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Stimulus blocking during compound discrimination training with pentobarbital and visual stimuli

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To investigate blocking by drug-induced stimuli and visual stimuli, three groups of rats were trained to escape shock in a T-maze by choosing the left or right arm. During the first phase of the experiment, the rats in Group 1 (drug group) were trained to make one response in the presence of the drug stimulus (10 mg/kg of pentobarbital) and the other response in the absence of the drug stimulus. The rats in Group 2 (light group) were trained to make one response in the presence of the light stimulus and the other response in the dark. During the second phase, rats in both groups were trained with both drug and light stimuli relevant. During the third phase, generalization tests with various doses of pentobarbital were conducted in both the light and dark conditions in all three groups. Training with drug as opposed to nondrug discrimination attenuated the stimulus control by the light added in the second phase; training with light as opposed to darkness discrimination attenuated the stimulus control by the drug added in the second phase. Rats in Group 3 (control group) were trained to make one response in the presence of the drug stimulus and the other response in its absence, but there was no systematic relationship between the light as opposed to darkness and the conditions of reinforcement. This yielded dose-generalization gradients that were not significantly different in the light and dark conditions. The results were similar to those which demonstrate blocking in experiments using exteroceptive stimuli.

Because the major difference between drug stimuli and conventional exteroceptive stimuli, such as light and sound, is the route of administration (Catania, 1971), one would expect that many of the phenomena observed during conditioning with externally applied sensory stimuli would also be observable with internally applied drug stimuli. Indeed drug discrimination studies provide supporting evidence for this expectation. For example, drug stimuli and sensory stimuli can act concurrently to control behavior (Colpaert, Niemegeers & Janssen, 1978; Järbe, Sterner, & Hjerpe, 1981; Watanabe, 1983). Variation in the salience of drugs can be produced by variations in drug dose (i.e., intensity; see Colpaert & Janssen, 1982; Colpaert, Niemegeers, & Janssen, 1980; Overton, 1979; Swedberg & Järbe, 1982, 1985). With an unequal probability of reinforcement of a response in drug and nondrug conditions, animals have a bias to make the response with the higher probability of reinforcement (Colpaert & Janssen, 1981; De Vry, Koek, & Slangen, 1984; Koek & Slangen, 1982a, 1982b; McMillan & Wenger, 1984). Procedures similar to those used to study overshadowing with exteroceptive stimuli (Heinemann & Chase, 1975; Mackintosh, 1974) have been used to determine whether drug stimuli may overshadow exteroceptive stimuli, or vice versa (Järbe & Johansson, 1984; Järbe, Laaksonen, & Svensson, 1983). In these studies, rats were trained to discriminate between compound stimuli (such as light plus drug as opposed to darkness plus nondrug). The degree of behavioral control by the drug stimulus was positively related to the dose of the drug.

In overshadowing experiments, the compound stimulus (A and B) is presented from the beginning of conditioning. In blocking experiments, initial training occurs with one stimulus (A) and then the compound stimulus (A and B) is presented. The purpose of the present study was to test whether an exteroceptive stimulus would block a drug stimulus after initial training with the exteroceptive stimulus, and to test whether a drug stimulus would block an exteroceptive stimulus after initial training with the drug stimulus. The stimuli consisted of the presence or absence both of light and of 10 mg/kg of pentobarbital.

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The response was an escape into the left or right arm of a T-maze. During the first phase of training, rats in the experimental groups were trained on a discriminative escape task with either the drug or the exteroceptive stimulus. During the second phase of training, they were trained with a compound of both types of stimuli (such as drug plus light as opposed to nondrug plus darkness). During the third phase, the relative strengths of stimulus control on the part of the visual, exteroceptive stimuli and the drug stimuli were measured in all groups, by means of generalization tests with various dosages of drugs in combination with the two visual stimuli. The prediction was that initial discrimination training with drug cues would decrease the amount of control subsequently acquired by exteroceptive cues, and that initial discrimination training using visual cues would attenuate the amount of control later acquired by drug cues. A control group of rats discriminated between the drug and nondrug states in both exteroceptive contexts (light and darkness). Since in this group, the visual cues provided no information about which response would be reinforced, no control by either the presence or the absence of light was expected.

