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Pimozide Attenuates Conditioned Taste Preferences Induced by Self-Stimulation in Rats

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ETTENBERG, A. AND N. WHITE. *Pimozide attenuates conditioned taste preferences induced by self-stimulation in rats*. PHARMAC. BIOCHEM. BEHAV. 15(6)915–919, 1981.—Conditioned taste preferences (CTPs) were observed in rats who drank flavored water followed by a session of self-stimulation. Control groups that did not self-stimulate did not exhibit CTPs. Other taste/SS pairings conducted under the influence of the dopamine receptor antagonist pimozide (0.1 or 0.3 mg/kg, IP) resulted in dose-dependent reductions in the size of the CTPs. No evidence of any aversive effects (conditioned tast aversions) of the pimozide treatment were observed in the no-stimulation control groups. These data suggest that, in addition to its effects on responding, low doses of pimozide reduce the rewarding properties of self-stimulation.

Pimozide Brain-stimulation reward Conditioned taste preferences Dopamine Self-stimulation

CONDITIONED taste preferences (CTPs) have been demonstrated in rats following the pairing of a novel taste with a session of self-stimulation [10, 14, 28]. Since the size of these preferences varies systematically as a function of the stimulation intensity the CTP paradigm has been employed as a measure of brain-stimulation reward (BSR) [7, 8, 14].

The most conventional measure of BSR is the rate at which animals respond to obtain brief trains of stimulation. Reward value is inferred from response rate: the higher the rate the more rewarding the stimulation is assumed to be. However, response rates are inappropriate in certain psychopharmacological studies because they cannot distinguish changes in a subject's ability to respond from changes in the rewarding properties of the stimulation. An example of such a problem is the interpretation of the reduction in response rate for brain stimulation reward (BSR) during dopamine receptor antagonism. Some workers have argues that the reductions reflect a reduction in the reward strength of the stimulation [16–19], and others have suggested that dopamine receptor antagonism interferes with an animal's ability to initiate or maintain responding [9, 11, 15, 24, 25]. In the present experiment the CTP paradigm was applied to the study of this phenomenon.

Earlier work demonstrated that high-intensity performance-debilitating stimulation which reliably reduced response rates for BSR, did not reduce the size of the CTP [8]. Furthermore, there was no correlation between the rate of responding or the number of stimulations received and the size of CTPs [7]. These results suggest it is the quality of the stimulation (i.e., the reward strength) and not the quantity that produces taste preferences. The CTP paradigm may, therefore, be a useful rate-independent measure of the reward magnitude of BSR. If dopamine receptor antagonism reduces the reward strength of brain stimulation it should reduce the size of the CTPs produced by that stimulation.

EXPERIMENT 1

METHOD

Animals

The animals were 48 male albino rats (300–350 g) which were individually housed and given ad lib access to food. The rats were each handled for several minutes every day during the seven days prior to surgery.

Surgery

A bipolar stimulating electrode (Plastic Products Co. 0.25 mm dia.) was implanted in each of 24 rats under 50 mg/kg sodium pentobarbital anesthesia. The electrodes were aimed at the lateral hypothalamus using the following coordinates: 0.8 mm posterior to Bregma; 1.5 mm lateral to midline; 8.6 mm ventral to the skull surface; the tooth-bar of the stereotaxic instrument was set at 3.2 mm above the interaural line. The remaining 24 rats were anesthetized but did not undergo surgery.

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Apparatus

Training and testing for each animal were conducted in one of four identical self-stimulation chambers. The chambers each consisted of a small Plexiglas cubicle (30.5×30.5×18 cm) with a metal grid floor and one aluminum plate wall. A metal lever protruded from the middle of the aluminum wall at a height of 5.0 cm above the grid floor. Every lever press resulted in the delivery of a 500 msec train of 60 Hz sine wave stimulation through the implanted electrode. The self-stimulation chambers were located inside individual sound-attenuating boxes equipped with 6 W lamps and loud speakers that provided constant masking noise.

Procedure

Self-stimulation training. One week following surgery the rats with electrodes were trained to lever-press for intracranial stimulation during single 30 min sessions. Stimulation current intensities were adjusted for each animal to produce steady rates of responding (mean current intensity 33 uA RMS). The unimplanted animals were similarly placed in the test apparatus for 30 min. The next day the implanted rats lever-pressed for 15 min, and the unimplanted rats were each placed in the apparatus for an equivalent time. The self-stimulation rates attained in these sessions were used to assign animals to experimental groups so that the mean rates of the rats in each group were approximately equal.

