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Oral Intake of Sweetened or Sweetened Alcoholic Beverages and Open-Field Behavior

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NADAL, R. A., M. PALLARÉS AND N. FERRÉ. *Oral intake of sweetened or sweetened alcoholic beverages and open-field behavior.* PHARMACOL BIOCHEM BEHAV **54**(4) 739–743, 1996.—The relationship between the intake of sweetened alcoholic beverages and individual differences in an open field was assessed using an oral self-administration procedure in male Wistar rats (n = 41). After four sessions in the open field, rats were gradually reduced to 80% of their ad lib body weights over a 10-day period. Rats were then allowed to drink an alcohol-containing solution (10% v/v ethanol, 3% w/v glucose) (experimental group: n = 20) or a solution of glucose (3% w/v glucose) (control group: n = 21) for 1 h/day during 9 consecutive days. Experimental rats were divided into two groups on the basis of the mean daily ethanol dose ingested (g/kg/h) in the nine sessions. The high ethanol-consuming (HEI rats), when compared with the low ethanol-consuming rats (LEI rats), only showed a tendency (p = 0.062) towards fewer global number of rearings in the open field. No relationship between open-field defecation and ethanol intake was observed. With regard to the control rats, the higher consuming also showed lower number of rearings in the open field, similarly to the experimental rats. When we divided all experimental or control rats into two subgroups on the basis of the mean daily tap-water ingested during 23 h/day, no differences in the number of rearings were found. The results suggest that rearing in a novel environment could be a predictor of susceptibility to reinforcement by sweetened or palatable beverages.

Voluntary alcohol drinking Palatability Activity Open field

IT is well accepted that humans demonstrate different tendencies to drug consumption, and the relationship between individual differences (personality) and drug intake has been extensively studied (4,15,17). Although an individual's vulnerability to addiction is a factor of great interest with implications for the development of preventive strategies, the importance of individual differences has often been neglected in animal studies, except for the data obtained from genetically selected animals.

Two of the most studied individual differences in rats have been the emotional reactivity and activity-exploration in a novel environment such as an open field (OF) (6). Previous data have shown a positive relation between emotional reactivity and alcohol (ETOH) intake in genetically selected rats (13,23), although contradictory data have also been obtained (5,22,24). More conflicting data have been shown with regard to the relationship between drug addiction and activity in a novel environment, since positive (14), negative (5), and no relation (3,21) has been reported.

In our previous data (16), conditioned place preference for ETOH (a measure of drug rewarding properties) was only found in the rats that showed a high number of defecations (measure of emotionality) and a low number of rearings (measure of activity) in a low-frightening OF. The low-frightening OF (8) is a test with low intensities of sound or light, in which the fear responses decrease and do not seem to interfere with exploratory behavior, not showing correlation between defecation and ambulation measures. Our aim is to further assess the relationship between individual differences in OF and intake of sweetened alcoholic beverages using our oral self-administration procedure (18) in nongenetically selected rats.

METHOD

Subjects

Forty-one naive male Wistar rats (Charles River), which were about 100 days old at the beginning of the study with

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an average weight of 450 g (SD = 43), were used. They were individually housed with food and water ad lib until the start of the study, in a temperature-controlled room (22–24°C) on a 12 L:12 D (0800–2000) schedule.

Apparatus

A locally manufactured circular OF was used, with a pink noise generator (60 Hz) and a timer. The OF was 81.5 cm in diameter and 31.5 cm high. The floor was made of plywood with three concentric circles divided into 19 sections, each of the same area. The source of the light was a 100-W lamp, hanging 1.20 m above floor level.

Drugs

ETOH solution for oral self-administration was prepared daily from 99.9% ETOH (Normasolv, Barcelona, Spain), glucose (Panreac, Barcelona, Spain), and diluted in distilled water. Fluids were presented in glass bottles with a valve that prevented spillage and evaporation.

