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Effects of Ethanol and Sherry on the Performance of a Conditioned Response in the Rat[1,2]

J. A. IZQUIERDO, ALICIA B. MERLO[3], ELBA CHEMERINSKI AND MAGDA BILLIET

Department of Experimental Pharmacology, Facultad de Farmacia y Bioquimica
Junin 956, Buenos Aires, Argentina

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IZQUIERDO, J. A., A. B. MERLO, E. CHEMERINSKI AND M. BILLIET. *Effects of ethanol and sherry on the performance of a conditioned response in the rat.* PHARMAC. BIOCHEM. BEHAV. 2(3) 317–323, 1974. – The effect of beverages containing ethanol was studied in male adult (Wistar derived) rats subjected to avoidance learning in a shuttle-box. Five sessions, each of 50 trials were held every 24 hr for 5 days. The following groups of rats drank ad lib during the 3 days prior to the first avoidance session and throughout the test period: (1) A control group which drank 1/3 isocaloric (related to alcoholic beverages) glucose in tap water. The animals drank about 3 volumes glucose related to one volume of alcoholic beverages. (2) Ethanol (17% v/v plus 2.0% glucose). (3) Sweet sherry (17% ethanol v/v plus 2.0% reducing sugars). (4) Artificial sherry made by mixing the volatile components (methanol, acetaldehyde, ethanol, n-propanol, ethylacetate, iso-butanol and iso-amylic alcohol) of sherry in water plus 2.0% glucose. (5) Artificial sherry without ethanol. (6) A control group which drank only water. No statistically significant difference was observed between the control groups on the avoidance learning task. Ethanol, sherry and artificial sherry significantly increased the performance level over that of the controls. The effect of ethanol was observed in the 5 sessions; that of sherry and artificial sherry after the third session. This difference is attributed to the absence of volatile congeners in the ethanol solution. In rats drinking for 19 days prior to the first avoidance trial and throughout the test period, neither ethanol nor sherry improved the performance level. When naive rats with an initially low performance level drank ethanol from the end of the first session and up to the last session their performance level was significantly increased. This effect was not observed when the rats drank sherry. After having drunk ethanol for 24 hr, the individual progress of rats with a low performance level was significantly increased until the last session. However, after having drunk sherry, individual progress was significantly favored in the 2nd session only.

Ethanol Avoidance learning Sherry Rat

THE EFFECTS of ethanol on animal and human behavior depend upon experimental conditions [3,10]. Some authors [4, 5, 13, 15] agree that administration of ethanol in high doses produces avoidance impairment in rats, a condition which might be related to an impairment of their motor or sensory capacity [3]. Reynolds and van Sommers [13] observe that avoidance behavior in rats is favored by low doses of ethanol, whereas high doses depresses it. According to Pawlowski *et al.* [12], intake of ethanol for 20 days prior to the avoidance test produces a slow-down of running in rats.

Research work on the effects of ethanol only, may well contribute more if the ethanol used were to include the other components of alcoholic beverages [2]. According to Aschkenasy-Lelu [1], a prolonged intake of wine in rat produces a more intense delay of acquisition and increased latency of avoidance conditioned response than the intake of a pure ethanol solution of the same alcoholic content, in the same conditions. Murphree *et al.* [11] pointed out that in man, there is a difference between the effects of vodka which contains a very small amount of volatile congeners, and bourbon which is rich in volatile congeners.

The alcoholic beverage used in this series of experiments was the commonly used Argentine sweet sherry. The effects of this beverage and the influence of volatile congeners were studied together with those of an ethanol solution in the same concentration. Beverages were ingested ad lib for 3 or 19 days prior to the first trial and throughout the conditioning period. The effect of alcohol beverages was also studied in low performance rats.

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[3] Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas. Argentina.

GENERAL METHOD

Analysis of the Volatile Components of Sherry

The volatile components of sherry were analyzed through gas chromatography using a Varian Aerograph chromatograph with flame ionization detector. A 5 ft. × 1/8 in. column packed with Porapak Q was used. Sherry was distilled and then injected in the chromatograph. The injector temperature was stabilized at 200°C.

