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Effects of Duration and Timing of Environmental Enrichment on Voluntary Ethanol Intake in Rats

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ROCKMAN, G. E. AND J. E. M. GIBSON. *Effects of duration and timing of environmental enrichment on voluntary ethanol intake in rats.* PHARMACOL BIOCHEM BEHAV 41(4) 689–693, 1992.—The effects of exposure to five environmental rearing conditions on subsequent voluntary ethanol intake was examined. Male weanling rats were reared for 60 days in either an enriched environment, individually, or in a smaller enriched environment (quasienriched). The quasienriched environment was employed to allow for a group measurement of ethanol intake. Following the initial 60-day environmental exposure period, the three initial groups (Enriched, Isolation, Quasienriched) were randomly subdivided into five groups (Enriched/Isolation, Isolation, Isolation/Quasienriched, Quasienriched, Quasienriched/Isolation) and exposed to increasing concentrations of ethanol (3–9% v/v) in a free choice with water. Results indicated that exposure to an enriched environment for 60 days does not alter ethanol intake. In contrast, rats exposed to the quasienriched environment while having access to ethanol demonstrated a significant increase in voluntary ethanol intake as compared to all other groups. Exposure to different environmental conditions while having access to ethanol was not by itself sufficient to alter ethanol intake. These data are discussed in terms of the amount and timing of exposure to an enriched environment necessary to alter voluntary ethanol intake.

Enrichment Ethanol Intake

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 (5). 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 (1,7). 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 (4).

Recent studies have yielded a more consistent effect of environmental factors on ethanol intake. Specifically, it has been shown that exposure to an enriched environmental condition produced a marked increase in ethanol consumption in some animals (2). 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 (3).

Studies in this laboratory have demonstrated that rats reared in an enriched environment consume significantly more ethanol than nonenriched rats (8–10). 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 (9). In addition, recent results (10) suggest that continued exposure to the enriched environment following initial 90-day exposure plays an important role in maintaining the observed increases in voluntary ethanol intake. Considered together, 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.

The present study was designed to examine whether a reduced amount of exposure to an enriched environment alters the increase in ethanol intake that has been reliably observed after exposure to 90 days of enrichment (8–10). As well, this study was designed to examine the viability of a "group measurement technique" for voluntary ethanol consumption. As

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noted in previous studies (8–10), to assess individual ethanol intake it was necessary to remove animals daily from the enriched environment and place them in individual cages from 1700–0900 h. While previous data suggest that this procedure does not itself alter ethanol intake (9), a method *not* requiring the placement of animals in individual cages would be preferable.

METHOD

Subjects

Male Sprague-Dawley rats (Charles River suppliers) 21 days old (55–65 g on delivery) were used. Animals were randomly divided into three initial environmental conditions as illustrated in Table 1. One group was raised in an enriched environment (Enriched) and the second group was raised in a quasienriched environment (Quasienriched) as described below. The third group was raised individually (Isolation) in standard cages located in a separate room. All groups of rats had access to water and food ad lib and a 12 L:12 D cycle (lights on at 0700 h).

Enriched Condition

The enriched environment used in this study was similar to the enriched environment described in detail in previous studies (8–10). Twenty-five male and five female rats were housed in a (1.8 × 1.8 × 1.2 m high) pen. A ratio of males to females was maintained at 5:1. Female rats were replaced by naive female rats every 18 days so that pups were not introduced into the environment. 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 one water spout for every two rats. This housing density was maintained throughout the entire study and was similar to the housing densities reported previously (8–10).

