12-1976

Alcohol Addiction & the Rat as a Possible Animal for the Study of Alcoholism

John Graham
Western Kentucky University

Graham,

John D.

1976

Alcohol Addiction and the Rat as a Possible Animal Model for the Study of Alcoholism

A Thesis

Presented to

the Faculty of the Department of Psychology

Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

John D. Graham

December 1976

Alcohol Addiction and the Rat as a Possible
Animal Model for the Study of
Alcoholism

Recommended December 17, 1976
(Date)

Richard L. Mills
Director of Thesis

Daniel L. Koenke

Leroy Metz

Approved 12-20-76
(Date)

Edmund Gleason
Dean of the Graduate College

Table of Contents

List of Tables and Figures

Alcohol Addiction and the Rat as a Possible Animal Model for the Study of Alcoholism

John D. Graham December 1976 35 pages

Directed by: Richard Miller, Leroy Metze, and Daniel Roenker

Department of Psychology Western Kentucky University

An attempt was made to produce an animal model for the study of alcoholism. It was hypothesized that the laboratory rat could be brought to physical dependency and maintained in that state under a free-choice circumstance. Two groups of 60-day-old Max hooded rats, consisting of 8 males and 8 females per group, were established to serve as experimental and control subjects. Two experiments were conducted. The intention of the first experiment was to install physical alcohol dependency in the experimental subjects with the use of a totally liquid diet. After the first experiment a 6-hr. period of total abstinence was used for all subjects to determine the severity of withdrawal reactions in the experimental subjects. The second experiment attempted to determine the free-choice consumption rates of alcohol by physically dependent and non-dependent subjects. A daily increase in the ethanol concentration of the alcohol-choice liquid was used for the presentation of alcohol during the free-choice situation. Alcohol preference was determined by an analyses of variance of a mixed-design with repeated measures on consumption across days. A measure of the gram of ethanol

per kilogram of body weight was used as the dependent variable. The results indicated a significant difference in alcohol consumption between alcohol exposed and naive subjects. Sex proved to be non-significant in regard to either group. The daily increase in the ethanol concentration of the alcohol-choice liquid during free-choice proved to be a significant factor in alcohol preference by both the experimental and the control subjects. Based on the observations between the two groups during free-choice, an attempt was made to equate alcohol preference with earlier state dependent learning variables.

Introduction and Literature Review

The etiology of alcoholism is a complex issue. Its developmental sequences are probably as diverse as the various individual personalities involved in the condition. Indeed, there seems to be no limit or restriction to any social class, racial group, or geographic area in which its effects are seen. In view of the wide social complexities and the magnitude of personal misery, tragedy, and suffering, the challenge that alcoholism projects to those interested in the study of human behavior is overwhelming.

Alcoholism is a chronic disorder involving a complex interaction of physical impairments, sociological factors, and underlying psychopathologies. It is characterized by such an overwhelming preoccupation with alcohol that all other activities are arranged around its consumption. In effect, the alcoholic loses control over drinking. He may experience minor periods of control but these will be followed by periods of relapse (Shearer, 1967).

If this chronic disorder is to be completely reversed, all psychological and sociological factors must be treated. Before such a task can be attempted, however, the alcoholic must be able to regain control over alcohol consumption or the resulting physical impairments will constantly interfere with the

psychotherapy. The physical disabilities, impaired emotions, etc. may indeed play a role in the development of alcoholism, but the physiological process then takes control and, in turn, plays a role in the development of other impairments. For this reason, abstinence is called for, but due to the severity of the neuroanatomic conditions of dependency in the alcoholic, this initial step in treatment becomes a very complex problem.

The psychodynamics leading to alcoholism are very complex and have a high emotional component. Whether the individual is faced with anxiety, guilt, hostility, a sense of inferiority, rage, depression, or a general state of emotional immaturity, he learns through a successive series of trials that the basic pharmacological depressant effect of alcohol on the central nervous system relaxes psychic and physiologic tensions. Since alcohol proved to be rewarding under these conditions, an alcohol craving may develop and induce drinking if an anxious, bored, or otherwise stressful situation should recur (Ludwig, 1974).

The Establishment of Addiction

With the early periods of consumption, the body gradually begins to adapt, at a cellular level, to the presence of alcohol. As this adaptation increases, tolerance to the effect of alcohol appears. In time tolerance gives way to dependency. The body now requires certain levels of alcohol to function on a normal level. With a long period of dependency, impaired major metabolic pathways and physical damage

to the brain and vital organs develop (Shearer, 1967).

It is important to note this developmental sequence from intolerance to tolerance to dependency by cellular adaptation because this description allows alcoholism to be defined in terms of the acceptable pharmacological criteria for addiction (Mendelson, 1964). Faser, Wikler, and Johnson (1957) expressed tolerance as the need to utilize increasing dosages of alcohol after repeated administrations with an original dose in order to produce the original effects. Dependency has been defined as a state produced by chronic alcohol administration which is revealed by physiologic dysfunctions when the alcohol is removed, and reversed with the readministration of the drug (Schuster & Johanson, 1974). Dependency, unlike tolerance, is not dose dependent since withdrawal symptoms may be seen whenever the blood alcohol level begins to drop (Mello & Mendelson, 1969).

The Physiology and Biochemistry of Addiction

The exact method of cellular adaptation involved in the establishment of tolerance and dependency has as yet remained unsolved. However, due to the requirements of chronic consumption and elevated blood alcohol levels, it has been assumed by many neurophysiologists and neurochemists that ethanol adaptation is a result of a change in neuronal membrane structure, and thus its metabolic functions (Axelrod, 1968; Goldstein & Goldstein, 1968; Israel & Kalant, 1965; Mendelson, 1970).

Ethanol, the primary alcohol of consumption, consists of an -OH end group bonded to a simple, short, double carbon chain (CH_3CH_2OH) which remains undissociated under normal physiological conditions. The terminal hydroxy group does, however, give ethanol a slightly polar activity very similar to that of water which very easily forms hydrogen bonds in solution. This is in contrast to the nonpolar properties of the hydrocarbon chain to which the hydroxy ion is bound. Actually, it seems to be just this dualistic propensity that allows ethanol to exert its effect on cellular membranes.