The column temperature was held at 160°C for 2 min and then programmed at 8°C/min up to 184°C, then at 4°C/min up to 204°C, which was held from the 10th to 13th min. From this point, it was again programmed at 10°C/min up to 234°C and held for the remainder of the analysis.

Purity of ethanol used to prepare 17% v/v ethanol solution as well as artificial sherry was checked by gas chromatography.

Conditioning

Adult male (Wistar derived) rats were fed Forramez balanced food, ground, mixed in 33% water and kept in individual boxes at 24°C with a 12 hr light/darkness cycle. At the beginning of the first avoidance test, the rats were about 3 months old and weighed 140–200 g. The training box was divided into 2 compartments connected by a small opening allowing the passage of the rat from one compartment to the other. Each half of the box could be lighted independently by a 15 W lamp. Three habituation sessions were held every 24 hr during which the rats were submitted to handling for 2 min; immediately after, 5 electric shocks (2.5 mA; 50 Hz; 5 sec) were applied to the feet at intervals of 0.5–1.0 min.

Three days after the last habituation session, learning tasks were carried out daily between 9 a.m. and noon, for 5 consecutive days. Every day, 6 to 12 rats, including treated and controls belonging to the same experiment, were conditioned. Each rat was tested at the same time each day.

The training consisted in placing the rat in one compartment of the box in the darkness which was then lighted. If the rat did not pass into the dark compartment within 5 sec after the light was switched on, it received an electric shock of the same intensity and frequency as in the habituation sessions until it moved into the dark compartment. In each daily session, 50 light stimuli were given at intervals varying from 0.5–1.0 min.

The beverages tested were given ad lib and were the only source of liquid. The body weight and the food and drink intake were measured daily.

EXPERIMENT 1

Procedure

Groups 1 to 5 of rats drank the test solution from the end of the 3rd habituation session, that is for 3 days prior to the first avoidance trial and throughout the test period: (1) 1/3 isocaloric (related to alcoholic beverages) glucose in tap water (glucose): control (n = 14). Since animals drank about 3 volumes glucose (about 30 ml/100 gw/day) related to 1 volume of beverages containing ethanol, the isocaloric glucose solution (28%) was diluted 3 times. (2) Ethanol in tap water (17% v/v plus 2.0% glucose): ethanol (n = 10). (3) Sweet sherry (17% ethanol v/v plus 2.0% reducing sugars): sherry (n = 14). (4) Artificial sherry (by mixing the volatile components of sherry in tap water, in the same proportion as determined by gas chromatography of natural sherry plus 2.0% glucose): artificial sherry (n = 13). (5) Artificial sherry without ethanol (prepared in the same way as artificial sherry without ethanol) (n = 13). (6) One control group (n = 16) drank water only.

In addition 3 other groups drank for 19 days before the first avoidance session including habituation and avoidance test period: (a) Glucose (n = 23) as in Group 1. (b) Ethanol (n = 15) as in Group 2. (c) Sherry (n = 20) as in Group 3.

EXPERIMENT 2

Procedure

In the second experiment the following 4 groups of naive rats with an initially low performance began drinking at the end of the first learning session and up to the last one: (a) Glucose (n = 13) as Group 1. (b) Ethanol (n = 12) as Group 2. (c) Sherry (n = 14) as Group 3. (d) One control group (n = 12), a different one than that used in Experiment 1, drank water only, like Group 6.

The performance of these rats was under 20% correct conditioned responses in the first session compared to the average of the population: about 40%.

RESULTS

Analysis of the Volatile Components of Sherry

An analysis of the volatile components of sherry used, revealed the following (ml percent v/v):

Methanol: 0.005; Acetaldehyde: 0.017; Ethanol: 16.900; n-Propanol: 0.026; Ethylacetate: 0.015; iso-Butanol: 0.003; iso-Amyl alcohol: 0.034.

In ethanol, no volatile congeners could be detected.

EXPERIMENT 1

Conditioning

Latency to perform the conditioned response was similar in the different groups and varied between 2.5–4.2 sec in the 1st block of 10 trials in the initial session. The latency was smaller in the 5th block of 10 trials in each daily session and it was 1.0–2.7 sec at the end of the last session. During the initial trials, when rats did not perform the correct avoidance response, the necessary punishment time for escape was as long as 15 sec but decreased with the number of trials carried out. When punished, animals attaining high performance levels in the 1st session, escaped immediately. In all cases, the avoidance response was made immediately after the middle of the second session. With shorter punishment time, there was also a concomitant decrease in piloerection, vocalization and defecation.

