Ph.D. Psychology

STRAIN, SEX AND ALCOHOL INTAKE IN THE LABORATORY RAT

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Abstract

The effects of prolonged exposure to ethanol in 96 rats, males and females of four rat strains, was investigated. In Experiment I, it was found that the sex-strain subgroups differed in the highest concentration of ethanol drunk in a free-choice situation.

The 75-day free-choice exposure to ethanol of Experiment II increased the level of alcohol-directed behavior in all strains, but the size of the increase differed among the strains. The increases in preference were found to be stable, in spite of several manipulations of the ethanol solutions used for testing. The sex-strain subgroups did not differ in their responses to these manipulations.

It is argued that the increase in ethanol-directed behavior following exposure reflects physiological changes resulting from chronic ingestion of ethanol which require that larger amounts of the drug must be ingested by alcohol-exposed rats than naive rats in order to obtain the same pharmacological effects.

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by

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Preface

Research on alcohol intake in animals has been motivated largely by the desire to understand the causes and treatment of alcoholism in humans. For the most part, however, the research has failed to reach this goal. The major reason for this failure is the fact that laboratory animals do not drink ethanol to an extent which approximates that of man. The result is that it has proved impossible to produce an "alcoholic" animal. On the other hand, a large body of research has accumulated concerning factors which are important in determining ethanol preference in animals.

The first group of variables which are important in determining the level of alcohol-directed behavior includes those described as "constitutional": Those variables which describe an animal's biological individuality, such as species, strain, sex, and age. In the first place, the species to which an animal belongs may determine his response to ethanol. Man is the only species of animal that will drink large quantities of ethanol voluntarily. Chimpanzees and orangutans

(Fitz-Gerald, Barfield & Warrington, 1968), cats (Masserman & Yum, 1946) and guinea pigs (Arvola & Forsander, 1961) all show an aversion to ethanol, while the hamster avidly drinks 10% ethanol.

Mice and rats are the most commonly used laboratory subjects, and it appears that the strain of these subjects is important in determining ethanol preference. Among the many mouse strains, the C57BL substrains consistently show high preference for ethanol (McClearn & Rodgers, 1959). In general, other mouse strains, and all rat strains do not show a preference for solutions of ethanol of concentrations higher than seven to ten per cent (McClearn & Rodgers, 1961; Richter, 1956; Richter & Cambell, 1940). Rat strains which have been bred selectively for some behavioral characteristic, such as the Maudsley Reactive and Non-reactive strains, have also been shown to differ in their response to ethanol (Brewster, 1968). In addition, it has been possible to breed selectively for extremes in ethanol preference (Eriksson, 1968; Mardones, Segovia & Hederra, 1953).

The sex of the animal also appears to be an important constitutional variable in determining alcohol-directed behavior. Although mice do not show significant sex differences (McClearn & Rodgers, 1959), female rats generally show higher preference for ethanol than do males (Brewster, 1969; Eriksson & Malmstrom, 1967).

Finally, age is an important constitutional variable: Rats show their highest preference for ethanol between three and five months of age (Goodrick, 1966; Parisella & Pritham, 1964).

Constitution-related differences in preference for ethanol are correlated with differences in some aspects of alcohol-related physiology. The best example of this type of correlation is that between preference for ethanol and activity of the enzyme primarily responsible for ethanol metabolism: Mice which show high preference for ethanol show higher enzymatic activity than mice which prefer ethanol to a lesser degree (McClearn, Bennett, Hebert, Kakihana & Schlesinger, 1964). Wistar female rats, which show a higher preference for ethanol than do Wistar males, also eliminate ethanol from

the body at a faster rate than do the males (Eriksson & Malmstrom, 1967).

Several manipulations have been used in an attempt to modify the ethanol intake of animals. Many of these manipulations have been suggested by clinical findings in man. One class of such manipulations involves alterations in the physiological state of the subject. Dietary (Mirone, 1956; Westerfeld & Lawrow, 1953) and endocrinological manipulations (Goldberg & Stortebecker, 1953; Richter, 1956) have not produced consistent changes, nor have the administration of drugs (Rogers & Pelton, 1958), nor interventions in the central nervous system (Amit, Stern & Wise, 1970; Cicero & Myers, 1969). The success of physiological manipulations often depends on the constitution of the subject (Brown, 1969).

The "stress-reduction hypothesis" of human alcoholism has prodded many researchers to attempt to confirm this hypothesis in animals. Although some stressful situations produce increases in ethanol preference (Brown, 1968), most investigators have not been able to demonstrate stress-related increases in

ethanol preference in rats (Persensky, Senter & Jones, 1969; Senter, Smith & Lewin, 1967).

Several important changes in physiological functioning have been reported to occur after long-term exposure to ethanol, such as increased rate of ethanol metabolism (Hawkins, Kalant & Khanna, 1966) and elevated levels of enzymatic activity (Khanna, Kalant & Bustos, 1967). However, these findings have not been replicated by all experimenters (Figueroa & Klotz, 1962a).

Behavioral changes have been shown to occur following prolonged exposure to ethanol. First, consumption itself is increased in both forced-choice (Richter, 1957) and free-choice (Veale & Myers, 1969) situations. Second, the phenomenon known as "behavioral tolerance" has been demonstrated a number of times: The behavior of an animal chronically exposed to ethanol is less disrupted in a given task by a challenge dose of ethanol than is that of an animal without prior exposure to ethanol. Behavioral tolerance has been demonstrated in a wide variety of tasks, ranging from

avoidance learning (Moskowitz & Wapner, 1964) to the ability to remain on a rotating rod (Kinard & Hay, 1960).

Subjects differing in constitutional factors have only rarely been used in investigations of behavioral changes resulting from long-term exposure to ethanol, in spite of the fact that constitutional factors are important in determining preference for ethanol. In addition, most studies designed to study the effects of prolonged exposure to ethanol have relied on the administration of ethanol by forced-choice, intragastric infusion, or injection. This thesis will deal with these two points: (1) Providing long-term exposure to ethanol in a free-choice situation, and (2) observing differences in the effects of this exposure on subjects of different constitutions.

The subjects for the experiments were males and females of four rat strains, two of which, Wistar and Hooded, are commonly used in psychological research. The other two strains were descendents of the Tryon Maze-Bright and Maze-Dull strains. These strains were originally bred for errors in a multiple T-maze (Tryon,

1929), but have since been shown to differ in a wide variety of learning tasks (Wehmer & Markowitz, 1964; Wolfer, Reid & Porter, 1963). In addition, these strains have been shown to differ in the level of neural transmitters (Bennett, Crossland, Krech & Rosenzweig, 1960). They also appear to differ in their response to stress (Wolfer et al., 1963), and in basic metabolic features (Wolfer, Reid, Gledhill, & Porter, 1964). These strains, differing as they do in so many characteristics, are ideal subjects for studying interactions between constitutional variables and prolonged intake of ethanol.

The preceding description of research on ethanol intake in animals is only an outline of a large, and often contradictory, body of knowledge in this area. The topics and issues raised are discussed in greater detail in the Historical Introduction which follows.

Historical Introduction

Alcoholism is a major health problem in North America. It has been viewed as a sociological, psychological, medical, and even a political problem. Alcoholism is also a problem only for man. As a result, early attempts to study the problem of alcoholism had to rely on information from clinical observations of alcoholics, since one cannot ethically induce alcoholism in a human being for experimental purposes.

More recently, the study of alcoholism has come to the attention of biochemists, pharmacologists and physiological psychologists who, using animals as subjects, have been able to contribute important information concerning certain aspects of the action of alcohol on biological organisms.

It has been found that, after oral ingestion, absorption of alcohol into the blood takes place almost immediately. Thirty per cent of the alcohol is absorbed from the stomach, the rest from the intestines. Alcohol is distributed throughout the body roughly in proportion to the water content of the various parts (Sardesai, 1969). The greatest proportion of ethanol metabolism takes place

in the liver, while the brain does not participate significantly in the process (Masoro & Abramovitch, 1953).

The major metabolic pathway of alcohol is well established (Mardones, 1963). Alcohol is oxidized to acetaldehyde by the enzyme alcohol dehydrogenase (ADH). The rate of metabolism increases linearly with blood-alcohol concentration until the concentration of alcohol in the blood reaches 20-25 mg/ml of blood (Westerfeld & Schulman, 1959). Acetaldehyde is in turn oxidized very rapidly to acetate by the enzyme aldehyde dehydrogenase (ALDH). The acetate thus formed may be used in the body in the same manner as acetate from any other source, by conversion to fatty acids or by oxidation in the Krebs cycle, resulting ultimately in carbon dioxide and water.

Because of its rapid absorption and metabolism, alcohol is an efficient source of energy, able to supply 7 cal/gm as compared with 4 cal/gm supplied by carbohydrates. The energy which it produces can substitute for the energy derived from fats, carbohydrates and proteins, but alcohol cannot provide amino acids, vitamins or minerals.

The most familiar effect of alcohol is a depression of the central nervous system. In rats, this depression manifests itself in many forms. When forced to drink

ethanol, rats decrease their level of spontaneous activity, as measured in a running wheel (Richter, 1926). Carpenter and MacLeod (1952) found that, among rats trained to run a maze, significantly more failed to run the maze after having been given ethanol than after water. It has been found that the angle of a tilted plane on which a rat can remain without slipping off decreases with increasing doses of ethanol (Arvola, Sammalisto & Wallgren, 1958). When individual behaviors in an open field were observed, Eriksson and Wallgren (1967) found that different responses were selectively affected at different dosages of ethanol.

Despite these widespread actions of ethanol on organisms, no significant effects on growth or behavior can be observed in rats given a choice between ethanol and water from weaning to maturity (Richter, 1926; Mirone, 1962).

The use of animals in the study of alcoholism has had another important result: the generation of interest in the factors which influence or modify the preference for ethanol in animals as a problem for study on its own merits. It is with some of these factors that this thesis is concerned.

Constitutional Variables and Ethanol

The most basic factors that determine the behavioral or physiological reaction of an animal to ethanol are the constitutional factors inherent in the animal: those variables that naturally prevail in an organism prior to any manipulation or experimentation. They include species, strain, sex, age, and hormonal state. Two types of material relating to these variables will be discussed: (1) the relation of constitutional variables to voluntary intake of ethanol, and (2) the interactions of constitutional variables and physiological reactions involving ethanol.

Species

Within the animal kingdom, man is virtually unique in ingesting large quantities of alcohol voluntarily. Most animals will reject all but the very lowest concentrations offered, and some reject it altogether. There is evidence, however, that for any given species there is a characteristic level of preference, and it is these levels that will be discussed in this section.

Most sub-human primates will not voluntarily ingest large quantities of alcohol. Pigtail monkeys, when tested for preference between water and 5%, 10% or 20%

ethanol, generally prefer water or 5% ethanol, and drink very little of the solutions of higher concentrations (Anderson & Smith, 1963). Clark and Polish (1960), while investigating the consumption of 20% ethanol during avoidance conditioning in rhesus monkeys, found that the pre-training preference for ethanol in these animals was very low. Apes drank significantly less fruit juice in the form of a 10% solution of alcohol than fruit juice without alcohol (Fitz-Gerald, Barfield & Warrington, 1968). In this study, chimpanzees often drank enough of the alcohol solution to become intoxicated, while orangutans never became intoxicated.

Several other species which have been studied also show patterns of alcohol intake indicative of low preference. Masserman and Yum (1946) observed that, prior to the induction of experimental neurosis, cats drank very little of a 5% solution of ethanol in milk, in comparison with their intake of milk without alcohol. Arvola and Forsander (1961) observed that hedgehogs preferred water to a 10% solution of ethanol, although the intake of the alcohol solution was relatively high, accounting for roughly one third of the total fluid intake. Rabbits seemed indifferent to the alcohol, drinking about one half of their fluid

consumption in the form of the alcohol solution.

Rodents are the most common group of animals used in studies of alcohol drinking and many different species have been investigated. Hamsters and Guinea Pigs present an interesting contrast (Arvola & Forsander, 1961). When given a choice between water an a 10% solution of ethanol, the Guinea pig displayed a marked aversion to the ethanol, while hamsters showed an equally marked preference for the ethanol, taking 88% of daily fluid consumption in the form of this solution. When hamsters were offered ethanol solutions of concentrations as high as 60%, the animals drank some of these solutions, even when water was present (Arvola & Forsander, 1963).

The standard rat strains used in laboratory work (Wistar, Sprague-Dawley, Long-Evans Hooded) in general show very low preference for alcohol, usually rejecting concentrations above 7 or 10 percent. (Richter & Campbell, 1940; Richter, 1956), although marked individual differences have been reported (Mendelson & Mello, 1964). Several more "specialized" strains of rats have been bred, and the voluntary intake of these strains will be discussed below. It is difficult to make a general statement about the laboratory mouse because of the magnitude of the strain

differences. These will also be discussed below.

Since laboratory strains of rats and mice are most commonly used in alcohol research, it is important to compare them with their wild relatives. In early work it was found that wild Norway rats, if forced to drink ethanol over a long period of time, developed a marked preference for ethanol (Richter, 1957). This effect has been impossible to demonstrate in the laboratory rat. In fact, Eimer and Senter (1968) found that they were not able to replicate Richter's findings with wild pack rats. Boice and Aspey (1968), however, pointed out that the pack rat differs from the Norway rat in many important behavioral and physiological respects, and conclude that the work of Eimer and Senter did not constitute a valid replication of Richter's work. Studies of a different species of rat, the cotton rat, (Emerson, Brown, Nash & Moore, 1952) show that these animals display relatively low preference for 10% ethanol. These same experimenters also studied wild deer mice and found a relatively high preference for ethanol in this species.

Strain

Special strains of rats and mice have been bred to meet the needs of experimenters in all areas of biological

research. Although these strains are usually specifically bred for only one or a few characteristics, it is usually found that the resulting strains show differences in many other characteristics. Accordingly, large bodies of research have accumulated concerning patterns of differences between strains. A common difference which is investigated is preference for ethanol. In addition, several variables associated with the metabolism of ethanol have been found to correlate with preference. This section is devoted to a discussion of some of these strain differences in behavioral and physiological responses to ethanol.

That strains of mice differ in their voluntary intake of ethanol has been repeatedly demonstrated (McClearn & Rodgers, 1959). Given a choice between water and a 10% solution of ethanol, C57BL mice preferred the ethanol solution, whereas C3H/A, BALB, and DBA mice preferred water (McClearn & Rodgers, 1961). In addition, the results of this experiment suggested that genetic factors might play a role in ethanol preference in that the F_1 offspring of crosses between the C57BL mice and mice of each of the other strains demonstrated a preference for ethanol which was intermediate between that of the two parental strains. The data from this experiment were

reexamined by Brewster (1968). His calculations confirmed a genetic involvement, with heritability estimates which suggested that approximately 80% of the variability in ethanol preference was due to hereditary factors.

Using a different experimental technique, Rodgers and McClearn (1962) demonstrated that the different strains preferred very different concentrations of ethanol. During a three-week period, mice of four strains were presented with a choice between water and a solution of ethanol, the concentration of which varied between 2.5% and 15%. The C57BL mice showed the strongest preference for 12.5% ethanol. BALB/c and A/Crgl/3 mice totally rejected all concentrations presented to them. The C3H/2 mice, however, showed little preference for ethanol at the beginning of testing, but increased their intake considerably by the end of the testing period.

Behavioral differences between strains of mice seem to be correlated with certain differences in physiological characteristics. Liver tissue taken from C57BL mice contained more alcohol dehydrogenase (ADH) than did that from DBA mice (McClearn, Bennett, Hebert, Kakihana & Schlesinger, 1964). The livers of F_1 offspring of crosses between these two strains contained concentrations of this enzyme

which were midway between the concentrations of the parental strains (Sheppard, Albersheim & McClearn, 1968).

The activity of aldehyde dehydrogenase was studied in the livers of C57BL and DBA mice and in the F_1 offspring of a cross between these two strains (Sheppard et al., 1968). The results indicated that the concentration of this enzyme in livers of C57BL mice was 250% greater than that of the DBA mice. The livers of the F_1 offspring showed values intermediate between those of the parental strains. In addition, CBA/2 mice showed significantly higher acetaldehyde levels after an injection of ethanol than did C57BL mice.

These data suggest that differences in preference of the two strains might be due to differences in the rate at which alcohol is metabolized. The differences in ALDH concentration suggest that concentrations of the toxic acetaldehyde may never be high enough in the C57BL mice to produce noxious symptoms after the drinking of ethanol.

Another mechanism to account for strain differences in preference for ethanol has been proposed by Kakihana, Brown, McClearn and Tabershaw (1966). These investigators injected C57BL/Crgl and BALB/Crgl mice with a dosage of ethanol sufficient to induce a comatose state in both strains. It was found that C57BL mice regained conscious-

ness significantly sooner than the BALB mice. Blood and brain alcohol levels were significantly higher at waking in the C57BL mice than in the BALB mice. When these levels were measured in the two strains at the same intervals after the injection, regardless of the length of time the animals had been awake, no significant strain differences appeared. The authors concluded that the two strains of micediffered in their sensitivity to ethanol, and that this difference might determine preference for ethanol, since no differences in rate of ethanol metabolism could be found.

Preference for ethanol in several strains of laboratroy rats has also been investigated. Myers (1962) found that G-4 rats (Gruneburg, 1949) showed a higher preference for ethanol solutions which ranged in concentration between 1.25% and 20% than did Wistar rats. Ethanol preference and amino acid excretion were studied in six strains of rats (Reed, 1951). The six strains studied showed significant differences in both preference for 10% ethanol and excretion of certain amino acids, but no correlations were found between the strain differences in preference and in amino acid excretion.

Several attempts have been made to breed rats

selectively for extremes of ethanol intake. Eriksson (1968), using Wistar rats as the parental stock, found significant differences in ethanol consumption in the F_8 generation when the animals were bred on the basis of intake of 10% ethanol in a free choice situation. The pattern of preference in the two strains of rats is described by Eriksson (1969). Those from the sub-strain selected for low preference drank virtually no ethanol. On the other hand, the high preferring strain was clearly not homogeneous with respect to ethanol preference, showing a great deal of individual variability. The average preference of members of this sub-strain was, however, higher than that of the parental stock.

