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Rapid Acquisition of a Two-Drug Discrimination: Time of Day Effect Upon Saline State

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SCHECHTER, M. D. *Rapid acquisition of a two-drug discrimination: Time of day effect upon saline state.* PHARMAC. BIOCHEM. BEHAV. 14(3) 269–271, 1981.—Rats were rapidly trained to discriminate between 0.8 mg/kd *d*-amphetamine and 6 mg/kg pentobarbital in a two-lever food-motivated operant task by imposing the drug states from the earliest stage of training, i.e., at the initiation of shaping to lever-press. Once trained, rats were administered each of the training drugs and were allowed to lever press without reinforcement until 10 responses were made on the lever that was not their first choice lever selection. By employing this extended schedule of responding in extinction, the amphetamine-induced interoceptive cue was observed to produce equivalent perseverance as that produced by pentobarbital. However, the administration of saline, the non-drugged state, produced significantly more pentobarbital-appropriate responding than amphetamine-appropriate responding when tested during the daytime, whereas it produced random responding when tested during the night. The results suggest that the arousal state of the rat, a nocturnal species, may differentially influence saline tests in the daytime and in the night time.

Drug-induced stimuli Amphetamine Pentobarbital Extended schedule of responding

THE production of discriminative stimuli by drug administration is a behavioral technique that has been extensively used as evidenced by the recent publication of several books on this subject [2, 4, 5]. Most research in this area has involved the training of centrally-active drugs vs. the non-drugged state, imposed by the administration of saline. However, there have been few drug vs. drug training studies [3, 8, 10] and even fewer drug vs. drug vs. saline experiments [6,7]. In one of these studies [10] drug vs. drug discrimination was shown to be more readily learned than either of the drug vs. saline discriminations.

Many techniques have been used to train rats to discriminate between drug and saline conditions. A recent report [9] indicated that employing a fixed-ratio 10 (FR10) schedule and reinforcing on the lever that was appropriate to the drug or non-drug state condition from the very earliest stage of training produced the fastest acquisition of drug-state discrimination. The present study employed this technique to train rats to discriminate between 0.8 mg/kg *d*-amphetamine and 6 mg/kg pentobarbital. In addition, a recently reported [11] modification, viz., extended schedule performance (as described in detail, below), was employed to investigate the perseverance of the rats to each drug state in order to indicate which drug, at the training dose used, produced a stronger discriminative stimulus.

METHOD

Sixteen experimentally-naive, ARS/Sprague-Dawley rats, weighing 220±10 g at the beginning of experimentation, were used. The drug discrimination procedure employed has been described in detail elsewhere [1]. In brief, materials consisted of standard animal test cages fitted with 2 levers and a food cup and programmed by solid-state logic modules located in an adjacent room. Rats were maintained, on a natural day-night cycle, at approximately 85% of their free feeding weights as ascertained by daily weighing of a free feeding rat obtained from the supplier (Zivic-Miller, Allison Park, PA) at the same time. Eight rats were administered 0.8 mg/kg *d*-amphetamine sulfate (as base) intraperitoneally (IP) and, 30 min later, were shaped to press the left lever to receive food reinforcement (45 mg Noyes pellet) under a continuous reinforcement schedule (FR1), whereas the other half of the rats were administered 6 mg/kg sodium pentobarbital (as base; IP) and, 30 min later, were trained to press the same lever under the FR1 schedule. The FR schedule was increased as training continued until the rats were pressing the drug-appropriate lever under a FR10 schedule. The number of consecutive, daily sessions conducted to reach FR10 responding was kept constant (13 sessions). Once the 2 groups of rats were observed to press the first lever under the FR10 schedule, as indicated by delivery of a minimum of 50 pellets (500 responses), they were injected with the other drug, i.e., the amphetamine-trained rats received pentobarbital and the pentobarbital-trained rats received amphetamine, and were required to press the opposite lever under a FR1 schedule. Training continued in daily 15 min sessions until the second lever was pressed under a FR10 schedule and the number of sessions to the second lever FR10 criterion was kept constant (9 sessions) for all subjects.

