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Drug Discrimination in Rats Successively Trained to Discriminate Diazepam and Pentobarbital[1]

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NIERENBERG, J. AND N. A. ATOR. *Drug discrimination in rats successively trained to discriminate diazepam and pentobarbital.* PHARMACOL BIOCHEM BEHAV **35**(2) 405–412, 1990.—In Phase 1, rats were trained to discriminate either diazepam or pentobarbital from the no-drug condition. Diazepam, pentobarbital, triazolam, meprobamate, and zopiclone occasioned 100% drug-lever responding in tests under both training conditions; but the generalization gradients determined under the pentobarbital training condition were shifted to the right of those determined under the diazepam training condition. In Phase 2, the training drugs were reversed for the two groups, as well as which lever was paired with drug or no drug, in an effort to produce greater specificity of the Phase 2 discrimination. In Phase 2 tests, the Phase 1 training drug occasioned responding on the Phase 2 drug lever in all rats, suggesting that retraining overrode the Phase 1 discrimination. There were indications, however, that Phase 1 training influenced Phase 2 responding: 1) Rats ceased responding partway through no-drug training sessions using the former drug lever, and criterion performance was somewhat more difficult to maintain in Phase 2. 2) In Phase 2, dose-effect curves determined under pentobarbital training were shifted even further to the right of those determined under diazepam training than in Phase 1.

Drug discrimination Behavioral history Pentobarbital Diazepam Zopiclone Triazolam Meprobamate Rats

IN the attempt to characterize differences between the discriminative stimulus effects of drugs which substitute for each other after drug (D) versus no-drug (ND) discrimination training, investigators have turned to alternative methodologies aimed at highlighting such differences. Rats can be trained to discriminate between closely related drugs (e.g., D versus D discriminations) such as pentobarbital versus chlordiazepoxide (11), even though both drugs usually occasion the "drug response" when either drug is discriminated from the ND condition. Overton (15) showed that the specificity of the phenobarbital cue, in terms of those compounds which occasion the phenobarbital response, can be systematically varied depending upon the types of compounds which are paired with the "other" lever during discrimination training.

In a different context, multiple drug discrimination training using dissimilar drugs can broaden the range of drugs occasioning the "drug response." Using a two-lever D vs. ND procedure, Overton, Merkle and Hayes (16) found that a drug which had acquired discriminative control (termed D1) continued to do so even after later retraining with a pharmacologically unrelated compound (D2). Retraining did not disrupt the original discrimination; instead, both D1 and drugs from the same classes as D1 and D2 occasioned drug-lever responding after retraining while drugs from other classes occasioned responding on the no-drug lever. Thus, the rats learned specifically to discriminate the presence or absence of each training drug.

The present study employed a successive D versus ND training procedure, but with D1 and D2 from the same general pharmacological class, that is, the depressants pentobarbital and diazepam. In previous drug discrimination research, pentobarbital occasioned drug lever responding in rats, monkeys, and pigeons trained to discriminate diazepam from ND and vice versa (7–9, 18). The present study used a cross-over design in which for half the rats diazepam was D1 (i.e., the training drug in Phase 1) and pentobarbital was D2 (i.e., the training drug in Phase 2); for the other half, pentobarbital was D1 and diazepam was D2. In the study by Overton *et al.* (16), the designated drug lever was not changed when the rats were retrained with D2. In the present study, however, in order to be able to observe whether D1 would continue to control responding after training with D2, the ND lever from Phase 1 became the D lever for Phase 2. It was hypothesized that if the original discrimination were not disrupted by retraining, administration of D1 in Phase 2 would occasion responding on the

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original drug lever. In other words, prior training with D1 would have increased the specificity of the D2 discrimination by producing a D2 versus D1 or N discrimination.

A second purpose of the study was to determine generalization to other sedative/anxiolytic drugs before and after retraining with D2. Triazolam (an hypnotic benzodiazepine), meprobamate (a classic nonbenzodiazepine anxiolytic), and zopiclone (a nonbenzodiazepine hypnotic that binds the benzodiazepine receptor) were chosen because it has been shown that these compounds occasion the drug response in rats trained with sedative-hypnotic drugs. Previous studies have shown that pentobarbital-trained rats showed generalization to meprobamate (5,12), triazolam (10), and zopiclone (4). In previous studies with diazepam-trained rats, generalization to triazolam (17) and partial generalization to meprobamate (13) were indicated, but data on zopiclone have not been reported.

In the event that the original discrimination from Phase 1 remained intact in Phase 2 (i.e., D1 still occasioned responding on the D1 lever), it was of interest to determine whether meprobamate, triazolam, and zopiclone would occasion responding on the "benzodiazepine" or "barbiturate" lever. In the event that the original discriminations were disrupted in Phase 2 (i.e., responding after high D1 doses occurred on the D2 lever), it was of interest to determine whether the generalization gradients differed as a function of the previous training condition.

METHOD

Subjects

Six male Sprague-Dawley rats (*Rattus norvegicus*; Blue Spruce Farms, Altamont, NY) were individually housed with a 12-hr light/dark cycle. The rats were experimentally naive and 8 to 10 weeks of age at the beginning of drug discrimination training. Weights were permitted to increase gradually and then held stable at 340 ± 10 g by feeding daily rations of rat chow (15 to 20 min after experimental sessions).

Apparatus

The apparatus has been described in detail previously (3). Six experimental chambers were used. Two rodent levers were mounted 13 cm apart and 5 cm above the grid floor; identically colored jewel lights (GE 1828, 37.5 V, 0.05 amp) were centered over each lever. A food cup was centered on the opposite wall. A pellet feeder delivered 45-mg food pellets. White noise served to mask extraneous sounds. Experimental conditions were controlled and data collected by a PDP8A computer programmed in SUPERSKED®.

