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Interaction of the Discriminative Stimulus Properties of Diazepam and Ethanol in Pigeons[1]

T. U. C. JÄRBE[2] AND D. E. McMILLAN[3]

University of North Carolina Medical School, Department of Pharmacology, Chapel Hill, N. C. 27514

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JÄRBE, T. U. C. AND D. E. McMILLAN. *Interaction of the discriminative stimulus properties of diazepam and ethanol in pigeons.* PHARMACOL BIOCHEM BEHAV **18**(1) 73–80, 1983.—One group of pigeons (n=5) was trained to discriminate between the effects induced by 5.6 mg/kg of diazepam (DZP) and the vehicle whereas other pigeons (n=5) had to discriminate between 3.0 g/kg of ethanol (ETOH) and the vehicle, administered intragastrically (IG) 10 and 40 min prior to the training sessions respectively. Once trained, the pigeons were tested with either diazepam or ethanol alone and in combination. The birds trained to discriminate between DZP and the vehicle mostly performed non-drug associated responses when tested with ETOH (0.56 to 3.0 g/kg). Tests with other doses of DZP (0.3 to 3.0 mg/kg) in the diazepam-trained birds resulted in an ED_{50} value of 1.4 mg/kg. The birds trained to discriminate between ETOH and the vehicle generalized DZP to ETOH, the ED_{50} value for diazepam being 3.0 mg/kg . Tests with other doses of ETOH (0.56 to 2.0 g/kg) in this latter group resulted in an ED_{50} value of 1.3 g/kg. Tests with combinations of DZP and ETOH produced a shift of the dose-response curves to the left indicating drug additivity. The discrimination of 5.6 mg/kg of IG administered DZP but not that of ETOH (3.0 g/kg) was attenuated by injections of the analeptic bemegride (ED_{50}=5.5 mg/kg), thus suggesting a difference in the cueing processes of the two drugs. When tested singly, bemegride induced non-drug responding or complete suppression of responding in the birds at the doses of 3.0 and 10.0 mg/kg respectively. In conclusion, the discriminable effects of DZP and ETOH are additive or even supra-additive, but the stimulus properties of the two drugs are not identical.

Drug discrimination Diazepam Ethanol Drug Combination Generalization Antagonism Pigeons

THE combination of certain drugs may result in a greater effect than when these drugs are given singly. In general, two outcomes have been considered: (1) the combined effect of two drugs is equal to the sum of their individual effects, which may be termed drug addition or summation, and (2) a supra-additive effect where the effects of the combination are greater than the effects of the individual drug.

When analysing such interactions at a behavioral level it has been difficult to establish if the interaction is due to the drugs combining along a similar dimension, or if the increase is due to different modes of action, thereby increasing toxicity or behavioral impairment. In order to differentiate between the "similar" versus "different" dimensional drug interaction, a procedure is required where the drug dimension is defined and, against which other drugs can be evaluated. The drug discrimination procedure fulfills this requirement. In this procedure, drug effects are established as discriminative stimuli in much the same way as external stimuli traditionally have been used to control behavior. The presence and absence of the effects of the training drug indicate to the animal which of two alternative responses will be reinforced during a particular training session. When tested with new compounds, responses learned to the training drug will generalize to sufficiently similar drugs but not to pharmacologically dissimilar drugs [1].

The benzodiazepine diazepam (DZP) is one of the most frequently prescribed psychopharmacological agents. As occurs with other depressant drugs (e.g. [31,32]) the combination of diazepam with ethanol (ETOH) can create unexpected impairment of motor coordination, increased drowsiness, and other symptoms which interfere with skilled activities. The nature of the interaction between ethanol and diazepam is unclear [4, 8, 9, 10, 29, 33].

In the present experiments diazepam and ethanol were chosen as discriminative stimuli to analyze the effects of combinations of these two drugs in the drug discrimination model. Separate groups of pigeons were trained to discriminate betwen either diazepam and its injection vehicle or ethanol and water and were then tested with varying doses of the drugs both singly and in combination. A shift to the left of the

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[2]Present address: Torbjörn U. C. Järbe, University of Uppsala, Department of Psychology, P. O. Box 227, S-751 04 Uppsala, Sweden.

[3]Present address: Donald E. McMillan, University of Arkansas for Medical Sciences, Department of Pharmacology and Interdisciplinary Toxicology, 4301 West Markham Street, Little Rock, AR 72201.

dose-response curves is expected if the interaction of the two drugs occurs along a similar stimulus dimension. The dependent variable is the discriminative response.

METHOD

The subjects were 10 male White Carneau pigeons (Palmetto Pigeons Plant, Sumter, S. C.), experimentally naive at the beginning of these experiments. The animals were maintained at about 80% of their free-feeding weights by the food presented during the sessions and by post-session supplemental feeding. Water and oyster shell grit were always available in the home cages. The average free-feeding weight after 10 days in the laboratory was 544 grams (SD±34.9).