Groups drinking for 3 Days Before the 1st Learning Session and Throughout the Test Period

The number of conditioned responses ($\bar{x} \pm$ S.E.) of each group per block of 10 trials is represented in Fig. 1, with *t* test for statistical significance. The 2 control groups do not differ significantly. The results of the remaining groups were compared with the glucose control.

Performance level during the 5 learning sessions is significantly increased in rats drinking ethanol. This effect is observed for all the 10 trials blocks beginning at the 3rd session until the last one.

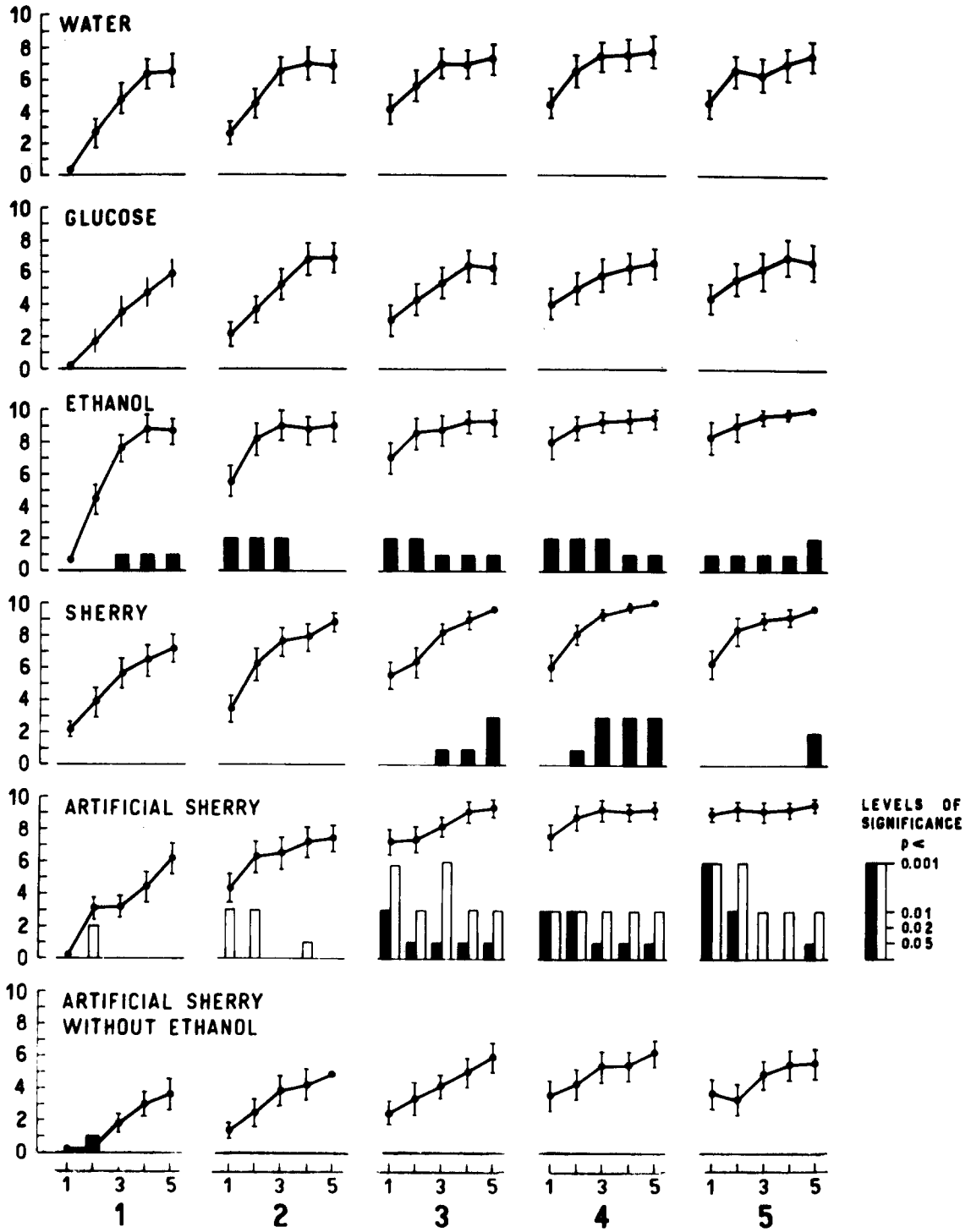

FIG. 1. Performance of drinking groups for 3 days before the 1st trial and throughout the test period. Ordinates: number of conditioned responses ($\bar{X} \pm$ S.E.). Abscissae: top – blocks of 10 conditioned stimuli. bottom – sessions. Black bars: levels of significances (t test), in relation to glucose control. White bars: levels of significances between artificial sherry and artificial sherry without ethanol.

Groups drinking sherry and artificial sherry did not modify their performance significantly during the first 2 sessions, in the 3rd and 4th sessions, the performance level of both groups improves significantly, but more so with artificial sherry. At the beginning of the last session the facilitating effect of artificial sherry is still marked; sherry, only improves the performance at the end of it.

The group drinking artificial sherry without ethanol tends to attain performance levels lower than the control rats; the difference is significant only in the 2nd block of 10 trials in the initial session. The group treated with artificial sherry carried out a number of conditioned responses significantly higher than those with artificial sherry without ethanol in the 5 sessions. The intake of beverages and food of all the groups is shown in Table 1. In glucose-drinking animals, urination was intense.

Groups Drinking for 19 Days Before the 1st Learning Session and Throughout the Test Period

The number of conditioned responses ($\bar{X}$ ± S.E.) of each group per block of 10 trials is shown in Fig. 2.

Control groups (water and glucose) do not differ significantly between each other or with those of ethanol and sherry.

The intake of beverages and food of the groups is shown in Table 2. The intake of glucose caused intense urination without hyperglycemia or glycosuria, at random determination, after the end of the last session.

