*Pharmacology Biochemistry & Behavior*, Vol. 8, pp. 695–699. Printed in the U.S.A.

# Bimodal Distributions of Highest Ethanol Acceptance Concentrations in Two Strains of Rats[1]

J. ALLEN GOAS AND ARNOLD S. LIPPA

*Central Nervous System Disease Research Section, Lederle Laboratories*
*Pearl River, NY 10965*

(Received 13 March 1978)

GOAS, J. A. AND A. S. LIPPA. *Bimodal distributions of highest ethanol acceptance concentrations in two strains of rats.* PHARMAC. BIOCHEM. BEHAV. 8(6) 695–699, 1978. – Two groups of non-deprived male Wistar rats and one group of male Sprague-Dawley rats were offered a choice of water and daily-increasing concentrations of ethanol. Each group's distribution of highest ethanol acceptance concentrations approximated a bimodal distribution with respect to concentration. Further, rats in each group which drank ethanol at high concentrations maintained relatively constant intakes of pure ethanol. These results are discussed in terms of taste and olfaction, central nervous system sensitivity and emotionality.

Ethanol acceptance  Ethanol rejection  Wistar  Sprague-Dawley  Bimodal distribution

LABORATORY rats typically display individual differences in their preferences of various ethanol concentrations over water [3,13]. In light of this variability, the practice of using one arbitrary ethanol concentration (e.g., 10%) for all animals in free-choice studies is questionable. Such variability is particularly confounding in studies which investigate techniques for increasing consumption of non-preferred ethanol solutions, as a single concentration is rarely equally aversive to all animals.

Thus, a method was proposed [5] for identifying single equally aversive ethanol concentrations for individual rats based on each animal's ethanol selection threshold. Rats were offered a choice of water or ethanol with ethanol beginning at 3% (v/v) and increasing by 1% each day. Since rats typically prefer ethanol over water at low concentrations of ethanol [13], each animal's test concentration was identified as that concentration which was three percentage points above the upper threshold of ethanol preference over water. Although this procedure does yield ethanol rejection concentrations for most rats, it does not necessarily identify the lowest concentration which each animal will reject.

A more promising procedure [2] was designed to identify the lowest ethanol concentration which a rat would completely reject in favor of water. Rats were given a choice between water and ethanol which was started at 3% and was increased by 1% each day until a concentration was reached which the animal rejected. This concentration was then offered for an additional two days. If the animal completely rejected this concentration in favor of water for three successive days, it became that animal's rejection concentration.

The present paper, which was to be the first stage of a larger study, reports the data from an attempt to determine ethanol rejection concentrations in rats by means of a slight modification of the latter procedure. As the ethanol rejection determinations were being made, however, it became increasingly clear that not all animals would reject ethanol in the 9–35% concentration range as previously suggested [2]. These ethanol drinking data are presented below.

## METHOD

### *Animals*

Forty-eight male Wistar rats (Royalhart Farms) and 20 male Sprague-Dawley rats (Holtzmann Co.) were used. All rats weighed between 240–260 g at the beginning of the experiment.

### *Apparatus*

All animals were individually housed in galvanized steel cages, 26 x 33 x 20 cm, with wire mesh floors and fronts. Each cage was equipped with two, 100 ml glass Kimax Richter-type drinking tubes, mounted on the front of each cage. The tubes were 8 cm apart and projected 3 cm into the cage, 1 cm above the floor.

[1] The authors gratefully acknowledge the assistance of Messrs. Marvin E. Miller and Harvey Coleman during the course of this experiment. They also acknowledge the assistance of Dr. Thomas Hoffman during data analysis.

Ethanol solutions were prepared from 95% ethanol (Alcohol, U.S.P., 190 proof) and mixed with tap water to achieve the desired concentrations (v/v).

*Procedure*

Upon arrival, the animals were placed in the individual test cages for approximately one week during which time the two drinking tubes were always filled with water. This was done to allow the animals to acclimate to the cages and to the drinking tubes. Purina Laboratory Chow was available ad lib for the duration of the experiment.