Mardones, Segovia and Hederra (1953) were able to breed "drinker" and "non-drinker" strains. The rats from these strains differed in their voluntary intake of ethanol when their diet was deficient in Factor N_1, which is a particular component of the B-complex vitamins. It was found (Segovia-Riquelme, Vitale, Hegsted & Mardones, 1956; Segovia-Riquelme, Campos, Solodowska, Gonzales, Alvarado & Mardones, 1962.) that the two strains of rats metabolized ethanol at the same rate.

Nichols and Hsaio (1967) selectively bred Sprague-

Dawley rats for susceptability to morphine addiction. When naive females of the two sub-strains were subjected to the procedure used to induce addiction, but with ethanol replacing the morphine, it was found that subjects of the substrain which had demonstrated high susceptability to morphine drank significantly more ethanol than subjects from the strain resistant to morphine addiction.

Brewster (1969) studied differences in ethanol preference and intake of absolute alcohol between the Maudsley Reactive (MR) and Maudsley Nonreactive (MNR) strains. These strains were bred for extremes of defecation in the open field (Broadhurst, 1960). In one experiment, six-month-old rats of both strains were given a choice between water and one of seven concentrations of ethanol. The MR subjects showed a significantly higher preference for the 5% and 10% solutions than did the MNR subjects. There were no significant strain differences in the intake of ethanol, however. In a second experiment, a choice was offered between water and a 5% solution of ethanol to four-month-old rats of each strain. In contrast to the previous study, the MNR animals showed both a higher preference for the alcohol solution and drank more of it than did the MR animals. The author (Brewster, 1969) suggests that the different results from the two experiments

can be explained by the age difference of two months between the subjects of the two experiments.

Brewster (1968) further analyzed the possible genetic mechanisms involved in ethanol preference and intake in the Maudsley strains, using a diallele cross method. It was found that the heritability estimates for both measures were quite high, hereditary factors accounting for about 70% of the variability.

Broadhurst and Wallgren (1964) injected rats of the MR and MNR strains with ethanol and measured blood-alcohol levels. There were no significant strain differences on this measure.

The Roman High Avoidance (RHA) and Roman Low Avoidance (RLA) strains were selectively bred for performance in avoidance conditioning (Bignami & Bovet, 1965). The alcohol preference for and intake by these strains of alcohol solutions of four concentrations were investigated by Brewster (1969). The RHA animals showed significantly higher preference and intake than the RLA animals, but only when the concentration of the alcohol solution was 10%.

Sex

Significant differences in the preference for alcohol

of male and female animals have been reported. In reviewing these data, however, no consistent pattern emerges which would indicate that one sex has a higher preference for, or intake of alcohol.

Male chimpanzees (Fitz-Gerald et al., 1968) and hamsters (Arvola & Forsander, 1963) showed higher preference for ethanol than did females of the same species. Clay (1964) and Schadewald, Emerson and Moore (1953) found that male rats drank more ethanol than females.

On the other hand, Eriksson and Malmstrom (1967), found that female Wistar rats had higher preference ratios for ethanol than did males of the same strain, and also consumed more ethanol per gram of body weight. Females of both the preferring and non-preferring strains bred by Eriksson drank more ethanol than did the males of the same strain (Eriksson, 1968).

Consistent sex differences were found by Brewster (1969) in his work with inbred strains. In all experiments with the Maudsley strains, the females drank significantly more alcohol for their body weight than did the males. In both of the Roman strains, the females showed higher preference for four concentrations than did the males. Their intake of 1% and 10% ethanol was also significantly

higher than that of the males.

In those studies with mice in which the role of sex was investigated, no significant differences were found (McClearn & Rodgers, 1959; Rodgers & McClearn, 1962).

The hormonal state of females is constantly changing, and voluntary consumption of ethanol varies in response to changes in hormonal state. Aschkenasy-Lelu (1962) monitored the preferences exhibited by male and female rats for water and 5% ethanol for 20 days. It was found that there were no sex differences in the overall preference for ethanol, but the females exhibited a pattern of preference which was correlated with the oestrus cycle. Thus, every four days, the females dropped to a lower level of preference for 24 hours, during which time they were in estrus. Pregnant hamsters raised from weaning with a choice between water and alcohol decreased their intake of 10% ethanol shortly before parturition (Carver, Nash, Emerson & Moore, 1953). During this period, there was also a proportionate decrease of the water intake, so that there was no change in the animal's preference for ethanol. During lactation, there was a decrease both in intake and preference.

Sex differences have been found to be present in

two types of physiological reactions to alcohol: elimination or metabolism of ethanol, and reactions to toxic doses. Eriksson and Malmstrom (1967) found that female Wistar rats eliminated a test injection of ethanol more rapidly than did males. Broadhurst and Wallgren (1964) injected males and females of both Maudsley strains with one of six dosages of ethanol. When the concentration of alcohol in the blood was later measured, it was found that the blood-alcohol levels in the males were significantly higher than those in the females.

Abderhalden and Wertheimer (1927) injected male and female mice with ethanol, and observed the resistance of the animals to the injections. All doses were sufficient to produce a comatose state in the animals. The injections were given daily until the animals died. It was found that the females outlived the males in all cases, and in many cases survived a week of daily injections after the males died.

Age

Several studies of differences in ethanol preference as a function of age have been reported. It is often difficult, however, to separate changes in ethanol preference in animals of different ages from age-related changes in other activities of the organism. Several alternatives

have been suggested, such as changes in exploratory behavior with age, but there is also evidence that there are changes in physiological reactions to alcohol which occur as a function of age.

Goodrick (1967) studied the preference for water and four concentrations of ethanol in Sprague-Dawley rats at 1, 3, 5, 10, 15, and 24 months of age. Up to five months of age, the rats showed an increase in preference with increasing age. The level of preference declined at 10 months and 15 months, but increased again at 24 months. Goodrick points out the similarity between curves relating ethanol preference and exploration to age (Goodrick, 1960) implying that young animals may be responding to an ethanol solution as a novel stimulus. Thus, as an animal's responsiveness to novel stimuli declines, the ethanol preference also declines. In addition, Goodrick attributes the increase in ethanol preference at 24 months to a decrease in sensitivity of the taste buds resulting from the aging process. Parisella and Pritham (1964) found that young adult (3 - 4 months of age) Wistar rats showed higher preference for 8% ethanol than did rats which were younger (1 - 2 months) or much older (10 - 15 months), thus confirming Goodrick's (1967) findings.

Wallgren and Forsander (1963) reported a significant increase in both preference and intake in rats which had been forced to drink 10% ethanol for one year beginning at 540 days of age. In contrast, 90 day-old rats given a choice between 10% ethanol and water for 75 days and then forced to drink the ethanol for an additional 60 days did not show significant increases over rats allowed a free choice between 10% ethanol and water during the entire experimental period.

The fact that there were differences between the groups in older rats but not the younger ones was interpreted as an effect of age. But, because the procedures used in studying the two age-groups of animals differed in such important factors as length and nature (free or forced choice) of the exposure to ethanol, it is difficult to accept the conclusion that this experiment demonstrates age-related differences in voluntary intake of ethanol.

Age-related changes in preference for 10% ethanol were studied in BALB/c mice (Kakihana & McClearn, 1963). This is a very low-preferring strain, some adults rejecting concentrations as low as 2.5%. The preference for 10% ethanol was measured in different subjects ranging in age from ? to 30 weeks. Young pups showed consistently higher preference than adults until week 9, when the preference

ratios began to approach adult values. When the same subjects were tested at 4 and 16 weeks, the preference ratios (proportion of total fluid intake consumed as ethanol solution) dropped from .50 at week 4 to .12 at week 16.

Age-related changes in physiological reactions to ethanol have been easier to demonstrate and interpret than behavioral reactions. Chesler, LaBelle, and Himwich (1942) studied the effects of toxic doses of ethanol on mortality in fetal, newborn and adult rats. Newborn rats were markedly less sensitive to toxic doses than either fetal or adult rats, surviving significantly longer after the injection.

Raiha, Koskinen and Pikkarainen (1967) studied levels of ADH in the livers of animals of different ages. This enzyme was first detectable in the livers of fetal rats at day 18 of gestation. At birth, the level of activity of ADH was 25% of the adult level. The adult level was reached at 18 days of age.

An apparent interaction between age and sex is reported by Wallgren (1959). The behavioral tolerance of males to injections of ethanol is constant between 14 and 22 weeks. At 14 and 18 weeks of age, however, the females showed a greater degree of tolerance to ethanol

than the males when tested on a tilted plane. During the period when the females were more tolerant, there were no sex differences in blood-alcohol levels measured 90 minutes after the injection.

Summary

Organismic variables have been shown to play an important role in determining behavioral and physiological responses to ethanol. Several of these factors will also be shown to be important in determining an animal's response to long-term exposure to alcohol, and in the animal's responses to manipulations in experiential or physiological conditions. Several examples could be given to illustrate the existence of interrelations between physiological and behavioral reactions. Physiological evidence suggests that young animals are more resistent to the effects of ethanol, and the behavioral evidence has demonstrated that in general, the younger animal shows greater preference for ethanol. C57BL mice show consistently higher preference for ethanol than other mouse strains, and the physiological evidence demonstrates that the livers of C57Bl mice contain a higher concentration of alcohol dehydrogenase, and that these mice are somewhat more tolerant to ethanol injections than are low preferring strains.

Different species of animals also show differences in preference for ethanol. Metabolic pathways vary from species to species. In addition, different strains of rat also possess different biochemical "personalities". Even though many of these differences are unrelated directly to the metabolism of ethanol, they may be important in determining an animal's response to ethanol. "It can be said as a general statement that the differences in preference for alcohol among different animals and different species basically depend on differences in chemical individuality" (Forsander, 1966, p. 526).

Modification of Alcohol Preference

Once the level of preference for ethanol has been established, it is desirable to investigate the conditions which result in a change in preference. Many of the experiments that have been performed in an attempt to discover these conditions have been inspired by reports of clinical observations. When these conditions have been clearly defined, they may be useful in prevention and treatment of alcoholism in humans.

The following discussion of research attempting to modify alcohol preference will be presented in two sections. The first section will deal with manipulations

of the physiological state of the animal, the second with manipulations which are mainly on a level which might be called "psychological". Research on these latter manipulations has utilized various learning paradigms, whereas research on the former has made use of dietary, endocrinological and neural interventions.

Physiological Manipulations

Several studies have been performed which show the effects that diets deficient in one or more components have on an animal's behavioral and physiological responses to alcohol.

Starvation seems to affect both preference for, and the metabolism of, ethanol. Westerfeld and Lawrow (1953) found that rats restricted to 50% of their normal caloric intake increased their preference for 10% ethanol, while those restricted to 75% of the normal ration, and those on normal ration did not. Several researchers have found a slowing of ethanol metabolism during fasting. Kerner and Westerfeld (1943) found that the activity of hepatic ADH in rats starved for seven days was 50% of the normal level. Liver slices from starved rats metabolized ethanol only half as fast as those from adequately fed rats (Smith & Newman, 1959).

Mirone (1957) studied the effects of manipulations

of the various components of the diet on ethanol intake of mice. Subjects maintained on a diet that was either rich or deficient in protein increased their voluntary intake of ethanol. Mice showed a significant decrease in their preference for ethanol when they were fed a high fat diet, while there were no differences in ethanol preference between subjects fed a fat-free diet and those fed a normal diet. No differences in ethanol consumption were found between mice fed normal or high-carbohydrate diets.

The vitamins in the B-complex are the vitamins most frequently studied in relation to alcohol intake because of their importance in metabolism. The majority of investigators have found that a deficiency of B-complex vitamins induced the subjects to increase their intake of ethanol. Rats fed a diet deficient in B vitamins increased their preference for ethanol when offered a choice between water and 20% ethanol (Brady & Westerfeld, 1947). Similar increases were found with mice fed a vitamin-deficient diet (Mirone, 1957).

An interesting sex difference in mice in response to a deficiency of thiamine (vitamin B_1), has been reported by Brown (1969). Brown found that female mice on this diet showed higher preference for 10% ethanol than normally-fed

females, whereas the preference for the ethanol solution shown by males on the vitamin-deficient diet was not significantly different from that of normally-fed males.

The endocrine glands and the hormones they secrete perform vital and complex functions. Since these glands are involved in so many activities, it is reasonable to ask whether voluntary intake of ethanol can be modified by manipulation of endocrine function. Several experiments have been performed in an attempt to answer this question.

The role of pancreatic function in voluntary alcohol intake has been investigated by Forsander, Kohonen and Soumalainen (1958), who found that the administration of insulin increased the consumption of 15% ethanol by rats. The role of the thyroid has also been investigated by Richter, (1956), and by Zarrow and Rosenberg (1959). These investigators found that levels of preference for ethanol solutions were negatively correlated with thyroid activity.

The relationship between ethanol and activity of the adrenal cortex seems to be reciprocal in that, while manipulation of the adrenal system affects ethanol consumption, ethanol in turn affects the activity of the adrenals. Conditions producing "stress" in an animal, such as grouping in C57BL/Crgl mice (Thiessen & Rodgers, 1965), produced both

a significant increase in adrenal activity and a significant decrease in preference for ethanol. In a strain of mouse which does not display adrenal hyperactivity in response to grouping, there was no significant change in ethanol preference associated with the grouping (Thiessen & Rodgers, 1965). Exposure to extreme cold, which also increases adrenal activity, produced a significant increase in preference for 10% ethanol (Zarrow, Aduss, & Denison, 1960).

The steroid output of the adrenals has been found to increase in response to an intoxicating dose of ethanol, but the effect seems to be dependent on the dosage and the route of administration (Kalant, Hawkins, & Czaja, 1963). A dose of ethanol given intraperitoneally will significantly elevate the steroid output of the adrenals, but the steroid output will not increase when the same dose is given by intubation. A divided dose of ethanol effects adrenal activity less than does the same dose given in a single administration. The dosages of ethanol required to produce an adrenal steroid response are extremely high and are much higher than a rat will voluntarily drink under normal circumstances. This reciprocal relationship between ethanol intake and adrenal activity, however, should be noted.

Sex differences in ethanol preference in intact

subjects have been described above. In addition, several experiments have manipulated the level of gonadal hormones to investigate the role of these endocrines in reactions with ethanol. Schadewald, Emerson, Moore and Moore (1953) allowed male and female Sprague-Dawley rats to choose between water and 10% ethanol for 55 days, finding that males preferred the ethanol solution more than females. The five highest-preferring males and the five lowest-preferring females were gonadectomized. In 35 days of postoperative testing, these 10 animals showed no significant change in preference, but there was a slight tendency for the difference in preference between the two sexes to become smaller.

Goldberg and Stortebecker (1943) injected castrated female rabbits with either ethanol or a combination of ethanol and an estrogen hormone. There were no differences in the rate of metabolism of ethanol between the two groups of subjects, but the rabbits given the hormone recovered from the ethanol-induced intoxication more quickly than the rabbits not given the hormone. During recovery from intoxication, the order in which the various reflexes reappeared was different in the treated and non-treated subjects.

Since the liver is the primary site of alcohol metabolism, it is reasonable to investigate the effects of hepatic pathology on ethanol preference. Sirnes (1953)

injected rats subcutaneously with carbon tetracholoride over a period of four months producing cirrhosis of the liver. During this period, the rats were offered a choice between water and 20% ethanol. It was found that the cirrhotic rats drank four times as much of the ethanol solution as did the control rats. Campos, Solodowska, Munoz, Segovia-Riquelme, Cembrano and Mardones (1964) found, however, that the rate of eliminated labeled CO_2 in rats with CCl_4-induced liver cirrhosis was significantly slower than that of rats with normal livers four to six hours after an injection of labeled ethanol.

It has been well documented that the hypothalamus is intimately involved in the monitoring and regulation of levels of various substances in the body. Several investigations have been performed to explore the degree to which changes in hypothalamic function might alter voluntary intake of ethanol. Marfaing-Jallat, Larue and Le Magnen (1970) found increased preference for 8% ethanol in rats in which lesions had been made in the ventromedial hypothalamus. Cholinergic stimulation of hypothalamic sites which produced polydipsia for water caused rats to reject all concentrations of ethanol, including those which had been preferred before the stimulation (Cicero & Myers, 1969). Using electrical

stimulation of the hypothalamus in rats, Amit, Stern and Wise (1970) were able to induce a long-term change in preference for solutions of ethanol, the concentrations of which were above the rejection threshold of the subjects at the beginning of the experiment. The increases in preference were extremely persistent; the animals drank at the same high level after a period of withdrawal, and they continued to drink ethanol even when it was adulterated with quinine.

Intraventricular infusion of ethanol allows the investigation of the question of whether or not persistent increases in the level of ethanol in the central nervous system leads to alterations in the neural mechanisms controlling the level of ethanol in the blood. Myers (1963) was the first to report an increase in ethanol preference in rats following infusions with ethanol, and these findings were replicated by Myers and Veale (1969). This technique has also been used with dogs (Jones, Essig & Creager, 1970) and monkeys (Koz & Mendelson, 1967), but the effect could be produced with neither species. Thus, while providing quite provocative results in rats, it appears that the effect may be specific to this species.

Attempts to alter voluntary intake of ethanol by means of drugs which have behavioral effects have been

unsuccessful. Rogers and Pelton (1958) measured the relative intake of water and 10% ethanol by rats before and during the addition of several tranquilizers, stimulants and LSD to the rats' food. The tranquilizer Sparine was the only drug which increased alcohol intake. This drug is bitter resulting in a decreased food intake. Thus it might be that the rats drank the ethanol for its nutritional effects. Moore, Moore, Nash and Emerson (1952a) found no significant change in preference between water and 5% ethanol in rats given amphetamine.

It was found that when normal gustatory sensations have been bypassed by intragastric infusion, rats did not show the patterns of preferences and aversions to sweet and salty solutions which appeared when these solutions were ingested orally (Borer & Epstein, 1965). It is possible, then, that rats avoid concentrations of ethanol higher than seven to ten percent (Richter & Campbell, 1940) because the smell and taste of ethanol is aversive. Several experiments have been performed to test this hypothesis.

