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Dose-Related Facilitation by Alcohol of Avoidance Acquisition in the Goldfish[1]

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PETTY, F., R. C. BRYANT AND W. L. BYRNE. *Dose-related facilitation by alcohol of avoidance acquisition in the goldfish.* PHARMAC. BIOCHEM. BEHAV. 1(2) 173–176, 1973.–The present study was designed to examine the effect of alcohol on the early phase of dark-avoidance acquisition in the goldfish. Fish were immersed in water solutions of ethyl alcohol (428, 628, or 856 mg/100 ml) for 3 hr before receiving 20 trials of active dark-avoidance training in individual shuttleboxes containing the same concentration of alcohol as that of the pretraining immersion. A dose-related facilitation of acquisition performance was found in fish treated with alcohol compared to fish treated similarly but not exposed to alcohol. Levels of responding calculated as [Total Shuttle Responses - Correct Responses] were not different among the 4 groups.

Alcohol Learning Goldfish

ALTHOUGH the effects of alcohol on learning have usually been studied in connection with state-dependent or dissociated learning phenomena, the question of alcohol's effects on initial acquisition is of interest in itself.

Alcohol has been reported either to impair acquisition [12,18] or to have no effect on it [8, 17, 18, 19]. Goodwin *et al.* [12] found that alcohol impaired learning of verbal material by humans; and Y-maze acquisition in the goldfish has been reported to be retarded in the presence of rising blood alcohol levels [18]. However, among studies finding no statistically reliable differences between alcohol-treated and control animals, several did find suggestions of facilitation of acquisition by alcohol [8, 17, 19]. Thus, while there is little reason to doubt that alcohol in sufficient doses will impair acquisition, the question of whether, under appropriate conditions, it will facilitate acquisition performance has not been answered.

Why the effects of alcohol on acquisition have not been less equivocally demonstrated is not clear. Ryback [17, 18, 19] used a task in which goldfish were trained to criterion on a left-right discrimination in a continuous Y-maze. The fish was punished for an incorrect choice only by bumping into a transparent barrier in the incorrect arm of the maze; no shock, food, or other reinforcement was used. A procedure of this type, while perhaps involving minimal stress to the animal, depends greatly on the fish's continuous spontaneous activity in the apparatus. A procedure which has been more widely used to examine the effects of various experimental treatments on learning is active avoidance conditioning reinforced by electric shock in a two-compartment shuttlebox. Using active conditioned-avoidance training with rats, Crow [8] found that alcohol-treated animals were slightly, but not significantly, better in initial acquisition.

Another problem in studies with a drug such as alcohol is that the blood levels of the drug usually cannot be easily controlled and are likely to be changing during training; studies with rats [8] and humans [12] face this difficulty. Fortunately, the goldfish, whose utility has been shown in a wide variety of pharmacological and behavioral work [2, 3, 4, 5, 6, 9, 10, 13, 16], offers a potential solution to this problem. Levy and Gucinski [15] have shown that the absorbing membranes of a goldfish immersed in a drug solution have permeability characteristics similar to other biologic membranes. In addition, Ryback *et al.* [20] have shown that equilibrium is obtained between the alcohol concentration in the medium and that in the blood of a fish immersed in the medium; equilibrium is reached by 3 hr, with blood alcohol concentration being approximately 85% of that in the surrounding water. It is therefore possible to study the effects of alcohol on various behaviors in the goldfish under steady-state pharmacologic conditions. However, several studies using the goldfish [17, 18, 19] apparently produced a rising blood-alcohol level during

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initial training, either by allowing insufficient pretraining immersion time or by training the fish in a solution of alcohol more concentrated than that during pretraining immersion.

The present study was designed to examine the effects of various concentrations of ethyl alcohol on the early phase of acquisition. We have employed an active-avoidance task reinforced with electric shock and have used procedures to maintain stable blood-alcohol levels during training. In addition, we obtained a general measure of inappropriate and escape responding during training, a measure not reported by other workers [8, 17, 18, 19].

