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Variable Interval Schedules of Timeout From Avoidance: Effects of Ethanol, Naltrexone, and CGS 8216

MARK GALIZIO,[1] MICHAEL PERONE* AND BARBARA A. SPENCER

*Department of Psychology, University of North Carolina at Wilmington, Wilmington, NC and *Department of Psychology, West Virginia University, Morgantown, WV*

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GALIZIO, M., M. PERONE AND B. A. SPENCER. *Variable interval schedules of timeout from avoidance: Effects of ethanol, naltrexone, and CGS 8216.* PHARMACOL BIOCHEM BEHAV 25(2) 439–448, 1986.—Four rats were trained on concurrent schedules of shock avoidance and timeout from avoidance, where responses on one lever postponed shock and responses on another lever occasionally (VI 45 sec schedule) produced a 2-min timeout during which the avoidance schedule was suspended. These procedures maintained stable rates of responding on both levers, providing a baseline for studying the effects of drugs on behavior under different types of aversive control (shock avoidance and timeout from avoidance). In the first experiment the effects of ethanol (0.5, 1.0, 1.5, and 2.0 g/kg) and an opiate antagonist, naltrexone (1 mg/kg) were assessed alone and in combination. Ethanol produced a dose-dependent decrease in avoidance characterized by increased shock rates and decreased response rates. At the same time, however, responding on the timeout lever generally increased relative to avoidance lever rates. All of these effects were largely confined to the early parts of the 2-hr session, when blood-ethanol levels were relatively high. Naltrexone had no effect on performances and did not interact with ethanol. In a second experiment, the effects of the benzodiazepine antagonist CGS 8216 were studied alone, and in combination with ethanol. CGS 8216 (5 mg/kg) generally disrupted both avoidance and timeout responding but did not reverse ethanol actions.

Ethanol Naltrexone CGS 8216 Concurrent schedules Timeout from avoidance Aversive control Opiate antagonists Benzodiazepine antagonists

THE effects of ethanol on aversively-motivated behavior have been of considerable interest because of hypotheses which emphasize the stress- or tension-reducing properties of ethanol as critical to human self-administration and dependence on the drug [30]. However, research support for such hypotheses has been mixed at best [8,22]. For example, studies of the effects of ethanol on continuous avoidance have left some confusion, with one study reporting that ethanol increased avoidance responding at moderate dose levels in rats [29], but others finding decreases in responding across the entire effective dose range [18, 19, 23]. Although there were a number of methodological differences between these various studies, the determinants of ethanol-stimulation of continuous avoidance remain unclear. It should be noted that ethanol has often been reported to stimulate responding in discrete-trial avoidance conditioning studies [2,16]. Thus, one purpose of the present study was to examine the effects of various doses of ethanol on behavior maintained by a newly developed procedure involving aversive control which was designed to permit more sensitive measurement of drug effects.

Our procedure involved training rats on concurrent schedules where responses on one lever postponed shock according to a Sidman avoidance schedule, and responses on another lever produced brief periods of signaled timeout from the avoidance schedule. Previous studies have shown that timeout from avoidance can serve as a reinforcer [11, 17, 27, 33], and the two lever timeout procedure appears to have promise as a technique in behavioral pharmacology for several reasons. For example, the interpretation of drug effects on simple avoidance schedule performance is complicated by difficulties in differentiating the effects on unconditioned reactions to shock from conditioned reactions. Thus, a drug which depresses responding might act by producing analgesia, ataxia, a reduction in the conditioned aversiveness of the situation, or other mechanisms. By comparison, a key advantage of the present procedure is that responses on the timeout lever have the sole effect of terminating stimuli associated with the avoidance situation, and are thus presumably maintained by the conditioned aversive properties of that situation. Of interest is whether ethanol might have differential effects on behavior related directly to shock (avoidance responding) versus behavior maintained by termination of conditioned aversive stimuli

[1]Requests for reprints should be addressed to Mark Galizio, Department of Psychology, University of North Carolina at Wilmington, Wilmington, NC 28403.

(timeout responding). An additional advantage of the procedure is that timeout can be scheduled in basically the same ways as appetitive stimuli, thus allowing more direct comparison between drug effects on aversive and appetitive schedule performance.

A second major purpose of the present study was to determine whether ethanol effects were mediated by specific receptor sites. In Experiment 1 the theory that the opiate receptor may represent a common biochemical link between ethanol and opioid compounds [1, 4, 20, 25] was tested by attempting to reverse the effects of ethanol with the opiate antagonist naltrexone. Reversal of a variety of ethanol effects by opiate antagonists has been reported [9, 21, 28], but a recent study from our laboratory [18] showed that an effective dose of naltrexone did not reverse (and tended to enhance) the effects of ethanol on avoidance responding in rats. Naltrexone was studied alone, and in combination with ethanol, to determine whether a different outcome might occur with the concurrent procedure.

EXPERIMENT 1

METHOD

Subjects

Four experimentally naive male Sprague-Dawley rats served in the operant procedure. The animals were 100–110 days old and weighed 350–400 g at the outset. All were individually housed, were allowed free access to food and water, and were maintained under continuous lighting. In addition, 20 female rats, between 200–300 g, were used to determine blood-ethanol concentrations.