Training Sessions

Each rat was assigned to an individual chamber, in which all experimental sessions were conducted. In Phase 1, rats (R) were trained to discriminate the no-drug condition (ND1) from D1, which was either diazepam 1.0 mg/kg IP (R1, R2, and R3) or pentobarbital 10.0 mg/kg IP (R4, R5, and R6). Sessions were preceded by a 15-min presession timeout that served as the drug pretreatment time. During timeout the chambers were dark and lever responses were counted but had no programmed consequences. At the end of the presession timeout, the two jewel lights (which were the sole source of chamber illumination) were turned on. Illumination of the house lights was correlated with food pellet availability. In Phase 1, the right lever was the D1 lever for R1, R2 and R3, and the left lever was the D1 lever for rats R4, R5, and R6. Lever pressing was shaped on the ND1 lever for each rat and the response requirement was gradually increased from 1 to 10. Training sessions with D1 began with the response requirement again at 1 and shaping was used, if necessary, to obtain responding on the other lever. Alternation of D1 and ND1 training sessions began after two or three consecutive D1 sessions, with the response requirements again increasing to 10 across sessions. A brief timeout was introduced after each pellet delivery and lengthened to 10 sec. Under the final training conditions, food delivery depended upon completion of 10 consecutive responses on the lever appropriate to the D1 or ND1 training condition in effect. Responses on the alternate lever reset the response requirement. Sessions were 20 min long and were conducted daily, Monday through Friday. The ND training sessions were conducted without vehicle injections because previously it was found that the injection procedure per se does not serve as a basis for D/ND discriminations under a two-lever procedure (2,14). Instead, test sessions with vehicle injections confirmed that the injection procedure per se did not occasion the D response.

In Phase 2, the training conditions were reversed for the two groups. Training procedures were comparable to those used at the beginning of Phase 1, except that shaping was not necessary in five of six rats, and D and ND sessions alternated from the beginning, with response requirement beginning at 1 and increasing to 10 across sessions. The rats (R1, R2 and R3) previously trained to discriminate diazepam as D1 were retrained to discriminate pentobarbital 10.0 mg/kg as D2 from the no-drug condition (ND2), and the rats (R4, R5, and R6) previously trained to discriminate pentobarbital as D1 were retrained to discriminate diazepam 1.0 mg/kg as D2 from the no-drug condition (ND2). These training doses were chosen not only because they had been used in Phase 1 but also because under test conditions in Phase 1, pentobarbital 10 mg/kg occasioned D1-lever responding in all diazepam-trained rats and diazepam 1.0 occasioned D1-lever responding in all pentobarbital trained rats. In Phase 2, D2 was paired with the lever that had been the ND1 lever in Phase 1; thus, the D1 lever from Phase 1 became the ND2 lever in Phase 2.

In Phase 1, a diazepam-trained rat (R1) began showing unreliable training session performance. It seemed possible that this was due to poor drug absorption resulting from frequent IP injections with the viscous diazepam vehicle (see below); tests with oral diazepam (1.0 mg/kg) in the diazepam-trained rats showed criterion level performance. A similar problem had occurred with IP lorazepam previously and the problem had been resolved by changing route of administration to PO (1). For consistency in training conditions, diazepam was administered PO in diazepam training sessions that followed completion of the diazepam dose-effect curves for diazepam-trained rats in both Phases 1 and 2 (see Table 1).

In Phase 2, two (R4, R6) of the three rats for which diazepam was D2 did not reliably meet the training criteria in training sessions interspersed between tests (see Results) and procedural manipulations were attempted to reinstate criterion performance. For R4, the response requirement was permanently increased from 10 to 15 (during the diazepam dose-effect curve), with no change in training session response rates. For R6, this manipulation (during the triazolam dose-effect curve) did not reinstate criterion performance and the response requirement remained 10.

Test Sessions

Experimental conditions during test sessions were identical to those in training sessions except that completing the required number of consecutive responses on either lever produced food. At the beginning of Phases 1 and 2, the first test sessions conducted were with the training drug dose and its vehicle. These tests were conducted after the response requirement had been raised to 10 and criterion performance had been shown in four consecutive training

sessions across which D and ND sessions alternated. A training session was said to meet criterion if: 1) 96–100% of the total responses and 98–100% of the total completed ratios were on the reinforced lever, and 2) at the beginning of the session, the first *five* completed ratios were completed on the reinforced lever. When criterion performance was shown in the four consecutive training sessions and in the two test sessions with vehicle and the training dose, then tests with novel doses of the training drug began. During Phase 1, sessions were scheduled according to the sequence ND, D, T, D, T, D, ND, T, ND, T. During Phase 2, sessions were scheduled according to the sequence ND, D, T, D, ND, T, ND, D, T. After dose-effect determinations were completed with a drug, criterion performance had to occur in two consecutive D and ND training sessions and then also in test sessions with the training drug dose and with the vehicle for the next test drug before dose-effect determinations with doses of the novel drug began. Whenever criterion performance was not shown, the next test session did not occur until the performance criteria were met in four consecutive training sessions.

In Phase 1, after dose-effect curves were determined for D1, pentobarbital dose-effect curves were determined for the diazepam-trained rats and diazepam dose-effect curves were determined for the pentobarbital-trained rats. Tests with triazolam, zopiclone, and meprobamate then followed. Doses were given in a generally ascending order for each rat. In Phase 2, dose-effect curves were determined in the same sequence as in Phase 1 (i.e., tests with novel doses of the new training drug, D2, were conducted first, followed by tests with the other drugs). With all drugs, repeated test sessions were conducted with intermediate drug doses to obtain a better estimate of the drug stimulus threshold, in view of the small N for each group.