Drug administration. One week after self-stimulation training, water was removed from all cages for 48 hrs. Eight implanted and eight unimplanted rats were then injected with 0.1 mg/kg of the dopamine receptor blocker, pimozide. The rats in two additional groups of eight were injected with 0.3 mg/kg of pimozide. The pimozide was dissolved in a vehicle solution of tartaric acid and injected intraperitoneally in a volume of 1.0 ml/kg body weight. The remaining two groups of eight rats with and without electrodes were injected with the tartaric acid vehicle alone.

Taste/stimulation pairing. Four hrs post-injection, each subject was put into the test apparatus which contained a Richter tube in place of the lever. The rats with electrodes were connected to the stimulator. All animals drank a solution of 2 mg/ml instant decaffeinated coffee in cold tap water for 10 mins. At the end of the drinking period the Richter tube was removed and the animals with electrodes were permitted to self-stimulate for 15 mins. The unimplanted animals were left alone in the test chamber for 15 mins after drinking the coffee.

Preference test. Immediately following the pairing procedure all animals were returned to their home cages without water for 24 hrs. They were then given access to water in their home cages for 20 mins, followed by an additional 24 hr period of deprivation. Each rat was then given a two-bottle preference test in the test chamber used for its pairing with the lever removed. Each test chamber contained two Richter tubes, one containing the coffee solution, the other containing water. Half the animals in each group were initially presented with the coffee tube on the left side and water on the right. The opposite was true for the remaining rats. After 10 mins the tube positions were reversed for an additional 10 mins. To ensure that all animals tasted the contents of both tubes during the test, the tubes were presented as follows: for each 10 min portion of the test, the left tube was presented alone until the rat licked its contents. The tube was then withdrawn and the right tube was presented. Once the rat had licked its contents it was removed. After approximately 10 sec both tubes were presented simultaneously. The amounts of coffee and water drank during this free-choice situation were recorded after each 10 min period.

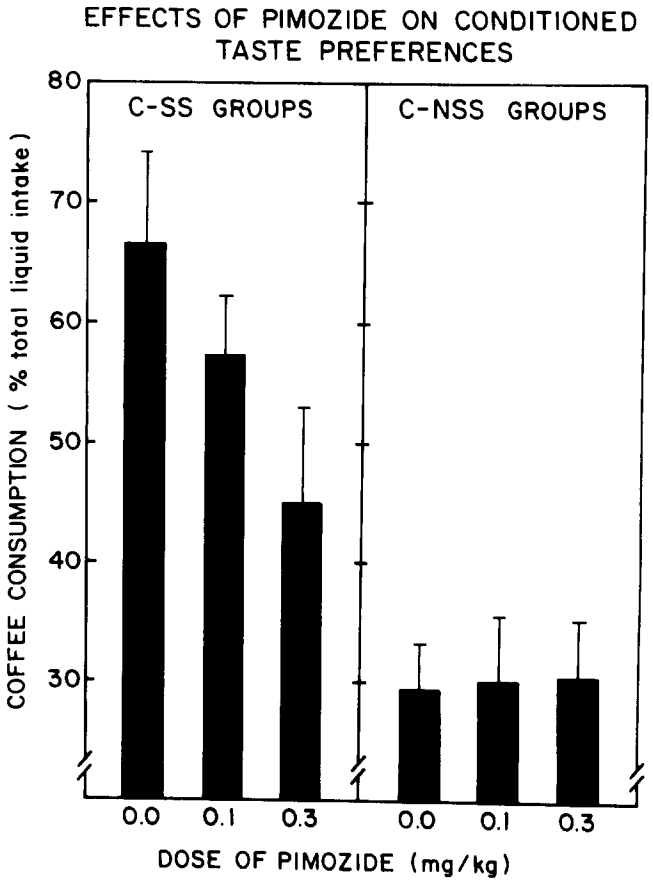

FIG. 1. Effects of the dopamine receptor antagonist pimozide on conditioned taste preferences induced by BSR. Coffee preference was reduced in a dose-dependent manner by pimozide in the coffee/self-stimulation (C-SS) paired groups. The drug had no effect in the no stimulation (C-NSS) groups. There were eight rats in each group.

Histology. Following the preference test the rats with electrodes were killed with an overdose of chloral hydrate. The animals were perfused with physiological saline followed by 10% Formalin. The locations of the electrode tips were subsequently determined from 40μ thionin-stained frozen sections.