METHOD

Subjects

Thirty experimentally naive, male Sprague-Dawley rats were purchased from Anticimex AB (Sollentuna, Sweden). The animals were acclimated to the laboratory for 2 weeks before the beginning of discrimination training. They were housed individually, with continuous access to tap water and pellet food (type R3, Ewos, Södertälje, Sweden), a 12:12-h light:dark cycle (lights on at 7 am and off at 7 pm), a relative humidity of approximately 55%, and a temperature of 20-22° C. The mean weight of the rats at the beginning of the experiment was 357.5 g; the standard deviation was 10.5 g.

Drugs

Sodium pentobarbital was administered intraperitoneally 15 min prior to some sessions. The vehicle injections were isotonic saline. The solutions were prepared freshly each day; the concentration of all injected solutions was 1 ml/kg.

Apparatus

A T-shaped two-choice shock-escape maze was used, with a floor plan similar to that reported by Järbe & Henriksson (1974). The maze was constructed of wood and painted gray. The central start alley was 15×30 cm. A choice alley (15×15 cm) was at the junction of the central start alley and the left and right side alleys (each one 15×45 cm). An exit alley (15×2.5 cm) was connected to each side alley. An acrylic lid covered the maze; the distance from the grid floor to the lid was 7 cm. A flexible sheet of fluorescent paper hung near the middle of each side alley, which permitted the investigator to record responses in the dark when the rats brushed against the paper. Barrier doors that were not visible from the choice point could be inserted into either exit alley (or both of them). The rats could escape from the maze by jumping off the grid floor into a cage placed just beneath the grid floor at the end of the exit alley. During light sessions, a 60-W light bulb was placed 30 cm above the choice area. During darkness sessions all the lights were turned off. Thus each entire session was conducted either in the presence or absence of light, which was intended to be analogous to the sustained presence or absence of the drug stimulus. Electric foot shock (0.3 mA, AC) was delivered to the grid floor throughout the maze by a LeHigh-Valley shock generator (Model no. 113-02).

Procedure

Experimental design. The experimental design is outlined in Table 1. The three groups had 8, 10, and 10 subjects, respectively. During the first phase, the rats in the two experimental groups (Groups 1 and 2) received discrimination training with only one of the stimulus modalities (drug or light). The animals in Group 1 were trained to discriminate pentobarbital (10.0 mg/kg) as opposed to saline; the animals in Group 2 were trained to discriminate the exteroceptive conditions (light versus darkness). During the second phase, the other stimulus modality was added as a relevant discriminative stimulus. Thus, the treatment of the two experimental groups during the second phase was identical, but a different added stimulus provided the redundant discriminative cues. The rats in the control group (Group 3) were trained to discriminate pentobarbital (10.0 mg/kg) as opposed to saline in Phases 1 and 2, but the training took place in both the light and darkness conditions, which were not systematically related to the conditions of reinforcement. In Phase 3, the continuing training trials were interspersed with test trials; during the first test series, all animals received compound cues of various doses of pentobarbital (3.0, 5.6, 7.5, and 10.0 mg/kg), in either light or darkness.

Training (Phases 1 and 2). Each training session consisted of five trials. In each group, the discriminative conditions (and the correct response) alternated on successive sessions. The rats were trained once a day, between 3 pm and 6 pm, 5 days per week. In each training session, the animals were first injected (i.p.) with the appropriate solution (pentobarbital or saline), and they then remained in the colony room for about 13 min before being transferred to the experimental room, where the appropriate exteroceptive stimulus

Table 1
Interoceptive (Drug) and Exteroceptive (Light) Stimuli during Training, and the Reinforced Escape Response (Right or Left)

