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Brief communication

Oral ethanol self-administration in inbred Roman high- and low-avoidance rats: Gradual versus abrupt ethanol presentation

Lidia Manzo [a], Mª José Gómez [a], José E. Callejas-Aguilera [a], Alberto Fernández-Teruel [b], Mauricio R. Papini [c], Carmen Torres [a,*]

[a] Universidad de Jaén, Spain
[b] Universitat Autònoma de Barcelona, Spain
[c] Texas Christian University, USA

HIGHLIGHTS

► Genetics and experiential factors affect ethanol consumption.
► Rats selected for high avoidance performance consume more ethanol than low-avoidance rats.
► At low ethanol concentrations, high-avoidance rats prefer ethanol over water.
► At low ethanol concentrations, low-avoidance rats prefer water over ethanol.

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ABSTRACT

Outbred Roman high-avoidance rats are known to consume more ethanol than inbred Roman low-avoidance rats. To determine whether ethanol consumption in inbred strains could be modulated by experiential factors, preference for a target 10% ethanol concentration was tested after either the gradual introduction of ethanol in increasing concentrations or the abrupt introduction of the target concentration. Whereas high-avoidance rats consumed more ethanol at lower concentrations, consumption and preference for ethanol over water were not differential across strains and administration procedure (gradual vs. abrupt). At the 4% concentration, ethanol was preferred over water by Roman high-avoidance rats, but water was preferred over ethanol by Roman low-avoidance rats. Ethanol consumption and preference for a 10% concentration appear to be immune to modification by either the gradual or abrupt ethanol presentation.

Ethanol is a type of alcohol ready for consumption derived from the fermentation of glucose present in food. Ethanol has rewarding properties that can lead to consumption in wild animals [1–3]. Under natural conditions, self-exposure to ethanol resulting from the consumption of fermented foods is probably characterized by variable and relatively low concentrations. Despite its natural occurrence, attempts at developing laboratory models of oral ethanol self-administration in rodents have encountered two major problems. First, neophobia—the reluctance to consume novel flavors. Neophobia is particularly strong when rodents are exposed to moderate-to-high doses of ethanol [4,5]. Two strategies have been used to circumvent this problem: gradually increasing the concentration of ethanol [6–8], and pairing ethanol with a palatable substance (e.g., sucrose) that then is gradually withdrawn [9]. The second problem for developing lab models of ethanol self-administration is individual differences in the preference for ethanol [10,11]. Individual differences have been exploited to develop rat strains based on their degree of ethanol preference [12–15]. Interestingly, in addition to their differences in ethanol preference, these strains exhibit correlated differences in their preference for sweet solutions, aversion for bitter solutions, and activity in novel situations [16,17]. These problems are compounded by the effects of specific testing conditions on behavior. For example, preference for ethanol occurs at relatively low concentrations in some strains (e.g., less than 6%; [18–20]), but at relatively high concentrations in others (e.g., 14% or higher; [21,22]).

The present research was designed to provide information on the effects of two different procedures of introducing a relatively high concentration (10%) on oral ethanol self-administration in inbred rat strains. The goal was to determine which of these procedures for introducing ethanol, if any, would maximize strain differences. Different groups were given access to 10% ethanol either after a gradual increase in the concentration or abruptly without prior exposure. In addition, inbred Roman High- and Roman Low-Avoidance inbred strains (RHA-I and RLA-I), derived from the respective RHA/Verh and RLA/Verh outbred rat lines [23], were used in this study. Although the Roman rat strains/lines were selected on the basis of their high (RHAs) vs. low (RLAs) acquisition of the two-way active avoidance task, respectively [23], they exhibit a variety

* Corresponding author at: Departamento de Psicología, Universidad de Jaén, 23071 Jaén, Spain.
E-mail address: mctorres@ujaen.es (C. Torres).

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of correlated changes in their response to appetitive stimuli [24], to novel situations [24,25], and to abuse substances [26–31]. Strain differences in dopamine release in the nucleus accumbens shell may underlie differences in addictive behavior. For example, dopamine release is greater in outbred RHA than RLA rats [31,32], a mechanism that could explain the tendency of RHA rats to prefer ethanol and other rewarding substances more than RLA rats [24,33]. The present design provided a direct comparison of the gradual vs. abrupt introduction of a 10% ethanol concentration in both inbred Roman rat strains. In addition, it made it possible to compare the rate of ethanol consumption in RHA-I and RLA-I rats exposed to increasing concentrations in the gradual condition and to determine the range of ethanol concentrations within which strain differences were observed. Both self-administration and preference in two-bottle tests were measured. It was predicted that, compared to RLA-I rats, RHA-I rats (1) would consume more 10% ethanol, (2) would consume more after a gradual procedure than after the abrupt introduction of the 10% concentration, and (3) would be less sensitive (i.e., less discriminating) in their preference for ethanol at lower concentrations.

1. Method

1.1. Subjects

The subjects were 32 male rats (16 RHA-I and 16 RLA-I) obtained from Universidad Autónoma de Barcelona, Spain (AF-T). Rats were 4 months old and weighed an average of 400 g at the start of the experiment. Animals were housed individually with free access to food and water throughout the experiment, in a room kept at 22–23 °C, and subjected to a 12:12 h light cycle (lights on at 08:00 h).

