Toward an analogue of alcoholism in mice: Criteria for recognition of pharmacologically motivated drinking

(C57BL/6J Mouse/voluntary alcohol consumption/animal model/ethanol)

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ABSTRACT   Two criteria of alcoholic drinking behavior—inelasticity of demand and dissociation of intake from normal eating and drinking—are illustrated by study of alcohol-preferring C57BL/6J mice. Although these mice drink enough to become intoxicated for brief periods each night, they do not meet the more rigorous criteria for pharmacologically motivated drinking. Their intake of alcohol was dramatically decreased when they were offered diets augmented with sugar or Crisco, and the temporal pattern of drinking was correlated with the intake of food. Thus, their motivation for drinking alcohol is related to nutrition and is not drug-seeking comparable to that of human alcoholics. Since the tests are simple and decisive, it might be useful to apply them to all putative models of alcoholism.

Mice of the C57BL strain consume larger quantities of alcohol per unit of body weight than do the most advanced human alcoholics (1, 2). The animals do this voluntarily while being provided with a nutritionally adequate diet and with unlimited amounts of plain water as an alternative to the alcohol solution. Moreover, monitoring the concentration of alcohol in their blood reveals peaks occurring several times each night following episodes of drinking (3). Although these peaks of blood alcohol concentration are transient because of the speed of clearance, the maximal concentrations are in a range that would be seriously intoxicating for a human.

In these respects the animals resemble human alcoholics and appear to provide an opportunity to analyze the disease under laboratory conditions. On the other hand, there are reasons to question the relevance of these phenomena to the human disease. Heavy drinking does not harm the animals in any obvious way. It neither disables them acutely, nor does it produce significant organ damage (4), even after months of ingesting the equivalent for humans of two quarts of whiskey per day. The rapid metabolism of alcohol in mice protects them from the toxicity that a man would experience from comparable drinking. While it is conceivable that mice might be found that would drink enough alcohol voluntarily to produce the toxic effects that are so prominent in human alcoholics, it is perhaps unnecessary to demand this of a model since toxicity is a result, not the cause, of excessive drinking.

Essentially, the problem in alcoholism is abnormal appetite—a compulsion to drink alcohol at inappropriate times and to continue drinking to the stage of gross impairment. Of course, not all motivation to drink alcohol is pathological. It is a food as well as a drug (5, 6), and studies of drinking by animals must make this distinction (7, 8). For human alcoholics, the substance appears to act as an addictive drug, while the caloric effects are motivationally irrelevant. The question

implicit in any proposed model of the disease is whether the animal's motivation is similarly drug-oriented or simply nutritive.

The present report discusses two tests that make this distinction: elasticity of appetite for beverage-concentration alcohol and temporal correlations of eating and drinking. Elasticity is exhibited by a reduction in the quantity of alcohol consumed when alternative sources of calories are provided. Correlations compare intake patterns of food and drink. By both criteria, the mice in the present study appeared to be consuming alcohol as a food rather than a drug. It is possible that other strains or species being studied as models of alcoholism would exhibit evidence of pharmacologically motivated drinking when evaluated by these criteria. Since such testing is not difficult, and the result is critical to issues of clinical relevance, it might be useful to subject all putative models to these criteria.

MATERIALS AND METHODS

Male mice of the C57BL/6J strain were housed individually in plastic cages, located in a temperature-controlled (22 ± 1°C) room with a light/dark cycle of 12:12 hr. The equipment used for continuous monitoring of fluid consumption and details of feeding and animal care have been described (9).

In addition, a device for monitoring consumption of dry food was developed for the present study. The standard feeding bottles, which consist of a wide-mouthed glass jar with a cylindrical metal screen in the central axis (Wahmann individual mouse feeder LC-207/A), were modified by provision of a second metal cover above the regular one and insulated from it. When a mouse crawls into the jar to feed, its hind feet make electrical contact with the upper plate while its forefeet grip the metal screen. A second switch, in series with this device, closes once per second. Thus, while the animal is feeding, the monitoring microprocessor receives signals at a 1/sec rate. These indicate when and for how long the animal is in the feeding bottle but do not provide a reliable measure of the quantity of food consumed, since the rate of consumption varies with the physical nature of the feed and the amount in the jar. For the present purpose, however, the device was adequate, since correlation of time series requires only determination of the times of feeding and drinking and the relative amounts consumed in each interval. The frequency of pulses was chosen arbitrarily to yield daily scores comparable to those produced by the lick detectors.

