End bottle preferences of inbred mice during alcohol preference and fluid intake multiple-bottle test procedures

CHARLES L. GOODRICK
Gerontology Research Center, Baltimore City Hospitals, Baltimore, Md. 21224

Two mouse strains (C57BL/Crgl females and C3H/Crgl/2 females) were tested for alcohol preference by the use of a seven-bottle choice situation with bottles in fixed or varied sequences. The results were partially dependent upon a tendency for end-bottle preferences in the sequence. Tests using high alcohol concentrations in the test bottles (0%-30%) or water in all the test bottles confirmed the finding of a strong tendency for end-bottle preferences for these groups. Further data on water intake in five- and seven-bottle tests were obtained for male and female mice (A/J, C3HeB/FeJ, C57BL/6J), with results showing strain and sex differences in end-bottle preference. Due to these differences, the two-bottle test should normally be used in studies of the genetics of preference behavior.

Investigators who have studied the role of heredity in alcohol preference behavior have generally used a two-bottle choice situation (McClearn & Rodgers, 1959, 1961). However, Fuller (1964) has suggested that a test which used a greater number of concentrations of alcohol was more appropriate on the grounds that the resultant preference ratios resulted in fewer scaling difficulties than did the two-bottle test. Both Fuller (1964) and Rodgers & McClearn (1962) had used multiple-bottle test procedures in the study of the genetic factor in alcohol preference. The present experiments determined the effect of bottle position upon alcohol preference and proportion of fluid intake.

EXPERIMENT 1

An initial experiment examined alcohol preference differences for two mouse strains by the use of a seven-bottle test situation with varied or constant position of alcohol concentrations.

Subjects

Sixteen C57BL/Crgl (Cancer Research Genetics Laboratory, Berkeley, Calif.) and 16 C3H/Crgl/2 6-month-old female mice were Ss. Both groups had extensive test experience with two bottles, with C57 groups high in alcohol preference (70%-90% preference for 10% alcohol) and C3H groups normally intermediate in alcohol preference (30%-50% preference for a 10% alcohol concentration). A prior forced 10% alcohol ingestion series had established an aversion to alcohol for C3H but not for C57 groups.

Procedure

The mice were caged in groups, eight of the same strain per cage, in Wahmann suspended cages (10 x 16 in., Baltimore, Maryland). Rodgers & McClearn (1962) used a similar procedure. For the two test cages of each strain, bottles were placed in either the same order (ascending series, left to right) or a varied order (using a table of random numbers) for six 3-day sessions. Concentrations of 0.0%, 2.5%, 5.0%, 7.5%, 10.0%, 12.5%, and 15.0% alcohol were used. Every 3 days, the amount of liquid ingested was measured from each bottle and the bottles were cleaned and refilled. The bottles were then replaced, in the same sequence for one group or in a randomly determined sequence for the other group. Results were tabulated in terms of proportion in each concentration ingested over the 18-day period.

Results

The results are shown in Table 1. Both fixed-order groups had side preferences, but the effect was much greater for the C57 group (high alcohol preference) than for the C3H group (low alcohol preference). The probability of obtaining this distribution of bottle preferences by chance is .0016[1] (Position 1 > 2 > 3 > 4 < 5 < 6 < 7). The varied-order groups show the expected distribution of alcohol ingestion for alcohol preferring and nonpreferring groups (Fuller, 1964; Rodgers & McClearn, 1962). The only conclusions regarding optimal concentration for varied-order groups were that C57 mice preferred high percentages more than low percentages and that this particular group of C3H mice preferred water over all alcohol percentages.

EXPERIMENT 2

The unusual result for the fixed-order C57 and C3H groups may be due to mouse preferences for the end bottles when confronted with a series of tubes spaced on the front of the cage. To test this hypothesis, the C57 groups with high alcohol preference were tested with both highly preferred and nonpreferred alcohol solutions in a fixed-order sequence. Also, C57 and C3H groups were tested for normal fluid intake with water in all seven drinking bottles.

Subjects

Sixteen naive 6-month-old female mice (8 per cage) of the C57BL/Crgl strain were tested at high alcohol concentrations in the seven-bottle test. Ss for normal fluid intake were the same mice used in Experiment 1, 16 C3H females and 16 C57 females.