Quasienriched Condition

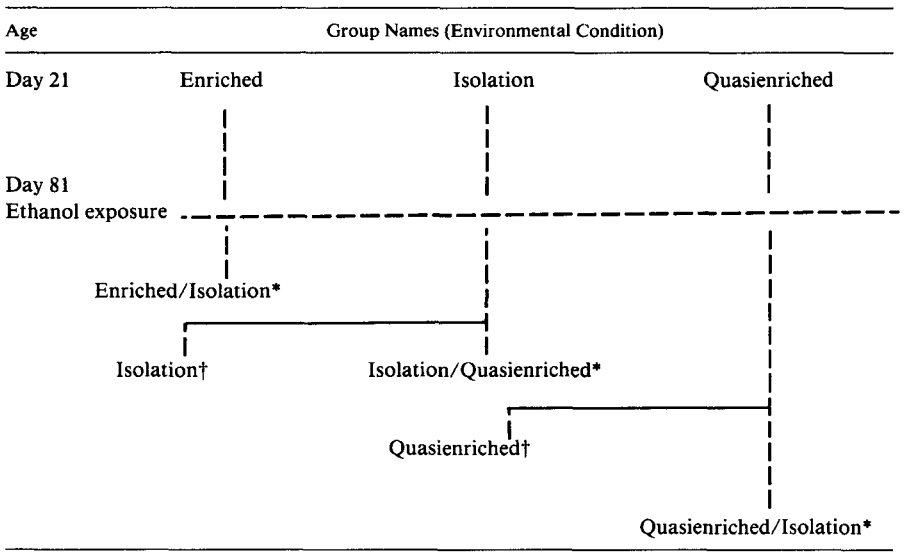
The quasienriched condition was employed to assess whether a "smaller" enriched environment would alter ethanol intake in a similar fashion as the "standard" enriched condition (8–10). As well, this smaller group housing situation was used to assess the viability of a group measurement technique for voluntary ethanol intake.

Thirty male rats were housed in six (0.91 × 0.78 × 0.91 m high) wire mesh pens (5 rats per pen). Several "toys" were placed in each pen. These included one running wheel (0.3 m in diameter), a tin can (0.05 × 0.30 m), two metal shelves (0.18 × 0.78 and 0.14 × 0.61), and a metal ladder. This housing density was maintained throughout the entire study to maintain a similar housing density as the enriched condition. A radio played music (55 dB) from 1900–0700 h. The room temperature was held at 24 ± 2°C.

Ethanol Exposure

After the initial 60-day environmental exposure period, the Quasienriched, Isolation, and Enriched groups were further randomly divided into the following groups as illustrated in Table 1: Enriched/Isolation, Isolation, Isolation/Quasienriched, Quasienriched, and Quasienriched/Isolation (each group total n = 15). Therefore, three of the six groups of five animals raised in the quasienriched pens were placed in individual cages nightly (1700–0900 h) and returned to their environments daily (Quasienriched/Isolation). Likewise, half the animals previously reared individually were exposed nightly (1700–0900 h) to the quasienriched pens and returned to their individual cages daily (Isolation/Quasienriched). To measure ethanol consumption, 15 animals in the enriched condition were randomly chosen and removed from the enrichment pen and placed in standard laboratory cages (Enriched/Isolation) with food available ad lib daily from 1700–0900 h in a similar manner as described previously (9,10). The Isolation and Quasienriched groups remained in their respective environments throughout the study.

TABLE 1



*Change in environment for ethanol exposure.
†No change in environment for ethanol exposure.