Drugs

The following drugs were kindly donated: diazepam (Hoffmann-La Roche, Inc., Basel, Switzerland), meprobamate (Wyeth Laboratories, Philadelphia, PA), triazolam (Upjohn Co., Kalamazoo, MI), and zopiclone (Rhone-Poulenc, Vitry-sur-Seine, France). Pentobarbital sodium was purchased from Abbott Laboratories, Chicago, IL and Sigma Chemical Co., St. Louis, MO. All drugs were administered IP except that, as described above, diazepam was administered IP during initial training and PO in later training, but not test, sessions (see Table 1). Oral diazepam was administered 5 min before the 15-min presession timeout, while all other drugs were administered immediately before the presession timeout.

The vehicle for IP meprobamate and PO diazepam was a cornstarch suspension (3% cornstarch, 5% polyethylene glycol 200, 0.336% Tween 80, q.s. sterile water). The other vehicles were: 0.9% saline for pentobarbital; 1% lactic acid in saline for zopiclone; 40% propylene glycol, 10% ethyl alcohol, and 50% sterile water for IP diazepam; 50% each of propylene glycol and saline for triazolam. All drug solutions or suspensions were prepared immediately before administration except that, based on previous findings, triazolam and IP doses of diazepam were maintained for up to one month in the vehicle without the aqueous component, and up to 5 days after dilution. Volume of delivery was 1.0 ml/kg. Doses are expressed in terms of the free drug, except for pentobarbital which is in terms of the salt.

Data Analysis

The distribution of responses between the two levers was expressed as the percentage of total session responses that occurred on the drug lever, excluding responses during timeouts.

TABLE 1

SESSIONS TO CRITERION (STC), RELIABILITY OF CRITERION PERFORMANCE IN SUBSEQUENT TRAINING SESSIONS, AND RESPONSE RATES (R/SEC) UNDER EACH TRAINING CONDITION IN PHASES 1 AND 2

			% Sessions Not Meeting Criterion*		Mean Response Rates (± SD)	
Rat	Phase	STC	ND	D	ND	D
			Diazepam Training Conditions†			
1	1	23	11	15	2.1 (0.3)	1.7 (0.1)
2	1	21	3	14	3.9 (0.5)	3.0 (0.5)
3	1	31	29	13	2.7 (0.4)	2.4 (0.2)
4	2	20	51	32	3.6 (0.2)	4.5 (0.6)
5	2	34	15	30	0.9 (0.1)	1.5 (0.4)
6	2	23	14	36	2.9 (1.3)	2.0 (0.7)
			Pentobarbital Training Conditions			
1	2	38	3	20	0.8 (0.3)	0.8 (0.1)
2	2	27	6	6	1.4 (0.9)	0.8 (0.6)
3	2	40	3	9	1.3 (0.6)	0.7 (0.2)
4	1	21	6	22	3.2 (0.4)	2.4 (0.3)
5	1	23	3	9	2.6 (0.7)	2.2 (0.5)
6	1	20	12	3	3.5 (0.5)	3.6 (0.5)

*For training sessions after those included in the STC measure. The range of sessions used to calculate individual ND and D percentages was 30–67 in Phase 1 and 53–121 in Phase 2.

†Change from IP to PO diazepam in training sessions (see text) occurred after the following dose-effect curves: R2 and R5—diazepam, R4 and R6—pentobarbital, R1—triazolam, R3—zopiclone.

Percentage of drug-lever responses in a test session was not included in the figures or in calculations of the group mean for that dose unless at least one reinforced sequence of responses was completed. Response rates were calculated for total session responses on both levers, excluding timeouts. The ND training sessions immediately preceding test sessions served as control sessions for evaluating drug effects on response rates. Two indices of discriminability were computed for each rat. The number of training sessions to criterion (STC) was scored beginning with the ND session prior to the first D session (i.e., from the onset of discrimination training). The STC measure includes all training sessions until criterion performance was shown in four consecutive training sessions, with the response requirement at 10, in which D and ND sessions alternated, but excludes any sessions in which shaping procedures were used or in which no responding occurred. After the training criteria were met initially in each phase, performance reliability was quantified by calculating the percentage of total training sessions in which criterion performance did not occur. Individual dose-response curves were approximated by least-squares regression lines from which the slope (M) was calculated and the ED_{50} inferred for statistical analyses. Group means for these measures were compared using two-tailed *t*-tests.

RESULTS

Training Session Performance

Table 1 presents the STC and reliability of criterion performance measures for all rats in Phases 1 and 2. In Phase 1, there was no significant difference between diazepam- and pentobarbital-trained rats in either of these measures. In Phase 2, the mean