One approach to this problem has been the determination of ethanol preference of subjects in which the normal olfactory or gustatory cues are bypassed or eliminated. The drug methylpentynol reduces gustatory sensitivity in

rats, and rats given it significantly increased their preference for high concentrations of ethanol (Dicker, 1958). In this experiment, several rats showed very high preference for ethanol under non-drug conditions. When methylpentynol was given to these rats, they refused to drink the ethanol solution, again suggesting that the taste of ethanol is used by rats as a distinctive stimulus for either preference or rejection.

Smell also seems to play an important role. Rats made anosmic by the removal of the olfactory bulbs drank ethanol solutions at concentrations which would be rejected by normal rats (Kahn & Stellar, 1960). These anosmic rats, however, were indifferent to concentrations low enough to be preferred to water by normal rats, again indicating that the smell of an ethanol solution may be a distinctive cue for the acceptance or rejection of certain concentrations.

Amit and Stern (1969) bypassed both olfactory and gustatory cues in rats by delivering ethanol by ingragastric infusion. These experimenters found that rats would ingest significantly more 17% ethanol via this route than via the oral route.

Further evidence for the importance of taste in

alcohol preference is provided by the observations of Le Magnen and Marfaing-Jallat (1961). These experimenters separated rats into groups of high and low ethanol drinkers. Thresholds for the rejection of quinine were then established, and it was found that the low drinkers had significantly lower quinine-rejection thresholds than did the high drinking group. In addition, a significant correlation was found between alcohol and quinine rejection thresholds (Le Magnen & Marfaing-Jallat, 1961).

The role of taste has also been investigated by observing changes in ethanol preference when a sugar solution is offered in addition to ethanol and water. The most common finding is that the availability of sugar solutions lowers the preference for ethanol shown under two-choice conditions (Mardones, Segovia-Riquelme, Hederra & Alcaino, 1955; Rodgers & McClearn, 1964). That this change is the result of gustatory rather than nutritional factors is demonstrated by the fact that the intake of ethanol also decreases when a solution of saccharin, which has no food value, is offered as a third choice (Lester & Greenberg, 1952). In addition, it is known that the addition of sugar to an ethanol solution increases consumption of the ethanol solution

(Rodgers & McClearn, 1964).

Psychological Manipulations

Attempts to induce permanent increases in ethanol intake by psychological manipulations in animals has had two objectives. The first has been to confirm the "tension reduction" hypothesis of the etiology of human alcoholism. Since this approach has not been particularly successful, the second objective has been to produce an "alcoholic" animal which might then be used to determine alternative hypothesis of the etiology and treatment of human alcoholism.

In man, alcohol abuse has been characterized as repetitive use of alcohol resulting from its "tension reducing" properties. The alcoholic is said to experience a decrease in anxiety after drinking, and this reduction reinforces the use of alcohol in anxiety-generating situations. To confirm this hypothesis in animals, it must first be demonstrated that ethanol possesses the ability to reduce experimentally induced conflict. Then it must be demonstrated that the drinking of ethanol is reinforced by this reduction in stress. Evidence that the reduction in tension produced by ethanol is reinforcing would consist of an increase in voluntary intake of ethanol in animals

under stress.

That ethanol is able to reduce "anxiety" in rats has been demonstrated several times. Masserman and Yum (1946) found that cats in which experimental neuroses had been induced began to function more normally after the administration of ethanol. Conger (1951) produced approach-avoidance conflict in rats by shocking them in the area of a straight alley in which food was to be found. He found that the administration of ethanol produced a reduction in the strength of the avoidance tendency, resulting in approach towards the food. Korman (1960), however, found that the ability of ethanol to reduce conflict in rats was dependent on the degree of emotionality of the animal; alcohol was effective in reducing tension only in subjects who were described as being low in emotionality before the experiment began. Weiss (1958) induced conflict in hungry rats by putting them in an open field in the middle of which food was placed. There were no significant differences in the ability to resolve the conflict (i.e. to leave the edge of the field and go to the food) between rats injected with ethanol and those given water.

That reduction of anxiety may reinforce the drinking of ethanol has been demonstrated by some authors. Moore, Moore, Nash and Emerson (1952b) found that repeated presentation of auditory stimuli which caused audiogenic seizures produced a significant increase in the preference of rats for 5% to 10% ethanol. Being spun on a 78 rpm turntable increased the preference of BALB/cJ mice for 5% ethanol (Brown, 1968). Cicero, Myers and Black (1968) determined baseline alcohol preference levels for a number of hooded rats, and observed the effect which avoidance training, unavoidable shock and cued unavoidable shock had on these levels. They found that neither avoidance conditioning nor unavoidable shock alone had an effect on level on preference for ethanol. When, however, the unavoidable shocks were preceded by a cue similar to the warning stimulus used in the avoidance conditioning, the rats showed a significant increase in their levels of preference for ethanol.

On the other hand, many experimenters have been unable to confirm experimental hypotheses derived from the "anxiety reduction" notion of alcoholism. Casey (1960) did find that the stress of electric shock increased

the preference of Sprague-Dawley rats for ethanol but the increase did not appear during the period of stress, but only after the stress had been terminated. Senter, Smith and Lewin (1967) required rats to drink 7% ethanol to avoid shock, thus, presumably, providing an association between alcohol and the offset or absence of shock. During the experimental sessions, the rats drank very large amounts of ethanol but when they were returned to their home cages, they did not demonstrate an increase in their preference for ethanol as compared with pre-experimental levels. Myers and Holman (1967) also found no differences in preference for ethanol between shocked and non-shocked subjects. Persensky, Senter and Jones (1969) found that when rats which had previously learned a discrimination task on the Lashley jumping stand were presented with an insoluble problem, they tended to perseverate in jumping to the same side. These animals demonstrated significantly lower levels of preference for 7% ethanol than animals which were given a soluble problem.

These conflicting data seem difficult to reconcile. It is worth asking, however, whether the experimental stress was, in fact, stressful to the subject. In no case

were independent measures of the degree of stress taken into account. In the studies of the effect of random unavoidable shock (Casey, 1960; Cicero et al., 1968; Myers & Holman, 1967) the shock alone did not increase ethanol preference. Brady (1958) demonstrated that unavoidable electric shock was not sufficiently stressful in itself to produce gastrointestinal lesions in monkeys. Perhaps, in rats, electric shock is not sufficiently stressful to provide negative reinforcement of ethanol drinking. Autopsy of the subjects of these experiments would provide evidence regarding the severity of the experimentally-induced stress.

In the experiments of Senter, Smith and Lewin (1967), the subjects ingested large quantities of ethanol in order to avoid shock and, in fact, became intoxicated. But the response of ethanol drinking did not seem to be reinforced, as shown in the lack of change in home-cage preference. This was interpreted as negative evidence for the anxiety reduction hypothesis. We have seen, however (Kalant et al., 1963), that large doses of ethanol are themselves stressful, in the sense that adrenal steroid output increases. Thus, the fact that

preference for ethanol does not increase may result from the fact that the high levels of ethanol may punish the drinking response.

These comments are speculative, but indicate the need for independent measurements of the degree of stress induced by and experimental procedure in order to assess accurately the role of "tension reduction" in the change of preference for ethanol in rats.

There have been several attempts to elevate alcohol preference in rats by means other than the relief of anxiety. These attempts have been guided by the notion that an animal drinking increased amounts of ethanol is in some sense like the human alcoholic. By studying such an animal, one may be able to observe, in simplified form, the development of the human illness and its treatment. Several techniques have been used to coerce the subjects to drink alcohol, either by positively reinforcing the drinking of ethanol, or by replacing water with ethanol in a schedule-induced polydipsia experiment.

Senter, Smith and Lewin (1967) required hungry rats to drink ethanol to get a food reward. When these rats were offered ethanol in their home cages, their

preference for ethanol showed a transient increase, lasting only one or two days. Further experimentation has indicated that if the animals remained in the experimental chambers during the post-experimental choice period, the increase in preference persisted for as long as 14 days (Senter & Persensky, 1968). These results were interpreted as evidence that alcoholism results from positive rather than negative reinforcing properties of alcohol.

Several investigators have studied changes in alcohol intake of rats using the method schedule-induced polydipsia (Falk, 1961). This phenomenon appears when hungry rats receive food pellets on a random, non-contingent schedule in the presence of water. Under these conditions rats drink excessive amounts of water in the periods between the delivery of food. Lester (1961) demonstrated that rats would drink enough ethanol in this situation to become intoxicated, but the excessive drinking did not appear unless the animal was required to bar-press for the food pellets. Senter and Sinclair (1967) replicated the experiments of Lester, finding, however, that the experience with ethanol in the experimental situation did not change the preference for ethanol after the experimental

sessions were terminated.

Since the changes in preference did not persist, this technique does not suggest an obvious model of human alcoholism. Methodologically, however, this technique poses intriguing questions, as shown by the work of Freed (1968), who produced intoxication in rats by means of schedule-induced polydipsia in an experiment designed to test the ability of ethanol to reduce approach-avoidance conflict in rats. The results were less dramatic than those resulting from intoxication produced by injection of ethanol. It is interesting that the oral route of administration, that used almost exclusively by man, should only produce mild tension reduction in rats.

The Effects of Prolonged Exposure to Ethanol

One conspicuous feature of alcoholism is that it requires a certain amount of time and exposure to alcohol in order to develop. Several behavioral and physiological changes occur concommitantly with the development of alcoholism. Thus, it is reasonable to ask whether prolonged intake of ethanol by animals, which do not develop "alcoholism", would produce changes in alcohol-related physiology or behavior. This section is devoted to a discussion of the effects of long-term exposure to ethanol.

Several aspects of the behavior of the rat change after prolonged exposure to ethanol. Of primary interest are the changes in preference for and intake of ethanol after a period of exposure. Exposure is also able to modify the behavior of rats in certain types of learning situations.

There have been several reports that rats exposed to ethanol either under free or forced choice conditions increase their preference for an ethanol solution. The most dramatic increase was that reported by Richter (1957) who found that wild Norway rats which had been forced to drink ethanol for some time, when given a choice between ethanol and water, drank only ethanol. Wallgren and Forsander (1953) reported that Wistar rats drank a larger proportion of their daily fluid intake in 10% ethanol after one year of forced exposure to ethanol. Arvola and Forsander (1961) reported changes in preference for 10% ethanol in rats and mice, although no details of the magnitude or direction of the changes were given. Rick and Wilson (1966) forced Wistar rats to drink one of several concentrations of ethanol over a six-month period. When these rats were given a choice between water and ethanol, they drank enough ethanol to provide

the same volume of absolute alcohol as they had consumed during the forced-choice condition.

Myers (1961) forced hooded rats to drink 5% or 20% ethanol for 10, 30, or 90 days. A 24-hour deprivation schedule was then imposed, and the subjects were permitted to bar-press for food, water, or an ethanol solution of the concentration which they had been forced to drink. During this testing, the group maintained on 5% ethanol showed a higher preference for this solution than did controls maintained on water. Animals maintained on 20% ethanol preferred water.

Veale and Myers (1969) induced a persistent change in preference for ethanol solutions of relatively high concentrations by means of systematic exposure of these solutions in a free-choice situation to rats over a considerable length of time. These experimenters devised a sequence of solutions of concentrations ranging from 3% to 30%. A new concentration was presented each day for 9 days in order of increasing concentration. When this sequence had been repeated several times, rats increased their intake of solutions of all concentrations. This increase was stable throughout

a period of five months with no exposure to alcohol.

Prolonged exposure to ethanol also alters performance of animals in certain kinds of learning situations. In a study extending over five months, Denenberg, Pawlowski and Zarrow (1961) investigated the effects of forced intake of 5% to 10% ethanol on acquisition and extinction of a bar-pressing response. These investigators found that rats receiving ethanol during the acquisition of the response were slower learners than rats not receiving ethanol, although there were no differences between the two groups in extinction of the response. These same investigators (Pawlowski, Denenberg & Zarrow, 1961) found several differences in the acquisition and extinction of a shuttle-box escape problem between rats which had been forced to drink 10% ethanol for 100 days and rats without exposure to ethanol. The alcohol-maintained subjects took longer to learn the response, required less time for its extinction, and took longer to re-learn the response than did rats maintained on water.

Another type of alcohol-related behavior which

requires prolonged exposure to ethanol for its development is the alcohol deprivation effect (ADE). In 1967 Sinclair and Senter demonstrated that subjects which had had access to ethanol for long periods of time and were then denied access to the ethanol for six days would show elevated levels of preference when alcohol was made again available. It was also found that one day of deprivation was not sufficient to produce the effect, but that the ADE appeared with deprivation periods of 7 or 21 days (Sinclair & Senter, 1968). A similar deprivation effect could not be produced with a solution of saccharin (Sinclair & Senter, 1968), suggesting that the effect is due to a pharmacological rather than a gustatory effect of ethanol. Senter and Richman (1969) used the alcohol deprivation effect to induce rats to drink alcohol in concentrations which were sufficiently high that they were usually rejected. After one week of deprivation, rats which had been given a choice between water and 6% ethanol for six months drank significantly more 20% ethanol than rats which had been similarly maintained but switched to 20% ethanol without deprivation.

The ADE suggests the possibility that the deprivation period is producing an abstinence syndrome in the rat. The experimenters demonstrating this effect, however, do not report the appearance of any signs of distress in their animals during the deprivation period which might be interpreted as symptoms of an abstinance syndrome. Acute distress is known to occur in man upon the withdrawal of alcohol, and abstinence syndromes have been observed to occur in the mouse (Freund, 1969) and in the dog (Essig & Lam, 1968). With the exception of one incidental report of distress appearing after withdrawal of alcohol (Amit, et al., 1970), there have been no reports of a true abstinence syndrome in the rat.

The fact that withdrawal of ethanol after prolonged exposure does not produce symptoms of distress in rats does not imply that no physiological changes have taken place as a result of the exposure. Evidence that changes have in fact taken place is provided by the phenomenon known as "behavioral tolerance". Behavioral tolerance implies that repeated exposure to ethanol renders the subject less sensitive to behavioral deficits caused by a given dose of ethanol.

In two studies (Isbell, Fraser, Wikler, Belleville & Eisenman, 1955; Wikler, Pescor, Fraser, & Isbell, 1956), human subjects were given large quantities of ethanol over a period of several months. The experimenters wished to maintain a constant level of intoxication in the subjects. In order to do this, it was necessary to increase the dosages several times, because, although the blood-alcohol levels of the subjects remained high, the level of behavioral intoxication decreased after several days at the same dosage.

A similar result was observed by Mirsky, Piker, Rosenbaum and Lederer (1941). These investigators gave repeated intoxicating doses of ethanol to humans, and observed the recovery of both behavior and blood-alcohol levels. It was found that over the course of the experiment the doses of ethanol had to be increased to produce the same level of intoxication, and that blood-alcohol levels were higher during the recovery from intoxication than they were when the subjects showed the same degree of behavioral impairment during the induction of intoxication. Goldberg (1943) found that a given dose of alcohol produced a greater degree

of intoxication in abstainers than in heavy drinkers.

Behavioral tolerance can also be demonstrated in animals, and a large variety of tasks have been used to show the reduction of ethanol-produced behavioral impairment as a result of prolonged exposure to ethanol. Hogans, Moreno and Brodie (1961) administered 2g/kg of ethanol to monkeys, and observed the impairment of an avoidance response produced by the ethanol. After several days of chronic intoxication, the impairment of the response disappeared, and the monkeys responded in a normal fashion. When blood-alcohol levels were measured, it was found that these were as high after the development of tolerance as they were at the beginning of the exposure period. This result is consistent with the work on man cited above.

Troshina (1959) gave ethanol to rats by stomach intubation and observed the length of time which the rats were able to remain on a suspended bar. Initially, performance was severely impaired, but after six months of exposure to ethanol, it was not different from that of non-exposed rats. Moskowitz and Wapner (1964) were not able to replicate Troshina's (1959) results, but

found evidence for behavioral tolerance using a different paradigm. These experimenters exposed rats to ethanol for 30 weeks. These rats, and rats which had had no exposure to ethanol were trained to pull a chain to avoid electric shock. On test days, all rats were given ethanol before being placed in the avoidance apparatus. It was found that the rats having experience with ethanol performed at normal levels, while the rats without prior experience with ethanol showed a high level of impairment on the avoidance task.

Simple motor tasks also seem to be less sensitive to impairment by a challenge dose of ethanol following prolonged exposure to ethanol. Eickholt, Schillaci and Searcy (1967) found that rats given chronic exposure to ethanol showed less impairment on the tilted plane test than rats without prior exposure to ethanol. LeBlanc (1968) tested rats exposed daily and rats not exposed to ethanol on a treadmill following a challenge dose of ethanol. He found that the alcohol-treated rats performed significantly better than those which had had no experience with ethanol. Likewise, subjects given daily injections of ethanol for 10 to 12 weeks showed

less impairment on a rotarod test following a challenge dose of ethanol than did rats with no prior exposure to ethanol (Kinard & Hay, 1960).

Since the metabolism of ethanol takes place primarily in the liver, changes might be expected to occur in this organ resulting from prolonged exposure to ethanol. Lieber and Davidson (1962) reviewed research on fatty infiltration of the liver and concluded that prolonged exposure to ethanol produces an increase in this form of hepatic pathology. Using electron microscopy, Kiessling and Pilstrom (1966) found evidence of structural pathology of hepatic cells of rats forced to drink ethanol for five months. Metabolic activity in the liver is also depressed after prolonged exposure to ethanol (Kiessling & Tilander, 1961; Kiessling & Tilander, 1963).

Similar measurements have been made on brain cells of rats maintained from weaning on 15% ethanol (Kiessling & Tilander, 1963). No changes in metabolic activity were found resulting from the 7 to 12 months of exposure to ethanol. That changes in the CNS do occur, however, is suggested by the studies demonstrating

an increased theshold for electroconvulsive shock resulting from prolonged exposure to ethanol (Allan & Swinyard, 1949; McQuarrie & Fingl, 1958; Zarrow, Pawlowski, & Denenberg, 1962).

Since physiological changes appear to occur as the result of prolonged exposure to ethanol, a mechanism must be found to account for the changes. Two possible mechanisms have been suggested, and each has stimulated a great deal of research. The first mechanism which has been postulated to account for the changes brought about by exposure to ethanol is an increase in the rate at which ethanol is eliminated from the body. The second mechanism through which changes in alcohol-related activities might be mediated is an increase in the amount and activity of the enzyme alcohol dehydrogenase. These mechanisms are not completely independent. A review of the research concerning these two mechanisms, and their interactions follows.