1.2. Apparatus

Access to ethanol was provided in the home cage, in 24-h cycles. Home cages were 32×15×30 cm (L×H×W), made of Plexiglas, with a wire lid. The floor was covered with saw dust. Each cage was equipped with two glass bottles and an area to store food pellets on the wire lid. Fluid consumption was measured by weighing the bottles before and after each 24-h cycle with a Cobos JT-300C digital scale. The different concentrations of ethanol used during the experiment were diluted from an original concentration of 96% (Panreac, Castellar del Vallés, Spain). Animals were weighed daily with a Baxtran (model BS3) scale.

1.3. Procedure

On days 1–4, animals were exposed to the two-bottle procedure with both bottles containing tap water. Each bottle had a stainless steel sipper tube equipped with a ball to minimize spillage. Water consumption was registered daily. Within each strain, animals were then matched by weight and randomly assigned either to the gradual (RHA-I/G and RLA-I/G) or to the abrupt (RHA-I/A and RLA-I/A) ethanol exposure group ($n=8$).

On days 5–12, animals were exposed to the gradual or abrupt procedure. The gradual ethanol exposure procedure involved the presentation of an increasing ethanol concentration in one of the bottles and water in the other. The concentrations used were 2, 4, 6, 8, and 10%, prepared by mixing 96% ethanol with tap water on a v/v basis. Each concentration was presented for 2 consecutive days, except the 10% concentration, which was available for 6 consecutive days. The relative position of each bottle in the cage was exchanged daily for each animal. Animals in the abrupt condition received water in both bottles during days 5–12 (the same as during the initial 4 days).

On days 13–18, all animals were exposed to 10% ethanol in one of the bottles and water in the other. All other conditions were as in the previous phase.

The following dependent variables were analyzed:

(1) Body weight (g)
(2) Food consumption (g)
(3) Water consumption (ml)
(4) Ethanol consumption (ml)
(5) Total fluid consumption (ml): the sum of ethanol plus water consumption
(6) Ethanol preference: observed ethanol consumption minus expected ethanol consumption (expected ethanol consumption was total consumption divided by 2). With this difference score, a positive number reflects preference for ethanol over water, a negative number reflects preference for water over ethanol, and zero implies no detectable preference for either fluid.

Weight measurements were taken every day between 09:00 and 12:00 h (at about the same time for each particular animal). Fluids and food were replenished at that time daily. Cages were cleaned every 2 days. Analyses of variance were computed for each dependent variable with an alpha value set at the 0.05 level.

2. Results

2.1. Weight

Average weights for the entire experiment were 391.7, 392.4, 397.0, and 398.7 g for groups RHA-I/G, RLA-I/G, RHA-I/A, and RLA-I/A, respectively. Weights were averaged in blocks of 2 days (Blocks 1–2 correspond to pretraining, Blocks 3–6 to testing 2–8% ethanol, and Blocks 7–9 to testing 10% ethanol), and subjected to a Strain (RHA-I, RLA-I)×Group (G, A)×Block (1–9) analysis of variance, with the latter as a repeated-measure factor. There was a significant increase in weight across blocks, $F(8, 224)=17.73$, $p<0.001$, and a significant strain by block interaction, $F(8, 224)=2.31$, $p<0.03$. None of the other factors or interactions was significant, Fs<1. The interaction was produced by inconsistent group orderings across blocks. However, pairwise LSD tests with the error term from the main analysis indicated nonsignificant strain differences for all the blocks, Fs<1.

2.2. Food consumption

Means for food consumption for the entire experiment were 23.4, 25.0, 24.8, and 25.2 g for groups RHA-I/G, RLA-I/G, RHA-I/A, and RLA-I/A, respectively. Amounts consumed were averaged for every block of 2 days over 9 blocks and subjected to a Strain×Group×Block analysis, as described above. There were significant interactions between strain and block, $F(8, 224)=9.77$, $p<0.001$, and between group and block, $F(8, 224)=2.02$, $p<0.05$. None of the other factors or interactions was significant, Fs<1.69, ps>0.20. Pairwise LSD comparisons with the error term from the main analysis indicated the following results. The source of the strain by block interaction was in the relative amounts eaten during the first and last blocks. RHA-I rats ate significantly more than RLA-I rats during the first block, $F(1, 28)=29.92$, $p<0.001$, but the opposite was the case for the last block, $F(1, 28)=6.89$, $p<0.02$. The source of the group by block interaction was restricted to Block 5, when rats in the gradual conditions (tested with 4% ethanol) ate significantly less than rats in the abrupt condition (not given access to ethanol), $F(1, 28)=8.83$, $p<0.01$.

2.3. Water consumption

Fig. 1 shows water consumption in 2-day blocks for each group. The two groups that received abrupt access to the 10% ethanol concentration were not exposed to ethanol during Blocks 3–6 and therefore consumed more water than the two groups given gradual access to ethanol.