Procedure

After a period of 3 days, in which rats were only weighed, the OF testing was repeated for 4 consecutive days for 2 min once a day. The procedure was similar to that used previously (7). The rat was placed in the middle of the OF and only one subject was tested at a time. Testing took place at the same hour every day, at about 0900–1000 h. The measures were ambulation (number of floor areas entered), defecation (number of fecal boluses), rearing (every time the rat stood on its hind legs), and crossing (when the animal moved across the diameter of the apparatus). When the rats moved across the diameter, besides the number of ambulations, the crossings were also recorded, and when the rat did not move across the diameter, the number of areas crossed was the only measure recorded (ambulation).

Following OF testing, all rats were gradually reduced to 80% of their ad lib body weights (80ABW) over a 10-day period. The procedure used for food deprivation during these 10 days was the following: (a) 48 h without food, (b) 5 g per day until the subject reached the 80ABW. For the following days of the experiment, rats had access once a day to a food ration that was computed according to the formula: food (g) = [(80ABW − PW) × 80]/100, with PW being the present subject's weight in g. Moreover, when food was any quantity below 5 g, we gave 5 g of food to the subject.

When the 80ABW was achieved, rats were randomly divided into two groups. In their home cages experimental animals (n = 20) had access to a solution of ETOH (10% v/v) and glucose (3% w/v) and to their daily food ration (to maintain the 80ABW) for 1 h/day. Control animals (n = 21) had access to a solution of glucose (3% w/v) together with their daily food ration for 1 h/day. When the solution bottle was removed, all subjects had only free access to tap-water, without food, for the rest of the day. During the 1 h/day of access to the solution, tap-water was not available. This procedure lasted for nine consecutive sessions and solution intake took place at the same hour every day, at about 1000–1100 h. Consumption of ETOH, water and body weight were recorded every day.

Experimental subjects were divided into two subgroups on the basis of the mean daily ETOH dose ingested in the nine experimental sessions, below or above the median. Half of the animals were classified as low ETOH intake rats (LEI rats) and the other half as high ETOH intake rats (HEI rats). Control rats were also divided into two subgroups on the basis of the mean daily volume of the dissolution of glucose consumed. This classification was used for the study of all the variables.

Statistics

Statistical computer package programme SPSS was used. Normality distribution of the data was verified using Kolmogorov-Smirnov test. For comparison between groups we used a mixed one-way analysis of variance for repeated measures (ANOVAs) using Student's t-test as a post hoc analysis. The homogeneity of variances was tested by means of Boxs test. For the ANOVA analysis the between-group factor was the degree of ETOH dissolution ingested (with two levels: HEI or LEI rats) for the experimental rats, or the degree of glucose dissolution ingested (with two levels: low- or high-glucose consuming) for the control rats. The within-group factor was one of the behavioral measures across the four sessions of OF testing (rearing, ambulation, crossing or defecation) and the within-group analysis made was a Polynomial contrast. When repeated measures were not studied and only one variable had been considered the between-groups analysis was made by means of a Student's t-test, using corrected degrees of freedom when the Levene's test of homogeneity of variances was significant. Correlational analysis was made with the Pearson correlation coefficient (two tailed). The level of significance was set at 0.05. The significances ranging from 0.05 to 0.10 were also reported as a tendency to be significant.

RESULTS

The averaged body weight during the nine experimental sessions for the experimental rats was: mean = 78.1% (SD = 2.3) of their ad lib body weight (LEI rats: mean = 79.7%, SD = 1.5; HEI rats: mean = 78.7%, SD = 1.5); and for the control rats was: mean = 79.9%, SD = 3.0 (low-glucose rats: mean = 80.7%, SD = 1.5; high-glucose rats: mean = 79.2%, SD = 3.8). ANOVA analysis showed no differences in this measure.