The first mechanism which might account for changes in ethanol preference resulting from exposure to ethanol is an increase in the rate of ethanol metabolism.

Thus, if the metabolism of the subject were to become more efficient, then the subject would be able to ingest more ethanol with fewer aversive effects. Evidence for exposure-produced increases in the rate of ethanol metabolism had been found in mice, rats, and man.

In man, it was found that alcoholic prisoners, who had abstained from drinking for some time, eliminated radioactively labeled carbon dioxide more rapidly after a period of daily alcohol consumption than before the drug treatment period (Mendelson, Stein & Mello, 1965). Schlesinger, Bennett and Hebert (1967) found that C57BL mice forced to drink 10% ethanol for 90 days metabolized ethanol at a faster rate than did mice of the same strain given only water. Wistar rats were given alcohol by intubation and by forced choice for three to six weeks, at which time the rate of disappearance of a challenge dose of ethanol was measured (Hawkins, Kalant & Khanna, 1966). It was found that alcohol disappeared from the blood significantly faster from rats maintained on ethanol than from those with no prior experience with ethanol.

These data suggest that prolonged exposure to

ethanol is able to change the subject's ability to metabolize the ethanol. Other experimenters have not been able to demonstrate this phenomenon, however. Mendelson (1968) found that there was no difference in the rate at which labeled CO_2 was expired by alcoholics and nonalcoholics given labeled ethanol. Segovia-Riquelme, Vitale, Hegsted and Mardones (1956) found that the rate of ethanol metabolism of several strains of rats did not change following 60 days of exposure to a free choice between water and 10% ethanol.

The lack of unequivocal results in this area of research does not invalidate the premise that alcohol-related physiology may contribute to the level of voluntary intake. It may be that many of the pharmacological effects produced by ethanol to which an habituated animal responds may be independent of, or causally unrelated to, the rate of ethanol metabolism or blood-alcohol levels. That this proposition may be true is suggested by the data on behavioral tolerance, in which behavior is less inpaired at a given blood-alcohol level following a period of exposure to ethanol.

Further evidence for this statement is to be found

in studies of correlations between preference for alcohol and efficiency of ethanol metabolism. Strains bred selectively for ethanol preference (Mardones et al., 1953) did not differ in their rate of ethanol metabolism (Segovia-Riquelme et al., 1956; Segovia-Riquelme et al., 1962). Strains of mice differing in their preference for ethanol did not differ in the rate at which alcohol disappeared from the blood (Kakihana et al., 1966). Female rats were less affected by an injection of ethanol than were male rats but there was no difference between the blood-alcohol levels of the two sexes 90 minutes after the injection (Wallgren, 1959).

An increase in the level of the enzyme alcohol dehydrogenase has been postulated as a route through which changes in alcohol-related reactions might occur. Increases in the level of this enzyme resulting from prolonged exposure to ethanol have been reported by many experimenters.

Mirone (1965) found a significant increase in liver ADH in C57BL/6J mice forced to drink 15% ethanol from weaning. Similar results were found in subjects of another substrain of C57BL mice given 10% ethanol as the sole fluid for three months as adults (Schlesinger,

Bennett, Hebert & McClearn, 1966). Abe (1963) found significant increases in ADH in both brain and liver in rats given ethanol daily for one year.

Hawkins, Kalant and Khanna (1966) administered massive doses of ethanol daily to Wistar rats by intubation. Under these conditions, increases in ADH were apparent after only two weeks of treatment. These results were replicated by Khanna, Kalant and Bustos (1967), who also found no significant differences in the changes induced in male and female rats by the alcohol treatment.

Dajani, Danielski and Orten (1963) studied in detail the time course of changes in enzyme levels in male Sprague-Dawley rats. The subjects were forced to drink 10% ethanol for six weeks, at which time the concentration of the ethanol solution was increased to 20% for the remainder of the experiment. The greatest difference between these subjects and those drinking water occurred at 25 weeks. Aldehyde dehydrogenase levels in the experimental rats showed the same pattern of differences as that shown by ADH.

McClearn, Bennett, Hebert. Kakihana and Schlesinger (1964)

explored the effects of prolonged exposure to ethanol on enzyme levels in high- and low-preferring strains of mice. They found that levels of ADH were higher in both C57BL and DBA mice following a period of forced intake of ethanol. The pattern of differences between the two strains remained constant during the experimental treatment: the ADH levels of the C57BL mice were higher than those of the DBA mice both under conditions of forced ethanol and no ethanol.

On the other hand, several reports have appeared which have shown no change or a decrease in ADH following prolonged exposure to ethanol, both in man and in rats (Figueroa & Klotz, 1962a, 1962b, 1962c). It may be possible to explain these contradictory results by noting that they have all been reported by the same two authors, and that perhaps some aspect of their experimental methodology may differ from that of other reported research. More difficult to explain, however, is the functional significance of a change in ADH levels. Intuitively, it would seem that such a change would be reflected in a change in the metabolic rate of ethanol or lowered blood-alcohol levels. These changes, however,

have not always been found.

A change in enzyme level might be reflected in more subtle aspects of alcohol metabolism. For instance, the maximum amount of ethanol which an animal can metabolize in an hour might be increased. However, this research has not been attempted.

The problem of the functional significance of ADH levels extends as well to research demonstrating correlations between ADH levels and preference for ethanol. Wilson (1967) found that the rate of metabolism of ethanol did not differ between C57BL and C3H mice. These strains have been shown to differ both in their preference for ethanol in the level of hepatic alcohol dehydrogenase (Rodgers, McClearn, Bennett & Hebert, 1963).

The Tryon Rats

It has been shown that the level of voluntary alcohol intake in an intact organism depends on many constitutional factors such as sex, strain and species. Also it has been shown that the level of intake can be modified by physiological and by psychological manipulations, and that the degree of success of a particular manipulation often is determined by the constitution of the subjects. Studies have shown that long-term exposure to ethanol

affects the physiological and behavioral reactions to alcohol. One question which remains to be asked concerns the effect of long-term exposure on voluntary intake of rats of differing constitutions. Ideally, several aspects of the behavior and physiology of the subjects for this investigation should have been studied previously, and the differences between the strains should be quite marked.

The Tryon Maze Bright and Maze Dull strains appear to be suitable subjects for this experiment. In 1929, Tryon reported preliminary results of a selective breeding program in rats based on errors in a 17-unit T-maze, one trial per day being given for 19 days. Beginning with a heterogenous parental stock, Tryon mated rats on the basis of their own error scores, error scores of their relatives, fertility, and coat color. In each generation, half of the breeders for each strain were mated with their siblings, and half with more distant relatives. Significant differences in the maze performance of the two strains were apparent as early in the program as the F_2 generation. By the F_7 generation, the distributions of error scores of the two strains no

longer showed any overlap. Selective breeding was continued through the F_{18} generation, but no further separation was obtained (Tryon, 1940).

Tryon also observed that there was a high correlation between the learning abilities of rats of both strains when young and at maturity. The brights tended to be more emotional in response to handling, but also to be more "efficient" learners than the dulls, as shown by differences in running time, hesitation time and errors (Tryon, 1940).

The Tryon Maze-Bright and Maze-Dull strains have stimulated the curiosity of several researchers, most of whom have tried to characterize the differences between the two strains on some dimension. The earliest and most complete study was that of Searle (1940). Searle studied the differences between the two strains on a battery of tests: open field, alley maze, water mazes, running wheel, successive discriminations and several types of elevated mazes. In all cases, the scores of the two strains were compared to the scores of a heterogenous median group.

The picture which emerged from these studies was

that the strains did not differ on the dimension of "brightness" except when tested in a maze identical to the one used by Tryon. Rather, the differences between the strains could be characterized as being "motivational" in nature. The brights, in general, scored above the median group in all tests involving food deprivation. The dulls performed at the same level as the median group on these tasks. The dulls, however, seemed less motivated to eat than the median group, in that they had longer latencies to start eating in the goal box, and often did not consume all their daily ration. The brights, on the other hand, were at the same level as the median group on these measures.

The brights were less active and the dulls more active than the median group in the running wheel. That activity which the brights did show was concentrated in the two hours before the daily feeding, while that of the other two groups took place at night. The differences in emotionality between the two strains were confirmed, both in response to handling and in the open field. While the brights were more emotional in response to these situations, the dulls seemed to be extremely disturbed by mechanical features of several of the mazes.

Recently, several other experimenters have studied the descendents of the Tryon Maze Bright (S_1) and Maze Dull (S_3) strains in many different kinds of learning situations. The S_1 subjects have been shown to make significantly fewer errors in Hebb-Williams, Dashiell and Lashley III mazes (Rosenzweig, Krech & Bennett, 1960). Jennings (1960) and McGaugh, Jennings and Thomson, (1962) found that S_3 subjects given massed practice in a Lashley III maze showed poorer performance than S_1 subjects given massed practice. There were no strain differences in performance with spaced trials. Wehmer and Markowitz (1964) required thirsty rats of both strains to remain motionless for a certain period of time in order to receive a water reinforcement. It was found that the S_1 subjects were better able to suppress their behavior in this situation than were the S_3 subjects. When an unsolvable problem was presented in the Krech Hypothesis Apparatus, both strains showed an initial tendency to form a visual rather than a spatial hypothesis. The S_1 subjects, however, formed new hypotheses after fewer trials than did the S_3 subjects (Rosenzweig, Krech & Bennett, 1958).

Strain differences in performance in learning problems using aversive motivation has been studied by several investigators. Subjects of the S_1 strain learned to swim through a Lashley III maze in fewer trials than did subjects of the S_3 strain (Wolfer, Reid & Porter, 1963). Tapp (1964) found that the S_1 subjects were able to learn a conditioned emotional response faster than were subjects of the S_3 strain. S_1 subjects required fewer trials than did S_3 subjects to learn a horizontal - vertical striation discrimination in a Thompson apparatus (Fehmi & McGaugh, 1961), although the S_3 subjects performed significantly better at light-dark reversals in a Krech Hypothesis Apparatus when motivated by shock (Markowitz, Sorrells & Harris, 1964).

The strains have also been found to differ in the concentrations of several neural transmitters and their associated enzymes. Pryor (1965) found that both the concentration and absolute amount of the neural transmitter serotonin was higher in subjects of the S_1 strain than in those of the S_3 strain. In addition, the principle enzyme in serotonin metabolism, monoamine oxidase, was

more concentrated in the S_1 than in the S_3 rats.

Bennett, Crossland, Krech and Rosenzweig (1960) found that the brains of S_1 rats contained significantly higher concentrations of acetylcholine than did the brains of S_3 rats. The enzyme cholinesterase (ChE) was also found in higher concentrations in the brains of S_1 than S_3 rats (Krech, Rosenzweig & Bennett, 1958; Bennett, Diamond, Morimoto & Hebert, 1966). In addition, a cross between the S_1 and S_3 strains showed concentrations of brain ChE midway between that of the two parental strains (Rosenzweig et al., 1960). The concentration of ChE in both cortex and subcortex in both strains increases with age to 80 - 100 days and then declines. The brains of the S_1 subjects, however, have higher levels of ChE at all ages (Bennett, Rosenzweig, Krech, Karlsson, Dye & Ohlander, 1958). It appears that the differences between the two strains in ChE activity may be particularly significant to the behavioral differences between the two strains since strain differences are not apparent in other enzymes important for metabolism but not for behavior, such as lactic dehydrogenase (Bennett, Krech, Rosenzweig, Karlsson, Dye & Ohlander,

1958).

Another characteristic of the central nervous system in which the two strains differ is in their sensitivity to electroshock seizures. Woolley, Rosenzweig, Krech, Bennett and Timiras (1960) found that the threshold for electroshock convulsions was significantly lower in the S_1 than in the S_3 subjects. It was also found that the extensor tonic phase of the seizure was shorter in the S_1 than in the S_3 subjects.

The S_1 and S_3 subjects have also been reported to respond differently to manipulations of early environment. Rosenzweig, Krech and Bennett (1964) found that the differences in cortical weight between groups of subjects of the S_1 strain reared in enriched and impoverished environments was greater than the difference between groups of S_3 subjects reared in the same two environments. On the other hand, no differential effects of rearing environment on open field behavior or conditioned avoidance responding were found in the two strains (Powell & Leach, 1967). In addition, Pryor (1965) failed to find differences in serotonin concentration in subjects of either strain reared in

different environments.

The Tryon strains have been used by McGaugh and his associates in their study of drug facilitation of learning. The interest of these workers is more in the investigation of learning facilitation than in differences between the Tryon strains. Their work suggests that the observed differences in learning ability are due to the shorter time required for consolidation of learning in the S_1 subjects. It was predicted that if neural activity could be stimulated in the S_3 subjects shortly after a learning trial, the learning by these subjects would be facilitated, and that the differences in learning ability between the two strains would decrease. These predictions were confirmed using strychnine sulphate (Ross, 1959; McGaugh, Thomson, Westbrook & Hudspeth, 1962). It was also predicted that if neural activity were disrupted shortly after a learning trial, the learning of the S_1 subjects, with their more rapid consolidation, would be disrupted to a lesser degree than that of the S_3 subjects. This hypothesis was confirmed by Thomson, McGaugh, Smith, Hudspeth and Westbrook (1961) using electroconvulsive shock.

A final point to be made about facilitation of learning by strychnine sulphate concerns the dose required to demonstrate facilitation. Petrinovitch (1967) compared the effects of the drug on learning in the two Tryon strains and in Long-Evans Hooded rats. It was found that, while the minimum lethal dose of the drug was the same for the three strains, the dose which produced optimal facilitation of learning was significantly lower for the Hooded than for the Tryon strains. Thus, not only are the Tryon strains different from each other in respect to drug facilitation of learning, they also are different from another strain of rat.

Powell, Martin and Kamano (1967) studied the effects of amobarbital on conditioned avoidance responding. In earlier work with Wistar rats (Kamano, Martin & Powell, 1966) it was found that a dose of 20mg/kg of this drug facilitated avoidance responding, while a dose of 40mg/kg disrupted it. These dosages were used with the Tryon rats, and it was found that the drug produced the same pattern of effects in the S_1 subjects as in the Wistars. The avoidance responding in the S_3 subjects, however, was

disrupted at both dosage levels, again demonstrating differences both between the two Tryon strains and between these strains and another strain of rat.

An attempt has been made to characterize the differences between the two strains as a difference in sensitivity to stress. In the Lashley III swimming maze experiment mentioned above (Wolfer et al., 1963), several of the S_3 subjects had to be rescued in the middle of a trial or they would have drowned. Stress provided by a very severe food deprivation schedule produced higher mortality in the S_3 subjects than in the S_1 subjects (Wolfer, Reid, Gledhill & Porter, 1964), and also decreased the weight of the thymus glands to a greater extent in the S_3 subjects (Jencks, Gortatowski & Porter, 1965).

According to this characterization of the difference between the two strains, stress provided by psychological experimentation causes greater disruption of behavior in subjects of the S_3 strain than in those of the S_1 strain. This characterization is more successful than other attempts in dealing with some of the contradictory data which have been published about these strains, but it

is unable to explain why the S_3 subjects sometimes perform better than do the S_1 subjects.

In addition to the behavioral and physiological differences between the two strains which have already been mentioned, there is a basic difference in their metabolism. For example, when subjected to a severe food deprivation schedule, there are no significant differences in the food intake of rats of the two strains, but the S_3 subjects lose weight significantly faster than do the S_1 subjects (Wolfer et al., 1964). The S_3 subjects also have a higher basal metabolic rate than do the S_1 subjects. They consume more water and pass more urine than do rats of the S_1 strain. In addition, chromatographic analysis of urine from rats of the two strains shows different patterns, indicating the presence of an unidentifiable substance in the urine of the S_3 subjects which is not present in the urine of subjects of the S_1 strain (Jencks et al., 1965). This observation led the authors to comment that, "the dulls appear to have a hereditary metabolic disease." (p. 164).

These two strains of rats, originally bred for

errors in a multiple T-maze, seem to be very different from one another, not only in behavioral traits, but also in physiological characteristics such as response to drugs, seizure thresholds and basic metabolic features. Attempts to characterize the two strains in general terms have, by and large, been unsuccessful. But, since they have been shown to differ in so many characteristics, it is likely that they will be found to differ in still others, although it is not possible to predict the nature or direction of the difference.

The Problem

Several variables have been shown to be important in determining an animal's response, both physiological and behavioral, to ethanol. Some of these variables will be important in the research described in this thesis.

In the first place, both ethanol preference and physiological responses to ethanol seem to depend on constitutional variables. The two constitutional variables most commonly discussed are sex and strain. The differences in response to ethanol between strains of mice are very dramatic (McClearn & Rodgers, 1959), some strains showing very low preference for ethanol, and the C57BL

strains showing consistent high preference. In these strains, the concentration of alcohol dehydrogenase and sensitivity to ethanol are correlated with preference. No differences between the sexes have been found in mice, however, (Rodgers & McClearn, 1962).

In the rat, strain differences in preference for ethanol are also very marked, especially among strains which have been bred for some particular behavioral characteristic, such as the Maudsley strains (Brewster, 1969). In contrast to mice, however, sex differences in preference for ethanol are commonly found in the rat, although one sex does not consistently show higher preference than the other (Schadewald, Emerson & Moore, 1953; Clay, 1964; Eriksson, 1968). Female rats, however, seem to be able to eliminate ethanol from the body more rapidly than males (Broadhurst & Wallgren, 1964; Eriksson & Malmstrom, 1967).

The first problem with which this thesis deals is that of determining further sex and strain differences in ethanol preference in rats. Four strains were used, two of which, Wistar and Hooded, are commonly used in laboratory research. The other two strains which were

studied are the Tryon strains which, although they were originally bred for errors on a multiple T-maze, have been shown to differ in many other behavioral and biochemical variables as well. In addition, both sexes of these four strains were studied in order to examine interactions between sex and strain. The method of measuring preference was an adaptation of the method of Cicero and Myers (1968), in which the highest concentration of ethanol which the subjects will voluntarily drink was measured.