Apparatus

The two experimental chambers used were sound-attenuating and ventilated. The response keys, 2 cm in diameter and dimly illuminated with white light, were mounted 10 cm apart on the front panel in the pigeon chambers, each about 19 cm above the chamber floor. The minimum force to operate the keys was about 15 g. Opening of the key-contacts defined the key-pecking response. The food magazine was located in between the response keys, 4 cm above the floor of the chambers. The reinforcer was 4-sec access to mixed grain (Purina pigeon grain). The key light and house light went off simultaneously with the 4-sec operation of the grain magazine and illumination of the food by the magazine light. The chambers were illuminated by 7.5 W, 110 V A. C., bulbs.

White noise was present in the chambers at all times. Chambers were ventilated by exhaust fans. Conventional relay programming and recording apparatus, located in a room adjacent to that of the chambers, was used.

Discrimination training and testing. The birds were trained initially to respond on either of the two keys to obtain food according to a FR 1 schedule of reinforcement; the requirement for obtaining the food was then gradually increased until a FR 15 schedule was in operation, i.e. the birds had to peck the key 15 times in order to get reinforced. When solutions were given before a session, the inappropriate key (left or right) for a given training condition was covered with tape during 5 drug sessions and 5 vehicle sessions, after which the free choice discrimination training commenced with both keys available. The pigeons had to respond on the appropriate key to produce food. Which key was correct was dependent upon whether the training drug or the vehicle had been administered prior to the start of the sessions. Responses on the inappropriate key had no programmed consequences. The sequencial order of the discrimination training sessions was similar to the protocol outlined by Colpaert *et al.* [7]. The pigeons were trained once a day, 5 to 6 days per week, for a maximum of 20 min per session or, until grain had been presented 35 times. For one group of pigeons (n=5), the drug training condition (D) consisted of an intragastric (IG) administration of 5.6 mg/kg of diazepam (DZP) and the non-drug training (N) condition was 2 ml/kg of the injection vehicle, administered 10 min prior to the training sessions. For the remaining pigeons (n=5), the final training of ethanol (ETOH) was 3.0 g/kg and the administration was IG, 40 min prior to the start of the sessions (cf. [21]. The birds were placed in the chamber immediately after the administration of diazepam or, in the case of ethanol, 30 min after administration, and remained in the chambers for 10 min until the session started, which was signalled by the onset of the house light and illumination of the response keys. A masking noise and exhaust fans were always in operation.

When the pigeons selected the correct key (left or right) at the onset of each training session during at least 8 out of 10 consecutive training days, the animals were switched from the training procedure to the test procedure. Subsequently, the animals were given two IG administrations per training occasion. The diazepam-maintained birds were given distilled water (ETOH vehicle), 40 min prior to the start of the training sessions in addition to the training dose of diazepam (5.6 mg/kg), or its vehicle (2 ml/kg). The ethanol trained birds were correspondingly administered the diazepam vehicle 30 min after ethanol, or distilled water, during the training sessions. During the test sessions, the pigeons could obtain 18 reinforcements if all responses (270 pecking responses) were directed to the key on which the bird first completed 15 responses. Once one key was selected, pecking on the other, non-selected key, did not activate the food magazine. Test sessions were preceded by at least one drug and one non-drug training session. Tests were not conducted unless the performance during the preceding training sessions had been on the correct manipulandum.

Drugs. Ninety-five percent ethanol was diluted with distilled water to yield a concentration of 10% (w/v) ethanol and intubations were made at the opening of the proventriculus. Dosages were adjusted by varying the volumes administered. Diazepam was dissolved in a vehicle supplied by Hoffman-La Roche Inc. (0.4 ml propylene glycol, 0.1055 mg ethyl alcohol, 0.015 ml benzylalcohol, 48.8 mg sodium benzoate, 1.2 mg benzoic acid and 1.0 ml water) and polysorbate-80. The suspensions, prepared shortly prior to use, contained 20% of the diazepam-vehicle and 4% polysorbate-80 and, physiologic saline; the volume administered was 2 ml/kg IG. Bemegride (Aldrich) was dissolved in saline shortly prior to usage. Bemegride was administered intramuscular (IM, 3 mg/kg in 1 ml/kg and, 10 mg/kg in 2 ml/kg).