Apparatus

Standard operant chambers (Lehigh Valley Electronics) with stainless steel end panels and Plexiglas sidewalls were used. Two levers were spaced 10 cm apart and mounted 8 cm above the floor grids. Foot-shock was generated by a Lafayette shock generator (Model 82400-SS) which delivered scrambled shock (Lafayette Model 5820 neon grid scrambler) through 0.5 cm-diameter stainless steel grids spaced 1.6 cm apart. The chambers were housed in sound-attenuating boxes (Lehigh Valley Electronics), equipped with a fan for ventilation and a white noise generator which delivered 80 dB masking noise. A single house light was mounted behind a sidewall. Control and recording operations were accomplished with a microcomputer (Tandy, TRS-80 Model III) connected to the chamber by a commercial interface (Alpha products, Interfacer 80) and electromechanical components using software described elsewhere [26].

Drug Preparation

A 10% ethanol solution (wt/vol) for injection was prepared by adding 66 ml 95% grain alcohol (Everclear) to 434 ml isotonic saline solution. Naltrexone HCl (provided courtesy of the National Institute of Drug Abuse) was in a 1 mg/ml concentration.

Procedure

Preliminary training. With the left lever retracted, four rats were trained with a shaping procedure to avoid shock by pressing the right lever (avoidance lever). Control was then transferred to a Sidman avoidance schedule where each response produced a 0.5 sec offset of white noise (feedback stimulus) and postponed the next shock for 30 sec (response-shock interval). In the absence of responding, shocks were presented at 5 sec intervals (shock-shock interval). Shock intensity was 1.0 mA and duration was 0.5 sec. Activation of the noise generator and house-light signalled the onset of each 2-hr session, and these events terminated at the end of the session. Training involved 21–23 daily sessions until the rats consistently avoided at least 90% of the shocks scheduled by the response-shock interval and response rates met a 10 session stability criterion. The criterion required that the difference between the means of the first 5 and last 5 sessions was within 10% of the grand mean.

Multiple schedule training. A multiple schedule was used to establish a discrimination between periods of avoidance and timeout from avoidance. The 10 min components of timeout (houselight and white noise off, shock schedule suspended) alternated with avoidance components signaled by the onset of houselight and white noise. A correction procedure ensured that the timeout component could not end within 1 min of a press on the avoidance lever. Training on the multiple schedule continued until virtually no responding was observed during timeout components (30–31 sessions).

Concurrent schedule training. The left lever (timeout lever) was inserted into the chamber for the first time, and pressing it resulted in immediate retraction of the lever, termination of houselight and white noise, and suspension of the shock schedule for 5 min. Three of the four animals acquired the timeout lever response during the first session. However, one animal (X2) showed a strong position preference and eventually required the retraction of the avoidance lever before responding on the timeout lever. When subjects showed consistent responding on the timeout lever the timeout duration was gradually reduced to the terminal value of 2 min. The schedule was then gradually changed so that within 5 to 7 sessions the terminal schedule of variable-interval 45 sec (VI 45 sec) was reached. Subjects were trained daily on this baseline concurrent schedule (right lever: Sidman avoidance; left lever: VI 45 sec timeout) until they attained a 10 session, 10% stability criterion for response rates (avoidance and timeout) and percent avoidance. After reaching stable performance levels (23–27 sessions) the drug probe procedures were introduced.

Drug probes. Drug sessions were scheduled three times per week (Mon, Wed and Fri), and sessions were conducted under baseline conditions on the remaining four days of the week (drug baseline sessions). On drug days subjects received two injections prior to testing. A subcutaneous injection of naltrexone (1 mg/kg) or equivalent volume of saline (IP for Rat X2) was administered 15 min before session onset, and was followed 5 min later by an IP injection of one of the ethanol doses (0.5, 1.0, 1.5) or a volume of saline equivalent to that of the highest dose. The schedule of drug conditions was randomly generated for each rat with the constraints that the highest two ethanol doses were never administered on consecutive drug days, and that the end of each cycle of the drug regimen was completed before beginning the subsequent cycles. The first drug session for each rat was a test administration of 1.0 g/kg ethanol and data from this initial session was disregarded and the condition replicated. This procedure was followed in order to eliminate "novelty" effects which make the first exposure to ethanol non-representative. Each subject was exposed to 3 sessions under each drug condition except for Rat Y1 who displayed

TABLE 1
MEAN DATA FOR DRUG BASELINE CONDITIONS

Subject	Avoidance Resp./Min	Timeout Resp./Min	Shocks/Min	Relative Rate
		Warm-up		
Y1	11.2	2.9	0.01	0.21
Y2	5.7	1.1	0.31	0.15
X1	5.4	4.0	0.35	0.44
X2	4.8	5.2	0.26	0.52
		Main Session		
Y1	9.7	2.6	0.03	0.22
Y2	5.2	2.2	0.11	0.30
X1	4.8	3.3	0.30	0.41
X2	5.5	5.7	0.14	0.51

an apparent insensitivity to the effects of ethanol. In the case of this animal, the 0.5 g/kg and 1.0 g/kg ethanol conditions were discontinued after the second replication, and 2.0 g/kg conditions were added. In addition, if an animal was so impaired during a drug session that it failed to respond for 250 sec, the session terminated automatically, data from the session were disregarded, and the condition was replicated. Such aborted sessions occurred at the initial 1.5 ethanol-saline session and the second 1.0 ethanol-naltrexone session for Subject X1, and at the first 1.5 ethanol-saline and 1.5 ethanol-naltrexone, and the second 1.0 ethanol-saline conditions for subject X2.