The First Experiment. This experiment tested the elasticity of appetite for alcohol and was designed as a series of crossover trials. After 4 weeks of residence in the laboratory with water and 10% alcohol as drinking fluids and powdered chow (Purina Lab Chow 5001) as the basal diet, 12 mice were divided into two stratified groups: the mice were ranked by alcohol consumption in the last week of the preliminary
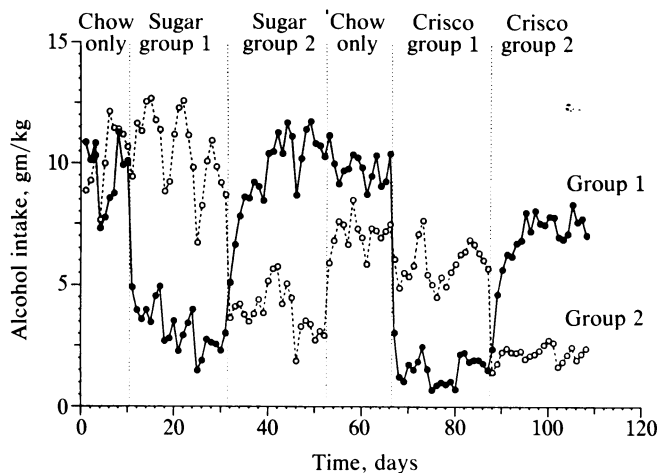
FIG. 1.   Elasticity of appetite for alcohol. Two groups of six mice each were given supplementary sugar or fat in a cross-over test, with constant access to 10% alcohol and water. The ordinates indicate average intake of alcohol (gm/kg of body weight per day).

period, and then members of each consecutive pair were assigned to the two groups.

The experiment was divided into six periods (Fig. 1). In the first period, all animals continued on the basal chow regimen; in the second period (designated "Sugar 1"), animals of group 1 were provided with a mixture of sucrose and chow (1:1 by weight) in place of their usual feed, while the animals of group 2 continued on the basal regimen; in the third period ("Sugar 2"), the diets were reversed, group 2 animals now receiving the sugar chow mixture while group 1 returned to the basal diet; in the fourth period, all animals were fed basal chow; in the fifth period ("Crisco 1"), animals of group 1 were provided with a supplement of Crisco in addition to chow; and in the sixth period ("Crisco 2"), the diets were reversed.

Throughout the experiment two drinking fluids, 10% alcohol and plain water, were constantly available, with bottles in the same positions. This invariant configuration avoided the confound between position, taste, and nutritional effects that occurs when different substances are added to the drinking fluids in the course of an experiment or the position of the bottles is varied.

The Second Experiment. The temporal association of feeding and drinking was studied in 12 mice. As usual, the drinking fluids were 10% alcohol and plain water. Licks at the fluid spouts and time spent in the feeder were recorded in consecutive 6-min intervals over a period of 2 weeks. The data were accumulated by a microcomputer, stored on disks, and later plotted and analyzed to determine cross correlations. For comparison, the temporal association of feeding and drinking was later obtained from six of these mice given a choice of 5% sucrose and water instead of 10% alcohol and water.

RESULTS

Elasticity of Demand for Alcohol. Consumption of alcohol dropped sharply when the basal chow diet was supplemented with either sucrose or fat (Fig. 1 and Table 1). Reversibility of these effects was demonstrated by crossover; alcohol intakes returned promptly to a high level when the supplement was discontinued and fell in the other group when supplement was added. All of the ethanol-preferring animals (11 of the 12) responded in this way.

This elasticity is consistent with a caloric motivation for consumption of alcohol and is quite different from the inelastic response that would be expected from an alcoholic human. Caloric intake and energy expenditure appeared to be balanced throughout the experiment since body weights were not significantly affected by the changes in diet. The contribution of alcohol to the total intake was high only during periods of basal chow feeding. At these times, alcohol provided approximately 10% of the daily caloric intake.

