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Strain Differences in Alcohol Preference of Guinea Pigs and Experimental Manipulation of Preference by Forced Alcohol Consumption

Gerald L. Appelman
Western Michigan University

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STRAIN DIFFERENCES
IN ALCOHOL PREFERENCE OF GUINEA PIGS
AND EXPERIMENTAL MANIPULATION OF PREFERENCE
BY FORCED ALCOHOL CONSUMPTION

by

Gerald L. Appelman

A Thesis submitted to the
Faculty of the School of Graduate
Studies in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
December, 1966
ACKNOWLEDGEMENTS

The investigator wishes to express his appreciation for the assistance of his advisor Dr. Thor, and his committee members Dr. Koronakos, and Dr. Asher.

Gerald L. Appelman
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INTRODUCTION

The study of the determinants of alcoholism, one of the most perplexing diseases of mankind, entered its most fruitful era of discovery in the laboratory of the animal researcher. A pioneering study of the determinants of alcohol consumption by Richter (1940) indicated that laboratory rats show a preference for alcohol over water beginning at 1.8 per cent ethanol (v/v) solution, and continue to prefer alcohol over water up to a 6 per cent ethanol (v/v) solution. Richter made two important contributions to alcohol research which had broader implications than the presentation of an index of alcohol consumption in rats. First, he hypothesized that the amount of alcohol consumed was determined by the nutritional needs of the organism, and, second, he pointed out that there existed a large degree of variation between the drinking habits of individual rats.

Interests in the study of alcoholism were given impetus by Williams and his co-workers (Williams, 1947; Williams, Berry & Beerstecher, 1949; Williams, 1951). They interpreted results of biochemical-genetic studies as indicating that the genetic development of each individual, as it affects metabolism and enzymatic
patterns, is distinctly different from every other individual. Williams *et al.* (1949) dealt with alcoholism as a "genetotrophic" disease, implying an interaction between genetic and nutritional factors in the etiology of alcoholism. Individual differences in dietary needs, metabolism, and consumption were taken as confirmation of their genetotrophic hypothesis. Further support of the significance of individual differences (Segovia-Riquelme, Vitale, Hegsted, & Mardones, 1956) came from a comparison of rats on a free-choice schedule. Rats which drank 4 to 10 grams of alcohol per kilo of body weight per day were grouped as drinkers (D), and those which drank less than 1 gram per kilo of body weight per day as non-drinkers (ND). Metabolism of alcohol was measured by the radioactivity of the carbon dioxide which was collected after injections of various doses of grams of alcohol per kilo of body weight. They found no differences in oxidation rate between D and ND groups. However, they did find individual variations which led to the acceptance of the genetotrophic concept.

The significant strain differences in ethanol consumption have been found by Rodgers, McClearn, Bennett, & Harbert (1963) employing a food deprivation schedule with a free choice of drinking water and a 10 per cent (v/v) ethanol solution. The difference in ethanol consumption was assumed to indicate strain differences in the ability to utilize ethanol as a source of calories. Schlesinger (1964) supported the finding by Rodgers *et al.* (1963) by determining the rate of ethanol $14^C$ metabolism for mice which had been selected as drinkers and non-drinkers (C57Bl/crgl and DBA/ 2crgl). The high preference strain exhibited a significantly higher ethanol metabolism rate.
Nutrition and alcoholism have also been studied primarily as a function of the effects of deficiency in B complex vitamins upon the voluntary consumption of alcohol (Mardones, Segovia, & Onfray, 1946). A diet deficient in factor N₁ (a combination of several unnamed B vitamins) increased the voluntary ethanol drinking of rats. Second generation rats raised on a diet deficient in factor N₁ (Mardones, Segovia, & Hederra, 1953) voluntarily drank significantly greater amounts of ethanol than their parents raised on the same diet. It is difficult to isolate any differences due to genetic in-breeding without respect to vitamin deficiencies.

Genetics were initially assumed operative in alcoholic preference because of observed individual differences in animals of the same strain which were tested under identical environmental conditions. It was suggested that these differences might be explained by "genetic unlikeness" (Mardones, 1951, p. 570). Second generation male rats which had been forced to drink a 5 per cent ethanol (v/v) solution from weaning showed a significant increase in growth and ethanol consumed, and a decrease in total fluid intake, over control animals (Mirone, 1952). This study supports an earlier finding by Williams (1947) which was not carried beyond the parent animals.