EXPERIMENT 2

Conditioning

Daily average latencies of the different groups to perform the conditioned response vary from 2.0 to 5.0 sec in the 1st session, from 1.0 to 3.5 sec in the 2nd session and from 1.0 to 3.3 sec in the remaining ones. Latencies within the same session vary for each animal; no evident modifications due to the effect of beverages have been observed. The escape reaction to the electric shock is similar to that of Experiment 1.

For evaluation of the results we considered the following:

Performance Level

According to our selection, the performance of the 4 groups in the 1st session is similar. By relating through the *t* test the number of correct responses per block of 10 conditioned stimuli in the following sessions, it is to be seen that there are no sifnificant differences between controls (water and glucose). The remaining groups were compared with the glucose control (Fig. 3). Ethanol significantly improves performance from the 2nd to the 5th session. The sherry-drinking group show a tendency to improve its performance level but this is not statistically significant.

Percentage of Errors Since the Second Session

Considering the total number of failures of each rat in the first session to be 100%, we estimated for each the percentage of failures in the following 4 sessions, comparing in this way individual improvement during the course of learning with respect to the first session.

The results shown by the groups are compared from the 2nd to the 5th session by means of the Mann-Whitney U test [9]. This statistical nonparametric test, using ranks instead of absolute values, was considered appropriate to compare our results with respect to a conventional pattern (number of failures of the 1st session = 100%).

It is seen that: (1) there are no significant differences from the 2nd to the 4th session between controls (water and glucose). (2) ethanol intake significantly decreases the percentage of errors with respect to glucose from the 2nd to the 5th session (α, for a one-tailed test, = 0.001 for the 2nd session and $\alpha < 0.01$ for the remaining 3 sessions). (3) sherry intake significantly decreases the percentage of errors in the 2nd session ($\alpha < 0.01$) but not in the remaining ones.

The intake of beverages and food of the groups is shown in Table 3.