Whittaker (1968) reviewed several theories concerning the actual structure of cellular membranes (micellar, mosiac, and double-layer sheets). All of these theories are based on the general conclusion that an arrangement of lipids interact with proteins and glycoproteins in such a manner as to form a highly stable, yet plastic structure. These lipids are very similar in structure to ethanol, possessing an actively polar hydroxy end group and a nonpolar hydrocarbon tail structure. The major difference between the two compounds seems to be the greater length of the hydrocarbon tail in the lipids. Their carbon atoms are usually numbered between sixteen and eighteen compared to only two in ethanol (Lehninger, 1970). The longer hydrocarbon tail tends to promote a more stable, nonpolar quality to the lipids.

Because of its close structure and similar chemical properties, ethanol may nonspecifically insert itself into

this plastic structure between the lipid arrangement and thereby disrupt the normal formation within the cellular membrane by forcing a shift in hydrogen bonding interactions with the available proteins, glycoproteins, and water molecules which associate with the lipid layers (Seeman, 1966a; Seeman & Weinstein, 1966).

The effect of this ethanol insertion on nerve cell membranes seems to be quite significant in regard to possible shifts in the action potentials and the resulting discharge capacity within neurons. Neuronal activity is basically regulated by a series of changes in membrane permeability to an influx of sodium ions and an efflux of potassium ions. When the neuron is in a resting state, sodium and chloride ions are in high concentrations in the interstitial fluid while the cytoplasm has a high concentration of potassium and various large molecular organic anions. This ion distribution gives the neuron a more negative inner charge in comparison to the greater positive outer charge. It is this characteristic that is described as the polarized resting potential. The excitability of the nerve cell is directly related to the measure of this polarization (Woodbury, 1965).

When the neuronal receptor is sufficiently stimulated, a brief period of sodium influx will begin, thus depolarizing the cell membrane and increasing the excitability towards an action potential or nerve impulse. Repolarization to the resting state begins as the sodium influx is reversed

and potassium efflux is increased to balance off the inner sodium concentration. A more complete and specialized description of this process has been outlined sufficiently by Woodbury (1965).

With the application of ethanol, the ability to influx sodium is greatly reduced, while the ability to move potassium out of the cell is virtually unaffected (Armstrong & Binstock, 1964; Moore, 1966). The effect on the action potential is seen as a slow depolarization towards a weaker nerve impulse. Once repolarization begins, however, the return to a resting state may appear faster in relation to total nerve action. Inoue and Frank (1967) have shown that as little as 1.0% ethanol is sufficient to reduce the action potential and neuronal excitability in general.

Israel, Kalant, and LauFer (1965), Israel, Kalant, and LeBlanc (1966), Israel and Salazar (1967), and Jarnefelt (1960) have attempted to describe the exact action of this partial neuronal depolarization as the result of alcohol's interference with the various enzymes associated with cellular membranes. By allowing the nonspecific insertion of ethanol into the lipid structure of neuronal membranes, they have found normal ATPase activity to be significantly depressed. The neuron's normal ability to move sodium out of and potassium into the cell is regulated by energy from ATP cleavage by this enzyme complex, ATPase.

Ethanol's apparent ability to combine and alter neuronal

membranes thus seems to be the key to understanding the process towards alcohol addiction. By combining the acute, nonspecific enzyme interference for alcohol metabolism with the obvious possibilities of greater, specific enzymatic shifts in general metabolism, the explanation for tolerance following chronic alcohol consumption seems to increase in validity (Goldstein & Goldstein, 1968).

Dependency, the consequences of this cellular adaptation towards tolerance, is manifested in the failure of vital neurons during their excitation cycle to allow proper propagation of nerve impulses. These consequences are usually hidden until the alcohol source is removed and the resulting effects on the neurons is manifested in terms of withdrawal signs. Delirium, hallucinations, seizures, and hyperirritability are all considered as signs of withdrawal. These behaviors may be the result of an overcompensation by the nerve for the depressed conductance induced during tolerance stages and/or the result of an increased overall efficiency in synaptic transmission (Mendelson, 1970).

This description of withdrawal symptoms is a modification of the theory of "denervation supersensitivity" promoted by Cannon and Rosenblueth (1949). It basically explains denervation as a form of disuse or depressed neuronal activity. When alcohol is removed, the very rapid rise to normal use results in a form of rebound hypersensitivity to neuronal

excitability. Rebound conductance with an increase in synaptic transmission would be more than enough to explain most withdrawal symptoms.

The Neuroanatomic Effects of Alcohol

What is needed now is a specific understanding of which neuronal pathways are most sensitive and which specific central nervous system sites are responding to specific stages of tolerance and withdrawal. The difficulty present in this line of investigation is basically sustained by the very general, nonspecific action of integration ethanol seems to employ. Its chemical bonding properties allow it to associate freely with all hydrogenated body fluids and membranes. In essence, ethanol's access to bodily distribution is very close to that of water, our primary cellular fluid (Davson & Danielli, 1952; Pappenheimer & Heisey, 1963). The main restricting force regulating ethanol distribution to various body tissues seems to be access to the circulatory system. Kety (1951) and Price (1963) have shown this restriction to be a measure of intercapillary distance and the volume of blood flow proportional to the unit mass of any tissue in question.

In an attempt to determine alcohol's primary site of action, Crone (1965) showed that within ten seconds following administration to the cardiovascular system, an equivalent of 90% of the available concentration of blood alcohol left the blood in the first pass over the cerebral capillary bed. The

total picture of alcohol's predominate influence on behavior is made much clearer when this information is combined with the knowledge that the alcohol level in cerebrospinal fluid is 20% higher than that of the blood (Harger, Hulpieu, & Lamb, 1937). This concentration in the cerebrospinal fluid gives ethanol its predominate access to nerve cells throughout the central nervous system.

