

# Effects of Environmental Enrichment on Voluntary Ethanol Intake in Rats

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ROCKMAN, G. E., J. E. M. GIBSON AND A. BENARROCH. *Effects of environmental enrichment on voluntary ethanol intake in rats*. PHARMACOL BIOCHEM BEHAV 34(3) 487-490, 1989. —The effects of exposure to four environmental rearing conditions on subsequent voluntary ethanol intake were examined. Male weanling rats were reared in either an enriched environment or individually for 90 days. After the 90-day environmental exposure period, the two initial groups (Enriched and Isolated) were randomly subdivided into four groups (Enriched, Enriched/Isolated, Isolated, and Isolated/Enriched) and exposed to increasing concentrations of ethanol (3% to 9% v/v) in a free choice with water. Therefore, half the animals raised in the enriched environment were permanently placed into individual cages (Enriched/Isolated) for the remainder of the study. Likewise, half of the animals previously reared individually were exposed daily (0900-1700) to the enriched environment (Isolated/Enriched). Results indicated that the enriched animals consumed greater amounts of ethanol as compared to all other groups. In contrast, rats placed in isolation following 90 days of enrichment demonstrated significant reductions in voluntary ethanol intake. The data suggest that rearing in an enriched environment for 90 days and continued exposure following 111 days of age, are necessary to enhance voluntary ethanol consumption.

Enrichment      Ethanol      Intake

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EXAMINATION of the effects of various environmental conditions on the consumption of ethanol has yielded interesting but contradictory results. For example, it was initially observed that rats reared in an enriched environment did not differ in terms of their ethanol consumption when compared to rats reared individually or in an impoverished environmental condition (7). However, other studies examining ethanol intake in rats reared either individually or in groups showed that rats reared individually consume significantly more ethanol than group-housed animals (2,8). In contrast, it has also been demonstrated that rats exposed to a moderately crowded environment consumed more ethanol as compared to rats in an uncrowded or highly crowded housing situation (5).

Recent studies have yielded a more consistent effect of environmental factors on ethanol intake. More specifically, it has been shown that exposure to an enriched environmental condition produced a marked increase in ethanol consumption in some animals (3). A subsequent study suggested that some rats exposed to an enriched condition consumed quantities of ethanol sufficient to produce withdrawal symptoms when access to ethanol was withdrawn (4).

Studies in this laboratory have demonstrated that rats reared in an enriched environment consume significantly more ethanol than nonenriched rats (9,10). In this latter study, male rats were reared in either an enriched environment which included female rats, with a male "partner," with a female "partner," or individually. Results from this study indicated that animals reared in the enriched environment consumed significantly more ethanol as compared to all other groups. It is important to note that the enhanced ethanol consumption observed in the enriched rats

seemed *not* to be a result of handling, exposure to females or the placement in individual cages for the purposes of ethanol exposure. Rather, it was suggested that the enriched environment *per se* (additional sensory and physical stimulation) resulted in changes in these animals as reflected by their ethanol consumption (10). These results serve to raise some important questions regarding the timing and amount of exposure to the enriched environment necessary to produce the observed increases in ethanol consumption.

In the present study, 2 groups of rats were reared in an enriched environment for 90 days and were then either permanently housed individually or continued to be exposed to enrichment daily while being presented with increasing concentration of ethanol. Conversely, 2 additional groups of rats housed individually for 90 days were then either exposed to an enriched environment for 8 hr daily or remained individually housed while receiving exposure to ethanol.

## METHOD

### *Subjects*

Male Sprague-Dawley rats (Charles River) 21 days old (55-65 g) on delivery were used. These animals were randomly divided into two initial groups. One group was raised for 90 days in an enriched environment (Enriched) as described below. The other group was raised individually (Isolated) for 90 days in standard cages in a separate room. All rats in all housing conditions had access to water and food ad lib with a 12/12 light/dark cycle (lights on at 0700 hr) throughout the entire experiment. Ethanol exposure, as described below, was provided only in individual cages and

always with food and water ad lib.

