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Comparison of Four-Drug Discriminations in Training Compartments With Four Identical Levers Versus Four Different Response Manipulanda

DONALD A. OVERTON[1] AND C. FRANK SHEN

Departments of Psychiatry and Psychology, Temple University, Philadelphia, PA 19122

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OVERTON, D. A. AND C. F. SHEN. *Comparison of four-drug discriminations in training compartments with four identical levers versus four different response manipulanda.* PHARMACOL BIOCHEM BEHAV **30**(4) 879–888, 1988.— Rats were trained to discriminate between four dissimilar drugs (phenobarbital, nicotine, fentanyl, and methylphenidate) in compartments which contained either four identical levers or four dissimilar response manipulanda. During successive training sessions, the drug condition was cycled through the four training conditions. The objective was to compare speed of acquisition and asymptotic accuracy of discriminative control in the following types of compartments: (1) Undecorated compartments with four identical levers; (2) Compartments with four dissimilar response manipulanda (lever, wheel, nosepoke, panel); (3) Four-lever compartments with a unique sensory environment surrounding each lever; (4) Compartments with four dissimilar manipulanda, each surrounded by a unique sensory environment. The required four-drug discriminations were learned in all training compartments. Independent variables that produced statistically significant effects on speed of acquisition and/or asymptotic accuracy included drug, dosage, use/nonuse of four dissimilar response manipulanda, and presence/absence of environmental decorations around each manipulandum. Although the use of four different response manipulanda and/or the use of distinctive decorations surrounding each of the four manipulanda did increase speed of acquisition, these manipulations also resulted in biases towards/against particular individual environments or manipulanda during the acquisition phase of the experiment. Such biases can complicate the interpretation of results of conventional drug discrimination studies, especially if they persist into the asymptotic accuracy phase, which was not observed in the present study.

Four-drug discrimination Response manipulanda Drug discrimination

THE drug discrimination (DD) paradigm is often employed as a method for investigating the effects of psychoactive drugs. Most studies utilize D vs. N (drug versus no drug) discrimination training followed by tests with various novel drugs (X,Y,Z). With this procedure, if an investigator wishes to determine how the effects of a particular novel drug (X) relate to those of several preexisting drugs (A,B,C . . .), it is common practice to train several groups of subjects to discriminate A vs. N, B vs. N, and C vs. N, etc. Such experiments require a considerable amount of effort devoted to training the required subjects.

For some types of investigations, it appears that the desired results might be obtained more rapidly if subjects were trained to simultaneously discriminate several different drugs using paradigms such as the following: D vs. A vs. B vs. C; D vs. A vs. B vs. N; D vs. A vs. B vs. (N or C or E or F . . .). In a pilot study, we found that rats could learn the first type of discrimination in compartments which contained four levers, and that the number of training sessions before discriminative control reached criterion was somewhat reduced if each lever was surrounded by a unique sensory environment which distinguished the four levers from one another; the environments were created by the use of unique floor textures and wall coverings in the vicinity of each lever.

The present study sought to extend this line of investigation by testing the speed of acquisition of four-drug DDs in compartments which contained four different manipulanda (e.g., lever, wheel, panel, nose poke manipulanda). Specifically, rats were trained in compartments which contained (A) four identical levers with no explicit sensory decorations, (B) four identical levers with unique sensory environments surrounding each lever, (C) four different manipulanda with no other unique sensory decorations surrounding these manipulanda, (D) four different manipulanda, each surrounded

[1]Requests for reprints should be addressed to Donald A. Overton, Department of Psychology, Weiss Hall, Temple University, 13th Street and C. B. Moore Avenue, Philadelphia, PA 19122.

