior. A reconciliation of these two effects may eventually emerge from the idea that the neural mechanisms of response production and of reinforcement are closely related.

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