STC for rats (R4, R5, R6) retrained with diazepam (26) did not differ from that for rats (R1, R2, R3) retrained with pentobarbital (25) nor from the STC for diazepam training in Phase 1 (Table 1). The mean STC (35) when rats (R1, R2, R3) were retrained with pentobarbital was significantly different, however, from the STC (22) when pentobarbital had been D1, $t(4)=3.3$, $p=0.03$. Once retrained, however, performance of the rats for which pentobarbital was D2 was quite reliable in training sessions interspersed between test sessions (Table 1). In contrast, the rats (R4, R5, R6) for which diazepam was D2 showed significantly more unreliable performance in training sessions between tests compared to their own performance in Phase 1, $t(2)=15.6$, $p=0.004$. Further analysis showed that the probability of not meeting criterion in D sessions was greater in Phase 2 compared to Phase 1 for R4, R5, and R6, $t(2)=3.2$, $p=0.08$, but comparison of ND sessions across the two phases showed no difference. Comparison of the pentobarbital and diazepam training conditions in Phase 1 showed that probability of meeting criterion in the training sessions between tests did not differ, but in Phase 2, when this measure was evaluated for pentobarbital- and diazepam-trained rats, the performance of the diazepam-trained rats was significantly more unreliable than that of the pentobarbital-trained rats, $t(4)=3.6$, $p=0.02$. Again, this difference resulted from poorer performance in D2 sessions for the diazepam-trained rats compared to the D2 sessions for the pentobarbital-trained rats, $t(4)=4.6$, $p=0.01$. Finally, reliability of training session performance in D sessions was significantly less when diazepam was D2 than when it was D1, $t(4)=10.1$, $p=0.0005$, but not when pentobarbital as D1 was compared with pentobarbital as D2.

Another way in which Phase 2 training session performance differed from Phase 1 performance was that response rates (Table 1) were markedly lower in ND2 sessions than in ND1 sessions for four rats (R1, R2, R3, R5). Moreover, during ND2 training sessions (in which the ND2 lever was the former D1 lever), three rats (R1, R3, R5) developed an unusual but characteristic response pattern. That is, 5 to 15 min of steady responding on the ND2 lever was followed by several (usually <10) responses on the D2 lever, and then responding ceased entirely. This pattern did not occur in any training sessions in Phase 1, nor did it occur in D2 training sessions; and, as noted above, ND2 session performance reliably met the training criterion.

Test Session Performance

For each drug, the lowest dose at which there was predominantly (>50%) drug-lever responding for an individual rat will be referred to as the cross-over dose. At cross-over doses of some of the drugs studied in both Phases 1 and 2, performance seemed to reflect a drug stimulus at threshold [cf. (1)]. Individual rats frequently completed response requirements on both levers; or, if multiple determinations were done at a dose, the rat might have responded 100% on the drug lever on one occasion and 0% on the drug lever on another occasion. Response rates at cross-over doses typically were similar to those in ND control sessions. Responding on both levers also occurred, but much less often, at high drug doses; usually, but not always, response rates were greatly decreased at those high doses.

Diazepam and Pentobarbital Generalization

When rats were both trained and tested with diazepam, all showed predominantly drug-lever responding at doses 0.25 to 0.5 $\log_{10}$ units below the 1.0 mg/kg diazepam training dose, regardless of whether diazepam was D1 or D2 (Fig. 1, open and closed squares). The mean ED_{50} (mg/kg, ±SE) when diazepam was D1

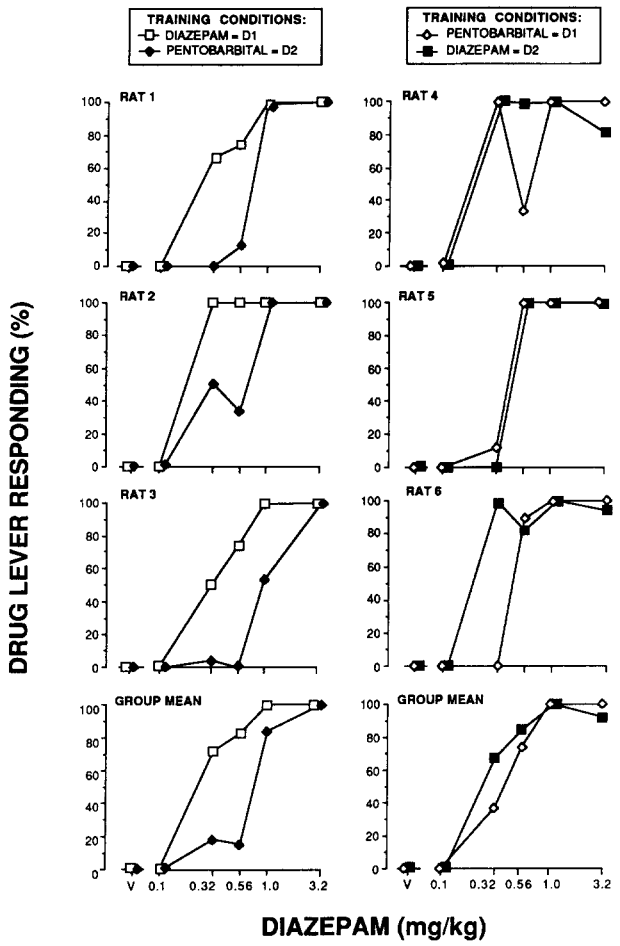

FIG. 1. Diazepam generalization. Percentage of total responses on the drug lever in test sessions after diazepam or its vehicle (V) under pentobarbital (10 mg/kg) and diazepam (1.0 mg/kg) training conditions. The dose-effect curve was determined twice for each rat, once during Phase 1 (open symbols) in which the rat was trained to discriminate a drug (D1) from the no-drug (ND1) condition and again in Phase 2 (closed symbols) after the same rat was retrained to discriminate another drug (D2) from the ND2 condition. (Under the D2 training condition, the ND1 lever was paired with D2 and the D1 lever became the ND2 lever.) Each point represents responding in a single test session, except that multiple determinations were made at some doses and the point represents the mean of two to four determinations (D1: 0.32, 0.56 mg/kg—R1, R3, R4; D2: 0.32, 0.56—R2, R6; 1.0—R1, R3, R4, R6; 3.2—R4, R6).

was 0.24 (0.04); it was 0.26 (0.07) when diazepam was D2. When rats were trained and tested with pentobarbital, the cross-over dose was more variable among rats, regardless of whether pentobarbital was D1 or D2, but most rats showed predominantly drug-lever responding at a dose less than 0.25 $\log_{10}$ units below the 10 mg/kg training dose (Fig. 2, open and closed diamonds). The mean ED_{50} (±SE) when pentobarbital was D1 was 6.3 (1.5); it was 7.0 (1.4) when pentobarbital was D2.

