Polydipsia-Induced Alcohol Dependency in Rats: A Reexamination

Abstract. *Seven Holtzman rats were kept on a polydipsia-induced schedule of alcohol consumption for 3 months in a replication of a 1972 study by Falk and colleagues. Contrary to their results, there was no evidence of alcoholism.*

Animals can be induced to self-administer large quantities of liquid when small quantities of food are administered at intermittent intervals (*1*). This technique, known as polydipsia, has been employed in attempts to produce an animal model of human alcohol dependence. In these polydipsia studies, in which an ethanol solution instead of water was used as the available fluid, results concerning alcohol dependence have been equivocal. Several investigators (*2*) who employed the schedule-induced polydipsia technique with rats found no evidence of physical dependence on ethanol. In a study by Ogata *et al.* (*3*), mice were maintained on a polydipsia-inducing schedule with ethanol solutions as the only available liquid, but no indication of physical dependence when alcohol was withdrawn was reported.

Falk *et al.* (*4*), however, report unequivocal evidence of physical dependence and death from tonic-clonic seizures in polydipsia-maintained rats upon removal of access to ethanol. Tremors, spasticity, clonic head movements, and seizures were reported as indices of withdrawal symptoms.

The development of an animal model reflecting the major psychological and physiological features of the human alcoholic would facilitate the study of alcoholism. However, there is no agreement about exactly what syndrome constitutes an indication of alcohol dependence. According to Cicero and Smithloff (*5*), alcohol dependence should be characterized not only by a physiological dependence on alcohol as evidenced by withdrawal symptoms, but also by a "psychological dependence" on alcohol as indicated by the animals' self-selection of alcohol to avoid a withdrawal syndrome or, at least, a willingness to perform work in order to obtain alcohol. Ratcliffe (*6*) emphasizes that rats' usual unwillingness to consume ethanol in order to prevent withdrawal symptoms raises questions concerning the efficacy of using rat drinking behavior as a model for human alcoholism. However, Falk *et al.* (*4*) regard sound-induced convulsive behavior as a sufficient criterion for evidence of physical dependence.

Three reasons prompted us to repeat the Falk *et al.* study (*4*). The first was the desirability of substantiating the effectiveness of a technique reported to lead to unequivocal establishment of alcohol dependency in rats. The second was our belief that the inclusion of additional indices of alcohol dependency might provide more substantial data concerning the existence or quality (or both) of any alcoholic syndrome which the animals might develop. Specifically, we believed that voluntary consumption of supernormal amounts of freely available ethanol solution—termed "psychological dependence" by Cicero and Smithloff (*5*)—is a circumstance necessary to the proper identification of an alcoholic syndrome in laboratory animals. Finally, because Falk *et al.* (*4*) had reported no pretreatment testing of their animals for natural susceptibility to audiogenic seizures, we wondered whether such susceptibility had been present in their animals and accounted for their results. The present study not only includes as exact as possible a replication of the Falk *et al.* procedure, but also includes a pretreatment screening test for seizure susceptibility among the experimental animals and observations of the rats' posttreatment "psychological dependency" as evidenced by consumption of freely available alcohol.

We tested eight male Holtzman rats upon receipt for convulsive tendencies by housing them in individual cages and shaking keys proximal to each rat individually for 5 minutes. No signs of hyperactive or convulsive behavior were observed.

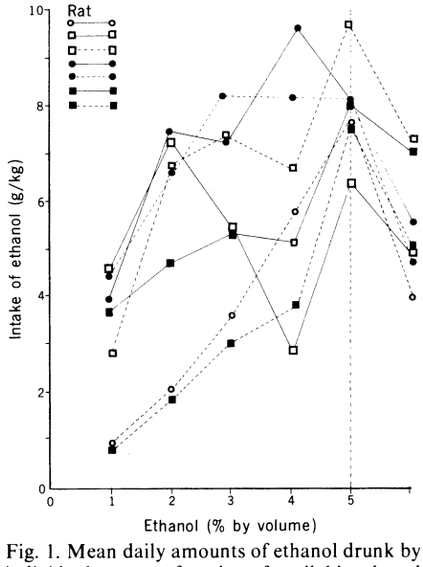

Fig. 1. Mean daily amounts of ethanol drunk by individual rats as a function of available ethanol concentration.

