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Volitional Ethanol Consumption as a Function of Auditorily Induced Stress

A Thesis

Presented to

the Faculty of the Department of Psychology The University of the Pacific

## In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

Rolando Roberto Henry

August 1973

This thesis, written and submitted by

Rolando Roberto Henry

is approved for recommendation to the Committee on Graduate Studies, University of the Pacific. Department Chairman or Dean: Manual Manual Manual Thesis Committee:

Koz Chairman 20 beach 7/30/25 Dated

#### Acknowledgments

The author would like to express his thanks to all of the persons who provided help and guidance in the preparation of this thesis. In particular, he is indebted to Dr. Roseann Hannon, committee chairwoman, Dr. Martin T. Gipson and Dr. Kenneth L. Beauchamp for their wise and patient guidance. The author is also grateful to Ms. Debbie Weeb for her assistance in prehandling the subjects and to Ricardo A. Henry for his help in the building of the apparatus. To Ms. Anna Kusick goes a special thanks for her typing of this thesis.

## Volitional Ethanol Consumption as a Function of Auditorily Induced Stress

The literature on alcohol is replete with studies attempting to determine whether or not the relief of tension (i.e., certain hypothesized aversive states such as fear, anxiety, and frustration, which can influence behavior) plays a role in the etiology of moderate and excessive drinking by humans. The classic presentation of the tension reduction hypothesis (TRH) by Conger (1956) provided the impetus for the analysis of chronic alcohol consumption using animal subjects. By applying established behaviorist principles to the problem of chronic excessive drinking he developed a theory which accounts for this behavioral phenomenon. According to his theory, the response of drinking alcohol is one of many possible tension reducing responses in the organism's repertoire. This theory suggests that the human user of alcohol exhibits the drinking response as a consequence of some tension state and that the sedative action of alcohol serves as a reinforcer of the response by reducing the tension state.

The TRH has been investigated under different types of

tension states (e.g., Barry & Miller, 1965; Korman, Knopf, & Austin, 1960; Masserman, 1962). The intent of much of this experimentation has been to determine whether or not animals will succumb to the anesthetic properties of alcohol as an escape from some tension state by associating the ingestion of alcohol with the reinforcement resulting from stress escape. Such experiments, however, fall short of providing conclusive evidence for the etiology of the alcoholic syndrome. First, many of the findings are contradictory. Second, the studies do not adequately approximate a human model of alcoholism.

While some investigators have found that animals increase their ingestion of alcohol when exposed to stressful stimuli (Baum, 1970; Clark & Polish, 1960; Freed, 1967; Greenberg & Lester, 1953), others have not (Chittal & Sheth, 1963; Harris, Piccolino, Roback, & Sommer, 1964; McMurray & Jaques, 1959). This inconsistency is due in part to past investigators' neglect in considering the temporal relationship between the drinking response and the reinforcement of tension reduction. When the depressant properties of alcohol serve as the single avenue for stress escape, the temporal distance between the operant (drinking) and the reinforcement may be long and variable, due to intervening metabolic processes. It would therefore be difficult for an animal to associate operant and reinforcer with sufficient strength to form a persistent habit.

The second shortcoming of previous research, provision of an approximation of a human model of the alcoholic in animals,

has recently been given attention by Falk, Samson, & Winger (1972). They state that one of the problems has been that the conspicuous behavioral requirements of such a model are very demanding:

> (i) Animals should orally ingest ethanol solutions excessively and chronically in a pattern that increases the concentration of blood ethanol analogous to that in the alcoholic; (ii) unequivocal physical dependence on ethanol must be demonstrated; (iii) food and ethanol should be available from sources physically separate so that the factors determining ethanol intake are not inextricably bound to those primarily concerned with meeting nutritional requirements; (iv) the experimental arrangement should retain an elective aspect to the ethanol ingestion by not programming extrinsic reinforcing events (for example, shock avoidance, food pellet delivery) contingent upon drinking ethanol (p. 811).

Since the stressful environment of the human alcoholic is continuous and prolonged, the additional requirement of a continuous and prolonged tension state appears to be essential in establishing such a model. Senter, Smith & Lewin (1957), for example, found that the stress used in their study (shock) did not enhance subsequent ingestion of alcohol. However, they have suggested that the stress used was, for each day acute rather than chronic. "It is possible that continuous prolonged exposure (days or weeks) to such stress, with escape available only through alcohol intake, might produce different results (p. 292)." Investigations by Clark & Polish (1960), Myers & Holman (1967), and Mello & Mendelson (1966) have attempted to ascertain what role prolonged periods of stress play in the etiology of the alcoholic syndrome, if any. The present investigation was designed to develop an approximate model of the human alcoholic rats by investigating:

(a) The development of preference for alcohol.

(b) The development of excessive consumption of alcohol.

(c) The effects of chronic stress on alcohol consumption.(d) The extent to which alcohol ingestion in the home cage is attenuated by aversion conditioning in a non-home situation.Development of Preference for Alcohol

Some techniques and procedures have been utilized to induce voluntary oral consumption of alcohol in rats (e.g., Myers & Holman, 1966; Richter & Campbell, 1940). A three part study by Veale & Myers (1969) investigated alcohol preference in rats to determine whether or not prolonged exposure to alcohol would affect their level of preference. To minimize the dehydrating effects of alcohol, water was made available to the animals during all determinations of alcohol preference.

Part I of their study exposed one group of rats to water only for 10 days (water group) and a second group to a 12% alcohol solution only for the same period of time (forced alcohol group). For the next 27 days all animals were offered water and an alcohol solution in a free-choice situation. The alcohol was presented in an ascending sequence in which the concentration was increased every third day. The concentrations were 3, 5, 7, 9, 12, 15, 20, 25, and 30%. The results show that rats having no prior exposure to alcohol

prefered alcohol at low concentrations but rejected it as the concentration was sequentially increased. In contrast, rats having prior exposure (forced alcohol group) to alcohol drank less alcohol at every concentration.