Once consistent FR10 responding was observed to occur on the second lever, each group of rats received either amphetamine (A) or pentobarbital (P) on a 2 week alternating

TABLE 1

EXTENDED SCHEDULE PERSEVERANCE AFTER 6 mg/kg PENTOBARBITAL, 0.8 mg/kg AMPHETAMINE AND SALINE ADMINISTRATIONS AT 1400–1500 HOURS

Group	Treatment	Mean Responses on PL Prior to 10 Presses on AL	Mean Responses on AL Prior to 10 Presses on PL
A-P*	Pentobarbital	218.7	0.4
	Amphetamine	0.3	200.4
	Saline	71.4	3.2
P-A†	Pentobarbital	181.5	0.9
	Amphetamine	0.3	198.3
	Saline	32.0	8.4
Combined	Pentobarbital	200.8	0.6
	Amphetamine	0.3	199.3
	Saline	51.8	5.8

*Eight rats initially shaped to lever press with 0.8 mg/kg *d*-amphetamine and then shaped on opposite lever with 6 mg/kg pentobarbital.

†Eight rats initially shaped with pentobarbital; then with *d*-amphetamine.

TABLE 2

LEVER SELECTION AND PERSEVERANCE OF AMPHETAMINE-PENTOBARBITAL TRAINED RATS AFTER SALINE ADMINISTRATION AT 1400–1500 AND 0200–0300 HOURS

	A-P Group	P-A Group	Combined
1400–1500 hour			
PL Selection (%)	87.5	75.0	81.3
Mean Perseverance on PL	71.4	32.0	51.8
Mean Perseverance on AL	3.2	8.4	5.8
0200–0300 hour			
PL Selection (%)	56.2	50.0	53.1
Mean Perseverance on PL	10.8	12.4	11.6
Mean Perseverance on AL	19.6	12.3	15.9

schedule: P-A-A-P-P; A-P-P-A-A. The lever first pressed 10 times was designated as the "selected lever" and training on the drug-appropriate lever continued for 15 min. After a 2 week period, 15 min maintenance training sessions, with either 0.8 mg/kg *d*-amphetamine or 6 mg/kg pentobarbital, were continued on Mondays, Wednesdays, and Fridays. On Tuesdays and Thursdays, each group of rats received either saline, the training dose of pentobarbital, or the training dose of amphetamine on 2 occasions each. During these sessions, subjects were allowed to lever press, in extinction, until 10 responses were made on the lever that was *not* their first choice lever selection (extended schedule responding; [11]). Thus, if a rat was administered amphetamine and it pressed the amphetamine-correct lever 10 times, that rat was allowed to continue pressing (without reinforcement) until 10 presses were made on the pentobarbital-correct lever. The number of lever presses made on the amphetamine-correct lever before 10 presses were accumulated on the pentobarbital-correct lever was recorded. Likewise, after pentobarbital administration the rats were allowed to press, in extinction, until 10 presses were made on the amphetamine-correct lever. After saline administration, the number of presses on each lever before 10 presses on the opposite lever were made was recorded. Whereas the amphetamine and pentobarbital training doses were administered and tested in extended schedule sessions between 1400–1500 hours, saline was similarly tested on 2 sessions each at 1400–1500 hours and at 0200–0300 hours. The experimenter (technician) had no knowledge of the substance administered in any of the extended schedule sessions, and all administrations were made at a constant volume of 1 ml/kg body weight. Each extended schedule session was preceded by one pentobarbital and one amphetamine maintenance session.

RESULTS

Rats trained to discriminate between amphetamine and pentobarbital by administering these drugs from the earliest stage of conditioning selected the appropriate lever on a minimum of 9 of the first 10 training sessions. Having reached criterion performance in only one 2-week alternating schedule of administration, these well-trained rats were exposed to the extended schedule to test discriminative perseverance.

Table 1 indicates the results of testing the 2 groups of rats, i.e., those first shaped with amphetamine and then with pentobarbital (A-P) and those initially shaped with pentobarbital and then with amphetamine (P-A), with 6 mg/kg pentobarbital, 0.8 mg/kg *d*-amphetamine and saline in extended schedule sessions at 1400–1500 hours. After the administration of pentobarbital, the A-P group rats all selected the pentobarbital-appropriate lever (PL) first and continued pressing the PL for a mean of 218.7 responses before pressing the amphetamine lever (AL) 10 times. All the P-A group rats selected the PL first and the mean responses on the PL for the P-A group after pentobarbital was 181.5. After amphetamine administration, the A-P rats selected the AL first on all trials and continued pressing the AL for a mean of 200.4 responses before accumulating 10 presses on the PL, whereas the P-A group persisted on the AL for a mean of 198.3 responses. After saline administration, the A-P group of rats selected the PL on 14 of the 16 trials (87.5%) and persisted on this lever for a mean of 71.4 responses. The P-A group selected the PL first on 12 of 16 trials (75.0%) and persisted on this lever for a mean of 32.0 responses. When the groups were combined (n=16), mean perseverance on the PL after pentobarbital was 200.8 responses and, after amphetamine, mean perseverance on the AL was 199.3 responses. After saline, mean perseverance on the PL was 51.8 responses.