	Phase 1		Phase 2	
Group	Right	Left	Right	Left
1a	drug + light	saline + light	drug + light	saline + dark
1b	drug + light	saline + light	drug + dark	saline + light
2a	saline + light	saline + dark	drug + light	saline + dark
2b	saline + dark	saline + light	drug + dark	saline + light
3a	drug + (light/dark)	saline + (light/dark)	drug + (light/dark)	saline + (light/dark)
3b	drug + (dark/light)	saline + (dark/light)	drug + (dark/light)	saline + (dark/light)

Note—Groups 1 and 2 were trained to discriminate one stimulus condition in Phase 1. A second condition was added in Phase 2. The added discriminative condition (light vs. darkness in Group 1, drug vs. saline solution in Group 2) provided redundant information during Phase 2. Rats in the control group (Group 3) were not differentially trained with respect to the exteroceptive (light/darkness) stimulus condition. These rats received an equal number of light and dark sessions, each occurring twice in succession, in both the drug and saline conditions. Training conditions were the same in both Phases 1 and 2 for Group 3.

conditions had been established (light from a 60-W bulb, or total darkness). Two minutes later, the rat was placed on the electrified grid floor of the center alley with its nose pointing toward the choice area of the maze. The rat could escape from the electrified grid floor only by running to the correct goal alley, from which it could jump into a cage placed adjacent to the maze. Exit through the alley designated as incorrect was prevented by a barrier. A choice was recorded when the animal first left the choice area far enough to move one of the fluorescent papers, which were located at the midpoints of the side alleys. Unless otherwise noted, in the first two phases, the rats were trained for 20 sessions or to a criterion of a correct choice during the first trial in 8 out of 10 consecutive training sessions, whichever was greater. In Groups 1 and 3, all the rats achieved this criterion within 20 sessions. In Group 2, during the first phase (light versus darkness discrimination conditions), three rats required 27–28 sessions to achieve criterion. To keep training in the various groups somewhat synchronized, the number of sessions in the second phase was reduced to 16 for these 3 animals. In Group 2, the mean numbers of training sessions (±SEM) were 22.3 ± 1.2 sessions in the first phase and 18.8 ± 0.6 sessions in the second phase. The animals were trained in batches of 3 to 5, and the order in which they were trained was changed both within each session and between sessions to control for possible order and odor cues (Extance & Goudie, 1981).

Testing (Phase 3). The test sessions were generally scheduled three times during each 2-week period; they were interspersed between continuing training sessions. Each test session consisted of two trials, during which the rats could escape by turning to either side of the alley. Each condition (drug dose and light condition) was tested once, and the interspersed training sessions were scheduled so that each test condition was preceded by at least one training session in each stimulus condition. In the first test series, four doses of pentobarbital sodium (3.0, 5.6, 7.5, and 10.0 mg/kg) as well as saline (1 ml/kg) were used. The order of doses was randomized for each rat. After the first test series was completed, additional tests were conducted in Groups 1 and 2 in the nondrug-associated stimulus condition, with doses of 13.75 and 17.5 mg/kg—that is, doses higher than those used in training. Group 3 also received these doses: half the subjects were tested in the light and half in darkness.

RESULTS

Acquisition and Asymptotic Accuracy

Figure 1 shows the mean percentages of correct first-trial choices in the three groups during the first two phases, and during the training sessions of the third phase. (The means of Phase 3 are based on 27 to 36 training sessions for each animal.) There were no significant differences among the groups in the mean percentages of correct first-trial choices in any phase of training [$F(2,26) < 1$, $F(2,26) = 1.96$, $F(2,25) = 1.00$, $p > .05$, for the three phases].

The mean numbers of sessions (±SEM) to the beginning of criterion performance of 8 correct first-trial choices in 10 consecutive training sessions were calculated for each group in the first and second phases. In Group 1, the sessions to criterion were 7.8 (2.3), and 1.6 (0.4) in Phases 1 and 2, respectively. In Group 2, they were 10.4 (1.9) and 1.0 (0) in Phases 1 and 2, respectively. In Group 3, the value of sessions to criterion was 10.6 (2.1) in the first phase. One animal in Group 1 became sick early in training and was not included in the analysis. A second animal in Group 1 was not included because it failed to maintain reliable discriminative performance (<80% correct first-trial responding) in the third phase.