Blood-ethanol determination. Twenty additional rats were exposed to one of the four alcohol doses used in the experiment. Rats were sacrificed and cardiac blood samples were taken at 30-min post-injection for 11 animals (3 per group except for the 0.5 g/kg dose where n=2), and at 90 min for the other 9 animals (3 per condition). Blood samples were analyzed within 4 hr after collection using gas chromatography techniques described by Frye *et al.* [16]. Fifty microliters of blood were diluted with 50 microliters of distilled water containing tert-butanol, which was used as the internal standard. Five-microliter aliquots of diluted samples were injected into the unit (Gowmac Series 150 Thermal Conductivity Detector equipped with a 0.25 in. 4 ft. Carbowax 20 M column).

RESULTS

All four animals developed patterns of responding on both avoidance and timeout levers which resulted in infrequent contact with shock and which remained stable throughout the experiment. Table 1 summarizes mean data from the drug baseline sessions. Data were analyzed in 20 min segments across the 2-hr session, but since the only intrasession changes occurred early in the session, data are presented for the first 20-min bin (warm-up), separately from the final 100 min (main session). In addition to response and shock rates, Table 1 presents a measure of timeout responding relative to avoidance responding (relative rate). This measure was determined by dividing the number of timeout responses by the total number of timeout and avoidance responses. It is apparent from analysis of Table 1 that responding on the timeout lever was maintained at a reasonably high rate for all four animals with the concurrent avoidance-timeout schedule.

Ethanol alone resulted in a disruption of avoidance responding in a dose-dependent fashion. This was most evident when considering shocks received by each animal at the various doses of ethanol, shown in Fig. 1. In general, there were trends for increases in shocks received as ethanol dose increased. These trends were most pronounced early in the session (warm-up), and in general, were not reversed by naltrexone (open circles). All rats showed clear increases in shocks received at the higher ethanol doses (1.5 or 2.0 g/kg), and some (Y1 and X2) showed impairment at the intermediate 1 g/kg dose during warm-up. Naltrexone did not diminish the effects of ethanol in any consistent way, and appeared to have enhanced them in some subjects (Y1 and X2).

To assess the statistical reliability of these findings, an analysis of variance was conducted on the shock rate data, with Ethanol Dose (0, 0.5, 1.0, and 1.5 mg/kg), Naltrexone Dose (0 and 1.0 mg/kg), and Session Segment (warm-up vs. main session) as the independent variables. To adjust for individual differences in baseline rates, the data were converted to difference scores by subtracting the rates on the saline control days (this adjustment procedure was used in all subsequent statistical analyses as well). The analysis verified the description of the findings based on the performances of the individual rats: there was a significant main effect of Ethanol, $F(3,9)=10.79$, $p<0.01$, and a significant Ethanol × Session Segment interaction, $F(3,9)=8.09$, $p<0.01$, but none of the effects involving Naltrexone were significant, with F's <1 for Naltrexone, Naltrexone × Ethanol, and Naltrexone × Ethanol × Segment. The remaining test, Session Segment, also was non-significant.

The avoidance impairment effects of ethanol were also observable to some extent in avoidance response rates (Table 2). Generally ethanol depressed rates in a dose-dependent fashion during the warm-up period, but no consistent effects can be seen during the main session. Again there was no evidence of naltrexone-reversal of ethanol effects. Statistical analysis of the adjusted avoidance rates confirmed the reliability of these trends observed in the individual performances, with significant effects of Ethanol, $F(3,9)=13.21$, $p<0.01$, Session Segment, $F(1,3)=12.8$, $p<0.05$, and the Ethanol × Segment interaction, $F(3,9)=12.12$, $p<0.01$. Again, none of the effects involving Naltrexone was significant.

A surprising finding is revealed in Fig. 2 which shows timeout responding relative to avoidance responses. The main findings are shown in the left-hand panels which show warm-up performances. All four rats showed a trend toward increased timeout responding relative to avoidance responding as a function of ethanol dose. The trend was apparent whether naltrexone (open circles) or saline (solid circles) was presented with ethanol. Although there was substantial range overlap across the various ethanol doses, comparisons between at least the highest ethanol doses and no-ethanol control conditions revealed clearly reliable effects in three animals (X2, Y1, and Y2) and comparable trends with range overlap in the fourth (X1). These effects dissipated during the course of the session, since they are not evident in the main session data (right-hand panels). In line with this account, statistical analysis of the adjusted relative timeout rates confirmed the effect of Ethanol, $F(3,9)=9.57$, $p<0.01$, and the interaction between Ethanol and Session Segment,

