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A Critical Evaluation of the Use of Animal Models in Alcohol Research: An Examination of Voluntary Alcohol Consumption in Rodents

Kathryn June Gill

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the degree of Doctor of Philosophy at
Concordia University
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ABSTRACT

A Critical Evaluation of the Use of Animal Models in
Alcohol Research:
An Examination of Voluntary Alcohol Consumption in Rodents

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Concordia University, 1989

The ongoing controversy regarding the validity of animal models of alcoholism has been a conspicuous aspect of alcohol research for many years. There is a pervasive notion that animals do not voluntarily drink sufficient alcohol to serve as useful tools in the study of the factors involved in the development of alcoholism. This thesis is devoted to a critical evaluation of this notion, and to an examination of voluntary alcohol intake in rodents.

In Experiment 1 the distribution of alcohol intake in a large sample of outbred, genetically heterogeneous Long Evans rats was examined. Animals displayed a wide range of alcohol intake ranging from 0 to 8 grams absolute alcohol/kilogram body weight/day. The temporal pattern of food, water, and alcohol consumption in Long Evans rats and genetically selected alcohol preferring (P) and Nonpreferring (NP) rats was examined in Experiment 2 A computerized drinkometer system was designed to continuously monitor feeding and drinking behavior with the aim of providing a more detailed analysis of the pattern of alcohol intake. A comparison of drinking in the outbred Long Evans and genetically selected P/NP rats indicated that high drinking animals of both strains consumed alcohol in a similar pattern. Alcohol drinking was further examined in Experiment 3 using the computerized drinkometer. The results demonstrated that rats consume considerable

volumes of alcohol in discrete, short bouts mainly during the lights-out cycle. There was a great deal of individual variation in alcohol intake in terms of the patterns of intake and the size and frequency of bouts of drinking. Simulation of a single alcohol drinking bout was carried out by limiting access to a short time period each day in Experiment 4. Detectable levels of blood and brain ethanol were observed following these individual drinking bouts. Behavioral evidence was obtained indicating that rats drink alcohol in quantities sufficient to produce pharmacological effects. In the final experiment the potential role of the alcohol metabolizing enzymes, Catalase and Aldehyde Dehydrogenase, in the regulation of voluntary alcohol intake was examined. The results indicated that a significant proportion of the variance in drinking behavior can be predicted by a knowledge of the activities of these enzymes in brain.

Taken together these data suggest that the voluntary consumption of alcohol in rodents can provide a useful animal model in the elucidation of the behavioral and underlying biological determinants of drinking behavior with potential for understanding the factors involved in the development of alcoholism.

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INTRODUCTION

It appears to be a well accepted fact that animals, including rats, do not drink alcohol. This fact is restated in the introduction to an enormous number of research papers in the alcohol literature (e.g. Asin, Wirstshafter & Tabakoff, 1985; Grant & Johanson, 1988; McBride, Murphy, Lumeng & Li, 1989; McCusker & Bell, 1988; Ritz, George & Meisch, 1989), with reference made generally to a polemic written by Cicero (1980) on animal models of alcoholism The concensus that animals do not drink alcohol has persisted in the literature, despite the best efforts of those who regularly measure the voluntary intake of alcohol in rats to convince the alcohol research community to the contrary (Amit, Smith & Sutherland, 1987; Gill, France & Amit, 1986; Samson, Pfeffer & Tolliver, 1988).

The ongoing controversy regarding the validity of animal models of alcoholism, particularly the voluntary oral "preference" paradigm in rats, has been a conspicuous aspect of alcohol research. In the preference paradigm animals are given a choice between an alcohol solution and water, and the alcohol preference ratio is determined by calculating the amount of alcohol consumed as a percentage of the total daily fluid intake. The absolute amount of alcohol consumed (grams alcohol/kilogram body weight/day) is often considered along with the preference ratio, as a measure of alcohol-oriented behavior. In general, various critiques of the preference paradigm have suggested that the majority of animal species drink very small quantities of alcohol, insufficient to produce intoxication, which are most likely ingested as a source of calories (Cicero, 1980; Deitrich & Melchior, 1985; Dole, Ho & Gentry, 1985; Kulkosky, 1985, Lester & Freed, 1973). The apparent limit on the intake of alcohol by rodents has been suggested to be due to the aversive post-ingestional consequences of intoxication, or possibly the aversive taste of

standard laboratory alcohol solutions (Deitrich & Melchior, 1985; Kulkosky, 1985; Mello, 1973; Woods & Winger, 1971)

This controversy has important implications for alcohol research. One of the major effects is the rapidly developing concensus that the only viable means of examining oral alcohol self-administration in rodents will be through the use of genetically selected high drinking strains. This view is reinforced by statements such as "only one line [of rats] thus far has been established that satisfied all the criteria for an animal model of alcoholism and that would be suitable for studying the biological basis of ethanol preference" (McBride et al., 1989). There are, worldwide, currently four lines of genetically selected alcohol drinking rats, as briefly described in Table 1. None of these lines are readily available to the research community at large This faith in the genetic model is echoed in most recent reviews of the alcohol literature (Kalant, 1987, Koob & Bloom, 1988, Tabakoff & Hoffman, 1987). Ironically, it was the voluntary oral "preference" paradigm that was used to develop these selected strains of rats, thus confirming the genetic basis for differences in alcohol intake previously observed in inbred strains of mice (Williams, Berry & Beerstecker, 1949)

It is the aim of the introduction of this thesis to critically evaluate the controversy over an animal model of alcoholism The disagreements in the literature concerning the adequacy of alcohol drinking paradigms appeared to be rooted in two factors 1) The consistent failure to establish alcohol as a reinforcer in paradigms commonly used to assess the reinforcing properties of drugs (e g intravenous self-administration, conditioned place preference), has led to the conclusion that alcohol is a weak reinforcer in rodents 2) Animal paradigms have consistently failed to meet a set of criteria that outlined the necessary features of an "adequate" model of oral self-administration in animals (Cicero, 1980, Dole, 1986, Lester & Freed, 1973) It is argued that these criteria

TABLE 1

GENETICALLY SELECTED RAT STRAINS

UChA/UChB

University of Chile A and B rats. Maintained in Santiago, Chile. Selected for voluntary consumption of a 10% alcohol solution (Mardones, Segovia & Hederra, 1953).