Ingestion of dry sucrose in the sugar periods caused a marked increase in drinking of water, presumably to help dissolve the sugar before absorption. Interestingly, the marked increase in fluid intake came entirely from the water bottle. The consumption of 10% alcohol decreased to about one-third that taken in the basal diet period. Thus, while alcohol is utilized as a source of calories when animals are restricted to chow, animals made thirsty by increased sugar intake do not choose the alcohol solution as a source of water, although the total water available from a 10% solution of alcohol (water content plus water of metabolism) is equal to that of plain water.

Correlation of Intakes. The times of eating and drinking alcohol by animals on basal diet were highly correlated, as is evident in plots of the two time series (Fig. 2). Numerically, the correlation coefficient of data smoothed by 30-min moving averages ranged from 0.68 to 0.89 with a mean of 0.81 ± 0.02 (SEM). The consumption patterns of plain water and alcohol also were correlated, further emphasizing the integration of alcohol intake into the normal feeding and drinking pattern.

In an independent test of this technique, not involving alcohol, the same mice were kept on basal chow and monitored while having a choice of 5% sucrose solution and water as drinking fluids. The volume of sucrose consumed was more than 3 times the volume of alcohol solution that had been ingested by these animals in a preceding period. Nevertheless, the patterns of eating and drinking remained closely correlated (Fig. 3), again showing the concordance of intake patterns when a substance is consumed as a food or fluid. Despite their great liking for this sweet fluid, the mice's consumption of sucrose solution was in keeping with the normal circadian rhythms of eating and drinking. The mean correlation coefficient of the 30-min smoothed data was 0.87 ± 0.03. Thus, the correlation technique correctly identified sugar as simply a food or preferred fluid rather than a drug, even when it was avidly ingested.

Table 1.   The mean daily intake of alcohol in C57BL/6J mice*

Group	Periods					
	1	2	3	4	5	6
1 (*n* = 6)						
Treatment	Chow	Sugar	Chow	Chow	Crisco	Chow
EtOH intake, g/kg.	9 + 0.5	2.6 + 0.1	11.0 + 0.2	9.7 + 0.3	1.8 + 0.1	7.6 + 0.2
2 (*n* = 6)						
Treatment	Chow	Chow	Sugar	Chow	Chow	Crisco
EtOH intake, g/kg	11.4 + 0.2	9.9 + 0.4	3.1 + 0.2	7.2 + 0.12	6.3 + 0.2	2.2 + 0.1

*The mice were given unlimited access to powdered lab chow (Chow) or to lab chow supplemented with either sucrose (sugar) or fat (Crisco). Data are means ± SEM in g/kg of body weight.
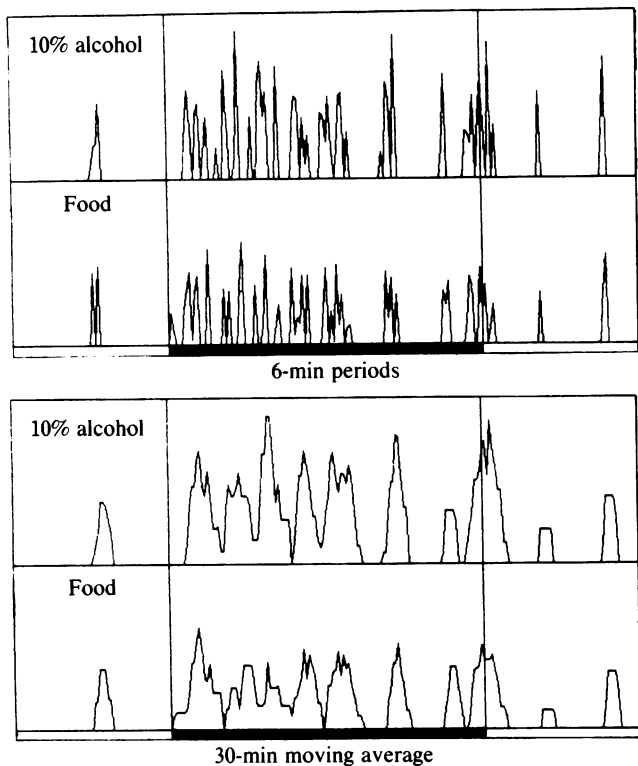
FIG. 2.　Association of alcohol consumption and feeding over a 24-hr period in one animal. (Upper) Pair of curves showing the intakes of 10% alcohol and dry diet, monitored in 6-min intervals. (*Lower*) Pair of curves displaying the same data after smoothing with a 30-min moving average. Coefficients of correlation were 0.48 and 0.88 for the first (*Upper*) and second (*Lower*) pair, respectively. (Ordinate scales are in arbitrary units, adjusted for convenience in comparison.)