At the end of the acclimation period, one of the two drinking tubes for each animal was filled with a 1% ethanol concentration while the other was filled with tap water. The ethanol concentration was then increased by 1% each day until a concentration was reached which an animal would not drink. If this rejection of an ethanol concentration persisted for three successive days, it became that animal's rejection concentration, with the preceding concentration declared as the animal's highest acceptance concentration.

Data were collected each morning between 8:00 and 11:00 a.m. Ethanol evaporation control tubes were placed on empty cages adjacent to those placed on occupied cages in order to determine daily volume loss due to evaporation alone and to correct the data for this loss. After the daily data were collected, the drinking tubes were rinsed, refilled and replaced on the cages with the left-right positions reversed from the previous day. The data were collected in three successive replications consisting of two groups of 28 and 20 Wistar rats, respectively, and one group of 20 Sprague-Dawley rats.

During this data collection period an attempt was made to determine whether the concentrations of various ethanol solutions remained constant in Richter tubes for 24 hr periods. Ethanol concentrations of 20, 40 and 60% were placed on empty cages in Richter tubes for 24 hr after which they were submitted for gas chromatographic analysis. None of the samples tested differed significantly from control samples. These results validate the procedure of determining pure ethanol intakes by calculation from intakes of ethanol solutions.

## RESULTS

Each animal's daily ethanol intake (corrected for evaporation loss) and water intake were recorded until each rat rejected an ethanol concentration for three successive days. As the ethanol concentrations increased above 70%, however, the evaporation control data became unstable and erratic thus precluding accurate measurement of the ethanol intakes of the rats who had not rejected by that point. Therefore each animal's highest ethanol acceptance concentration (that concentration immediately preceding the concentration rejected for three successive days) became the primary end-point rather than lowest rejection concentrations. Rats which had not rejected ethanol by 70% were given a highest concentration score of $\geq 70\%$. Individual highest acceptance concentrations were then tallied with respect to rat group and summarized in the form of histograms which are shown in Fig. 1.

The distributions of highest ethanol acceptance concentrations for the two groups of Wistar rats and the one group of Sprague-Dawley rats approximated bimodal distributions. The first group of Wistar rats (Wistar Group I; N = 28) had one lower mode in the 10 to 19% concentration interval with 28.5% of the animals showing highest acceptance concentrations in this interval. This group also had a higher mode in the $\geq 70\%$ concentration interval with 50% of the animals showing highest acceptance concentrations in this interval. The second group of Wistar rats (Wistar Group II; N = 20) had one