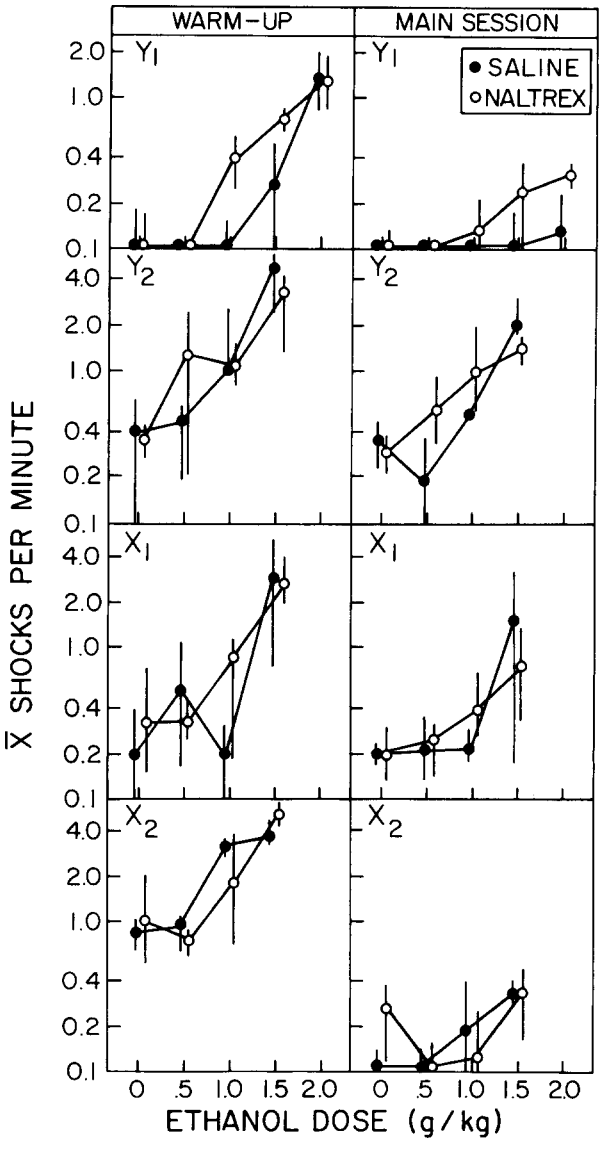

FIG. 1. Mean shock rates for warm-up (left panels) and main session (right panels) for subjects exposed to ethanol (solid circles) and ethanol with naltrexone (open circles). Shocks are plotted on a logarithmic scale and the various doses of ethanol are indicated in g/kg on the abscissa. Vertical lines passing through the circles indicate the range of values.

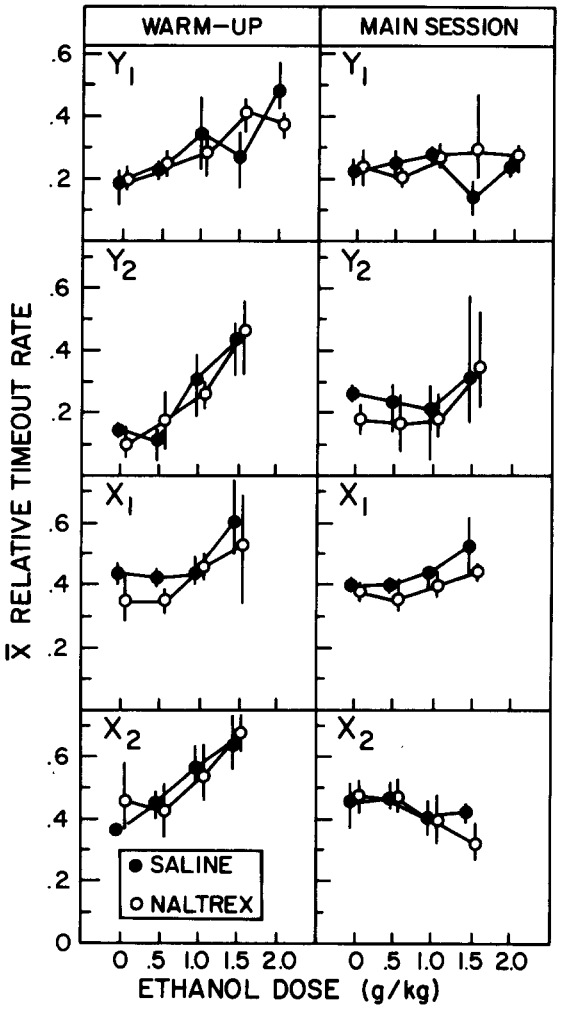

FIG. 2. Relative timeout rates for warm-up (left panels) and main session (right panels) for subjects exposed to ethanol (solid circles) and ethanol with naltrexone (open circles). The proportion of timeout responses relative to avoidance responses are plotted on the ordinate and the various doses are indicated in g/kg on the abscissa. Vertical lines passing through the circles indicate the range of values.

$F(3.9)=9.65$, $p<0.01$. Again, none of the effects involving Naltrexone was significant.

Thus, ethanol enhanced responding on the timeout lever, at least relative to avoidance responding, during the early part of the session. This effect could have been due to increased responding on the timeout lever, or reduced responding on the avoidance lever, or both. Table 3 presents response rates on the timeout lever. These rates were generally stable across the range of conditions, and statistical analysis of the adjusted rates revealed no reliable effects associated with Ethanol, Naltrexone, Session Segment, or the interaction of these variables. These findings, in conjunction with the avoidance data presented above, reveal that the increased relative rates were due to decreased avoidance responding while timeout responding remained constant.

The results of the blood-ethanol determination helped to clarify the dissipation of ethanol effects noted above. Table 4 displays the means and ranges of the blood-ethanol concentrations for the various alcohol doses both 30 min and 90 min following injection. As seen in Table 4 the blood-ethanol level increased with increasing ethanol dose, and the concentration declined substantially by 90 min. These results were confirmed statistically by factorial analysis of variance with Ethanol dose and Time as the main factors. There was a significant main effect for Ethanol, $F(2,12)=74.2$, $p<0.01$,

