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Rewarding and Aversive Effects of Stimulant Drugs in Infant Rats[1]

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SMITH, C. A. AND E. W. HOLMAN. *Rewarding and aversive effects of stimulant drugs in infant rats.* PHARMACOL BIOCHEM BEHAV **26**(2) 211–215, 1987.—Four experiments investigated the reinforcing effects of cocaine, amphetamine, and phenylpropanolamine using odor as the conditioned stimulus and infant rats as the subjects. At the age of 2 days the rats were injected with saline or with one of the three drugs and placed for 30 min in a plastic bag with almond-scented shavings. At the age of 18 days the rats were deprived of water for 12 hr and then given two tests that required different responses. In the consumption test they were given 30 min access to two water spouts, one surrounded by almond-scented cotton and the other surrounded by plain cotton. In the place test, they spent 5 min in a shuttle box with almond-scented shavings under one side and plain shavings under the other side. Compared to saline, all three drugs decreased water intake from the almond-scented spout and increased time spent over the almond-scented shavings.

Conditioned place preference Conditioned flavor aversion Reinforcement Odor Amphetamine Cocaine Phenylpropanolamine Infant rats

MANY drugs can induce a conditioned flavor aversion, including those that support self-injection or a conditioned place preference. Drugs that have been shown to produce both effects simultaneously in the same rats include amphetamine [14, 17, 26], morphine [16,24], and apomorphine [22,26]. Such effects are paradoxical in the sense that the same drug treatment acts as either a positive or a negative reinforcer, depending upon the stimuli associated with the drug and the responses tested. So far in the research on these drugs, the positive effects have been shown to external stimuli with motor responses and the negative effects to flavor stimuli with ingestive responses.

Several experiments have manipulated various procedural parameters, including time between drug injection and stimulus presentation [14, 16, 17], and position preference, baseline intake, and dose level [20]. None of the investigated variables proved to account for the paradoxical drug effect. There is, however, evidence for an anatomical separation between the rewarding and aversive effects of both amphetamine [25] and apomorphine [22].

The present research approached paradoxical drug effects from three different directions. The first approach was behavioral. In previous experiments, the stimuli (external or flavor) associated with the drug have been confounded with the responses (motor or ingestive) observed in the test. The present experiments held the stimulus constant and varied the response. An odor stimulus was used, since odors can control both motor and ingestive responses in rats.

The second approach was developmental. There is evidence that learning about different reinforcers appears at different stages of maturation. For example, rats can learn to avoid an odor paired with electric shock at 2 days of age if the shock is delivered intraperitoneally but not before 10 days of age if the shock is delivered peripherally [7]. Studies of drug reinforcement have shown that lithium chloride can produce a flavor aversion when delivered 2 days prenatally [19] and an odor aversion at 2 days postnatally [15]; amphetamine can produce a flavor aversion at 18 days [9], the earliest age investigated with this drug. Although rats can learn a preference for an odor paired with milk at the age of 1 to 3 days [10,11], there are no reports on rewarding effects of drugs in neonatal rats. The present experiments therefore investigated whether the positive as well as the negative reinforcing effects of drugs can be conditioned at similarly early ages.

The third approach was pharmacological. The drugs used in reinforcement studies have other effects besides reinforcement. Amphetamine, for instance, has stimulant and anorectic properties, which have been demonstrated in infant as well as adult rats [2,13]. To explore possible relationships between reinforcement and other drug effects, the present experiments used not only amphetamine, but also two other drugs, cocaine and phenylpropanolamine (PPA), that differ widely in the relative prominence of their stimulant and anorectic effects.

Griffiths, Brady and Snell [5] directly compared the

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anorectic and rewarding properties of cocaine and eight phenylethylamines, including amphetamine and PPA, in baboons. PPA reduced food intake but did not support self-injection at any dose tested; cocaine had the greatest rewarding effect relative to its anorectic effect of all the drugs; and amphetamine fell between these extremes. In rats, PPA has been shown to reduce food intake and (at higher doses) to increase locomotor activity [8]; there is one report of a flavor aversion with PPA [23] but no reports of positive reinforcement. Cocaine is known to increase motor activity and stereotypy and also to produce a slight decrease in food intake at high doses [21]; strong positive reinforcing effects of cocaine have been shown using both self-injection [12] and place preference [18]; flavor aversions have also been demonstrated [1,4], although they were relatively weak and required several trials to establish. In summary, the three drugs can be arranged on a continuum according to their effects, with anorexia, flavor aversion, and PPA toward one end, psychomotor stimulation, positive reinforcement, and cocaine toward the other end, and amphetamine toward the middle.