RESULTS

The electrodes were located in the area of the lateral hypothalamus slightly dorsolateral to the fornix. The results of the 20 min preference test are illustrated in Fig. 1. A two factor analysis of variance, with self-stimulation vs no-self-stimulation as one factor and the three doses of pimozide as the other factor, was computed on the arc-sin transformed data. The animals that experienced pairings of coffee and self-stimulation drank a significantly greater proportion of their intake from the coffee tube than the animals that drank coffee without self-stimulation, $F(1,42)=8.41$, $p<0.01$. This

TABLE 1

MEAN (±S.E.M.) TOTAL LIQUID INTAKE (COFFEE + WATER) OF EACH GROUP DURING THE 20 MIN PREFERENCE TEST (ml)

	Dose of Pimozide (mg/kg)		
	0.0	0.1	0.3
Coffee/BSR	14.3 (±2.1)	16.1 (±1.9)	13.1 (±1.6)
Coffee/no BSR	15.8 (±2.6)	13.8 (±2.4)	12.7 (±3.1)

TABLE 2

MEAN COFFEE CONSUMPTION (±S.E.M.) OF EACH GROUP DURING THE 10 MIN PORTION OF THE TASTE/BSR PAIRING (ml)

	Dose of Pimozide (mg/kg)		
	0.0	0.1	0.3
Coffee/BSR	9.7 (±1.4)	10.1 (±1.9)	8.1 (±2.0)
Coffee/no BSR	10.1 (±1.8)	8.9 (±2.3)	7.8 (±2.1)

is a replication of our previous demonstration of the CTP [14]. The analysis also revealed a reliable effect of pimozide on coffee consumption, $F(2,42)=3.68$, $p<0.05$, reflecting the dose-dependent decrease in coffee intake observed in the C-SS groups. The significant interaction between the self-stimulation and drug variables, $F(2,42)=6.70$, $p<0.01$, confirms the impression that the drug affected the size of the CTP in the self-stimulating rats in a dose dependent manner, while having no effect on the coffee intake of the no-self-stimulation rats.

These differences in coffee consumption cannot be explained by differences in the amounts of liquid drunk by the rats during the preference test (Table 1). The differences in consumption of coffee during the 10 min drinking portion of the pairing were also small, indicating that Pimozide did not reliably attenuate drinking in 48 hr water-deprived rats (Table 2).

One hypothesis that can account for the reduction in the size of the CTP associated with pimozide is that, by blocking DA neurotransmission, the drug may reduce the reward value of each train of stimulation that the animal receives. Pairing stimulation of reduced reward value with coffee would reduce the size of the CTP. However, pimozide produced a dose-dependent decrease in the mean number of trains of self-stimulation obtained by the rats in the three groups (0.0 mg/kg=787.6 responses; 0.1 mg/kg=698.4 responses; 0.3 mg/kg=242.5 responses; $F(2,21)=5.85$, $p<0.01$). Therefore, rather than reducing the reward value of the stimulation, the drug may have interferred with responding itself, reducing the number of trains of stimulation received by the rats in the drug groups. Pairing a reduced number of trains of normally rewarding self-stimulation with coffee can also account for the reduced size of the CTP.

In Experiment 2 we tested the hypothesis that the size of the CTP that results from pairing a normal taste with self-stimulation varies as a function of the number of trains of stimulation.

EXPERIMENT 2

METHODS

Subjects

Forty rats similar to those used in Experiment 1 served as subjects. The rats were housed, fed, watered and handled as previously described.

Surgery

Thirty-two rats were implanted with stimulating electrodes as in Experiment 1. The eight remaining rats were anaesthetized but did not undergo surgery.

Procedure

All procedural details (e.g. test apparatus, recovery from surgery, self-stimulation training) were identical to those described in Experiment 1. Each implanted rat drank coffee for 10 mins followed by a session of self-stimulation. For eight rats the ICSS consisted of lever-pressing for 150 500 msec trains of 60 Hz sine wave stimulation. Three additional groups, of eight rats each, obtained 300, 500 or 1000 trains of stimulation. The eight unimplanted rats remained in the SS chambers for 15 mins after their 10 mins of coffee consumption. Following the pairing procedure each rat was returned to its home cage with no liquids available, and returned to its ICSS chamber 2 hrs later where a 20-min coffee/water preference test was conducted as described in Experiment 1.

Following the experiment the electrode placements were confirmed using standard histological techniques.

RESULTS

The results of the histological analysis confirmed that the electrodes were located in the area of the lateral hypothalamus dorsolateral to the fornix as in Experiment 1. The mean coffee consumption (expressed as a percentage of total liquid intake) is presented for each group in Fig. 2. The coffee/no stimulation group did not show a CTP, consuming only 31% of its total liquid intake from the coffee tube during the preference test. The four coffee/SS groups all showed CTPs, consuming between 58% and 73% of their total liquid intake from the coffee tube. Varying the number of trains of stimulation did not have a consistent effect on CTP size. An analysis of variance on the arc-sine transformed data for the four experimental groups revealed no significant differences in their coffee consumption, $F(3,28)=0.96$, $p>0.05$. There were no consistent differences among the groups in the total amounts of liquid consumed either during the preference test. This experiment shows that, within the range tested, CTP size is not a function of the number of trains of self-stimulation that are paired with the novel taste. This finding is in line with the hypothesis that the reduction in CTP size associated with pimozide in Experiment 1 was caused by a reduction in the reward value of the stimulation, rather than by a simple reduction in the number of trains of stimulation the rats received.

