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The Effect of Genetic and Environmental Stress Factors on Alcohol Consumption in Rats

Sharon E. Pryor

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The Effect of Genetic and Environmental Stress Factors

on Alcohol Consumption in Rats

(TITLE)

BY

Sharon E. Pryor

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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The Effect of Genetic and Environmental Stress Factors on Alcohol Consumption in Rats

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Psychology Department

Eastern Illinois University
Dedication

To the "Little Ones", the laboratory rats who served as subjects in this study. This thesis is their memorial.
Acknowledgments

Credit is due the many individuals who have helped me with this thesis. I wish to thank Dr. Eleanor Midkiff, my thesis advisor, for her guidance and patience; Dr. William Kirk and Dr. Russell Gruber, members of my thesis committee, for their encouragement; Dr. John Rearden for helping me with the data analysis; Dr. Ed Sanders, for his advice and support; Mickey Carroll, for her help in printing; Judy Craig, for the art work; Chuck Bowles, for his assistance in the rat lab; and my husband, Jessy Pryor for his help and encouragement.
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The etiology of alcoholism continues to be investigated. Heredity and environment have both been implicated as causes; however, the reason some people drink more than others has not been determined by science. Twin and adoption studies in humans yield evidence for genetic factors in alcoholism. Studies of possible markers in humans and studies of laboratory animal models have produced further evidence for the genetic transmission of alcohol preference. This animal study was done to further investigate the effects of heredity and environment on preference for alcohol in laboratory rats. Two genetic strains of rats, Wistar and Fischer-344, (n=39) were given a choice of a sweetened water solution or a sweetened, 10% alcohol solution. After a two week baseline period the rats were randomly assigned to an experimental group and a control group. The experimental animals were subjected to stress by enclosure for one hour each day for a two week period. During the third phase of the study the animals were not stressed. Food, sweetened water, and alcohol were consumed ad lib throughout the six week study. The amount of each solution consumed was measured daily. The alcohol intake of the animals varied over the three phases of the study, however, there was a significant difference between strains in the consumption of alcohol over the entire study. The response to stress was inconclusive.
The Effect of Genetic and Environmental Stress Factors on Alcohol Consumption in Rats

Chapter I

Statement of the Problem

The cause of alcoholism is unknown. Why some people are more susceptible to alcoholism than others is also unknown (Li and Lockmuller, 1989). Alcoholism is considered by some to be genetic and by some to be caused by environmental factors. Still others believe the cause to be a combination of genetics and environment. Many different scientific disciplines are working to find the cause of this disease. Progress has been made but the investigation is far from complete (Goodwin, 1985, Li and Lockmuller, 1989).

Many scientific theorists have attempted to explain alcoholism. Psychodynamic theorists view the alcoholic as an oral-dependent person who uses alcohol as a means of coping with conflicts and anxieties (Price, 1972). According to Miller and Chappel (1991) no evidence shows that psychodynamic factors explain the origin of alcoholism.

Behavior theorists believe that alcoholism is learned through reinforcement. The narcotic value of alcohol provides primary reinforcement while companionship, group acceptance, and interpersonal warmth in drinking situations provide secondary reinforcement (Price, 1972). Lewis (1990) examined reinforcement mechanisms in alcohol abuse and alcoholism. The euphoric and anxiolytic effects of alcohol
were found to be the basis of positive reinforcement and aversive consequences the basis of negative reinforcement.

Several other theories of alcoholism can be found in the literature. Humanists regard alcoholism as a form of escape from the confrontation of self and the existential anxiety of life (Price, 1972). Cognitive social learning theory has established the effectiveness of social models in increasing the likelihood of a behavior. This study of the genetic and environmental factors that influence the development of alcoholism is an attempt add to the understanding of this problem.

Alcoholism is a serious problem that affects about 10 percent of the adult population of the United States (U.S. Department of Health and Human Services, 1990). A study using data from a survey of drinking practices and problems in the United States in 1967, 1969, and 1984 showed relative increases in the prevalence of alcoholism from 53% to 200% during this time span (Hasin, Grant, Harford, Hilton, & Endicott, 1990). Since then there has been a gradual, consistent decline in per capita consumption of alcoholic beverages in the United States. The per capita consumption of alcohol in 1987 was estimated at 2.54 gallons of pure alcohol. Alcohol is still used by more people than any other drug including tobacco (USDHHS, 1990). A study by Rearden and Markwell (1989) found that 31% of lower division college students at a midwestern university were heavy drinkers. An increasing proportion of heavy drinkers are in
their twenties. The increase in the incidence of heavy drinking in these age groups makes the question of the causes of alcoholism an important area of research (USDHHS, 1990).
Chapter II
Review of Related Research
Familial Tendency