Due to the magnitude of ethanol interaction with the total nervous system, it has been extremely difficult to localize the primary site of involvement in the state of overall addiction, or in any of the developmental stages from intolerance to total dependency. Himwich, Diperri, David, and Schweigerd (1966) and Kalant (1961) have suggested that the depressant effects of ethanol are greatest in the cortical associational areas and followed very closely by the reticular formation and then by the somatosensory cortex. Israel (1970), however, reminds us that the physiological and behavioral effects of intoxication are probably produced by the combined interaction of larger nervous subsystem. His suggestion is based on the findings that alcohol concentrations between .05% and .3% may produce intoxication effects before the 1.0% concentration that is necessary for affecting individual cell excitability, impulse conduction, and transmitter release is reached. This seems to be compatible with the findings by Kalant (1970) and Wallgren and Barry (1970) that polysynaptic reflexes

are much more sensitive to ethanol than are the simple monosynaptic reflexes.

Animal Subjects and Addiction

From the information available it is obvious that the exact description of the neurochemical and neurophysiological processes that produce behavioral dependency on alcohol has not been developed. Due to the inherent nature of the techniques necessary to investigate these physiological processes, such studies have been hampered because living alcoholics are needed for study subjects. Before adequate forms of treatment can be developed for the alcoholic, these basic physiological questions must be answered. The obvious answer seems to be in the use of animals for this needed research. The difficulty in such an approach is that to date it appears as though man is the only animal which will self-administer alcohol in adequate quantities over a sufficient period of time to develop the state defined as alcoholic.

If the question of psycho-social development can be put aside while emphasis is placed in the physiology, then it may be possible to produce an animal model for studying the conditions of alcohol tolerance and dependency. The use of animal data in regard to the human conditions of alcoholism seems encouraging in light of the fact that 1) drugs which are known to be reinforcing to animals have also proven to be reinforcing to man, and 2) the toxic effects of self-administered drugs in man are also found in animals (Schuster

& Johanson, 1974). Due to past availability, economics, and acceptance of the rat in the research environment, it would prove most beneficial if the rat could be used for the animal model.

Before acceptance as a model can be made, however, certain criteria must be considered. If a complete model for alcoholism were to be developed, then many of the restrictions reviewed by Lester and Freed (1973) would have to be met. This would include the totally voluntary initiation of consumption of alcohol ultimately leading to dependency. Inasmuch as there appears to be a marked inequality in the environment of a rat and man, no meaningful comparison could be gained by incorporating such a condition to this model. However, two conditions stated by Lester and Freed should be met. First, the rat must be brought to a state of physical dependency on alcohol. This method may be voluntary or involuntary in nature since only the dependency is important for research purposes. Second, the rat must show <u>voluntary</u> continuance of alcohol consumption after dependency is reached in order to avoid withdrawal effects.

To meet the first requirement, an appropriate method must be employed in order to induce the rat to consume large amounts of alcohol until dependency is reached. Several different techniques which have been employed to obtain this goal are shown on Table 1. All of these techniques have proved useful in creating tolerance as well as various degrees

Table 1

Techniques Employed in the Attempt to Establish Alcohol Dependency in the Rat

Researchers	Techniques
Lester & Greenberg (1952)	Sweetened ethanol solutions as only liquid
Mello & Mendelson (1965) Senter, Smith, & Lewin (1967) Keehn (1969)	Delivery of food contingent on alcohol consumption
Cicero, Myers, & Black (1968)	Avoidance of electric shock contingent on alcohol consumption
Myers & Veale (1969)	Direct injection of ethanol into the brain
Amit, Stern, & Wise (1970) Wayner, Greenberg, Carey, & Nolley (1971b)	Electric stimulation of the hypothalamic center to regulate drinking of ethanol solutions
Branchley, Raucher, & Kissen (1971) Freund (1969) Ogata, Ogata, Mendelson, & Mello (1972)	Ethanol diluted liquid diets as the only nutrient source
Falk, Sampson, & Winger (1972) Lester (1961)	Schedule induced polydipsia with ethanol solutions as only liquid
Carey (1972) Ratcliffe (1972)	Ethanol as only liquid
French & Morris (1972)	Ethanol vapor inhilation

of withdrawal symptoms to indicate various states of dependency. None, however, have been successful in fulfilling the second criteria which calls for self-administration of ethanol in a free-choice setting after dependency has been established. Of these techniques, those similar to the approach of Falk et al (1972) which have managed to maintain a high blood alcohol level throughout each 24-hr. cycle, produced the most measureable states of dependency. The state of dependency was usually so strongly established in these cases that the severity of withdrawal frequently resulted in tonic-clonic seizures and death through respiratory failure (Walker, Hunter, & Riley, 1975).

Summary Statement

The use of alcohol may prove rewarding in certain stressful conditions. With chronic use during the recurrence of such conditions, a physiological process develops at the cellular level resulting in a loss of the individual's control over drinking. This physiological process is characterized as a transition from alcohol tolerance to dependency. The behavioral, anatomical, and biochemical descriptions of tolerance and dependency are necessary for a complete understanding of the major role physical addiction plays in alcoholism. Following this physiological emphasis, the use of animal subjects becomes a viable alternative to humans in alcohol studies. Based on previous research with rats in this area, the potential exists for the establishment

of an animal model for the human alcoholic.

Statement of Problem

With careful consideration given to the above information, it was this author's intention to first establish a state of dependency based on the liquid diet procedure outlined by Walker et al (1975). This method seemed to serve as the most efficient and economical in comparison with those outlined previously since it provided for an uncomplicated route of ethanol administration as well as maintenance of an elevated blood alcohol concentration throughout an extended period of time. Following establishment of the aformentioned dependency, a modified Begleiter (1975) method consisting of a stepwise increase in the ethanol concentration of the daily alcohol-choice solution was employed to determine alcohol preference and consumption in a free-choice setting.