The second topic with which this thesis will be concerned is an attempt to modify the preference for, and intake of, ethanol in these groups of rats. It has been shown that although various manipulations of diet, hormonal state and neural function do not reliably produce persistent changes in the level of alcohol-directed behavior, the nature of those changes which are produced by these manipulations often depends on constitutional differences in the subjects (Goldberg & Stortebecker, 1943; Brown, 1969; Jones & Essig, 1970).

Long-term exposure to ethanol has been shown to result in changes in alcohol-related physiology

and behavior. The majority of such changes which have been reported have resulted from forced exposure to ethanol. Two studies (Sinclair & Senter, 1967; Veale & Myers, 1969), however, have shown that prolonged exposure to ethanol in a free-choice situation can result in an increase in preference for ethanol. In addition, the possibility that subjects which differ constitutionally would be affected to different degrees by prolonged exposure to ethanol has not been investigated. Thus, the second question which the research reported in this thesis was designed to explore was whether there would be differences in the amount of change in alcohol-directed behavior of males and females of the Wistar, Hooded, and Tryon strains following a period of exposure to ethanol.

The Present Investigation

Experiment I was designed to determine the highest concentration of ethanol which rats of both sexes of several strains would drink voluntarily. This concentration was used to calculate the concentration (MC) of a solution of ethanol used throughout Experiment II. In Experiment II, the MC was offered to some of the subjects on a long-term basis, in order to investigate the degree to which rats would change their preference for it as the result of exposure to ethanol.

The subjects for both Experiments I and II were 96 rats, all of which were born in the animal colony in the Psychology Department of McGill University. After weaning, the Ss lived in plastic cages measuring 12" x 14" x 6.5" with three or four other rats of the same sex and strain. The Ss were 80 to 110 days of age at the beginning of Experiment I.

The individual cages in which the animals were housed for both Experiments were made of sheet metal and ½" wire mesh. They measured 8" x 8" x 10".

Two 100 ml Richter drinking tubes were mounted on the outside of each cage. The spouts protruded through the front of the cages, 1½" above the floor. Purina Rat Chow was available to the rats at all times.

The ethanol solutions presented to the subjects were prepared from 95% ethanol and tap water, volume/volume. Thus, 100 ml of a 10% solution would be prepared by adding 89.44 ml of tap water to 10.56 ml of 95% ethanol.

The experimental room was illuminated in a 12 hours on, 12 hours off cycle. The temperature in the room was maintained between 70° and 72°F.

Experiment I

Methods

Subjects. The 96 subjects were 12 male and 12 female rats of four strains: Wistar albino rats, Hooded rats of the Royal Victoria Hospital strain, and descendents of the Tryon Maze Bright (S_1) and Maze Dull (S_3) strains. The original breeding stock of the Wistar and Hooded animals was obtained from

Quebec Breeding Farms, St-Constant, Quebec; that of the Tryon animals was obtained from Dr. Gordon Pryor of the Stanford Research Institute, Menlo Park, California.

Procedure. The rats were weighed at the time they were placed in their individual cages for preference testing beginning at 80 - 100 days of age. They were allowed to become accustomed to their new quarters for two days. During this time, both Richter tubes contained water. On the third day, a 4% solution of ethanol was offered in one of the tubes. On the next day, and on each subsequent day, the amount of fluid consumed from both bottles during the previous 24 hours was recorded, and the concentration of the ethanol solution was increased by 2%. In addition, the position of the tube containing the ethanol solution was alternated daily from side to side to avoid the confounding effects of position preferences. This procedure was continued until the S did not drink any of the ethanol solution on two consecutive days.

Results

The highest concentration of ethanol that a rat would voluntarily drink was termed his Final Acceptance Concentration (FAC). The means and standard deviations of FACs for all eight sex-strain subgroups appear in Table I. It can be seen by reference to Table I that there was a wide range of the mean FAC for the different groups, ranging from 11.67% in the S_3 females to 87.33% in the S_1 females. Table I also shows that not only were there strain differences in FAC, but that in most cases there were also sex differences within strains. In addition, it can be seen that the direction of the sex differences in FACs was not the same in the four strains. That is, whereas the female subjects of the Wistar and S_1 strains reached much higher FACs than the male subjects of these strains, the FACs for the males of the S_3 strain were higher than those of the S_3 females, and there were virtually no sex differences in FACs of the Hooded subjects.

Strain and sex differences in FACs were evaluated statistically by an analysis of variance, the results

Table I

Means and Standard Deviations of Final Acceptance Concentrations (% volume/volume) of male and female subjects of four rat strains. (n=12)

Subjects	Mean	Standard Deviation
Wistar		
Males	23.50	18.95
Females	59.33	12.32
Hooded		
Males	22.67	22.95
Females	23.83	13.87
S_1		
Males	59.50	6.16
Females	87.33	3.55
S_3		
Males	33.67	7.67
Females	11.67	5.03

of which appear in Table II. The results of this analysis indicated that there were significant differences in the mean values of the FACs between the four strains, and also between the sexes. The interaction between these two variables was also highly significant.

The nature of the sex x strain interaction is presented graphically in Figure 1. This interaction was analyzed in two ways: comparisons of FACs between the males and females of each strain and comparisons between the strains within each sex. All comparisons between sex-strain subgroups were made using the method of Scheffé (Winer, 1962).

Examination of the comparisons between the males and females of each strain reveals that male and female Ss of the Wistar and S_1 strains differed strongly ($p<.01$), those of the S_3 differed somewhat ($p<.05$) while those of the Hooded strain were not significantly different. Examination of the strain-within-sex comparisons reveals that, for female Ss, all strain groups differed significantly from one another at the .01 level except

Table II

Results of a two-way analysis of variance of Final Acceptance Concentrations of 96 rats.

Source	df	MS	F	p
Strain (S)	3	13,658.00	80.063	.01
Sex (G)	1	2,688.17	15.758	.01
S x G	3	4,007.28	23.491	.01
Within	88	170.59		
Total	95			

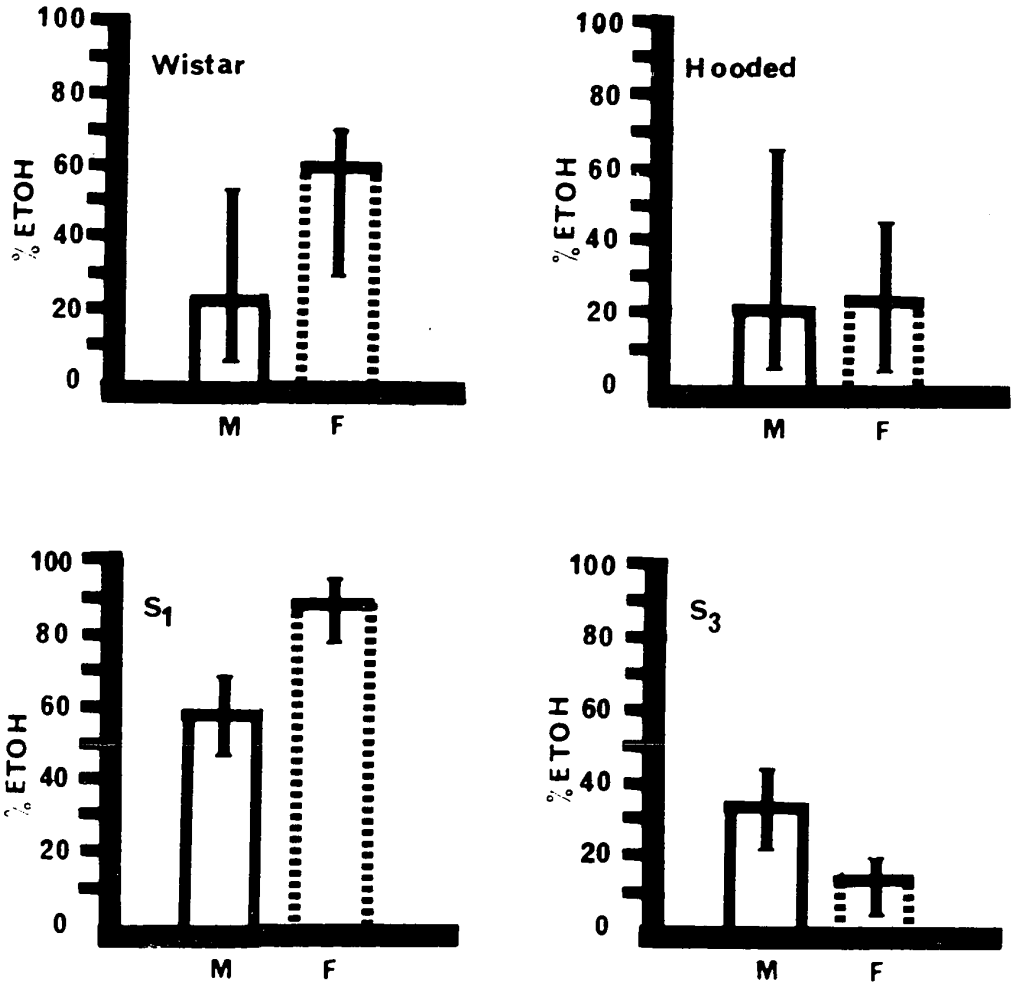

Figure 1. Mean Final Acceptance Concentrations of male and female rats of four strains. Vertical lines indicate ranges. (n=12)

the S_3 from Hooded; the S_1 males showed significantly (.01) higher FACs than the males of all other strains, which did not differ from one another.

Experiment II

Method

Subjects. Each sex-strain subgroup from Experiment I was further divided into two groups, each containing 6 Ss: an Experimental (E) group, and a Control (C) group. Because the experiment was replicated twice, it was not possible in all cases for the number of subjects in the E and C conditions to be equal in the two replications. Whenever possible, assignment of an S to one of the two conditions was done in such a way as to make the average FACs obtained in Experiment I as comparable as possible in the E and C groups in Experiment II. A reexamination of the data from Experiment I by a three-way analysis of variance with sex, strain and experimental condition as main effects shows that the FACs in the E and C conditions did not differ significantly. The F associated with experimental condition was not

significant at the 25% level ($F = .032$, $df = 1/80$). In addition, the interactions of experimental condition with strain ($F = .026$, $df = 3/80$) and with sex ($F = .0008$, $df = 1/80$) are well below the 25% probability level, as is the three-way interaction with sex and strain ($F = .0437$, $df = 3/80$).

Procedure. Each S began Experiment II on the day it completed the preference testing in Experiment I. On the first and third days, all animals were presented with a choice between water and an ethanol solution of a particular concentration, the Maintenance Concentration (MC). This concentration was 80% of the FAC, and it was calculated individually for each S. Thus an animal whose FAC had been 40% would be presented with an MC of 32%. The Richter tube containing the ethanol solution appeared once in each of the two cage positions. On the second and fourth days, both tubes contained water.

On alternate days from day 5 to day 75, the Ss in the Experimental group were given a free choice between water and their MC solution. A choice between two tubes containing water was presented on the

intervening days. The side of the cage on which the tube containing the ethanol solution appeared alternated every day on which the ethanol was offered.

The Ss in the Control group were presented with two tubes containing water from day 5 to day 72. The positions of the tubes were changed every second day. On days 73 and 75, the C Ss were again allowed a free choice between water and their MC solution, the tube containing the ethanol being presented once on each side of the cage. Two tubes containing water were presented on day 74.

From day 76 to day 113, the MCs of all Ss were altered in several ways. These alterations, suggested by Amit (1970), were designed to test the stability and persistence of the S's level of ethanol intake. Seven tests in all were performed. A summary of the experimental design and testing schedule is presented in Table III.

Beginning with Free Choice I, each test was performed in a four-day cycle. Within each cycle, the appropriate ethanol solution was presented on days 1 and 3, with the drinking tube containing the ethanol

Table III

Schedule of Experimental Periods and ethanol solutions associated with each.

Day	Experimental Period	Available Solution (+ water)
1-4	Initial Choice	Maintenance Concentration (MC)
5-72	Experience	Experimentals: MC Controls: water
73-75	Final Choice	MC
76-89	Withdrawal	water
90-93	Free Choice I	MC
94-97	Metering I	M- (90% of MC)
98-101	Metering II	M+ (110% of MC)
102-105	Free Choice II	MC
106-109	Quinine	Q (.05% quinine solution in MC)
110-113	Free Choice III	MC

solution appearing once on each side of the cage. Water only was offered on days 2 and 4.

Ethanol was withheld from all Ss for two weeks (Withdrawal), from day 76 to day 89. During this period two Richter tubes filled with tap water only were available to the S. The positions of the two tubes were alternated daily. Following the period of Withdrawal, the animal was again given access to his MC (Free Choice I).

The concentration of the alcohol solution was then altered to observe the degree to which the animal was able to maintain a constant level of intake of absolute ethanol. The concentration of the ethanol solution was first lowered (M-) to 90% of the MC (Metering I.), so that the M- for a S with an MC of 40% would be 36%. The concentration was then raised (M+) to 110% of the MC (Metering II). Thus an animal having an MC of 40% would have an M+ of 44%. These periods were followed by a period of access to the MC itself (Free Choice II).

The amount of the MC which an S would drink when a highly aversive dose of quinine had been added

was measured (Quinine). A quinine solution (Q) was prepared for each S by adding 50 mg of quinine hydrochloride to 100 ml of the MC, and this was offered to the S. Finally, the rats were again allowed to choose between water and the MC (Free Choice III).

Results

Two measures of ethanol-directed behavior were used in the statistical analysis of the results of this experiment: proportion of daily fluid intake taken in the form of ethanol solution (preference ratios) and volume of absolute ethanol consumed per one hundred grams of body weight. These two measures were chosen because they yield values which are independent of the weight of the subject. This precaution was necessary due to the large variation in body weight among the subjects.

The data for the two days of any experimental period in which alcohol was available were averaged to give one score for each period.

In many cases in the analysis of the data from this experiment, it was found that the error variances

tended towards heterogeneity. Because of this trend a relatively rigid criterion for significance was used. When the analysis of variance was applied to these data, only those F-ratios reaching significance at the .01 level of probability were subjected to further statistical analysis. When necessary to meet the assumptions of the analysis of variance, the data were transformed before analysis or an alternate form of statistical analysis was used.

Experience. The effects of the Experience period were assessed by comparing the preference ratios and amount of absolute ethanol consumed during Initial Choice to those during Final Choice. An analysis of variance design was used in the analysis of the data from both measures which permitted the inspection of the interrelations between the effects of sex, strain and experimental condition in the amount of change between Initial and Final Choice.

Mean preference ratios during the Final Choice period for the sex-strain subgroups in the two experimental conditions are found in Table IV. It can be seen that in every case, the mean preference ratios

Table IV

Mean Preference Ratios ($\frac{\text{ml MC}}{\text{ml MC + ml } H_2O}$) of the sex-strain subgroups within each experimental condition during Final Choice. (n=6)

Subjects	Experimental	Control
Wistar		
Males	.748	.106
Females	.308	.037
Hooded		
Males	.623	.047
Females	.402	.040
S_1		
Males	.370	.074
Females	.254	.051
S_3		
Males	.446	.052
Females	.610	.187

of the groups which had been exposed to ethanol during the Experience period were higher than the corresponding groups without exposure. Reference to Table IV also indicates differences in preference ratios among the sex-strain subgroups.

The results of the statistical analysis of the preference ratios are found in Table V. The significant condition effect indicates that the experimental group showed a higher preference for ethanol than did the control group.

The nature of the highly significant Condition x Period interaction is presented graphically in Figure 2. When the preference ratios shown by the subjects in the two experimental conditions during the Initial Choice period are compared by an F-test, no significant differences are found ($p > .05$). The increase in preference for ethanol shown by the E Ss between Initial and Final Choice, and the decrease shown by the C Ss between the same two periods are both highly significant ($p < .01$).

The nature of the significant sex x strain interaction is presented graphically in Figure 3. The differences between the males and females of each strain were analyzed by t-tests. These analyses

Table V

Results of an analysis of variance performed on the Preference Ratios at Initial and Final Choice of 96 rats.