GENERAL DISCUSSION

Pairing a novel tasting substance with a session of self-stimulation resulted in conditioned preferences for the novel taste. Pimozide-induced dopamine receptor antagonism attenuated the size of these CTPs in a dose-dependent manner, and this attenuation was not a result of the simple reduction

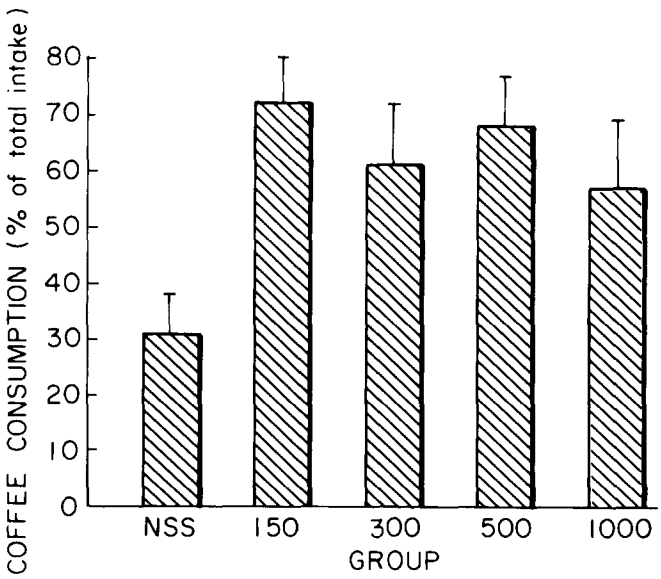

FIG. 2. Mean coffee consumption of each group during a single 20 min coffee vs water preference test. NSS=coffee/no ICSS group. The remaining groups are designated by the number of 0.5 sec trains of rewarding brain stimulation each group obtained.

in the number of trains of stimulation received by the rats in the drug groups. Although these results are consistent with the notion of a central dopamine involvement in the neural mediation of BSR, a number of other explanations must be considered.

Pimozide, may have aversive effects. The decrease in the size of the CTPs might have been a result of the algebraic sum of the reward value of the stimulation and the aversion of the pimozide. However if pimozide had such aversive effects a conditioned taste aversion (CTA) [20, 26, 27] should have been observed in the two drug-treated no-stimulation (C-NSS) groups. However, there is clearly no evidence of such a CTA in the present experiment even though the rats were tested in a two bottle situation and drank 30 percent of their total intake from the coffee tube. It seems unlikely, therefore, that the reduction in CTP size can easily be explained by a simple drug-aversion hypothesis.

Another possible explanation for the present results, is that pimozide blocks the animal's ability to form associations among various stimuli (e.g., taste and reward in the present experiment) and thereby reduced the CTP size. This argument suggests that the brain stimulation is still rewarding but that as the dose of pimozide was increased an increasing number of animals failed to make the association between the BSR and the coffee. As a result the mean coffee consumption during the two bottle test was reduced. However, a series of papers by Beninger and his associates [3–5] appears to demonstrate that dopamine receptor antagonism does not block the learning of associations between pairs of stimuli [3–5]. Other investigators have similarly reported that neuroleptics do not block classical conditioning [23], the acquisition of defensive burying of a metal prod previously paired with electric shock [3], latent learning [1], or short term memory [2]. In view of this evidence it seems unlikely that pimozide prevented the rats in the present study from associating the novel taste and the rewarding properties of the brain stimulation.

Perhaps the most reasonable explanation for the present results is that pimozide at these low doses reduced the rewarding properties of the self-stimulation. This conclusion is consistent with numerous papers implicating dopamine in the mediation of BSR [6, 13, 18, 30]. This does not, however, exclude the possibility that pimozide also alters the performance capabilities of treated animals. Once again there is an equally impressive literature demonstrating just that point [4, 9, 15, 24, 25, 27]. It has been shown that lever-press rates, unlike CTPs [8], are particularly susceptible to disruption by response-debilitating treatments [8, 21, 22]. It is, therefore, conceivable that pimozide's effect on response rates is at least in part due to the drug's performance-altering properties. This view might be supported by the poor correlation between response rate and CTP size ($r=0.13$, $p>0.05$). Another way of accounting for both the performance and reward deficits observed during dopamine receptor blockade involves the suggestion that the performance of the response may contribute to the rewarding properties of intracranial stimulation (e.g., [12]). In this sense the response debilitation may itself produce some of the reward reductions. This interpretation is based upon the notion that reward and responding are in fact two aspects of the same behavioral function mediated by dopamine neurotransmission.

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