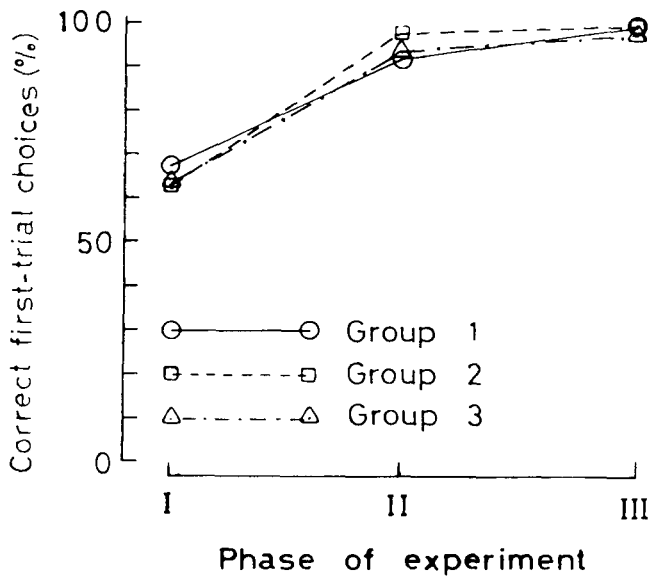

Figure 1. Mean correct first-trial choices during the three phases of the experiment.

There were no significant differences within groups in the mean number of sessions to the beginning of criterion performance. The *A* test for paired contrasts was used to compare data within the groups (cf. Järbe & Johansson, 1984; Järbe, Laaksonen, & Svensson, 1983); this is equivalent to the *t* test for pairs (McGuigan, 1965, p. 149). The value of *A* was greater than 1 for all comparisons except the contrast in Group 3 between drug mean (0.56) and saline mean (0.67), where $A = 0.39$. Thus, there were no significant differences in sessions to criterion due to drug as opposed to nondrug training sessions, or to light as opposed to dark sessions.

Within-Group Comparisons During the Test Trials of Phase 3

The effect of drug dose and visual conditions (light versus darkness) on the percentage responding to the drug position is shown for Groups 1, 2, and 3 in Figures 2, 3, and 4, respectively.

In Group 1, there was a small, but significant, difference between the number of drug-appropriate responses during tests in the drug-associated and the nondrug-associated exteroceptive test conditions ($A = 0.340$, $p < .05$; one-tailed test). A least-squares regression analysis showed that the slopes of both regression lines differed significantly from zero ($p < .01$). The results from the tests with 13.75- and 17.5-mg/kg doses in the nondrug-associated condition, as shown in Table 2, were not included in the calculation of the regression line, since these data were not on a linear portion of the line. The ED50 values were 5.1 mg/kg under the drug-associated stimulus and 6.1 mg/kg in the nondrug-associated stimu-

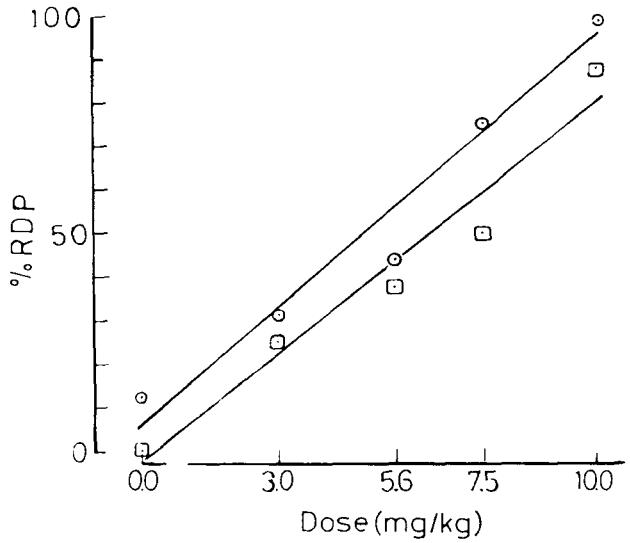

Figure 2. Mean percentage responding to the drug position (% RDP) as a function of dose of pentobarbital in Group 1 under the exteroceptive stimulus associated with the drug during Phase 2 (circles) and the exteroceptive stimulus that was associated with saline during Phase 2 (squares).