The results of administering saline during 2 sessions at 0200–0300 hr, as compared to administering saline at 1400–1500 hr, is shown in Table 2. Data for the 1400–1500 hr administrations indicate that response perseverance on the PL for the A-P rats was greater than that observed in the P-A group. When the groups are combined (n=16), the PL was

first pressed 10 times on 81.3% of the trials and the perseverance on the PL was greater than the perseverance on the AL. However, at 0200–0300 hours, the A-P rats selected the PL on 9 of 16 trials (56.2%) and their mean perseverance on the PL (10.8) was similar to their mean perseverance on the AL (19.6). The P-A rats selected the PL on 8 of 16 trials (50%) and their mean perseverance on that lever (12.4) was similar to that measured on the AL (12.3). When the groups are combined, the PL was selected first on 53.1% of trials and mean perseverance on the PL (11.6) was similar to that recorded on the AL (15.9).

DISCUSSION

The results indicate that arranging a FR10 schedule of reinforcement for the lever responses that are appropriate to each of the drug states imposed from the very earliest stage of training produces rapid acquisition of a drug vs. drug discrimination. Overton [9] reported that this technique produces rapid acquisition of a drug vs. saline discrimination and the present study extends these findings to the discriminative training of drug vs. drug. Although the literature on drug-induced discriminative stimuli contains a paucity of drug vs. drug studies, one such study [10] indicated that rats learn to discriminate between amphetamine and pentobarbital more rapidly than they learn to discriminate between either amphetamine and saline or pentobarbital and saline.

The administration of drugs and saline at 1400–1500 hr to rats trained to discriminate between 0.8 mg/kg *d*-amphetamine and 6 mg/kg pentobarbital produced interesting and, perhaps, surprising results. The extended schedule performance after amphetamine and pentobarbital indicated a similar perseverance on the appropriate lever, i.e., a mean of 199.3 responses on the AL after amphetamine and a mean of 200.8 responses on the PL after pentobarbital. This suggests that, when all 16 rats are considered, the training doses of the drugs employed produced what appears to be equivalent discriminative stimulus "strengths". The extended schedule performance technique [11] allows testing for the possible occurrence of drug transfer "overinclusiveness", a term that describes the suggestion that transfer tests reveal results indicating that drugs are similar when, in fact, they differ. In a transfer test in rats trained to discriminate drug from saline, the rat is asked to indicate which of the 2 trained conditions is most similar to the test drug state and since the rat must make one of 2 choices, the drug state (being a "stronger" state) is most often chosen. In the present design, the strength of the pentobarbital-induced interoceptive cue was shown to be essentially equivalent to that of the amphetamine-induced cue. However, when the animals first trained with amphetamine (A-P group) were compared to those first trained with pentobarbital (P-A group), the administration of saline produced greater perseverance on the PL in the former group and a greater perseverance on the AL in the latter group (Table 1) suggesting that the last trained drug is capable of influencing saline-induced (non-drug) interoceptive cueing to a greater degree than the first trained drug.

The observation that the 16 rats first selected, and persevered upon, the pentobarbital-lever to a greater degree than the amphetamine-lever after saline administration at 1400–1500 hr is in contradiction to previous reports. Richards [10] trained rats to discriminate between 1 mg/kg *d*-amphetamine and 15 mg/kg pentobarbital on different schedules of reinforcement (i.e., DRL-15 and VI 60) than that employed in the present study and observed that saline produced approximately random lever choices, whereas Duncan and Kao [3] trained rats with 0.8 mg/kg *d*-amphetamine and 10 mg/kg pentobarbital and observed that saline treatment produced responding more similar to amphetamine than to the barbiturate. The results of the present study indicate the possibility that rats perceive the non-drug state as more sedating (pentobarbital-like) than stimulating (amphetamine-like) when saline was tested at 1400–1500 hours.

The administration of saline at 0200–0300 hr produced equivalent selections and perseverance on the 2 levers. This suggests that, at that nighttime hour, rats are less "sedated" than at 1400–1500, a tenable conclusion when one considers that the rat is a nocturnal species in nature.

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