Source	df	MS	F	p
Between-Subject				
Strain (S)	3	1187.88	3.102	.05
Sex (G)	1	1011.09	2.640	ns
Condition (C)	1	25268.00	65.983	.001
S x G	3	2388.13	6.236	.001
S x C	3	1044.75	2.728	.05
G x C	1	1467.44	3.832	ns
S x G x C	3	556.36	1.453	ns
Ss Within Groups	80	382.95		
Within-Subject				
Period (P)	1	3326.67	28.937	.001
S x P	3	104.69	0.911	ns
G x P	1	328.13	2.854	ns
C x P	1	13296.70	115.667	.001
S x G x P	3	101.96	0.887	ns
S x C x P	3	47.06	0.409	ns
G x C x P	1	320.33	2.786	ns
S x G x C x P	3	96.69	0.841	ns
P x Ss Within Groups	80	114.96		
Total	191			

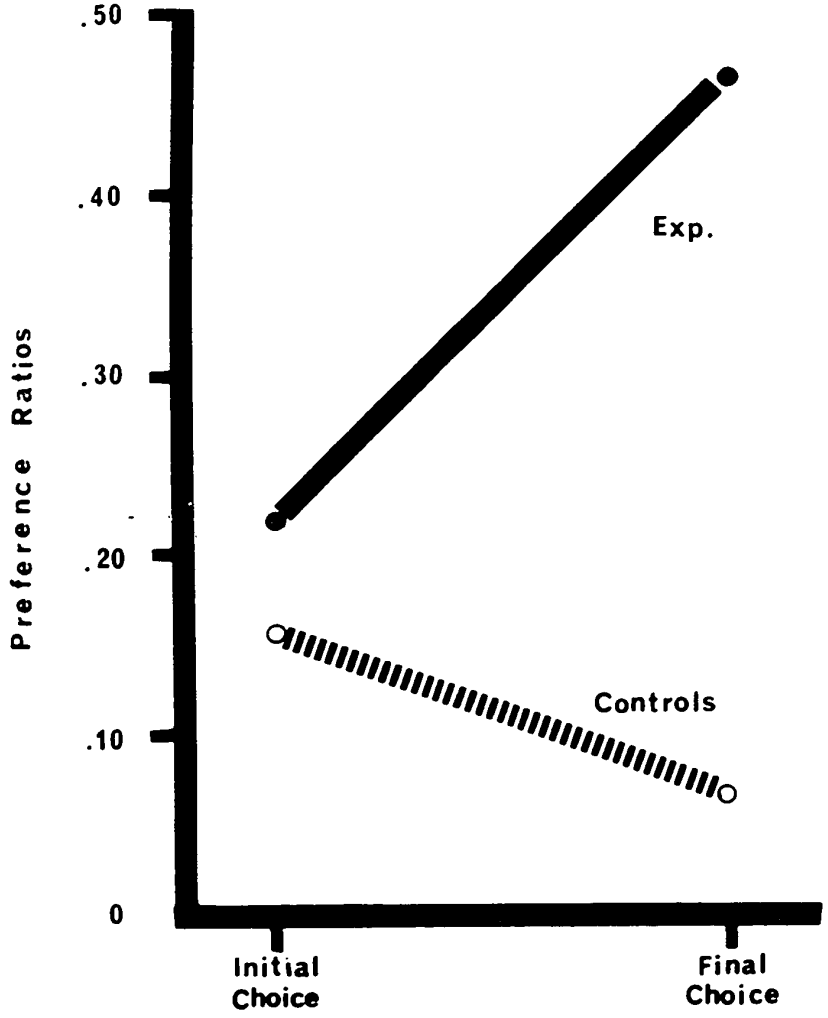

Figure 2. Mean Preference Ratios ($\frac{\text{ml MC}}{\text{ml MC + ml } H_2O}$) for the Maintenance Concentration at Initial and Final Choice shown by subjects of the Experimental and Control groups. (n=48)

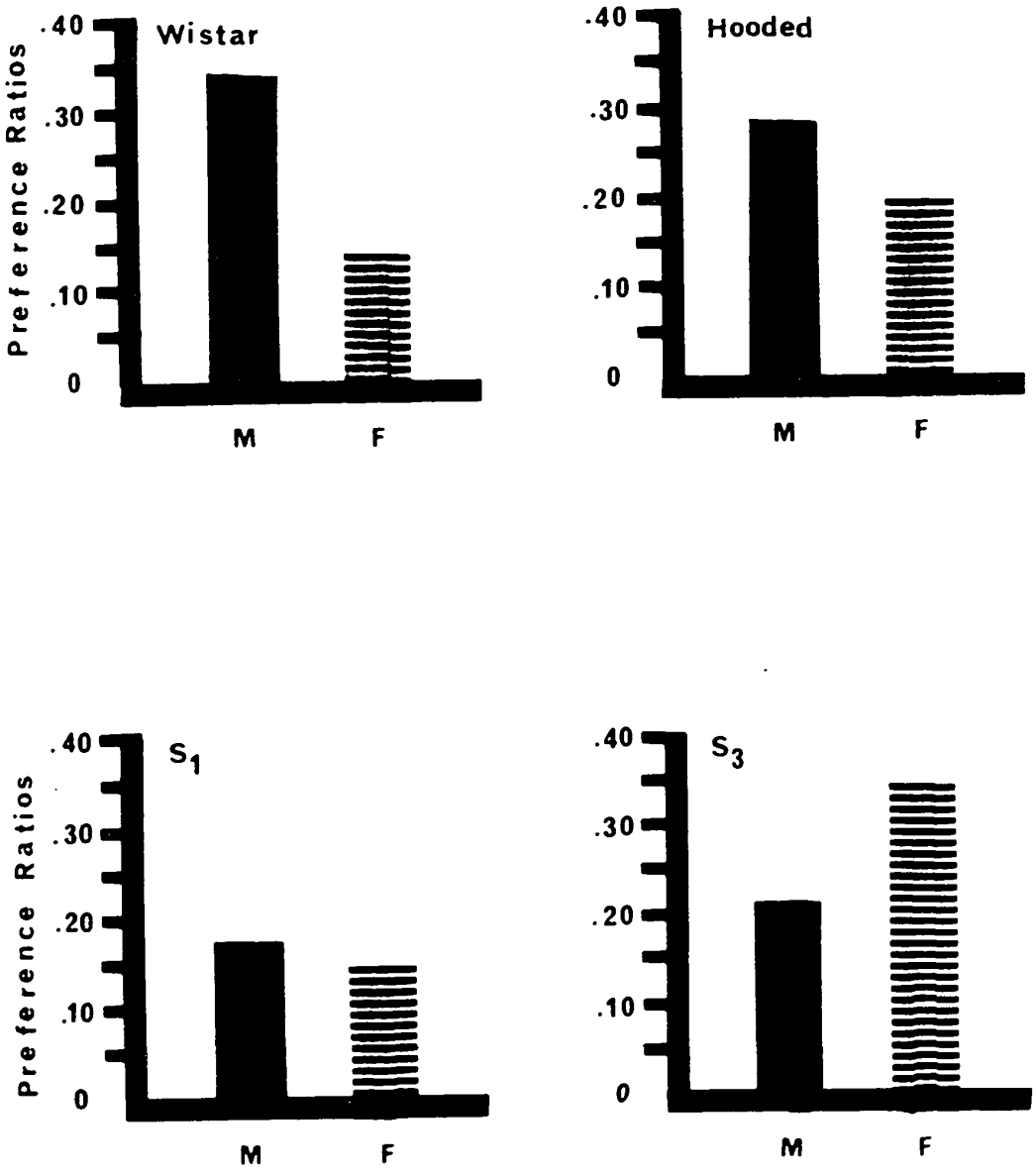

Figure 3. Mean Preference Ratios $\left(\frac{\text{ml MC}}{\text{ml MC + ml } H_2O}\right)$ for the Maintenance Concentration shown by the sex-strain subgroups during the Experience Period. (n 1.)

indicated that the females of the Wistar and Hooded strains showed lower preference for ethanol than the males of the same strains, while the females of the S_3 strain preferred ethanol to a greater extent than did the S_3 males. All of these differences are significant at the .005 level of probability. The difference between the preference ratios for the males and females of the S_1 strain was not significant ($p > .05$).

The mean intake of absolute ethanol per 100 grams of body weight during Final Choice for each of the sex-strain subgroups in each condition is presented in Table VI. It can be seen that the intake of subjects exposed to ethanol was considerably higher than that of subjects without exposure. This is true in every sex-strain subgroup. In addition, it can be seen that, within each strain, there are differences between the sexes, and also that the pattern of these differences is the same as that found in the FACs of Experiment I.

The results of the analysis of variance performed on the absolute ethanol intake data are shown in Table VII. The results of the analysis of the intake data are similar to the results of the analysis of the preference ratios in several respects. The E Ss consumed signif-

Table VI

Mean intake of absolute ethanol (ml ETOH/100 gm body weight) of the sex-strain subgroups within each experimental condition during Final Choice. (n=6)

Subjects	Experimental	Control
Wistar		
Males	.653	.098
Females	.885	.132
Hooded		
Males	.593	.052
Females	.605	.101
S_1		
Males	.933	.227
Females	1.475	.312
S_3		
Males	.867	.125
Females	.738	.142

Table VII

Results of an analysis of variance performed on intake of absolute ethanol at Initial and Final Choice of 96 rats.

Source	df	MS	F	p
Between-Subjects				
Strain (S)	3	1.934	14.46	.001
Sex (G)	1	0.703	7.29	.01
Condition (C)	1	6.042	62.67	.001
S x G	3	0.464	4.81	.01
S x C	3	0.004	0.04	ns
G x C	1	0.061	0.63	ns
S x G x C	3	0.093	0.97	ns
Ss Within Groups	80	0.096		
Within-Subjects				
Period (P)	1	0.127	3.02	ns
S x P	3	0.008	0.20	ns
G x P	1	0.111	0.26	ns
C x P	1	5.542	131.70	.001
S x G x P	3	0.019	0.45	ns
S x C x P	3	0.376	8.93	.001
G x C x P	1	0.028	0.65	ns
S x G x C x P	3	0.055	1.32	ns
P x Ss Within Groups	80	0.042		
Total	191			

icantly more absolute ethanol ($\bar{X}$ = .648) than did the C Ss ($\bar{X}$ = .293). The nature of the highly significant C x P interaction is shown in Figure 4. Again, the difference between the E and C Ss on Initial Choice was not significant ($p>.25$), while the increase in absolute ethanol intake in the E Ss and the decrease in the C Ss between Initial and Final Choice period are both significant ($p<.01$).

The S x G interaction is illustrated in Figure 5, where the intake data for each of the sex-strain subgroups are presented graphically. The Wistar and S_1 females drank more absolute ethanol than did the males of those strains ($p<.01$). The female S_3 subjects, however, drank significantly less ethanol than the S_3 males ($p<.05$), while there were no significant differences in ethanol intake between the sexes of the Hooded strain ($p>.25$).

An important difference between the results of the analyses of the intake and preference data is found in the strain x condition x period (S x C x P) interaction. This interaction is not significant for the preference data, but is highly significant for

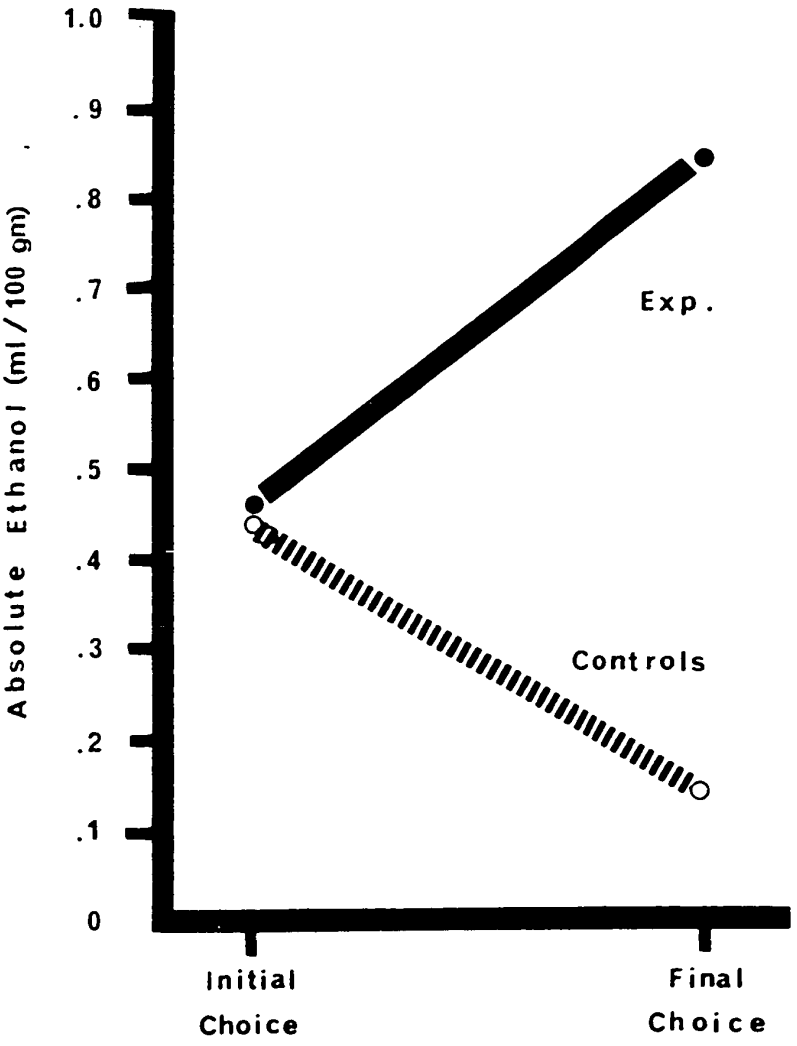

Figure 4. Mean intake of absolute ethanol (ml ETOH/100 gm body weight) at Initial and Final Choice of subjects of the Experimental and Control groups. (n=48)

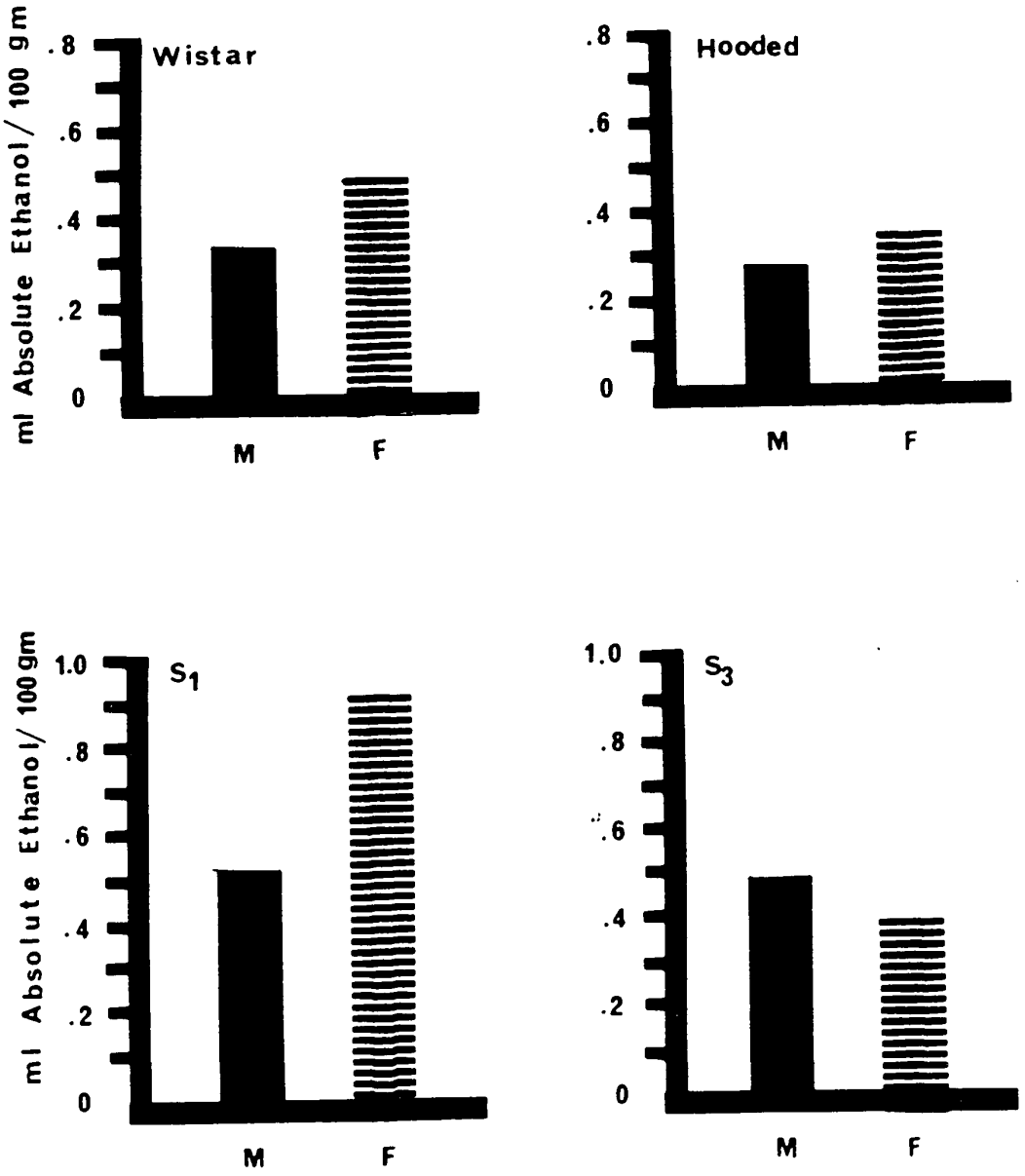

Figure 5. Mean intake of absolute ethanol (ml ETOH/100 gm body weight) of the sex-strain sub-groups during the Experience Period. (n=12)

the intake data. The nature of the interaction was examined in two ways. First, it was determined whether the C x P interactions for each strain were significantly greater than zero. Second, the sizes of the C x P interactions of the different strains were compared with each other.

The form of the C x P interactions for each strain are presented in Figure 6. To determine whether the interactions were significantly greater than zero, a sum of squares for each strain was calculated based on the degree to which the experimental Ss increased and the control Ss decreased their intake of absolute ethanol between the two periods. An F ratio was constructed from this value. The F was significant for all strains except Hooded beyond the .01 level of probability, indicating that for the Wistar, S_1 and S_3 strains, the tendency for the E Ss to increase and the C Ss to decrease their intake of absolute ethanol between Initial and Final Choice was significantly greater than zero.