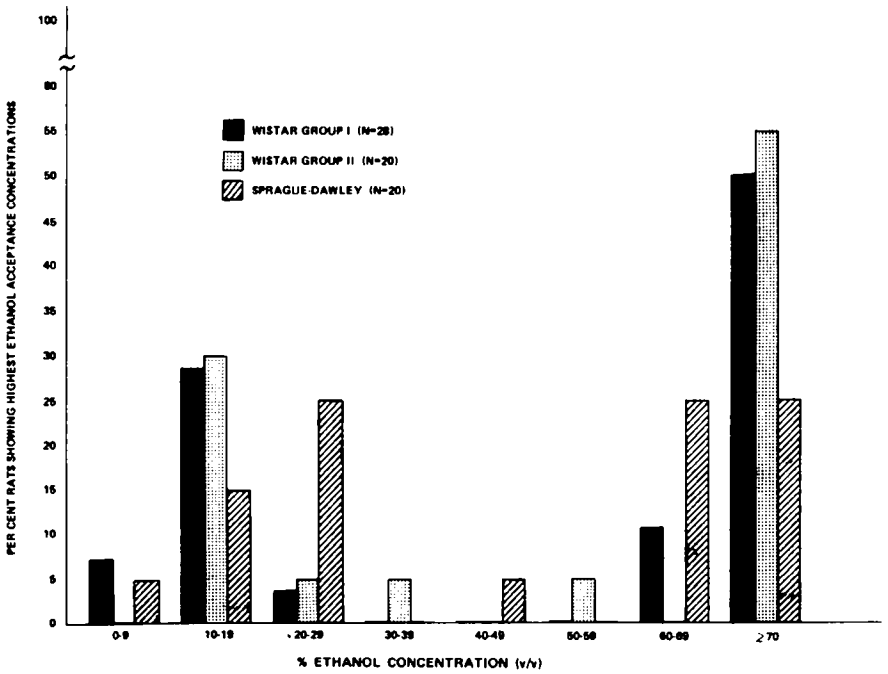


FIG. 1. Bimodal distributions of highest ethanol acceptance concentrations for two groups of Wistar rats and one of Sprague-Dawley rats.

lower mode in the 10 to 19% concentration interval with 30% of the animals showing highest acceptance concentrations in the interval. This group also had a higher mode in the >70% concentration interval with 55% of the animals showing highest acceptance concentrations in this interval. The Sprague-Dawley group (N = 20) had one lower mode in the 20 to 29% concentration interval with 25% of the animals showing the highest acceptance concentrations in the interval. This group also had a higher mode in the combined 60 to 69% and >70% concentration intervals with 50% of the animals showing highest acceptance concentrations in these combined consecutive intervals.

The Kolmogorov-Smirnov test [6] was used to analyze the raw data from which these three distributions were derived. There was no statistically significant difference between the Wistar Group I and the Wistar Group II bimodal distribution ($T = 0.1212$, $p > 0.10$). Because of the lack of a significant difference between the two Wistar groups, their distributions were combined and tested against the Sprague-Dawley distribution. Again, no statistically significant difference was found between the Wistar and Sprague-Dawley rats ($T = 0.2708$, $p > 0.10$).

Mean intake of pure ethanol (calculated from the intakes of ethanol solutions) were plotted at each ethanol concentration for each mode group within the Wistar Group I, the Wistar Group II, and the Sprague-Dawley groups of rats (Fig. 2). This was done in order to examine the patterns of pure ethanol intake across increasing concentrations for the various groups of rats. As can be seen in Fig. 2-A, the largest mean volume of pure ethanol consumed by the lower mode (low accepting) rats of Wistar Group I (N = 11) was about 1.0 ml/24 hr, consumed as 3 and 4% ethanol. As the concentrations increased above 4%, the mean daily intakes of pure ethanol approached zero. The pattern of mean pure ethanol intakes for the higher mode (high accepting) animals of Wistar Group I (N = 17) showed that as the ethanol concentrations increased from about 1% to about 30%, the mean intakes of pure ethanol increased to about 3.0 ml/24 hr. As the ethanol concentrations further increased from 30% to 70%, the mean daily pure ethanol intakes remained at a fairly constant level of about 3.0 ml/24 hr, dropping off slightly above 65%.

The drinking patterns of the Wistar Group II rats were similar to those of the Wistar Group I rats. As can be seen in Fig. 2-B, the largest mean volume of pure ethanol consumed by the lower mode (low accepting) rats of Wistar Group II (N = 8) was about 0.7 ml/24 hr, consumed as 3% ethanol. As the concentrations increased above 3%, the mean daily intakes of pure ethanol gradually declined to zero. The pattern of mean pure ethanol intakes for the higher mode (high accepting) animals of Wistar Group II (N = 12) showed that as the ethanol concentrations increased from 1% to about 30%, the mean intakes of pure ethanol increased to about 2.0 ml/24 hr. As the ethanol concentrations further increased from 30% to 70%, the mean daily pure ethanol intakes remained at a fairly constant level of about 2.0 to 2.5 ml/24 hr.