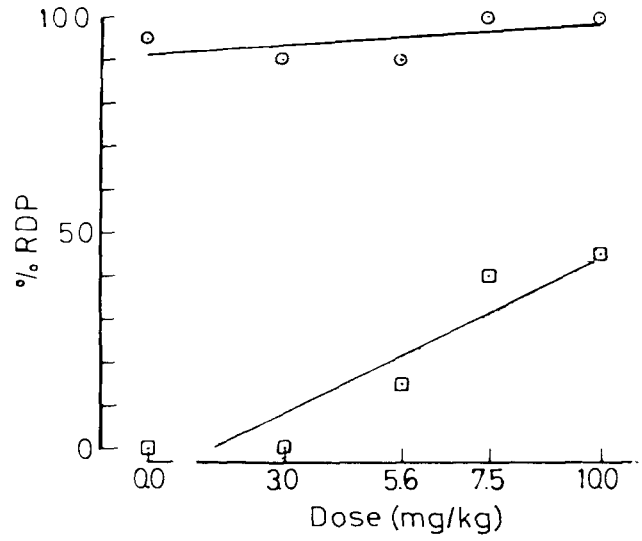

Figure 3. Mean percentage responding to the drug position (% RDP) as a function of dose of pentobarbital in Group 2 under the exteroceptive stimulus associated with the drug during Phase 2 (circles) and the exteroceptive stimulus that was associated with saline during Phase 2 (squares).

lus. These data suggest that the rats were slightly more inclined to report presence of drug in the drug-associated than in the nondrug-associated exteroceptive condition.

In Group 2, there was a significant difference in the number of drug-appropriate responses during testing in the drug-associated and nondrug-associated exteroceptive conditions ($A = 0.109$, $p < .001$; one-tailed test). The slope of the least squares regression line in Figure 3 that was fitted to the drug-associated data (circles) did not differ significantly from zero [$r = 0.573$, $t(3) = 1.210$, $p > .05$]. The slope of the regression line fitted to the nondrug-associated data (squares) differed significantly from zero [$r = 0.934$, $t(3) = 4.513$, $p < .05$; see Hays, 1963, p. 521]. The ED50 was 10.0 mg/kg for the nondrug-associated condition. When results from tests with 13.75 and 17.5 mg/kg of pentobarbital in the nondrug-associated condition (see Table 2) were included in the regression calculation, the slope of the regression line steepened [$r = 0.968$, $t(5) = 8.559$, $p < .001$]. The ED50 estimate was 9.0 mg/kg for these nondrug-associated data. The ED50 was not calculable for the drug-associated test condition since the line did not cross the 50% level. These data suggest a clear difference in the control of choice behavior by the pentobarbital stimulus in the drug-associated and the nondrug-associated exteroceptive conditions.

There were no drug-associated or nondrug-associated exteroceptive stimuli in Group 3 (control), so Figure 4 shows separately the mean percentages of drug-position responses in tests conducted in the light and in the dark. Results of the A test were not significant ($A = 10.5$, $p > .05$). The two regression lines fitted to these data were almost identical, and the slopes of both lines were significantly different from zero ($p < .002$). Data from tests with the 13.75 and 17.5 mg/kg of pentobarbital (see Table 2) were not included in the regression calculations. The ED50 estimates were 4.1 mg/kg (light) and 4.2 mg/kg (darkness).