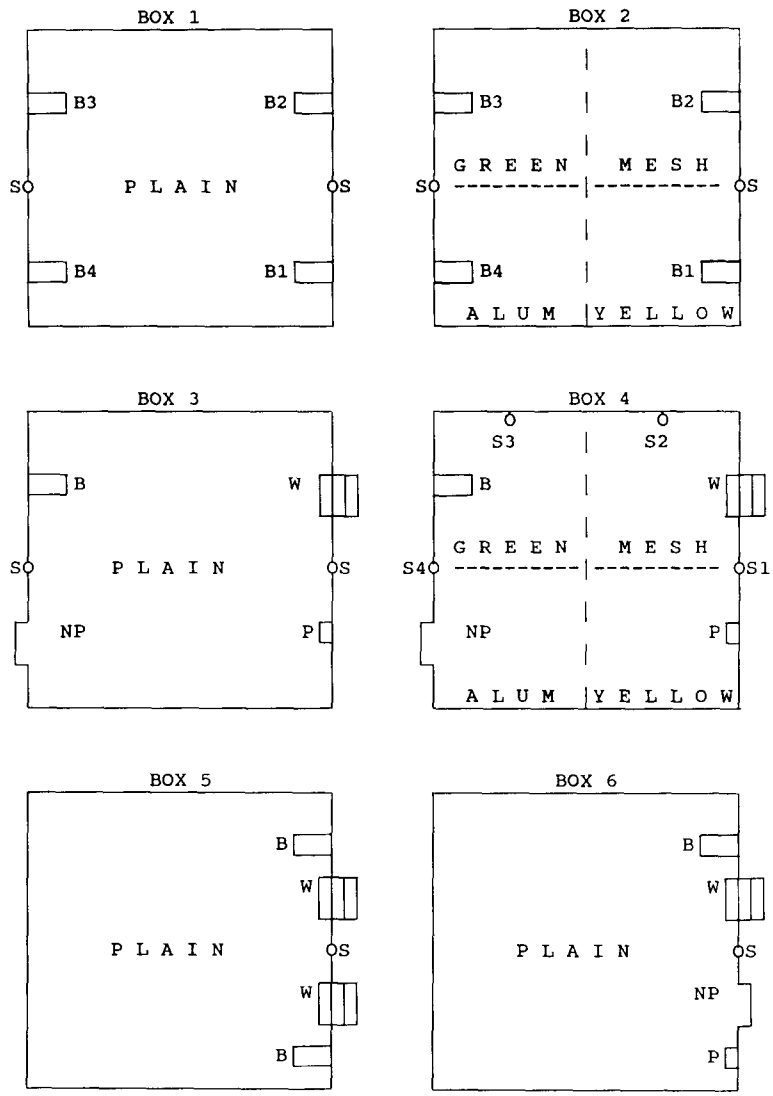

FIG. 1. Floor plans of training boxes used in the experiment. B=Conventional rat lever. P=Vertical panel to be pushed. W=Wheel to be rotated. NP=Hole into which the nose is pushed. S=Gravity feed solenoid which dispensed 0.1 cc sweetened water reinforcer. PLAIN=Wire grid floor and sheet aluminum ceiling and walls. ALUM=sheet aluminum environment. MESH=Wire mesh environment. GREEN=Green plastic environment. YELLOW=Yellow checkerboard environment.

by a unique sensory environment. In each of these four compartments, one manipulanda was located in each quarter of the training compartment as diagrammed in the top four panels of Fig. 1.

Previous studies have shown that when two levers are mounted on opposite walls of a training compartment, two-drug DDs can be learned only if sensory decorations are included to provide "orientational cues" which allow the rats to distinguish one side of the compartment from the other [1]. However, if the two levers are mounted side by side on a single wall of the training compartment, the "right-handed" versus "left-handed" position of the levers apparently provides very salient orientational cues making unnecessary the inclusion of other explicit sensory decorations. To evaluate the strength of right vs. left position cues, the present study tested the ease with which four-drug discriminations could be learned in training compartments which utilized four response manipulanda all located on a single wall of the compartment as shown in the two bottom panels of Fig. 1.

In summary, this study was designed to evaluate the relative utility of using unique, sensory environments around each of several identical response manipulanda, as opposed to the alternative of using several different response manipulanda. Additionally, the study was designed to investigate whether the placement of four manipulanda on a single wall of the training compartment constituted a usable training environment.

METHOD

Subjects

Male hooded rats of the Long Evans strain were purchased from Blue Spruce Farms, quarantined for one week, and placed on a water deprivation schedule which allowed ad lib access to water during 15 minutes each day. The rats were individually housed with dry food continuously available in the home cages, and weighed about 375 g at the beginning of the experiment. Six animals were trained in each of six different training compartments.

Drugs

In most animals the four discriminable drug conditions were produced by sodium phenobarbital 35 mg/kg, nicotine 0.8 mg/kg, fentanyl 0.04 mg/kg, and methylphenidate (Ritalin) 5 mg/kg. All injections took place 15–20 minutes before the beginning of a training session. Drugs were dissolved in isotonic saline in concentrations such that a 1 ml/kg injection volume was required. Fentanyl was injected subcutaneously and the remaining drugs intraperitoneally.

Discrimination Training Procedure

During each DD training session, the first two responses after the rat was placed in the training compartment were disregarded by the SKED process control software. After these two presses, a "test ratio" occurred which was immediately followed by a 15-min training session. The test ratio lasted until 10 responses had occurred on any one of the four manipulanda. Responses during the test ratio occurred before any reinforcer had been delivered in the current session, and were reinforced only if the animal completed 10 responses on the correct (drug-appropriate) manipulandum before it completed 10 responses on any of the other manipulanda. During the remainder of the training session after the test ratio, responses on the correct manipulandum were reinforced on an interlocked FR-10/FI-90 sec schedule. Under this schedule, a maximum of 10 responses on the correct manipulandum was required to earn reinforcement, and the number of required responses was decremented by 1 every 10 seconds down to a minimum FR-1 response requirement. With this schedule of reinforcement, the temporal patterning of responding approximates that seen with a conventional FR-10 schedule of reinforcement. Responses on the other three (incorrect) manipulanda were recorded, but had no programmed consequences. The reinforcer was 0.1 cc of saccharin-sweetened water delivered via the gravity feed solenoid valve closest to the correct manipulandum.

One training session occurred each day, six or seven days per week. Under each training drug condition, a different manipulandum was assigned as 'correct,' and only responses on the correct manipulandum were reinforced. Assignment of the correct manipulandum under each training drug was imperfectly balanced across the six animals trained in each compartment. After the initial shaping sessions (see below), the imposed drug condition was varied in a regular sequence during successive session (i.e., ABCDABCD, etc.) during most of the experiment.

To evaluate the possibility that animals might learn the sequence (across sessions) in which different manipulanda were reinforced, a pseudorandom sequence of drug conditions (and reinforced manipulanda) was employed subsequent to session 90. During this period of training, the assignment of drug conditions to reinforced manipulanda remained unchanged, but the sequence in which the drug condition (and reinforced manipulanda) were altered across sessions was pseudorandom instead of orderly.

Pretraining and Shaping Procedures

Before the beginning of drug discrimination training, a series of pretraining sessions were conducted to accustom the animals to responding on each of the response manipulanda that would subsequently be used during discrimination training. These sessions took place in four compartments (30 cm wide $\times$19 cm deep $\times$ 18 cm high) each of which contained a single manipulandum and a single water-delivery solenoid. Each pretraining session lasted 12 hours. After responding had begun, ratio schedules of increasing difficulty (FR-1, FR-4, FR-8, FR-16, FR-32) were employed during successive sessions until each animal had completed two sessions with each type of manipulandum during which more than 100 reinforcements were earned with an FR-32 schedule. After this criterion had been met, animals were entered into discrimination training.

