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BRIEF COMMUNICATION

Stability of the Stimulus Properties of Drugs Over Time

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SCHECHTER, M. D., S. A. SIGNS AND J. W. BOJA. *Stability of the stimulus properties of drugs over time.* PHARMACOL BIOCHEM BEHAV 32(1) 361–364, 1989.—Three separate groups of rats were trained to discriminate the stimulus effects of either 600 mg/kg ethanol (n=5), 0.8 mg/kg *d*-amphetamine (n=8) or 1.0 mg/kg 1-(3-trifluoromethylphenyl)piperazine (TFMPP; n=10). Once criterion performance was attained, each group was tested with various doses of the drug used in their training, thus allowing for calculations of dose-response curves and ED_{50} values. A second dose-response relationship was established at a later time, averaging over a year later, and this result was compared to the initial curve. In none of the three groups was there a substantial change in the sensitivity of the rats to different doses of the drug used in training as indicated by similar ED_{50} values. These results suggest that the drug discrimination procedure is stable over a period of continuous training and testing.

Drug discrimination Amphetamine Dose-response Ethanol TFMPP Age effects

DRUG discrimination has proven to be a powerful tool in behavioral pharmacology. This technique has provided information concerning drugs of numerous pharmacological classes and its use has generated abundant suggestive evidence regarding the mechanism of action of many centrally-active drugs. Some of the drugs that have been shown to be capable of controlling discriminative performance in animals are known to produce tolerance. Tolerance being defined as an increased requirement of drug to produce an equivalent effect following repeated exposures to the drug. Having been the site of many investigations employing this behavioral paradigm, we were concerned about the possible changes in the discriminative sensitivity of animals to the drug used in their training following months of drug maintenance sessions, as well as deleterious effects produced by intervening test drugs. Tolerance or, indeed, supersensitivity could develop from the repeated administration of the trained and/or test drug as these treatments may affect the perception of the discriminable cue as reflected by changes in dose-response experiments. Although researchers on drug discrimination have informally noted the stability of this behavioral paradigm, there is but one formal report (1) attesting to this observation.

A series of dose-response experiments were conducted in three groups of rats who had previously undergone dose-response testing with the drug used in their training, as well as extensive subsequent testing with novel agents. Analysis and comparison of the dose-response curves generated at these two times (which actually were conducted an average of a year apart) would provide us with information regarding the possible intervention of changes in sensitivity to the trained drug condition over time.

METHOD

The subjects for all of these experiments were ARS/Sprague-Dawley rats (Zivic-Miller Laboratories, Allison Park, PA) who were kept at 80–85% of their expected free-feeding weights by daily food rationing. Water was freely available to the rats as they were housed in individual cages. The experimental space consisted of 10 identical standard rodent operant test cages (Lafayette Instrument Corp., Lafayette, IN) equipped with two operant levers and a food receptacle. Solid-state equipment (Med Associates, E. Fairfield, VT) used to control and record the sessions was located in an adjacent room.

The procedure used to train rats to discriminate between a drug and its vehicle has been described in detail elsewhere

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(4–8). In brief, daily discrimination training started after initially training the rats to lever-press on both levers on a food-reinforced fixed-ratio schedule of 10 (FR10). Subsequently, the rats were injected intraperitoneally (IP) with either the drug or an equal volume of its vehicle (distilled deionized water). Depending on whether the rat was administered drug or vehicle, it obtained reinforcement by pressing either the drug-appropriate lever or the vehicle-appropriate lever, respectively. After every tenth press (FR10) on the appropriate lever, a 45 mg Noyes pellet was delivered through the food receptacle. Responses on the incorrect lever were recorded but produced no programmed consequence.

Each rat was run once each weekday for a daily session of 15 min duration. Drug (D) or vehicle (V) injection were initially given according to the daily two-week sequence: D-V-V-D-D; V-D-D-V-V. The training criterion was reached when the animals correctly pressed the appropriate drug- or vehicle-appropriate lever on 8 of 10 consecutive training sessions.

