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Extended Schedule Transfer of Ethanol Discrimination

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SCHECHTER, M. D. *Extended schedule transfer of ethanol discrimination.* PHARMAC. BIOCHEM. BEHAV. 14(1) 23–25, 1981.—Stimulus control was established in rats with ethanol (600 mg/kg) and saline by employing a two-lever response choice task and an FR10 schedule of food reinforcement. Subjects were then tested with an extended schedule procedure in which lever selection and its perseverance were measured under the training conditions and after the administration of pentobarbital at doses of 2 to 12 mg/kg. With decreasing doses of pentobarbital, drug-lever selection was observed to decline. The dose at which initial lever selection was evenly distributed between the two levers (ED50) was determined to be 4 mg/kg. However, at this dose the perseverance on the ethanol-appropriate lever was not significantly different than that observed after the training dose of ethanol. In addition, the perseverance of saline-lever selection produced by saline was observed to be greater than that produced by the training dose of ethanol on the ethanol-lever. The advantages inherent in employing the extended schedule performance procedure in transfer experiments are discussed.

Extended schedule transfer Pentobarbital Ethanol Drug-induced stimulus control

DRUG discrimination research measures the ability of centrally-active drugs to exert discriminative stimulus control over specific behavioral responses. Various procedures for the training and testing of the discriminative stimulus effects produced by drugs have been employed [2, 10, 13] and the two-lever, food-motivated fixed-ratio schedule procedure has been most successful [3]. Once rats are trained to discriminate between the effects of the drug and saline (non-drug), other drugs can be administered to determine if the rats generalize (transfer) the effects of the training drug with the effects of the test drug. This transfer of discriminative effects has been tested in extinction, i.e., as the first lever pressed ten times (selected lever) in the absence of reinforcement [11] or by reinforcing selected lever responding for a fixed amount of time [4]. Drug transfer studies have allowed drugs to be classified as similar or dissimilar on the basis of their discriminative stimulus properties [1]. However, stimulus generalization of a test drug with a training drug may often be partial in that only some rats will generalize, whereas other rats will not. Since the rat has been trained to emit a series of 10 responses on one lever on the FR10 schedule, all responses generally continue to be made on the first-chosen lever. Thus, transfer experiments often yield intermediate results since only a quantal measure of the number of rats first selecting the drug-appropriate lever can be made, and this necessitates a large number of rats for precise, reliable results. All too often these intermediate results are unfortunately ignored [13].

The present study sought to employ a well-established drug generalization and measure the perseverance or strength of the transfer effect. Thus, the often-replicated ability of ethanol-trained rats to generalize to pentobarbital [8,9] was investigated in an attempt to observe how persistent the rats were in their selected lever choice.

METHOD

Subjects

The subjects were 6 experimentally-naive male ARS/Sprague-Dawley rats weighing 160 ± 10 g at the beginning of experimentation. They were housed in individual living cages and their weights were adjusted (by daily rationing of rat chow) to approximately 85 ± 5% of their free-feeding values as determined by daily weighing of a control free-feeding rat purchased from the supplier (Zivic-Miller, Allison Park, PA) at the same time. Water was continuously available.

Apparatus

The experimental space was a standard rodent Skinner test cage (Lafayette Instrument Co.) equipped with 2 operant manipulanda (levers) placed 7 cm apart and 7 cm above the grid floor. A food pellet receptacle was mounted 2 cm above the grid floor at an equal distance between the levers. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and house light. Solid-state programming equipment (LVB Corp.) was used to control and record the sessions and was located in an adjacent room.

Training Procedure

The procedure used to train rats to discriminate between ethanol and saline has been described in detail elsewhere [4]. Daily discrimination training started after initial shaping to lever-press on both levers on a FR10 schedule of food reinforcement. Fifteen minutes prior to placement into the test chamber, the rats were injected intraperitoneally (IP) with either 600 mg/kg ethanol (10% v/v solution in 0.9% saline) or an equal volume of saline. Depending on whether the rat was

TABLE 1

EXTENDED SCHEDULE TRANSFER OF ETHANOL DISCRIMINATION TO PENTOBARBITAL

Treatment	Dose (mg/kg)	EL Responses Prior to 10 Presses on SL (±SD)	No. EL Selections/ No. Trials Conducted	SL Responses Prior to 10 Presses on EL (±SD)
Ethanol	600	26.8 (11.2)	12/12	0
Saline	—	0	0/12	58.0 (30.5)
Pentobarbital	12	27.0 (17.2)	11/12	6.5 (3.1)
	10	22.7 (21.5)	9/12	7.8 (4.9)
	8	19.3 (8.2)	7/12	10.8 (10.4)
	6	19.9 (12.9)	7/12	7.4 (5.7)
	4	19.1 (14.8)	6/12	8.1 (3.5)
	2	5.6 (3.4)*	3/12	23.4 (12.9)

*Significant difference from EL responses prior to 10 presses on SL after 600 mg/kg ethanol (two-tailed t-test; $p<0.01$).