Data analyses. The data recorded and the calculations made were: position selection (left or right key) expressed as the number of tests where the pigeons selected the drug associated position (symbol: DP selected); accuracy of the selection which is expressed as the number of responses emitted before the first reinforcement (median plus range, symbol: FRF; possible values, 15 to 29); the percentage of responding on the selected key out of the total number of responses emitted during a session (median plus range, symbol: % on selected position). The mean percentage of responses on the drug associated position out of the total number of responses during a session is symbolized by % RDP. Response rate (mean±1 SEM) is expressed as the quotient between the most recently preceding vehicle session and the drug session. A score above 1.0 means an increased response rate during drugged sessions. It should be noted that latency scores (time in seconds to initiate the first key-pecking response) were subtracted from the total session time since in this situation the latency to initiate responding may depend on a generalized depressant effect by the drugs and/or a decision making period. The statistics used are indicated in the appropriate sections.

RESULTS

The mean percentage of pecking responses on the drug associated key, % RDP, is presented graphically in Fig. 1 for

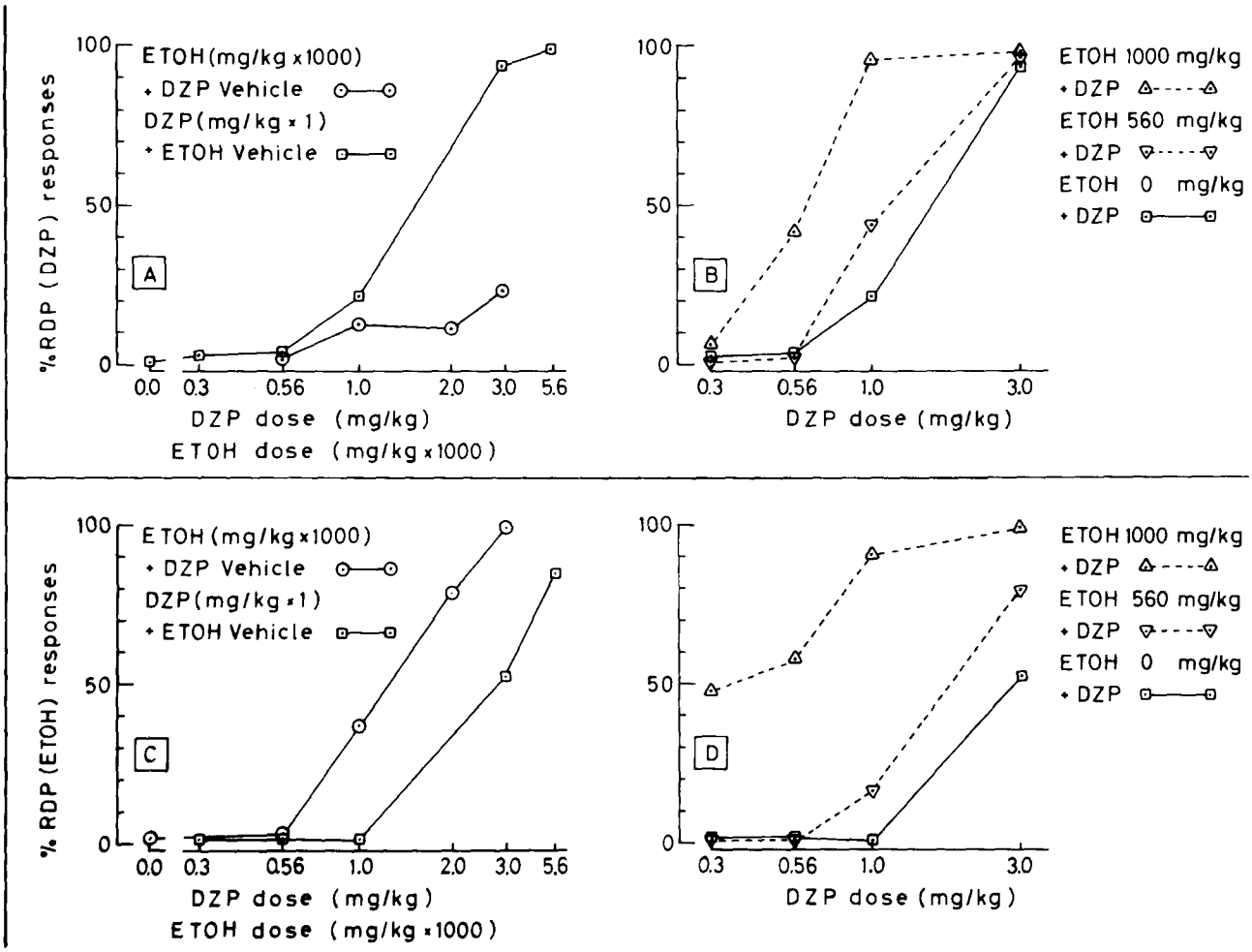

FIG. 1. Graphical presentation of mean percentage of responses at the drug position for an entire session (%RDP). Separate groups of pigeons were trained to discriminate between the presence and absence of diazepam (DZP) effects (n=5) or, the presence and absence of ethanol (ETOH) effects (n=5). The training doses were 5.6 mg/kg of DZP and 3000 mg/kg of ETOH, administered IG respectively 10 and 40 min prior to the training sessions. Graph A: the effects of testing different doses of DZP in the diazepam trained birds as well as the effects of testing ETOH singly in these birds. Graph B: the effects of testing combinations of DZP and ETOH in the diazepam trained birds. Ordinate, percent DZP appropriate responses (%RDP); abscissa, doses in mg per kg. Graph C: the effects of testing different doses of ETOH in the ethanol trained birds as well as the effects of testing DZP singly in these birds. Graph D: the effects of testing combinations of ETOH and DZP in the ethanol trained birds. Ordinate, percent ETOH appropriate responses (%RDP); abscissa, doses in mg per kg. For comparison the dose-effect curve after tests with DZP alone is also presented in graphs B and D for the two groups of pigeons respectively. Note that the dose of ETOH (mg/kg) is to be multiplied by a factor of 1000. Each datum point was determined on a separate day.

all test conditions, except for the studies on the interaction with bemegride.