METHOD

Animals

One hundred and twenty 3 -4 in. common goldfish *(Carassius auratus)* were used; they were obtained from Ozark Fisheries, Stoutland, Missouri, U.S.A. After arrival in the laboratory, fish were placed in a 1600 l aerated holding tank and were subsequently placed, 1 day prior to the start of the experiment, in 4 l of water in shallow home tanks, 2 fish per tank. Fish were housed under constant illumination and were fed commercial fish food (Shrimpellets) daily except during and on the day prior to training. Fish were obtained and used during the months of September and October.

Apparatus

Training was carried out in a specially designed fish training and testing apparatus [5]. The apparatus consisted of 10 clear polycarbonate plastic tanks (28.5x18x12.5 cm, lwd) with associated, operationally silent, electronic circuitry controlling the stimulus presentations and recording responses. Each shuttlebox was halved by an opaque partition allowing 3 cm clearance underneath. Passages completely under the partition (shuttle responses) were monitored by 2 photocell units mounted on either side of the partition outside the tank. Shock electrodes (monel wire mesh) covering the ends of the tank and both sides of the partition could supply electric shock in one end of the tank (7 V for 0.1 sec, pulsed once each 1.0 sec). Clear stimulus lamps (G.E. 313 lamps operated at 12 V) were mounted outside the tank at each end, 10 cm from the floor of the tank. During training, the shuttleboxes were under covers, with flat black interiors, that isolated the shuttleboxes from each other and from outside activity. Each shuttlebox had a houselight (G. E. 313 clear lamp operated at 12 V) mounted centrally in the cover 24 cm from the surface of the water.

Procedure

Goldfish were randomly assigned to one of 3 groups. Three hr prior to training, half of the fish in Group 1 (randomly sorted) were taken from home tanks and placed in tanks containing 4.25 l of water containing 24 ml of 95% ethanol; the rest of the Group 1 fish received no ethanol but were placed in similar pretreatment tanks. In Group 2, half of the fish were placed in tanks containing 4.25 l of water containing 32.5 ml of 95% ethanol, with the remainder of Group 2 receiving no ethanol. Similarly, Group 3 received 48 ml ethanol in 4.25 l, or water only. Each experimental group subsequently was trained concurrently in the apparatus with its own control group. Thus, there were 6 groups of 20 fish each: 3 alcohol-treated groups and 3 control groups. Alcohol concentrations were 428 mg%, 628 mg%, and 856 mg% in the water containing the various alcohol-treated groups. Each fish remained in the pretreatment tanks for 3 hr after which it was immediately placed in the apparatus for training.

Each fish was placed for training into one of the individual shuttleboxes of the training apparatus in the same concentration of alcohol as during pretraining immersion. All fish received 20 trials of active dark-avoidance training. A trial of dark-avoidance training consisted of 10 sec of darkness in the compartment occupied by the fish (i.e., the stimulus light in the unoccupied compartment was illuminated), followed by the addition of electric shock (in the compartment initially occupied by the fish) for 50 sec, after which darkness was again presented to the fish in the occupied compartment, initiating the next trial. The stimulus light in the compartment into which the fish swam to escape or avoid shock remained on during the entire 60-sec trial. The house light was on only before the beginning of the first trial and after the last trial of a session. A correct response was counted if the fish avoided shock by swimming out of the darkened compartment within 10 sec of the beginning of a given trial. Active dark-avoidance training was used to examine drug effects on learning since we as well as others [2] have found it a more difficult task for fish to learn than active light-avoidance. In addition to correct responses, total shuttle responses were recorded for each fish.

RESULTS

Figure 1 (Panel A) presents the mean levels of correct dark-avoidance responding for fish treated with each concentration of alcohol and for the nonalcohol-treated controls. Also shown in Fig. 1 (Panel B) are the mean levels for each group of a score calculated for each fish as [Total Shuttle Response - Correct Responses]. Since shock remained on in the darkened compartment until the beginning of the next trial, this score should not be considered a conventional activity score; rather it indicates the level of escape responding and inappropriate responding during the training session. Response levels from the 2 measures shown in Fig. 1 were very similar among the 3 control groups; therefore, after one-way analysis of variance of the 3 control groups (Correct Responding: $F = 0.127$, df 2/57, $p = 0.882$; Total - Correct: $F = 0.744$, df 2/57, $p = 0.479$), these groups were pooled for further analysis.