Since ancient times the belief that alcoholism runs in families has been a part of our folk wisdom (Goodwin, 1985). Recent scientific studies have also implied a familial tendency in the development of alcoholism. Dragun (1990) found that a family history of alcoholism was an important factor in the development of a rapid course of early alcoholism. A study of young men in Prague showed that early and serious alcohol abuse was more strongly related to parental alcoholism than was alcohol abuse in later life (Kubicka, Kozeny, & Roth, 1990). Pandina & Johnson's (1990) study of 1,380 New Jersey youth found that rates of serious alcohol problems were about twice as great for individuals coming from a family background of alcoholism as for those from nonalcoholic families.

The familial nature of alcoholism is not conclusive proof that it is totally inherited (Anthenelli & Schuckit, 1990). It sounds reasonable that environmental factors also play a part, but sounding reasonable does not make it true. If a tendency to become alcoholic is inherited there must be some basic biochemical difference between those who are prone to be alcoholic and those who are not (Kenney & Leaton, 1987); however, a specific gene and mechanism of transmission of alcoholism have yet to be identified (USDHHS, 1990).
The familial tendency for alcoholism may be genetically transmitted or the result of the shared social environment of the alcoholic family. Since most children are reared by their biological parents, scientists have had the task of separating nature and nurture. Researchers have taken several different approaches in assessing the role of genetics in alcoholism. Human twin and adoption studies are two classical methods of minimizing the environmental variables (Kenney & Leaton 1987, USDHHS, 1990).

Twin Studies

Identical (monozygotic) twins share 100% of the same genetic material; fraternal (dizygotic) twins share only 50% of their genes. Therefore, identical twins should have a higher level of concordance for this disorder than fraternal twins if genetic factors alone explain the tendency toward alcoholism. If the risk is determined only by childhood environmental factors, identical and fraternal twins reared by their biological parents should be equally concordant in their alcoholism (Anthenelli & Schuckit, 1990, Kenney & Leaton, 1987). Schuckit (1981) found that the majority of twin studies show a significantly higher concordance for alcoholism in identical twins of alcoholics than that found in fraternal twins.

Many twin studies have been done in Scandinavia because these countries have complete records of family and medical data (Kenney & Leaton, 1987). A Swedish investigator, Kaij (1960), in one of the earliest twin studies found that the
more severe the alcoholism the greater the concordance of alcoholism in twin pairs. A study of Australian twin pairs estimated the heritability of frequent drinking of alcohol to be 66% in women and 42-75% in men. The heritability estimates for quantity of drinking was 57% in women and 24-61% in men (Heath, Meyer, Jardine, & Martin, 1991). These studies suggest that a tendency to be alcoholic is genetically transmitted but environmental factors cannot be ruled out. Rose, Kaprio, Williams, Viken, and Obremski, (1990) concluded that "all investigators should stop pitting genes against experience and call off the nature-nurture warfare". Their twin studies did, however, provide more evidence to link alcohol consumption to heredity.

Adoption Studies

Researchers have attempted to separate the role of environment from genetic transmission of alcoholism by studying children who were adopted out of the families of known alcoholics at an early age. Goodwin, Schulsinger, Hermansen, Guze, and Winokur (1973) studied Danish children who were separated before the age of six weeks from their biological parents and reared by nonrelatives. They found that the natural children of alcoholic parents were more likely to have alcohol problems than the children of nonalcoholics, even though they had no contact with their natural parents after separation. In a later study these researchers compared sons, adopted in infancy, from homes of alcoholic parents with their brothers who were reared by
their natural parents to determine if length of exposure to an alcoholic parent was associated with the development of alcoholism in the children. Their main finding was that living with an alcoholic parent appeared to have no relationship to the development of alcoholism. The conclusions of this study contradict the assertion that alcoholism is caused by the interaction of genetic and environmental factors. They found that severe or classic forms of alcoholism may have a genetic basis uninfluenced by environment (Goodwin, Schulsinger, Moller, Hermansen, Winokur, & Guze, 1974).

