A Method to Measure Interactions of Various Agents and Ethanol on Behavioral Performance in Rats (35227)

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Since alcoholism is a major health problem, a suitable test to quantify the effects of various agents as possible antagonists of ethanol-induced changes in the behavioral performance of laboratory animals has seemed desirable. Arvola *et al.* (1) have described the use of a tilted plane to assess ethanol intoxication in the rat, and pointed out that the method seemed to measure principally the effects on equilibrium and motor coordination. Gibbins *et al.* (2) employed a test, based on the classic one of a person walking a straight line, that involved a belt-treadmill over an electrified grid to quantify the degree of ethanol intoxication in small animals. Reynolds and Van Sommer (3) reported that ethanol affected avoidance behavior.

The present report describes our experience in the development of a model, based on trained rat performance, to identify compounds which might antagonize the depressant effect of ethanol on behavior. The method separates such responses as stimulation, depression, loss of coordination, confusion, and disruption of training, while providing for observations of peak effects, duration of action, and cumulative differences.

Methods. Adult, male Wistar rats,[2] weighing 400–650 g, were used in these studies. The operant behavior chambers[3] contained avoidance and escape levers, mounted on the left and right, respectively, of a side wall. The shock (350 V and 0.6 mA short circuit current) was provided by a shocker-scrambler circuit connected to the chamber's grid floor.

The continuous avoidance schedule used was essentially that described by Scheckel and Boff (4). Briefly, the rats were trained individually to avoid a shock that was programmed to occur at 10 sec intervals and thus delay the succeeding shock for 40 sec. By pressing the avoidance lever before 40 sec elapsed the rat could again postpone the next shock for 40 sec. If he failed to press the avoidance lever, the shock began and persisted for 5 sec unless the rat pressed the escape lever to terminate the shock.

The parameters recorded were the number of avoidance lever presses (A), total escape lever presses, excess escape lever presses (EE), avoidance + excess escape lever presses (AEE), shocks (S), shocks terminated and shocks not terminated (SNT). This last parameter was found to give a useful quantitative assessment of the differences in behavioral performance by a rat receiving a test agent + ethanol versus the same rat receiving saline + ethanol.

To enable a rat to serve as his own control each rat was trained individually. The rats were fasted overnight prior to any training, control or test session. Each rat was treated according to the protocol shown in Table I. During the 1-hr adjustment period (Table I) in the operant chamber it was possible to determine whether the rat was, in general, performing normally. All performance sessions began at zero time (Table I) and continued for 5 hr thereafter. Thus, the total elapsed time in a complete test was 6.25 hrs.

The ethanol-tolerance dose was determined for each rat individually (ethanol control, Table I). The initial dose of 1250 mg/kg was increased in 250 mg/kg increments until SNT was a reproducible number. The tolerance dose was defined arbitrarily as the ip dose which yielded an SNT of approx-

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TABLE I. Treatment Protocols in Ethanol Behavior Performance Studies in Male Rats.

Treatment[a]	Animal group (at indicated times [min])		
	Saline control	Ethanol control	Test
Rats placed in operant chamber	—75[b]	—75	—75
Saline	—15 and 0	—15	NA[c]
Test compound	NA	NA	—15
Ethanol	NA	0	0

[a] All injections were given intraperitoneally.
[b] Minutes.
[c] Not applicable to this group.

imately 240–260. The ethanol doses were calculated as milligrams of pure ethanol, using appropriate amounts of 95% ethanol diluted in 0.9% NaCl solution since it rendered the ethanol less irritating than did distilled water. All doses were administered in 10-ml/kg volumes.

The effects of test compounds on ethanol-tolerance-dose-induced changes in behavior were determined according to the protocol shown in Table I. Experiments using saline + ethanol were conducted before and after each test drug to assure that a rat response to his tolerance dose of ethanol was reproducible. Approximately every seventh week a saline + saline control test was conducted to verify that the rat was performing at his original level. No rat was used more frequently than once every 5 days.

Calculations of test agent effect as rideouts (R), based on SNT, were made from the following equation:

$$\left[\frac{SNT_{te}}{(SNT_{eb} + SNT_{ea})/2}\right] \times 100 = R,$$

where SNT_{te} = SNT with test agent + ethanol; SNT_{eb} = SNT with saline + ethanol before test agent; SNT_{ea} = SNT with saline + ethanol after test agent; and R = rideouts (as a %).

Results. How the data can be plotted to document an individual rat performance is shown in Fig. 1 for rat No. 8458 that had received DL-threonine (1 g/kg) in addition to ethanol (1.75 g/kg). During the first 2 hr the amino acid reduced the depression induced by ethanol to the degree represented by the shaded areas in both the A and AEE portions, either as the cumulative or percentage difference from the saline + saline control. But by comparing the A, AEE, and EE portions of Fig. 1 it is apparent that DL-threonine had its prime effect on the more critical avoidance behavior.

Beyond the shaded area (*i.e.*, beyond 2 hr) performance after DL-threonine + ethanol treatment paralleled that after saline + ethanol treatment. Also the amino acid depressed both S and SNT to essentially the same degree, indicating improved performance in these parameters compared with ethanol alone.

The range of effects of test agents on R can be seen in Table II. At one extreme D-fructose and sodium pyruvate reduced R to 42 and 52%, whereas at the other nicotinic acid and *p*-chlorophenylalanine increased R to 140 and 180%, respectively, of control. Certain other compounds that caused marked decreases in R included L-alanine, L-asparagine, and L-serine which in limited tests yielded R values of approximately 45–70.