The initial 24 discrimination training sessions were "shaping" sessions; during these sessions more lenient schedules of reinforcement were employed (FR-2, FR-5), session durations were extended (120, 60, 30 minutes), and each animal completed two or three training sessions with reinforcement on a particular manipulandum before the drug and reinforced manipulandum were switched. Drugs were injected prior to all shaping sessions, and a typical sequence of drug conditions (A,B,C,D) and reinforced manipulandum assignments (1,2,3,4) during these 24 shaping sessions was A1 A1 A1 B2 B2 B2 C3 C3 C3 D4 D4 D4 A1 A1 B2 B2 C3 C3 D4 D4 A1 B2 C3 D4. Throughout the subsequent presentation of the results of this experiment, the instances in which two or more successive sessions employed the same drug and manipulandum assignment will be treated as a single prolonged session, and only data from the first session of the sequence will be reported. Hence, instead of describing the number of training sessions to reach a given criterion level of discrimination, this paper will report the number of training drug state changes before the specified criterion level of performance was achieved.

Apparatus

The six groups of rats received DD training, respectively, in the six training compartments diagrammed in Fig. 1. Each compartment was 50$\times$50$\times$29 cm high. Except as otherwise described, the walls, door, and ceiling were plain aluminum, and the floor was wire grid (15 cm squares). In boxes 1–4 two manipulanda were mounted 25 cm apart on the right wall and another two manipulanda were mounted on the left wall. A water delivery spout was centered between the manipulanda on the right and on the left walls. A 5-watt houselight was mounted near the top edge of the left wall with a shield immediately below it so that light from the houselight did not project directly down onto the two manipulanda mounted on the left wall. Masking noise was provided by a loudspeaker.

Four different home built response manipulanda were employed in the study. Lever manipulanda were horizontal aluminum panels, 4.3 cm wide which projected 3.1 cm out of the compartment wall and were activated by a vertical force of 20 g. Panel manipulanda were flat sheets of aluminum (painted black) 5 cm wide and 13 cm high attached to the wall of the compartment by a hinge located at the top edge of the panel. A horizontal force of 20 g directed toward the wall of

TABLE 1

INDICES OF SPEED OF ACQUISITION AND ASYMPTOTIC ACCURACY IN EACH TYPE OF TRAINING COMPARTMENT PRESENTED SEPARATELY FOR EACH LEVER

(A) Box No.	(B) Correct Manipulandum	(C) Sensory Environment	(D) (No. learning)/ (No. trained)	(E) SAI	(F) SAC	(G) No. Rats in STC Indices	(H) Asymptotic Accuracy (AA)	(I) No. Rats in AA Indices
1	Lever 1	plain		32.1		5	79.6	6
	Lever 2	plain		40.7		5	84.2	6
	Lever 3	plain		26.3		5	88.1	6
	Lever 4	plain		30.3		5	86.3	6
	Average		5/6	32.0	78.0	5	84.5	6
2	Lever 1	yellow		16.7		6	81.8	5
	Lever 2	mesh		14.7		6	91.4	5
	Lever 3	green		28.4		6	80.2	5
	Lever 4	aluminum		24.5		6	93.3	5
	Average		6/6	20.6	42.8	6	86.7	5
3	Panel	plain		27.0		6	85.2	4
	Wheel	plain		21.1		6	86.8	4
	Lever	plain		12.1		6	93.3	4
	Nose Poke	plain		4.3		6	96.4	4
	Average		6/6	14.5	51.2	6	90.4	4
4	Panel	yellow		13.3		6	88.9	5
	Wheel	mesh		7.4		6	81.4	5
	Lever	green		18.5		6	85.4	5
	Nose Poke	aluminum		22.6		6	92.1	5
	Average		6/6	14.8	48.0	6	87.0	5
5	Lever	plain		35.2		4	81.4	5
	Wheel	plain		7.0		4	94.9	5
	Wheel	plain		26.6		4	90.9	5
	Lever	plain		26.0		4	88.7	5
	Average		5/6	21.7	61.8	4	89.0	5
6	Panel	plain		23.2		5	81.4	5
	Nose Poke	plain		11.7		5	94.7	5
	Wheel	plain		24.5		5	94.9	5
	Lever	plain		16.4		5	94.2	5
	Average		6/6	18.5	43.4	5	91.3	5

Indices in columns E, F, and H were computed from performance during the test ratios of all sessions in which the lever specified in column B was the correct lever, averaged across the different drugs that were injected prior to these sessions in different rats.

SAI values were obtained from the inverse logarithm of mean ln(SAI + 10). The values for individual levers were obtained by averaging ln(SAI + 10) across animals. The average values per box were obtained by averaging the four mean values for individual levers.

SAC values are simple arithmetic averages across animals of the individual values of the index.

Number of rats in SAI and AA indices reflect the deletion of some rats from the analyses due to death or excessively low dosages of one or more drugs.

SAI is the No. of training sessions (minus 3) on an individual manipulandum before the beginning of a criterion string of sessions on that manipulandum during which the sliding average of % correct responses during the test ratio exceeded 70%.

SAC is the No. of training sessions (minus 12) before the beginning of a series of 20 sessions during which the rat concurrently achieved the SAI criterion on each manipulandum.