Experiment 1

Method

Subjects. An initial group of thirty-two 60-day-old Max hooded rats were paired by sex and weight. From this initial group, two new groups consisting of sixteen subjects each were formed by a random selection of eight males and eight females for placement in each group. Pairing by weight was necessary in the initial group in order to assure a balanced weight distribution within each final group. The animals to be used for the establishment of alcohol dependency were labeled the experimental group, while those animals serving as comparison subjects (no alcohol exposure) were labeled the control group.

Procedure. All subjects were individually housed in 20.32 cm. x 25.40 cm. x 20.32 cm. metallic cages in a room automatically regulated on a 12-hr. light-dark cycle. For the first five days, all subjects were placed on a deprivation diet in order to reduce them to approximately 80% of their free-feeding weight. They were then placed on a totally liquid diet. The liquid diet consisted of 50 ml. per day of a chocolate flavored Nutriment drink, fortified with .1 ml. of a Poly-Vy-Sac Vitamin solution. The commercially available Nutriment provided all the daily nutrition

and caloric requirements in combination with a flavor highly acceptable to the animals. No other liquid or solid food was available for consumption.

The control subjects received the liquid diet for the next twenty-eight days. For the experimental subjects, the liquid diet contained 8% ethanol on day 1 and was increased 2% every four days until 20% was reached on the 25th day and continued at 20% until the 28th day. All alcohol solutions were prepared from a 95% stock ethanol and distilled water solution. The presentation of 8% to 20% ethanol solutions represented an increase from 35% to 48% in total daily calories present in the form of ethanol calories.

Precise consumption volumes for all subjects were determined by pre- and post-weight comparisons of dietary delivery bottles at the beginning and end of every 24 hr. period. All weight determinations were made with the use of a Sartorius Model 2255 electronic balance made available by Preiser Scientific. At the end of the twenty-eight days, all subjects were placed on total deprivation for a six hour period and their resulting behavior recorded to determine the states of physical alcohol dependency.

Results

The control subjects maintained a daily pattern of consuming approximately 45 ml. of the Nutriment solution. All appeared to be in good health, remaining active and alert and spending much of their time in grooming activities. By the

end of the twenty-eight day period, the control subjects had moved to within 90% of their free-feeding weight.

During the first four days the experimental subjects were presented with an 8% ethanol solution. No noticeable differences in behavior were observed between the experimental and control subjects throughout this four day period. However, when the experimental subjects were subjected to a 10% ethanol solution during the second four day period, their behavior was characterized by hyperirritability, repeated cage chewing and pacing activities in contrast to the control subjects. The experimental subjects increased their number of consumption periods but consumed less volume during any one period of consumption.

During the third four day period, the introduction of a 12% ethanol solution precipitated a decline in activity in the experimental subjects. More time seemed to be spent in sleeping, and the animals engaged in fewer feeding periods although these periods were characterized by longer durations and tongue-chewing activities. When confronted with a 14% ethanol solution, the experimental animals manifested a noticeable change in grooming. More time was spent in the back of the cage with most rats preferring to sleep curled up and resting on their heads rather than on their sides as was characterized by the control animals.

Consumption of the 16% ethanol solution produced sluggish behavior during feeding but general activity seemed to

improve comparable to observed behavior at the 14% ethanol solution. General characteristics of behavior consisted of approaching the front of the cage and engaging in periodic cage chewing.

Although the experimental subjects seldom abstained from the available liquid solution, one female experimental subject when presented with the 16% ethanol solution refused to drink for several hours and exhibited signs of withdrawal. It became necessary to intubate approximately 7 grams of ethanol per kilogram of body weight (g./kg. ethanol) for four days until unaided drinking was continued at a rate comparable to the other experimental animals.

Concentrations of 18% and 20% ethanol produced little change in behavior, although the experimental animals appeared more passive in nature. The ability of the experimental subjects to consume the available solution was frequently hampered by a deterioration in motor coordination necessary for consumption. Most of their time was spent alternating between eating and sleeping.

Throughout the twenty-eight day period, average consumption for all experimental subjects was 14.59 g./kg. ethanol. This rate is comparable to that reported by Walker et al (1975), and is notable when compared to the daily ethanol metabolism rate of 7 g./kg. ethanol for the rat.

During the six hour withdrawal period following the 28th day, all experimental subjects showed extreme signs of hyper-

excitability with tremors and spastic movements. Cage chewing became very intense. When a slight puff of air was blown on a chewing rat, it demonstrated a squeeling and jumping behavior. At approximately four hours into withdrawal a key-rattling episode brought about a very violent period of jumping, rapidly followed by a period of tremors and seizures for 75% of the females and 38% of the males. Respiratory failure occured in two of the females. One was saved by means of a manual chest pumping technique but the other failed to regain breathing. After the fourth hour of withdrawal the behavior for all experimental subjects was characterized by tremors and convulsions. One male subject was observed engaging in a behavior characteristic of defending himself against attack. This behavior was characterized by rearing motions and attacking with his front paws. No comparable behavior was observed in the control animals. A key-rattling episode prior to Experiment 1 showed no unusual behavior in either the experimental or the control subjects. The limitations of such behavioral observations indicates the need for a simple, yet quantifiable, method for the determination of the degree of dependency in alcohol addiction.

Experiment 2

Method

Subjects. The fifteen surviving Max hooded rats from the experimental group in Experiment 1 and the sixteen Max hooded rats from the control group in Experiment 1 were used in Experiment 2 and each retained its group classification.

Procedure. The experimental and control subjects were placed in an alcohol preference situation. Two liquid delivery bottles were placed in each cage every twenty-four hours. One bottle contained 50 ml. of a Wyler's cherry drink solution while the second bottle contained 50 ml. of the same Wyler's cherry drink and increasing concentrations of ethanol. Purina Rat Chow was freely available at all times. Bottle placement was random for each subject on each day throughout the experiment.

Alcohol preference was measured for eight days on a presentation format similar to that described by Begleiter (1975). The alcohol-choice bottle contained 6% ethanol on day 1 and was increased 2% each day until 20% was reached on the 8th day. Consumption rates were measured daily by electronic balance as described in Experiment 1.