TABLE 2

MEANS AND RANGES OF AVOIDANCE RESPONSE RATES

Condition*		Rat X1	Rat X2	Rat Y1	Rat Y2
S-0.0	wu†	6.38 (4.7–7.7)	6.90 (5.5–8.2)	15.32 (11.0–23.6)	6.66 (6.1–7.2)
	ms	5.18 (5.0–5.4)	6.22 (4.8–7.8)	9.46 (8.3–10.3)	4.91 (4.4–5.0)
N-0.0	wu	6.70 (4.6–9.6)	4.07 (3.0–5.6)	11.85 (8.4–16.8)	7.65 (6.9–8.3)
	ms	5.25 (4.2–6.0)	5.94 (4.6–8.2)	8.25 (7.4–9.8)	5.35 (4.7–6.3)
S-0.5	wu	6.09 (5.8–6.2)	5.16 (3.4–6.5)	13.87 (12.4–15.3)	5.58 (4.5–6.5)
	ms	4.85 (4.2–5.4)	5.53 (4.7–6.6)	10.83 (9.2–12.4)‡	5.42 (5.3–5.6)
N-0.5	wu	6.32 (5.4–7.6)	5.49 (4.1–7.6)	8.54 (7.6–9.4)	4.04 (3.2–5.5)
	ms	6.06 (5.3–7.0)	5.92 (4.4–6.9)	8.88 (8.7–9.0)‡	5.36 (4.9–5.6)
S-1.0	wu	5.43 (4.5–7.0)	2.30 (2.2–2.4)	7.95 (6.3–9.6)	4.84 (3.7–6.6)
	ms	4.80 (4.3–5.4)	6.79 (6.3–7.6)	7.98 (7.0–8.8)‡	5.40 (4.1–6.9)
N-1.0	wu	4.27 (3.2–5.8)	2.99 (2.1–4.1)	9.22 (7.7–10.7)	4.79 (3.8–5.7)
	ms	5.96 (5.0–6.9)	6.50 (5.2–8.1)	6.45 (5.5–7.4)‡	5.01 (3.9–6.7)
S-1.5	wu	2.77 (1.2–3.7)	1.50 (1.1–2.1)	8.50 (5.4–12.3)	1.55 (1.4–1.7)
	ms	3.80 (1.9–4.9)	6.28 (5.0–7.1)	12.85 (9.8–18.2)	3.44 (2.4–5.0)
N-1.5	wu	1.75 (1.4–2.2)	.97 (0.51–1.2)	5.01 (3.0–7.4)	2.01 (1.0–3.4)
	ms	4.58 (4.1–5.2)	7.48 (5.3–8.9)	7.10 (4.7–8.9)	3.35 (2.9–3.8)
S-2.0	wu			5.10 (3.0–7.4)	
	ms			8.65 (8.0–9.0)	
N-2.0	wu			4.99 (4.3–5.8)	
	ms			5.41 (4.9–5.9)	

*Condition is reported in g/kg ethanol. S = Saline; N = Naltrexone.
†wu = warm-up; ms = main session.
‡Subject was run only twice in these conditions.

TABLE 3

MEANS AND RANGES OF TIMEOUT RESPONSE RATES

Condition*		Rat X1	Rat X2	Rat Y1	Rat Y2
S-0.01	wu†	4.54 (4.3–4.9)	4.08 (3.4–4.8)	3.12 (2.8–3.3)	1.15 (0.99–1.4)
	ms	3.43 (3.2–3.6)	3.67 (1.1–5.3)	2.59 (2.3–2.9)	1.74 (1.6–1.9)
N-0.0	wu	3.37 (2.5–3.7)	3.58 (2.4–5.0)	2.76 (2.1–3.0)	0.86 (0.56–1.3)
	ms	3.13 (2.8–3.6)	5.38 (4.5–6.4)	2.44 (1.8–3.0)	1.15 (0.97–1.3)
S-0.5	wu	4.39 (3.9–5.0)	4.04 (3.1–5.2)	4.02 (3.9–4.1)	0.66 (0.21–1.0)
	ms	3.17 (2.8–3.5)	4.94 (4.0–5.8)	3.41 (3.1–3.6)‡	1.70 (0.87–2.2)
N-0.5	wu	3.40 (2.6–3.9)	4.00 (3.4–4.2)	2.77 (2.5–3.0)	0.80 (0.43–1.1)
	ms	3.28 (3.2–3.3)	5.50 (5.0–6.0)	2.18 (1.9–2.4)‡	1.03 (0.47–1.9)
S-1.0	wu	4.38 (3.0–5.5)	3.02 (2.3–3.5)	3.97 (2.7–5.2)	2.08 (1.5–2.4)
	ms	3.78 (3.0–4.3)	4.69 (3.4–5.4)	2.90 (2.9)‡	1.35 (0.36–1.9)
N-1.0	wu	3.65 (2.7–5.0)	3.24 (2.8–3.5)	3.15 (1.9–5.3)	1.69 (1.6–1.7)
	ms	3.83 (3.7–3.9)	4.63 (3.8–5.1)	2.40 (2.3–2.4)‡	1.08 (0.82–1.5)
S-1.5	wu	3.82 (3.4–4.2)	2.65 (2.2–3.0)	2.88 (2.0–4.1)	1.17 (0.80–1.4)
	ms	3.94 (2.8–5.2)	4.27 (4.0–4.4)	2.11 (2.0–2.3)	1.61 (0.60–3.0)
N-1.5	wu	2.00 (1.1–3.0)	1.81 (1.3–2.2)	3.57 (2.7–4.3)	1.51 (1.3–1.6)
	ms	3.46 (3.0–4.1)	3.22 (3.1–3.3)	2.96 (1.9–4.1)	1.90 (1.2–3.1)
S-2.0	wu			4.41 (3.9–5.3)	
	ms			2.64 (2.3–2.9)	
N-2.0	wu			2.96 (2.5–3.2)	
	ms			2.01 (1.7–2.3)	

*Condition is reported in g/kg ethanol. S = Saline; N = Naltrexone.
†wu = warm-up; ms = main session.
‡Subject was run only twice in these conditions.