In part II 3 groups of naive rats were given three 9-day test periods. For the first 9-day test period all subjects received alcohol solutions which were increased daily in the following sequence: 3, 5, 7, 9, 12, 15, 20, 25, and 30%. During the second 9-day test period one group was given water only (water group), a second group was restricted to a 15% alcohol solution as the sole fluid (forced alcohol group), and a third group was given the water versus the 3 to 30% alcohol ascending sequence. In the third 9-day period all subjects were again given the alcohol ascending sequence. When 3 successive 3 to 30% sequences were given, the mean daily alcohol intake significantly increased between the first and third sequences for all animals. The mean daily alcohol intake for the water group also increased significantly between the first and third sequences. However, the forced alcohol group did not increase their mean daily alcohol intake significantly from the first to third sequences.

Part III of the study entailed the use of 6 naive rats. Alcohol concentrations were increased daily in the following sequence: 3, 4, 5, 6, 7, 9, 12, 15, 20, 25, and 30%. This 11-day ascending sequence was repeated at the following different intervals, with water given <u>ad lib</u>. between sequences:

(a) Three repetitions of the sequence each

(b) Two additional repitions separated by 2 weeks
(c) Two additional repetitions separated by 6 weeks
(d) Two additional repetitions separated by 5 months
(e) Two additional repetitions separated by 1 day
Animals repeatedly exposed to this 11-day ascending sequence
consumed two to three times more alcohol in the seventh
sequence than in the first. This significant elevation in
preference for alcohol was evident not only at low concentrations
but at the higher concentrations as well.

In summary their results show: (a) naive rats prefered alcohol at low concentrations and rejected it as the concentrations increased sequentially; (b) rats forced to drink a non-prefered concentration of alcohol drank less alcohol than control animals in a subsequent free-choice test; (c) rats repeatedly exposed to an ll-day sequence in which the alcohol concentration was sequentially increased from 3 to 30% consumed two to three times more alcohol in the seventh sequence than in the first; (d) within a free-choice situation the adaptation effect occured only when water was constantly available. These results suggest that "the determining factor in the selection or rejection of alcohol appears to be the specific conditions of exposure to alcohol, i.e., the presence or absence of water in a choice situation and the length of time during which alcohol is consumed (p. 363)."

From these findings the following three points appear to be crucial in the development of an alcohol preference in the

rat: (a) Some prior exposure to alcohol must be experienced if high concentrations are to be freely consumed. (b) Repeated exposures to alcohol in a manner that increases the concentration gradually, enhances alcohol preference. (c) Forced alcohol administration (i.e., a lack of an alternate fluid, e.g., water) reduces preference for alcohol.<sup>1</sup> Development of Excessive Consumption of Alcohol

## By making a positively reinforcing stimulus a consequence of the selection and ingestion of an alcohol solution, a relatively persistent drinking behavior can be established (e.g., Falk, 1961; Persensky, Senter, & Jones, 1968; Senter, Smith, & Lewin, 1967). This technique however, is not useful in evaluating the reinforcing properties of alcohol consumption under stressful environments. The TRH assumes the sedative action of alcohol to be the positively reinforcing stimulus which follows the selection and ingestion of alcohol under stressful situations. When an additional positive reinforcer (e.g., food pellet) is presented concurrently with the reinforcing sedative action of alcohol, it becomes difficult

<sup>1</sup>An explanation for this is that an animal restricted to a forced choice situation in order to survive is unable to dilute the alcohol solution with another fluid. As the period of forced alcohol consumption continues, the animal could become dehydrated (Essig, 1968). Results of other investigators also support these findings (Kahn & Stellar, 1960; Mardones, 1960; Myers, 1961).

to determine which of the reinforcers actually produced the increase in consumption.

An ingenious method of separating positive stimuli from the selection and ingestion of alcohol has been presented by Falk, Samson, & Winger (1972). They have shown that rats maintained on an intermittent food schedule, with an available ethanol solution, drink excessively (avg. of 11-15 g per Kg of body weight daily). Food pellets were delivered every 2 min during 1-hr feeding periods that were separated by 3-hr intervals. Thus, there were 6 feeding periods in a 24-hr cycle.

Stein (1964) in evaluating Falk's findings (1961) of excessive drinking suggests that increases in fluid consumption are due to the intake of a thirst provoking stimulus (dry food pellet). Normally, rats have a strong inclination to drink after a meal. On <u>ad lib</u>. feeding, rats eat a few relatively large meals and the fluid intake is fixed by amounts drunk during the small number of drinking periods. Since rats do not compensate for this increase in the number of drinking periods by reducing their intake per period (Stein, 1964), a pattern of excessive drinking is exhibited. A similar explanation for the development of polydipsia has recently been reported by Lotter, Woods, & Vasselli (1973).

#### Stressful Stimuli and Alcohol Consumption

There have been a great many behavioral indices used in evaluating the effects of stress on alcohol consumption.

The majority of these indices, however, have dealt with the effects of acute stress rather than chronic stress.

Acute stress. One of the most dramatic of all behavioral indices used to measure tension states is the audiogenic seizure. Greenberg & Lester (1953) exposed rats to an intense noise of a bell. The effect of such stimulation was a precipitation of frenetic activity followed by a convulsion and a catatonic state. Their results show that voluntary consumption of alcohol (in nonintoxicating amounts) reduced the incidence of audiogenic seizures. Similar results were reported by Dember, Ellen, & Kristofferson (1953).

Another index of behavior subjected to evaluation is that of conflict. Conger's (1951) well known study best exemplifies the findings in this area which support the TRH. His results show an injection of 1.2 g per Kg of ethanol to be effective in restoring approach and eating responses which had been inhibited by punishment. It was also demonstrated that a dose of alcohol which significantly weakened avoidance behavior had little effect on approach. Conger therefore concluded that fear reduction was the mechanism of conflict resolution. In a systematic replication of Conger's study, Freed (1967) drew the same conclusions. Freed provided for dose-response data by using groups receiving 0, 0.5, 1.0, and 1.5 g of ethanol per Kg of body weight. No control rats resolved the experiment induced conflict but a significant number of alcohol treated rats did. Contradictory results however, have been reported by Barry, Wagner, & Miller (1962).