Results

Solid Food Consumption. Throughout the free-choice

period, it was observed that the experimental and control subjects consumed an average of 15 g. of Rat Chow per day. Comparable data (Barnett, 1975) indicates that this is approximately 50% of the rat's normal daily intake of solid food.

Ethanol Consumption. Table 2 contains the analyses of variance summary data for a Group x Sex x Day (2 x 2 x 8) mixed-design with repeated measures on consumption across days. Substitution of the mean score for all female experimental subjects was used for the missing scores of the deceased female experimental subject. Male and female consumption trends were not significantly different, F (1,28) < 1.0. However, ethanol consumption was significantly affected by the group membership of the subjects (experimental vs. control), F (1,28) = 11.88; $p < .01$. The alcohol presentation scheme employed across days used in the free-choice situation was a significant factor in the alcohol consumption rate for all subjects regardless of group membership, F (7,196) = 22.20; $p < .005$. However, the interaction effect of group membership across days showed a significant effect on alcohol consumption, F (7,196) = 3.08; $p < .01$. Figure 1 graphically represents the analyses of variance results. The mean trend for each classification is shown in comparison to the 7 g./kg. daily metabolism rate for ethanol in the rat. As shown on Figure 1, a pattern of divergence emerged between experimental and control subjects above the 16% ethanol

Table 2

Analyses of Variance:

Experiment 2

Source	SS	df	MS	F	ω^2
$Subjects_b$	3,733.80	31	120.45	1.30	
Group (G)	1,100.43	1	1,100.43	11.88*	.25
Sex (S)	14.32	1	14.32		
G x S	25.77	1	25.77		
$Error_b$	2,593.28	28	92.62		
$Subjects_w$	4,418.61	224	19.73	1.68	
Days (D)	1,819.82	7	259.97	22.20**	.41
D x G	252.16	7	36.02	3.08*	.06
D x S	25.94	7	3.71		
D x G x S	26.13	7	3.73		
$Error_w$	2,294.56	196	11.71		

*$p < .01$

**$p < .005$

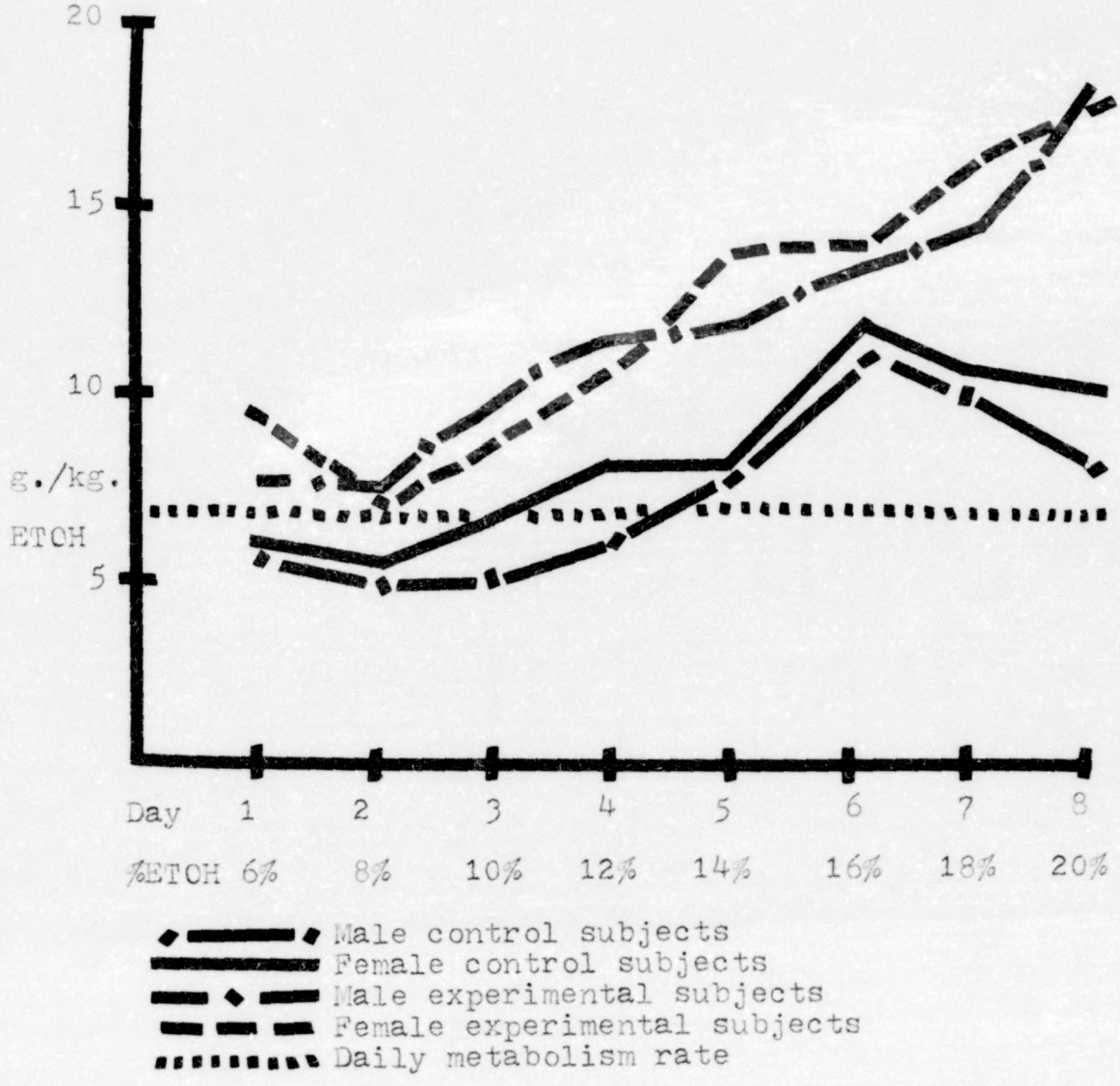

Figure 1

g./kg. Ethanol consumption for all subjects in free-choice

presentation. This trend was analyzed by means of a Newman-Keuls' post-hoc comparison for all individual points between subjects in each group for each day of alcohol presentation.