The present interest in the genetics of alcoholism stems from the work of McClearn and Rodgers (1959). These investigators pointed out that the determination of ethanol preferences among inbred strains of animals would have two important implications. "First, it would make possible the study of genetic mechanisms of alcoholic preferences, using generations derived from strains of different phenotypes" (1959, p. 691).
Second, it would allow researchers who are looking for other determinants of ethanol preference to select animals with predictable levels of consumption for their studies. The study by McClearn and Rodgers (1959) was a measure of the daily free-choice consumption of a 10 per cent ethanol (v/v) solution in four mouse strains. C57bl mice drank significantly more ethanol solution than did the three strains which exhibited low preference, and did not differ significantly from each other. Results of a second study (McClearn and Rodgers, 1961) showed that in almost all crosses of the C57bl strain with strains of non-preferring animals the ethanol preference of the offspring was significantly higher than that of the non-preferring parent. It was concluded that the study of the physiological and behavioral causes of ethanol preferences can be profited by genetic manipulation.

The work which has been presented above views the etiology of alcoholism or "preference" for alcohol as a function of metabolic functioning, nutritional requirements, and genetics. The studies cited have used amount of consumption as a measure of "preference" and have attempted changing this "preference" by applying various procedures. From the results of these studies it is questionable whether they are actually manipulating "preference." It has been suggested (Forsander, 1962) that it would be more correct to speak of the somatic limitations of alcohol consumption, instead of the degree to which the animals, including man, "crave" alcohol. Animals evolutionarily lower than man are evidently limited in alcohol consumption by certain physiological conditions inherent in their species. It
appears reasonable to consider these conditions as an explanation for variance in human consumption as well as in animals. The limitation of ethanol consumption is, in this instance, the amount of alcohol tolerated before it has a deleterious effect upon an animal's metabolism. The critical value is different for various species and individuals because of genetic variability.

The studies cited above were confined exclusively to either rats or mice and all have used the amount of a 10 per cent ethanol (v/v) solution as the criterion of preference. Studies of ethanol preferences in other species are rare and only three have been found by this writer. Anderson & Smith (1963) found that monkeys drank equal amounts of water and a 10 per cent ethanol (v/v) solution. Arvola and Forsander (1961) presented a choice between water and a 10 per cent ethanol (v/v) solution to six species of animals: hedgehog, golden hamster, guinea pig, rabbit, rat, and mouse. Their results indicated that rabbits were either unable to discriminate, or had no preference for ethanol or water, exhibiting a preference ratio of .483. The other animals were obviously able to make the discrimination in preferring water to ethanol, except the hamsters which had a preference ratio of .880. The guinea pigs had the lowest preference ratio at .108. Arvola and Forsander (1963) demonstrated distinct sex differences in the alcohol preferences of golden hamsters, with males preferring water over all concentrations of ethanol.

A pilot study by the author investigated the alcohol preference of guinea pigs. The subjects were 3 animals from an Empire strain, 2 animals from an Abyssinian strain, and 2 animals from a Peruvian
strain. All animals were maintained on a diet of green vegetables and standard, guinea pig, food pellets. The first 6 days all animals were offered a choice to drink ad lib. from a bottle containing water or a bottle containing a 1 per cent ethanol (v/v) solution. The next 6 days all animals were offered an ad lib. choice between water and a 2 per cent ethanol (v/v) solution. The last 4 days the Empire and Abyssinian strains were offered a choice between water and a 3/4 per cent ethanol (v/v) solution, and the Peruvian strain had a choice between water and a 3 per cent ethanol (v/v) solution. Measurements were recorded daily from each cage. Results indicated strain differences with the Peruvian strain consuming more ethanol (v/v) solution at each concentration. The preference ratio for the Peruvian strain was over 5,000 on each concentration offered. The other two strains consumed small volumes of ethanol (v/v) solutions and did not differ from each other.

The presence of a threshold is determined by presenting a choice between water and increasing or decreasing concentrations of ethanol (v/v) solutions on successive days or blocks of days. A difference between the amounts of ethanol and water consumed between two test days indicates the threshold for the discrimination, physiological limitations, or taste preference of the two fluids. Below this threshold point there is no difference between the amount of ethanol consumed and the amount of water consumed. Above this point an animal will exhibit a preference for one liquid over the other. This threshold in rats has been measured at 1.8 per cent ethanol (v/v) concentration (Richter and Campbell, 1940), and at a .0039 per cent ethanol (v/v)
concentration (Kahn and Stellar, 1960). The discrepancy lies in the interpretation of graphic presentation, and the definition of threshold. The first study presented results in the form of a ratio of the volume of ethanol consumed over total volume of liquid. The second study presented results as separate graphs of volumes of ethanol and water consumption.