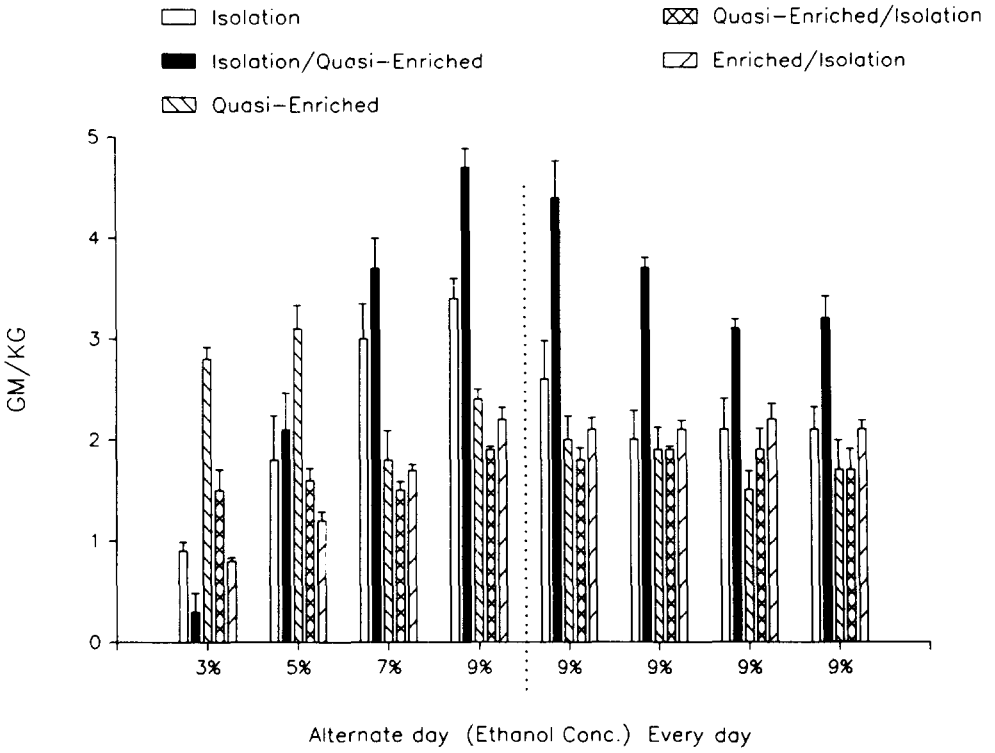


FIG. 1. Ethanol intake in terms of mean grams per kilogram at each ethanol concentration for male rats in the Isolation, Isolation/Quasienriched, Quasienriched, Quasienriched/Isolation, and Enriched/Isolation groups.

The Enriched/Isolation, Quasienriched/Isolation, and Isolation groups (15 animals per group) received the following schedule of ethanol exposure at night while in individual cages. Two calibrated drinking tubes were attached to the left front of each cage. One contained tapwater while the other initially contained a 3% (v/v) solution of ethanol. The concentration was presented every alternate day over an 8-day period, that is, every other day 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 (3–9% v/v) on 4 occasions, totaling 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, rats were exposed to ethanol for a grand total of 32 days.

The Quasienriched and Isolation/Quasienriched groups were exposed to ethanol in a similar manner as the other groups with the exception that these animals were exposed to ethanol while in the group pens. Since five animals were housed in each quasienriched pen, five pairs of drinking bottles were mounted on the side of each pen. Of each pair of drinking bottles, one contained tapwater while the other contained either water or a solution of ethanol corresponding to the kind and concentration of solution to which animals in the other groups were exposed.

During the ethanol exposure period, all animals were tail-marked. Animals in the Enriched/Isolation and Quasienriched/Isolation groups were placed back in the enrichment pens each day from 0900–1700 h. Similarly, the Isolation/Quasienriched animals were also returned to their individual cages daily (0900–1700 h). Animals in the Quasienriched group remained in their environment throughout the study. All animals in all groups were handled twice per day and weighed every second day. Daily fluid consumption of both ethanol and water were measured.

Statistical Analysis

Ethanol consumption was calculated in terms of mean grams per kilogram per day (g/kg/day). For data presentation and statistical analysis, ethanol consumption was divided into eight periods of four days, each corresponding to each ethanol concentration. Ethanol intake for those rats in the quasienriched condition was determined as follows: Each group of five animals in the quasienriched pens was treated as an $n = 1$ (i.e., each group was treated as a single subject), totaling 3 subjects (three pens with five rats per pen) for the Quasienriched group and three subjects (three pens with five rats per pen) for the Isolation/Quasienriched group. As a result, total volume of ethanol and water and total body weight for all rats in each pen was used to calculate daily ethanol intake. This procedure approximates a procedure (6) whereby groups of rhesus monkeys were treated as single subjects in a repeated-measures design.

Similarly, ethanol intake for the remaining groups was calculated by taking the individual data from the Enriched/Isola-

tion, Quasienriched/Isolation, and Isolation groups and averaging a mean grams per kilogram per day score from each random set of five animals in each group (three sets of five rats per group). As a result, all groups in this study were treated as having an $n = 3$ (three sets of five rats per group).

Ethanol intake data was analyzed by a repeated measures analysis of variance (ANOVA) [group (Enriched/Isolation, Quasienriched, Quasienriched/Isolation, Isolation/Quasienriched, Isolation) × time period (periods 1-8)], appropriate posthoc (Tukey) tests, and simple main effects analysis when interactions were significant.