In addition, differences between strains in the size of the interactions calculated above were analyzed

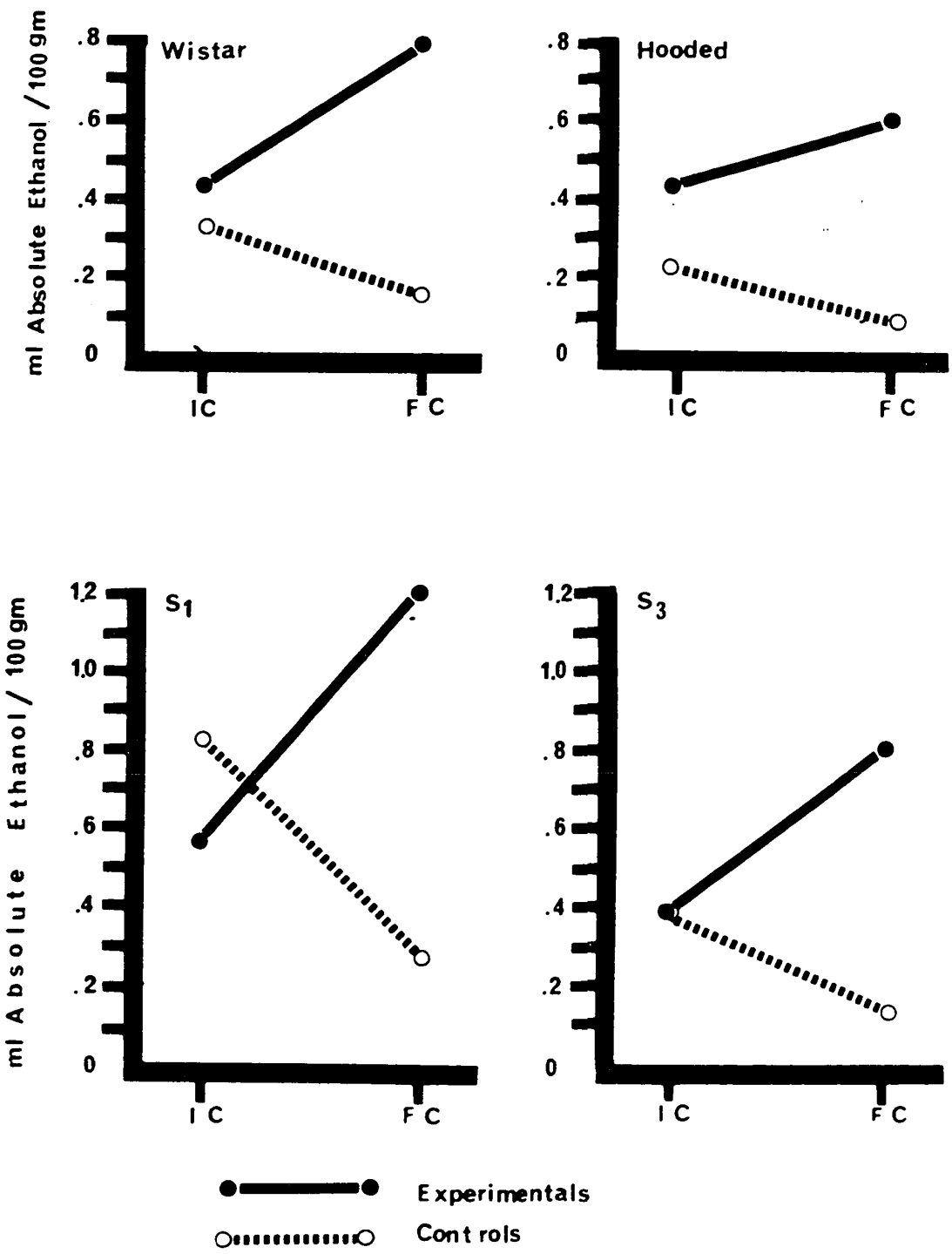

Figure 6. Mean intake of absolute ethanol (ml ETOH/100 gm body weight) at Initial Choice (IC) and Final Choice (FC) of the Experimental and Control groups within four rat strains. (n=12)

using probability values suggested by Scheffé (Winer, 1962). It was found that the interaction in the S_1 strain was significantly greater than that of the Hooded ($p<.01$) and the Wistar ($p<.05$) strains. No other differences were significant.

Withdrawal. In order to test the effects of a two-week period of withdrawal from ethanol on ethanol-directed behavior, the preference ratios and intake data from the Final Choice and Free Choice I periods were subjected to an analysis of variance of the same design as that used to test the effects of the Experience period. The results of the analysis of the preference ratios appear in Table VIII.

The significant condition effect results from higher preference shown by subjects in the experimental group. The Condition x Period interaction was analyzed using an F test based on sums of squares calculated in the same manner used in analyzing the C x P interaction above. A significant difference ($p<.001$) was found between the Ss in the two experimental conditions during the Final Choice, indicating a higher baseline level in the E subjects. The E subjects showed a

Table VIII

Results of an analysis of variance performed on Preference Ratios at Initial Choice and Free Choice I of 96 rats.

Source	df	MS	F	p
Between Subjects				
Strain (S)	3	1588.06	2.62	ns
Sex (G)	1	1729.20	2.85	ns
Condition (C)	1	85033.60	140.06	.001
S x G	3	3581.80	5.90	.01
S x C	3	1340.58	2.21	ns
G x C	1	2466.77	4.06	.05
S x G x C	3	996.49	1.64	ns
Ss Within Groups	80	607.12		
Within-Subjects				
Period (P)	1	248.89	8.75	.01
S x P	3	76.11	2.68	ns
G x P	1	69.36	2.44	ns
C x P	1	300.50	10.57	.01
S x G x P	3	20.46	0.72	ns
S x C x P	3	17.60	0.62	ns
G x C x P	1	42.75	1.50	ns
S x G x C x P	3	37.79	1.33	ns
P x Ss Within Groups	80	28.44		
Total	191			

significant increase in preference following Withdrawal ($p < .001$). The preference ratios of the C subjects during the Final Choice and Free Choice I, however, were not significantly different ($p > .20$). These results are presented graphically in Figure 7A.

The sex x strain (S x G) interaction was again examined by comparing the means of the males and females within each strain. The nature of the interaction is shown in Figure 8. Significant sex differences were found in all strains. The probability levels associated with the differences between the sexes in the Wistar and S_3 strains were less than .001, while the level for the difference in the Hooded strain was .01 and that of the S_1 strain was .05.

In the analysis of the amount of absolute ethanol ingested, which appears in Table IX, the condition and period variables show significant differences in the same direction as those resulting from the analysis of the preference ratios. The Condition x Period interaction, which is presented graphically in Figure 7B, shows a pattern of significant differences identical to that of the same interaction in the analysis of

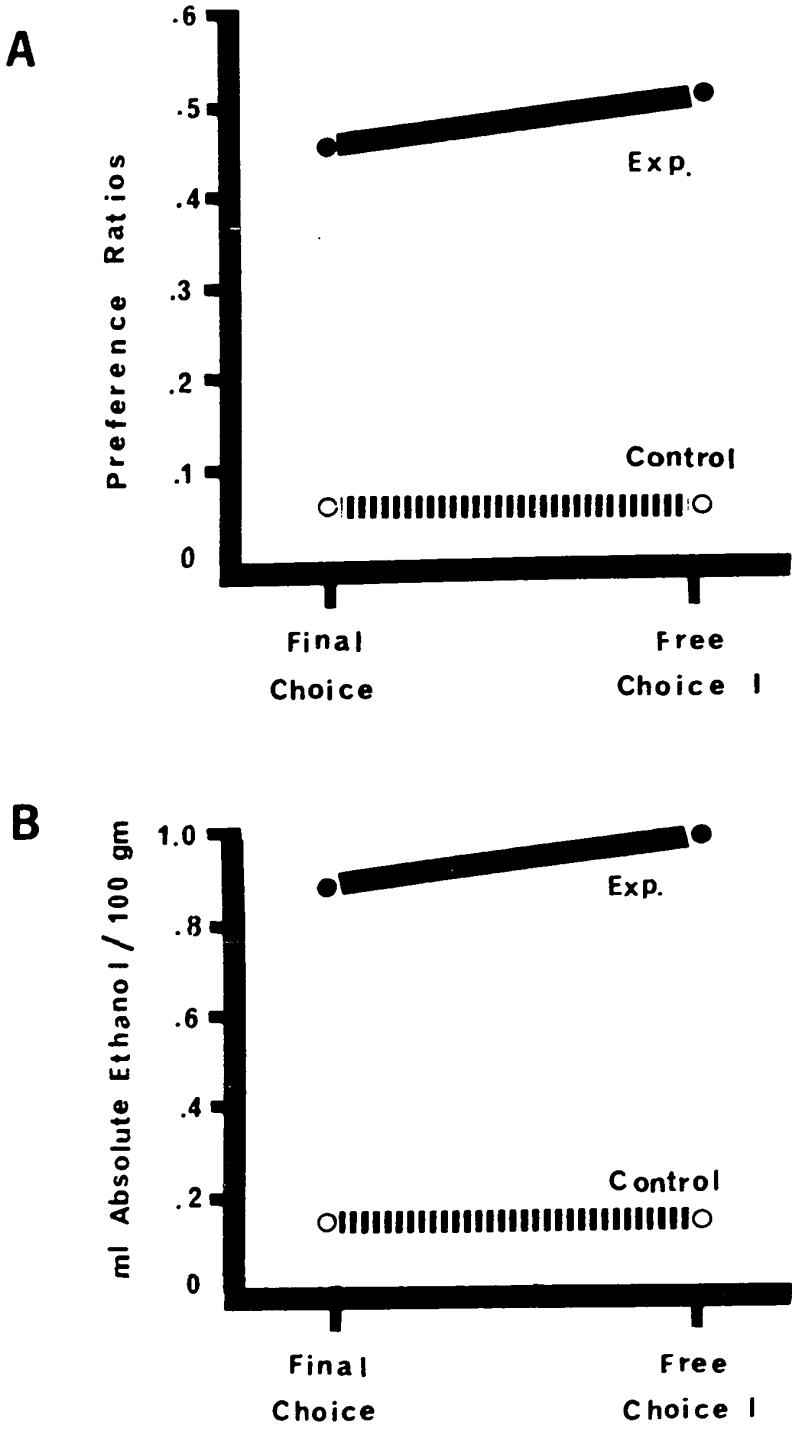

Figure 7. Mean Preference Ratios (A) and intake of absolute ethanol (B) of the Experimental and Control groups before (Final Choice) and after (Free Choice I) a two-week period of Withdrawal. (n=48)

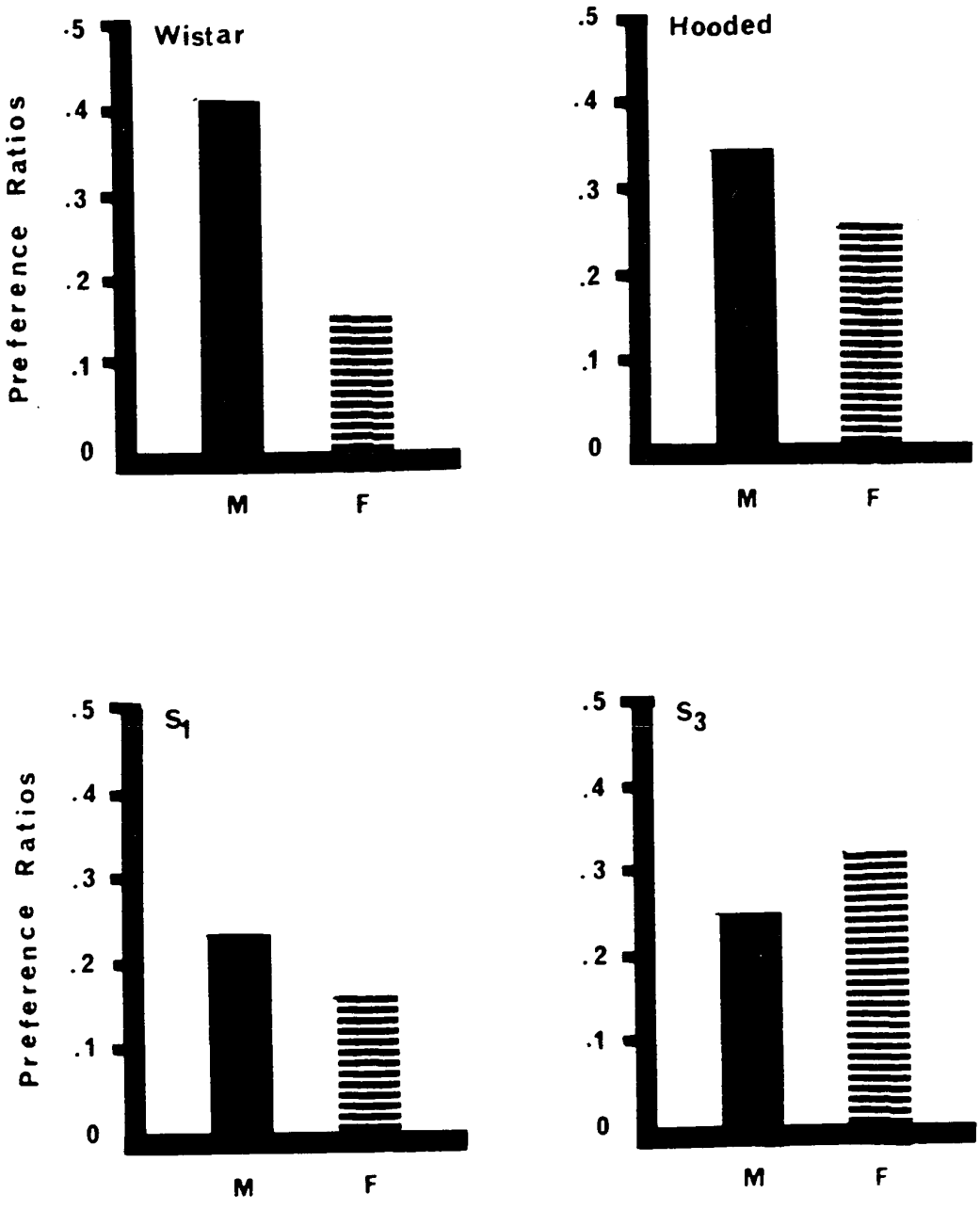

Figure 8. Mean Preference Ratios ($\frac{\text{ml MC}}{\text{ml MC + ml } H_2O}$) of the sex-strain subgroups during the Withdrawal Period.

(n=12)

Table IX

Results of an analysis of variance performed on the intake of absolute ethanol at Final Choice and Free Choice I of 96 rats.

Source	df	MS	F	p
Between-Subjects				
Strain (S)	3	1.951	11.10	.01
Sex (G)	1	0.869	4.95	.05
Condition (C)	1	27.984	159.21	.001
S x G	3	0.438	2.49	ns
S x C	3	0.512	2.92	.05
G x C	1	0.342	1.94	ns
S x G x C	3	0.293	1.67	ns
Ss Within Groups	80	0.176		
Within-Subjects				
Period (P)	1	0.183	11.43	.01
S x P	3	0.060	3.75	.05
G x P	1	0.040	2.48	ns
C x P	1	0.228	14.30	.01
S x G x P	3	0.039	2.47	ns
S x C x P	3	0.016	0.99	ns
G x C x P	1	0.030	1.85	ns
S x G x C x P	3	0.017	1.05	ns
P x Ss Within Groups	80	0.016		
Total	191			

ethanol preference.

Metering. The ability of a subject to maintain a constant level of ethanol intake was analyzed by comparing the mean of the subject's consumption of the ethanol solution in the four days in which the Maintenance Concentration was available in the Free Choice periods (I and II) which preceded and followed the metering with consumption in the two Metering periods.

The data from the Metering period indicated that the Ss in the Experimental group were better able to monitor their intake of absolute ethanol than were the Control Ss.

The data on absolute ethanol consumption did not satisfy the assumption of homogeneity of variance for use in parametric statistical analysis. In order to satisfy this assumption, the data were transformed by computing the standard deviation of the intake during the three conditions for each subject. This score was used as an index of the variability of intake. Thus, the score of a subject ingesting a constant amount of absolute ethanol would be small, while that of a subject which did not respond consistently would be large.

The results of a three-way analysis of variance

of these variability scores are presented in Table X. The results of this analysis indicate significant effects of sex, strain and experimental condition. They indicate, in addition, a significant interaction between sex and strain (S x G). Experimental condition (C), however, did not interact significantly with either sex or strain.

When the significant condition effect is examined, it is seen that the variability scores of the Ss in the Experimental group ($\overline{X}$ = .1547) were significantly lower than those of the control group ($\overline{X}$ = .3456). The reliability of this difference may be seen by referring to Table XI, where the means of the variability scores of the E and C groups of each of the sex-strain subgroups are presented. It can be seen that in all of the sex-strain subgroups the C Ss had higher variability scores than did the corresponding E Ss.

The nature of the sex x strain interaction is seen in Figure 9, where the variability scores are presented graphically for each of the sex-strain subgroups. Multiple comparisons by t-tests reveal that the variability of the females of the Wistar, Hooded

Table X

Results of an analysis of variance performed on the variability scores of 96 rats.

Source	df	MS	F	p
Strain (S)	3	.3566	15.078	.01
Sex (G)	1	.3290	13.911	.01
Condition (C)	1	.8746	36.979	.01
S x G	3	.2329	9.850	.01
S x C	3	.0479	2.024	ns
G x C	1	.0275	1.161	ns
S x G x C	3	.0427	1.806	ns
Within	80	.0236		
Total	95			

Table XI

Mean Variability Scores of the sex-strain subgroups within each experimental condition during the Metering Period. (n=6)

Subjects	Experimental	Control
Wistar		
Males	.1594	.3629
Females	.3819	.5481
Hooded		
Males	.1854	.2200
Females	.2194	.3342
S_1		
Males	.3300	.4051
Females	.4510	.6382
S_3		
Males	.2766	.4656
Females	.1816	.3169

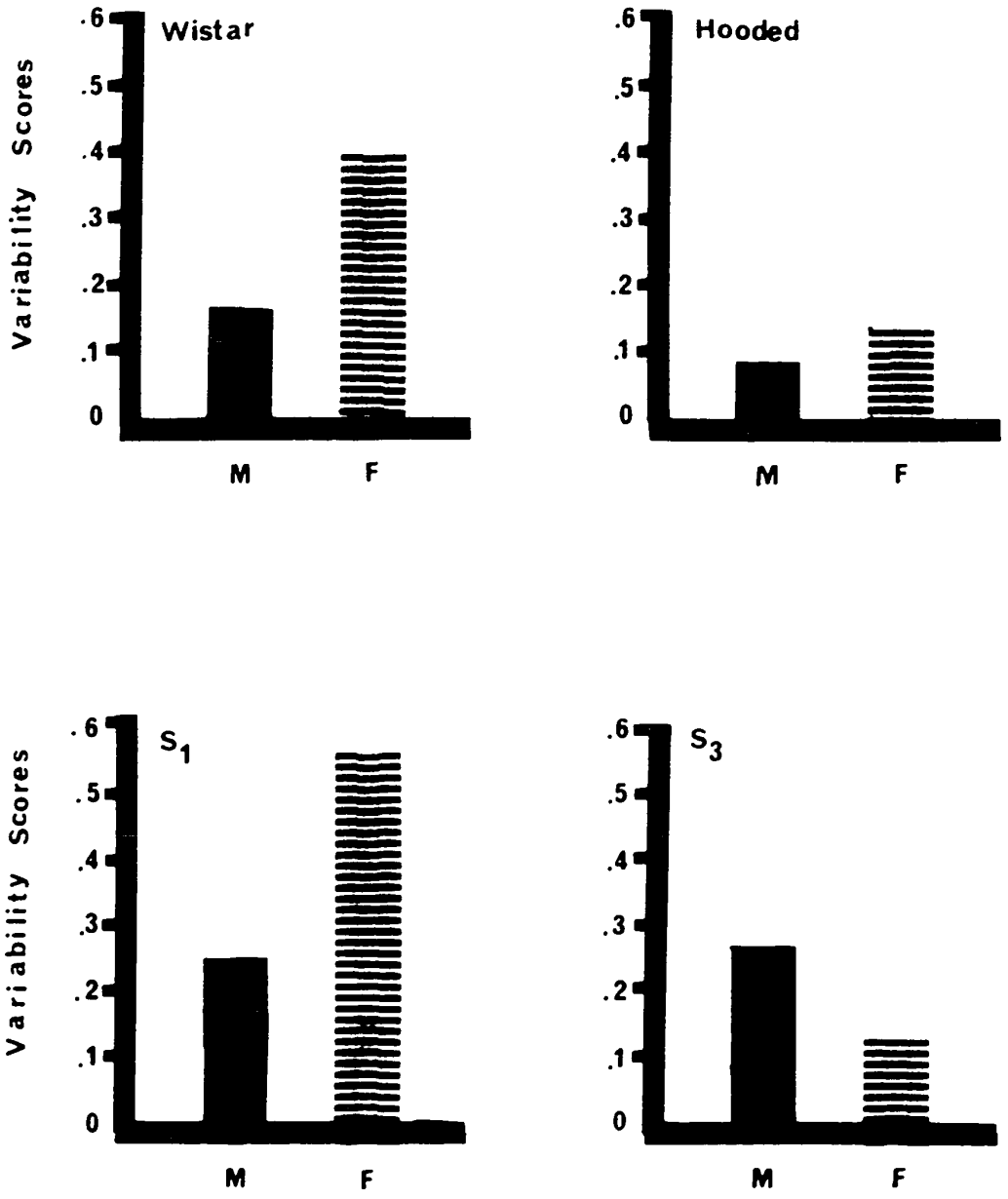

Figure 9. Means of variability scores of the sex-strain subgroups during the Metering Period. (n=12)

and S_1 strains was significantly larger than that of the males of the same strains at the .01 level of probability. The males of the S_3 strain, however, were significantly more variable than were the S_3 females ($p<.01$).