(All reported probabilities for F values for given degrees of freedom were obtained by evaluation of a continued fraction of the incomplete beta function [1]. For values of F and degrees of freedom found in standard tables, the calculated probability is, of course, the same as the tabled probability.)

One-way analysis of variance of the correct dark-avoidance responses yielded an F of 16.03 (df 3/116, $p = 0.0001$). Comparison of the mean of each of the 3 alcohol-treated groups with the water control by use of the Dunnett t [21] showed that the 628 mg% group and the 856 mg% group were significantly greater than the control (628 mg%: $t = 5.91$, df 116, $p<0.01$ two-tailed; 856 mg%: $t = 4.65$, df 116, $p<0.01$, two-tailed). Standard deviations for each group were: control, 3.41; 428 mg%, 2.90; 628 mg%, 5.16; 856 mg%, 9.29. One-way analysis of variance of the scores calculated as [Total Shuttle Responses - Correct

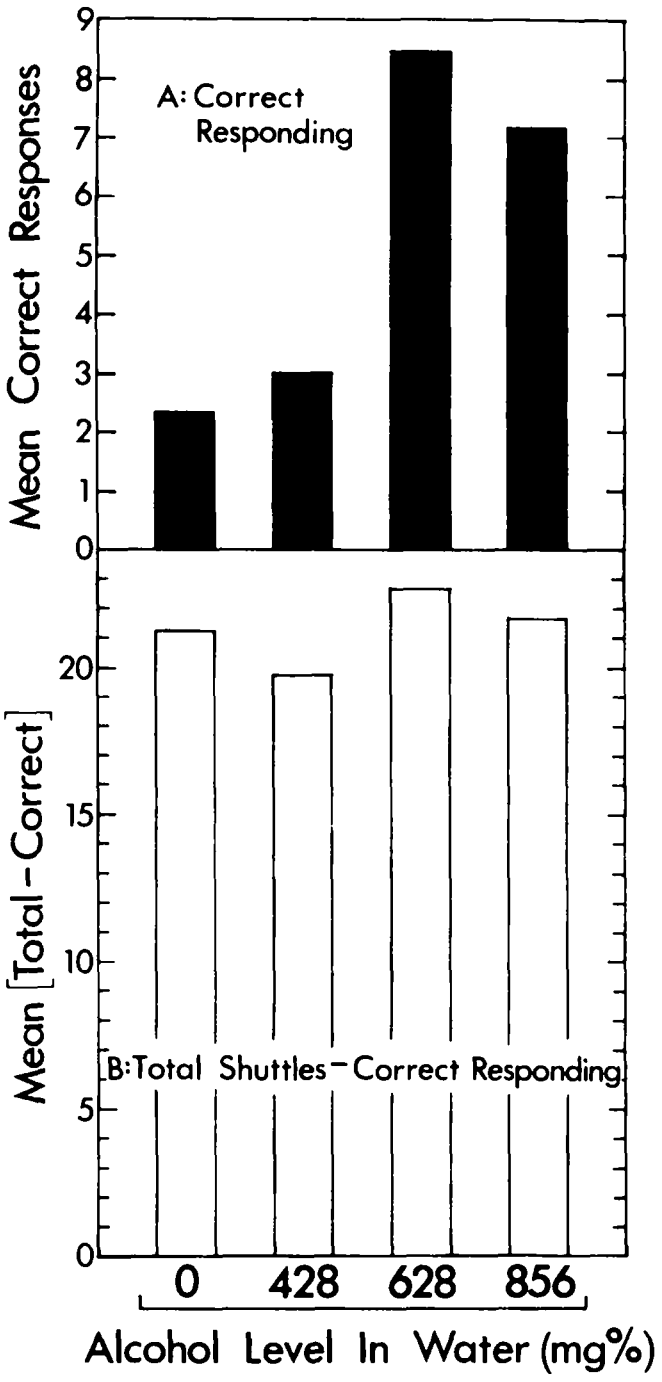

FIG. 1. Mean levels of correct dark-avoidance responding and mean levels of [Total Shuttle Responses - Correct Responses], for 4 groups of goldfish pretreated and trained in water containing various concentrations of ethyl alcohol. Concentration given as mg alcohol per 100 ml solution (mg%). Group O (no alcohol), N = 60; Groups 428, 628, and 856 (various concentrations of alcohol, mg%), N = 20 each group. For correct responses (A), overall F = 16.03, *df* 3/116, $p = 0.0001$; for [Total Shuttle Responses - Correct Responses], overall F = 0.28, *df* 3/116, $p = 0.834$.

Responses] for each fish showed an F of 0.28 which was not significant. Standard deviations for each group were: control, 10.82; 428 mg%, 9.09; 628 mg%, 9.90; 856 mg%, 9.29.

The preceding analyses were performed on actual scores. For the small whole number scores obtained for correct responses, square root transformation ($\sqrt{X + 0.4}$; [7]) gave analyses yielding the same conclusions for pooled control groups (F = 0.28, *df* 3/116) and for correct response levels among treatments (F = 14.48, df 3/116, $p = 0.0001$).

As shown in Fig. 1, there was a dose-related facilitation of acquisition performance. The lowest concentration used (428 mg%) did not significantly facilitate acquisition; however, both higher doses did so. The facilitation appeared to be strongest at the intermediate concentration (628 mg%). However, the effect was also seen at the highest concentration used (856 mg%). No differences in the level of [Total Shuttle Responses - Correct Responses] were seen among groups.