TABLE 4
MEANS AND RANGES OF BLOOD-ETHANOL CONCENTRATIONS (mmol/ml)

	Ethanol Dose (g/kg)			
Time	0.5	1.0	1.5	2.0
30 minutes	5.2(2.0–8.3)*	13.1(9.7–15.1)	23.7 (21.1–26.4)	34.7 (33.1–37.1)
90 minutes	—	3.8 (2.4–4.6)	14.5 (12.5–17.5)	19.0 (15.0–21.1)

*Mean based on only two determinations.

and Time, $F(1,12)=84.8$, $p<0.01$, but no significant Ethanol × Time interaction. Since in the experiment proper, alcohol was administered 10 min prior to the onset of the session, the warm-up period represents the time when blood-alcohol levels were at their highest. By the final 30 min of the avoidance sessions it may be assumed that blood levels would have been as low or lower than those noted in Table 4. Thus, the decline in ethanol effects seen later in the avoidance sessions was likely due to the decline in the functional dose of ethanol over time.

DISCUSSION

Consistent with the results obtained by Galizio *et al.*[18] alcohol impaired avoidance performance in a dose-dependent fashion as indexed by lower response rates and higher shock rates. Also consistent with that study, naltrexone failed to reverse ethanol effects. However, the present study eliminated two possible explanations for the lack of naltrexone reversal. In the Galizio *et al.* [18] study a high dose of naltrexone which had intrinsic action was used (3.0 mg/kg) while the present study used a lower dose without effects on its own. Also, in the present study naltrexone was administered before ethanol; in the other study the order was reversed. Thus, the present findings argue strongly against the notion that ethanol effects on avoidance behavior are reversible by opiate antagonists. Such findings are inconsistent with the theory that the actions of ethanol on aversively-motivated behavior are mediated by activity involving the opioid receptor system.

A second major finding concerned the stimulatory effects of ethanol on the proportion of timeout responses. Although these effects were limited to the early part of the session, when blood-ethanol concentrations were high, it was consistently observed in all 4 animals. The effect was surprising since, given the reputation of ethanol as an anxiolytic drug, one might expect it to reduce the aversiveness of the avoidance situation and thus reduce the reinforcing properties of timeout from avoidance. In view of the counter-intuitive nature of the finding, we sought to replicate the ethanol effects in Experiment 2, and in addition to examine the effects of the benzodiazepine antagonist CGS 8216 alone, and in combination with ethanol.

EXPERIMENT 2

Recent reviews have noted the similarities between pharmacological and behavioral effects of ethanol and the benzodiazepine tranquilizers and suggested the possibility of a common mechanism of action [7,32]. The development of specific benzodiazepine (BZ) antagonists, such as CGS 8216, raises the question of whether such drugs might also antagonize the effects of ethanol. Few studies have been reported which have examined the interaction between ethanol and BZ antagonists. Bonnetti *et al.* [6] noted that the BZ antagonist Ro 15-1788 did not reverse ataxia produced by ethanol on the horizontal wire test but did reverse diazepam's actions on this test. Similarly Ro 15-1788 reverses the anti-conflict actions of diazepam, but not of ethanol [12,24] and it does not affect ethanol self-administration [3]. Despite these negative outcomes there is still reason to re-examine the question. Several studies have shown that Ro 15-1788 is not a pure BZ antagonist, but rather may possess mixed agonist-antagonist activity [10,13]. Thus, it is possible that the agonistic actions of Ro 15-1788 may have obscured possible reversal of ethanol effects. However, another BZ antagonist, CGS 8216, has been shown to reverse the major pharmacological and behavioral effects of benzodiazepines without possessing agonist properties (see [5]), and the possibility that it might also reverse ethanol effects was examined in the present study.

METHOD

Subjects and Apparatus

Three of the rats studied in Experiment 1, X1, Y1, and Y2, served in the present study. The apparatus was identical to that used in Experiment 1.

Drug Preparation

Forty mg of CGS 8216 was added to a vehicle of 20 drops of Tween and 20 ml isotonic saline and placed in suspension with ultrasound. Ethanol solution was prepared as in Experiment 1.

Procedure

The procedures of Experiment 2 followed Experiment 1 immediately for two subjects (X1 and Y1), while a behavioral manipulation intervened for Y2. This procedure involved exposing Y2 to the concurrent schedule under conditions where responses on the timeout lever led to termination of the house light and white noise, but did not suspend the avoidance schedule. Under these conditions the timeout response extinguished, showing that it was the timeout from avoidance and not merely stimulus change which was maintaining the behavior. Identical procedures led to extinction of timeout responding in the other subjects after the present experiment had been completed [27]. Baseline performance was recovered for Y2 before beginning the present experiment.