AA/ANA

Aiko, alcohol accepting and non-accepting rats. Maintained at the Research Laboratories of the State Alcohol Monopoly, Finland. Selected for voluntary intake of a 10% solution following 1 week of forced alcohol intake (Erikkson, 1968).

P/NP

Preferring and non-preferring rats. Maintained at the Indiana University Medical Center. Selected for voluntary intake of a 10% alcohol solution following 3-4 days of forced alcohol intake (Lumeng, Hawkins & Li, 1977).

HAD/LAD

High and low alcohol drinking rats. Selected and maintained as per P/NP rats (Lumeng, Doolittle & Li, 1986).

were overly restrictive and based on an inadequate description of the phenomenon of "alcoholism" in the human population. It is the contention of the present author that this controversy has resulted in the diversion of research interest away from the necessary development, improvement and standardization of the voluntary "preference" paradigm and away from the study of the indigenous preference of rats for alcohol.

The data reported in this thesis provide a detailed analysis of the alcohol drinking behavior of rats. These data were obtained from an examination of the diurnal/nocturnal patterns of food, water and alcohol consumption in the outbred strain of Long Evans rats, as well as the genetically selected line of P (Preferring) and NP (Non-preferring) rats. A computerized data collection system was designed in order to continuously monitor feeding and drinking behavior, providing measures of fluid bout size, frequency and duration, as well as permitting the examination of the relationship between food, water and alcohol intake. It is the aim of the research presented in this thesis to demonstrate that oral alcohol consumption in rats is a valid model of alcohol "drinking" in the human population. An animal model is described that will be useful in the examination of the underlying biological mechanisms regulating alcohol intake. Furthermore, the author maintains that the current focus on "genetic" models of drinking may not be the most productive research strategy for uncovering the underlying biological contribution to alcoholism.

WHY STUDY ALCOHOL INTAKE IN RODENTS?

Alcohol is a strong oral reinforcer in the human population. In various forms, strengths and flavours, it is the most commonly used drug in North America (Schuster, 1987). Alcohol abuse has been ranked as the third major health problem following cancer and heart disease and the most common psychiatric illness (Mello, 1987, Mello & Griffiths, 1987). The medical, psychiatric and

social complications resulting from alcohol abuse are extensive. They exact an enormous toll on society in economic terms. From figures in the Sixth Special Report to the U.S. Congress on Alcohol and Health, it has been estimated that annual cost of alcohol abuse in the U.S. is $117 billion, accounted for by health care, lost employment and reduced productivity (Casement, 1987). Equally important is the toll that alcohol use and abuse exacts in terms of the quality of life; for among others, the victims of motor vehicle accidents involving alcohol and the families of alcohol abusers.

There is no concensus among clinicians and researchers with regards to the cause of alcohol abuse. A 1980 monograph produced by the National Institute on Drug Abuse presented 43 different theories on the development of drug abuse (Lettieri, Sayers & Pearson, 1980). As shown by a sample of short titles--Metabolic Deficiency Perspective (Dole & Nyswander, 1980), Social Influence Theory (Becker, 1980), Disruptive Environment Theory (Chein, 1980) and Personality-Deficiency Theory (Ausubel, 1980), researchers are clearly dealing with the problem of drug abuse from very diverse and often incompatible theoretical perspectives. These theories could be classified broadly as representing biological, social, family and intrapsychic orientations. More recent publications concerned with theories of drug abuse showed a similar broad range of orientations (Baker, 1988). In general, there is considerable discussion in the clinical literature about the initiation, maintenance and escalation of alcohol abuse, as well as the diagnosis and clinical course of alcoholism; much of this has a bearing on the development of animal models of alcoholism.

THE SUBJECT OF THE MODEL: HUMAN ALCOHOL USE AND ABUSE

The Alcohol Research Group in Berkeley California has conducted surveys of the drinking patterns, consumption levels and alcohol-related problems of the American general population since 1964 (Hilton, 1987). Their most recent survey of 2081 males and 3097 females indicated that 24% of the male and 36% of the female population were abstinent. Alcohol consumption in the remaining population was rated on a quantity-frequency scale into 7 categories. The highest drinking category--frequent heavy drinkers--consumed 5 or more drinks/episode, once per week or more often. These individuals comprised 18% of the male and 5% of the female population. The largest proportion of problematic drinking behavior (e.g. loss of control, binge drinking), physical dependence symptomology (e.g. tremor, early morning drinking) and adverse consequences of drinking (problems with spouse, job, police and health) were reported in this group of drinkers (Hilton, 1987). The remaining population--roughly 57% of the males and 59% of the females--exhibited a wide range of "social" drinking with corresponding lower alcohol intake and fewer reported alcohol related problems. It would appear from such figures that alcohol consumption is widely distributed in the human population, ranging from abstainers to those individuals with frequent heavy intake.