Alcoholism is not a single disease entity. In 1960 Jellinek identified two different subgroups of alcoholics. He found one group who had an "inability to abstain entirely" from alcohol and a group who exhibited a "loss of control." The individuals in the latter group could abstain for long periods of time but could not stop once they had started on a drinking binge (Cloninger, 1987). Goodwin et al. (1974) also identified two separate forms of alcoholism, one familial and one non-familial. A slightly different typology was found by Bohman and Cloninger in their Stockholm adoption studies. These studies identified two types of alcoholic susceptibility that correspond to Jellinek's two subgroups of alcoholism: type 1 or milieu-limited and type 2 or male-limited (Cloninger, 1987, & USDHHS, 1990). Type 1, "loss of control" affects both men and women and is found only in certain environments.
Genetic predisposition plus environmental provocation are necessary to increase susceptibility to this type of alcoholism. Type 2, "inability to abstain" is highly heritable from father to son regardless of environment (Sigvardsson, von Knorring, Bohman, & Cloninger, 1984, Cloninger, 1987).

Daughters of Alcoholics

Studies of sons of alcoholics have indicated that they are four times more likely to be alcoholic than the sons of nonalcoholics, but what of the daughters? Less evidence for the risk of alcoholism in women is available because men have constituted the majority of the subject samples in studies of alcoholism (Radomsky, 1992). In phase III of the Stockholm Adoption Study the investigators found that the sons of type 2 alcoholics are prone to alcoholism and petty criminality, but the daughters do not tend to be alcoholic. The daughters are predisposed to a type of hypochondriasis known as diversiform somatization (Bohman, Cloninger, von Knorring, & Sigvardsson, 1984). Cloninger found that "women develop loss of control (type 1) alcoholism predominantly." This is characterized by later onset and rapid progression of complications associated with guilt and depression (Cloninger, 1987). In another study of adopted-out daughters of alcoholics 49 daughters of alcoholics were compared to 47 daughters of nonalcoholics. The numbers who were alcoholic in both groups were above the expected rate of alcoholism among women but the study was considered to be
inconclusive. These daughters of alcoholics had no more depression than the controls (Goodwin, Schulsinger, Knop, Mednick, & Guze, 1977). Radomsky (1992) studied daughters of parents who were alcohol dependent and found that they had more chronic illness then those with nonalcoholic parents. Depression represented 26% of the chronic illness reported by these women; however, they had minimal problems with alcohol use. To control for families where alcoholism does not exist but where the psychosocial milieu is disturbed, Radmowsky studied daughters of families who were harsh, rigid, or difficult. These women also had increased reports of chronic illness, including depression, but 7% less than the daughters of alcoholics, and no significant difference in alcohol use. These findings support the study by Fulton and Yates (1990) who found no significant differences in the number of drinking problems between the children of alcoholics and nonalcoholics and limited support for the concept that they are impaired in personality, life adjustment, or have more psychopathology. At this time there is evidence that the tendency to be alcoholic is genetically transmitted from fathers to sons of type 2 alcoholics but genetic transmission of alcoholic tendency to daughters is less certain. While the daughters escape becoming alcoholic they tend to manifest other psychopathology.
Markers

Human studies in the laboratory also indicate a biological etiology for alcohol dependence (Hwu, Yeh & Yeh, 1990). Potential markers of susceptibility to alcoholism are being investigated by alcohol researchers. Two types of markers are being sought: those that predispose or are directly involved in the development of alcoholism and factors that are correlated with these predisposing factors. These markers may be behavioral, physiological, or biochemical. It is necessary to determine if the markers are those of true predisposition (trait markers) or the result of prolonged alcohol use (state markers).

An electrophysiological marker that has been identified is the P3 wave of the electroencephalographic tracing (EEG). EEG tracings of identical twins are more alike than those of fraternal twins. This suggests a genetic basis for the P3 response. Reduced amplitude of P3 waves have been found in young non-drinking sons of alcoholics. Alcoholic fathers and sons were found to have excessive Beta wave activity compared to controls (USDHHS, 1990).

Levels of cortisol and prolactin have been selected as possible endocrinological markers (USDHHS, 1990). A decreased cortisol response to alcohol has been found in sons of alcoholics by Shuckit (1984). They also found that after a similar rise, alcoholics had a more rapid drop in prolactin levels; however, these results were not confirmed in a 1989 study by Moss, Yao, & Maddock.
Monoamine oxidase (MAO) has been investigated as a possible biochemical marker. Statistically significant decreases in platelet MAO levels compared to controls have been found in type 2 alcoholics. It is not clear, however, whether this is a state or a trait marker (USDHHS, 1990). A study by Pehl & Peterson (1990) found sons of male alcoholics to have lower platelet (MAO) activity than controls and that his finding correlated with the frequency of family history of alcoholism. Another possible biochemical marker is the platelet enzyme adenylate cyclase (AC). The low responsiveness of platelet AC to stimulation in alcoholics may be a genetically influenced marker.