The general procedures for data collection were the same as in Experiment 1, except that injections of CGS 8216 (5 mg/kg) or Tween 20 vehicle preceded the injection of ethanol

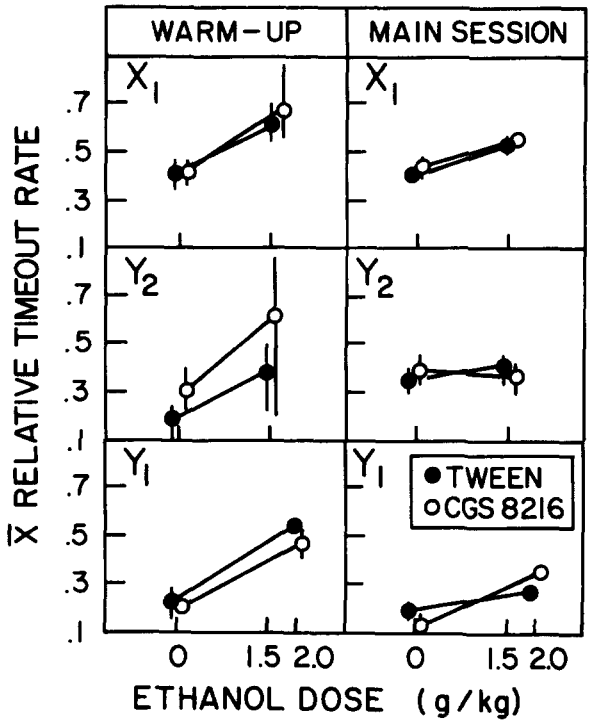

FIG. 3. Relative timeout rates for warm-up (left panels) and main session (right panels) for subjects exposed to ethanol-CGS 8216 combinations (open circles) and ethanol-tween combinations (solid circles). The proportion of timeout responses relative to avoidance responses are plotted on the ordinate and presence or absence of ethanol (1.5 g/kg for X1 and Y2; 2.0 g/kg for Y1) on the abscissa. Vertical lines passing through the circles indicate the ranges.

or saline control. The dose of CGS 8216 was chosen on the basis of demonstrated long-acting reversal of benzodiazepine effects [5,31], and on the demonstration in our laboratory that 5.0 mg/kg CGS 8216 reversed the actions of up to 30 mg/kg of chlordiazepoxide on the same concurrent schedule studied in the present experiment [17]. Only one dose of ethanol was studied: 1.5 g/kg for X1 and Y2, and 2.0 g/kg for Y1. Three drug probe sessions were conducted for each condition for X1 and Y2, but only two for Y1.

RESULTS AND DISCUSSION

Avoidance and timeout response rates, and shock rates are summarized in Table 5. The effects of ethanol noted in Experiment 1 generally were replicated in the present study. Consider the comparison between the Tween and Tween-Ethanol conditions (1.5 g/kg for X1 and Y2; 2.0 g/kg for Y1) of Table 5. Avoidance rates were depressed and shock rates increased by ethanol, and both effects were confined to the warm-up segment of the session. Statistical analysis (Ethanol Dose × CGS 8216 Dose × Session Segment) of the adjusted avoidance and shock rates confirmed the reliability of the Ethanol × Segment interaction for both measures (avoidance: $F(1,2)=30.93$, $p<0.05$; shock: $F(1,2)=85.35$, $p<0.05$). Timeout responding tended to increase with ethanol during warmup, but the effect was not apparent in the main session data, as reflected in the Ethanol × Segment interaction effect on adjusted timeout rates, $F(1,2)=21.41$, $p<0.05$.

Table 5 shows that CGS 8216 tended to depress both avoidance and timeout responding, and to increase shock rates. Statistical confirmation of these trends was clearest for the depression of avoidance, where there was a significant main effect of CGS, $F(1,2)=21.47$, $p<0.05$. For the shock and timeout rates, statistical reliability of the effect of CGS was marginal (shock: $F(1,2)=14.40$, $p=0.06$; timeout: $F(1,2)=14.40$, $p=0.06$). When combined with ethanol, CGS 8216 showed no tendency to reverse any of the above effects (tests of the CGS × Ethanol interaction were non-significant for all dependent measures). Interestingly, there is some evidence to suggest that CGS tended to potentiate the effects of ethanol on shock rates, as indicated by the increased shock rates during warm-up in the CGS 8216-Ethanol condition and the statistical test of the CGS × Ethanol × Session Segment interaction, $F(1,2)=14.33$, $p=0.06$.

The effects of CGS 8216 observed here suggest that the compound does have some intrinsic actions which influence avoidance performance. This is particularly noteworthy in view of the recent report [14] that CGS 8216 may possess some properties of an inverse agonist since it shows anxiogenic action under some conditions. Perhaps the effects of CGS 8216 seen in the present study reflect such action manifested by disruption of avoidance. Complicating this interpretation are the results of another study in which CGS 8216 failed to affect avoidance [17]. Although the drug dose and operant baselines in the two studies were identical, one potentially important difference was that the rats in the present study had an extensive history of exposure to ethanol before they were exposed to CGS 8216. In view of studies which noted long-term effects of ethanol exposure on the GABA-BZ receptor complex (see [32] for a review), it may be that chronic exposure to ethanol is necessary for the intrinsic actions of CGS 8216 to be observed in our procedure.

Figure 3 shows the relative rates of timeout and avoidance responding. As in Experiment 1, ethanol enhanced relative timeout responding in all three animals during warm-up (left-hand panels of Fig. 3). Although there was range overlap in the case of Y2, the trend was still evident, and the one rat who showed range overlap in Experiment 1 (X1) showed a more robust and reliable effect here. As in Experiment 1, the effect dissipated as the session continued (right-hand panels). In line with this description based on the individual performances, statistical analysis of the adjusted relative rates confirmed the effects of Ethanol, $F(1,2)=28.74$, $p<0.05$, and the interaction between Ethanol and Session Segment, $F(1.2)=30.97$, $p<0.05$. In general CGS 8216 did not affect relative rates with the possible exception of Y2 during warmup, and again there was no tendency for CGS 8216 to reverse the action of ethanol. None of the statistical tests of the effect of CGS on adjusted relative rates was significant (CGS, CGS × Ethanol, CGS × Segment, CGS × Ethanol × Segment). Thus, the present results did not support the notion that benzodiazepines and ethanol act through a common mechanism.