Among those individuals seeking treatment for alcohol abuse there is also a great deal of individual variability. Griffith Edwards has written extensively about the complexity of the clinical spectrum of "alcoholism". He wrote "if the diagnostician goes about his business with open eyes he will not see what is before him in terms of a limited typology, but only in terms of great and continuously unfolding variation" (Edwards, 1982). The variability that Edwards referred to has been observed in the amounts and patterns of alcohol drinking

(i.e. continuous, intermittent or binge drinking), the duration of problem drinking, the development of physical dependence and the amount of damage to health and social functioning (Edwards, 1982; 1986; Kivlahan, Sher & Donovan, 1989; McMahon, Gersh & Davidson, 1989). Babor, Kranzler & Kadden (1986) noted in their historical review on the diagnosis of alcoholism that "there has been a shift in the emphasis from the unitary construct "alcoholism" to a more heterogeneous aggregation of alcohol-related problems." In addition, through extensive individual case studies Edwards and colleagues have described alcohol abuse as "cyclical" with fluctuating periods of heavy drinking interspersed with periods of controlled drinking or voluntary abstinence (Edwards, Brown, Duckitt, Oppenheimer, Sheehan & Taylor, 1986).

In summary, it is very clear from surveys of the general population, that alcohol is consumed in widely varying amounts and patterns. Despite the fact that large numbers of individuals regularly ingest alcohol, the majority are able to regulate their intake, and only a small proportion abuse the drug. Among those individuals who abuse alcohol there also appeared to be wide variability. There is a general concensus among clinical researchers that not only do "alcoholics" differ quantitatively on a number of dimensions (Kivlahan et al., 1989; Naranjo, 1985; Sanchez-Craig, 1986; Skinner, 1985), they also appear to vary over time. Alcoholics do not invariably continue on progressive course, but may exhibit cyclical variations in their alcohol intake, level of physical dependence on alcohol and the severity of alcohol-related social problems (Edwards, 1982, 1986; Hill, 1985; Mello & Mendelson, 1972).

The variability in human alcohol use and abuse has considerable significance for the development of animal models of alcoholism. Naranjo (1985) described alcoholism as ranging from early stage problem drinkers to severely dependent "classical alcoholics". Naranjo suggested that perhaps "too

much attention has been paid to the 5% of classic alcoholics to the detriment of the other subjects." Naranjo (1985) went on to state that the wide clinical spectrum of alcohol-related problems has not been taken into consideration in the development of animal models of alcohol drinking behavior, and as such has served as a serious impediment to progress in areas related to the development of pharmacotherapies and to the discovery of underlying neurobiological controls of drinking. The lack of progress has been echoed in several recent reviews of the current trends within alcohol research. As stated by Kalant in his 1987 review, there has been considerable progress in understanding the physiological effects of alcohol on membranes, on the development of tolerance and in the development of alcohol-induced pathology (i.e. cardiovascular disease). However, Kalant noted that "regrettably little progress has been made in understanding of the basic nature of the effects which reinforce the self-administration of ethanol." This was attributed to the lack of a suitable model of alcoholism in experimental animals. Similarly, in their review of current alcohol research, Tabakoff and Hoffman (1987) delineated progress in a number of areas related to tolerance and physical dependence, stating however, that little is known about the reinforcing properties of alcohol self-administration, in part due to the difficulty of developing animal models of self-administration.

THE PROBLEM OF INADEQUATE MODELS OF ALCOHOLISM

The development of an adequate animal model of alcoholism has been a continuous preoccupation of the alcohol research community for many years (Erikkson, Sinclair, & Kiianmaa, 1980; Lester & Freed, 1973; Mello, 1973; Samson & Li, 1988). The notion of a "model" of alcoholism, as typically used in the alcohol research field, can best be described as an animal analogue of the human condition, to be used as an experimental tool in the study of the

behavioral, biochemical and neurophysiological concomitants of alcohol consumption.

While clinicians such as Naranjo (1985) have exhorted researchers to enter the alcohol field in order to study the neurobiological underpinnings of alcohol self-administration, there would appear to be few well validated, well accepted tools with which to tackle the job. In contrast, in areas of behavioral pharmacology examining the intake of drugs such as opiates, cocaine, amphetamine and benzodiazepines, there appears to be a well developed technology for studying self-administration through the intravenous route of administration (Deneau, Yanagita & Seevers, 1969; Yokel, 1987), and more recently through the intracranial route of administration (Bozarth, 1987b). As noted by Mello (1987) there is general agreement in the research community that self-administration of drugs by animals bears a striking resemblance to that of humans. Drugs that are abused by humans have been shown to be self-administered in various animal preparations; thus animal models appear to be very good indicators of the potential abuse liability of drugs (Bozarth, 1987a; Brady & Fischman, 1985; Deneau, Yanagita & Seevers, 1969; Johanson, Woolverton & Schuster, 1987).

Other methods considered to be useful for assessing the reinforcing effects of drugs such as cocaine, amphetamine and opiates, include paradigms such as conditioned place preference (Bozarth, 1987c; Nomikos & Spyraki, 1988) and brain stimulation reward (Hubner & Kornetsky, 1988; Schaefer & Michael, 1987; Wise & Routtenberg,1983). Extensive research examining the neuroanatomical and neurochemical substrates of drug self-administration is being carried out using these and other techniques (for recent examples see Hoffman & Beninger, 1989; Pettit, Batsell & Mueller, 1989). Most recently, animal

preparations are increasingly being used to determine the efficacy of various potential treatments for drug abuse (Mello, 1987; Johanson et al., 1987).