The mean free-feeding weight of these rats (when fed Purina Rat Chow) was 341.62 g. Each rat was reduced to 80 percent of its free-feeding weight by limiting food rations and then was housed in an individual chamber under constant illumination. A Noyes animal food pellet (45 mg) was delivered automatically every 2 minutes during 1-hour feeding periods separated by 3-hour nonfeeding intervals. There were, therefore, six feeding periods in each 24-hour cycle, during which a total of 180 pellets were delivered.

In the initial phase of the polydipsia treatment, water was the only available fluid. Each day the fluid intakes of each animal were recorded, and the animals were given quantities of food necessary to maintain their weights at 80 percent of the free-feeding level. After the establishment of schedule-induced polydipsia, increasing concentrations of ethanol solution were substituted for the water. The original 1 percent (by volume) ethanol concentration was increased in 1 percent increments every 6 to 8 days until a level of 5 percent ethanol was reached. The animals were continued on the polydipsia regime for 3 months after the 5 percent level was reached. As in the original Falk *et al.* study (*4*), when the animals began drinking ethanol additional food supplements were no longer given since the animals' weights began to increase.

The mean daily water intake at the end of the initial phase of the experiment was 61.18 ml, which compares favorably with the intake reported in the original Falk *et al.* study (64.0 ml). The mean daily self-administered dose of ethanol is shown in Fig. 1. Although the average ethanol intake increased from the 1 percent to the 2 percent condition, there is apparently no marked tendency for the animal to "drink to excess" in these circumstances.

At no time during the incremental alcohol phase of the experiment did the animals appear intoxicated. All motor responses were quick and aggressive. Handlers reported no signs of docile or ataxic behavior.

For the last 10 days of the experiment (5 percent condition) Falk *et al.* (*4*) reported an average ethanol intake of 13.1 g per kilogram of body weight. During the same period (last 10 days of experiment) in the present study, we found an average ethanol consumption of 11.71 g/kg, which is consistent with the average reported in the original study.

At the conclusion of the polydipsia treatment phase of the experiment, all seven surviving animals were removed from the experimental situation and placed in individual observation cages. These cages

contained a water bottle but provided no access to alcohol. One hour after the animals were placed in them, all observation cages were placed on a large table and keys were shaken above the cages for 5 minutes. No unusual behavior, other than curiosity, was observed. No seizures nor partial seizures were observed.

Three hours after removal from the polydipsia regime, the same procedure was repeated. At the sound of the shaking keys, rats 4, 5, and 6 leaped out of their observation cages and ran about on the laboratory floor. However, no convulsions or tonic-clonic seizures occurred. After the key shaking, all rats were given the amount of pellets that would have been dispensed in the experimental situation during an equivalent time period.

Seven hours after removal, the same procedure was repeated once more. Rats 4, 5, and 6 duplicated their previous behavior; again, however, there were no convulsions or seizures, or any other indication of withdrawal symptoms.

Immediately following this phase of the experiment, all rats were placed in home cages with access to both water and 5 percent ethanol. With unlimited food supply and free access to water, the animals failed to maintain their previous level of ethanol consumption (Fig. 1). Average ethanol intake during this period was only 5.46 g/kg.

Replication of the Falk *et al.* procedure (*4*) did not replicate the previously reported results. Falk *et al.* interpreted their findings as being unequivocal evidence of ethanol dependence in rats by virtue of convulsions following withdrawal of the animals from alcohol. We found no indication of such behavior after identical subject treatment.