The drinking patterns of the Sprague-Dawley rats were also similar to those of both Wistar groups. As can be seen in Fig. 2-C, the largest mean volume of pure ethanol consumed by the lower mode animals of the Sprague-Dawley group (N = 10) was about 1.2 ml/24 hr, consumed as 7% ethanol. As the concentrations increased above 7%, the mean daily intakes of pure ethanol gradually dropped to zero. The pattern of mean pure ethanol intakes

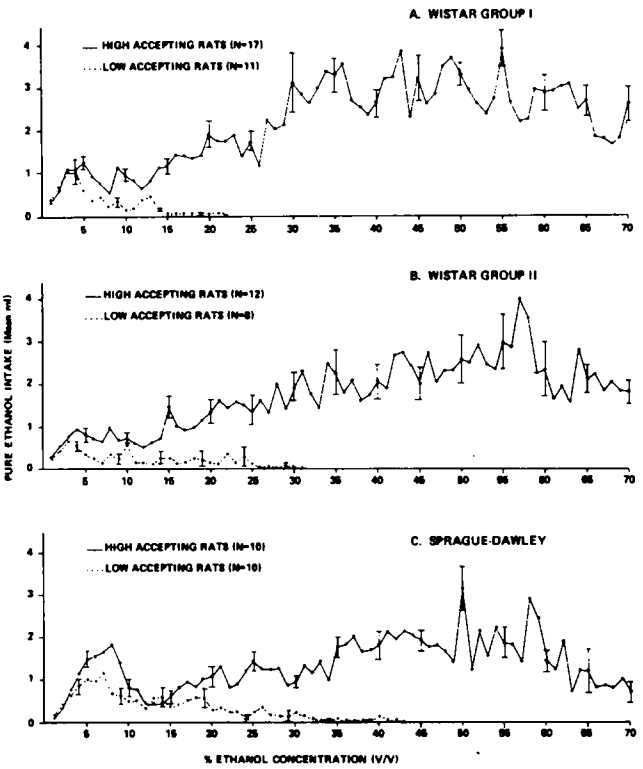


FIG. 2. Mean 24 hr consumption of pure ethanol (ml, calculated from solution intakes) as a function of ethanol concentration (± 1 standard error) for the high accepting (high mode) and low accepting (low mode) rats in the three groups of rats.

consumed by the higher mode rats of the Sprague-Dawley group (N = 10) showed that as the ethanol concentrations increased from 1% to about 8%, the mean intakes of pure ethanol increased to about 1.8 ml/24 hr. The daily mean intakes of pure ethanol then decreased to about 0.4 ml/24 hr at 13% followed by an increase in mean pure ethanol consumption to about 2 ml/24 hr as the ethanol increased to 35%. As the ethanol concentrations further increased from 35% to 60%, the mean daily pure ethanol intakes remained at a fairly constant level of about 2.0 ml/24 hr, followed by a gradual decline in pure ethanol intake above 60%.

## DISCUSSION

In contrast to an earlier report [2], the results of the present study suggest that it is difficult if not impossible to arrive at valid ethanol rejection concentrations for all experimental rats using the procedure described here. This is based on the finding that almost half of all the rats studied in the present experiment failed to reject ethanol by the time the concentration reached 70%. Above 70%, the data was confounded by unstable evaporation control data. These data lend support rather, to the conclusion [23] that the determination of preference-aversion functions for ethanol in the rat might be impossible. In that study, when Sprague-Dawley rats were given a choice between increasing concentrations of ethanol and water in a sequence similar to that used in the present experiment, a large number of animals continued to drink a significant

volume of ethanol when the ethanol concentration reached upwards of 77%. The present study confirms and extends this finding, circumventing the criticism [1] that the drinking tubes used by previous investigators [23] had stainless-steel ballpointed spouts which have a tendency to leak fluid. The present study used the more reliable glass Richter tubes.

The most surprising aspect of the present results was that the distributions of highest ethanol acceptance concentrations approximated bimodal distributions for all three groups of rats. Differential ethanol intakes between animals of different strains have been reported for selected strains of mice [16] and rats [17] with the latter reporting one rat strain whose females freely consumed ethanol up to a concentration of 85%, when the concentration began at 4% and increased by 2% daily. However, the present experiment shows that volitional intake of high concentrations of ethanol is possible in a significant number of animals within two commonly used, presumably homogeneous strains of albino rats. The nature of the obtained bimodal distributions suggests the possibility that each presumed homogeneous strain may actually contain two relatively distinct populations defined by some parameter governing ethanol acceptance.