Previous studies have demonstrated that it is possible to alter the threshold of rats and mice by forced ethanol drinking. However, there are conflicting results as to the direction of change (Williams, et al., 1949; Powell, Ramano, & Martin, 1966). The demonstration of changes in both directions has been presented in one study (Mendelson and Mello, 1964). There was an increase in consumption in all groups of rats after an initial forced drinking period, and a decrease in consumption in 7 out of 10 groups after a second forced drinking period. The increase after the second forcing may be explained by increase in age or length of exposure, thereby developing a taste preference or a metabolic tolerance. Walgren and Forsander (1963) tested the preference for a 10 per cent ethanol (v/v) solution in 90- and 540-day old rats after 61 and 350 days of restricted drinking respectively. The first group did not alter their preference; however, the second group increased their consumption. There was no control for age, and the authors concluded that the change in group 2 was due to increased exposure in the forced drinking stage. Kakihana and McClearn (1963) investigated preference as a function of age in BALB/c mice and demonstrated that increased age resulted in a decrease in consumption of a 10 per cent ethanol (v/v) solution. Their study
employed a strain of mice which had been shown to avoid concentrations as low as 2.5 per cent (Rodgers and McClearn, 1962). Failure to identify the strains of animals employed as drinkers and non-drinkers may account for the contradictory results in the studies cited above.

The study by Kakihana and McClearn (1963) is an indication that any change in preferences due to forced drinking is dependent upon initial level of preference, or the age of the animals at the start of the test. It was expected, on this basis, that low-preferring animals will lower their preference, and high-preferring animals will raise their preference. This hypothesis has repeatedly been confirmed (Mardones, 1960; Rodgers et al., 1963). The first part of the present study was an attempt to identify the threshold for the discrimination of ethanol and water in two inbred strains of guinea pigs. All animals were offered a free choice of drinking water or an ethanol (v/v) solution. The second part of this study remeasured the threshold after a period of forced drinking of an ethanol solution. It was hypothesized that there would be significant strain differences both in the initial level of alcohol discrimination and in the remeasurement of the level of discrimination. It was also hypothesized that there would be significant strain differences in ratio of ethanol preference.

METHOD

Subjects and Apparatus

The subjects were 20 male guinea pigs. Ten animals were from an
inbred colony of the Peruvian strain (purchased from the Henry Rabbit Farm, Sangus, Mass.) and 10 animals were from an inbred colony of the Empire strain (purchased from the Reynolds Guinea Pig Ranch, Battle Creek, Mich.). All animals were housed individually in standard laboratory cages and watered from bottles with the tips inserted into the sides of the cages.

Procedure

All animals were allowed to feed *ad libitum* on Purina Guinea Pig Chow throughout the study. The first part of the study (A) was to determine the initial level of discrimination between water and ethanol (v/v) solution by *ad lib* free-choice drinking. The concentration of ethanol (v/v) solution was increased every day, beginning with a .002 per cent (v/v) solution. Measurements were taken between 7 P.M. and 9 P.M. each day from all the bottles for 15 days.

During the second part of the study (B) all animals were forced to drink a 7 per cent ethanol (v/v) solution *ad lib* without being offered the water bottle. Measurements were taken from the bottles for 15 days.

The third part of the study (C) was to remeasure the threshold. Part C was carried out in the same manner as Part A.

RESULTS

Figure 1 illustrates the daily mean preference ratio for both strains during part A of the experiment. Predicted strain difference in ratio of ethanol preference was not significant at the .05 level
with an $F(1, 18) = 2.00$ [refer to Table 1]. The analysis of variance for concentration with an $F(14, 252) = 2.60$ was significant at the .01 level, indicating a decrease in preference as the concentration of ethanol (v/v) solution increased.

Figure 2 illustrates the mean daily consumption of ethanol (v/v) solution and water for the Empire strain. The analysis of variance, with an $F(1, 18) = 9.00$ significant at the .01 level [refer to Table 2] shows that the Empire strain preferred water to ethanol during Part A. The data indicated that some value between an .08 per cent ethanol (v/v) solution and a .10 per cent ethanol (v/v) solution may be considered the preference threshold for this strain when an ascending series of concentrations is employed. The t value on the tenth day, at a .10 per cent ethanol (v/v) solution, was 3.56, significant at the .005 level.