In attempting to analyze the data in terms of the proportion of the total daily fluid intake taken in the form of the ethanol solution, no index could be found which would satisfy the assumption of homogeneity of variance for the analysis of variance. Therefore, an index of variability of response for each subject was formed by calculating the difference between the largest and smallest observed preference ratios and dividing this difference by the free choice preference ratio. These scores were then ranked, and the difference between the experimental and control groups was analyzed by means of the Mann-Whitney U. The results of this analysis showed that the control subjects were significantly more variable than the experimental subjects at the .001 level of probability.

Quinine. All 48 subjects in the experimental group and none of the subjects in the control group drank ethanol on at least one of the days during which

it was adulterated with quinine. A Chi-Square performed on these data is significant beyond the .001 level of probability.

The effects of adulteration of the MC by quinine were analyzed by comparing the preference and intake shown by the 48 E Ss during Free Choice II with the same measures during the Quinine Period. Again, the effects of sex and strain were included as variables in the analyses of variance. The results of these analyses are to be found in Table XIIA and B.

On both measures there was a significant decrease between Free Choice II and Quinine. Both analyses reveal a sex x strain interaction which is significant at only the .05 level of probability.

When the strain effect, significant only with the data from consumption of absolute alcohol, was analyzed by the procedure of Newman-Kuels (Winer, 1962), it was found that subjects of the S_1 strain ingested significantly more ethanol than subjects of the other three strains ($p<.01$), which did not differ from each other ($p>.10$).

Free Choice III. All subjects in the experimental

Table XII

Results of analyses of variance performed on the Preference Ratios (A) and intake of absolute ethanol (B) at Free Choice II and Quinine of 48 subjects of the Experimental Group.

A Source	df	MS	F	p
Between-Subjects				
Strain (S)	3	547.38	1.27	ns
Sex (G)	1	972.83	2.25	ns
S x G	3	1765.92	4.08	.05
Ss Within Groups	40	432.59		
Within-Subjects				
Period (P)	1	22983.50	79.46	.001
S x P	3	703.66	2.43	ns
G x P	1	1906.38	6.59	.05
S x G x P	3	891.27	3.08	.05
P x S Within Groups	40	289.24		
Total	95			

B Source	df	MS	F	p
Between-Subjects				
Strain (S)	3	3.572	10.05	.01
Sex (G)	1	1.777	5.00	.05
S x G	3	1.035	2.91	.05
Ss Within Groups	40	0.356		
Within-Subjects				
Period (P)	1	3.737	67.31	.01
S x P	3	0.020	0.35	ns
G x P	1	0.070	1.27	ns
S x G x P	3	0.148	2.66	ns
P x S Within Groups	40	0.056		
Total	95			

group and 20 of the Ss in the control group drank the ethanol solution on at least one of the days in the Free Choice III period. A Chi Square calculated from these data is significant beyond the .001 level, indicating a greater tendency for the experimental subjects to drink ethanol when unadulterated ethanol was made available following the substitution of quinine-adulterated ethanol.

For each subject in the experimental group and the 20 subjects in the control group which drank during Free Choice III, the change in both the preference ratio and intake of absolute alcohol between Free Choice II and Free Choice III was calculated. The algebraic size of these differences were ranked and analyzed by means of a Mann-Whitney U. In both the case of preference and of intake, the experimental group was different from the control group at the .01 level of significance. Thus, even among those control Ss which did drink ethanol during Free Choice III, the decrease from Free Choice II was greater than that of the experimental subjects.

The changes between Free Choice II and Free Choice III in preference and intake for the subjects in

the experimental group were subjected to analyses of variance using the same design used to analyze the effects of quinine. The results of these analyses appear in Table XIIIA and B.

The results from the analysis of the preference data show no significant differences except a S x G interaction significant at only the .05 level.

Absolute ethanol intake was significantly lower during Free Choice III than during Free Choice II. In addition, an analysis of the significant strain effect using the Newman-Kuels method shows the intake of the S_1 subjects to be higher than that of the other three strains ($p < .01$), which do not differ from each other ($p > .10$).

Summary. The data from the experimental periods in Experiment II indicate consistent differences between the Control and Experimental groups. As a result of a 75-day exposure to ethanol, the Ss in the Experimental Group showed a significant increase in preference and intake of ethanol, while Control Ss, without exposure to ethanol showed a significant decrease in both measures. Following a two-week withdrawal period, preference for,

Table XIII

Results of analyses of variance performed on Preference Ratios (A) and intake of absolute ethanol (B) at Free Choice II and Free Choice III of 48 subjects of the Experimental Group.

A Source	df	MS	F	p
Between-Subjects				
Strain (S)	3	2895.40	2.62	ns
Sex (G)	1	3546.59	3.20	ns
S x G	3	3206.47	2.94	.05
Ss Within Groups	40	1106.75		
Within-Subjects				
Period (P)	1	87.21	1.04	ns
S x P	3	58.77	0.70	ns
G x P	1	234.06	2.80	ns
S x G x P	3	170.37	2.04	ns
P x Ss Within Groups	40	83.53		
Total	95			

B Source	df	MS	F	p
Between-Subjects				
Strain (S)	3	2.435	6.26	.01
Sex (G)	1	1.688	4.34	.05
S x G	3	0.622	1.60	ns
Ss Within Groups	40	0.389		
Within-Subjects				
Period (P)	1	0.218	7.91	.01
S x P	3	0.072	2.62	ns
G x P	1	0.054	1.95	ns
S x G x P	3	0.013	0.49	ns
P x Ss Within Groups	40	0.028		
Total	95			

and intake of, ethanol increased in the experimental Ss, but not in the Control Ss. Ss in the experimental group were better able to monitor their intake of absolute ethanol than were Ss in the control group. When the ethanol solution was adulterated with quinine, none of the control subjects and all of the experimental subjects drank some of the ethanol solution. Finally, when unadulterated ethanol was again offered, subjects in the experimental group showed a smaller decrease from pre-quinine intake and preference than did subjects in the Control group.

Discussion

This research has shown that a period of exposure to ethanol in a free-choice situation is able to produce a marked and persistent change in ethanol-directed behavior of male and female subjects of four strains of rats. In the previous section, the results were presented individually for each experimental period. It is the purpose of this section to discuss several patterns which emerge when the results of the individual periods are viewed together. In addition, a possible mechanism which might account for the findings will be proposed, and lines of future research suggested.

The present research was designed to study the effects which constitutional variables (sex and strain) have on the free selection of ethanol by rats when the ethanol is available on a long-term basis. The results of Experiment I demonstrated clearly that sex and strain were important in determining the highest concentration of ethanol which a rat would drink voluntarily. The results of Experiment II showed that rats given a free choice between

ethanol and water over a prolonged period of time increased their preference for, and intake of, an ethanol solution. In addition, Experiment II confirmed the effects of constitutional variables on alcohol-directed behavior. When the results are studied closely, however, two features of the data relating to the role of sex and strain become apparent. The first feature is the difference between the results obtained from the analysis of the data provided by two measures of alcohol consumption which were used. The second feature is the fact that constitutional factors did not produce differences in responses to some of the manipulations.

The two measures which were used, preference ratios and intake of absolute ethanol per 100 grams of body weight, were chosen because the values obtained were independent of the weight of the subjects. The calculation of preference ratios, however, must take the amount of water ingested into account, while the calculation of ethanol intake does not require this. Since the two measures are calculated using different information, it is not surprising that statistical

analyses using the two measures should yield different results. Differences found in the present research were not random; rather, they followed a consistent pattern.

The analyses of intake data indicated significant differences between sexes and/or strains, but only in the Experience period and the Metering period was there a significant sex x strain interaction. In the analyses of the preference ratios, on the other hand, the effects of constitution on alcohol preference were seen in the form of sex x strain interactions. Sex and strain alone were never significant as main effects. These differences between the two measures of ethanol-directed behavior pose a methodological problem: What is the most valid measure to use in self-selection studies in animals? These are only two of the many measures of alcohol-directed behavior which have been used, and it is possible that many inconsistencies in the literature on self-selection of ethanol by animals might be resolved if the same measures were used consistently. It should be emphasized that the results derived from analyses of both

measures indicate that sex and strain are important determinants of alcohol-directed behavior in the rat. The difference in form of the results should not be allowed to obscure the general picture of the importance of constitutional variables demonstrated by the present research.

During the Experience period, subjects in the experimental group increased, and subjects in the control group decreased, their levels of alcohol drinking. During this period, the groups of the S_1 subjects showed a greater degree of divergence between Initial Choice and Final Choice than did the two groups of the Wistar and Hooded subjects (Figure 6). Alterations of the Maintenance Concentration after Final Choice, such as Withdrawal, Metering and Quinine, however, seemed to affect all sex-strain subgroups in the same way. That is, these alterations had no differential effects on subjects which differed constitutionally. This fact was shown in two ways.

The first source of support lies in the absence of significant interactions between either sex or strain and experimental condition. The differences in

response to alterations of the Maintenance Concentration between experimental and control groups were quite marked, but in no case were there significant strain- or sex-produced differences in the magnitude or direction of these changes in responding. This fact suggests that when a level of consumption of the MC which is characteristic of a sex-strain subgroup has been established following 75 days of exposure to ethanol, any short-term alterations of this concentration do not differentially affect the ethanol consumption of these groups.

The second source of support lies in a comparison of the pattern of the sex x strain interactions. In Experiment I, it was found that the S_1 and Wistar females had significantly higher FACs than the males of the same strain; that there were no sex differences in the Hooded strain; and that the S_2 males had significantly higher FACs than the S_3 females. This same pattern of sex differences within strains was found in the results of the analysis of the intake data following Experience (Figure 5) and in the analysis of the Metering period in Experiment II (Figure 9).

The consistency of the pattern again suggests the absence of differential effects of manipulations of the MC upon subjects of different constitutions.

In addition to the exposure to ethanol during the 75-day Experience period, the subjects were also exposed to ethanol during the preference testing in Experiment I. The method of testing for ethanol preference used in Experiment I was based on the method of Cicero and Myers (1968). In the present study, the concentration was increased until the animals refused the ethanol solution, instead of stopping when the subjects drank less than fifty per cent of their daily fluid intake in the ethanol solution. In many cases, this testing was quite prolonged, and this period of exposure resulted in the drinking of abnormally high concentrations of ethanol by subjects of both the experimental and control groups. The elevation of drinking was temporary, however, as shown by the significant decrease in preference and intake of the control subjects between Initial and Final Choice.

The existence of the alcohol deprivation

effect was confirmed by the results from the Withdrawal period. When ethanol was withdrawn for two weeks, the experimental Ss showed higher preference and intake when the ethanol was made available again, while the control subjects showed no change.

It has been shown that a 75-day period of exposure to ethanol significantly increases preference for, and intake of, ethanol. Three of the sex-strain subgroups of the experimental condition, in fact, showed a clear-cut preference for their Maintenance Concentration over water, as shown in Table III. The S_3 females indicated a preference for their MC over water. The mean MC for this group is, however, within the range of concentrations which are normally used in ethanol research with animals. Both the Hooded and Wistar males showed clear preference for the ethanol solutions to which they were exposed. In the case of these groups, however, the mean MC was above 18%. It is an important finding that rats will develop strong preferences for high concentrations of ethanol with no experimental intervention except access to an ethanol solution.

The problem which remains is to account for this change in ethanol-directed behavior. When one examines the previously reported literature, a possible explanation presents itself.

In the first place, while most rats will voluntarily drink some ethanol when the concentration is less than 10%, this drinking appears not to affect their behavior. For example, activity in a running wheel does not change when rats are permitted a free choice of ethanol and water (Hausmann, 1932). By contrast, forced consumption of ethanol produces a decrease in running wheel activity (Hausmann, 1932; Richter, 1926). No changes in functioning in learning situations have been reported to occur after prolonged voluntary intake of ethanol; reports of positive findings of this type have resulted from experiments using forced exposure to ethanol (Denenberg, Pawlowski & Zarrow, 1961; Pawlowski, Denenberg & Zarrow, 1961). In short, it has proven impossible to demonstrate any effect which alcohol, in the amounts which are drunk voluntarily by rats, has on the behavior of rats.

One must presume, however, that since rats drink ethanol, the drug must produce positive effects, even though these effects are not directly observable.

Another source of information which is useful for the purpose of finding an explanation for the results of the present research is the data on increased "behavioral tolerance". These data show that long-term forced administration of ethanol produces two types of changes in response to ethanol: The behavior of an ethanol-exposed subject is less affected by a given dose of ethanol than is that of a subject not exposed to ethanol, and in order to produce the same level of behavioral impairment, the administration of increasingly larger doses is required. The latter class of changes has been demonstrated in man (Isbell et al., 1955). These experimenters wished to administer enough alcohol to human subjects to maintain a constant level of behavioral intoxication. It was found that it was necessary to increase the amount of ethanol given daily in order to accomplish this. It is not known,

however, whether the amount of ethanol taken in voluntarily by rats is sufficient to produce increased behavioral tolerance.

If a rat does, in fact, drink ethanol for some positive effect, and if the ethanol solution is available over a prolonged period of time, it may be that the rat must ingest increasing amounts of the drug to experience the same positive effect. At the same time that the intake is increasing, the rat would also learn to regulate the rate of intake so that the effects of the ethanol do not become noxious.

This hypothesis is supported by much of the data from the present research. It is supported most firmly by the increase in intake by the experimental group following 75 days of exposure to ethanol. Also, the metering condition indicated that rats with ethanol-drinking experience were better at maintaining a constant intake of absolute ethanol than were rats without this experience. That is, they were able to ingest enough absolute ethanol in spite of the change in the taste of the ethanol solution, to produce the positive effects derived from ethanol. At the same

time, they did not drink too much, thereby producing noxious effects.

A program of experimentation should be undertaken to investigate the possibility that a change in sensitivity to toxic doses of ethanol occur following prolonged exposure to ethanol. In this research, two groups of rats would be given the same treatments as the Exerimental and Control groups of the present research. After the Experience period, the subjects would be tested to determine the degree of their sensitivity to ethanol. The hypothesis suggested in this thesis would predict a decrease in sensitivity to ethanol in the subjects given exposure to ethanol.

That there were significant strain and sex differences in the concentrations of ethanol rejected by the subjects indicates differences among animals of differing constitutions in sensitivity to ethanol. Kakihana et al. (1966) showed that subjects of a mouse strain which showed higher preference for ethanol were less sensitive to the effects of an injection of an intoxicating dosage of ethanol than were mice of a strain which showed low preference for ethanol. It may

be predicted that subjects of a high drinking strain of rats, for example the S_1, would be less sensitive to intoxicating dosages of ethanol than, for example, subjects of the S_3 strain. Research similar to that of Kakihana et al. should be performed using the Tryon strains to confirm this prediction.

Long-term exposure to an ethanol solution in a free-choice situation increases the preference for and intake of that solution. The increase is so marked that the question should be asked whether changes in behaviors unrelated to ethanol ingestion, such as learning, have occurred, as is the case with prolonged forced intake. It may be that, even though the effects of short-term voluntary intake on behavior are not observable, long-term exposure may produce changes which could possibly indicate the nature of the "positive effect" produced by ethanol.

Summary

Interactions of constitutional variables with prolonged experience with ethanol were investigated in 12 male and 12 female rats of four strains: Wistar, Hooded, S_1, and S_3. In Experiment I, the sex-strain subgroups were found to differ in the highest concentration of ethanol drunk in a free-choice situation.

In Experiment II, each of the sex-strain subgroups was further divided: Half of the Ss, the Experimental (E) group, were given free access to an ethanol solution on alternate days for 75 days. The Control (C) group had access only to water during this period. The 75 days of exposure to ethanol produced a significant increase in preference for, and intake of, ethanol. In addition, the Ss in the E group drank increased amounts of the ethanol solution following a two-week Withdrawal period, and continued to drink the ethanol solution when quinine was added, while all C Ss rejected the quinine-adulterated solutions. Finally, the Ss in the E group were better able to monitor their intake of absolute ethanol

than were the C Ss.

It was found that the exposure to ethanol affected the four strains to different degrees. The sex-strain subgroups, however, did not differ in their responses to the withdrawal of ethanol, changes in concentration of the ethanol solution, or the addition of quinine.

It is argued that the increase in ethanol-directed behavior following exposure reflects physiological changes resulting from chronic ingestion of ethanol which require that larger quantities of the drug must be ingested by alcohol-exposed rats than naive rats in order to obtain the same pharmacological effects.

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