The present research included separate experiments with cocaine, amphetamine, and PPA. Each experiment used four groups of rats: low, medium, and high dose, and saline control. In order to associate a drug state with an odor cue, 2-day-old rats were injected with the drug (or saline) and placed in a bag containing wood shavings treated with almont scent for 30 min. Control injections were performed on the next day, with no further drug-odor pairings. At the age of 18 days, the rats were tested for preference or aversion to the odor in two tests: place preference, with a procedure similar to that of Rudy and Cheatle [15]; and water consumption, with a procedure similar to that of Hankins, Garcia and Rusiniak [6]. An identical regimen was used for each of the three drugs.

In a fourth, no drug, experiment, the rats were trained and tested in exactly the same way as the previously described animals, except that both the Day 2 and Day 3 injections were of saline, and the animals were not exposed to the almond scent during training. This was an additional control for both drug and odor effects.

METHOD

Subjects

The subjects were male and female albino rats bred in the lab from Sprague-Dawley stock originally purchased from Simonsen Laboratories Inc. (Gilroy, CA). At the time of the initial training the rats were 2 days of age and weighed about 8 g each. The litters were not culled, except that 2 pups weighing less than 6 g were not used; litter size ranged from 5 to 14 pups. A total of 234 rat pups were used in the four experiments.

Drugs and Doses

The medium doses of amphetamine and cocaine were chosen from the literature as approximately average doses in rat experiments. For PPA, however, adult doses proved to cause excessive mortality in infant rats; therefore, the medium dose chosen was approximately one-tenth the average adult dose. The high doses were determined by going up about half a log unit from the medium doses, and likewise the low doses were determined by going down about half a log unit from the medium doses. Table 1 presents the doses used for each drug.

TABLE 1

DRUG DOSAGE (mg/kg) FOR EACH GROUP

	Drug		
Dose	Cocaine	Amphet	PPA
Low	3.16	0.44	0.15
Medium	10.00	1.40	0.50
High	31.60	4.44	1.50

TABLE 2

NUMBER OF ANIMALS INJECTED (AND DEATHS) IN EACH GROUP

	Treatment			
Dose	No Drug	Cocaine	Amphet	PPA
Saline	24 (0)	16 (2)	18 (3)	20 (3)
Low		18 (1)	14 (3)	18 (2)
Medium		16 (1)	17 (2)	17 (5)
High		15 (2)	16 (4)	25 (8)

TABLE 3

MEAN WEIGHTS (g) ON TEST DAY FOR EACH GROUP

	Treatment			
Dose	No Drug	Cocaine	Amphet	PPA
Saline	32.6	29.0	27.7	25.4
Low		28.6	27.5	26.0
Medium		29.2	27.2	24.4
High		28.6	24.6	23.3

All compounds used were in HCl salt form and were dissolved in isotonic saline, which was also used for the control injections. The injections were administered intraperitoneally, in a volume equal to 2% of body weight, using a 0.25 ml glass syringe and 27 gauge disposable needles.

Apparatus

The rat pups were exposed to the drug-odor pairing by placing them, after drug injection, in a 35×36×24 cm polyurethane bag that contained 500 ml of pine shavings scented with 2.5 ml of Schilling almond extract.

The rat pups were tested for place preference in a 30×20×10 cm Plexiglas compartment with a fine mesh screen floor overlying a slightly larger pan that contained 500 ml of shavings with 2.5 ml of almond extract on one side and 500 ml of shavings with 2.5 ml of water on the other side. The experimenter observed the animals' movements and recorded them by pressing switches that controlled a timer and an event recorder.