Two additional indices used to assess the effects of acute stress on alcohol consumption are avoidance and escape performance. Weak support of the TRH is given by Baum (1970). His results show that the escape latency of rats increased in a dose-related manner on the first trial of shock avoidance training. Ethanol however, did not have any effect on subsequent avoidance training. Negative outcomes have also been reported. In a study by McMurray and Jaques (1959), 1.0 g of ethanol per Kg of body weight failed to affect rats' avoidance behavior in a shuttle-box, even though other drugs were found to be effective. Chittal & Sheth (1963) studied the effects on avoidance of 0.4, 0.8, 1.2, and 1.6 g of ethanol per Kg of body weight. Performance was unaffected except at the highest dose.

<u>Chronic stress</u>. In addition to their use as behavioral indices in evaluating the effects of acute stress on alcohol consumption, avoidance and escape performance have been used in studies investigating the effects of long term stress on alcohol consumption. Clark & Polish (1960) concurrently investigated alcohol self-administration and bar-press avoidance in monkeys over an extended period of time. They report that animals prefer higher concentrations of alcohol under stressful conditions than under non-stressful conditions. These findings suggest that animals drink in part to attain emotional relaxation and further that the greater the need for emotional relaxation the more alcohol will be consumed. Myers & Holman (1967), however, report contradictory findings.

Their results show a 14-day period of stress produced by intermittent shock delivered to the floor of rats' cages to have had no significant effect on ethanol intake when compared with the intake of a control group. Mello & Mendelson (1966) also report contradictory results with monkeys.

#### Aversion Conditioning and the Consumption of Alcohol

Sobell & Sobell (1972) have presented several necessary characteristics of a behavioral treatment of alcoholism in humans. However, in developing an animal approximation of the human alcoholic only two of these characteristics are relevant:

> 1. Treatment sessions should deal directly with the behavior itself, namely drinking, and should be conducted under stimulus conditions which simulate as closely as possible the setting events which have preceded and accompanied heavy drinking in the past.

2. All treatment conditions should be designed so as to maximize generalization of the treatment effects as much as possible (p. 12-13).

Both of these characteristics entail the concept of generalization. That is, the organism, after having learned to emit a given response to a given situation having certain stimulus cues, emits the learned response in a new situation as a direct function of the number of stimulus cues common to both the old and new situation. The greater the number of stimulus cues common to both the old and new situations the greater the likelihood of emitting the given response in the new situation without re-training. New situations in which the stimulus cues are exactly the same as those of the situation in which the response was learned will have the maximal probability of causing the organism to exhibit the learned response.

The most recent behavioral approach to the treatment of alcoholism, aversion conditioning, incorporates this concept of generalization. In essence, aversion conditioning associates the drinking response with some unpleasant stimulation (chemical or electrical). It is hoped that a connection between the drinking response and the unpleasant stimulation will develop thereby reducing or suppressing the occurrance of the response (e.g., Blake, 1965, 1967; Hsu, 1965; Rachman, 1965; Vogler, Lunde, Martin, & Johnson, 1970). These studies, however, are plagued with the problem of specific discriminations. For example, subjects conditioned with one alcoholic beverage did not display any suppression of the drinking response when stimulated with other alcoholic beverages. The effectiveness of the technique then, appears to be dependent on specific taste stimuli being paired with the unpleasant stimulus. In their review of the treatment of alcoholism by chemical aversion, Voegtlin & Lemere (1942) quote an early French report in which patients conditioned with wine acting as the conditioned stimulus developed an aversion to this drink but not for other types of alcoholic beverages. More recent examples can be found in MacCulloch, Feldman, Orford, & MacCulloch (1966) and Quinn & Honbest (1967). Thus if all the stimuli contained in the non-treatment situation (i.e., all alcoholic beverages) are not present in the treatment situation the effectiveness of the treatment appears to be diminished.

1.2

This problem of limited generalization is not unexpected if drinking is considered to be an operant, i.e., controlled by stimulus conditions which precede, follow, and/or accompany the response. Many of these stimulus conditions are an integral part of the human society and are usually absent in a treatment environment. In relation to the TRH, the presence of a tension state (a stimulus condition which has preceded and/or accompanied the drinking response in the past) during treatment appears to be crucial in maximizing the effect of the treatment. If the organism is to suppress the drinking response under stressful situations following treatment, the organism must be conditioned to suppress the response under stressful situations during treatment.

#### Method

#### Subjects

The subjects were 40 female hooded rats obtained from Blue Spruce Farms (Altamont, New York). They were approximately 75-80 days old at the onset of the experiment. Each subject was individually housed in a 24.76 cm X 18.42 cm metal cage and given <u>ad lib</u>. access to Purina rat chow and water. Upon arrival at the laboratory subjects were randomly assigned to two experimental conditions (stress, n = 20; non-stress, n = 20).

#### Apparatus

<u>Stressful stimulus</u>. A noise produced by a 10.16 cm open gong electric bell (Trine Manufacturing Co., No. 174, New York, New York) served as the stressful stimulus. The noise was analyzed by an Oscilloscope (Tektronix Inc., Type 516, Portland, Oregon) to have two main sound components: 1.8 KHz and 2 KHz. The noise was recorded on a Revox tape recorder, model A77 (Willi Studer; Zurich, Switzerland), using a 3-min variable interval schedule (VI-3) which was repeated every 4 hours. The recorder was wired in conjunction with a Heathkit preamplifier, model WAPZ, and amplifier, model 44 AM (Heath Co., Benton Harbor, Michigan) in order to increase the intensity of the noise to a level of 110 db. The noise was delivered through a 38.10 cm Jensen speaker (Chicago, Ill.) and was of 1 sec duration.

Aversive stimulus. A unijunction transistor circuit (See Appendix 1.) wired to the drinking spout of the animal's ethanol bottle administered a .25 mA electric shock of 300 msec duration to the subject's tongue. The subject activated the circuit by stepping on a switch located on the floor of the cage 6.4 mm away from the drinking spout. The switch consisted of two 5.22 cm X 17.18 cm stainless steel plates separated by a 52.2 mm X 1.9 mm piece of plexiglass. The subject's body weight was sufficient to cause the two plates to make contact with each other allowing the circuit to build up the .25 mA shock. Discharge of the shock was delayed for approximately .5 sec after the animal stepped on the plates allowing the animal time to taste the solution before being If the subject remained on the plates without drinking shocked. shock was repeatedly discharged through the drinking spout with approximately .5 sec separating each shock.