This description should not suggest that there is complete agreement about the validity of these various techniques in measuring drug reward. There has been a great deal of debate regarding the validity of the conditioned place preference paradigm for example (Bozarth, 1987b) or the interpretation of changes in the rate of operant behavior following pharmacological manipulations (e.g. Porter & Villanueva, 1989; Trujillo, Belluzzi & Stein, 1989). The point to be made here is, that despite differences in interpretation of data, there have been reliable, measurable effects of many drugs of abuse in these paradigms; with the exception of alcohol.

In his review of intravenous self-administration (IVSA), Yokel (1987) included alcohol on his list of compounds with equivocal reinforcing properties. In monkeys, alcohol has been reported to support the acquisition of IVSA (Deneau, Yanagita & Seevers, 1969; Mello et al., 1988; Winger, Young & Woods, 1983). However, in some cases animals were trained to self-administer drugs such as cocaine, before they would maintain responding for alcohol (Woods, Ikomi & Winger, 1971). Both Deneau et al. (1969) and Woods et al. (1971) noted that monkeys became physically dependent on alcohol, going through voluntary withdrawal in a cyclical pattern. Although IVSA in monkeys appeared to provide a good model with which to study alcohol self-administration, Mello (1976) noted a reluctance towards the use of this paradigm due to the fact that alcohol was not administered through the "oral" route. Another factor mediating against the development of an IVSA model is that in rats, a more commonly used experimental animal, it has been exceedingly difficult to produce consistent IV self-administration of alcohol. No reliable IVSA in rats was observed across doses ranging from 0.1 to 360

mg/kg/infusion (Collins, Weeks, Cooper, Good & Russell, 1984; DeNoble, Mele & Porter, 1985). Numan (1981) has reported low rates of alcohol IVSA following multiple cycles of forced intravenous infusion, while naive animals failed to acquire the operant response. Prior history with voluntary alcohol drinking failed to increase the likelihood of animals acquiring IVSA behavior (Gill, Boyle & Amit, 1989). Sinden & LeMagnen (1982) reported that low doses of alcohol supported IVSA in rats, however, two laboratories have failed to replicate these findings (Gill et al., 1989; Numan, Naparzewska & Adler, 1984).

Similar to the situation with IVSA, the conditioned place preference (CPP) paradigm has not been shown to be a useful method of measuring alcohol effects. In the CPP procedure, drug administration is paired with a distinctive environment; the subsequent avoidance or preference for that environment is taken as measure of aversion or reward produced by the drug. An early report suggested that alcohol was able to produce a CPP in rats (Black, Albiniak, Davis & Schumpert, 1973). Numerous subsequent studies however, have failed to confirm this initial finding despite extensive effort; a variety of dose regimens, routes of administration and experimental conditions have been employed (Asin et al., 1985; Cunningham, 1981; Stewart & Grupp, 1989; Van der Kooy, O'Shaughnessy, Mucha & Kalant,1983). An alcohol-induced CPP was reported following a history of forced alcohol ingestion in rats (Reid, Hunter, Beaman & Hubbell, 1985). However, an extensive series of studies in this laboratory has failed to find alcohol-induced CPP in animals with a prior history of alcohol intake, under a variety of experimental conditions (Gill, unpublished observations).

Similar to the effects with IVSA and CPP, there have been few reports of facilitation of brain stimulation reward (BSR) following alcohol administration. In the BSR paradigm, animals are given the opportunity to perform an operant

response to receive electrical stimulation to the brain. Facilitation of BSR occurs when a drug increases the rate of responding for BSR, or when there is a reduction in the number of electrical pulses or the amount of electrical current necessary to maintain the bar pressing response. A great deal of work has been carried out in which variables related to the alcohol (i.e. dose, route of administration, experimenter vs self-administered alcohol), the experimental design (i.e. type of operant performed) as well the dependent measure (i.e. changes in rate of responding vs threshold for BSR), have been manipulated with few positive results (Carlson & Lydic, 1976; Kornetsky et al., 1988; Musgrave, Randolph, & Freedman, 1989; Schaefer & Michael, 1987). Thus far it would appear that low doses of self-administered alcohol are most likely to produce a facilitation of BSR (Kornetsky et al., 1988). It is clear however, that the phenomenon is not robust or easily measurable (Gill, unpublished observations). Without considerable research this technique is unlikely to be widely used to study the reinforcing properties of alcohol.

In comparing alcohol as a reinforcer to other drugs of abuse, Winger et al. (1983) noted that the most salient feature of alcohol is its very low relative potency. Because alcohol is a liquid that cannot be administered at high concentrations, a limit is placed on the rate at which it can be delivered to the brain. Rapid rate of onset has consistently been identified as an important characteristic of drugs that are highly self-administered by animals (Winger et al., 1983). Lack of potency is believed to have contributed to the difficulty in measuring consistent, robust alcohol-induced effects in the paradigms listed above--paradigms that have previously been shown to be sensitive measures of the effects of other drugs of abuse.

THE ADEQUATE ANIMAL MODEL OF ALCOHOLISM--THE ORAL ROUTE OF ADMINISTRATION

As discussed above, the second major factor contributing to the lack of adequate research tools in the alcohol field has been the set of criteria outlining the necessary features of an "adequate" animal model of alcoholism. This set of criteria were formally outlined by Lester and Freed in 1973 and expanded and codified by Cicero in 1980 (see Table 2). The first criterion, and as implied by Lester and Freed (1973), the most important criterion, is that alcohol should be self-administered through the oral route. The criteria are unduly restrictive, promoting a definition of alcoholism that has not been supported by empirical evidence.