administered ethanol or saline, it obtained reinforcement by pressing either the "ethanol lever" (EL) or the "saline lever" (SL), respectively. After every 10th press (FR10) on the appropriate lever, a 45 mg Noyes pellet was delivered through the food receptacle. Responses on the incorrect lever (i.e., on the SL after ethanol administration or on the EL after saline administration) were recorded but produced no programmed consequence. To compensate for possible position preference, the lever assignments were EL left, SL right for half of the rats and EL right, SL left for the other half and these assignments remained constant throughout the experimentation. The number of responses made on either lever before the first food pellet (FFP) was obtained, and, thus before 10 responses were made on the correct lever, was recorded. The FFP, therefore, reflects the accuracy of the rats' lever selection and the number with which the FFP exceeds 10 equals the number of incorrect responses made before the first reinforcement.

Every week, each rat was run once a day, on 5 consecutive days, in a daily session of 15 min duration. Daily ethanol (E) and saline (S) injections were given according to a 2 week alternating sequences: E-S-S-E-E and S-E-E-S-S. The training criterion was reached when the FFP of the animals did not exceed 12 on 10 consecutive training sessions.

Extended Schedule Discrimination

Once all rats attained the training criterion, testing and training sessions of 15 min duration, with alternating administration of 600 mg/kg ethanol and saline, were continued on Mondays, Wednesdays and Fridays. This procedure endeavored to insure and maintain behavioral discrimination to the trained drug conditions and it was intended that if a rat was observed to fall outside the criterion of FFP≤12 on these maintenance sessions, the data on the rat's performance would be deleted from the results. This, however, did not occur.

On Tuesdays and Thursdays, the rats were injected IP with different doses of sodium pentobarbital (2–12 mg/kg in saline, as base) and, 15 min later, they were placed into the experimental chamber and were allowed to lever press (for a maximum of 15 min) in extinction until 10 responses were made on the lever that was not the first lever selected. Thus, for example, when a rat pressed the EL 10 times that lever was designated the "selected lever" and the rat was allowed to continue pressing, without reinforcement, until it accumulated 10 presses on the SL. The number of lever presses made on the EL prior to 10 presses on the SL was recorded. Likewise, if the SL was pressed 10 times first (the selected lever), the rat was allowed to continue pressing until 10 responses were made on the EL.

Each pentobarbital dose was administered in a random order on 2 occasions with each test dose session preceded by one saline and one ethanol maintenance session. In this way, the animal's experience on days preceding pentobarbital test days was counterbalanced with respect to any possible after-effects that might have been produced. In addition, on 2 test sessions each 600 mg/kg ethanol and saline were administered and the rats were tested in extinction to observe their perseverance to the selected lever during trained conditions. All administrations were made without the experimenter (technician) knowing the substance administered.

RESULTS

The 6 rats trained to discriminate 600 mg/kg ethanol from saline required a median of 46 training sessions (23 sessions with each condition) in order to meet the criterion of FFP≤12 in 10 consecutive sessions. During the extended schedule transfer experiments, this high level of accuracy to the training conditions during interspersed maintenance sessions persisted for all rats. Table 1 presents the results of testing rats in extinction with 600 mg/kg ethanol, saline and 2–12 mg/kg pentobarbital. Ethanol administration resulted in 100% EL selection and produced a mean of 26.8 responses on the EL before 10 responses were made on the SL. The SL was first pressed 10 times by all rats after saline administration and they continued pressing the SL for a mean of 58.0 responses before pressing the EL 10 times.

Doses of pentobarbital produced EL selection in a dose-dependent manner with 12 mg/kg producing 91.7% selections

on the EL and 2 mg/kg eliciting 25%. The ED50 for pentobarbital, i.e., the dose at which half the rats pressed the EL 10 times first, was 4 mg/kg. However, at this dose, the mean number of responses on the EL before 10 responses were made on the SL (19.1) was not significantly different from the mean EL responses after 600 mg/kg ethanol (26.8). The 2 mg/kg pentobarbital dose was observed to produce significantly fewer responses on the EL than recorded after 600 mg/kg ethanol administration.

DISCUSSION

The observation that rats trained to discriminate the interoceptive cues produced by ethanol will generalize (transfer) to pentobarbital has been made by various investigators. Thus, Kubena and Barry [9] trained food-deprived rats to discriminate between 1200 mg/kg ethanol and saline administered IP and found an ED50 for pentobarbital of 7 mg/kg, whereas Krimmer [8] trained rats with 1000 mg/kg ethanol and observed an ED50 for pentobarbital of 5.4 mg/kg. In the present study, using 600 mg/kg as the ethanol training dose, a pentobarbital ED50 of 4 mg/kg was observed when lever selection was the measurement. These observations support the notion that the ED50 is dependent upon the drug dosage used to initially train rats in this behavioral procedure [10,15]. This transferability between the effects of ethanol and a barbiturate appears to reflect the human condition in which the behavioral effects of ethanol have been reported to be similar to those produced by some barbiturates [6,12] and other reports that these two drugs of abuse are often used interchangeably [5,7].