Once all rats attained the training criterion, sessions of 15 min duration with alternating administrations of drug and vehicle were continued on Mondays, Wednesdays and Fridays. This procedure endeavored to ensure and maintain behavioral discrimination to the trained drug conditions. It was intended that if a rat was observed to fall below the 8 of 10 criterion on these maintenance days, the data on that rat's performance would be eliminated from the results. This, however, did not occur. On Tuesdays and Thursdays, the rats were injected with doses of the training drug differing from that dose used in training. Following the same time-course after injection that was used in training, they were placed into the experimental chamber and were allowed to lever press without reinforcement until 10 responses were made on either of the two levers. When these 10 responses were completed on either lever, the rat was immediately removed from the experimental chamber to preclude training at a drug dose other than that which it was originally trained. The lever first pressed 10 times was designated as the "selected" lever. Each novel drug dose was administered in a random order on two occasions with each test session preceded by one vehicle and one drug maintenance session.

The percentage of rats selecting the lever appropriate for the training drug condition was the quantal measurement of discrimination. Quantal data are presented as percent correct first choice responding on the drug-correct lever. The quantal data were subjected to the Litchfield-Wilcoxon procedure (3) that employs probits vs. log-dose measurements. A Chi^2 best-fitted line yielded an ED_{50} value for each dose-response experiment and tests for parallelism between curves were analyzed by computer (9). In addition, the total number of lever presses on both levers made before 10 lever presses on either lever constituted the quantitative measurement. This measurement is derived by dividing the number of responses made on the drug lever by the number of responses on both levers upon fulfillment of the "selection" criterion.

The drug used in each group of rats (n), their doses, time between administration and testing, the time in weeks between the initial and second dose-response curves and the reference (in parentheses) to detail the novel test drugs used in each group are: ethanol, 600 mg/kg (v/v), 5 rats, 10 min, 56 weeks, (8); *d*-amphetamine, 0.8 mg/kg, 8 rats, 20 min, 62 weeks (5); 1-(3-trifluoromethylphenyl)piperazine (TFMPP), 1.0 mg/kg, 10 rats, 20 min, 39 weeks (4).

RESULTS

The comparisons of drug discrimination between two dose-response curves performed numerous months apart for the three drugs appears in Table 1. Thus, Table 1A indicates the effects of ethanol on rats (n=5) who were trained to 600 mg/kg ethanol and subsequently tested with 4 different doses (150–900 mg/kg). The training dose maintained discrimination where rats chose the ethanol-appropriate lever on 87.5% of all trials, whereas the same lever was selected on 8% of all trials after administration of vehicle (or the vehicle-appropriate lever was selected on 92% of trials after vehicle administration). Administration of decreasing doses of ethanol resulted in decreasing discriminative performance of both quantal and quantative measurements and the initial dose-response curve (DR1) yielded a quantal ED_{50} of 330 mg/kg. The second dose-response curve (DR2), derived from dose-response experiments conducted 56 weeks following the first series and after multiple maintenance and test sessions (8), yielded an ED_{50} of 280 mg/kg.

Likewise, Table 1B illustrates the previously published (5) dose-response curve in animals trained to discriminate 0.8 mg/kg *d*-amphetamine from its vehicle. The earlier ED_{50} was 0.31 mg/kg, whereas the latter dose-response curve, following multiple novel drug testing (5,6), indicates a similar quantal ED_{50} of 0.25 mg/kg.

The serotonergically-specific drug TFMPP, in 10 rats, was observed to produce a dose-response relationship which yielded an ED_{50} of 0.27 mg/kg (4). A second dose-response relationship, generated after 39 weeks of testing in these animals, yielded a similar ED_{50} of 0.19 mg/kg. In this latter case, an additional lower dose of 0.125 mg/kg TFMPP was needed to be tested as the 0.25 mg/kg dose produced a quantal measurement above the 50% level.

DISCUSSION

Rats trained to discriminate each of three different drugs from vehicle were tested to determine if the sensitivity of these animals changed over the course of many months of experimentation. No substantial change between the initial dose-response curve and the second one generated (in two cases over a year) later could be demonstrated. These results suggest that the sensitivity of rats trianed to discriminate a drug from its vehicle does not change with continued exposure to the drug used in training over this time period. It, however, does not address the possibility that tolerance may have developed to the training drug as it was being trained.