AA is average asymptotic accuracy computed as the arithmetic mean across sessions and across animals of % correct presses during the initial ratio of the 16 sessions before, and sessions 17 through 32 after the onset of random drug sequence.

the compartment at the bottom edge of the panel was required to operate the switch located behind the panel manipulandum. The nose poke manipulandum was a round hole in the wall of the compartment 5 cm in diameter which constituted the entrance to a 6 cm square box behind the compartment wall. Insertion of the snout into the aperture was detected by a beam of light which passed vertically just behind the circular aperture to strike a photocell. To activate the nose poke manipulandum, it was necessary for the rat to insert its head 2 cm into the aperture and to then withdraw its

head. Alternately, the rat could insert its snout and wiggle it from side to side. Wheel manipulanda were constructed from mouse running wheels which were 12 cm in diameter and 6.5 cm wide. The wheels rotated around an axle which was mounted horizontally, parallel to and 3 cm behind the compartment wall; this allowed about 1/4 of the wheel to project into the compartment. Access to the sides and inside of the wheel was prevented by aluminum panels. Rats operated the wheel manipulandum by pressing downward on its exposed surface, thereby rotating the wheel about its axle. Each 180 degrees of rotation caused a microswitch contact closure which was recorded as a response. Rotation of the wheel was essentially frictionless, except for the force required to activate the microswitch.

In boxes 2 and 4, four different sensory "environments" were provided in the quarters of the box where each manipulandum was located to assist the animals in distinguishing between the four different manipulanda. We will describe the environmental decorations used in box 2 in detail. In box 2, lever 1 was surrounded by a "yellow" environment in which the walls and ceiling were painted with yellow and black 2.5-cm wide horizontal stripes, and the floor was a yellow plastic "checkerboard" 0.6-cm thick with alternate 1-cm squares cut out to produce square holes. Lever 2 was surrounded by a "mesh" environment in which the white walls and ceiling were covered with black 0.5-cm mesh (steel lath), and the floor was wire mesh with 1.5-cm square holes. Lever 3 was surrounded by a "green plastic" environment in which the floor and walls were covered with green corrugated plastic (roofing material with parallel 'waves' in it; each wave was 1.5-cm high and wave peaks were 7-cm apart, center-to-center). The waves on the plastic floor were parallel to the side wall and the waves on the walls were vertically oriented. The ceiling was painted green. Lever 4 was surrounded by an "aluminum" environment in which the floor, walls and ceiling were undecorated sheet aluminum.

Referring to Fig. 1, box 1 was the control box. It contained 4 identical lever manipulanda and no explicit decorations designed to provide orientational cues. Box 2 contained 4 levers, and unique sensory decorations in the vicinity of each lever, as just described. Box 3 contained four different response manipulanda, and no explicit sensory decorations. Box 4 contained four different response manipulanda and unique sensory decorations in the vicinity of each manipulanda (the same as in box 2). Boxes 5 and 6 contained response manipulanda along one wall of the compartment and no sensory decorations. In box 5, only two different types of manipulanda were employed, whereas four different types of manipulanda were employed in box 6. It was our expectation that durg discriminations would be learned most easily in box 4, less easily in boxes 3 and 2, and with most difficulty in box 1.

Data Analysis

Training was conducted and data collected by SKED software operating in a Digital Equipment Corporation PDP-12 computer [3]. The software recorded the number of responses on each manipulandum during the test ratio at the beginning of each session, and during each 1.5-min interval of the subsequent 15-min training session. The software also recorded number of reinforcements earned, latency to start responding, latency to complete the test ratio, session duration, responses before and after the session, and other parameters of interest.

To quantify the speed of acquisition of discriminative control, we computed sessions to criteria using two types of criteria based on 5-day sliding averages. For the first index of speed of acquisition (SAI) the criterion was five consecutive sessions under an individual training drug during which the average percent correct responses during the test ratio exceeded 70 percent; each rat received four such SAI scores, one for each training drug. SAI scores were not normally distributed, so inferential statistics were carried out on the transformed values ln(SAI + 10). The second index required concurrent discrimination of all four drugs. Each rat was assigned one SAC score reflecting the number of training sessions before it concurrently achieved the 70 percent 5-day sliding average criterion under each of the four training drugs. No transformation of SAC scores was necessary. Note that each of these scores excludes the pretraining sessions and the 12 shaping sessions during which the correct manipulandum was the same as that reinforced during the immediately preceding session. Also, both schemes count the number of sessions prior to the beginning of the criterion string, rather than the number of sessions to its completion.

Percent responses on the correct manipulandum during the test ratio was used as an index of asymptotic accuracy (AA) of discrimination, and to show the effect of switching from regular assignment of drug conditions during successive sessions to a random sequence of drug conditions. For each rat, 12 average percentages (3 for each drug) were computed from percent correct responses during the test ratios of the 16 sessions prior to the onset of random sequence, the 16 sessions immediately subsequent to the onset of random sequence, and the 16 remaining sessions before training was terminated.

A priori, it appeared possible that the rats might prefer (or avoid) certain of the sensory environments and/or certain of the manipulanda, that discrimination of some drugs (and/or dosages) might be more rapid (or accurate) than discrimination of others, and that the overall average level of discriminative control might differ in boxes 1–6. Because the assignment of drugs to manipulanda (and of drugs to environments, and of manipulanda to environments) could not be completely balanced using six subjects per box (or any feasibly small number of rats and compartments), simple marginal averages could not provide maximum likelihood estimates of the effect of training drug or dosage, the effect of sensory decorations, or of the effect of the various types of manipulanda. To obtain such estimates, we used multiple regression with dummy variables. Additionally, since the assignment of some independent variables was not balanced, we could not simply perform a six-factor ANOVA to test the effects of all of the independent variables at once. Instead, we adopted the tactic of concurrently estimating the effects of all independent variables using multiple regression, subtracting several of these effects from the data using a covariance-like adjustment procedure, and then performing ANOVAs on the residual scores. These one- or two-factor single or repeated measures ANOVAs were followed by post hoc or planned contrast tests. All analyses were based only on data from boxes 1–4, except for the final analysis (below) which specifically deals with the data from rats trained in boxes 5 and 6.