#### Enriched Condition

Male and female rats were housed in a ( $1.8 \times 1.8 \times 1.2$  m) high pen constructed out of plywood covered in galvanized steel. The floor of the pen was covered with approximately 8 cm of wood shavings which were changed regularly. Several "toys" were placed in the pen. These included two running wheels (0.3 m in diameter), 12 different sized sheet metal stove pipes (0.3 m to 1 m in length), two Plexiglas ( $0.15 \times 1$  m) cylinders, and a small metal table ( $1 \times 1$  m) with an attached metal ladder. These objects were wired or hung together in various ways, and changed every other day to create different bridges, tunnels and hills. A radio played country music (approximately 55 dB) from 1900 to 0700 hr. The room temperature was held constant at  $24 \pm 2^\circ\text{C}$ . As mentioned above water and food were available ad lib. Feeding stations were provided on each side of the enriched environment which provided easy and ample access to both food and water. Specifically, this arrangement provided 1 water spout for every 2 rats. The ratio of males to females was held constant at 5:1. The female rats were replaced by naive female rats every 18 days so that pups were not introduced into the environment. The housing density was maintained at approximately  $0.13 \text{ m}^2$  per rat throughout the entire study and was similar to the housing densities reported previously (9,10).

#### Ethanol Exposure

After the 90-day environmental exposure period, the two initial groups (Enriched and Isolated) were further randomly divided into four groups (Enriched, Enriched/Isolated, Isolated, and Isolated/Enriched). Therefore, half of the animals raised in the enriched environment were permanently placed into individual cages (Enriched/Isolated) for the remainder of the study. Likewise, half of the animals previously reared individually were exposed daily (0900–1700) to the enriched environment (Isolated/Enriched). In order to measure ethanol consumption, the animals in the two enriched groups (Enriched, Isolated/Enriched) were removed from the enrichment pen and placed in standard laboratory cages with food available ad lib daily from 1700 to 0900 hr in a similar manner as described previously (10). All groups received the following schedule of ethanol exposure. Two calibrated drinking tubes were attached to the left front of each cage. One contained tap water while the other initially contained a 3% (v/v) solution of ethanol. This concentration was presented *every alternate day* over an 8-day period; that is, every other day the rats received two tubes of water and on intervening days, they received one tube of water and one tube of ethanol. The position of the ethanol tube was changed upon each presentation to eliminate the possibility of formation of a position preference by the rats. The same alternate day presentation was continued for ethanol concentrations of 5%, 7% and finally 9% (v/v). Therefore, during this initial ethanol exposure phase rats were exposed to each concentration of ethanol (3% to 9% v/v) on four occasions totaling to 16 ethanol exposure days. At the end of the alternate day ethanol exposure procedure, all rats received the 9% (v/v) ethanol in an *everyday* free-choice with water for 16 consecutive days. Therefore, the rats were exposed to ethanol for a grand total of 32 days.

During the ethanol-exposure period all the animals were tail marked. The animals in the Enriched and Isolated/Enriched groups were placed back in the enrichment pen for 8 hr each day (0900–1700 hr). The remaining animals in the Isolated and

Enriched/Isolated groups were housed individually. All animals in all groups were handled twice per day.

Daily fluid consumption of both ethanol and water, and animals' weights were measured. Ethanol consumption was calculated both in terms of mean grams per kilogram per day and mean percentage of total daily fluid consumption. Finally, over the course of this study which lasted 4.5 months, a total of 6 animals that commenced this study either died or were sacrificed due to illness. The criterion for sacrificing was 2 consecutive days of less than 5 ml of total fluid (ethanol and water) consumed. This resulted in the following group sizes: Enriched,  $n=9$ ; Enriched/Isolated,  $n=10$ ; Isolated,  $n=9$ ; Isolated/Enriched,  $n=6$ . Only data from these animals are reported.