The shaking of keys after the removal of alcohol was reported by Falk *et al.* (*4*) to have triggered convulsive seizures that resulted in death for two animals; they exposed only three of the seven rats to the key-shaking stimulus. It seems questionable whether audiogenic seizures are indicative of alcohol dependence under these circumstances, and our failure to observe even these symptoms raises some question as to the reliability of this polydipsia procedure in the production of alcohol dependency in the rat. We believe that the convulsive behavior reported by Falk *et al.* may have been a result of the rats' inherent proneness to seizures. The animals' "natural" seizure susceptibility was not tested by Falk *et al.*; therefore some seizure-prone animals might have been included in the sample. We further believe, as do others (*5, 6*), that an appropriate criterion of an animal's dependence should include voluntary ethanol consumption maintained presumably to avoid withdrawal symptoms. This aspect of alcohol dependence was not examined by Falk *et al.*

In the present study, when rats were given free access to alcohol after a period of withdrawal, they failed to maintain their previous level of consumption. This can only suggest that these rats were not dependent on alcohol.

The incidence of death associated with seizures reported by Falk *et al.* (*4*) can possibly be explained in terms of the Selye general adaptation syndrome (*7*). In the last stage of this syndrome (exhaustion), the organism has theoretically consumed its internal resources for dealing with continued stress and further stress will often result in death. If we assume that Falk's sample of animals contained some rats naturally susceptible to audiogenic seizures, it is possible that the induction of seizures may have provided additional stress conditions which, when summed with physiological stress resulting from chronic high alcohol consumption, may have pressed the animals beyond the point of general adaptation exhaustion and, hence, resulted in death. It is possible, therefore, that the deaths observed by Falk *et al.* were not the result of withdrawal symptoms at all but were caused by a combination of stressors, chronic high-level ethanol consumption coupled with induced seizures.

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Adrenaline-Forming Enzyme in Brainstem: Elevation in Genetic and Experimental Hypertension

Abstract. *The adrenaline-forming enzyme (phenylethanolamine* N-*methyltransferase) was elevated in the* A_1 *and* A_2 *regions of the brainstem of 4-week-old spontaneously (genetic) hypertensive rats and in the* A_1 *region of adult experimentally (deoxycorticosterone acetate and sodium chloride) hypertensive rats. The administration of a phenylethanolamine* N-*methyltransferase inhibitor to experimentally hypertensive animals caused a reduction of the elevated blood pressure to normal values. These results implicate adrenaline-containing neurons in the brainstem in the development of hypertension.*

It has been recognized that peripheral and central noradrenergic nerves play a role in the regulation of blood pressure and in the expression of some forms of hypertension (*1*). Recent work has demonstrated the presence of other catecholamine-containing neurons (adrenaline) in the brain. The adrenaline-forming enzyme, phenylethanolamine *N*-methyltransferase (PNMT) (*2*), has been detected in certain areas of the brain by immunohistofluorescent techniques with the use of antibodies directed against bovine adrenal PNMT (*3*), as well as by the direct measurement of the enzyme (*4*). Adrenaline has also been detected in the same regions (*5*). Nerve tracts and cells containing PNMT were found to be highly localized in the A_1 and A_2 areas of the brainstem.

Table 1. Activity of phenylethanolamine *N*-methyltransferase (PNMT) in specific regions of the brainstem of spontaneously (genetic) hypertensive rats (SHR). Results are expressed as mean ± standard error of the mean (S.E.M.) of groups of 14 animals. Brain regions from individual rats were dissected and analyzed separately as described in the text.

Region	PNMT activity (picomoles per milligram of protein per hour)	
	Controls	SHR
A_1	23.2 ± 5.9	38.6 ± 3.5*
A_2	42.4 ± 6.0	65.8 ± 5.4**
Locus coeruleus	7.2 ± 1.3	6.4 ± 1.7

*Statistically significant $P < .05$ (Student's *t*-test) against control values. **Statistically significant $P < .01$ (Student's *t*-test) against control values.

The A_1 area of the rat brainstem contains the cell bodies of the catecholaminergic neurons that send their axons to the spinal cord (*6*). The A_2 area corresponds in part to the nucleus of the solitary tract in which the majority of the fibers of the carotid sinus nerve terminate (*6, 7*). It is densely supplied with noradren-