RESULTS

General Description of Performance

Most rats sooner or later learned the required 4-way discrimination as shown in column D of Table 1. The latency to

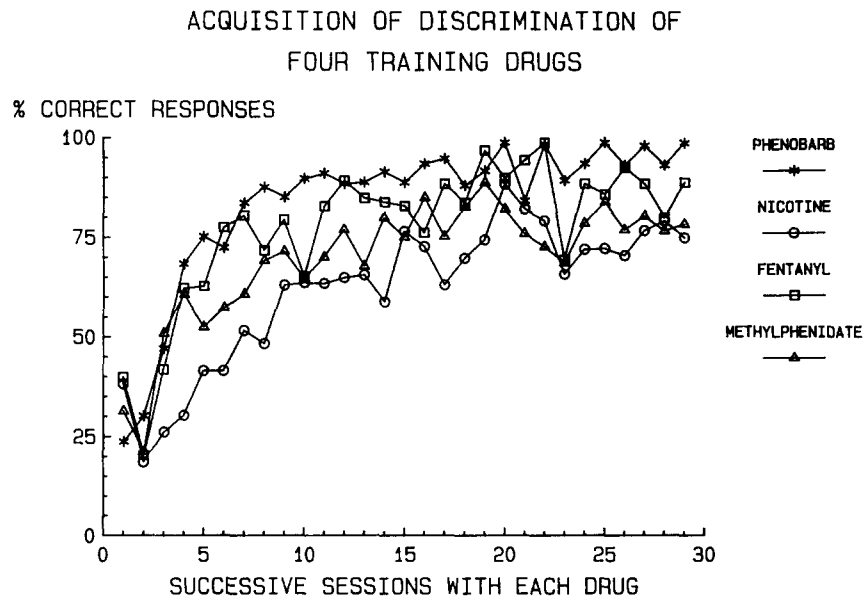

FIG. 2. Learning curves showing acquisition of discrimination in the 4-manipulandum, 4-drug discrimination task. Performance under each training drug is plotted separately. X-axis is successive blocks of 16 sessions (4 under each drug condition). Y-axis is average percent correct responses during the test ratio at the beginning of training sessions. Data are averaged across all rats trained in compartments 1–4.

start responding at the beginning of the sessions decreased during early training sessions and stabilized after 25 to 90 sessions, after which mean asymptotic latencies before the beginning of responding were 12 sec for phenobarbital, 44 sec for nicotine, 40 sec for fentanyl and 75 sec for methylphenidate. The total number of responses per session (and the number of reinforcements earned) stabilized after 30 to 90 sessions in most rats. The average number of training sessions before the beginning of SAI and SAC criterion strings, were 19.3 and 54, respectively. Average asymptotic accuracy was 86.9 percent (reduced to 82.2 percent immediately after randomization).

The most obvious and statistically consistent effect in the data was a difference between the four training drugs employed, with some drugs allowing faster acquisition and/or higher asymptotic accuracy than others. Figure 2 shows learning curves obtained by averaging percent correct responses during the test ratio across all animals in boxes 1, 2, 3 and 4 separately for each training drug; the figure shows the gradual acquisition of discriminative control, and the effect of the onset of the random sequence of drug conditions.

It turned out that the initially selected training dosages were sufficiently high to be incompatible with regular responding in a few rats. Therefore, during the first 75 sessions of training, rates of responding were monitored and training dosages were incrementally reduced until regular responding was obtained by each rat under each drug. The final average training dosages were phenobarbital 34.5, nicotine 0.77, fentanyl 0.033, and methylphenidate 3.96 mg/kg. Fortunately, the required dosage reductions were moderately randomly distributed with respect to the independent variables of interest.

Before analyzing data on speed of acquisition, we deleted data for one rat which showed more than four sessions during early acquisition in which a total of less than 50 responses occurred. The rationale for this deletion was that if the rat was virtually or totally failing to respond during several successive sessions conducted with fentanyl, for example, then little or nothing could be learned during these sessions, and the number of sessions to achieve the fentanyl discrimination would be correspondingly increased, thereby biasing our data sample if that rat were included in the analysis. The analysis of asymptotic accuracy omitted data for two rats which died before asymptotic accuracy was achieved, and two rats which ended up being trained with a dosage less than 50 percent of the initially assigned nominal training dosage with one or more of the training drugs. These decisions regarding data deletion were made before the data analysis was conducted by scorers who were blind to the effect of the deletions on the outcome of the subsequent data analysis.