DISCUSSION

The performance level of control group of rats drinking

TABLE 1

AVERAGE OF DAILY INGESTION OF BEVERAGES AND FOOD ($\bar{X}$ ± S.E.) PER 100 G. W. IN GROUPS DRINKING FOR 3 DAYS BEFORE TRIALS AND THROUGHOUT THE TEST PERIOD

Groups	PERIOD PRIOR TO TESTS		PERIOD OF TESTS	
	Beverage (ml)	Food (g)	Beverage (ml)	Food (g)
Water	19.6 ± 2.1	22.2 ± 3.3	18.7 ± 1.9	20.1 ± 2.5
Glucose	29.8 ± 2.6	12.1 ± 4.0	30.1 ± 3.0	12.8 ± 2.3
Ethanol	12.1 ± 2.5	13.1 ± 2.8	13.2 ± 2.8	14.0 ± 1.9
Sherry	10.8 ± 1.2	12.7 ± 3.1	11.7 ± 2.1	13.3 ± 3.6
Artificial Sherry	11.3 ± 1.6	15.0 ± 3.1	12.6 ± 1.9	14.8 ± 4.0
Artificial Sherry without Ethanol	12.6 ± 2.0	13.8 ± 5.1	12.0 ± 1.9	12.9 ± 4.0

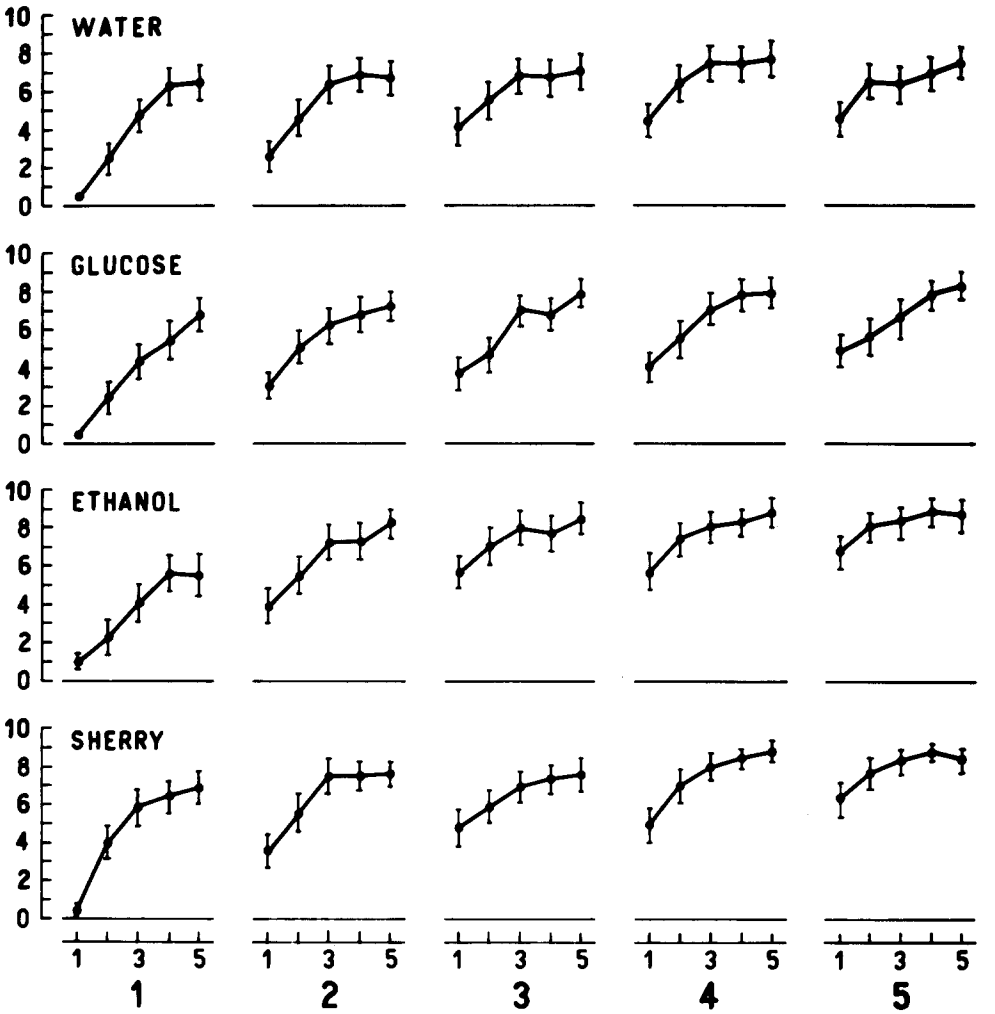

FIG. 2. Performance of drinking groups for 19 days before the 1st trial and throughout the test period. Ordinates: number of conditioned responses ($\bar{X} \pm$ S.E.). Abscissae: top – blocks of 10 conditioned stimuli. bottom – sessions.