THE CRITERION OF TOLERANCE

Both tolerance and physical dependence have been considered defining characteristics of chronic alcoholism (Lester & Freed, 1973). Tolerance resulting from chronic intoxication has been proposed to be a separate phenomena from physical dependence, mediated by different neuronal and neurochemical systems (Tabakoff & Hoffman, 1988; Tabakoff & Ritzmann, 1977). In 1979, Cappell and LeBlanc stated that "perhaps the most important question about tolerance to ethanol...is whether it affects [the] probability of self-administration."

In answer to this question, Tabakoff and colleagues proposed that individual differences in the rate of development of tolerance were genetically determined, thus altering an individual's response to both the aversive and reinforcing effects of alcohol and subsequent self-administration of the drug (Tabakoff & Hoffman, 1988). Similarly, Li et al. (1987) proposed that rapid development of tolerance to the sedating effects of alcohol would allow animals to experience more of the stimulatory, reinforcing effects of the drug. In support of these notions, Le and Kiianmaa (1988) demonstrated that the high drinking AA rats

TABLE 2

CRITERIA FOR AN ANIMAL MODEL OF ALCOHOLISM

(Adapted from Cicero, 1980; Dole, 1986; Lester & Freed, 1973)

1) Alcohol should be voluntarily self-administered through the oral route, in animals that are not food deprived.

2) Alcohol should be consumed in preference to other solutions.

3) Intake patterns should indicate that alcohol is consumed at times other than those associated with feeding i.e. bouts of alcohol drinking should be temporally dissociated from food bouts.

4) Must demonstrate that animals consume alcohol for its drug-related intoxicating properties. i.e. measure blood alcohol levels following drinking and determine whether they produce a pharmacological effect. Intoxication should be sustained over a long period of time.

5) Demonstrate that alcohol acts as a reinforcer i.e. animals will work for alcohol or overcome significant obstacles to obtain it.

6) Voluntary intake should lead to the development of tolerance and physical dependence with concomitant symptoms of withdrawal upon abrupt cessation of drinking.

developed tolerance more rapidly to the locomotor-impairing, hypothermic and hypnotic effects of alcohol, than the ANA. However, the low drinking UChA rats developed tolerance much more readily than the UChB to the hypnotic and hypothermic effects of alcohol (Quintanilla & Tampier, 1982; Tampier, Urrutia & Quintanilla, 1988). Thus, based on work with selected strains, one must conclude that there is no firm relationship alcohol preference and the capacity to develop tolerance to alcohol.

THE CRITERION OF PHYSICAL DEPENDENCE

In 1973, Mello wrote, "the crucial determinants in the development of alcohol addiction are unknown...it has been generally agreed that only the development of an alcohol dependent animal would permit the study of the natural history of the addictive process..." Consequently, efforts in the alcohol field were directed for many years at developing methods to produce physical dependence in animals by inducing larger and larger intakes of alcohol. Voluntary intake was increased through a variety of manipulations such as gradually increasing the alcohol concentration (i.e. Myers & Veale, 1972) or exposing animals to alcohol on an alternate day schedule (Amit, Stern & Wise,1970; Wayner et al., 1972). However, with such methods animals consistently failed to show signs of physical dependence following withdrawal of the alcohol.

Other techniques were developed such as forced administration (Cicero, Snider, Perez & Swanson, 1971), food restriction, food-reinforced drinking (Keehn, 1969), schedule induced polydipsia (Lester, 1961) and electrical stimulation-induced drinking (Amit et al.,1970). Methods were also developed to passively produce physical dependence in animals, such forced inhalation of alcohol vapour (Goldstein, 1974) and liquid diets (Lieber & DeCarli, 1989). These methods have been described extensively in many reviews (Amit et al., 1987; Cicero, 1980; Deitrich & Melchior,1985; Woods, Ikomi & Winger, 1971).

However, many of these latter methods have been judged inadequate due to the lack of "voluntary" ingestion of alcohol, the first criterion listed on Table 2.

In summary, many researchers demonstrated that animals would not voluntarily ingest enough alcohol through the oral route to become physically dependent upon alcohol (Mello, 1973; Pohorecky, 1981). Therefore, based on these two apparently incompatible criteria (Wise, 1975), it has been impossible, in practice, to develop an adequate animal model of alcoholism. The myth that animals do not drink alcohol has arisen as a direct consequence of the inability to voluntarily induce physical dependence. The fact was that animals did consume alcohol; however it was not in quantities that satisfied the physical dependence criterion.

REACTION TO THE CRITERIA FOR "ADEQUATE" ANIMAL MODELS OF ALCOHOLISM

The difficulty in developing an animal preparation that simultaneously displayed high "voluntary" oral intake and physical dependence led some researchers to seriously question the necessity of including physical dependence in a model of alcoholism (Amit et al., 1987; Amit, Sutherland & White, 1975; Wise, 1975). Objections were based primarily on the observation that physically dependent animals would periodically abstain from taking alcohol and undergo withdrawal despite the free availability of alcohol (Deneau et al., 1969; Woods et al., 1971). Similarly, in laboratory studies of chronic alcoholics researchers have demonstrated that physically dependent subjects will spontaneously go through periods of abstinence and withdrawal (Mello & Mendelson, 1972), or spontaneously "taper-off" their rate of alcohol consumption (Nathan, Titler, Lowenstein, Solomon & Rossi, 1970).