The human leukocyte antigen CW3 is frequently increased in alcoholics in comparison to controls. Alcoholics have also been found to have an increased frequency of particular blood group factors. These may prove to be serological markers of susceptibility to alcoholism (USDHHS, 1990).

Protective Factors

Another physiological difference in humans is an inborn protective factor against alcoholism. This is a dysphoric or flush reaction to alcohol ingestion exhibited by many Asians. This biochemical difference in the effect of alcohol tends to discourage alcohol consumption and may account for a lower incidence of alcoholism in affected individuals. The phenomenon is caused by a difference in alcohol metabolism. When alcohol is metabolized in the liver it is converted to acetaldehyde by the enzyme alcohol
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dehydrogenase. Acetaldehyde is oxidized to produce acetate. The body then converts acetate to carbon dioxide and water. A catalyst in the oxidation of acetaldehyde is an enzyme known as aldehyde dehydrogenase (ALDH). A form of ALDH, the isoenzyme ALDH2, is involved in most of this oxidation. An essentially inactive form of ALDH2 has been identified and correlated with the flush reaction observed in many Asians after they ingest alcohol (USDHHS, 1990). This aversive effect, caused by high levels of acetaldehyde discourages alcohol consumption (Kenny & Leaton, 1987). A molecular and genetic basis for ALDH2 deficiency has been scientifically determined (Li & Lockmuller, 1989).

Sex-linked Transmission

The sex-linked transmission of alcoholism has not been definitely established. In their study of alcohol abuse among grandsons of alcoholics Harford & Grant (1990) seemed to find evidence of sex-linked transmission of alcohol abuse. A higher level of alcohol abuse was found among maternal compared with paternal grandsons. However, a study by Tambs & Vaglum (1990) did not support the sex-linked transmission of alcoholism. Thus, the question of possible sex-linked transmission remains undecided.

High-risk Subjects

Subjective responses to alcohol in high-risk subjects are consistent with a genetic mechanism. Moss, Yao, & Maddock (1989) studied sons of alcoholic fathers and found that they exhibited different mood patterns from sons of
nonalcoholics in a drinking situation independent of the amount of alcohol they ingested. This lesser response to a single dose of alcohol is experienced as not feeling as high and having greater muscle relaxation after one drink than those without a family history of alcoholism (Kenny & Leaton, 1987). The difference was observed even when the high-risk subjects were served a placebo indicating that expectancy may play a part in this response as well as direct pharmacologic effects (USDHHS, 1990).

Just what is inherited by those at high risk for alcoholism is unknown. Instead of inheriting a deficiency they may possess a strength, the ability to hold their liquor too well (Kenney & Leaton, 1987).

Other researchers do not agree that alcoholism is a heritable disease. Orford & Velleman (1990) found only a modestly increased risk of drinking problems in young men from homes with parental drinking problems. Other studies (Harburg, DiFransceisco, Webster, Gleiberman, & Schork, 1990; Peele, 1990) have also cast doubt on the genetic origin of alcoholism.

Environmental Factors

Barnes and Welte (1990) concluded that family influences may have long-term consequences on drinking behavior. In their study parental drinking was found to be important for predicting heavy drinking in both males and females. In addition to parental role models, television may influence beliefs and behaviors through observational
Stress and Alcohol in Rats

learning. Beliefs concerning drinking acquired in childhood may influence drinking patterns at a later age when there are no legal or social restrictions on drinking (Wallack, Grube, & Madden, 1990). Lee, DeFrank, & Rose (1990) studied the associations between anomie, alienation, and alcohol use. Their 36-month study showed that alienation was not predictive of change in either alcohol abuse or consumption. In another study the belief that cognitive and motor skills are improved by alcohol was found to begin in adolescence and remain high in problem drinkers while decreasing in the general adolescent sample. This belief was subsequently discovered in 305 hospitalized alcoholics, suggesting that this expectancy may have prognostic and etiologic significance (Christiansen & Goldman, 1985). The influence of drinking companions in bars has also been investigated. A study of group participation on drinking behavior in public bars failed to replicate earlier studies in Canada, New Zealand, and Britain. The hypotheses that drinking in public bars occurs primarily in groups and that persons drinking in groups drink to keep up with the group were not supported (Sykes, Rowley, & Schaefer, 1990).