TABLE 2

AVERAGE OF DAILY INGESTION OF BEVERAGES AND FOOD ($\bar{X} \pm$ S.E.) PER 100 G. W. IN GROUPS DRINKING FOR 19 DAYS BEFORE TRIALS AND THROUGHOUT THE TEST PERIOD

	PERIOD PRIOR TO TESTS		PERIOD OF TESTS	
Groups	Beverage (ml)	Food (g)	Beverage (ml)	Food (g)
Water	20.3 ± 2.5	20.5 ± 3.2	18.7 ± 1.9	20.1 ± 2.5
Glucose	32.2 ± 3.1	13.2 ± 3.1	33.3 ± 2.6	12.6 ± 2.9
Ethanol	12.3 ± 2.3	11.9 ± 3.2	12.7 ± 1.9	13.3 ± 4.1
Sherry	13.0 ± 2.6	12.7 ± 4.0	13.1 ± 2.2	11.0 ± 4.1

water, chosen at random and conditioned inserted among others that drank other beverages, did not differ significantly from other control groups conditioned before and after the period of our test.

So we consider that the capacity of our strain to perform the conditioned response is not modified along the time.

Since glucose does not modify significantly the performance level in comparison to the water-drinking group and since the amount of food intake and the caloric contribution from the beverages per body-weight unit and per day are similar for those drinking alcoholic beverages and glucose, we consider the later to be the respective controls of the former. Performance is significantly improved when alcoholic beverages are given for 3 days before the 1st avoidance trial and throughout the test period. Taking into account the differences between the facilitating effect of ethanol, sherry and artificial sherry and the behavior of the group drinking artificial sherry without ethanol, we suggest that the volatile congeners (in very low proportions, in