#### Statistical Analysis

Ethanol consumption was expressed in terms of mean grams per kilogram per day (g/kg/day). For data presentation and statistical analysis, ethanol consumption was divided into 8 periods of 4 days each corresponding to each ethanol concentration. All ethanol intake data were analyzed by a repeated measures analysis of variance [group (Enriched, Enriched/Isolated, Isolated, Isolated/Enriched)  $\times$  time period (periods 1 to 8)], appropriate post hoc (Tukey) tests, and simple main effects analysis when interactions were significant.

#### RESULTS

Ethanol intake in terms of mean gram per kilogram for all groups is illustrated in Fig. 1. Analysis of variance with repeated measures yielded an overall significant group effect,  $F(3,30)=3.6$ ,  $p<0.05$ , a significant period effect,  $F(7,210)=15.6$ ,  $p<0.001$ , and a significant group  $\times$  period interaction,  $F(21,210)=9.1$ ,  $p<0.001$ . Post hoc Tukey tests indicated that during the initial periods of ethanol presentation there were generally no differences in ethanol consumption for all groups when given 3, 5, and 7% ethanol, with the exception of the Enriched-Isolation and Isolation-Enriched. The Enriched-Isolation group drank significantly more ( $p<0.01$ ) 5% ethanol than the Isolation-Enriched group. In contrast, during the last period of ethanol exposure, the Enriched group drank significantly more ( $p<0.001$ ) ethanol in the last period of continuous 9% than all the other groups. The Isolation-Enriched did not differ in terms of ethanol intake throughout the entire study. The Enriched-Isolation showed an initial increase (approaching significance) in ethanol consumption during the initial periods of ethanol exposure and then a significant ( $p<0.01$ ) drop towards the end. The Enriched group showed an opposite effect, drinking significantly ( $p<0.01$ ) more ethanol towards the end of the study. It is important to note that not only was the *mean* ethanol intake of the Enriched group greater than all the other groups, but the variability was considerably smaller. Specifically, it was noted that in the final ethanol (9% everyday) exposure period, all rats in the Enriched group consumed ethanol to the extent that it comprised in excess of 85% of their total daily fluid intake, whereas none of the other animals in any other group consumed more than 65%. No significant differences were observed between the body weights among the groups, nor did any of the animals show signs of ethanol-induced intoxication or withdrawal.

#### DISCUSSION

The present data show that rats reared in, and continually exposed to an enriched environment consume significantly more ethanol than rats reared in an enriched environment and then