Between-Group Comparisons during the Test Trials of Phase 3

The mean number of drug-appropriate choice responses by rats in the drug-associated and nondrug-associated test conditions (light and darkness in Group 3) was calculated during tests with pentobarbital doses of 0, 3, 5.6, 7.5, and 10 mg/kg. Since there was an unequal number of subjects per group, a two-way unsystematic analysis of variance was used (Snedecor & Cochran, 1967; p. 338). There were differences among the groups [$F(2,50) = 17.5$, $p < .01$]. The mean square within was used as the error term for determining the critical value in Dunn's equation, and we limited the number of comparisons to six (i.e., $C = 6$; see Kirk, 1968, pp. 79-81). The pattern of results is shown in Figure 5, and the following

Table 2
Tests with Doses Higher Than the Training Dose

Group	N	Dose (mg/kg)	%RDP
1	8	13.75	87.5
		17.5	100.0
2	10	13.75	95.0
		17.5	100.0
3	10	13.75	100.0
		17.5	100.0

Note—% RDP = percentage of responding directed towards the drug-(pentobarbital) associated position of the T-maze. The test protocol here was a simple cross-over design, and testing occurred only in the saline-associated exteroceptive condition (Groups 1 and 2). Since saline- and drug-associated had no meaning in Group 3, half the number of tests were carried out in the dark and the other half when the maze was lighted.

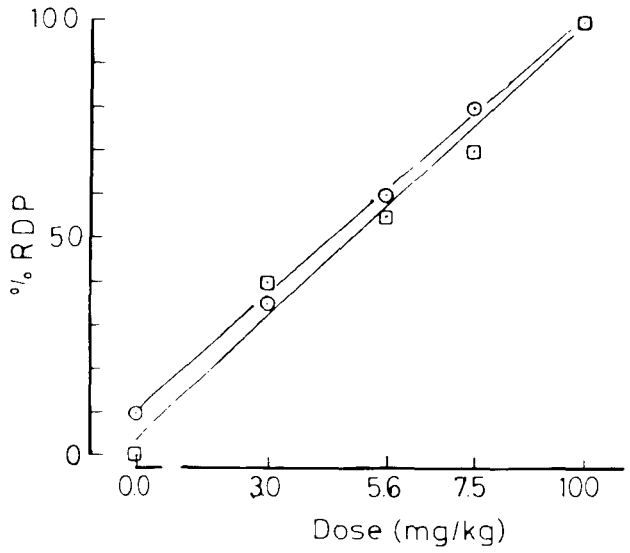

Figure 4. Mean percentage responding to the drug position (% RDP) as a function of dose of pentobarbital in Group 3 in the light (circles) and the dark (squares).

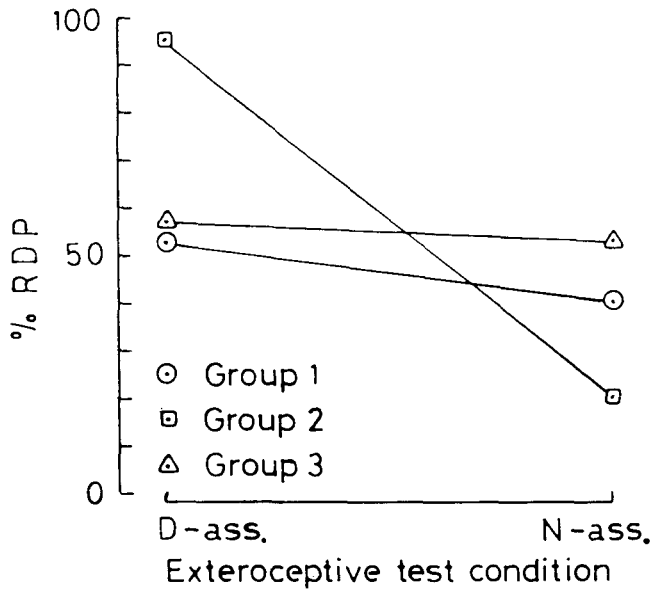

Figure 5. Summary of results. Mean percentage of responding to the drug position (% RDP) as a function of the association of the exteroceptive stimulus with the drug (D-ass.) and nondrug (N-ass.) conditions.

conclusions were reached with the probability of a Type 1 error set at .05: In the drug-associated condition, Group 1 differed significantly from Group 2, and Group 2 differed from Group 3; but Group 1 was not significantly different from Group 3. In the nondrug-associated condition, Group 2 differed significantly from Group 3, but Group 1 did not differ from either of them.