Glennon and Rosecrans (2) have discussed the high degree of specificity and sensitivity inherent in the drug discrimination paradigm. In addition, the stability of the drug discrimination has been regarded as a valuable asset though only limited evidence has supported this claim. Colpaert *et al.* (1) reported that the ED_{50} value of cocaine remained stable throughout a posttraining period as long as 8 months. In the present experimentation, we have again shown that this paradigm is stable and have, thus, demonstrated its reliability in the continued classification of centrally-active drug properties.

Of further significance is the age range over which the animals were observed to display no changes in their sensitivity to these drug-induced cues, especially in the case of ethanol discrimination (Table 1A). In these rats, there were no significant change in the potency between the dose-response curves that were derived 56 weeks apart and in animals whose age at the time of the second DR curve was

TABLE 1

A COMPARISON OF DRUG DISCRIMINATION BETWEEN 2 DOSE-RESPONSE CURVES PERFORMED BEFORE AND AFTER EXTENSIVE NOVEL DRUG TESTING

	DR1		DR2	
Dose (mg/kg)	Quantal	Quantitative (SD)	Quantal	Quantitative (SD)
		A. Ethanol, 600 mg/kg, n=5		
900	100.0	94.3 (0.0)	100.0	94.5 (5.0)
600	87.5	77.3 (12.5)	96.0	86.5 (8.1)
450	55.0	59.9 (26.1)	90.0	79.3 (7.9)
300	40.0	43.1 (33.5)	30.0	32.4 (9.7)
150	10.0	24.0 (6.5)	20.0	25.7 (10.8)
0.0 (veh.)	8.0	16.4 (14.6)	0.0	1.2 (1.8)
ED_{50} (95% conf. limits)	330 mg/kg (210–540)		280 mg/kg (188–430)	
		B. Amphetamine, 0.8 mg/kg, n=8		
0.8	100.0	91.9 (2.7)	96.9	93.4 (5.6)
0.4	72.2	69.5 (20.8)	75.0	68.1 (4.0)
0.2	22.2	30.5 (19.4)	37.5	42.5 (6.9)
0.0 (veh.)	0.0	6.8 (4.6)	6.3	16.7 (8.2)
ED_{50} (95% conf. limits)	0.31 mg/kg (0.2–0.47)		0.25 mg/kg (0.16–0.39)	
		C. TFMPP, 1.0 mg/kg, n=10		
1.0	90.0	78.2 (7.2)	100.0	97.1 (1.9)
0.5	85.0	77.9 (10.3)	90.0	80.7 (9.0)
0.25	40.0	48.6 (6.3)	60.0	58.0 (3.4)
0.125	ND		15.0	18.5 (10.8)
0.0 (veh.)	5.0	17.1 (2.5)	4.0	5.5 (5.7)
ED_{50} (95% conf. limits)	0.27 mg/kg (0.15–0.47)		0.19 mg/kg (0.09–0.32)	

approximately 2¼ years old. These results would suggest that drug sensitivity in a drug discrimination paradigm is independent of age-related pharmacokinetic and dynamic influences. However, the possibility exists that tolerance or supersensitivity (7) may have occurred only to be masked by age-related counterbalances in drug metabolism and/or neuronal responsiveness (10). It has previously been suggested that tolerance development may have been compensated for continued improvement in learning of the discriminative task (1).

In summary, animals trained to discriminate three different drugs were tested for dose-effect relationships on two occasions separated by many months. Analysis of results indicated that their sensitivity did not change over time and that the dose-response curves were not significantly altered by repeated treatment with the drug used in training. In addition, intermittent treatment with other drugs and/or aging appear to have little influence upon the animals' sensitivity to the drug-produced interoceptive cues. These results indicate the stability of the discriminative paradigm in assessment of effects of three drugs which may work by very different mechanisms of action, i.e., *d*-amphetamine acting upon dopamine neurons (6), TFMPP acting upon serotonergic systems (4) and ethanol affecting both (8).

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