#### Procedure

Period I: prehandling. Rats were prehandled in a 76.20 cm X 76.20 cm activity box 2 min a day for 9 days before the onset of the experiment. Each rat was picked up every 30 sec then placed back in the box near the center.

Period II: preference development. After handling, ethanol preference development was begun. The rat was offered an ethanol solution in a water-ethanol self-selection situation for 12 days. Starting with an ethanol solution of 1% by volume, the concentration was increased in 1% increments every 2 days until the solution was 6% by volume. Measurements of the amount of fluid ingested from each bottle were recorded each time the concentration was increased. Food was available ad lib. A record of the daily food consumption was maintained for each animal during this period and the subsequent 5% baseline period. To prevent the development of a position habit 3 bottles were attached to each cage (water, ethanol, and empty), and their relative positions were randomly altered every 2 days throughout the entire experiment (Myers & Holman, 1966).

Period III: five percent baseline. At the end of 12 days of preference development, all animals were offered a 5% solution of ethanol in a water-ethanol self-selection situation for 4 days. The 5% concentration was used throughout the remainder of the experiment. A 5% concentration was determined to be that concentration prefered to water by all rats (n = 6) in the pilot study conducted by the present

investigator (1973). Ad lib. feeding was available. Baseline measurements for the amount of water and ethanol ingested during this 4-day period were taken on the second and fourth days.

Period IV: excessive consumption. Following Period III all rats were maintained on an intermittent food schedule with a 5% ethanol solution and water available in a selfselection situation. The mean daily amount of food consumed by each rat, based on records taken during preference development, was divided into six equal portions and administered during six 1 hr free-feeding periods daily (8 am, 12 pm, 4 pm, 8 pm, 12 am, and 4 am). Each feeding period was separated by a 3-hr interval in which no food was available to the animal. This intermittent food schedule was maintained until the experiment was terminated. Measurements of the amount of water and ethanol ingested were taken every 2 days for a period of 8 days.

Period V: introduction of stress. Following Period IV the stress group (n = 20) was moved, with their home cages, to the stressful environment while those in the non-stress group (n = 20) remained in the animal colony. Rats in the stress environment were collectively exposed to the stressful stimulus (bell) administered on the VI-3 min schedule throughout a 24-hr cycle. The water and ethanol intake for both groups were measured and recorded every 2 days for a period of 8 days.

Period VI: aversion conditioning. At the end of Period

V, subjects in the stress and non-stress groups were divided into two equal groups each, which received aversion conditioning under stress or non-stress. This resulted in a total of four groups (n = 10), named according to the circumstances under which the animals experienced Periods V and VI of the experiment: 1) pre-aversion conditioning stress - aversion conditioning under stress (PS-ACS); 2) pre-aversion conditioning stress - aversion conditioning under non-stress (PS-ACN); 3) pre-aversion conditioning non-stress - aversion conditioning under stress (PN-ACS); 4) pre-aversion conditioning non-stress - aversion conditioning under non-stress (PN-ACN). Each rat was given an aversion conditioning session of 2 days, half of each of the previous groups receiving conditioning in the stressful environment and half in the natural laboratory environment. During the conditioning session the rat was shocked for drinking the ethanol solution. Shock was delayed until approximately .5 sec after the first lick on the spout allowing the rat to taste the solution, During this sequence measurements for the amount of water and ethanol consumed were taken every 4 hours.

Period VII: test. Following the period of aversion conditioning all animals were returned to the environment they lived in prior to the period of aversion conditioning, i.e., stress or non-stress environments. They remained in these environments for a period of 8 days. The amounts of water and ethanol ingested were measured and recorded every 2 days. The purpose of this design was to investigate whether aversion conditioning was more effective in reducing alcohol consumption if received in the same environment as pre-aversion conditioning or in a different environment. Figure 1 provides a diagram of the experimental procedure.

#### Results

Before determining whether or not stress or aversion conditioning had any effect on ethanol ingestion, ethanol and water raw data from the preference development, 5% baseline and excessive consumption periods were examined to determine whether or not subjects prefered ethanol to water prior to the introduction of stress.

The differences between the amount of ethanol and water ingested prior to the introduction of stress are illustrated by the preference-aversion curves in Figure 2. The mean ethanol and water intake during each 2-day period in grams per kilogram of body weight are plotted for each of the ethanol concentrations during the 12-day preference, the 4-day 5% baseline period, and the 8-day excessive consumption period. Related t-tests were performed comparing the mean amount of ethanol and the mean amount of water consumed by all animals during the preference development, 5% baseline, and excessive consumption periods. The two means of all 40 subjects' scores per 2 day period formed the 6, 2, and 4 pairs of scores for each of the three t-tests. Ethanol was consumed in significantly greater quantities than was water in all three periods (t = 63.23, df = 5, p < .001;



Figure 1. Schematic diagram of experimental procedure used.



Figure 2. Mean ethanol and water intake, for each ethanol concentration, measured during preference development, 5% baseline, and excessive consumption.

t = 14.40, df = 1, p < .05; and t = 137.40, df = 3, p < .001 respectively).

To determine whether or not the intermittent food schedule had any effect on fluid consumption, randomized block analyses of variance (mixed effects model; Kirk, 1968) were run on ethanol and water consumption data obtained from the measurements taken every 2 days during the 4-day 5% baseline period and the 8-day excessive consumption period by each subject. The treatment variable (B) for both analyses was the six measurements taken during these periods. There were 40 blocks of one subject (S) each, with repeated measures taken on each subject over days. Results of these analyses show no significant change in ethanol or water consumption over days (F = 2.16, df = 5/195, p > .05 and F = 1.29, df = 5/195,p >.05 respectively). This indicates that intermittent feeding had no effect on the fluid intakes of the animals. Differences between subjects were significant for both ethanol and water analyses (F = 29.44, df = 39/195, p < .001 and F = 3.91, df = 39/195,  $p \ll .001$  respectively). (Summaries of the respective analyses of variance are presented in Appendix 2.)