Procedure

Both alcohol test groups were presented with fixed-order alcohol concentrations double those used in Experiment 1 (0.0%, 5.0%, 10.0%, 15.0%, 20.0%, 25.0%, and 30.0% alcohol). The method of testing was the same as in Experiment 1. Normal fluid intake groups were tested for a 6-day interval with water in all seven bottles.

Results

The results for alcohol intake at high concentrations is shown in Fig. 1. A comparison fixed-order group (0%-15% range) from Experiment 1 is included. The basic form of the fluid intake function is similar for both groups in that end bottles are more preferred than center bottles. The results of water intake tests for the groups tested in Experiment 1 are given in Table 2. There is a clear preference for end bottles for these groups. The probability of obtaining these sequences by chance is .0016.

Table 1
Percentage of Fluid Ingested as a Function of Alcohol Concentration and Method of Bottle Placement for Female Mice

Alcohol Concentration	Same Order		Varied Order	
	C57	C3H	C57	C3H
0.0	23.0	70.1	6.2	73.6
2.5	6.4	12.6	7.0	6.6
5.0	3.0	5.4	6.5	3.6
7.5	3.0	0.9	19.6	3.2
10.0	9.9	2.0	21.2	4.7
12.5	16.0	2.7	18.1	6.4
15.0	39.5	6.3	21.4	1.9

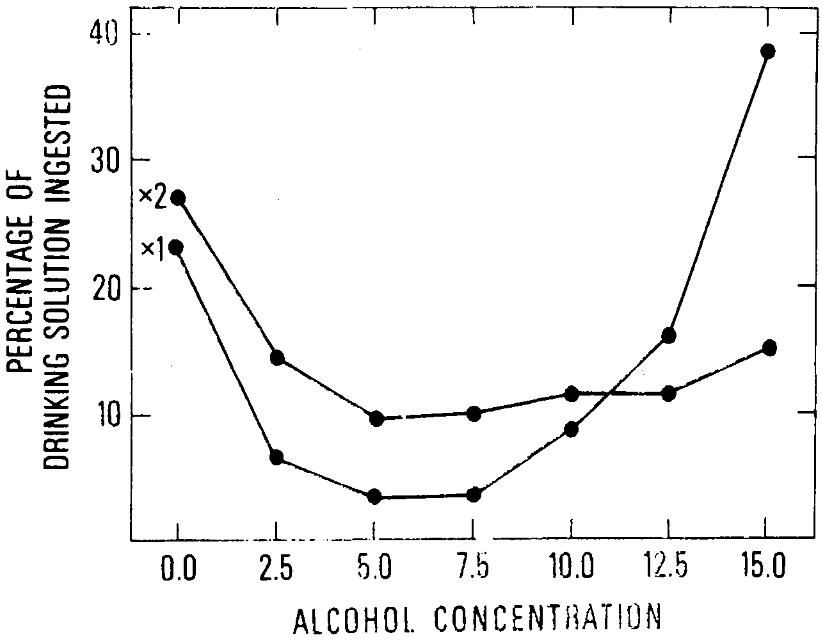

Fig. 1. Percentage of drinking solution ingested as a function of alcohol concentration (solutions placed left to right, 0% to 15% = x1 or 0% to 30% = x2).

EXPERIMENT 3

The next experiment examined the following possibilities: (1) Previous experience might have affected the bottle preference sequences obtained in Experiment 2; (2) end-bottle preference might be restricted to female mice; (3) strain or subline differences might exist in side preference tendencies; and (4) a more optimal number of bottles might exist. This experiment, which used male and female mice of three inbred strains from a different laboratory, obtained the proportion of fluid intake (tap water) from each bottle, using a five- or seven-bottle test situation.

Subjects

Mice were obtained from Jackson Memorial Laboratories (Bar Harbor, Maine), which normally score low (A/J), intermediate (C3H), or high (C57) on alcohol preference tests (Fuller, 1964; McClearn & Rodgers, 1961). Inbred mouse strains A/J, C3Heb/FeJ, and C57BL/6J were tested when 6 months old. Sixteen males and 16 females of each strain (8 per cage) were tested for a total N of 96.