Figure 3 represents the mean daily consumption of ethanol (v/v) solution and water for the Peruvian strain. The analysis of variance, with an $F(1, 18) = 4.17$ was significant at the .10 level [refer to Table 3], indicating that the Peruvian strain consumed more water than ethanol solution. The data indicated that a value between an .08 per cent ethanol (v/v) solution and a .10 per cent ethanol (v/v) solution may be considered the preference threshold for the Peruvian strain. The t value for the tenth day and a .10 per cent ethanol (v/v) solution was 2.16 and significant at the .025 level.
FIGURE 1 PER CENT ETHANOL (v/v) SOLUTION PER DAY

MEAN PREFERENCE RATIO

Peruvian 

Empire
Table 1
Analysis of Variance
in Ratio of Preference in Part A

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between subjects</td>
<td>1.848</td>
<td>19</td>
<td>.097</td>
<td></td>
</tr>
<tr>
<td>Strain (A)</td>
<td>.184</td>
<td>1</td>
<td>.184</td>
<td>2.00</td>
</tr>
<tr>
<td>Subjects within groups</td>
<td>1.664</td>
<td>18</td>
<td>.092</td>
<td></td>
</tr>
<tr>
<td>Within subjects</td>
<td>32.702</td>
<td>280</td>
<td>.117</td>
<td></td>
</tr>
<tr>
<td>Concentration (B)</td>
<td>4.009</td>
<td>14</td>
<td>.286</td>
<td>2.60</td>
</tr>
<tr>
<td>Interaction</td>
<td>.885</td>
<td>14</td>
<td>.063</td>
<td>.573</td>
</tr>
<tr>
<td>B x subjects within groups</td>
<td>27.808</td>
<td>252</td>
<td>.110</td>
<td></td>
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</table>
Figure 2: Per cent ethanol (v/v) solution PLR day for the Empire strain.
Table 2
Analysis of Variance
of the Empire Strain in Part A

<table>
<thead>
<tr>
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<th>MS</th>
<th>F</th>
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</thead>
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<tr>
<td>Between subjects</td>
<td>359,781</td>
<td>19</td>
<td>18,935</td>
<td></td>
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<tr>
<td>Liquids (A)</td>
<td>124,644</td>
<td>1</td>
<td>124,644</td>
<td>9.00</td>
</tr>
<tr>
<td>Subjects within groups</td>
<td>235,137</td>
<td>18</td>
<td>13,063</td>
<td></td>
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<tr>
<td>Within subjects</td>
<td>803,540</td>
<td>280</td>
<td>2,869</td>
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</tr>
<tr>
<td>Concentration (B)</td>
<td>20,149</td>
<td>14</td>
<td>1,439</td>
<td>.508</td>
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<tr>
<td>B x subjects within groups</td>
<td>714,341</td>
<td>252</td>
<td>2,834</td>
<td></td>
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</table>
FIGURE 3  PER CENT ETHANOL (v/v) SOLUTION PER DAY FOR THE PERUVIAN STRAIN
Table 3

Analysis of Variance

of the Peruvian Strain in Part A

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
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<tbody>
<tr>
<td>Between subjects</td>
<td>108,118</td>
<td>19</td>
<td>5,690</td>
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<tr>
<td>Liquids (A)</td>
<td>20,334</td>
<td>1</td>
<td>20,334</td>
<td>4.17</td>
</tr>
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<td>Subjects within groups</td>
<td>87,784</td>
<td>18</td>
<td>4,876</td>
<td></td>
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<tr>
<td>Within subjects</td>
<td>437,464</td>
<td>280</td>
<td>1,562</td>
<td></td>
</tr>
<tr>
<td>Concentration (B)</td>
<td>27,953</td>
<td>14</td>
<td>1,996</td>
<td>1.33</td>
</tr>
<tr>
<td>Interaction</td>
<td>32,732</td>
<td>14</td>
<td>2,338</td>
<td>1.56</td>
</tr>
<tr>
<td>B x subjects within groups</td>
<td>376,779</td>
<td>252</td>
<td>1,495</td>
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</table>
Figure 4 represents the mean daily consumption in cc's of a 7 per cent ethanol (v/v) solution for both strains during the 15-day forced drinking period. The means for the Peruvian strain are based on an N of 8 in Part B. Two of the animals stopped their fluid and solid intake and did not survive the forced drinking with cause of death undetermined. Figure 5 represents the mean daily total volume consumed by both strains in Part A. During the forced drinking there was a decrease in total liquid consumed daily, for both strains.