The rat pups were tested for water consumption with a two bottle choice test in standard individual rat cages, using two 25 ml glass micropipettes with 0.1 ml divisions. The glass tubes were wired to the front of the cages approx-

imately 8 cm apart. Each tube was fitted with a stainless steel drinking spout surrounded by a plastic cone. Each cone had a small wad of cotton that had been injected with 0.50 ml of almond extract for one tube or 0.50 ml of water for the other. The cotton was inserted into the cone in such a way that the animal was able to sniff the cotton when it approached the spout, but was unable to chew at it.

Procedure

The rats were maintained on a 12 hr light/dark cycle with constant room temperature at 24°C. Each litter was housed in an individual cage with its mother. The pups had continuous access to the lactating mother, except during the training or testing periods.

When the pups were 2 days of age (Day of birth=Day 0), the mother was removed from the home cage and placed in a holding cage. The pups were then weighed and marked with either a red, black, blue or green felt tip pen. Each treatment (low, medium, high drug dose, or saline) was assigned to a color. The experimenter was blind to the color-treatment code until the end of each experiment. After color coding, each pup was injected with the assigned solution. The entire litter was then placed in the plastic bag containing the almond-scented shavings for a period of 30 min. At the end of this training trial, the pups were cleaned of any almond-scented particles and placed back into the home cage with the mother. On the next day the pups were again removed from the home cage and injected with saline, except for the group that had saline the day before. This group was injected with the medium dose of drug, in order to control for any nonspecific developmental effects the drug might produce. The animals were then returned to the home cage. Thus, all animals were injected with the drug, but the saline group had no drug-odor pairing.

The training procedure in the no drug experiment was the same except that the color code was not used, both injections were of saline, and the shavings were unscented.

When the pups were 14 days of age, they were placed on a 4-day regimen in which water was continuously available in two accessible tubes. Dry food was also continuously available, but the mother was removed from the cage each morning and returned each evening. On the fourth evening the mother was left out of the cage, and the water bottles were removed. This training procedure ensured that the pups were able to drink out of the water tubes, and that they were sufficiently thirsty, but not dehydrated, for the testing on the morning of the next day.

When the pups were 18 days of age, the litter was placed in a holding cage. The pups were individually weighed and then tested for place preference and water consumption. Half of the animals in each drug group received the place preference test before the water consumption test, and the other half received the water consumption test before the place preference test. As no differences were noted, the two subgroups were combined in the analysis.

For the place test, the pups were placed, one at a time, in the center of the Plexiglas testing compartment for a period of 5 min. The amount of time spent over the almond-scented shavings was recorded in 0.2 sec intervals. The number of crossings from side to side, and the number of rears on each side, were also recorded.

For the consumption test, the pups were placed, one at a time, in a testing cage fitted with two graduated drinking tubes with plastic cones. After 30 min the animals were removed, and the amount of water consumed from each tube was measured.

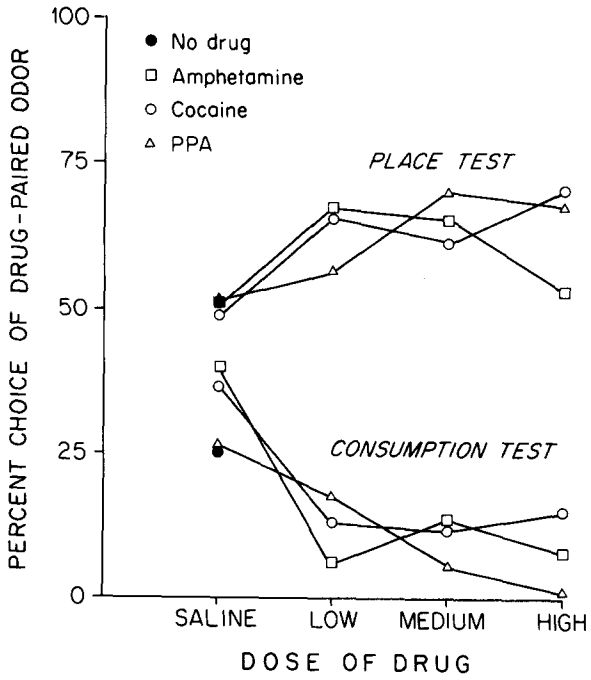

FIG. 1. Performance in tests as function of drug and dosage in training. Upper curves are mean percent of total time spent on almond-scented side in place tests. Lower curves are mean percent of total water intake from almond-scented tube in consumption tests.