GENERAL DISCUSSION

The present experiments did not support the idea of a common neurochemical link between alcohol and opiates since none of the effects of alcohol was reversed by naltrexone. Similarly the failure of the BZ antagonist, CGS 8216 to reverse any of the actions of alcohol was inconsistent with the notion that such actions are mediated by the BZ recep-

TABLE 5

MEAN AVOIDANCE RESPONSE RATE, TIMEOUT RESPONSE RATE AND SHOCK RATE FOR THE THREE SUBJECTS ACROSS THE CONDITIONS OF EXPERIMENT 2

	Avoidance Resp/Min	Timeout Resp/Min	Shocks/Min
Rat X1			
CGS 8216			
WU	4.19 (2.79–5.76)	3.17 (2.30–3.65)	1.02 (0.70–1.48)
MS	4.17 (3.66–4.58)	3.25 (2.92–3.66)	0.92 (0.39–1.28)
CGS 8216 Ethanol			
WU	1.91 (0.54–3.44)	3.66 (2.52–4.30)	2.95 (1.51–3.80)
MS	3.38 (3.09–3.66)	3.88 (3.25–4.75)	0.70 (0.69–0.71)
Tween			
WU	5.47 (4.62–7.07)	4.00 (3.87–4.06)	0.39 (0.34–0.45)
MS	4.58 (4.39–4.70)	3.61 (3.29–4.20)	0.09 (0.02–0.13)
Tween Ethanol			
WU	3.84 (2.80–5.84)	6.30 (5.34–6.97)	0.45 (0.32–0.51)
MS	4.02 (3.87–4.18)	4.22 (4.17–4.29)	0.32 (0.21–0.41)
Rat Y1			
CGS 8216			
WU	9.19 (8.26–10.11)	2.14 (2.02–2.26)	0.22 (0.17–0.27)
MS	15.01 (14.22–15.79)	1.90 (1.39–2.41)	0.05 (NONE)
CGS 8216 Ethanol			
WU	2.79 (1.39–4.18)	2.74 (1.01–4.47)	3.00 (0.58–5.42)
MS	5.16 (4.76–5.56)	2.77 (2.44–3.09)	0.55 (0.12–0.98)
Tween			
WU	16.33 (13.33–19.33)	4.22 (5.33–3.11)	0.00 (NONE)
MS	10.78 (9.75–11.81)	2.52 (2.38–2.65)	0.03 (NONE)
Tween Ethanol			
WU	4.53 (4.06–5.00)	5.00 (4.59–5.40)	0.36 (0–0.71)
MS	6.48 (6.28–6.67)	2.33 (2.27–2.38)	0.14 (0.08–0.19)
Rat Y2			
CGS 8216			
WU	3.32 (1.89–5.03)	1.47 (0.75–2.02)	3.14 (0.76–5.79)
MS	2.86 (0.92–4.56)	1.54 (0.72–2.21)	3.09 (0.92–7.34)
CGS 8216 Ethanol			
WU	1.76 (0–3.81)	2.46 (0.76–3.73)	2.61 (1.91–3.92)
MS	3.57 (3.47–3.75)	1.96 (1.31–2.45)	1.26 (0.63–2.11)
Tween			
WU	8.11 (6.76–9.70)	1.90 (1.03–2.39)	0.04 (0.0–0.12)
MS	4.80 (4.43–5.32)	2.51 (2.32–2.75)	0.17 (0.14–0.18)
Tween Ethanol			
WU	4.12 (1.52–5.86)	2.32 (0.77–4.68)	1.23 (0.22–2.87)
MS	3.47 (3.08–3.87)	2.34 (1.88–2.62)	0.89 (0.19–1.29)

tor. The major finding was that ethanol reliably enhanced relative rates of responding to produce timeout from avoidance. These effects were somewhat surprising given the idea that alcohol has anxiolytic actions. One might argue that an anxiolytic drug should reduce responding for timeout because that drug should attenuate the aversiveness associated with the avoidance schedule. However, in a recently completed study using this same procedure [17], we noted that the anxiolytic drug chloridazepoxide also stimulated timeout responding at doses which decreased or did not affect avoidance responding. There are a number of possible explanations for these counter-intuitive findings. One possibility is that the differential effects on timeout and avoidance were due to rate-dependency, since in general the response rates on the avoidance lever were higher than those on the timeout lever (but note that this was not the case for X1 in Experiment 2). However, an alternative explanation might be that since alcohol led to a deterioration of avoidance responding, perhaps due to generalized loss of motor control, the resultant increase in shocks received might increase the aversiveness of the situation. Thus, the increase in relative timeout rates might have reflected increased reinforcing properties of timeout when the situation increased in aversiveness. There are certainly other accounts of the effect

which might be developed, but much more work with this procedure will be necessary before the nature of this intriguing effect can be clearly defined. Research defining the effects of other drugs on timeout responding would help to clarify the theoretical significance of the ethanol effects. In any case the selective nature of the ethanol-effects observed in the present research suggests the potential value of the concurrent avoidance-timeout procedure as a tool in behavioral pharmacology.

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