Graph A in Fig. 1 shows the % RDP data from testing different doses of diazepam in the birds trained to discriminate between the presence and absence of 5.6 mg/kg of diazepam. Graph A also shows the effects of substituting ethanol for diazepam in these birds. A dose of 3.0 mg/kg diazepam appears to generalize to the training dose of diazepam, but doses of ethanol as high as 3.0 g/kg do not. Table 1, upper portion, shows that all birds selected the drug key when tested with 3.0 mg/kg of diazepam whereas with the dose of 1.0 mg/kg only one out of the five birds selected the drug position. The lower portion of Table 1 shows that when ethanol was substituted for diazepam, only one bird selected the DZP key when tested with 3 g/kg of ethanol. Note, however, the significant change in both the number of responses needed to produce the first reinforcement (FRF) and, the percentage of responding on the initially selected key out of the total number of responses emitted during the test session (% on selected position). Except for the highest test dose of ethanol (3 g/kg), the quotient between the most recently preceding vehicle session and the drug session exceeded 1.0 meaning that the rate of responding was higher during most drug sessions than during vehicle sessions.

When the diazepam maintained pigeons were tested with combinations of diazepam (0.3 to 3.0 mg/kg) and ethanol (560 and 1000 mg/kg) there was a shift to the left of the dose-response curves suggesting drug additivity (cf. Fig. 1, graph B). That is, the birds now selected the drug associated key with lower diazepam doses as compared to the testing of these animals with diazepam without ethanol. As shown in Table 2 there were many instances of increased rate of re-

TABLE 1

SUBSTITION TESTS WITH DIFFERENT DOSES OF DIAZEPAM AND ETHANOL

Drug 1	Dose (mg/kg)	Time (min)	Drug 2	Dose (mg/kg)	Time (min)	DP selected	FRF	% on selected position	Response rate
N:ETOH	0.0	40	DZP	0.0	10	0/20	15 (15–18)	100 (99.8–100)	—
D:ETOH	0.0	40	DZP	5.6	10	20/20	15 (15–15)	100 (100–100)	1.44 (0.17)*
T:ETOH	0.0	40	DZP	0.3	10	0/5	15 (15–18)	100 (94.5–100)	1.47 (0.22)
T:ETOH	0.0	40	DZP	0.56	10	0/5	15 (15–20)	100 (92.2–100)	1.21 (0.11)
T:ETOH	0.0	40	DZP	1.0	10	1/5	15 (15–25)	100 (97.6–100)	1.25 (0.13)
T:ETOH	0.0	40	DZP	3.0	10	5/5	15 (15–27)	95.4 (78.1–100)	1.42 (0.26)
N:ETOH	0.0	40	DZP	0.0	10	0/20	15 (15–16)	100 (100–100)	—
D:ETOH	0.0	40	DZP	5.6	10	20/20	15 (15–15)	100 (100–100)	1.34 (0.21)*
T:ETOH	560	40	DZP	0.0	10	0/5	15 (15–16)	99.1 (93.9–100)	1.29 (0.16)
T:ETOH	1000	40	DZP	0.0	10	0/5	15 (15–18)	100 (57.5–100)	1.36 (0.14)*
T:ETOH	2000	40	DZP	0.0	10	0/5	15 (15–26)	100 (45.5–100)	1.25 (0.27)
T:ETOH	3000	40	DZP	0.0	10	1/5	17.5 (15–20)*	96.4 (91.6–100)*	0.90 (0.38)

The pigeons were trained to discriminate between the vehicle (N) and 5.6 mg/kg of diazepam (D), administered IG 10 min prior to the training sessions. The ethanol vehicle (distilled water) was given 40 min prior to sessions. T=test condition. DZP=diazepam and, ETOH=ethanol. The abbreviations used are explained in the method section (data analyses). The number of pigeons used equals 5. Each datum point was determined on a separate day. *Significantly different ($p<0.05$) from the N-control data (A-test; 25).