All statistical tests were one-way analyses of variance with group as the independent variable and $p<0.05$ as the rejection criterion.

RESULTS

Unconditioned Effects

Table 2 presents, for each group, the total number of rats that were injected and (in parentheses) the number of rats that died between the time of injection and the day of testing. All the surviving rats were tested. It should be noted that except in the no drug experiment, the saline groups received a medium dose of a drug on Day 3.

The deaths were probably due to the anorectic properties of the drugs. Although the litters were not culled for size, no animal under 6 g was injected on Day 2, and no deaths were observed within 2 days of the injection. The animals that were found dead in the cages (during daily inspection of the animals) were without exception smaller than their littermates, and many showed signs of malnutrition (e.g., heads larger than bodies, prominent rib cages).

Because of the anorectic properties of the three drugs, it was necessary to record the weights of the animals on the day of testing. On this day all the rats were 18 days of age and had all been partially food and water deprived for 4 days according to the procedure previously described. Table 3 presents the mean weight for each group.

The mortality rate provided a good predictor of the weight data obtained on Day 18. The no drug group of rats, which had gone through the same injection and deprivation procedures as the experimental rats except that they did not receive a drug, had no deaths and the highest mean weight of

all the groups. Among the drug groups, the fewest deaths and highest weights were in the cocaine groups, the amphetamine groups were next, and finally the most deaths and lowest weights were in the PPA groups. The weights were consistent with the death rates and demonstrate the relative anorectic properties of the three drugs.

Consumption Test

As a measure of preference in the consumption test, the amount of water consumed by each rat from the scented tube was divided by the total amount consumed by that rat from both tubes. The lower part of Fig. 1 shows the mean of these percentages for each group.

All the groups drank less from the scented tube than from the unscented tube. There were only slight differences between the no drug control group, which had not been exposed to the odor at all, and the saline control groups from the three drug experiments. These differences were not significant, $F(3,66)=1.70$. Each of the control groups limited their intake from the scented tube to about one-third of their total intake, indicating an unconditioned aversion or neophobia to the almond odor.

Nevertheless, the mean percentage intake from the scented tube was higher in all the control groups than in any of the groups for which the odor had been paired with a drug. There were significant differences between groups for each drug: cocaine, $F(3,55)=3.25$; amphetamine, $F(3,49)=6.01$; PPA, $F(3,58)=11.25$. Thus, each drug caused a conditioned aversion to the odor in the consumption test.

To test for nonspecific effects on intake, the total consumption from both tubes was also compared across groups for each drug. There were no significant differences: $F<1$ for each drug.

Place Test

As a measure of preference, the upper part of Fig. 1 shows the mean percentage of total time spent on the scented side of the chamber by each group in the place test.

The no drug and saline groups spent approximately half of their time on the scented side of the chamber, indicating no preference. The differences between the control groups were not significant, $F<1$.

On the other hand, there was a preference for the scented side over the nonscented side by all the groups for which the odor had been paired with a drug. The differences between groups were again significant for each drug: cocaine, $F(3,55)=4.39$; amphetamine, $F(3,49)=5.27$; PPA, $F(3,58)=9.83$. Thus, each drug caused a conditioned preference for the odor in the place test.

The high dose of amphetamine resulted in a bimodal distribution with about one-third of the animals preferring the unscented side, which accounts for the relatively low mean score in that group. Apparently the dose was high enough to be aversive to some animals. All the other groups showed unimodal distributions.

While the animal was being timed as to which side it was on during the place test, the number of times the animal reared on either side was also being recorded, as was the number of times the animal crossed the midline from one side to another. These data proved to be quite variable, but the following trends were noted.