The mean daily ETOH dose ingested was: mean = 1.22 g/kg/h, SD = 0.64 (n = 20). For LEI rats (n = 10) was: mean = 0.68 g/kg/h, SD = 0.26; and for HEI rats (n = 10) was: mean = 1.78 g/kg/h, SD = 0.39. ETOH intake in HEI and LEI rats across the nine sessions is shown in Fig. 1. ETOH intake showed a significant lineal upward evolution across the sessions that was different [ANOVA, $F(8, 144) = 4.47, p < 0.001$] for the two subgroups [ANOVA, polynomial contrast first degree: LEI rats: $F(1, 144) = 26.18, p < 0.001$; HEI rats: $F(1, 144) = 196.56, p < 0.001$].

Figure 2 shows the volume of the glucose solution ingested for the control rats across the nine sessions. The mean daily volume of the dissolution of glucose ingested for the high-glucose consuming was (n = 11): mean = 55.97 ml/kg/h, SD = 7.77; and for the low-glucose consuming rats was (n = 10): mean = 27.69 ml/kg/h, SD = 12.80.

The mean daily glucose ingested for each subgroup was calculated. For LEI rats was mean = 0.26 g/kg/h, SD = 0. 10; for HEI rats was mean = 0.68 g/kg/h, SD = 0.15; for the low-glucose consuming control rats was mean = 0.83 g/kg/h, SD = 0.38; and for the high-glucose consuming control rats was mean = 1.68 g/kg/h, SD = 0.23. The HEI rats ingested more glucose than the LEI rats, $t(18) = 7.49, p < 0.001$, and the high-glucose more glucose than the low-glucose consuming control rats, $t(19) = 6.19, p < 0.001$, whereas the low-glucose

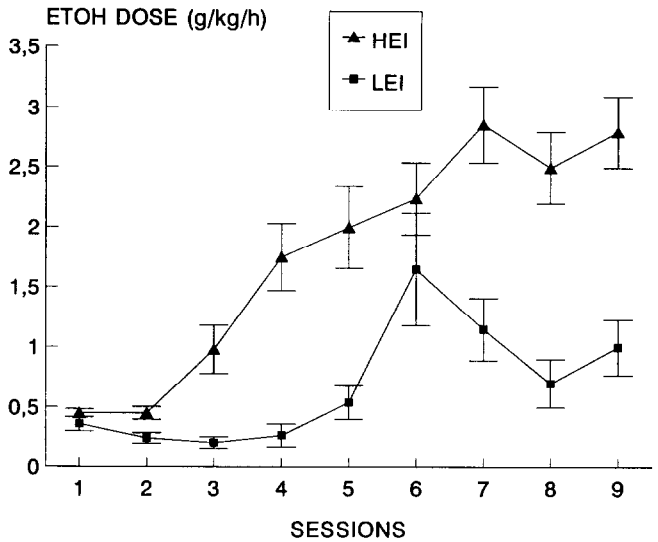

FIG. 1. Mean and standard error of ETOH dose (g/kg/h) in HEI rats (n = 10) and LEI rats (n = 10).

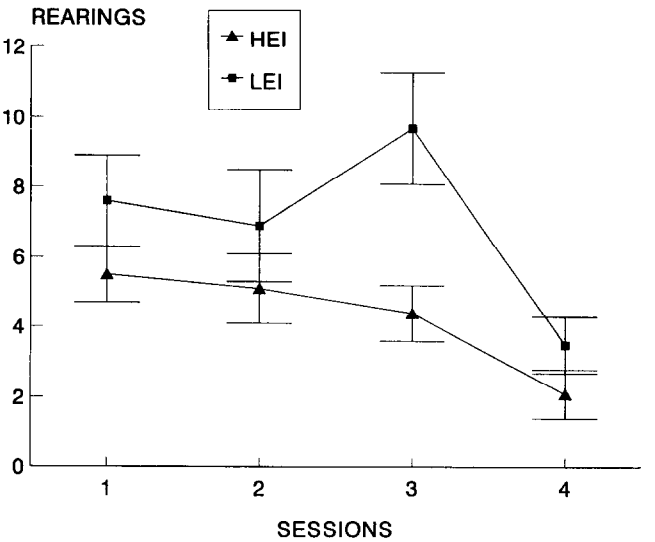

FIG. 3. Mean and standard error of rearings across the OF sessions in the experimental group, for HEI rats (n = 10) and LEI rats (n = 10).

consuming control rats ingested statistically the same amount of glucose as the HEI rats.