As discussed previously, clinical research has shown that "alcoholics" vary a great deal in terms of the development of physical dependence. Edwards

(1982, 1986) proposed that the development of physical dependence and tolerance to alcohol may contribute to the maintenance of problematic drinking at some points during an individual's drinking career. He was, however, cognizant of the fact that physical dependence was not a necessary nor sufficient cause of alcohol related problems. Edwards was careful to stress that many "alcoholic" patients show no signs of the alcohol dependence syndrome (Edwards, 1982, p. 43). In one of the first clinical studies on chronic drinking in human subjects, Isbell, Fraser, Wikler, Belleville and Eisenman (1955) demonstrated that there were large individual differences in the capacity to develop physical dependence to alcohol. The subjects were continuously intoxicated (for periods ranging from 7 to 87 days) with large doses of alcohol (average 266 to 489 milliliters of 95% alcohol/day), administered at intervals of 1-2 hours. Only those patients drinking at these high levels continuously for longer than 48 days developed severe signs of withdrawal.

In summary, the most important contributing factor in the development of physical dependence appears to be the maintenance of continuous, high blood levels of alcohol (Wallgren & Berry, 1970; Woods et al., 1971); a condition that few animals or humans are capable of maintaining consistently (Amit et al., 1975; Dole & Gentry,1984). As noted by Isbell et al. (1955) "a large proportion of the alcoholics admitted to general hospitals have been intoxicated only a brief time...only a few days duration; they cannot, therefore, be expected to develop serious manifestations of an abstinence syndrome..."

Despite the evidence that has continuously been mounted against the necessity of including the criterion of physical dependence, a persistent emphasis has been placed on this aspect of the animal model. As implied by Naranjo (1985), too much emphasis has been placed on the 5% of end-stage alcoholics who display evidence of physical dependence. While there has

been no clear resolution of the role of physical dependence in the maintenance of drug use (e.g. see Koob & Bloom, 1988), one important point can be made. Physical dependence can only develop after a considerable period of heavy alcohol use. It follows therefore, that drug use is initiated by factors independent from physical dependence.

In conclusion, the criterion specified in Table 2 are overly restrictive. These criteria demand that the voluntary oral drinking behavior of a randomly selected group of rats, should exhibit the characteristics of 5% of the human "alcoholic" population. The voluntary oral drinking behavior of a randomly selected group of humans would also provide a very poor model of alcoholism. Here, you would find that like rats, some do not drink alcohol, some drink a little and a few drink heavily--and there would be little evidence of physical dependence.

THE CRITERION OF INTOXICATION--THE CENTRAL ISSUE FOR THE DEVELOPMENT OF ANIMAL MODELS

It is interesting to note that Lester and Freed (1973) have argued, that tolerance and physical dependence are in fact sequels of addiction, stating that "given alcohol-directed behavior of an animal over a period of time, the remaining components of addiction will follow if substantial blood alcohol levels are maintained over a sufficient integrated intoxication time." In their paper they placed considerable emphasis on the initial motivation of animals and humans to ingest alcohol. This motivation (i.e. alcohol-directed behavior) was presumed to be mediated through the central positively reinforcing, pharmacological actions of alcohol.

There is a much cited claim in the literature, however, that animals do not voluntarily ingest quantities of alcohol, through the oral route, sufficient to produce intoxication (Cicero, 1980; Deitrich & Melchior, 1985; Lester & Freed, 1973; Woods & Winger, 1971). This point was particularly emphasized in

Cicero's (1980) critique of the preference paradigm. He stated that preference measurements are deceptive since they do not provide evidence that animals actually consume alcohol for its drug-related pharmacological properties. The assumption that rats do not achieve intoxication through voluntary oral intake is based on a consideration of the maximum oxidative capacity of the animal--roughly 300 mg alcohol/kg/hour (Wallgren & Berry, 1970). According to this method of calculation, an animal consuming less than roughly 7.2 g/kg/day would not show measurable blood alcohol levels and would not therefore become intoxicated by alcohol. However, it should be noted that in this calculation there is no consideration of the pattern of intake in terms of nocturnal vs diurnal distribution, or the size of individual bouts of intake. The daily intake of alcohol could be distributed irregularly throughout the day, so as to produce very large increases in blood alcohol levels.

Cicero (1980) stated that resolution of the issue of intoxiction would come about by measuring blood alcohol levels following drinking and to objectively evaluate whether these levels were "consistent with those required to produce a pharmacological effect." In general usage the term intoxication is used to denote states which range from euphoria to overt drunkeness. In humans the behavioral changes induced by alcohol have been measured in various tests of cognitive, social, affective and psychomotor functioning. There is an obvious difficulty in defining this multi-faceted phenomenon, and more importantly, in attempting to measure it in infra-human species. Defining the "critical" blood level necessary for pharmacological effects becomes strictly a technological problem concerning the sensitivity of one's instruments for measuring pharmacological effects. It has been exceedingly difficult to measure "euphoria" or intoxication in animals. The behavioral effects of intoxication--following the voluntary ingestion of alcohol--have in fact received little empirical investigation.

Issues concerning the pattern of intake and intoxication following voluntary consumption, will be addressed in Experiments 3 and 4 of the present investigation.

CURRENT USE OF THE PREFERENCE PARADIGM

It should be noted that criticism of the voluntary preference paradigm did not totally dissuade researchers from using the paradigm. Variations of the two bottle choice paradigm are currently used extensively in pharmacological studies aimed at determining the underlying neural mechanisms controlling alcohol drinking as well as in the search for agents that will decrease alcohol intake (Deitrich & Melchior, 1985). A wide range of pharmacological agents have been employed in these types of alcohol drinking studies--including those that affect the serotonin system (Gill & Amit, 1989), opiates and endorphins (Pulvirenti & Kastin, 1988), the renin-angiotensin system (Grupp, Perlanski & Stewart, 1989), and the GABAergic system (Daoust, Saligaut, Lhuintre et al., 1987).