The notion that the Wistar and Sprague-Dawley groups of rats may each have contained two populations defined by differential ethanol intake is further supported by the finding that the high ethanol accepting animals from each group appeared to maintain fairly constant pure ethanol intakes across increasing concentrations (Fig. 2). These stable mean ethanol intake patterns suggest that the high accepting rats began to regulate their volumes of pure ethanol intake at a moderate level (2 to 3 ml/24 hr) regardless of concentration, while the low accepting rats stopped drinking ethanol completely. These stable patterns of ethanol intake in the high accepting groups remained until the concentrations increased above 60%, at which point the mean pure ethanol intakes for all groups began to decline.

A number of possible explanations could account for these findings. One explanation involves a potential difference in taste and/or olfactory sensitivity to ethanol between the two modal groups of rats within each group. Kahn and Stellar [9] studied the effects of olfactory bulbectomies on rat ethanol intake. The authors reported that the anosmic rats' ethanol preference-aversion function shifted slightly toward the higher concentrations as compared to preoperative drinking patterns. Support for the taste sensitivity notion comes from a study [10] in which rats which were previously selected as either "drinkers" or "non-drinkers" of 6% ethanol were tested for rejection of various quinine solution concentrations. The results indicated that the ethanol drinkers had higher quinine taste thresholds than the non-drinkers. However, unpublished data from our laboratory suggest that there is no significant correlation between ethanol rejection and either quinine or saccharin rejection, using the basic procedure described here.

An alternative explanation is suggested by studies of differential ethanol effects in high- and low-drinking strains of mice. It has recently been reported [12] that C57BL mice, a high ethanol-preferring strain, have less CNS sensitivity to ethanol as measured by duration of ethanol-induced sleep time than the non-preferring BALB strain. Similar differences were reported in ethanol-induced deficits of a centrally mediated reflex in C57BL and non-preferring DBA mice [19]. Such differences in neural sensitivity, which cannot be accounted for by proposed differences in ethanol metabolism rates (in C57BL and DBA mice) [14,15] or liver alcohol dehydrogenase and aldehyde dehydrogenase activity (in selectively bred strains derived in part from C57BL, BALB and DBA mice) [8] may account for the difference in alcohol intake within the two rat strains reported here. Such an explanation would require empirical verification in the rat strains used in the present study.

A third possible explanation for the bimodal distributions involves a proposed difference in emotionality levels between the upper and lower mode rats within the three groups of rats. Researchers from two laboratories [4,18] investigated the relationship between volitional ethanol intake and emotional reactivity in two strains of rats selectively bred for differential open-field defecation scores. The results from both studies indicate that the high reactive rats consumed significantly more 5% and 10% ethanol solutions than the non-reactive rats, when given a choice between ethanol and water. These data are consistent with the finding that emotionality, as reflected by the proneness of rats to audiogenic seizures, seems to be positively related to the ingestion of 5% ethanol [7]. Thus, although differences in emotionality per se between rats within strains are rarely considered to be important determinants of behavior, such differences may be correlated with animals' tendencies to show highest ethanol acceptance concentrations at high or low concentrations, as in the present experiment. Such within-strain differences in emotionality might also explain reported findings of "high" and "low" ethanol-drinking rats in stress experiments (e.g., [11]), in operant experiments (e.g., [21]), and in post-experimental data (e.g., [20]).