Results obtained in limited testing of selected combinations of various effective compounds were not conclusive. With some combinations the effects were at least additive,

TABLE II. Effects of Selected Agents on Ethanol-Induced Changes in Behavioral Performance in Rats.[a]

Agent	Dose[b] (mg/kg)	No. of trials	Rideouts (R)[c]
D-Fructose	1550	6	42
Sodium pyruvate	500	6	52
L-Methionine	110	4	56
DL-Glutamine	1250	5	58
Sodium succinate	200	3	62
Choline bitartrate	85	13	70
L(—)-Carnitine	70	3	72
Sodium glutamate	1400	3	75
N-Acetyl glucosamine	200	4	104
Nicotinic acid	125	4	140
Desmethylcarnitine	150	2	142
p-Chlorophenylalanine	300	2	180

[a] Adult, male, Wistar.
[b] By intraperitoneal route.
[c] Calculated as described in text.

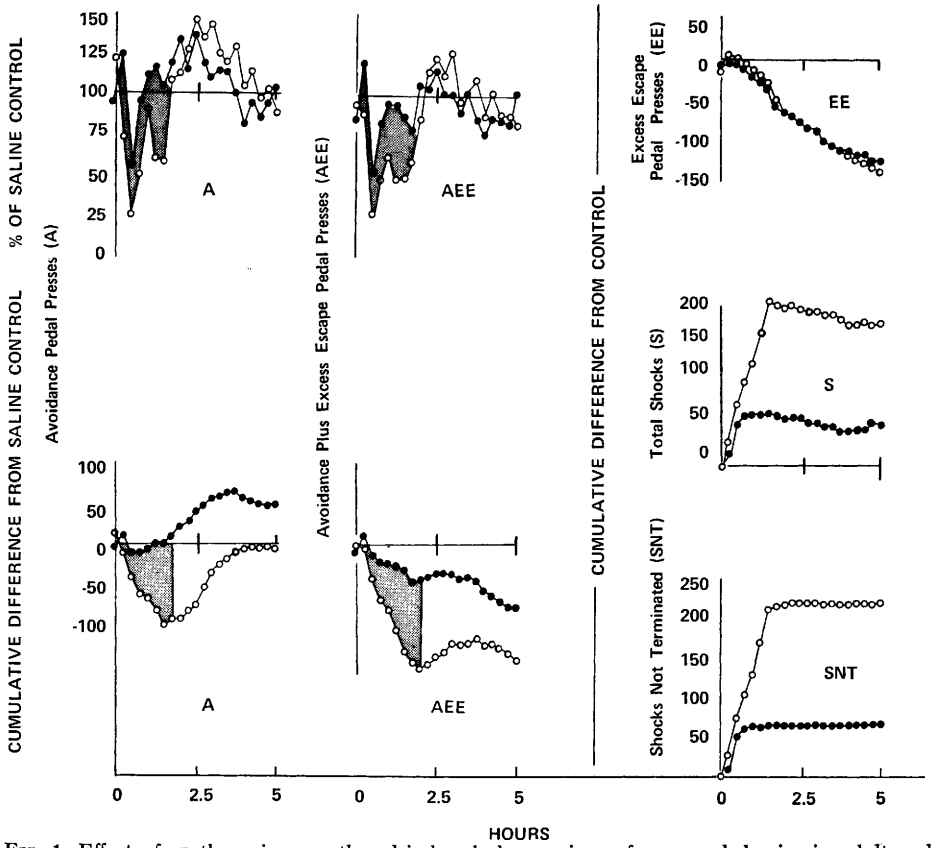

FIG. 1. Effect of DL-threonine on ethanol-induced changes in performance behavior in adult male rat over 5 hr; ○ = ethanol, 1.75 g/kg, ip; ● = DL-threonine (1 g/kg, ip) + ethanol.

even if not synergistic, whereas with others the effects were no greater than with either component alone. However, in none of the combinations tested did there seem to be any overt antagonism of one component by the other. The most effective combinations were those of L-methionine with either sodium pyruvate, L-threonine, or L-alanine where the *R*'s were 13, 17, and 27%, respectively.

Discussion. The comprehensive test described in this report offers a method whereby one can study possible ethanol antagonism in a meaningful way in the laboratory. A broad spectrum of effects of inhibitors, or even potentiators, of the ethanol-induced changes in behavioral performance can be quantified. The data generated are applicable to on-line, computer-programming as well as to statistical treatment. In particular the test provides a clue to the interaction of the test agent and ethanol on the rat's discriminatory, avoidance behavior beyond simple termination of shocks. However, the number of shocks not terminated offers a useful parameter for quantification of inhibitor, or potentiator, effects.

Summary. 1. A method, employing a continuous avoidance-type behavioral performance schedule in adult male rats, to measure the effects of test agents on ethanol-induced changes in animal performance is described. 2. The test measures discriminatory, drug-ethanol interactions on avoidance behavior, but the number of shocks not terminated was, overall, the most useful parameter to quantify the influence of a test agent on performance in ethanol-treated rats. This effect was calculated as a percentage value and termed "rideouts." 3. Of the test agents examined in this model those involved in metabolism were the most active inhibitors (*e.g.*, DL-threonine, D-fructose, sodium py-

ruvate, L-methionine, and DL-glutamine).

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