Figure 6 represents the daily mean preference ratio for Part C of the experiment. The sign test was applied to mean daily preference ratio scores for the last 5 days of testing in Parts A and C. It was found that the probability of change of preference due to forced ethanol drinking was insignificant for both the Empire (p = .180) and the Peruvian strain (p = .726).

The analysis of variance for Part C did not support the hypothesis of strain differences, in ratio of ethanol preference and threshold value. Significant threshold values were not found in Part C. The analysis of variance for concentration with an F (14,224) = 2.58 was significant at the .01 level [refer to Table 4], indicating a decrease in preference as the concentration of ethanol (v/v) solution was increased.

DISCUSSION

The presence of genetic mechanisms in determining the ethanol preference of guinea pigs was not supported by the findings of the present study. Strain differences were not significant in the
FIGURE 4  DAILY MEASUREMENTS AT A 7 PER CENT ETHANOL (v/v) SOLUTION

MEAN CONSUMPTION IN cc

Peruvian  Empire

1  2  3  4  5  6  7  8  9  10  11  12  13  14  15
FIGURE 5 DAYS IN PART A

MEAN TOTAL CONSUMPTION IN cc

Peruvian
Empire

170
155
140
125
110
95
80
65
50
35

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
FIGURE 6 PER CENT ETHANOL (v/v) SOLUTION PER DAY

MEAN PREFERENCE RATIO

Peruvian
Empire
Table 4

Analysis of Variance

of Ratio of Preference in Part C

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between subjects</td>
<td>1.64</td>
<td>16</td>
<td>.102</td>
<td></td>
</tr>
<tr>
<td>Strain (A)*</td>
<td>.38</td>
<td>1</td>
<td>.38</td>
<td>4.52</td>
</tr>
<tr>
<td>Subjects within groups</td>
<td>1.26</td>
<td>15</td>
<td>.085</td>
<td></td>
</tr>
<tr>
<td>Within subjects</td>
<td>28.72</td>
<td>238</td>
<td>.120</td>
<td></td>
</tr>
<tr>
<td>Concentration (B)*</td>
<td>3.76</td>
<td>14</td>
<td>.268</td>
<td>2.58</td>
</tr>
<tr>
<td>Interaction</td>
<td>1.45</td>
<td>14</td>
<td>.103</td>
<td>.990</td>
</tr>
<tr>
<td>B x subjects within groups</td>
<td>23.51</td>
<td>224</td>
<td>.104</td>
<td></td>
</tr>
</tbody>
</table>

* Empire n = 9

* Peruvian n = 8
comparison of ratio preferences and threshold values.

A significant ascending threshold value, between an .08 per cent ethanol (v/v) solution and a .10 per cent ethanol (v/v) solution, was determined for both strains. The forced drinking procedure did not change the ratio of ethanol preference in either strain. However, the forced drinking was responsible for the disappearance of threshold values. The negative results of the present study indicate that it may be more profitable to study strain differences in guinea pigs using a procedure similar to that employed by McClearn & Rodgers (1959), who measured the preference of a single ethanol concentration. This was the method used in the pilot study which resulted in finding strain differences in ratio of ethanol preference in guinea pigs.

Further research in this area should employ more control groups. Each strain should have one group tested under Part A and C only, in order to control for the effects of the testing procedure. The effects of forced drinking could then be more positively determined by having one group in each strain measured under Parts B and C only. A control for age (Kakihana & McClearn, 1963) may also be profitable by having groups in each strain measured under Part A only, and under Part C only. It is also possible that threshold values would be more accurate if determined from the differences in values which might be obtained from employing both an ascending and a descending scale of concentration (Meyers & Carey, 1961). One significant environmental variable not controlled in this study was room temperature (Meyers, 1962), which fluctuated daily according to the outside
temperature. The high outside temperature recorded during the experiment was 94 degrees Fahrenheit, and the low recording was 47 degrees Fahrenheit. This was more controlled in the original observations and may have accounted for some discrepancy in drinking behavior.

SUMMARY

Strain differences in ratio of ethanol preference and preference threshold in guinea pigs were measured using a free-choice drinking procedure for 15 days. The concentration of ethanol (v/v) solution was increased each day. Strain differences were not significant. A significant ascending preference threshold was determined for both strains. A tendency toward decreasing consumption of ethanol with increasing concentrations of ethanol (v/v) solution was significant at the .01 level.

Remeasurement of preferences after a forced drinking procedure did not indicate significant strain differences in ratio of preference or preference threshold. Forced drinking resulted in the disappearance of threshold values, but had no other apparent effects upon drinking behavior.
References