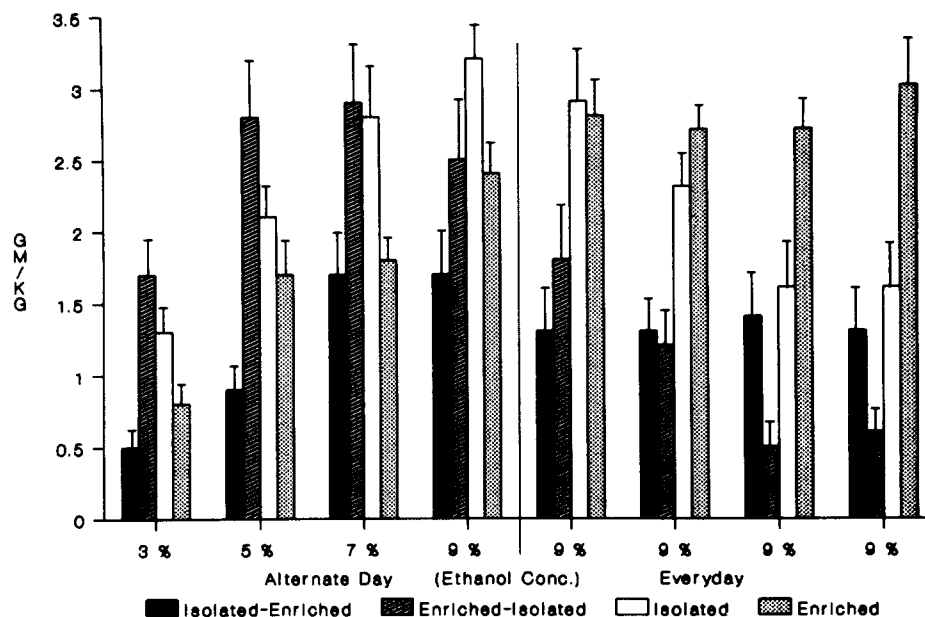


FIG. 1. Ethanol intake in terms of mean grams per kilogram at each ethanol concentration for male rats in the Enriched ( $n=9$ ), Enriched/Isolated ( $n=10$ ), Isolated ( $n=9$ ), and Isolated/Enriched ( $n=6$ ) groups.

placed in isolation, or rats which were either reared in isolation and then exposed to enrichment or left in isolation. Notably, while the enriched group did consume, on average, a greater amount of ethanol than the other groups, their drinking patterns differed as well. Specifically, all the animals in the Enriched group consumed ethanol such that it comprised at least 85% of their daily fluid intake. None of the other animals matched this percentage of ethanol intake. These figures unquestionably demonstrate that the enriched animals' ethanol-consuming behaviour was significantly different from that of all other rats. This finding is consistent with previous studies showing that environmental enrichment can influence ethanol consumption (3, 4, 9, 10).

The results from this study reveal some interesting patterns of ethanol consumption among the groups and must be interpreted in light of previous data suggesting that the enhanced ethanol consumption observed in the enriched rats seemed *not* to be a result of handling, exposure to females or the placement in individual cages for the purposes of ethanol exposure (10). Specifically, in the present study, those animals reared individually during the entire study (Isolated) demonstrated the expected pattern of ethanol consumption as observed in previous studies. That is, ethanol consumption increased with exposure to increasing concentration of ethanol presented every alternate day. When the schedule of ethanol presentation was switched to everyday exposure, a characteristic drop followed by stabilization was observed (9,10). It is interesting to note that the rats exposed to the enriched environment throughout the entire study (Enriched) did not show this pattern. These results further support the contention that the enriched animals' drinking behavior is quite different than that of individually housed animals.

The Enriched/Isolated group, while exhibiting a somewhat elevated ethanol intake initially, demonstrated a large decrease in ethanol consumption during the later stages of the study when ethanol concentration was highest (9% v/v). It is important to note that the only difference in treatment between the Enriched/Isolated

and Enriched groups is that the Enriched group continued to receive daily exposure to the enriched environment. These results seem to indicate that continued exposure to the enriched environment plays an important role in maintaining the observed increases in ethanol consumption as a consequence of exposure to an enriched environment. This view is supported by studies examining changes in brain weights, cortical thickness and brain chemistry as a consequence of exposure to an enriched environment followed by placement into an isolated condition. Specifically, it has been shown that many of the cerebral differences induced by enrichment dissipate when animals are switched to individual housing (1,6). This suggests that alterations in brain physiology and brain chemistry induced by exposure to an enriched rearing situation may be responsible for the observed patterns of ethanol consumption in the present study.

Animals reared individually for 90 days and then exposed to the enriched environment daily (Isolated/Enriched) showed no major changes in ethanol consumption over time. This suggests that, in comparison to the Enriched group, 8 hr of daily exposure to an enriched environment after 90 days of isolation is not sufficient to alter ethanol consumption.

Finally, it is important to note that alternative explanations for the above mentioned data are available. Specifically, it is possible that the animals' ethanol consumption reflects their response to placement in individual cages during the ethanol exposure and testing phases, housing densities and/or the presence of female rats in the enrichment pen. Consequently, it is conceivable that these methodological factors resulted in stress of sufficient magnitude to result in altered ethanol consumption. While previous data from this laboratory suggest that these factors seem not to contribute to the observed differences in ethanol consumption (10), the possibility clearly still exists. As a result, further studies are underway to determine whether these methodological issues, including the length of environmental enrichment, are responsible for the observed differences in voluntary ethanol consumption.

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