The caloric value of the solutions ingested by the subjects was also calculated, considering that ETOH has 7.12 kcal/g. and glucose 3.87 kcal/g. For the HEI rats the mean daily caloric value was mean = 5.89 kcal, SD = 1.62, for the LEI rats was mean = 2.18 kcal, SD = 0.79; for the high-glucose consuming control rats was mean = 2.16 kcal, SD = 0.23, and for the low-glucose consuming control rats was mean = 1.14 kcal, SD = 0.56. The HEI rats ingested more kcal than the high-glucose control rats, $t(9.33) = 7.22, p < 0.001$, and the LEI rats ingested more kcal than the low-glucose consuming control rats, $t(18) = 3.45, p < 0.010$, whereas the high-glucose consuming control rats ingested statistically the same amount of kcal as the LEI rats.

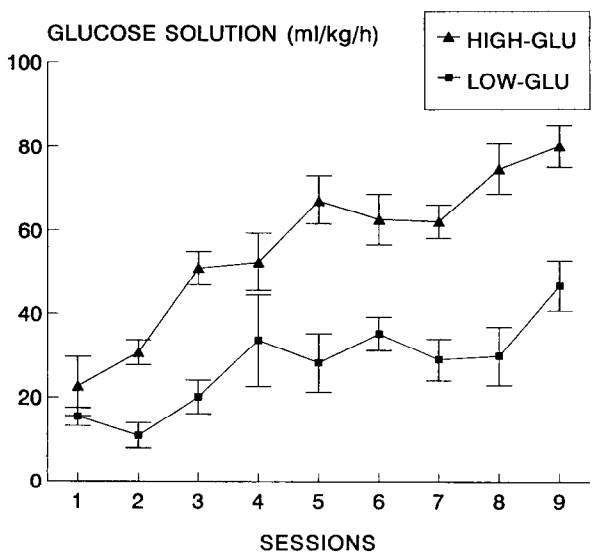

FIG. 2. Mean and standard error of glucose dissolution intake (ml/kg/h) for the high glucose-consuming rats (HIGH-GLU, n = 11) and the low glucose-consuming rats (LOW-GLU, n = 10).

For the total sample, we analyzed the correlations between the OF measures. Ambulation and rearings showed a $r = +0.393$ ($p < 0.05$), defecation and crossing showed a $r = +0.355$ ($p < 0.05$), and ambulation and crossing showed a $r = +0.316$ ($p < 0.05$); meanwhile, the other correlations were not significant (data not shown).

Before ETOH access, HEI rats showed a tendency towards fewer global number of rearings in the OF (Fig. 3) as compared to LEI rats [ANOVA: $F(1, 18) = 3.95, p = 0.062$]. Individual comparisons showed that differences were observed in the third OF session, $F(1, 18) = 8.41, p = 0.010$. The number of rearings across the four sessions of OF testing showed a significant lineal downward evolution both in HEI and in LEI rats [ANOVA, polynomial contrast first degree: LEI rats: $F(1, 54) = 6.55, p < 0.05$; HEI rats: $F(1, 54) = 8.58, p < 0.01$]. The lineal downward evolution in HEI rats was more pronounced, because there was a subgroup-session interaction [ANOVA: $F(3, 54) = 2.80, p < 0.05$]. No significant differences between HEI and LEI rats were noted with regard to other OF measures such as defecation, crossing or ambulation (data not shown).