Stress Factors

Human studies have also demonstrated an increase in drinking due to environmental stress (Cole, Tucker, & Friedman, 1990; Higgins & Marlatt, 1975; Tucker, Vuchinich, Sobell, & Maisto, 1980). A survey of health care employees found that stressful life events and employment insecurity
predicted a greater use of alcohol. However, workplace stressors did not contribute to the use of alcohol (Steffy & Laker, 1991). Watts and coworkers studied drinking and drug use by higher education faculty and staff and found work related stress and alcohol use to be indirectly related (Watts, Cox, Wright, Garrison, Herkimer, & Howze, 1991).

Other studies do not support the hypothesis that drinking is caused by stress. A study by Kahn & Cooper (1990) found that the dealers in financial markets in the City of London had a high intake of alcohol, but they associated it with the unique social structure of this environment.

Animal Studies

Twin and adoption studies provide evidence for a genetic predisposition to alcoholism in humans. The selective breeding of strains of alcohol preferring and nonpreferring rodents is also indicative of a genetic basis for the preference or avoidance of alcohol (Taylor, Harris, & Vogel, 1990). Models of alcoholism have been developed in laboratory animals. McClearn and Rodgers (1959) demonstrated that inbred strains of mice differed in their preference for alcohol. Crabbe, Merrill, Kim, & Belknap (1990) also noted a variation in alcohol preference among different genetic strains in mice. These differences provide evidence for a genetic influence for the trait of alcohol preference.
Spuhler & Detrich (1984) found that rat strains clearly differed in their response to alcohol. Rat lines have been selectively bred for difference in alcohol preference. In Chile, Mardons and coworkers developed the low alcohol preference UChA line and the high alcohol preference UChB line. An AA (high preference) and an ANC (low preference) line were developed in Finland. In the United States, high preference (P) and low alcohol preferring rats (NP) were developed from Wistar rats. After more than 20 generations of selective breeding, the P and NP lines showed a difference of more than six fold in their voluntary consumption of alcohol. In developing this animal model it was noted that the P rats consumed most of their alcohol and food intake during their dark cycle. The alcohol consumption occurred at irregularly spaced bursts of drinking. In this study both P and NP rats developed a preference for 2 and 5% alcohol solutions, but solutions of 10% and higher concentrations were aversive to the NP rats. The P rats obtained pharmacologically active blood alcohol concentrations as measured by sampling blood at regular intervals. These rats also developed a tolerance for alcohol as evidenced by a jumping test. Physical dependence was demonstrated by signs of withdrawal when ethanol was withheld after 20 weeks of self-administration. Thus the P line fulfilled all the criteria for an animal model of alcoholism (Li, Lumeng, McBride, & Murphy, 1987).
In studies of the neurochemical differences between P and NP rats the principal findings were a lowered content of Serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) in the thalamus and hypothalamus and a lowered content of Dopamine and Norepinephrine in the thalamus of the alcohol preferring animals. These differences were noted before the animals ingested alcohol. Support of the relationship between 5-HT and drinking behavior has come from neuropharmacological studies. In these studies Serotonin and Norepinephrine reuptake inhibitors were effective in reducing self-administration of alcohol by rats (Li, Lumeng, McBride, & Murphy, 1987).

Li and Lumeng's 1984 study of alcohol preference and voluntary alcohol intake of eight inbred rat strains and studies of the National Institutes of Health heterogeneous stock of rats support the conclusion that alcohol preference is significantly influenced by genetic factors. They found that within-strain variation was strain-dependent indicating that some strains are influenced more strongly by environmental factors than others. This study, however, did not seek to discover what environmental factors may have an influence on alcohol consumption in rats.

Stress

Taylor, Harris, & Vogel, (1990) found a definitive correlation among stress, anxiety, and alcohol consumption in laboratory rats. In a study of the effects of shock on alcohol preference in rats the investigators concluded that
alcohol was not consumed to alleviate stress through its pharmacological properties (Volpicelli, Ulm & Hopson, 1990).

Krishnan, Nash, and Maickel (1991) studied the relationship between stress and alcohol consumption. The role of stress as a causal factor in the development of alcoholism had been suggested by Pohorecky (1987) but the precise relationship was not defined. Nash and Maickel (1985) observed an increase in ethanol consumption during the post-stress period of their animal study. This finding contradicted the "tension reduction hypothesis" which suggests that animals drink alcohol during periods of stress to reduce tension.