DISCUSSION

Blocking clearly occurred. In Group 1, in which initial training involved either drug or no drug, the subsequently added stimulus of light as opposed to darkness acquired little discriminative control (Figure 1). In comparison, in Group 2, in which the initial training involved light or darkness, the subsequently added drug as opposed to nondrug discriminative conditions exerted only weak control. The control group (Group 3) showed discriminative control only for the drug as opposed to no-drug conditions. An additional possible control group, trained to discriminate light as opposed to darkness with the drug and nondrug conditions randomly related to reinforced choice, was not included in the present experiment.

Blocking is most easily observed if the relative salience of the initially and subsequently introduced pairs of discriminative stimuli are approximately matched. The 10-mg/kg dose of pentobarbital as opposed to saline stimuli used in the present experiment may have been slightly more salient than the stimuli of light and darkness used in the present experiment. During the first phase, the group with the drug as the discriminative stimulus (Group 1) learned the discriminative escape response more rapidly than the group with light as the discriminative stimulus (Group 2). In addition, a comparison of Figures 2 and 3 suggests that initial acquisition of the drug discrimination (Group 1) almost completely prevented subsequent acquisition of the light discrimination, but initial acquisition of the light discrimination (Group 2) only partially prevented subsequent acquisition of the drug discrimination. The only result not in accord with the hypothesis that the drug was the more salient stimulus was that during the first phase, the mean percentages of correct responses were approximately the same for subjects trained on the drug discrimination (Groups 1 and 3) and on the light discrimination (Group 2).

The present results can be contrasted to those of a previous study (Järbe, Svensson, & Laaksonen, 1983) that had a design similar to the present one, but with a higher dose of pentobarbital (17.5 mg/kg). In that study, the light as opposed to darkness discrimination conditions in Group 1 exercised no significant stimulus control, and the drug as opposed to no-drug stimulus conditions in Group 2 exercised a relatively greater degree of discriminative control than was observed in the present data. Apparently, the relationship between salience and dose obtained in overshadowing experiments involving externally and internally applied stimuli (Järbe, Laaksonen, & Svensson, 1983; Järbe & Johansson, 1984) also occurs in blocking experiments involving these same types of stimuli (Järbe, Svensson, & Laaksonen, 1983; cf. the present experiment).

In Group 2, during tests with doses higher than the training dose in the nondrug-associated exteroceptive condition, there was a dose-related increase in the percent-

age of drug choices. This indicates that the drug could acquire complete control over choice behavior. Apparently, the more intense drug effects overrode control by the exteroceptive cues. Although the designs differ greatly, this is reminiscent of Kamin's (1969) finding that unblocking occurred when the intensity of the added stimulus was increased. Thus, the seeming inattentiveness to the added stimulus was shown to be restricted to the initially conditioned stimulus intensity (see also Swedberg, 1985).

During tests with low doses of the drug (0 and 3 mg/kg), nearly all of the responding of the animals in Group 2 was controlled by the presence or the absence of the light. At higher doses, control of the drug stimulus over responding increased. This was evident only in the nondrug-associated condition, because there was a ceiling effect in the drug-associated condition (cf. Järbe & Johansson, 1984). This led to the regression lines in Figure 3 that are not parallel. In contrast, parallel regression lines occur when rats learn discriminations involving two drug stimuli of equal salience and the present exteroceptive cues of light and darkness (Järbe, Swedberg, & Hiltunen, 1984).

The present data, in conjunction with previous results, show that drug stimuli and exteroceptive stimuli interact in a predictable manner with respect to the control over behavior.

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