RESULTS

Ethanol intake in terms of mean grams per kilogram for all groups is illustrated in Fig. 1. ANOVA with repeated measures yielded an overall significant group effect, $F(4,10) = 30.6$, $p < 0.001$, a significant period effect, $F(7,70) = 28.8$, $p < 0.001$, and a significant group × period interaction, $F(28,70) = 12.1$, $p < 0.001$. Posthoc Tukey tests revealed several interesting patterns of ethanol intake. Specifically, the Isolation group initially exhibited an enhanced ethanol intake ($p < 0.05$) when ethanol (7 and 9%) was presented on alternate days. However, this transient increase was not evident once ethanol was presented daily. Similarly, the Quasienriched group showed an initial increased preference for ethanol ($p < 0.05$) that was observed only when exposed to 3 and 5% ethanol. The Isolation/Quasienriched group showed a consistent and significant increase in ethanol intake as compared to all other groups ($p < 0.01$). Finally, both the Enriched/Isolation and Quasienriched/Isolation groups did not exhibit any remarkable patterns of ethanol intake.

No significant differences were observed in total fluid intake (water and ethanol) or body weights between the groups, nor did any of the animals show any obvious signs of stress or excessive fighting among rats (i.e., body scars, hair loss).

DISCUSSION

The present data show that exposure to an enriched environment for 60 days does not alter voluntary ethanol intake in rats as compared to rats reared in isolation. This result was consistent for both the "traditional" Enriched group as well as for the Quasienriched group. It is important to note that previous studies have demonstrated that rats reared in an enriched environment for 90 days consume significantly more ethanol than rats reared in isolation (8-10). Consequently, it seems that 60 days of exposure to an enriched environment is insufficient to alter ethanol intake.

As illustrated in Fig. 1, the Isolation/Quasienriched group demonstrated a significant elevation in voluntary ethanol intake in comparison to all other groups. This group was initially housed in individual cages for 60 days. Following this initial exposure, these rats were placed daily (1700-0900 h) in the quasienriched environment while having access to ethanol. It is important to note that the combination of switching to the quasienriched environment and exposure to ethanol seems to have resulted in an increase in ethanol consumption. Other groups (Enriched/Isolation, Quasienriched/Isolation) subjected to a similar switching of environments did not show increases in ethanol intake. Similarly, the Quasienriched, having been continually exposed to this environment, did not show an elevated ethanol intake. As well, changes in ethanol intake do not seem to be a result of alterations in total fluid consumption (water and ethanol) or differences in body weight since no significant differences across groups in these measures were observed.

These results suggest that neither exposure to enrichment nor the switching of environments are sufficient to alter ethanol intake. Rather, it seems that the combination of these factors resulted in the observed increase in ethanol intake in the Isolation/Quasienriched group. Hence, this observation raises the issue of timing of the exposure to an enriched environment. Specifically, it is suggested that not only may the amount of exposure to an enriched environment play a role in increasing ethanol intake but also the time at which exposure occurs. In the present situation, the Isolated/Quasienriched group were exposed daily (1700-0900 h) to an enriched environment *following* 60 days of individual housing with ethanol available *only* in the quasienriched condition. It is suggested that this combination of exposure to enrichment and access to ethanol results in the observed enhanced voluntary ethanol intake.

While it is suggested that the additional sensory and physical stimulation provided in the quasienriched environment accounts for the observed increases in ethanol intake, other factors may have contributed to this observation. Specifically, it is possible that switching animals from isolation to the quasienriched environment resulted in stress of sufficient magnitude to result in altered ethanol consumption. However, it is important to note that the daily switching of environments per se does not seem to be sufficient by itself to alter ethanol intake. This view is supported by the data from the two other groups (Enriched/Isolation, Quasienriched/Isolation) in this study that were also exposed to daily switching of environments but did not result in increases in ethanol intake.

Animals reared in individual cages throughout the entire study (Isolation) demonstrated the standard pattern of ethanol intake (8-10), that is, these rats showed an initial increase followed by a drop in ethanol consumption once ethanol was presented daily.

In addition, the "group measurement" procedure for voluntary ethanol intake employed in the Quasienriched groups seems to represent an accurate and viable technique that warrants further use.

Results from this study suggest that 60 days of exposure to an enriched environment is, by itself, insufficient to result in altered ethanol consumption. In addition, the timing of the exposure to enrichment and exposure to ethanol intake seems to play a role in the observed increases in voluntary 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 phase, 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 (9), the possibility clearly still exists. As a result, further studies are underway to determine whether these methodological issues are responsible for the observed differences in voluntary ethanol consumption.

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