It is clear however, that the preference paradigm, as presently used in pharmacological studies, is poorly controlled for extraneous factors that might affect the drinking (Deitrich & Melchior, 1985; Mardones, Contreras & Segovia-Riquelme, 1988). Shortcomings outlined by Deitrich and Melchior (1985) included the observation that often drinking was not of sufficient magnitude to measure a significant, clinically relevant decrease and that often studies have not included a dose-response curve. An important question, often not addressed in pharmacological studies is whether the effects of any given manipulation are specific to alcohol (Gill, Amit & Ogren, 1985). The problem of specificity has important implications for the interpretation of data. How one interprets a decrease in alcohol consumption depends on whether the pharmacological manipulation affects concurrent behaviors such as locomotor

activity, feeding and water drinking, for example, or the consumption of other flavoured substances. The fact that the question of specificity is consistently ignored in studies using the preference paradigm is surprising, considering the fact that many of the systems being manipulated through pharmacological agents (i.e. serotonin, GABA, opiates, angiotensin, vasopressin) are known to interact with feeding and drinking mechanisms as well (Blundell, 1986; Gill & Amit, 1987; Kraly, 1984; Lopez-Sela et al., 1989; Neill & Cooper, 1989).

Recent examples illustrate the diversity of procedure and application of th preference paradigm. Svensson, Engel and Hard (1989) examined the effects of a single dose of the serotonin agonist 8-OH-DPAT on the absolute intake and preference for a 6% alcohol solution in Wistar rats. The animals were given 5 weeks exposure to alcohol and water prior to drug treatment. They reported that the drug reduced alcohol intake, and interpreted their data as support for the hypothesis that activation of the central serotonin system reduced the positive reinforcing effects of ethanol. However, the results are in fact difficult to interpret due to the well know effects of serotonergic manipulations on other behaviors such as feeding (Gill & Amit, 1987) and amphetamine self-administration (Leccese & Lyness, 1984) in rats. The effects of 8-OH-DPAT should have been interpreted in light of the other known behavioral and biochemical actions of serotonergic compounds.

In another recent study, Sprague-Dawley rats were exposed to 10% alcohol and water for 14 days, and then housed either singly or in pairs. The research was aimed at determining the effects of stress (i.e. the change in housing conditions) and stress hormones (ACTH and corticosteriod) on alcohol intake (Weisinger, Denton & Osborne, 1989). The change in housing conditions resulted in a significant increase in alcohol intake, starting approximately 5 days after the change in housing conditions. These results were difficult to interpret

in relation to the effects of stress hormones, due to a simultaneous observed decrease in body weight, food intake and water intake. A knowledge of the time course of these effects relative to one another may have aided in the interpretation of the increased alcohol intake. In addition, the baseline alcohol intake was extremely low (2-3 mls of 10% alcohol/day) and it is entirely possible that alcohol intake would have increased in these animals over time, due to habituation.

Variations in the preference paradigm center around procedural factors such as: the method of screening for alcohol intake, method of selection of animals, the length of exposure to alcohol, alcohol concentration used during baseline measures and the dependent measure (i.e. preference ratio vs the absolute alcohol intake). Despite the fact that questions have been repeatedly raised about procedural variations in the preference paradigm (Deitrich & Melchior, 1985; Gill & Amit, 1985; Mardones et al., 1988), there has been no attempt to standardize the paradigm and little attempt to find ways to improve the method.

THE PRESENT INVESTIGATION

To summarize the discussion thus far, the overall impact of a number of factors has contributed to the perception that animals do not drink alcohol, and that they do not drink sufficient alcohol to become intoxicated. The major contribution to this notion has been the experimental evidence that animals do not drink sufficient alcohol to consistently exceed their metabolic capacity (on a 24 hour basis) and as a consequence fail to display chronic intoxication, tolerance and physical dependence. In addition the difficulty in measuring alcohol effects in alternative paradigms (IVSA, CPP, BSR) has reinforced the perception that rodents are not reinforced by alcohol.

The net result of these developments is a paucity of valid, reliable tools that can be used to measure the behavioral effects of alcohol. One group of

researchers has consistently refused to consider the "preference" paradigm a valid or useful tool (e.g. Cicero, 1980). This should be compared to another group who use the preference paradigm as a model of alcohol drinking, frequently with poor, uninterpretable results. The uninterpretability of results emanating from the current use of the preference paradigm has only strengthened initial objections to it. As stated by Dole (1986) "...60 years of offering alcohol to animals has produced no fundamental insight into the causes...or even a convincing analogue of pathological drinking."

The research described in this thesis was aimed at addressing several of the issues raised above. One of the first questions addressed was: Do animals drink alcohol? In a large sample, what is the frequency of various consumption levels? Is it likely that animals consume enough alcohol to become intoxicated, do animals discriminate the effects of the alcohol they consume and how does it affect their behavior? What biological systems are involved in regulating the initiation, and cessation of drinking?

In Phase I of these studies, the alcohol drinking behavior of rats was examined using several experimental paradigms. First, the voluntary consumption of alcohol was examined using a traditional "preference" paradigm. The range of consumption in a large outbred sample of rats was determined, in terms of the preference for alcohol as well as the absolute alcohol intake. In the second and third studies, a more detailed analysis of drinking behavior was made using a computerized drinkometer system in which the consumption of food, water and alcohol could be monitored continuously over long observation periods. One of the primary aims of Experiment 2, was a comparison of the pattern of alcohol intake in outbred Long Evans rats compared to the genetically selected P and NP rats. In the fourth study animals were placed on a limited ethanol access schedule in order to simulate a single

ethanol drinking bout. Blood and brain alcohol levels and behavior were examined following these alcohol drinking bouts.