The rate of rearing on each side was calculated by dividing the number of rears on that side by the time spent on that side. In all groups, the mean rate of rearing was higher on the unscented side than on the scented side. Also, for each drug, the difference between the sides was less in the saline group than in any of the drug groups. These data are consistent with the observation that the animals explored with their noses close to the grating on the scented side, whereas on the unscented side, they spent more time on their hind legs sniffing the air. Thus, the observed differences in rearing may reflect a tendency of the odor to elicit investigation, particularly in the drug groups.

The number of crossings tended to decrease with dose, although the ordering was not perfect for any of the drugs. The implication is that the saline (and sometimes the low dose) groups explored more actively and ran from side to side, whereas the other groups lingered on one side, usually the scented side according to the preference data.

DISCUSSION

There are a number of alternative explanations that have to be considered when discussing these results. First, nonassociative effects can be discounted because of the design of the experiments. For each drug, the saline control group was treated the same as the medium dose group except that the drug was not paired with the odor. Nonassociative effects should therefore be the same in these groups, yet performance in the tests were different. Conversely, the three saline groups were similar in performance to each other and to the no drug group. This similarity also indicates that drugs not paired with the odor had no effect on performance in the tests.

Another possible artifact to be considered is that the test results were not a reflection of a choice in themselves, but rather a secondary effect of other responses (such as activity, escape, etc.). Additional behavioral measures (rears and crossings) were recorded in order to evaluate this possibility in the place tests. As already described, the results for rears and crossings are more easily explained as secondary effects of preference than the other way around. Although no additional measures were recorded in the consumption tests, the absence of differences in total intake provides indirect evidence against a substantial role for competing responses.

One further possibility to be considered is the concept of sensitization. Perhaps the conditioning procedure just enhanced the unconditioned response to the odor in the drinking test, because the control groups avoided the odor, and the degree of aversion was significantly greater in the drug groups than in the control groups. This argument would not apply to the place test, however, because the control groups evenly divided their time between the two sides.

In conclusion, it seems clear that the opposite effects obtained for the drinking and locomotion tests in response to a single stimulus were conditioned by the reinforcing properties of the drugs used in these experiments. The results thus confirm the paradox reported in the literature that the same drug treatment can have both rewarding and aversive properties. All three drugs used in this experiment, amphetamine, cocaine, and PPA, produced locomotor preference and ingestive aversion.

The present findings extend previous results in three ways. First, whether the conditioned effect of a drug was rewarding or aversive depended upon the response required in the test, with the stimulus held constant. Historically, the procedure for experiments of this nature has been to condition two stimuli that control different responses. Thus, the response requirement would vary according to which

stimulus was presented. With the present procedure of a single conditioned stimlus the drug paradox is localized to the response rather than the stimulus.

Second, both effects could be conditioned in a single trial in 2-day-old rats. This is the earliest age for reinforcement demonstrated by all three drugs. There was no evidence for developmental differences between positive and negative reinforcement. It is important to note that although the learning was at 2 days of age, the consequent performance occurred 16 days later. Despite the immaturity of the nervous system at the time of the initial drug-odor pairing, a memory of that pairing must have persisted until the next exposure to the odor, when the response, either locomotor or consummatory, was required.

Third, the effects occurred for each of three drugs that differed widely in the relative prominence of their stimulant and anorectic properties. Although it has previously been reported that a flavor aversion can be obtained with cocaine, and reports of rewarding effects of cocaine are legion, this series of experiments includes the first demonstration of a paradoxical effect for cocaine. Moreover, these experiments are not only the first to demonstrate a paradoxical effect for PPA, but also serve as the first demonstration of positive reinforcement for PPA. The similarity in the reinforcing effects of these different drugs suggests that positive and negative reinforcement may be neurochemically similar despite their anatomical and behavioral differences.

The results of the present experiments can be tentatively described in terms of the "gating" concept suggested by Garcia, Lasiter, Bermudez-Rattoni and Deems [3]. On the training trial, the animal senses an odor in combination with the various effects of the drug in many neural systems. The odor becomes associated with these effects. Later, when the animal is exposed to the odor in the presence of the drinking tubes, the odor is gated into the feeding system and aversion results. On the other hand, the odor in the shuttlebox is gated into the locomotor system where it elicits approach.

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