#### Period IV: Introduction of Stress

The mean amount of ethanol and water consumed by each subject in each 2-day period during the 8-day introduction of stress period are shown in Appendix 3 for each subject. Independent t-tests applied to the data contained in Appendix 3 show the difference between the stress and non-stress groups

for the amount of ethanol consumed to be reliable,  $\overline{X}_{stress}$ = 6.77 vs  $\overline{X}_{non-stress}$  = 4.75, (<u>t</u> = 2.49, <u>df</u> = 38, <u>p</u> < .01), while the difference for the mean amount of water consumed in a 2-day period was not found to be reliable,  $\overline{X}_{stress}$  = 2.97 vs  $\overline{X}_{non-stress}$  = 3.03 (<u>t</u> = 0.1, <u>df</u> = 38, <u>p</u> > .25).

## Period VI: Aversion Conditioning

CRF-22 analyses of variance (Kirk, 1968) were separately performed on the total amount of ethanol and water consumed during aversion conditioning by each subject (See Appendix 4.) to determine what effect, if any, pre-aversion conditioning environment and aversion conditioning environment had on the animals' fluid intakes during the aversion conditioning situation. The independent variables for both analyses were pre-aversion conditioning environment (stress vs non-stress) and aversion conditioning environment (stress vs non-stress). The results of these analyses show no differences between groups for either the amount of ethanol or water ingested during the aversion conditioning period (all <u>F</u>'s  $\leq$  1). All subjects terminated ethanol ingestion within 4 - 16 hours after the onset of aversion conditioning. (Appendix 5 presents summaries of the respective analyses of variance.)

#### Period VII: Test

Appendix 6 contains the mean amount of ethanol and water consumed in each 2-day period by each subject during the 8-day test period. CRF-22 analyses of variance (Kirk, 1968) were run on the data contained in Appendix 6. For both analyses the independent variables were aversion conditioning environment (stress vs non-stress) and pre-aversion conditioning - test period environment (stress - stress vs non-stress - non-stress). Figure 3 presents the mean 2-day ethanol intake for each of the 4 groups during the 8-day test period. The effect of preaversion conditioning - test period environment significantly affected the amount of ethanol ingested during the test period  $(F = 5.23, df = 1/36, p \ll .01)$ . Animals returning to a stressful environment consumed more ethanol than did animals returning to a non-stressful environment. However, the effect of pre-aversion conditioning - test period environment did not significantly affect water intake (F = 1.85, df = 1/36,  $p \ge .10$ ). Aversion conditioning environment did not significantly affect either ethanol or water intake (F = 1.49, df = 1/36, p > .25and F 🕊 1, respectively). Interactions between aversion conditioning environment and pre-aversion conditioning - test period environment were not significant for either ethanol or water (all F's a 1). (Appendix 7 presents summaries of the respective analyses of variance.)

## Discussion

The results of the comparison between the ethanol and water intake prior to the period in which stress was introduced clearly indicate that rats prefer ethanol to water in a selfselection situation (See Figure 2.). These findings support the generality of the conclusions drawn from previous research (e.g., Richter & Campbell, 1940; Rick & Wilson, 1966) that normal rats ordinarily prefer ethanol in low concentrations



Figure 3. Mean 2-day ethanol intake for the 4 groups during the Test period.

#### (1.4 - 6.5%) to water.

The finding that intermittent feeding did not increase the intake of either ethanol or water is contradictory to previous reports of schedule-induced polydipsia (e.g., Falk, Samson, & Winger, 1972; Falk, 1961; Stein, 1964). One explanation of this contradiction lies in the method used to induce polydipsia in the present study. The mean daily amount of food eaten by each animal was divided into six equal portions and offered on a fixed-interval 4-hr schedule. Food remained in the cage for a period of 1-hr after which all uneaten portions were removed. In attempting to explain the phenomenon of polydipsia, Lotter, Woods, & Vasselli (1973) concluded polydipsia to be a function of the number of bites the animal takes to complete a meal and not a function of the schedule used or the total amount of food consumed. A "bite" was defined as the amount of food consumed between drinks of The size of a bite varied with the amount of food water. available between drinks. Using varying numbers of food pellets (yielding varying bite sizes) as reinforcement for bar-pressing these investigators demonstrated that the rat drinks a fixed amount of water after every bite, independent of the size of the bite. The smaller the bite, therefore, the greater the total amount of water consumed. If the explanation presented by Lotter et al. is correct then the results of the present study could be explained in the following manner. Since each animal received the total amount of food to be eaten during each 1-hr meal session at the beginning of the

of the hour, there were no predetermined spaced bites within the hour. Presumably the animal could have eaten all of his food at one time or spaced it out over the hour. If the animal consumed all of his food at one time there was only one bite followed by a single drinking period. It seems reasonable to presume that under <u>ad lib</u>. feeding, normal animals have a minimum of 2 to 3 bites per day with subsequent drinking periods. It could be that the difference between the number of bites in <u>ad lib</u>. feeding and the number of bites in the schedule used in the present study is not large enough to produce a significant increase in fluid intake.

In previous studies water was the sole liquid used in the development of polydipsia (e.g., Falk et al., 1972; Falk, 1961; Lotter et al., 1973; Stein, 1964). Unlike the present study the animals used in these studies were placed on food deprivation prior to and during the period in which the polydipsic effect was acquired. Given the contradiction between the present finding and the findings of previous studies it is suggested that further investigations in the area of schedule-induced polydipsia be designed to investigate the effects a state of food non-deprivation has on increased water intake. This is especially important if the method of schedule-induced polydipsia is to be used in developing increased ethanol consumption in the rat. Knowledge of these effects, if any, are essential since it is generally accepted that ethanol has a high caloric content and might therefore be a contributing factor in the subject's selection of ethanol

in a free-choice situation if food deprived.

#### Period V: Introduction of Stress

Contrary to the findings of other investigators (e.g., Barry, Wagner, & Miller, 1962; McMurray & Jaques, 1959; Myers & Holman, 1967) the stressful environment in the present study increased ethanol consumption significantly without significantly increasing water consumption. This finding strongly supports the TRH as a contributing factor in the etiology of alcoholism.