Previous studies of the stress-induced biological changes that influence alcohol consumption had suggested involvement of the hypothalamus-pituitary-adrenalcortical axis (HPA). Both stress and alcohol activate the HPA resulting in increased release of adrenocorticotropic hormone (ACTH). Nash and Maickel (1988) administered repeated doses of ACTH\textsubscript{1-39} which produced the same effect as repeated stress. This effect was a post-treatment increase in alcohol consumption. Further testing suggested that the activity was located in the ACTH\textsubscript{4-10} fragment of the ACTH\textsubscript{1-39} molecule.

In 1991 Krishnan, Nash, & Maickel attempted to further describe the effect of ACTH. They administered ACTH\textsubscript{11-24} to adult Sprague-Dawley rats on a random schedule for eight days. This compound had no effect on the rats' free-choice drinking pattern.
alcohol consumption. In a second study they tested the activity of the ACTH$_{4-10}$ fragment. This resulted in suppression of alcohol consumption during the 8 day treatment period. In a third study the rats were stressed by immobilization for three periods consisting of four days of stress followed by resting periods of four days. The repetitive stress produced a decrease in alcohol consumption until the third stress period. By that time the rats appeared to have developed a tolerance to stress. When ACTH$_{4-10}$ was given to both the stressed and control animals a dramatic reduction in their alcohol consumption occurred. These results confirmed the hypothesis that ACTH$_{1-39}$, when released from the pituitary in response to stress or alcohol consumption, is the precursor of ACTH$_{4-10}$, the active fragment of the ACTH molecule. Exogenous administration of ACTH proved to be more effective than stress in decreasing the rats' alcohol drinking behavior. The presumption was that the exogenous dose was larger than the amount produced by the organism from ACTH$_{1-39}$ during stress. After stress there was a rebound increase in ethanol consumption which varied according to the baseline consumption of the animal.

These researchers hypothesized a stress/ACTH model for alcoholism. ACTH$_{1-39}$ release is induced by the consumption of alcohol. The pleasurable feelings aroused by activation of the brain reward circuitry in the presence of stress by the release of ACTH$_{1-39}$ and subsequent conversion by the organism to ACTH$_{4-10}$ primes the animal to continue alcohol
consumption after the stress has been discontinued. Thus the drinking of alcohol continues the pulsatile production of ACTH$_{4-10}$. In this study ACTH$_{4-10}$ was effective in all the subjects whether they were low medium or high alcohol drinkers.

One problem of applying animal work to human conditions is the impossibility of gaining access to the subjective experience of animals. However, drug-taking behavior in animals seems to parallel the mood states and subjective effects of drugs in human subjects (Stolerman, 1990). Using animal models researchers can amass critical data that can be applied to the study of alcoholism in humans. The use of animals allows controlled analysis of biological characteristics observed in human studies (Li, & Lockmuller, 1989).

In the present study two strains of laboratory rats were used to further investigate the effects of inherited characteristics and stress on alcohol consumption. Immobilization has been used previously (Sines, 1962 and Krishnan, 1991) to stress animals; therefore, immobilization stress was used in this study. The hypothesis was that there would be a significant difference between the strains and between the stressed and control animals in alcohol preference.
Chapter III

Procedures

Method

Subjects

The subjects were 18 adult, outbred, male Wistar and 21 adult, inbred, male Fischer-344 rats (313-610 g.) obtained from Harlan Sprague Dawley (Indianapolis, IN). These animals had not been bred to be either alcohol preferring or nonpreferring. They were housed in separate cages with ad lib access to food and fluids. The room was on a 12/12 light/dark cycle (lights on 0900-2100h). Once a week the cages were rotated to allow equal exposure to sunlight.

Equipment

The only special equipment was the device for stressing the animals. During the stress periods, the Fischer rats were restrained in sleeves obtained from Plas-Labs (Lansing, MI). These tubular, translucent, polyethylene sleeves; 22.5 cm in length and 6 cm in diameter; were each closed at one end by a 6.5 cm square of similar material. The other end of the sleeve was open to allow entrance of the animal and then closed by an opaque disk, 6 cm in diameter, that was fitted into the end of the sleeve. This disk was held in place by a screw. There were holes in each end of the sleeve and two slots down the length of the tubes (figure 1).
Figure Caption

Figure 1. Sleeve used to restrain Fischer rats.
Because the Wistar rats were too large to be placed in the sleeves, a substitute restraining device was necessary. After experimentation with other materials a device was improvised using a plastic box, the packaging for premoistened baby wipes. This was prepared by removing the wipes, rinsing the box with water, and punching holes for air in the sides, bottom, and lid. Each 22 x 11 x 9 cm plastic box was large enough to accommodate one Wistar rat but small enough to restrict his movement. The hinged lid was secured with duct tape and a 280-380 g weight placed on the lid to prevent escape of the animal (figure 2). Four polyethylene sleeves and three of the plastic boxes were used in stressing the animals requiring three shifts of one hour each per day to stress twenty animals.