FIG. 3.　Association of drinking sucrose solution and feeding in one animal. (*Upper*) Pair of curves showing data collected in 6-min periods. (*Lower*) Pair showing the same data after smoothing, as in Fig. 2. The correlation was 0.76 for the first (*Upper*) and 0.93 for the second (*Lower*) pair. (The ordinate scale for the sucrose solution was compressed by a factor of 4 relative to the alcohol scale of Fig. 2.)

DISCUSSION

Attempts to replicate alcoholic drinking in laboratory animals have included pharmacological manipulations (e.g., see ref. 10), the examination of existing strains for ethanol preference (e.g., see refs. 1 and 2), and selective breeding techniques (e.g., see refs. 11–13). Now, with a spectrum of neuropeptides and other neuroactive substances to be examined for behavioral effects, the possibility of linking high voluntary alcohol consumption to a specific neurochemical lesion has been enhanced. At the same time, however, there is increased danger that investigators might be misled by behavioral phenomena in animals that actually bear only a superficial resemblance to clinically important disorders.

The C57BL/6J mouse has provided an instructive case study on this point. Consuming an impressive amount of alcohol under conditions of free choice with unlimited availability of water and nutritionally adequate food, and repeatedly experiencing brief pulses of elevated blood alcohol as a consequence of voluntary intake of alcohol, the animal meets the most obvious criteria of alcoholism. It might be assumed, therefore, that studies of brain and organ function of these animals would illuminate the etiology of the human disease. This could be a mistake. The results presented here, and the findings of previous investigators (e.g., refs. 14 and 15), demonstrate that the high consumption of alcohol by C57 mice is contingent on a dietary restriction—not of quantity but of selection. Furthermore, the close correlation of food and alcohol consumption and their occurrence at normal times in the circadian cycle demonstrate that the intake of alcohol has remained subordinate to normal regulatory rhythms even under conditions that maximize the intake of
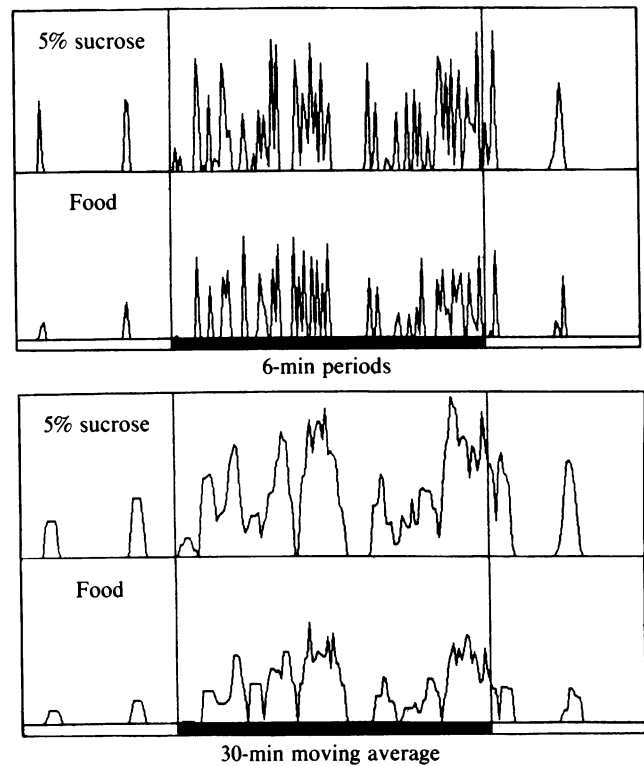
alcohol. But it is precisely in these respects that the humanalcoholic appears to be deviant. A dysfunctional appetite for alcohol is the abnormality that needs to be replicated if the behavioral problem of alcoholism is to be brought into the laboratory. Therefore, further progress toward a relevant analogue of alcoholism will require both (*i*) new techniques to produce drinking in animals that is inelastic and dissociated from food intake and (*ii*) quantitative studies of human alcoholics to improve the operational definition of the disease.

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