This analysis revealed a significant difference in alcohol consumption ($p < .01$) by experimental and control subjects during the presentation of 14%, 18%, and 20% ethanol solutions. The differences between the experimental and control subjects were characterized by a similar trend in alcohol consumption between the presentation of 6% and 12% ethanol solutions followed by a period of vascilation by the control subjects with the presentation of 14% and 16% ethanol solutions. With the presentation of 18% and 20% ethanol solutions, however, the experimental subjects actually increased their consumption of alcohol while the control subjects decreased their consumption rate to a range closer to the normal metabolization rate for ethanol in the rat.

A test of associative strength similar to that reported by Craig, Eison, and Metze (1976) was performed on the significant F values. The results indicated a high accountability for shared variance between day of alcohol presentation and alcohol preference, $\omega^2 = .41$, and between group membership and alcohol preference, $\omega^2 = .25$. The Group x Day interaction indicated a skewed variance accountability between the variables and alcohol preference, $\omega^2 = .06$.

Discussion

The establishment of a state of ethanol dependency based on the liquid diet procedure outlined by Walker et al (1975) satisfied the first basic criteria for an animal model for the study of alcoholism. The noted ability of the experimental subjects in Experiment 1 to successively accept the increasing ethanol concentrations while maintaining an average of twice the daily concentration of metabolizable ethanol more than indicates the probability of the transition from intolerance to tolerance for alcohol by means of cellular adaptation.

The behaviors described in Experiment 1 for the experimental subjects during the latter stages of acquisition also seem indicative of the trend toward alcohol tolerance. With the observed severity of convulsions, tremors, hypersensitivity, and seizures during the 6-hr. abstinence period, there seems to be no doubt that a high level of dependency had been established in the experimental subjects. This verification of tolerance and dependency is consistent with much of the reported research on alcohol dependency in the rat (Falk et al, 1972; French et al, 1972; Ratcliffe, 1972).

The modification of the Begleiter (1975) method for alcohol presentation showed that the amount of alcohol

consumption by the experimental subjects in a free-choice setting, after the establishment of alcohol addiction, was sufficient enough to avoid withdrawal symptoms. This withdrawal avoidance behavior satisfies the second criteria for an animal model for alcohol addiction and is consistent with the major restriction of voluntary continuance of alcohol consumption placed on such an animal model by Lester and Freed (1973).

In the free-choice situation, it was hoped that the presentation of the alcohol-choice liquid could be made in such a manner as to avoid any taste aversion while still allowing the subjects to associate withdrawal avoidance with alcohol-choice consumption. Two interesting observations were made as a result of the systematic elevation of the ethanol concentrations. First, the lower concentrations appeared to have minimal aversiveness for the control animals, as evidenced by their tendency to consume measureable quantities of alcohol similar to the experimental animals. This trend continued up to the presentation of a 14% solution. Second, only a small amount of solid food was consumed by all subjects (15 g./24 hrs.) during the free-choice setting even though the reported daily consumption concentration (33.5 g./24 hrs.) was readily available throughout Experiment 2 (Barnett, 1975).

As emphasis has been placed on the physiological factors for the establishment of an animal model for the general application to the human condition of alcohol addiction, it

has been summized by this investigator that an explanation for the observed behavior in alcohol and food consumption for both the experimental and control subjects during Experiment 2 could possibly rest in a state dependent learning situation that may have occurred during the presentation of the liquid diet in Experiment 1. Both the experimental and control subjects may have associated liquid consumption with proper satiation of the hypothalamic feeding centers.

Since the liquid diet in Experiment 1 contained all nutritional, caloric, and osmotic requirements, the receptor cells for thirst and hunger may have restricted the consumption of solid food. Following the description of state dependent learning by Rech and Moore (1971), the liquid diet served as one of the external cues while the hypothalamic satiation served as an internal cue as the subjects learned to drive reduce thirst and hunger. For the experimental subjects the presence of alcohol in the liquid diet would serve as an additional cue from the environmental set, as is reflected by the high trend of alcohol consumption in the free-choice situation for the experimental subjects when compared to the consumption of the control subjects.

The supposition that state dependent learning was responsible for the choice of a liquid for drive reduction of thirst and hunger by both experimental and control subjects increases in validity when viewed in comparison to other research reports on the rat's feeding behavior. Barnett (1975)

indicates that the selection of a food source is based on the rat's familiarity with the texture, odor, and flavor of the food item. However, given the choice between two choices of food, the rat will not be totally selective but will consume minimal quantities of the least familiar food source. Hausmann (1933) indicated a decrease in solid food consumption proportional to the caloric value of alcohol available to the rat.

When placed in the free-choice situation, the control subjects were inclined to consume a measureable quantity of ethanol as they had learned to drink for hunger as well as thirst drive reduction. The liquid available in the free-choice situation, however, did not provide all caloric requirements. The ethanol solution provided this caloric requirement when consumed in conjunction with the non-ethanol solution. The alcohol consumption trend is shown in Figure 1 for both control and experimental subjects. No significant difference between controls and experimentals were noted between 6% and 12%.

With the presentation of the 14% solution, a significant difference developed between control and experimental subjects' consumption of alcohol, indicating a possible difference between the groups in cellular adaptation for alcohol metabolism. Even though the control subjects were consuming relatively low amounts of alcohol at the 14% level, they had to experience some withdrawal reactions as their blood alcohol

concentration dropped during daily metabolism. It is summized that at 16% the withdrawal reactions greatly increased in degree of discomfort and the control subjects, who were as yet below the addiction level, withdrew from major consumption of alcohol. The experimental subjects, however, were operating under a greater degree of tolerance and dependency, and were as such, no longer "in control" of their alcohol consumption since they were maintaining a need to avoid withdrawal.