Effect of Training Drug

Figure 2 suggests that different speeds of acquisition and asymptotic accuracies were obtained with the four training drugs. To evaluate the statistical significance of the effect of training drug on SAI and AA scores, we first estimated (using regression and multiple regression) and then removed (using a covariance-like adjustment procedure) the effects of dosage, decorations (within and between boxes), and manipulanda (within and between boxes). ANOVAs applied to the residual scores for boxes 1–4 indicated highly significant overall effects of drug ($p=0.001$ for SAI and $p=0.004$ for AA scores). Table 2A shows the average levels of each index under each drug in the adjusted data and indicates the levels of statistical significance observed in post hoc tests. The

TABLE 2

INDICES SHOWING SIZE AND SIGNIFICANCE OF EFFECTS OBSERVED WITHIN BOXES

	(A)	(B)	(C)	(D)

A. Average Indices Showing Differences Between Training Drugs

	Training Drug			
	Phenobarb	Nicotine	Fentanyl	Methylphenidate
SAI	$12.2^{b,d}$	$33.0^{a,c,d}$	14.9^{b}	$21.1^{a,b}$
AA	$95.8^{b,c,d}$	80.7^{a}	87.9^{a}	83.1^{a}

B. Mean Slope of the Regression of ln(SAI + 10) and AA vs. ln(dose)

	Training Drug			
	Phenobarb	Nicotine	Fentanyl	Methylphenidate
Slope of SAI	−2.4*	−1.2	−0.7*	−0.4
Slope of AA	−3.6	9.9	4.9	26.9*

C. Averages Showing Differences Between Manipulanda

	Type of Manipulanda			
	Wheel	Nose-Poke	Lever	Panel
SAI	15.8	9.3^{d}	13.0	23.5^{b}
AA	82.2	91.1	91.7	85.7

D. Averages Showing Differences Between Decoration Types

	Decoration			
	Yellow	Green	Mesh	Aluminum
SAI	$11.1^{b,d}$	21.7^{a}	11.0^{d}	$29.8^{a,c}$
AA	86.7	82.0	88.9	90.1

*Slope is significantly different from zero, $p<0.05$.

$^{a}p<0.05$ vs. the results in column A; $^{b}p<0.05$ vs. the results in column B; $^{c}p<0.05$ vs. the results in column C; $^{d}p<0.05$ vs. the results in column D.

Before each of the means in this table was calculated, all other within-box effects (i.e., 3 out of 4 of the following; drug type, dosage, differences between manipulanda, differences between decorations) were removed by covariance-like adjustment of the data.

SAI and AA are defined as in the Table 1 notes.

table shows that nicotine produced slower acquisition (higher SAI scores) than the other drugs, and that asymptotic accuracy during phenobarbital training sessions was higher than observed with any of the other three training drugs.

Effect of Dosage

Table 2B shows that differences in training dosage were significantly correlated with SAI scores in phenobarbital and fentanyl data, and significantly correlated with asymptotic accuracy in the methylphenidate AA scores. To obtain these results, we estimated (by multiple regression) and removed from the data the effects of other independent variables including drug, decorations within and between boxes, and manipulanda within and between boxes. Then for each index and drug we computed the linear regression of the adjusted data values versus dosage to produce the results in Table 2B. Descriptively, the results show that decreased training dosage was associated with slower acquisition (higher SAI scores) and with lower asymptotic accuracy (lower AA scores) in the instances where statistical significance was obtained.

Preference/Avoidance of Manipulanda

Next we tested whether animals were "biased" towards or away from any of the training manipulanda that were employed; such a result was anticipated since the manipulanda required markedly differing response topographies. We tested for this effect using SAI and AA data from animals trained in boxes 3 and 4. After the effects of drug, dosage, and decorations were removed, the adjusted data values were subjected to ANOVAs which yielded an overall $p=0.04$ for SAI scores and $p=0.3$ for AA scores. Table 2C shows the mean levels of each index during sessions when each of the four manipulanda was correct, and shows the results of post hoc tests. The only significant effects were obtained with the SAI variable indicating very rapid acquisition of discrimina-

TABLE 3
MEANS SHOWING SIZE AND SIGNIFICANCE OF EFFECTS BETWEEN BOXES

	(A)	(B)	(C)	(D)

A. Averages Showing Effect of Presence/Absence of Decorations on Indices of Speed of Acquisition and Accuracy

	Boxes With Decorations		Boxes Without Decorations	
Box Number:	2	4	1	3
Mean SAI	15.9	18.9	24.3	20.4
Mean AA	88.2	85.6	85.9	88.6

B. Averages Showing Effect of Presence/Absence of Unique Manipulanda on Indices of Speed Acquisition and Accuracy

	Boxes With Unique Manipulanda		Boxes Without Unique Manipulanda	
Box Number:	3	4	1	2
Mean SAI	13.6‡§	16.3‡	27.6*†	23.3*
Mean AA	89.3	86.4	85.1	87.4

C. Averages Showing Combined Effect of Decorations (D) and Unique Manipulanda (M) on Indices of Speed of Acquisition and Accuracy

	Box Number			
	1	2	3	4
Cues in Box:	None	D	M	D + M
Mean SAI	30.7†‡§	20.7*	15.6*	14.3*
Mean AA	85.2	87.4	89.3	86.4

*$p<0.05$ when compared to results in column A; †$p<0.05$ when compared to results in column B; ‡$p<0.05$ when compared to results in column C; §$p<0.05$ when compared to results in column D.

The effect of all within box variables (i.e., dosage, drug type, manipulanda type, decoration type) were removed from the data by a covariance-like adjustment before any of the indices in this table were computed. Additionally, manipulanda effects (between boxes) were removed from data in Table 4A and decorations effects (between boxes) were removed from data in Table 4B.

SAI and AA are defined as in the Table 1 notes.

tive control by drugs associated with the nose poke manipulanda and relatively slow acquisition by drugs associated with the panel manipulanda. Quite possibly these results reflect the relatively easy response (wiggling the snout) required by the nose poke manipulanda and the relatively difficult response (pressing horizontally against a panel mounted on the wall) required by the panel manipulandum. Significant differences in asymptotic accuracy were not observed.