Design and Procedure

The animals of each genetic strain, Wistar and Fischer 344, were randomly assigned to experimental and control groups. Nine Wistar and ten Fischer rats were in the control group. Another nine Wistar and eleven Fischer rats were in the experimental group.

The rats were allowed ad lib access to food (Purina Rat Chow). Each animal was allowed to consume ad lib a .4% aqueous saccharin solution or a similar solution containing 10% ethanol. Each cage was equipped with two water tubes, one containing the sweetened water and the other containing the sweetened alcohol solution. The position of the tubes (left-right) were altered once a week to control for
Figure Caption

Figure 2. Device used to restrain Wistar rats.
positional preference. The rats consumed most of their alcohol and food intake in irregularly spaced bursts during their dark cycle as did the rats in Dr. Li's lab (Li, Lumeng, McBride, & Murphy, 1987).

The volume of solution remaining in each tube was measured and the amount consumed was calculated and recorded every 24 hours. A baseline level of ingestion of the sweetened water and alcohol solutions was measured and recorded for 14 days.

The animals in the experimental group were subjected to stress by immobilizing each rat in a restraining device for one hour a day for fourteen days. The animals were stressed in three shifts each day, four Fischer rats and three Wistar rats stressed at a time. The animals were given two days of no stress in the middle of the experimental phase of the study. The animals in the control group were kept in their cages and not stressed.

Observation of fluid intake was continued for another fourteen days after the stressing phase. The influence of genetic strain and stress on alcohol preference was measured by the difference in alcohol consumption of the rats in each group and each strain.
Chapter IV

Results

The method of statistical analysis of this mixed design repeated measures experiment was analysis of variance. Because of the difference in weight between the Wistar and Fischer strains the percent preference for alcohol was divided by the weight of the animal in this analysis. The analysis of the percent preference for alcohol revealed a significant main effect for strain difference ($F_{1, 35} = 16.22, p<.001$). This means that, on the average, the Wistar rats consumed more alcohol than did the Fischer rats (fig. 3). The main effect for stress was not significant; however, there was a significant phase effect ($F_{2, 70} = 16.07 <.001$). This indicates that there was an overall difference between the prestress, stress, and poststress phases. There was a significant day effect ($p <.05$), indicating significant changes within a phase over the 14 days. The phase by day effect was significant ($F_{26, 910} = 3.25, p <.001$). This effect indicates that the nature of the day effect depends on the phase in which it occurs. The four-way interaction of strain by stress by phase by day of percent preference for alcohol was significant ($p = <.05$), but became nonsignificant when adjusted for weight (fig. 4). Post-hoc tests on the difference between the stressed animals and the controls revealed no significant difference. This suggests that the
Figure Caption

*Figure 3.* Mean alcohol intake per week of Wistar and Fischer rats.
Figure Caption

Figure 4. Alcohol preference of Wistar and Fischer rats. Four-way interaction.
relationships studied are complex, and it is not possible to make any simple conclusions about the effect of stress on alcohol consumption.

There was a significant interaction between the two strains and the two conditions, stress and no stress (fig. 4). The Wistar rats, both the stressed and non-stressed, decreased their alcohol intake by the second week and then showed a gradual increase during the last three weeks of the study. The Fischer animals in the experimental group consumed less alcohol during the first week, dramatically increased their intake during the second week, and reverted to below baseline level by the third week (fig. 3).
Chapter V
Conclusions

The results of this study support the findings in the literature that suggest preference for alcohol is hereditary. Since the subjects in this experiment were not bred to be alcohol preferring or non-preferring the baseline preference of one strain over the other for alcohol is not explained. What this study does show is a difference between strains and makes the assumptions that this difference is hereditary.