TABLE 2

INTERACTION TESTS WITH NOVEL DOSES OF DIAZEPAM AND ETHANOL

Drug 1	Dose (mg/kg)	Time (min)	Drug 2	Dose (mg/kg)	Time (min)	DP selected	FRF	% on selected position	Response rate
N:ETOH	0.0	40	DZP	0.0	10	0/20	15 (15–18)	100 (100–100)	—
D:ETOH	0.0	40	DZP	5.6	10	20/20	15 (15–17)	100 (100–100)	1.37 (0.19)*
T:ETOH	560	40	DZP	0.3	10	0/5	15 (15–21)	100 (100–100)	1.34 (0.12)*
T:ETOH	560	40	DZP	0.56	10	0/5	15 (15–25)	98.9 (97.7–100)	1.41 (0.09)*
T:ETOH	560	40	DZP	1.0	10	2/5	15.5 (15–26)	94.2 (73.8–100)	1.53 (0.16)*
T:ETOH	560	40	DZP	3.0	10	5/5	15 (15–25)	100 (90.9–100)	1.42 (0.14)*
N:ETOH	0.0	40	DZP	0.0	10	0/20	15 (15–15)	100 (100–100)	—
D:ETOH	0.0	40	DZP	5.6	10	20/20	15 (15–17)	100 (100–100)	1.44 (0.20)*
T:ETOH	1000	40	DZP	0.3	10	0/5	16 (15–25)	100 (78.3–100)	1.75 (0.17)*
T:ETOH	1000	40	DZP	0.56	10	2/5	15 (15–26)	100 (78.8–100)	1.43 (0.19)*
T:ETOH	1000	40	DZP	1.0	10	5/5	15 (15–28)	100 (87.2–100)	1.37 (0.15)
T:ETOH	1000	40	DZP	3.0	10	5/5	15 (15–15)	100 (86.7–100)	1.24 (0.11)*

The pigeons were trained to discriminate between the vehicle (N) and 5.6 mg/kg of diazepam (D), administered IG 10 min prior to the training sessions. The ethanol vehicle was given 40 min prior to sessions. T=test condition. DZP=diazepam and, ETOH=ethanol. The abbreviations used are explained in the method section (data analyses). The number of pigeons used equals 5. Each datum point was determined on a separate day. *Significantly different ($p<0.05$) from the N-control data (A-test; 25).

sponding during these interaction studies. Median FRF and % on selected position did not differ significantly from the nondrug sessions.

Graph C in Fig. 1 shows the results of testing different doses of ethanol in the birds trained to discriminate between the presence and absence of 3000 mg/kg of ethanol. Tests with 2000 mg/kg of resulted in 78.5% ETOH appropriate responses and 1000 mg/kg produced less than 50% responses on the ETOH key. Graph C also shows the % RDP data for the tests where diazepam was substituted for ethanol. The 5.6 mg/kg dose of diazepam resulted in 84.5% ETOH appropriate responses and the 3.0 mg/kg dose produced intermediate (≈50%) responding. Table 3, upper portion, shows that four out of the five birds selected the drug key when tested with 2000 mg/kg of ethanol whereas the dose of 1000 mg/kg induced drug associated responding in two of the birds. None of the five birds selected the ETOH key when tested with 560 mg/kg of ethanol. The lower portion of Table 3 shows that when diazepam was substituted for ethanol four birds selected the ETOH key when tested with 5.6 mg/kg of diazepam; at the dose of 3.0 mg/kg of diazepam only two of the pigeons continued to select the ETOH associated key whereas with the dose of 1.0 mg/kg of diazepam none of the five birds selected the ETOH key. Rate of responding appears increased since most quotients were above 1.0 during the test sessions.