The choice made by the control subjects to decrease alcohol consumption at the 16% concentration in comparison to the experimental subjects' choice to increase alcohol consumption may indicate a possible area of shifting from a relatively low dependency state to a severe state representative of the move towards addiction. This critical point in the free-choice situation may explain the difficulty encountered by researchers interested in establishing alcohol addiction in the rat (Begleiter, 1975; Carey, 1972; Freund, 1969; Walker, 1975).

The verification of these summations may rest in the replication of these two experiments with more emphasis placed on behavior observations and identification of withdrawal reactions at all levels of alcohol exposure. Comparison of hypothalamic stimulation surveys with the liquid diet-polydipsia techniques may prove useful in quantifying the possibility of state dependent learning effects.

Overall, there seems to be two major issues involved in controlling the development of alcohol addiction. The influence of state dependent learning within specific environments seems to direct those specific consumption activities which, in turn, bring about the physiological adaptations which regulate addictive behavior. If this is true, the implications for alcoholic treatments may be the restriction to a specific sequence of actions. Regardless of the environmental, social, or psychological influences directing the state dependent learning situation, the physiological adaptations must be returned to normal by abstinence after controlled withdrawal. Then removal from those influences responsible for the first learning situation must be completed and substitution of new influences must be made.

The animal model would prove most beneficial in this regard by first allowing surgical and pharmacological experimentation on the alcoholic nervous system to investigate possible avenues for reversing the cellular adaptations involved in tolerance and dependency formations. From the social learning prospective involved in the etiology and maintenance of alcohol addiction, the animal model in the physiological addictive state may be used in various behavioral modification paradigms in an effort to isolate and to identify the key elements in the learning process.

References

Amit, Z., Stern, M. H., & Wise, R. A. Alcohol preference in the laboratory rat induced by hypothalamic stimulation. Psychopharmacologia, 1970, 17, 367-377.

Armstrong, C. M., & Binstock, L. The effects of several alcohols on the properties of the squid giant axon. Journal of General Physiology, 1964, 48, 265.

Axelrod, J. The addictive states. A. Wikler (Ed.), 46, 247-264, The Williams & Wilkins Co., Baltimore, 1968.

Barnett, S. A. The rat; a study in behavior, chapter 4, University of Chicago, Chicago, 1975.

Begleiter, H. Ethanol consumption subsequent to physical dependence. Alcohol intoxication and withdrawal; advances in experimental medicine and biology. M. M. Gross (Ed.), 59, 373-378, Plenum Press, N. Y., 1975.

Branchley, M., Rauscher, G., & Kissin, B. Modifications in the response to alcohol following establishment of physical dependence. Psychopharmacologia, 1971, 22, 314-322.

Cannon, W. B., & Rosenblueth, A. The supersensitivity of denervated structures, a law of denervation. The MacMillan Co., N. Y., 1949.

Carey, R. J. A decrease in ethanol preference in rats resulting from forced ethanol drinking under fluid deprevation. Physiology and Behavior, 1972, 8, 373-375.

Cicero, T. J., Myers, R. D., & Black, W. C. Increase in volitional ethanol consumption following interference with a learned avoidance response. Physiology and Behavior, 1968, 3, 657-660.

Craig, J. R., Eison, C. L., & Metze, L. P. Significance tests and their interpretation; an example utilizing published research and omega2. Bulletin of the Psychonomic Society, 1976, 7(3), 280-282.

Crone, C. The permeability of brain capillaries to non-electroytes. Acta Physiologica Scandinavia, 1965, 64, 407.

Davson, H., & Danielli, J. F. The permeability of natural

membranes, 2nd. ed., 80-94, University Press, Cambridge, 1952.

Falk, J., Sampson, H. H., & Winger, G. Behavioral maintenance of high concentrations of blood ethanol and physical dependence in the rat. Science, 1972, 177, 811-813.

Fraser, H. F., Wikler, A., Isbell, H., & Johnson, H. K. Partial equivalence of chronic alcohol and barbiturate intoxications. Quarterly Journal of Studies on Alcohol, 1957, 18, 541.

French, S. W., & Morris, J. R. Ethanol dependence in the rat induced by non-intoxicating levels of ethanol. Research Communications in Chemical Pathology and Pharmacology, 1972, 4, 221-233.

Freund, G. Alcohol withdrawal syndrome in mice. Archives of Neurology, 1969, 21, 315-320.

Goldstein, D. B. An animal model for testing effects of drugs on alcohol withdrawal reactions. Journal of Pharmacology and Experimental Therapeutics, 1972, 183, 14-22.

Goldstein, D. B., & Goldstein, A. Enzyme expansion theory of drug tolerance and physical dependence. The addictive states, A. Wikler (Ed.), 44, 265-267, The Williams & Wilkins Co., Baltimore, 1968.

Harger, R. N., Hulpieu, H. R., & Lamb, E. B. The speed with which various parts of the body reach equilibrium in the storage of alcohol. Journal of Biological Chemistry, 1937, 120, 689.

Hausmann, M. F. The behavior of albino rats in choosing foods. Journal of Comparative Psychology, 1933, 15, 419-428.

Himwich, H. E., Diperri, R., David, A., & Schweigerdt, A. Comparative susceptibility to alcohol of the cortical area and midbrane reticular formation of the cat. Psychosomatic Medicine, 1966, 28, 458.

Inoue, F., & Frank, G. B. Effects of ethyl alcohol on excitability and on neuromuscular transmission in frog skeletal muscle. British Journal of Pharmacology, 1967, 30, 186.

Israel, Y. Cellular effects of alcohol; a review. Quarterly Journal of Studies on Alcohol, 1970, 31, 293-316.

Israel, Y., & Kalant, H. Effect of ethanol on eletrolyte transport and electrogenesis in animal tissues. Journal of Cellular and Comparative Physiology, 1965, 65, 129.

Israel, Y., & Salazar, I. Inhibition of brain microsomal adenosine triphosphatases by general depressants. Archives of Biochemistry and Biophysics, 1967, 122, 310.