The phase effect indicates that the animals' alcohol preference varied over the three phases, baseline, stress and poststress. The interaction indicates a differential response during the three phases (figure 4). As illustrated in the graph (figure 3) the Wistar strain behaved in a similar manner to the animals in Krishnan, Nash, and Maickel's study. Their alcohol intake decreased in the stress period and increased in the post-stress period. The stressed Fischer rats showed an unexplained increase in alcohol consumption in the second week, a decrease in the third week, the stress period, and maintained a steady increase during the fourth through the sixth weeks. The stress effect obtained in the analysis of variance, however, was not significant. The effect of environmental stress on the alcohol consumption of these animals was, therefore inconclusive. The increase in alcohol consumption may be
explained by increased tolerance of the rats to the effects of alcohol.

Suggestions for Further Research

The failure to find a significant difference between the stressed and unstressed animals may be explained by the method used to apply stress. The most stressful part of the procedure was transferring the animals from their cages to the restraint devices. After the second day the Fischer rats, when placed on a table in front of the restraint sleeves ran into the sleeves, put their noses through the hole in the end, closed their eyes, and made no effort to escape. They seemed to the observer to be sleeping while in the restraints. The restraint sleeves were observed to be warm to the touch at the end of the stress period. This increase in heat was obviously due to the body heat of the animal and may have been soothing to the animal instead of stressful. The stressing was done during the warm season in the late afternoon when the laboratory was flooded with sunlight from the inadequately shaded, west-facing windows. The opaque nature of the restraint sleeves allowed the animals to be bathed in sunlight in contrast to the darkness of their cages. This exposure to sunlight may have further increased the heat in the devices. It was more difficult to remove the Fischer rats from the restraints than to induce them to enter. The "stressing" procedure did not seem to be very stressful for these animals.
The Wistar rats, who were restrained in the opaque boxes, exhibited more stress during this procedure as evidenced by chewing holes in the devices in an effort to escape and defecating during the procedure. These problems would seem to make the results of the effect of stress inconclusive. It is suggested, therefore, that in any replications of this study a different method of stressing the animals be used. The amount of defecation during the stressing procedure might be measured to determine the mood of the animal as an indication of the level of stress (Hirsjarvi, Junnila, & Valiaho, 1990). It is further suggested that the animals be matched for alcohol preference according to the results obtained in the baseline phase before the stress is applied.
Chapter VI
Discussion and Implications

The significant main effect for strain found in this study suggests that preference for alcohol is biological in origin. Although the effect of stress in the development of alcoholism cannot be ruled out the evidence from this study supports the conclusions from previous studies that biological factors play a greater role than environment in alcoholism. Grant & Johnstone (1990) have concluded that "the biological approach to the study of alcoholism is the most important direction in contemporary research which continues to move forward in relative isolation from other approaches" (p. 212). The purpose of alcohol studies is not to find a cure for alcoholism in rats, but to use animal models to understand alcoholism in humans. When the disease is better understood attitudes toward alcoholism may change for the better.

Alcoholism has been and still is considered by some to be a moral problem. According to a 1988 Gallop Poll the moral stigma of alcoholism still exists. When asked to describe their feelings about alcoholism 23% of the respondents thought it was a lack of willpower and 16% considered it a moral weakness (Miller & Chappel, 1991). Since Biblical times alcoholism has been considered a sin. In the early 19th century temperance became an ethic of middle-class morality, which attached an increased moral connotation to any drinking (Miller & Chappel, 1991).
However, some denominations of the Protestant church have shown certain concessions in response to scientific progress. They now accept the loss of control over drinking alcohol as an illness (Jellinck, 1960).

Alcoholism has been recognized as a disease for two centuries. According to the medical model the loss of control over drinking qualifies alcoholism as a disease state. The American Medical Association, which has always considered alcoholism a disease, clarified its position in 1956, urging hospitals to admit patients diagnosed as alcoholic (Miller & Chappel, 1991). Alcoholism is now treated by a combination of approaches including drug therapy, behavioral therapy, and referral to self help groups such as Alcoholics Anonymous (AA).

The disease concept of alcoholism has lagged behind that of medical diseases and psychiatric conditions (Miller, and Chappel, 1991). As more evidence is found for the biological origin of alcohol addiction, alcoholism may be accepted by the public as a legitimate medical disease like tuberculosis and cancer and the stigma may disappear. Establishing alcoholism as an organic disease rather than a psychogenic disease, character defect, or moral problem will be a step toward better treatment outcome for those who suffer from this condition.
References


people more susceptible to alcoholism? Alcohol Health and Research World, 13(4), 310-315.