TABLE 3

SUBSTITUTION TESTS WITH DIFFERENT DOSES OF ETHANOL AND DIAZEPAM

Drug 1	Dose (mg/kg)	Time (min)	Drug 2	Dose (mg/kg)	Time (min)	DP selected	FRF	% on selected position	Response rate
N:ETOH	0.0	40	DZP	0.0	10	0/15	15 (15–15)	100 (99.8–100)	—
D:ETOH	3000	40	DZP	0.0	10	15/0	15 (15–18)	100 (100–100)	1.18 (0.22)
T:ETOH	560	40	DZP	0.0	10	0/5	15 (15–24)	100 (97.8–100)	1.21 (0.16)
T:ETOH	1000	40	DZP	0.0	10	2/5	15 (15–25)	100 (84.1–100)	1.40 (0.14)*
T:ETOH	2000	40	DZP	0.0	10	4/5	15 (15–18)	100 (95.0–100)	1.39 (0.25)
N:ETOH	0.0	40	DZP	0.0	10	0/25	15 (15–15)	100 (100–100)	—
D:ETOH	3000	40	DZP	0.0	10	25/25	15 (15–17)	100 (99.7–100)	1.22 (0.28)
T:ETOH	0.0	40	DZP	0.3	10	0/5	15 (15–15)	100 (94.6–100)	1.24 (0.15)
T:ETOH	0.0	40	DZP	0.56	10	0/5	15 (15–24)	100 (98.1–100)	0.98 (0.14)
T:ETOH	0.0	40	DZP	1.0	10	0/5	15 (15–15)	100 (100–100)	1.44 (0.43)*
T:ETOH	0.0	40	DZP	3.0	10	2/5	15 (15–15)	100 (40.1–100)	1.32 (0.19)*
T:ETOH	0.0	40	DZP	5.6	10	4/5	15 (15–29)	89.6 (31.8–100)	1.21 (0.24)

The pigeons were trained to discriminate between the vehicle (N) and 3 g/kg of ethanol (D), administered IG 40 min prior to the training sessions. The diazepam vehicle was given 10 min prior to sessions. T=test condition. ETOH=ethanol and, DZP=diazepam. The abbreviations used are explained in the method section (data analyses). The number of pigeons used equals 5. Each datum point was determined on a separate day. *Significantly different ($p<0.05$) from the N-control data (A-test; 25).

TABLE 4

INTERACTION TESTS WITH DIFFERENT DOSES OF ETHANOL AND DIAZEPAM

Drug 1	Dose (mg/kg)	Time (min)	Drug 2	Dose (mg/kg)	Time (min)	DP selected	FRF	% on selected position	Response rate
N:ETOH	0.0	40	DZP	0.0	10	0/20	15 (15–17)	100 (100–100)	—
D:ETOH	3000	40	DZP	0.0	10	20/20	15 (15–16)	100 (100–100)	1.13 (0.24)
T:ETOH	560	40	DZP	0.3	10	0/5	15 (15–24)	100 (99.6–100)	1.23 (0.10)
T:ETOH	560	40	DZP	0.56	10	0/5	15 (15–17)	100 (100–100)	1.29 (0.13)
T:ETOH	560	40	DZP	1.0	10	1/5	15 (15–25)	100 (76.5–100)	1.41 (0.13)*
T:ETOH	560	40	DZP	3.0	10	4/5	15 (15–18)	100 (100–100)	1.41 (0.20)
N:ETOH	0.0	40	DZP	0.0	10	0/20	15 (15–15)	100 (100–100)	—
D:ETOH	3000	40	DZP	0.0	10	20/20	15 (15–16)	100 (96.0–100)	1.21 (0.16)*
T:ETOH	1000	40	DZP	0.3	10	2/5	15 (15–22)	100 (67.9–100)	1.62 (0.37)
T:ETOH	1000	40	DZP	0.56	10	3/5	17.5 (15–24)	97.6 (65.6–100)	1.60 (0.24)*
T:ETOH	1000	40	DZP	1.0	10	5/5	20 (15–28)	96.6 (68.0–100)	1.41 (0.17)*
T:ETOH	1000	40	DZP	3.0	10	5/5	15 (15–29)	100 (84.9–100)	1.18 (0.15)

The pigeons were trained to discriminate between the vehicle (N) and 3 g/kg of ethanol (D), administered IG 40 min prior to the training sessions. The diazepam vehicle was given 10 min prior to sessions. T=test condition. ETOH=ethanol and, DZP=diazepam. The abbreviations used are explained in the method section (data analyses). The number of pigeons used equals 5. Each datum point was determined on a separate day. *Significantly different ($p<0.05$) from the N-control data (A-test; 25).

When the ethanol trained pigeons were tested with combinations of ethanol (560 and 1000 mg/kg) and diazepam (0.3 to 3.0 mg/kg) there was a shift to the left of the dose-response curves suggesting drug additivity (cf. Fig. 1, graph D). That is, the birds now selected the drug associated key with lower doses of diazepam as compared to when these animals were tested with diazepam but without ethanol. As shown in Table 4 there were several instances where the rate of responding was above 1.0 signifying an enhanced response output during the drugged sessions as compared to the vehicle sessions. Other measurements and calculations were not significantly different from the vehicle data.

Table 5 summarizes the ED_{50} determinations, according to the procedure of Litchfield and Wilcoxon [23], for the % RDP data shown in Fig. 1. There is a progressive, dose-related decrease in the ED_{50} value for diazepam in both the DZP and ETOH maintained animals when diazepam (0.3 to 3.0 mg/kg) was tested together with ethanol (560 and 1000 mg/kg). Note the higher ED_{50} value for diazepam alone in the ETOH trained birds as compared to the ED_{50} value for diazepam in the DZP trained group.