When rats were trained with pentobarbital and tested with diazepam, the diazepam gradients were shifted to the right of those determined under the diazepam training condition for one rat (R6) for which pentobarbital was D1 (R6) and for all three rats for which pentobarbital was D2 (Fig. 1). For these three rats (R1, R2, R3), the mean ED_{50} (mg/kg, ±SE) for diazepam under diazepam

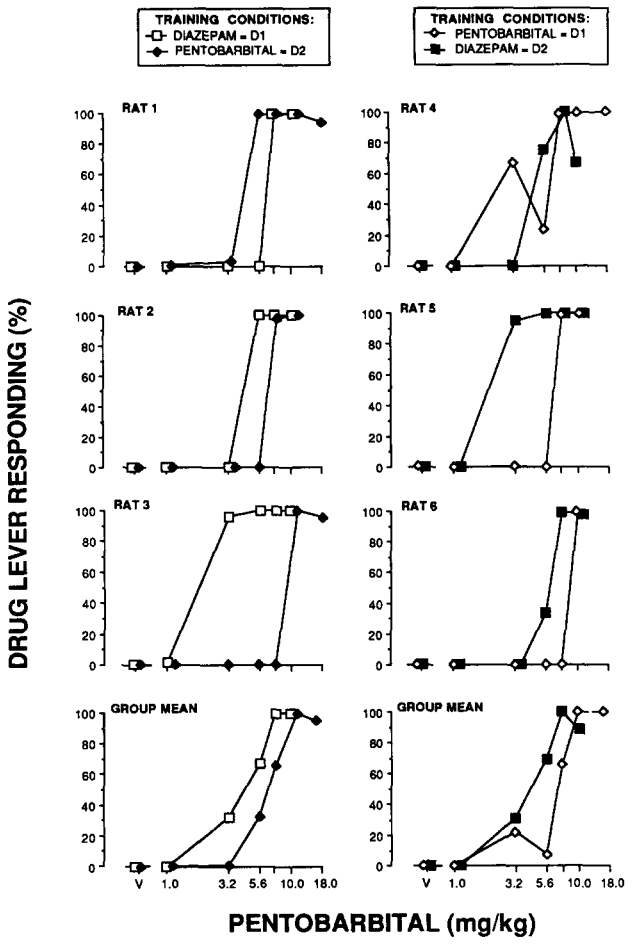

FIG. 2. Pentobarbital generalization. Percentage of total responses that were on the drug lever in test sessions after pentobarbital or its vehicle (V) under pentobarbital (10 mg/kg) and diazepam (1.0 mg/kg) training conditions. Multiple determinations (two to four) were made at some doses (D1: V—R1; 1.0—R4; 3.2 and 5.6—R1, R4; 7.8—R6; D2: 3.2—R5; 5.6—R2, R4, R6; 7.8—R2, R4; 10.0—R4). All rats were tested at 18 mg/kg under both D1 and D2 training, but percentage of drug lever responding was not included in the graphs for a test session if the rat did not complete a sufficient number of consecutive responses on one lever to obtain at least one pellet. All other details are as for Fig. 1.

(D1) training was 0.24 (0.04) compared to 0.76 (0.39) under pentobarbital (D2) training. Although these values are not significantly different ($p>0.05$) from each other, comparison of mean percentages of drug-lever responding at intermediate diazepam doses shows that drug-lever responding was significantly less under pentobarbital (D2) training than it was under diazepam (D1) training [0.32 mg/kg: $t(2)=8.31$, $p=0.01$; 0.056 mg/kg: $t(2)=21.0$, $p=0.002$]. The other three rats (R4, R5, R6) also showed less drug lever responding, on the average, under pentobarbital (D1) training than under diazepam (D2) training, but the mean ED_{50} ($\pm$SE) for diazepam under pentobarbital (D1) training, 0.34 (0.03), was not much higher than it was under diazepam (D2) training, 0.26 (0.07); and mean percentage of drug lever responding at intermediate diazepam doses for these rats did not differ ($p>0.05$) between pentobarbital and diazepam training conditions.

Only under Phase 2 pentobarbital training (i.e., in rats with a diazepam training history) did diazepam generalization reveal considerable differences between it and the other training conditions. As noted, the mean diazepam ED_{50} value when pentobarbital was D2 was approximately three times the values obtained under diazepam (D1 or D2) training and was more than two times the value observed when pentobarbital was D1. Percentage of drug lever responding at 0.56 mg/kg was also significantly lower under pentobarbital (D2) training than it was under both diazepam training in Phase 2, $t(4)=6.8$, $p=0.003$, and pentobarbital training in Phase 1, $t(4)=2.6$, $p=0.06$. Interestingly, on both occasions on which one of these Phase 2 pentobarbital-trained rats (R3) was given the diazepam dose (1.0 mg/kg) that had been the D1 training dose, this rat: 1) distributed responses evenly across both levers, receiving an almost equal number of pellets after responding on both levers, and 2) distributed responding such that following the first 5 minutes of the session, the ratio requirement was constantly reset and no further pellets were received.