Israel, Y., Kalant, H., & LauFer, I. Effects of ethanol on sodium, potassium, and magnesium stimulated microsomal ATPase activity. Biochemical Pharmacology, 1965, 14, 1803.

Israel, Y., Kalant, H., & LeBlanc, A. E. Effects of lower alcohols on potassium transport and microsomal ATPase activity of rat cerebral cortex. Biochemical Journal, 1966, 100, 27.

Jarnefelt, J. A possible mechanism of action of ethyl alcohol on the central nervous system. Annual of Medicinae Experimentalis et Biologiae Fenniae, 1960, 39, 267.

Kalant, H. The pharmacology of alcohol intoxication. Quarterly Journal of Studies on Alcohol, 1961, Suppl. No. 1, 1-23.

Kalant, H. Effects of ethanol on the nervous system. International encyclopedia of pharmacology and therapeutics, Section 20, 189-236, Oxford, Pergamon, 1970.

Keehn, J. D. "Voluntary" consumption of alcohol in rats. Quarterly Journal of Studies on Alcohol, 1969, 30, 320-329.

Kety, S. S. The theory and applications of the exchange of inert gas at the lungs and tissues. Pharmacological Reviews, 1951, 3, 1.

Lehninger, A. L. Biochemistry, the molecular basis of cell structure and functions. Worth Publishers, Inc., John Hopkins University, 1970.

Lester, D. Self-maintenance of intoxification in the rat. Quarterly Journal of Studies on Alcohol, 1961, 22, 223-231.

Lester, D., & Freed, E. X. Criteria for an animal model of alcoholism. Pharmacology and Biochemistry of Behavior, 1973, 1, 103-107.

Lester, D., & Greenberg, L. A. Nutrition and the etiology of alcoholism. Quarterly Journal of Studies on Alcohol,

1952, 13, 553-560.

Ludwig, A. M. Alcohol craving. Quarterly Journal of Studies on Alcohol, 1974 (Sept.), 35(3A), 899-905.

Mello, N. K., & Mendelson, J. Operant drinking of alcohol on a rate contigent ratio schedule of reinforcement. Journal of Psychiatric Research, 1965, 3, 145-152.

Mello, N. K., & Mendelson, J. H. Experimentally induced intoxication in alcoholics: a comparison between programmed and spontaneous drinking. Journal of Pharmacology and Experimental Therapeutics, 1969, 173, 101.

Mendelson, J. H. Experimentally induced chronic intoxication and withdrawal in alcoholics. Quarterly Journal of Studies on Alcohol, 1964, Suppl. No. 2, 235-239.

Mendelson, J. H. Biologic concomitants of alcoholism. New England Journal of Medicine, 1970, 283, 24-32.

Moore, J. W. Effects of ethanol on ionic conductances in the squid axon membrane. Psychosomatic Medicine, 1966, 28, 450.

Myers, R. D., & Veale, W. L. Alterations in volitional alcohol intake produced in rats by chronic intraventricular infusion of acetaldehyde, paraldehyde, or methanol. Archives of Internationales de Pharmacodynamie et de Therapie, 1969, 180, 100.

Ogata, H., Ogata, R., Mendelson, J. H., & Mello, N. K. A comparison of techniques to induce alcohol dependence in mouse. Journal of Pharmacology and Experimental Therapy, 1972, 8, 373-375.

Pappenheimer, J. R., & Heissey, S. R. Exchange of material between CSF and blood. Proceeds of the First International Pharmacology Meeting, 1963, 4, 95.

Price, H. L. Uptake and distribution of anesthetic agents. 123, McGraw-Hill, N. Y., 1963.

Ratcliffe, F. Ethanol dependence in the rat: its production and characteristics. Archives of Internationales de Pharmacodynamie et de Therapie, 1972, 196, 146-156.

Rech, R. H., & Moore, K. E. An introduction to psychopharmacology, chapter 6, Raven Press, N. Y., 1971.

Schuster, C. R., & Johanson, C. E. The use of animal models for the study of drug abuse. Research advances in alcohol and drug problems, R. J. Higgins (Ed.), 1, 3-33, John Wiley & Sons, N. Y., 1974.

Seeman, P. Erythrocyte membrane stabilization by steroids and alcohols: a possible model for anesthesia. Biochemical Pharmacology, 1966a, 15, 1632.

Seeman, P., & Weinstein, J. Erythrocyte membrane stabilization by tranquilizers and antihistamines. Biochemical Pharmacology, 1966, 15, 1737.

Senter, R. J., Smith, F. W., & Lewin, S. Ethanol ingestion as an operant response. Psychonomic Science, 1967, 8, 291-292.

Shearer, R. J.(Ed.), Manual on alcolholism of the Aerican Medical Association, 1967.

Walker, D. W., Hunter, B. E., & Riley, J. A behavioral electrophysiological analysis of ethanol dependence in the rat. Alcohol intoxication and withdrawal; advances in experimental medicine and biology, M. M. Gross (Ed.), 59, Plenum Press, N. Y., 1975.

Wallgren, H. Absorption, diffusion, distribution and elimination of ethanol. Effect on biological membranes. International encyclopedia of pharmacology and therapeutics. Alcohols and derivatives, J. Tremolieres (Ed.), 1, 161-188, Pergamon Press, 1970.

Wallgren, H., & Barry, H. Actions of alcohol, chapter 9, Elsevier, Amsterdam, 1970.

Wayner, M. J., Greenberg, I., Carey, R. J., & Nolley, D. Ethanol drinking elicited during electrical stimulation of the lateral hypothalamus. Physiological and Behavior, 1971(b), 7, 793-795.

Whittaker, V. P. Structure and function of animal cell membranes. British Medical Bulletin, 1968,24, 101.

Woodbury, J. W. Action potentials: properties of excitable membranes. Neurophysiology, T. C. Rush (Ed.), 41, W. B. Saunders Co., Philadelphia, 1965.