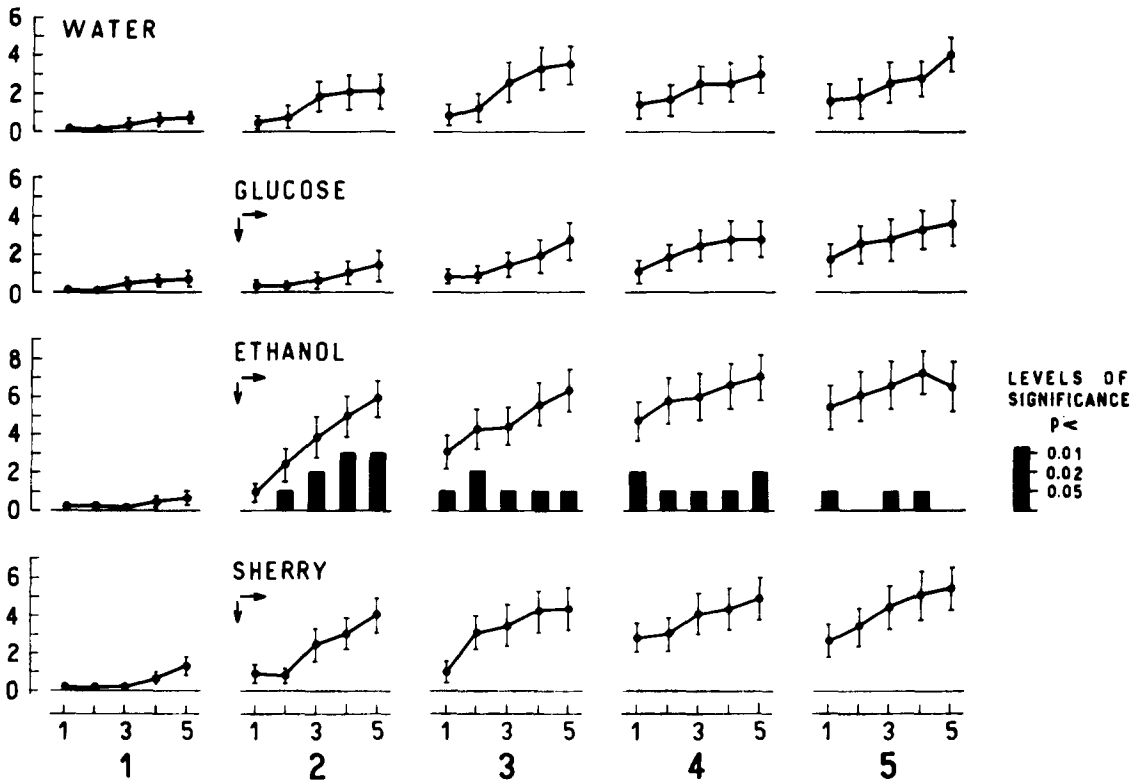

FIG. 3. Performance of groups with initially low performance having drunk from the end of the 1st session till the last one. Ordinates: number of conditioned responses ($\bar{x} \pm$ S.E.). Abscissae: top – blocks of 10 conditioned stimuli. bottom – sessions. Black bars: levels of significances (t test) in relation to glucose control.

TABLE 3

AVERAGE OF DAILY INGESTION OF BEVERAGES AND FOOD ($\bar{X} \pm$ S.E.) PER 100 G. W. FROM THE END OF THE 1ST LEARNING SESSION UNTIL THE LAST ONE IN GROUPS WITH INITIALLY LOW PERFORMANCE

Groups	Beverage (ml)	Food (g)
Water	20.6 ± 2.9	21.6 ± 3.3
Glucose	25.9 ± 2.4	14.0 ± 3.5
Ethanol	9.6 ± 1.7	13.5 ± 2.7
Sherry	9.4 ± 1.9	14.4 ± 4.0

sherry and artificial sherry) bring about their influence by off-setting the facilitating effect of ethanol, especially in the first two sessions. Although we cannot disregard the possibility of the influence of the non-volatile components of sherry on behavior, the vast coincidences between the performance levels of the groups drinking sherry and artificial sherry let us assume that volatile components as a whole bring out the most important influence.

The results obtained with rats having an initially low performance confirmed the facilitating effect of ethanol given for 3 days before the trials. No significant improvement is noticed in the performance level of the sherry-treated group under the same conditions. If we consider the individual progress of each animal in relation to the initial session, we observe a significant improvement during the whole intake time with ethanol, whereas sherry, is only effective in the 2nd session (24 hr after starting intake).

From what is expressed above, we consider that the effects of ethanol alone do not really show what could be expected by using alcoholic beverages and that the volatile congeners of these beverages which are more center-depressing and toxic than ethanol [11], must be taken into account while studying the effects of beverages upon behavior.

On the other hand, according to several authors [6, 7, 8, 14], rats develop tolerance to ethanol after a long intake period. Following the results of these authors, our rats drinking for 19 days would have developed tolerance. In them, the performance level does not differ from those of the controls. In these drinking animals, we did not observe a slowdown of speed in their response as did Pawlowski *et al.* [12].

According to Aschkenasy-Lelu [1], rats that drink wine or ethanol ad lib during 2.5 months previous to the 1st learning session and during the days when the following sessions were held, delay the acquisition and increase latency of avoidance conditioned response. These effects are more marked with wine than with the sole ethanol solution of the same concentration. The more prolonged

time of alcoholic intake of his rats would explain the differences with our results, besides the different strain, experimental conditions and beverages.

In the present work, the importance of ethanol and the volatile congeners of sweet sherry on conditioned avoidance in rats is shown when tolerance would have still not been developed. Tolerance development to alcoholic beverages would be the cause of the inefficacity to modify the performance during the test.

We disregard ethanol concentration in blood and tissues of our rats drinking different alcoholic beverages, during the trials, which were held under the effect of ethanol ingested ad lib during the 24 hr, with an average of 16 g/kg, which would most probably exceed the metabolic capacity. It is impossible to venture the effective concentration of ethanol in blood and tissues to modify acquisition in our rats, as was already stated by other authors [3], so as the effective concentration of volatile congeners that attenuate effects of ethanol.

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