Comparison of correct responding between all alcohol-treated and all control fish gave means of 6.20 and 2.32, respectively; this difference was highly significant ($t = 8.28$, $df = 118$, $p < 0.0001$, two-tailed). However, for the measure [Total Shuttle Responses - Correct Responses], means for all alcohol-treated and all control fish were 21.35 and 21.25; this difference was insignificant ($t = 0.05$, $df = 118$).

No fish died during the experiment, and all appeared healthy the following day. There were the expected gross behavioral changes with the higher doses of alcohol; e.g., slower righting reflexes and mild incoordination.

DISCUSSION

There was a clear facilitation by alcohol of initial acquisition performance in the present experiment, and this facilitation, in the procedure used, was not associated with increased levels of incorrect (inappropriate and escape) responding. Thus it appears, as suggested but not demonstrated by previous work [8, 17, 18, 19], that facilitation of initial acquisition by alcohol can be obtained.

We are reluctant to ascribe the observed facilitation to a direct effect on learning. Although such an interpretation cannot at this time be excluded, several other explanations may be considered. A change in sensitivity to light, electric shock, or both, could contribute to the effect observed. However, alcohol may act in a situation such as ours to reduce stress or arousal. As Jarvik [14] has pointed out, sedative-hypnotic agents, which might be expected to impair learning, may under some circumstances promote learning. For example, animals can learn a visual discrimination task with low levels of shock, whereas high shock levels disrupt acquisition; but with sodium amylbarbital, shock is effective at both levels [11]. Presumably, the drug shifts the arousal curve downward sufficiently to attenuate the disruptive effects of higher shock. Such an explanation might reasonably apply to the present finding inasmuch as shock-reinforced training, particularly during the initial phase, is presumably quite stressful. The experiment of Crow [8], which found slight but nonsignificant superiority of alcohol-treated rats on initial training, also used an active-avoidance task. On the other hand, a training procedure such as Ryback's [17, 18, 19], to the extent that it is less stressful, might also be less likely to show a facilitating effect of alcohol if such facilitation is due to a postulated reduction of stress or arousal. Finally, it is

possible that stable, as opposed to changing, levels of blood alcohol may contribute to the demonstration of the facilitation we observed, although the facilitation may not depend entirely upon this factor. In any case, the ease with which blood-alcohol levels may be manipulated in the goldfish recommends the goldfish for further work in this area.

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