When rats were trained with diazepam and tested with pentobarbital, the pentobarbital gradients were generally to the left of the pentobarbital gradients determined under the pentobarbital training condition (Fig. 2, squares). There was, however, no apparent contribution of previous training condition to this effect. The mean ED_{50} (mg/kg, $\pm$SE) for pentobarbital when diazepam was D1 was 4.5 (1.4); it was 3.7 (1.4) when diazepam was D2. These values were not significantly different ($p>0.05$) from each other or from ED_{50}'s under pentobarbital (D1 or D2) training (given above).

Slopes and ED_{50} values from regression analysis of diazepam and pentobarbital generalization gradients were consistently (i.e., for 70 to 75 of the cases), though not significantly, higher when pentobarbital was the training drug. Ratios of the training dose to training drug ED_{50} (i.e., 1.0/diazepam ED_{50}, for rats currently trained with diazepam; 10.0/pentobarbital ED_{50}, for rats currently trained with pentobarbital) were calculated as measures of quantitative specificity of the diazepam and pentobarbital discriminations. The value of this ratio is said to decrease with increasing specificity. In both Phases 1 and 2, group mean ratios for diazepam (4.4 and 4.1, respectively) were significantly higher than for pentobarbital (1.6 and 1.4, respectively) [Phase 1, $t(4)=3.2$, $p=0.03$; Phase 2, $t(4)=3.2$, $p=0.04$].

The lowest dose of diazepam to decrease response rates to less than 50% of the control (ND response rate means) was 3.2 mg/kg in both Phases 1 and 2. Pentobarbital at 18 mg/kg decreased response rates virtually to zero in all rats in both Phases 1 and 2, regardless of training drug. Thus, the primary effect of training drug condition and, arguably, training drug history was on the probability of drug-lever responding rather than response rate.

Triazolam

The generalization gradients for triazolam (Fig. 3) under the pentobarbital training condition (diamonds) were to the right of those obtained under the diazepam training condition (squares) in five of six rats, regardless of whether pentobarbital was D1 or D2. Mean percentage of drug-lever responding at 0.032 mg/kg triazolam in the rats for which pentobarbital was D2 was 0%, while the means at that dose under both diazepam training conditions were 100%. Response rates generally were decreased below ND control ranges at triazolam doses of 0.032 or 0.1 mg/kg.

Zopiclone

Zopiclone occasioned drug-lever responding in all six rats in Phase 1 and in three of the four rats tested in Phase 2 (Fig. 4). All generalization gradients determined under diazepam training were to the left of those determined under pentobarbital training.

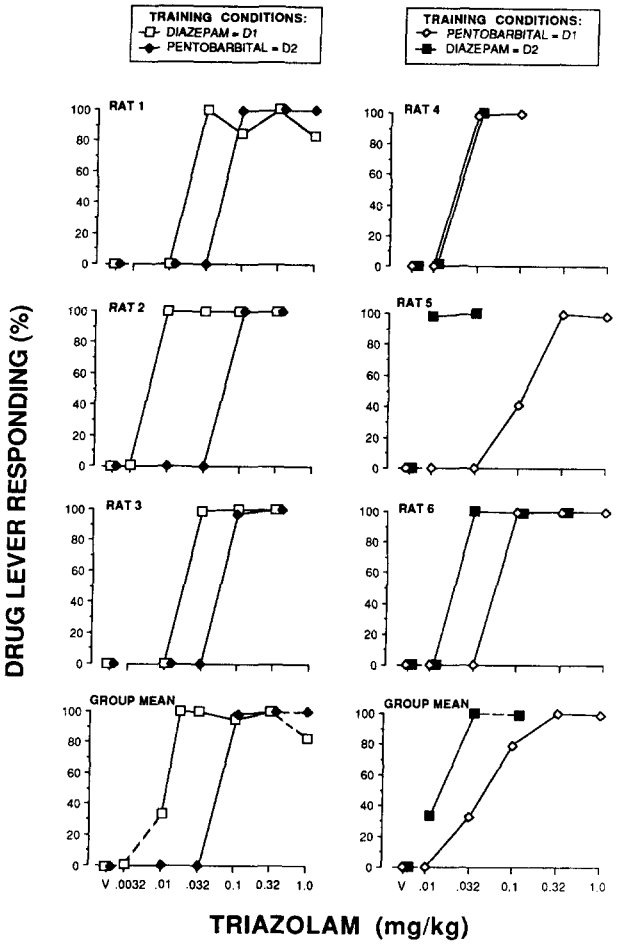

FIG. 3. Triazolam generalization. Percentage of total responses that were on the drug lever in test sessions after triazolam or its vehicle (V) under pentobarbital and diazepam training conditions. The points for individual rats represent single test sessions except that multiple determinations were made at some doses (D2: 0.032—R1, R2, R3; 0.1—R1, R3; 0.32—R2, R3). At some doses the number of consecutive responses sufficient to obtain at least one pellet was not made and no point is included in the graphs (D1 condition: 0.32—R4; D2 condition: 0.1—R4, R5). Dashed lines in the group mean curves indicate that data are only from one rat. All other details are as for Fig. 1.