Table 6 summarizes the results of testing bemegride (BMG) singly, and in combination with diazepam or ethanol, in three pigeons from each of the two groups. The upper portion of Table 6 shows that when tested singly, bemegride resulted in non-drug associated key selections or complete suppression of responding in the diazepam trained birds at the doses of 3.0 and 10.0 mg/kg respectively. When beme-

TABLE 5

SUMMARY OF PROBIT ANALYSIS

Drug 1 ETOH	Dose (mg/kg)	Drug 2 DZP	Dose (mg/kg)	ED_{50} (mg/kg)		% Change in ED_{50} value
Pigeons trained with 5.6 mg/kg of diazepam (DZP)						
	560 to 3000		0	—	ETOH	
	0		0.3 to 5.6	1.4	DZP	—
	560		0.3 to 3.0	1.2	DZP	−14
	1000		0.3 to 3.0	0.6	DZP	−57
Pigeons trained with 3000 mg/kg of ethanol (ETOH)						
	560 to 3000		0	1300	ETOH	
	0		0.3 to 5.6	3.0	DZP	—
	560		0.3 to 3.0	1.8	DZP	−40
	1000		0.3 to 3.0	0.4	DZP	−87

ED_{50} determinations (23) for diazepam (DZP) and ethanol (ETOH) when tested either singly or in combination in pigeons. Separate groups of birds were trained to discriminate between the presence and absence of the DZP (5.6 mg/kg) stimuluscomplex or the complex induced by 3 g/kg of ETOH.

TABLE 6

TESTS FOR ANTAGONISM BY BEMEGRIDE AGAINST DIAZEPAM OR ETHANOL

Drug 1	Dose (mg/kg)	Time (min)	Drug 2	Dose (mg/kg)	Time (min)	n	DP selected	FRF	% on selected position	% RDP	Response rate
N:DZP	0.0	10	0.0	0.0	10	24	0/24	15 (15–15)	100 (100–100)	0.0	—
D:DZP	5.6	10	0.0	0.0	10	24	24/24	15 (15–16)	100 (100–100)	100.0	1.29 (0.19)*
T:DZP	0.0	10	BMG	3.0	10	6	0/6	15 (15–16)	100 (100–100)	0.1	0.81 (0.11)
T:DZP	0.0	10	BMG	10.0	10	6	—	—	—	—	—
T:DZP	5.6	10	BMG	3.0	10	6	6/6	17.5 (15–28)	99.3 (78.4–100)	94.3	1.32 (0.15)
T:DZP	5.6	10	BMG	10.0	10	6	0/6	17.5 (15–24)	97.4 (81.2–100)	6.8	1.24 (0.13)
N:ETOH	0.0	40	0.0	0.0	10	18	0/18	15 (15–15)	100 (100–100)	0.1	—
D:ETOH	3000	40	0.0	0.0	10	18	18/18	15 (15–15)	100 (100–100)	100.0	1.23 (0.28)
T:ETOH	0.0	40	BMG	3.0	10	3	0/3	15 (15–16)	100 (100–100)	0.0	1.14 (0.44)
T:ETOH	0.0	40	BMG	10.0	10	3	—	—	—	—	—
T:ETOH	3000	40	BMG	3.0	10	6	5/6	15 (15–24)	99.6 (85.2–100)	79.7	1.31 (0.24)
T:ETOH	3000	40	BMG	10.0	10	6	3/3	15 (15–15)	100 (100–100)	100.0	1.13 (0.15)

Separate groups of pigeons were trained to discriminate either between 5.6 mg/kg of diazepam (D) and the vehicle (N) or, between 3 g/kg of ethanol (D) and the vehicle (N). The administration was IG 10 and 40 min prior to sessions respectively. Bemegride (BMG) was given IM 10 min prior to sessions. T=test condition. DZP=diazepam and, ETOH=ethanol. The number of tests conducted is symbolized by n and, the number of pigeons used from each of the two groups equals 3. Each datum point was determined on a separate day. The abbreviations used are explained in the method section (data analyses). *Significantly different ($p<0.05$) from the N-control data (A-test; 25). The mean rate±SEM during N-sessions were 1.24 (0.04) and 1.25 (0.05) responses per second for the diazepam and ethanol trained birds respectively.

gride was tested together with 5.6 mg/kg of diazepam an attenuation of the DZP induced discriminative control was apparent, the estimated ED_{50} value of bemegride being 5.5 mg/kg when based on the % RDP data. Bemegride induced selection of the non-drug associated key at the dose of 3.0 mg/kg and complete response suppression at the dose of 10.0 mg/kg in the ethanol trained birds tested. When given together, bemegride did not significantly attenuate the ethanol stimulus although on three test occasions the 10 mg/kg dose of bemegride when combined with 3 g/kg of ethanol suppressed responding completely during the 20 min test period. The other measurements did not differ significantly from the vehicle-control data.