The present investigation employed lever selection as the first measurement as to drug choice. In effect, it asks the animal subject to indicate which one of the 2 trained drug conditions is most similar to the test drug condition. In addition, the perseverance of lever selection gives a second measure of discriminative behavior by, in effect, asking how much like the test condition is the training condition. This strength of lever selection may help to interpret and expand the partial transfer effects seen in much of the earlier literature. Thus, if only lever selection were employed to indicate the similarity between 4 mg/kg pentobarbital and the training dose of ethanol in the present study, the pentobarbital dose would be considered dissimilar as it produced only 50% selection of the ethanol-correct lever. With the extended schedule, the results indicate that the perseverance of that choice is statistically similar to that seen after 600 mg/kg of ethanol. The 2 mg/kg pentobarbital dose, however, produced a significantly lower perseverance and this may be viewed as the breaking point for transfer of stimulus control [14].

Furthermore, the perseverence in 2 trials each with the training dose of ethanol and saline indicate that the saline condition produced a higher (58.0) mean response rate on the SL before 10 presses were made on the EL than were made on the EL after ethanol administration (26.8). This indicates that with the training dose of ethanol used, the ethanol (drug) condition was not observed to produce a "stronger" lever selection when compared to the saline (non-drug) condition. This suggests that at the training dose of ethanol used, drug "overinclusiveness" [10] may not have been observed in the transfer tests.

The present technique of extended schedule performance may be considered to have the following advantages: (1) It provides an additional behavior measurement to those studies that only employ lever selection in drug transfer experimentation and it obviates the possible bias inherent in continued reinforcement of the first lever selection during transfer drug tests [3]; (2) It indicates the possibility of drug transfer "overinclusiveness" [10] by providing a measurement of the strength or perseverence to the lever selection under each trained state; (3) In those experiments in which rats are trained to discriminate one drug from a second drug, the present technique will allow measurement of the strength of lever selection to each drug state and it may be used to indicate the possibility of equivalent discriminable drug dosages. Although preliminary in nature, the data presented here suggest a procedure that may add a new dimension to experimentation involving drug-induced stimulus control.

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REFERENCES

1. Barry, H., III. Classification of drugs according to their discriminable effects in rats. *Fedn Proc.* **33:** 1814–1824, 1974.
2. Barry, H., III and E. C. Krimmer. Pharmacology of discriminative drug stimuli. In: *Drug Discrimination and State-Dependent Learning*, edited by B. T. Ho, D. W. Richards, III and D. L. Chute. New York: Academic Press, 1978, pp. 3–32.
3. Colpaert, F. C. Some properties of drugs as physiological signals: The FR procedure and signal detection theory. In: *Stimulus Properties of Drugs: Ten Years of Progress*, edited by F. C. Colpaert and J. A. Rosecrans. Amsterdam: Elsevier/North-Holland Biomedical Press, 1978, pp. 217–242.
4. Colpaert, F. C., C. J. G. Niemegeers and P. A. J. Janssen. Theoretical methodological considerations on drug discrimination learning. *Psychopharmacology* **46:** 169–177, 1976.
5. Devenyi, P. and M. Wilson. Barbiturate abuse and addiction and their relationship to alcohol and alcoholism. *Can. Med. Ass. J.* **104:** 215–218, 1971.
6. Fraser, H. F., A. Wikler, H. Isbell and N. K. Johnson. Partial equivalence of chronic alcohol and barbiturate intoxications. *Q. Jl Stud. Alcohol* **18:** 541–555, 1957.
7. Glatt, M. M. The abuse of barbiturates in the United Kingdom. *Bull. Narc.* **14:** 19–38, 1962.
8. Krimmer, E. C. Drugs as discriminative stimuli. *Dissert. Abstr. Internat.* **35:** 4572-B, 1974.
9. Kubena, R. K. and H. Barry. Two procedures for training differential responses in alcohol and non-drug conditions. *J. Pharm. Sci.* **58:** 99–101, 1969.
10. Overton, D. A. Experimental methods for the study of state dependent learning. *Fedn Proc.* **33:** 1800–1813, 1974.
11. Schechter, M. D. Amphetamine discrimination as a test for anti-parkinsonism drugs. *Eur. J. Pharmac.* **44:** 51–56, 1977.
12. Wesson, D. R. and D. E. Smith. *Barbiturates: Their Use, Misuse and Abuse*. New York: Human Science, 1977.
13. Winter, J. C. Drug-induced stimulus control. In: *Contemporary Research in Behavioral Pharmacology*, edited by D. E. Blackman and D. J. Sanger. New York: Plenum Press, 1978, pp. 209–237.
14. Winter, J. C. Drug-induced stimulus control and the concept of breaking point: LSD and quipazine. *Psychopharmacology*, in review.
15. York, J. L. A comparison of the discriminative stimulus effects of ethanol, barbital and phenobarbital in rats. *Psychopharmacology* **60:** 19–23, 1978.