## REFERENCES


1. Amit, Z., S. Amir and M. E. Corcoran. A possible artifact in studies of alcohol consumption in rats. *Q. Jl Stud. Alcohol* **34**: 524–527, 1973.
2. Amit, Z., M. H. Stern and R. A. Wise. Alcohol preference in the laboratory rat induced by hypothalamic stimulation. *Psychopharmacologia* **17**: 367–377, 1970.
3. Arvola, A. and O. Forsander. Comparison between water and alcohol consumption in six animal species in free-choice experiments. *Nature* **191**: 819–820, 1961.
4. Brewster, D. J. Ethanol preference in rats selectively bred for behavioral characteristics. *J. Genet. Psychol.* **115**: 217–227, 1969.
5. Cicero, T. J. and R. D. Myers. Selection of a single ethanol test solution in free-choice studies with animals. *Q. Jl Stud. Alcohol* **29**: 446–448, 1968.
6. Conover, W. J. *Practical Nonparametric Statistics.* New York: John Wiley and Sons, Inc., 1971, p. 309.
7. Dember, W. N. and A. B. Kristofferson. The relation between free-choice alcohol consumption and susceptibility to audiogenic seizures. *Q. Jl Stud. Alcohol* **16**: 86–95, 1955.
8. Heston, W. D. W., V. G. Erwin, S. M. Anderson and H. Robbins. A comparison of the effects of alcohol on mice selectively bred for differences in ethanol sleep-time. *Life Sci.* **14**: 365–370, 1974.

9. Kahn, M. and E. Stellar. Alcohol preference in normal and anosmic rats. *J. comp. physiol. Psychol.* **53**: 571–575, 1960.
10. LeMagnen, J. and P. Marfaing-Jollat. The role of buccal afferent information in the determination of spontaneous drinking of alochol in the rat. *J. Physiol.*, Paris **53**: 407–408, 1961.
11. Myers, R. D. and T. J. Cicero. Effects of serotonin depletion on the volitional alcohol intake of rats during a condition of psychological stress. *Psychopharmacologia* **15**: 373–381, 1969.
12. Randall, C. L. and D. Lester. Differential effects of ethanol and pentobarbital on sleep time in C57BL and BALB mice. *J. Pharmac. exp. Ther.* **188**: 27–33, 1974.
13. Richter, C. P. and K. Campbell. Alcohol taste thresholds and concentration of solutions preferred by rats. *Science* **91**: 507–508, 1940.
14. Rodgers, D. A. Factors underlying differences in alcohol preference among inbred strains of mice. *Psychosom. Med.* **28**: 498–513, 1966.
15. Rodgers, D. A. Inherited characteristics of inbred mice as these relate to voluntary alcohol consumption. In: *Biological Aspects of Alcohol Consumption*, Vol. 20, edited by K. Eriksson and O. Forsander. Helsinki: Finnish Foundation of Alcohol Studies, 1972, pp. 105–112.
16. Rodgers, D. A. Factors underlying differences in alcohol preference of inbred strains of mice. In: *The Biology of Alcoholism*, Vol. 2, edited by B. Kissin and H. Begeiter. New York: Plenum Press, 1972, pp. 107–130.
17. Russel, D. E. and M. H. Stern. Sex and strain as factors in voluntary alcohol intake. *Physiol. Behav.* **10**: 641–642, 1973.
18. Santinder, K. P. Behavior-genetic-dependent self-selection of alcohol in rats. *J. comp. physiol. Psychol.* **80**: 422–434, 1970.
19. Schneider, C. W., S. K. Evans, M. B. Chenweth and F. L. Beman. Ethanol preference and behavioral tolerance in mice: Biochemical and neurophysiological mechanisms. *J. comp. physiol. Psychol.* **82**: 466–473, 1973.
20. Senter, R. J. and J. J. Persensky. Effects of environment on ethanol consumption in rats after conditioning. *Q. Jl Stud. Alcohol.* **29**: 856–682, 1968.
21. Sinclair, J. D. Rats learning to work for alcohol. *Nature (New Biol.)* **249**: 590–592, 1974.
22. Veale, W. L. and R. D. Myers. Increased alcohol preference in rats following repeated exposures to alcohol. *Psychopharmacologia* **15**: 361–372, 1969.
23. Wayner, M. J., I. Greenberg, R. Targaglion, D. Nolley, S. Fraley and A. Cott. A new factor affecting the consumption of ethyl alochol and other sapid fluids. *Phsyiol. Behav.* **8**: 345–362, 1972.