The control rats that ingested greater amounts of glucose solution across the nine sessions showed previously lower number of rearings in the OF, as compared to the lower glucose consumption rats, as shown in Fig. 4 [ANOVA: $F(1, 19) = 6.90, p < 0.05$]. These results are similar to the experimental HEI and LEI rats. The differences in rearings between the high and low glucose-consuming were observed in the first [$F(1, 19) = 5.43, p < 0.05$] and fourth [$F(1, 19) = 5.15, p < 0.05$] sessions of OF testing, this also being the tendency in the second [$F(1, 19) = 3.50, p = 0.078$], although not in the third session. The number of rearings across the four sessions of OF testing showed a significant lineal downward evolution, that was the same for the low and high glucose-consuming because no subgroup-session interaction was found [ANOVA, polynomial contrast first degree: $F(1, 57) = 12.72, p < 0.01$].

The averaged number of OF rearings across the four sessions was statistically the same for HEI rats (mean = 4.3, SD

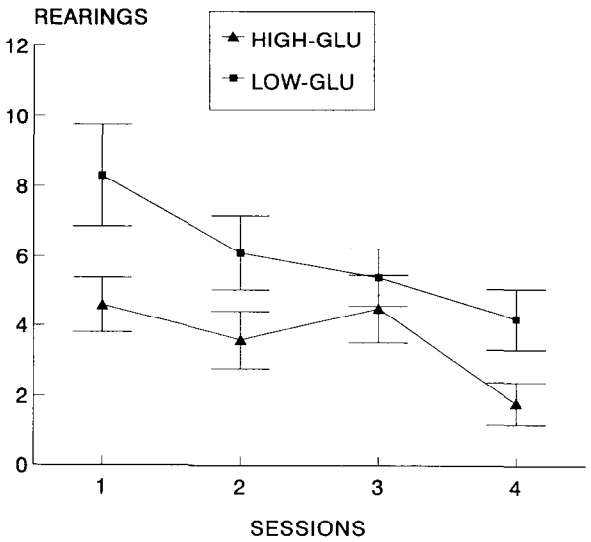

FIG. 4. Mean and standard error of rearings across the OF sessions in the control group, for the high glucose-consuming rats (HIGH-GLU, $n = 11$) and the low glucose-consuming rats (LOW-GLU, $n = 10$).

= 1.8) and the control rats that ingested greater amounts of glucose solution (mean = 3.6, SD = 1.8). The number of rearings was also equal for LEI rats (mean = 6.9, SD = 3.8) and the lower glucose consuming control rats (mean = 6.0, SD = 2.3), as revealed by a *t*-test analysis. The HEI rats and the low-glucose consuming control rats, which showed statistically the same glucose consumption, also showed statistically the same number of rearings. The LEI rats and the high-glucose consuming control rats showed statistically the same caloric value of the dissolution ingested, but the high-glucose consuming control rats made previously in the OF fewer rearings, $t(19) = 2.57$, $p < 0.05$).

For the experimental rats, the correlation of the global number of rearings with the dose of ETOH ingested (g/kg/h) and with the amount of glucose ingested (g/kg/h) only showed a tendency to be significant: $r = -0.392$ ($p = 0.088$). For the control rats, the correlation of the rearings with the amount of glucose ingested (g/kg/h) was of $r = -0.448$ ($p < 0.05$). Other OF measures did not show correlations with the amount of glucose consumed.

When we divided all experimental rats or control rats into two subgroups on the basis of the mean daily tap-water ingested during 23 h/day as a function of the median (data not shown), the two pairs of subgroups did not show significant differences neither in the number of rearings nor in ambulation, as revealed by ANOVA analysis. Water intake not showed significant correlations with open field measures, not only in experimental but also in control rats.