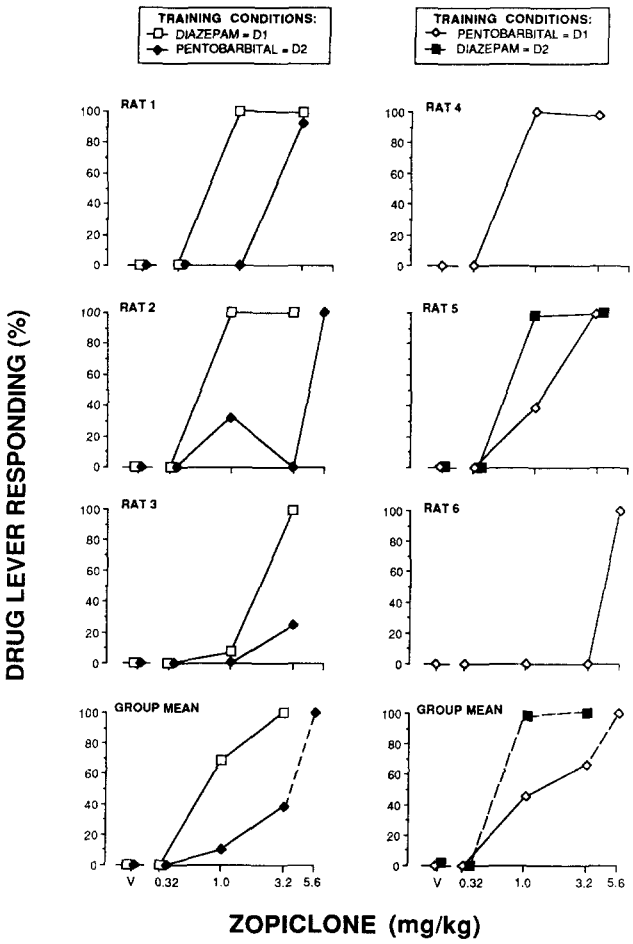

FIG. 4. Zopiclone generalization. Percentage of total responses that were on the drug lever in test sessions after zopiclone or its vehicle (V) under pentobarbital and diazepam training conditions. The points for individual rats represent single test sessions except that multiple determinations were made at some doses (D1: 0.32 and 1.0—R1, D2: 1.0—R1, R2, R5; 3.2—R1, R2, R3). R3 was given 5.6 mg/kg under D2 training, but no responses were made. Dashed lines in the group mean curves indicate that the data are only from one rat. All other details are as for Fig. 1.

Because two of the three rats for which diazepam was D2 died before the zopiclone condition in Phase 2, a meaningful comparison between phases can only be made between zopiclone gradients determined under pentobarbital training conditions. For these groups, percentage of drug-lever responding at intermediate zopiclone doses was lower under Phase 2, as compared with Phase 1, pentobarbital training. In fact, R3 showed no more than 25% pentobarbital-lever responding after any dose of zopiclone in Phase 2 (at 5.6 mg/kg there was no responding).

All four rats tested with zopiclone in both Phases 1 and 2 showed greater sensitivity to the rate-decreasing effects of zopiclone in Phase 1. Although there was a great deal of variability among rats in the dose which decreased response rates out of the control range, response rates for individual rats were decreased below the range by doses that were at least 0.5 $\log_{10}$ units lower than those producing the same effect in Phase 2 (i.e., 0.32–5.6 in Phase 1 and 3.2–5.6 in Phase 2).

Meprobamate

Meprobamate occasioned drug-lever responding in all rats in Phase 1 and in three of the four rats tested in Phase 2 (Fig. 5). In Phase 1, the group mean cross-over dose was 100 mg/kg under both the diazepam and pentobarbital training conditions (Fig. 5, bottom panels, open symbols). When R1, R2, and R3 were retrained with pentobarbital in Phase 2, the group cross-over dose was again 100 mg/kg, but one rat (R2) did not respond on the drug lever after any dose of meprobamate up to a dose (180 mg/kg) that completely suppressed responding. The group means of percentage of drug-lever responding at intermediate meprobamate doses, though lowest for the rats for which pentobarbital was D2, were not significantly different. Response rates were decreased below the ND control range by 56 or 100 mg/kg for all rats in Phases 1 and 2.

DISCUSSION

In Phase 1, all of the compounds investigated in the present

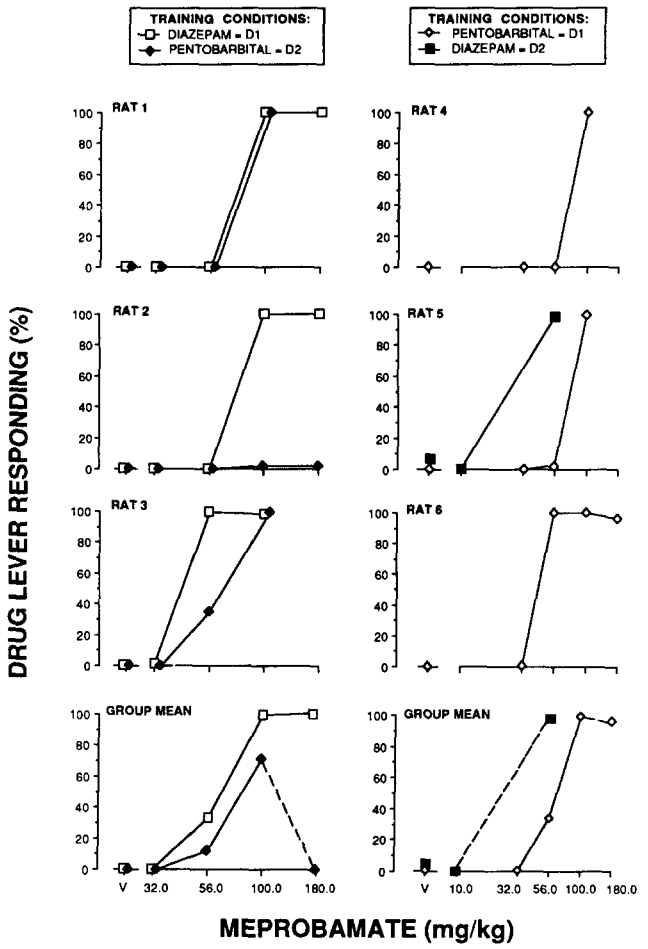

FIG. 5. Meprobamate generalization. Percentage of responding on the drug lever in test sessions after meprobamate or its vehicle (V) under pentobarbital and diazepam training conditions. The points for individual rats represent single test sessions except that multiple determinations were made at some doses (D1: 32—R3; D2: V—R1; 32—R3; 56—R1, R3; 100—R2, R3). In tests with 180 mg/kg, most rats did not complete a sufficient number of consecutive responses on one lever to obtain at least one pellet and percentage of drug lever responding was not included. Dashed lines in the group mean curves indicate that the data are only from one rat. All other details are as for Fig. 1.