Preference/Avoidance of Decorated Environments

Next, we tested whether rats were significantly biased toward or away from any of the four sensory environments that were used. To carry out this analysis we used data from boxes 2 and 4 from which we removed the effects of drug, dosage, and manipulandum type before submitting the adjusted SAI and AA data to ANOVAs which yielded an overall $p=0.0012$ for SAI scores and $p=0.4$ for AA scores. Table 2D shows the average adjusted indices for responding in each environment, and indicates the results of post hoc tests. The results indicate that animals acquired the discriminations that involved responding in the yellow and mesh environments more rapidly than they acquired discriminations that involved responding in the green and aluminum environments. It is difficult to guess the factors that may have led to these preferences; it may be relevant that the houselights were located in the green and aluminum environments which may have resulted in a preference for the right side of the compartments during early acquisition.

Overall Level of Performance in Boxes 1-4

Table 3A shows the results of an analysis intended to determine whether performance was significantly better in the two boxes which contained unique environmental decorations around each manipulanda than in the boxes which did not contain such decorations. SAI and AA data were adjusted to remove the effects of drug, dosage, decorations within boxes, manipulanda within boxes, and the between boxes effect of presence/absence of unique manipulanda. Then the residual data scores were submitted to one-way ANOVAs which failed to yield significant results for either SAI or AA scores. Although inspection of the adjusted mean SAI scores in Table 3A suggests that acquisition may have been slightly more rapid in boxes with decorations than in boxes lacking such decorations, this difference was not significant ($p=0.08$ for the contrast comparing boxes 1 + 3 versus 2 + 4) in the present data set.

Table 3B shows the results of an analysis designed to compare performance in boxes containing four different types of manipulanda with performance in boxes which contained four identical lever manipulanda. For this analysis, the effects of drug, dosage, manipulanda-within, and decorations-within were removed as described previously. Additionally, the between-boxes effect of presence/absence of decorations was removed. Then, the adjusted data were submitted to one-way ANOVAs which yielded a significant overall effect ($p=0.003$) for SAI scores and a nonsignificant effect for AA scores. Table 3B shows the mean adjusted values for each index in each box, and indicates the results of post hoc tests. The results indicate that rats acquired discriminations more rapidly in boxes which contained four different manipulanda than in boxes which contained four identical levers.

Table 3C shows the results of a reanalysis of the same effects designed to allow comparison of the relative size of the effect of using unique decorations with the size of the effect of using different manipulanda. For this analysis, within box effects were removed from SAI and AA data and residual scores were then submitted to a one-way ANOVA (box, 4 levels) followed by post hoc tests and planned contrasts. The mean adjusted indices in Table 3C suggest no significant differences in AA scores. The mean SAI scores suggest an orderly summation of effects with most rapid acquisition observed in box 4 which contained both dissimilar manipulanda and environmental decorations. The planned contrast comparing boxes 1 +2 (no manipulanda) versus 3 +4 (manipulanda) was significant ($p=0.0003$) confirming the significant result reported in Table 3B. The planned contrast comparing boxes 1 + 3 (no decorations) with boxes 2+4 (decorations) was significant only if one-tailed hypothesis is assumed ($p=0.08$) confirming the lack of strongly significant effects of presence/absence of decorations previously shown

TABLE 4

INDICES OF PERFORMANCE IN BOXES 5 AND 6 IN WHICH ALL MANIPULANDA WERE ON ONE WALL

A. Average Indices Allowing Comparisons Between Manipulanda Positions

	(A) Right Edge	(B) Right Center	(C) Left Center	(D) Left Edge
	Location of Manipulanda			
Mean SAI	23.1†	11.1*‡§	24.2†	28.3†
Mean AA	79.3†‡	99.7*§	101.4*§	83.6†‡

B. Average Indices Allowing Comparison With Other Boxes

	Box Number 5	6
Mean SAI	24.5	18.2
Mean AA	90.6	91.3

*$p<0.05$ when compared to results in column A; †$p<0.05$ when compared to results in column B; ‡$p<0.05$ when compared to results in column C; §$p<0.05$ when compared to results in column D.

Before average indices in this table were calculated, the data were adjusted to remove the effects of dosage, drug type and manipulanda type using estimates of the size of these effects obtained from data in boxes 1, 2, 3, and 4 during previous analyses.

SAI and AA are defined as in the Table 1 notes.

in Table 3A. The ordering of mean SAI values in Table 3C suggests that inclusion of decorations somewhat improved asymptotic accuracy, but that use of four different manipulanda had a stronger effect.

Effect of Manipulandum Position in Boxes 5 and 6

This analysis tested whether animals were biased toward the manipulanda that were closest to the reinforcement delivery solenoid in boxes 5 and 6. SAI and AA data for boxes 5 and 6 were adjusted to remove the effects of drug, dosage, and differences between manipulanda using estimates for the strength of these effects previously calculated from the data in boxes 1–4. Then the adjusted data for boxes 5 and 6 were submitted to a two-factor repeated measures ANOVA (factor 1=box, two levels; factor 2=manipulanda position in box, 4 levels, repeated measures). These ANOVAs yielded marginal overall significance ($p=0.04$) in SAI scores and showed highly significant differences in AA scores ($p=0.0003$). Table 4A shows the mean values of the adjusted indices, and indicates the results of post hoc tests. The table shows obvious differences in asymptotic accuracy with the animals showing much higher accuracy during sessions when the drug-appropriate manipulanda was located immediately adjacent to the solenoid than during sessions when the animals were required to respond on either of the more distal manipulanda. Hence the predicted bias toward the manipulanda closest to the reinforcement solenoid was observed (In one case, the adjustment process yielded a mean index greater than 100 which reflects the presence of some noise in the original data.) The marginally significant effect observed in the SAI score (very low SAI scores only on the manipulandum located just to the right of the solenoid) is difficult to explain.