There are at least two possible reasons why stress facilitated ethanol consumption. First, it could be argued that the stressful stimulus used in the present study was more stressful than stimuli used in previous research. Jamison (1950) in investigating auditory thresholds of the rat found adult rats to have an absolute intensity threshold of 38-47 db for a tone having a frequency of 2KHz. The intensity of the 2KHz sound component of the stressor used in the present study was determined to be 110 db. It was observed that upon delivery of the stressful stimulus the animals exhibited an aroused behavioral state. Generally, this state consisted of either the animal engaging in frenetic activity or the exhibition of a rigid body position with twitching of the head and ears. A small number of animals were observed to have mild muscle spasms during presentation of the noise and following its termination.

An alternative explanation might center on the fact that the average amount of time between presentations of the stimulus

was 3 min. The possible deficit in sleep caused by such a schedule as well as the frequent interruption of sleep might also have played a part in establishing a stressful environment. Also, the continuous presentation of the stressful stimulus throughout a 24-hr cycle may have contributed to the stressful situation.

Unlike the present study, previous studies investigating the effects of chronic stress on ethanol consumption report that random unavoidable shock (stressful stimulus) presented for prolonged period of time did not affect ethanol consumption (Mello & Mendelson, 1966; Myers & Holman, 1967).

Because of the many differences between those experiments and the present one, it is impossible to specify the cause of the contradictory results.

In view of the results of the present experiment it would seem reasonable to employ the rat in certain kinds of experimental situations assumed to be stressful in order to develop an animal approximate of the human alcoholic. Such experimental situations could then be used to test a theory of the etiology of alcoholism, or to investigate a treatment of this disease. The use of unavoidable auditory stimulation seems to constitute a reliable method whereby volitional ethanol consumption can be increased in an ethanol-water free-choice situation.

## Period VI: Aversion Conditioning

It was hypothesized that the four groups would be related in the following manner in regards to the mean

total amount of ethanol ingested during aversion conditioning: PS-ACS PS-ACN ? PN-ACS PN-ACN. In line with the experimental results obtained by Breuer & Goesling (1969), avoidance conditioning was acquired by the subjects in a relatively short period of time and no differences between groups for the mean total amount of ethanol consumed were found.

There are at least two tenable explanations of these results. First, the intensity of the shock to the tongue could have been of such a magnitude that it did not allow for differential responses between subjects. The second explanation deals with shock-object discriminability. Shock delivered through a highly discriminable object leads to a specific avoidance of that object. Blanchard & Blanchard (1970) report that subjects shocked by discriminable objects displayed reliably longer latencies to enter the shock situation than did subjects shocked by less discriminable objects. Subjects shocked by discriminable objects also acquired avoidance of the shock-object faster than did subjects shocked by less discriminable objects.

The fact that all animals greatly reduced ethanol consumption during the aversion conditioning period raises the question of the specificity of the use of ethanol consumption to reduce stress. It is reasonable to conclude that pain and fear induced by shock to the tongue is stressful. Why then did ethanol consumption not reduce stress due to shock induced pain and fear? A viable answer to this question may be that ethanol consumption is learned to reduce specific

tension states (in this case, auditory stress) and not any or all tension states. In terms of the present investigation, ethanol consumption was perhaps the only way to reduce stress during auditory stimulation. However, the behavior of not drinking from the ethanol drinking spout was an effective way to reduce the tension caused by shock to the tongue and also eliminated ethanol consumption as a way to reduce other tensions, as long as ethanol consumption produced shock. Period VII: Test

Generalization from the aversion conditioning environment to the pre-aversion conditioning - test environment was hypothesized to have a greater effect on the mean 2-day ethanol intake during the test period than was chronic stress. However, the results of the analysis on the test period data show that animals returning to the stress environment consumed more ethanol than did animals returning to the non-stress environment. It was therefore concluded that generalization from the aversion conditioning environment to the pre-aversion - test period environment had no effect on ethanol intake during the test period and that a chronic stress environment increased the rate of recidivism, regardless of the environment during aversion conditioning.

The implications of these findings are supportive of the TRH being a causative factor in the development of alcoholism. The TRH as a viable explanation of the etiology of alcoholism necessitates the presence of a tension state preceding and/or accompanying the drinking response prior to treatment.

Obviously, these same tension states which existed prior to treatment also exist after treatment if the organism has to return to the environment in which he acquired the drinking response. If the organism returns to the same stressful environment and if the negative feelings classically conditioned to alcohol consumption during aversion conditioning are not extremely intense, it is highly likely that the organism will resume his original drinking pattern, because it is still the only response in his repertoire which reduces the tension state. The effectiveness of a treatment situation. then, does not appear to be solely dependent on the organism being able to generalize negative feelings toward alcohol acquired during the treatment situation to the after-treatment situation. Rather, an effective treatment for the human alcoholic should incorporate an alternative response for dealing with the subject's tension state. thereby giving him an additional defense mechanism other than the ingestion of alcohol.

#### Summary

In light of the contradictory findings of previous research on the tension reduction hypothesis (TRH) this study investigated the effects of chronic stress on ethanol ingestion and the extent to which ethanol ingestion in the home cage is attenuated by aversion conditioning. Preference for a 5% ethanol solution, in a 3-bottle free-choice situation, was developed in rats following an 8-day period during which the concentrations of ethanol were systematically increased from 1-6%. When offered a 5% solution in a free-choice situation, and exposed to 3-min variable-interval auditory stimulation over 24-hrs, rats (stress group) learned to drink significantly more ethanol than rats not exposed to such stimulation (non-stressed group) during an 8-day period. Stress and nonstressed groups were divided into 2 groups each which were exposed to an environment like the environment in which stressed rats learned to drink (stress) or to an environment like the environment in which non-stressed rats learned to drink (non-stress). Aversion conditioning was given these 4 groups for 2 days. No difference in ethanol intake was found to exist between groups during aversion conditioning. To determine the effectiveness of aversion conditioning, rats were returned to the environment in which they learned to drink for an 8-day test period. Rats returned to a stress environment drank more ethanol than rats returned to a nonstress environment regardless of the type of environment (stress or non-stress) in which aversion conditioning was

received. In general, stress facilitated ethanol ingestion prior to and following aversion conditioning. Generalization from the aversion conditioning environment to the environment in which drinking was learned had no effect. These results suggest that tension reduction plays a role in the etiology of alcoholism and that merely simulating pre-aversion conditioning conditions in aversion conditioning does not increase the effectiveness of aversion conditioning if conditions are stressful after aversion conditioning.