study reliably occasioned drug-lever responding in all diazepam- and pentobarbital-trained rats. Pentobarbital (6–8, 17) and triazolam (17) previously were reported to occasion drug-lever responding in diazepam-trained rats, but the Phase 1 data of the present study are the first to show complete generalization to zopiclone and meprobamate in diazepam-trained rats. Only partial generalization to meprobamate previously has been reported in diazepam-trained rats (13); in those experiments, training doses (5 mg/kg and above) were considerably higher than that (1.0 mg/kg) employed in the present study. Generalization test results under the pentobarbital training condition are consistent with previous research. That is, diazepam (8,9), meprobamate (5), triazolam (10), and zopiclone (4) occasioned drug-lever responding in animals trained to discriminate pentobarbital.

In Phase 2, test results with the original training drugs (D1) suggested that discrimination training in Phase 2 largely overrode the discriminations trained in Phase 1, because D1 occasioned responding on the D2, rather than the D1, lever. The discriminability of D2 in terms of STC values was lower than D1 only for pentobarbital (i.e., for rats with the diazepam training history); but discriminative control in training sessions between tests was maintained better by pentobarbital than diazepam. Moreover, discriminative control in diazepam training sessions in Phase 2 appears to have been affected by prior training with pentobarbital as evidenced by the fact that the percentage of D sessions not meeting criterion for these rats was, on the average, two to three times that for rats in the other three training groups. That some effect of the previous training condition influenced responding in Phase 2 is suggested also by the fact that three of six rats developed a pattern of ceasing responding altogether partway through ND2 sessions (i.e., when responding on the D1 lever under ND conditions). Previous research in our laboratory with rats trained for comparably long periods of time with diazepam or pentobarbital, without a change in training conditions, has not shown such effects. A similar effect was observed in a study in which rats concurrently trained to discriminate two sedative drugs sharing discriminable effects (i.e., pentobarbital vs. chlordiazepoxide) did not respond in test sessions with either saline or low drug doses (11).

In the present study, pentobarbital was a considerably stronger discriminative stimulus than diazepam at the training doses used. Under the pentobarbital training conditions, generalization gradients for all drugs (significantly for doses of diazepam and triazolam) generally were shifted to the right compared to gradients under diazepam training conditions. ED_{50}'s inferred from regression analysis of diazepam and pentobarbital generalization were higher when pentobarbital was the current training drug in 75% of the cases. For each of the other drugs, at least 75% of the cases showed higher cross-over doses under pentobarbital training, as well.

Overton (15) suggested that the relative degree of "quantitative" specificity is estimated by comparing the percentage of drug choices at various doses of the training drug(s). By this estimate it may be suggested that the pentobarbital training condition showed greater specificity, in a "quantitative sense" (15), than the diazepam training condition. Moreover, regression lines derived from the diazepam and pentobarbital generalization gradients were steeper (i.e., values of M were higher) under pentobarbital training in approximately 70% of the cases. A specificity difference between training drugs would also be reflected by a difference in the ratio of training dose to cross-over dose, determined when rats were tested with the current training drug. When applied to our data, this measure revealed significant differences in specificity between diazepam and pentobarbital in both Phases 1 and 2 and further suggested that the discriminations learned in Phase 2 showed slightly greater specificity than those learned in Phase 1.

The fact that generalization gradients for diazepam, zopiclone, triazolam, and meprobamate were shifted further to the right for pentobarbital-trained rats with a diazepam training history than for pentobarbital-trained rats without a diazepam training history also suggests a difference in the pentobarbital training condition between Phases 1 and 2. In fact, two of three rats for which pentobarbital was D2 failed to show generalization to zopiclone and meprobamate, respectively. Moreover, the fact that significantly less drug-lever responding occurred at an intermediate dose of diazepam under Phase 2, relative to Phase 1, pentobarbital training supports this notion. The absence of this difference between Phase 1 and 2 pentobarbital training in terms of pentobarbital generalization does not negate this, but rather suggests that prior training with diazepam imparts a "qualitative" increase in the specificity of the pentobarbital discrimination (15).

Overton *et al.* (16) found that successive drug discrimination training with drugs from different pharmacological classes did not interfere with retention of control by the original training drug.

One might have predicted that under the present two-lever procedure, rats with a diazepam training history but currently trained with pentobarbital could have responded exclusively on the lever previously paired with diazepam when tested with diazepam. Under the present procedure, successive drug discrimination training with drugs from similar pharmacological classes apparently disrupted stimulus control by the original training condition.

A number of other experimental questions remain to be addressed, including which of the effects observed would be found if the D and ND levers were merely reversed without changing the training drug. In addition, it is of interest whether the stimulus effects of D1 which do not overlap with those of D2 would be revealed if the rats were trained to respond on a third lever under no drug conditions.

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