Procedure

Fluid ingestion measures for a 7-day period were obtained for either a seven- or a five-bottle test for each experimental group. Measures of fluid intake were obtained, and the bottles were washed, refilled, and randomly switched twice during the 7-day interval (on Days 3 and 5).

Table 2
Percentage of Total Water Ingested as a Function of Mouse Strain and Bottle Position for Female Mice

	C57BL/Crgl	C3H/Crgl/2
L	23.4	19.0
	12.7	12.2
	9.6	9.4
	6.6	8.1
	7.1	11.8
	14.4	16.1
R	26.2	23.4

Results

Results are shown in Table 3 for the proportion of fluid intake as a function of bottle position. There was a statistically significant tendency for C57 female, C57 male, and C3H female mice to drink from the end bottles (p = .0048, combined probability). In contrast, C3H male, A/J female, and A/J male mice had no consistent ingestion pattern.

DISCUSSION

The most important findings of this experiment were that there were mouse strain and sex differences in the tendency to ingest drinking fluid from end test bottles when five or seven test bottles were used. The use of a multiple-bottle test procedure failed to precisely determine relative preference for alcohol, although general tendencies were readily discernible. The failure to equate group variances with a two-bottle test procedure may be preferable to imprecise data obtained from five- to seven-bottle test procedures. An alternative possibility, which should be carefully explored, is to use a score based on a series of two-bottle tests with ascending alcohol concentrations similar to sequences used by Kahn & Stellar (1960) for rats.

Fuller (1964) concluded that the six-choice method placed strains in the same order of preference as the two-choice test, but the most important outcome was "... the demonstration of variability of mode of inheritance of alcohol preference between the ... F_1 crosses [p. 88]." However, McClearn & Rodgers (1961) had found a fair degree of consistency in the mode of inheritance for alcohol preference (10% alcohol, two-bottle tests); mean F_1 scores were generally intermediate at the midparent in value. This inconsistency between the two experiments may be due to strain differences in the tendency to select end bottles of a sequence during multiple bottle testing.

The reasons for strain differences in end-bottle preferences are not clear.

Table 3
Percentage of Fluid Ingested as a Function of Mouse Strain, Sex, and Bottle Placement for Seven- and Five-Bottle Tests

	C57		C3H		A	
	Female	Male	Female	Male	Female	Male
			Seven-Bottle Test			
L	22.1	21.0	16.5	11.4	14.2	14.5
	12.9	12.7	13.1	10.9	12.9	12.9
	10.4	12.7	11.7	14.3	21.1	18.6
	11.7	10.5	10.9	18.9	13.2	17.6
	13.2	10.8	14.5	13.1	11.4	16.4
	11.4	12.7	15.1	18.3	12.7	11.0
R	18.3	19.7	18.2	13.0	14.3	8.0
p	.048	.048	.048	—	—	—
			Five-Bottle Test			
L	27.0	23.9	24.2	20.9	17.8	15.6
	19.7	17.7	19.3	19.9	21.6	20.7
	17.4	16.1	18.0	20.6	17.2	24.4
	12.7	17.6	17.3	19.7	21.6	20.0
R	23.2	24.7	21.2	18.9	21.8	19.3
p	.10	.10	.10	—	—	—
Combined p	.0048	.0048	.0048	—	—	—

Strain A sublines are known to be inactive compared to highly active C57 strain sublines (McClearn, 1960). High activity is known to reduce the tendency of mice to remain nearthe sides of an open field (Ross et al, 1966). If the tendency to remain at the sides of the home cage is correlated with a preference for end bottles, then the present A/J and C57BL/6J group differences should be reversed. The determination of thecorrelates of end-bottle preferences may required direct observation of cage behavior for mice caged in groups.

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NOTE

1. The following probabilities were used. $p = 2[(n-1)/2]/n!$ denotes the probability of an unordered odd sequence (Experiments 1 and 2). For $n = 7$, $p = .0016$. For Experiment 3, the direct probability of obtaining high values for the end bottles was used to simplify outcomes; for seven bottles, $p = (2 \times 5!)/7! = .048$; for five bottles, $p = (2 \times 3!)/5! = .10$; and if both outcomes occurred, $p = .0048$.