DISCUSSION

Using an oral self-administration procedure, we have not obtained a relationship between OF measures and ETOH intake. Thus, we have only found that the animals with higher levels of ETOH intake (HEI rats) showed a nonsignificant tendency ($p = 0.062$) towards making fewer global number of rearings in the OF when tested previously. Our data obtained with regard to the rearings are in contrast with our previous study where rats that showed conditioned place preference for ETOH previously made a fewer number of rearings, as a measure of activity, in an OF (16). The relationship between higher levels of drug consumption and lower levels of activity (measured by ambulation) has also been obtained in the AA rats genetically selected for ETOH preference, although this strain does not seem to show any difference with regard to the ANA rats in the number of rearings (5). In contrast to these findings, higher levels of ambulation in a novel environment were reported in another strain selected for ETOH consumption, the P rats (14), and in Sprague–Dawley rats that rapidly develop intravenous self-administration of amphetamine (19). Furthermore, LAS rats (low-ETOH sensitive rat line) with greater ETOH preference (10), showed elevated ambulation and rearing scores in the OF (11). Contradictory results have also been obtained in Wistar rats with high sensitivity to the development of dependence on morphine, because these animals showed intensive ambulation and low frequency rearing in the OF (26). More recent studies have reported no relation between ETOH consumption and motor activity, in several types of genetically selected rats (1) and in nonselected Wistar rats (21), according to our results. Globally, it is very difficult to compare data between these experiments as the animal strains and procedures employed differed greatly.

In our study, a relationship between low levels of glucose consumption and high number of rearings was obtained for the control rats not drinking ETOH. In contrast, the relation observed between low consumption and high number of rearings in the OF is not extensible to water intake, not only in the experimental but also in the control rats. There could be an association between low rearing frequency and high consumption of a sweet fluid, being the presence of ETOH or the caloric value of the dissolution ingested by the subject not very related to the rearings made in the OF. The HEI rats and the low-glucose consuming control rats, which showed the same glucose consumption, also made the same number of rearings, the difference being that HEI rats ingested ETOH. But these two subgroups also differed in the caloric value of the dissolutions ingested by the subjects. The LEI rats and the high-glucose consuming control rats ingested isocaloric solutions, but the high-glucose consuming control rats made a fewer number of rearings. These two last subgroups differed in the glucose ingested and in the fact that LEI rats also ingested ETOH. Globally, these results suggest that the most important factor is the amount of glucose ingested and not the caloric value nor ETOH consumption. Anyway, we cannot reject the possibility that ETOH consumption (and not only sweetened beverages consumption) was related to individual differences. Future studies are needed with different groups using the same concentration of glucose and different ETOH concentrations to separate the amount of glucose ingested from the dose of ETOH consumed.

It has been proposed that activity in a novel low-frightening OF, such as that used in this study, could be an approximate analogue of the extraversion trait in humans (8). According to this hypothesis, the high-glucose consuming rats seem to be more introvert with regard to the low-glucose consuming rats. Thus, it could be that rats with weaker activity or exploration traits, as measured by the number of rearings (introverts), would be more vulnerable to sweetened beverages consumption. In this study, the number of rearings made in a low-frightening OF seems to be a measure of activity, correlated to ambulation, and the two measures are uncorrelated to emotional reactivity as measured by defecation, in agreement with

previous data (8,16). If future studies find an association between ETOH consumption and low levels of activity measures, such a result would agree with several human studies in which a relation between low levels of extraversion and alcoholism has been reported (17). However, the meaning of the rearings in a low-frightening OF and their equivalence regarding ambulation needs further study.

In our study, no positive relationship between OF defecation, as a measure of emotionality, and ETOH intake was observed, in contrast to previous data with genetically selected rats (13,23) and to our previous study in Wistar rats (16). However, other studies have found no relationship between OF defecation and ETOH intake in genetically selected rats (5,22) and yet others have described (24) a negative relationship between the two measures. In this regard, there are few studies in which nonselected rats are used. It has been observed (3) that behavioral emotionality, assessed by the measures of central arena exploration, did not correlate with ETOH intake in Wistar rats.

In nonselected rats, ETOH consumption and the preference for oral intake of sweet tastes such as saccharin are associated (2,9,12,25), and the use of sweet alcoholic solutions seems to be important to obtain an oral self-administration of toxic doses (20). Therefore, it could be expected to find a relation between activity measures in an OF and ETOH intake in other procedures to obtain ETOH consumption without food deprivation, using a two-bottle paradigm or with several ETOH concentrations. Meanwhile, this study supports a relationship between the preference for sweet tastes and activity measures in an OF.

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