Table 4B presents adjusted mean SAI and AA values averaged across the four manipulanda in boxes 5 and 6 in order to allow comparison of performance in these boxes with that observed in boxes 1–4; the values in Table 4B are most directly comparable to those observed in box 3 in Table 3C since boxes 3, 5, and 6 all contained different response manipulanda and no decorations, and the data from these boxes was subjected to the same adjustments before computation of the mean indices shown in Tables 3C and 4B. The mean indices suggest that SAI and AA scores were not significantly different in boxes 3, 5 and 6, and because of the many differences between these compartments we did not test the statistical significance of this conclusion.

Effect of Random Training Sequence

Figure 2 suggests that asymptotic accuracy transiently decreased when the sequence of training sessions was switched from regular to random. To statistically test this effect, average accuracy was computed for each rat and training drug during (A) the 16 sessions immediately preceding onset of random sequence, (B) the first 16 sessions after random sequence, and (C) the subsequent sessions (approximately 16) before the end of the experiment. After the effects of drug, decorations within box, and manipulandum type were estimated and removed, a repeated measures ANOVA indicated an almost significant effect of epoch ($p=0.06$). Mean adjusted AA scores during epochs A, B, and C were and 87.2, 81.7, and 86.7, respectively. Post hoc tests indicated a significant difference between epochs B and C ($p<0.05$) whereas the difference between epochs A and B failed to reach significance ($0.05<p<0.10$).

DISCUSSION

We attempted to select training drugs with salient and essentially nonoverlapping discriminable effects so that the discriminations would be easily formed. The significant differences between the speed of acquisition and asymptotic accuracy scores obtained with the various drugs apparently indicates that the drugs were not all equally discriminable at the dosages that we used.

The use of either unique sensory decorations around each lever (box 2) or four different manipulanda (box 3) significantly increased the speed of acquisition above that observed in the control box (box 1) which contained four identical levers and no explicit orientational cues. Acquisition in Box 4, which contained both sensory decorations and four different manipulanda, was nonsignific ntly faster than that observed in boxes 2 and 3.

From a practical point of view, the use of four different manipulanda does not appear to provide a significant advantage by comparison to the use of four levers. Discriminative control was more rapidly acquired in boxes that contained four different manipulanda, but the reduction in the required number of training sessions was offset by the increased number of pretraining sessions that was necessary to establish operant responding on all four manipulanda.

Although presence of at least some orientational cues appears to be necessary in boxes which have four identical manipulanda symmetrically placed (as in boxes 1 and 2), the presence of such decorations also raises the possibility that rats will specifically approach or avoid some of the sensory environments that are employed, thus introducing a bias into

the results obtained. We did obtain statistical evidence for such biases in our animals during the acquisition phase of training. The use of four different manipulanda also biased responding during acquisition, and the animals appeared to prefer the nose poke manipulanda and to avoid the vertical panel manipulanda. Such biases are generally undesirable in studies designed to obtain psychopharmacological data, and it is worth noting that such biases may occur whenever an asymmetrically decorated DD training compartment is employed. Nonetheless, in the present data set biases were only apparent during the acquisition phase of the experiment, and were too small to achieve statistical significance during the asymptotic phase, if indeed they were still present at all. Hence they may not pose a serious difficulty as long as some care is exercised in the selection of environmental decorations so to avoid decorations that rats will strongly prefer or avoid.

When four manipulanda were placed side-by-side on a single wall of the compartment, the animals were biased toward selecting the two center manipulanda (those closest to the reinforcement delivery solenoid) and this effect was still very evident in the asymptotic accuracy data. Hence this training compartment arrangement appears to be less than optimal. A similar bias may occur when three keys or levers are located side-by-side on one wall of the compartment, although this has not been reported by investigators using such compartments.

The disruption in discriminative control which accompanied the onset of randomization of the sequence in which training drugs were presented has not been previously reported. The rapid recovery of discriminative control suggests that only a small portion of response control was based on the orderly sequence of training states. A similar transient disruption of accuracy would presumably have been produced by the insertion of generalization test sessions inbetween training sessions.

Acquisition of the present four-way discriminations was slower than is usually reported when two-way D vs. N discriminations are learned in 2-lever boxes. After 80 shaping and training sessions, 80% of the animals in boxes 2, 3 and 4 had reached the beginning of the SAC concurrent discrimination criterion. To this we can add the 10 to 30 pretraining sessions and the 20 sessions which comprised the criterion string, yielding a total of 110 to 130 sessions before most rats were ready for use in tests for generalization to novel compounds. This is 2 to 3 times the number of sessions that would normally be required to discriminate a single drug during D vs. N discrimination training. Overall, the training procedure was sufficiently prolonged so as to appreciably reduce the amount of the rat's life span available for generalization tests after training was completed, a disadvantage that would be less serious in an animal with a longer life span.

In summary, rats could learn four-drug discriminations in four-lever and in four-manipulanda boxes. The number of training sessions required to learn such four-way discriminations was 2 to 3 times the number of sessions usually required to learn two-way D vs. N discriminations. Speed of acquisition was substantially enhanced by the use of four different manipulanda, but this compartment configuration also had the undesirable effect of biasing the animals so that they reached criterion more rapidly on some manipulanda than on others. Use of four dissimilar manipulanda did not decrease *total* sessions before criterion because the increased number of sessions required to shape responding on all four manipulandum was equal to the subsequent reduction in number of discrimination training sessions. Speed of acquisition was also enhanced by the provision of unique sensory environments in different sectors of the training compartment which presumably assisted the rats by providing orientational cues. The use of unique sensory decorations did not necessitate additional shaping sessions, and hence did reduce the total number of sessions before discriminative control was established.

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