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## Table 2.1

## Results of Analysis of Variance Effects of Intermittent Feeding Ethanol Data

Source of Variation	SS	df	MS	F
Between treatment (B)	23.33	5	4.67	2.16
Between blocks (S)	2480.16	39	63.59	29.44*
Residual	422.10	195	2,16	
Total	2925.59			

p < .01

## Table 2.2

Results of Analysis of Variance Effects of Intermittent Feeding Water Data

Source of Variation	SS	df	MS	F
Between treatment (B)	37.28	5	7.46	1.29
Between blocks (S)	883.86	39	22,66	3.91
Residual	1128.37	195	5.79	
Total	2049.51			

p < .01

******	Ctropped	(N = 20)	Non-stro	$r_{\rm ec} (N = 20)$	
	STRESS	IN = 207	NOII-511 6.	55 (11 - 207	
	Ethanol	Water	Ethanol	Water	
	6.46	3.88	6.33	3.12	
	6.93	2.27	9.34	2,56	
	3.86	6.30	5.33	4.29	
	3.25	8.02	6.15	1.68	
	10.36	.57	9.57	. 42	
	11.18	.92	7.98	.99	
	9.05	.66	3,38	3.44	
	5.43	3.09	5.28	3.42	
	9.44	.58	5,13	1,50	
	5.43	.70	5.86	2.72	
	8.91	3.06	3.21	3.24	
	8.26	1.39	3.42	2.24	
	5.42	5.17	4.27	2.34	
	6.59	2.68	8.92	1.83	
	6.46	3.90	.84	4.35	
	2.68	3.78	.90	6.23	
	6.21	3.52	3.00	3.61	
	4.06	6.57	1.40	4.10	
	7.70	1.30	1.06	4.71	
	7.73	.95	3.71	3.86	
	$\overline{X} = 6.77$	$\overline{X} = 2.97$	$\overline{X} = 4.75$	$\overline{X} = 3.03$	

## The mean $(\overline{X})$ 2-day ethanol and water intakes in g/Kg of body weight for the stress and non-stress rats during the introduction of stress period

	3-AC3 61000	(n = 10)		PS-ACN Group	(n = 10)
Subject #	Ethanol	Water	Subject #	Ethanol	Water
8	.40	7.61	2	.26	7.67
12	.40	7.54	3	.26	8.47
14	2.08	7.93	4	1.73	7.18
20	.40	7.23	9	.28	5,60
23	1.28	8.40	17	.38	7.73
26	1.35	7.46	18	2.48	7.17
27	.28	8.78	33	.14	8,18
30	1.55	7.99	37	1.46	7,86
36	.44	5.08	19	1.34	9.34
<b>x</b> =	.86	7.60	X =	.90	7.68
<u>F</u>	N-ACS Group	(n = 10)		PN-ACN Group	(n = 10)
	N-ACS Group	<u>(n = 10)</u> 10.98	6	PN-ACN Group	(n = 10) 9.08
1 5	<u>N-ACS Group</u> 5.20 .44	(n = 10) 10.98 7.44	6 7	<u>PN-ACN Group</u> .57 .78	(n = 10) 9.08 6.92
1 5 11	<u>N-ACS Group</u> 5.20 .44 .64	(n = 10) 10.98 7.44 7.65	6 7 10	<u>PN-ACN Group</u> .57 .78 .48	(n = 10) 9.08 6.92 5.88
1 5 11 13	<u>N-ACS Group</u> 5.20 .44 .64 1.48	(n = 10) 10.98 7.44 7.65 8.00	6 7 10 24	<u>PN-ACN Group</u> .57 .78 .48 2.38	(n = 10) 9.08 6.92 5.88 5.86
1 5 11 13 15	<u>N-ACS Group</u> 5.20 .44 .64 1.48 .35	(n = 10) 10.98 7.44 7.65 8.00 5.19	6 7 10 24 25	<u>PN-ACN Group</u> .57 .78 .48 2.38 .28	(n = 10) 9.08 6.92 5.88 5.86 9.05
1 5 11 13 15 16	5.20 .44 .64 1.48 .35 1.26	(n = 10) 10.98 7.44 7.65 8.00 5.19 6.72	6 7 10 24 25 28	<u>PN-ACN Group</u> .57 .78 .48 2.38 .28 1.19	(n = 10) 9.08 6.92 5.88 5.86 9.05 6.08
1 5 11 13 15 16 32	5.20 .44 .64 1.48 .35 1.26 2.71	(n = 10) 10.98 7.44 7.65 8.00 5.19 6.72 7.46	6 7 10 24 25 28 29	<u>PN-ACN Group</u> .57 .78 .48 2.38 .28 1.19 .28	(n = 10) 9.08 6.92 5.88 5.86 9.05 6.08 7.98
1 5 11 13 15 16 32 40	2N-ACS Group 5.20 .44 .64 1.48 .35 1.26 2.71 1.34	(n = 10) 10.98 7.44 7.65 8.00 5.19 6.72 7.46 6.07	6 7 10 24 25 28 29 34	<u>PN-ACN Group</u> .57 .78 .48 2.38 .28 1.19 .28 1.89	(n = 10) 9.08 6.92 5.88 5.86 9.05 6.08 7.98 9.61
1 5 11 13 15 16 32 40 21	5.20 .44 .64 1.48 .35 1.26 2.71 1.34 .56	(n = 10) 10.98 7.44 7.65 8.00 5.19 6.72 7.46 6.07 5.49	6 7 10 24 25 28 29 34 35	PN-ACN Group .57 .78 .48 2.38 .28 1.19 .28 1.89 .57	(n = 10) 9.08 6.92 5.88 5.86 9.05 6.08 7.98 9.61 6.93
1 5 11 13 15 16 32 40 21 22	5.20 .44 .64 1.48 .35 1.26 2.71 1.34 .56 .14	(n = 10) 10.98 7.44 7.65 8.00 5.19 6.72 7.46 6.07 5.49 6.72	6 7 10 24 25 28 29 34 35 38	PN-ACN Group .57 .78 .48 2.38 .28 1.19 .28 1.89 .57 .70	(n = 10) 9.08 6.92 5.88 5.86 9.05 6.08 7.98 9.61 6.93 6.73