DISCUSSION

Pigeons trained to discriminate between the presence and absence of effects induced by 5.6 mg diazepam (IG) did not generalize to ethanol in doses from 0.56 to 2 g/kg (IG), but one of the five birds generalized 3 g/kg of ethanol to diazepam. When ethanol (0.56 and 1 g/kg) was administered 30 min prior to diazepam (0.3 to 3 mg/kg) a progressive, dose-related decrease in the ED_{50} value of diazepam was recorded. Among the birds trained to discriminate between the presence or absence of effects induced by 3 g/kg of ethanol (IG), 4 out of the 5 animals selected the ETOH associated key when tested by substitution with the highest dose

of diazepam (5.6 mg/kg), indicating at least a partial (one-way) generalization between the compounds. When tested in combination, the dose response curve for diazepam was shifted to the left, indicating drug additivity.

The observation that combinations of the two drugs increased the number of drug appropriate responses suggest an enhanced stimulus effect when diazepam and ethanol are combined. The interaction occurred along a defined drug dimension. If based strictly on an operational definition, the interaction between diazepam and ethanol in the diazepam trained pigeons constitutes evidence for supra-additive effects since ethanol alone in these birds generally yielded responses appropriate for the vehicle condition; drug additivity cannot be predicted by an inspection of the separate drug curves in these animals. From an inspection of the curves for the ethanol trained birds, on the other hand, additive effects between ethanol and diazepam would seem plausible, since generalization of diazepam to ethanol occurred in these birds. Note, however, the comparatively higher ED_{50} value for diazepam in the ethanol trained birds.

Several mechanisms may explain the supra-additive effect. One possibility is that ethanol facilitates the absorption of diazepam within the gastrointestinal tract, as reported by Hayes *et al.* [12], MacLeod *et al.* [24], and Mörland *et al.* [27], although others have reported no change in the absorption of diazepam after ethanol ingestion in man [11,22]. Whitehouse *et al.* [34] and Paul and Whitehouse [30] concluded that the basis for the interaction between diazepam and ethanol in rats and mice was due to interference of the drug-metabolizing enzyme systems in the liver by ethanol, resulting in increased levels of active diazepam metabolites in brain. There was no enhanced absorption of diazepam after ethanol in mice [30] and the absorption of diazepam was delayed when co-administered with ethanol in rats [34]. Thus the case for ethanol-induced facilitation of the absorption of diazepam is not strong and therefore this factor appears insufficient to explain the effects of the interaction between diazepam and ethanol in the present study. A metabolic basis for the interaction between ethanol and diazepam is unlikely because of the short interval between the administration of diazepam and testing.

Central mechanisms may be responsible for the interactive effects between ethanol and diazepam. Neither chronic administration of ethanol [19], nor chronic administration of diazepam or phenobarbital [5,26] lead to changes in benzodiazepine receptor binding which, according to Karobath *et al.* [19], suggests that the interaction between diazepam and ethanol arise from additive but independent actions on separate receptors. That the stimulus-complexes are different is suggested by the present study, since the diazepam cue was attenuated by the analeptic bemegride whereas the ethanol stimulus was not. This corraborates previous observations in rats, gerbils and pigeons that the stimulus-complexes induced by benzodiazepines [2, 6, 18] and barbiturates [13, 17, 20, 28] are blocked by bemegride whereas the ethanol-produced cue is not convincingly antagonized by the same analeptic [14,20]. Likewise, diazepam or pentobarbital induced alterations of behavior in gerbils tested in an open-field can be normalized by bemegride [15] whereas tests with combinations of ethanol and bemegride yielded negative results [16].

One-way, asymmetrical transfer, similar to that between ethanol and diazepam in the present study, have been reported earlier for ethanol and barbiturates. Rats trained to discriminate between ethanol and no drug responded in a manner more appropriate for the drug training condition than for the saline condition when tested with barbital and phenobarbital. In other rats, trained to discriminate between baribturates and saline, no generalization occurred to ethanol [35]. Additional studies suggesting asymmetrical transfer between ethanol and other CNS depressant drugs have been reviewed by Barry and Krimmer [3]. Because the drug action is multidimensional, i.e. the drug initiates activity in several effector systems, York [35] speculated that asymmetrical transfer might be understood by considering that animals discrimination trained with drug 1, focused primarily on a particular pharmacologic effect, A, not shared by drug 2, whereas animals discrimination trained with drug 2 rather relied on pharmacologic effect B, which is shared by both drugs. Although these presumed subset of pharmacologic actions cannot be defined more specifically at the present, it is clear that the "primary" component of diazepam, but not that of ethanol, is suceptible to antagonism by bemegride.

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