•

Total ethanol and water intakes in g/Kg of body weight for all rats during the aversion conditioning period

## Table 5.1

## Results of Analysis of Variance Aversion Conditioning Period Ethanol Data

Source of Variation		SS	df	MS	F
Pre-aversion conditioning Environment	(A)	.78	1	.78	.80
Aversion conditioning Environment	(B)	.52	1	.52	.54
АХВ.		.76	1	.76	.78
Within cell		35.05	36	.97	
Total		37.11	39		

## Table 5.2

## Results of Analysis of Variance Aversion Conditioning Period Water Data

Source of Variation		SS	df	MS	F
Pre-aversion conditioning Environment	(A)	.25	1	.25	.15
Aversion conditioning Environment	(B)	1.20	1	1.20	.72
AXB		.07	1	.07	.04
Within cell		59.50	36	1.65	
Total		61.02	39		

	-ACS-TS Group	n = 10	PS	-ACN-TS Grou	1p (n = 10)
Subject #	Ethanol	Water	Subject #	Ethanol	Water
8	3.20	5.91	2	6.41	3.74
12	2.08	6.94	3	5.84	3.60
14	4.94	2,57	4	6.20	3.41
20	4.36	3.87	9	7.97	2.84
23	9.11	1.24	17	7.32	1.52
26	9.83	2,66	18	6.88	1.64
27	6.16	2.54	33	6.24	3,39
30	8.34	1.86	37	3.75	3.13
36	10.31	3.36	19	6.20	5.07
39	8,46	1.74	31	2.32	3,76
¥ -	6 68	3.27	<del>x</del> =	5.91	3.21
· · ·	0.05	2111			
<u>PN-</u>	ACS-TN Group	(n = 10)	<u>PN-</u>	ACN-TN Group	o (n = 10)
<u>PN-</u>	ACS-TN Group	(n = 10) 4.44	<u>PN-</u>	ACN-TN Group 3.76	o (n = 10) 5.12
<u>PN-</u> 1 5	ACS-TN Group 4.20 1.02	(n = 10) 4.44 3.13	6 7	ACN-TN Group 3.76 6.46	5.12 3.36
<u>PN-</u> 1 5 11	ACS-TN Group 4.20 1.02 5.53	(n = 10) 4.44 3.13 3.44	6 7 10	ACN-TN Group 3.76 6.46 5.85	5.12 3.36 3.51
<u>PN-</u> 1 5 11 13	ACS-TN Group 4.20 1.02 5.53 8.01	(n = 10) 4.44 3.13 3.44 4.27	6 7 10 24	ACN-TN Group 3.76 6.46 5.85 5.20	5.12 3.36 3.51 3.44
<u>PN-</u> 1 5 11 13 15	ACS-TN Group 4.20 1.02 5.53 8.01 4.32	(n = 10) 4.44 3.13 3.44 4.27 2.00	6 7 10 24 25	ACN-TN Group 3.76 6.46 5.85 5.20 2.50	5.12 5.12 3.36 3.51 3.44 4.51
<u>PN-</u> 1 5 11 13 15 16	ACS-TN Group 4.20 1.02 5.53 8.01 4.32 4.90	(n = 10) 4.44 3.13 3.44 4.27 2.00 2.85	6 7 10 24 25 28	ACN-TN Group 3.76 6.46 5.85 5.20 2.50 4.45	5.12 $3.36$ $3.51$ $3.44$ $4.51$ $2.61$
<u>PN-</u> 1 5 11 13 15 16 26	ACS-TN Group 4.20 1.02 5.53 8.01 4.32 4.90 3.31	(n = 10) 4.44 3.13 3.44 4.27 2.00 2.85 7.60	6 7 10 24 25 28 29	ACN-TN Group 3.76 6.46 5.85 5.20 2.50 4.45 4.18	5.12 $3.36$ $3.51$ $3.44$ $4.51$ $2.61$ $2.64$
<u>PN-</u> 1 5 11 13 15 16 26 22	ACS-TN Group 4.20 1.02 5.53 8.01 4.32 4.90 3.31 3.00	(n = 10) 4.44 3.13 3.44 4.27 2.00 2.85 7.60 4.15	6 7 10 24 25 28 29 34	ACN-TN Group 3.76 6.46 5.85 5.20 2.50 4.45 4.18 2.72	5.12 $3.36$ $3.51$ $3.44$ $4.51$ $2.61$ $2.64$ $3.38$
<u>PN-</u> 1 5 11 13 15 16 26 22 32	ACS-TN Group 4.20 1.02 5.53 8.01 4.32 4.90 3.31 3.00 6.84	(n = 10) 4.44 3.13 3.44 4.27 2.00 2.85 7.60 4.15 3.34	6 7 10 24 25 28 29 34 35	ACN-TN Group 3.76 6.46 5.85 5.20 2.50 4.45 4.18 2.72 1.23	5.12 $3.36$ $3.51$ $3.44$ $4.51$ $2.61$ $2.64$ $3.38$ $5.39$

Mean  $(\overline{X})$  2-day ethanol and water intakes in g/Kg of body weight for all rats during the test period

## Table 7.1

## Results of Analysis of Variance

Ethanol Data

Source of Variation		SS	df	MS	F
Aversion conditioning Environment	(A)	7.67	1	7.67	1.49
Pre-aversion conditioning - Test per Environment	iod (B)	27.44	1	27.44	5.23
AXB		.12	1	.12	.02
Within cell		185.56	36	5.15	
Total		220.79	39		

P < .01

## Table 7.2

Results of Analysis of Variance

## Water Data

Source of Variation		SS	df	MS	F
Aversion conditioning Environment	(A)	1.05	1	1.05	.48
Pre-aversion conditioning - Test per Environment	iod (B)	4.00	1	4.00	1.85
AXB		.72	1	.72	.33
Within cell		77.89	36	2,16	
Total		83.65	39		