In Phase II of this thesis, biological factors involved in the regulation of alcohol drinking were examined. There have been previous reports that the alcohol oxidizing capacity of the brain--as measured by the activities of the enzymes Catalase and Aldehyde Dehydrogenase--is related to levels of voluntary alcohol intake in rodents. This area of research was introduced in Phase II and in Experiment 5, voluntary alcohol intake in different groups of animals was examined in relationship to the activities of Catalase and Aldehyde Dehydrogenase.

EXPERIMENT 1

AN EXAMINATION OF THE ACQUISITION AND MAINTENANCE OF VOLUNTARY ALCOHOL INTAKE IN THE LONG EVANS STRAIN OF RATS

In early work on voluntary alcohol consumption, alcohol was offered to rats in an ascending series of concentrations with the aim of determining threshold for detecting the presence of alcohol in the solution (Richter, 1941). With this procedure, Richter (1941) was able to show that rats drank more alcohol than water at concentrations ranging from 2%- 6% and drank more water than alcohol at higher concentrations. Since that time many variations in the procedure for presenting alcohol to rats have developed. For example, DeWitte (1984) increased the concentration of alcohol from 1-10 % over a 5 week period, with 2-3 days at each concentration. In contrast, Daoust et al. (1987) used a procedure where animals received 12% alcohol as the sole source of fluid for 2 weeks, followed by free-choice between water and alcohol. Myers and Privette (1989) presented alcohol in concentrations ranging from 3-30 % over a 10 day period in a free choice with water.

Much of the subsequent work in rats utilized single concentrations of alcohol (often 10% solutions), a procedure that generated some controversy (Lester, 1966; Veale & Myers, 1969). Some experimenters demonstrated that animals drank more alcohol when exposed to an acclimation period of gradually increasing alcohol concentrations (Mendelson & Mello, 1964; Veale & Myers, 1969, Williams et al., 1949) compared to procedures where the animals were initially forced to drink alcohol. However, other research demonstrated that alcohol concentration during the acquisition period played no role in the final acceptance of a 10% solution (Erikkson, 1968). Wise (1973) examined several factors believed to contribute to alcohol intake--acclimation, forced vs free

choice and intermittent vs continuous access--using a single test concentration of 20% alcohol. He maintained that while animals exposed to an acclimation procedure initially consumed more 20% alcohol, the most important factor contributing to high stable consumption was intermittent exposure.

Despite the differences in screening procedures, maintenance concentrations etc., it is clear from many studies that animals do drink alcohol, in some cases considerable volumes in 24 hour access periods (e.g. Daoust et al., 1987). However, since the mean absolute alcohol intake, alcohol preference ratio and range of alcohol intake within random samples of rats is rarely reported, it has been difficult to gauge the distribution of alcohol consumption within the rat population.

The following study was carried out to determine the distribution of alcohol consumption in an outbred population of Long Evans rats. A screening procedure was chosen which combined gradual increases in the alcohol concentration and intermittent exposure. This population study could serve as a database by which to judge the alcohol intake of groups of rats screened by other techniques, batches of rats run in subsequent experiments, as well as a comparison to other strains.

MATERIALS AND METHODS

Subjects: Four hundred and eighty-five male Long Evans rats initially weighing 150-200g were used in this study. The animals were obtained from two separate Long Evans colonies maintained by Charles River. "New" colony animals were obtained from a colony maintained in St. Constant, Quebec, while the "old" colony animals were obtained from a second colony in Wilmington, Vermont. The animals were obtained from the breeder in a total of 25 batches, ranging in size between 15 to 30 rats/batch, at 1-2 month intervals. The rats were housed individually in suspended stainless steel cages in an animal room

batches, animals that failed to consume any alcohol were culled, and the remaining animals were maintained on 10% alcohol and used in various experiments. The data from these latter batches of animals were not included in this portion of the data analysis.

RESULTS

The raw data for daily alcohol and water intake were converted into two measures; GM/KG (grams absolute alcohol intake/kilogram body weight/day) and the preference ratio (total alcohol intake in mls/total fluid intake in mls). Statistical analyses were carried out using the statistical programs BMDP 2V (ANOVA with repeated measures) and BMDP 4F (frequency distributions). In a preliminary analysis, new and old colony animals were compared on both measures of alcohol intake. There were no significant differences between the groups in terms of GM/KG [$F(1,448)=0.19$, $p=0.66$] or the alcohol preference ratio [$F(1,448)=1.69$, $p=0.19$]. Therefore, data for both new and old colony animals were combined into one database for all subsequent analyses.

The group mean daily alcohol intake in terms of GM/KG across the range of alcohol concentrations used during the screening period is presented in Figure 1. There was a significant increase in alcohol intake over days [$F(8,3584)=3.39$, $p<0.00001$] with the animals reaching a mean daily intake of 2.32 ± 0.10 gm/kg at the 10% alcohol concentration. The alcohol preference ratio showed a significant decline over concentrations [$F(8,3584)=37.57$, $p<0.00001$] starting with a preference ratio of 0.70 ± 0.012 for 2% alcohol and ending with a mean preference ratio of 0.25 ± 0.011 at the 10% concentration. Figure 2, presents the distribution of alcohol preference ratios at the 2% and 6% alcohol concentrations. There was a clear change in the distribution of preference ratios from high values (> 0.70) at 2% alcohol to low values (< 0.30) at the 6% concentration [$X^2(9)=254.83$, $p<0.0001$]. There was an additional significant

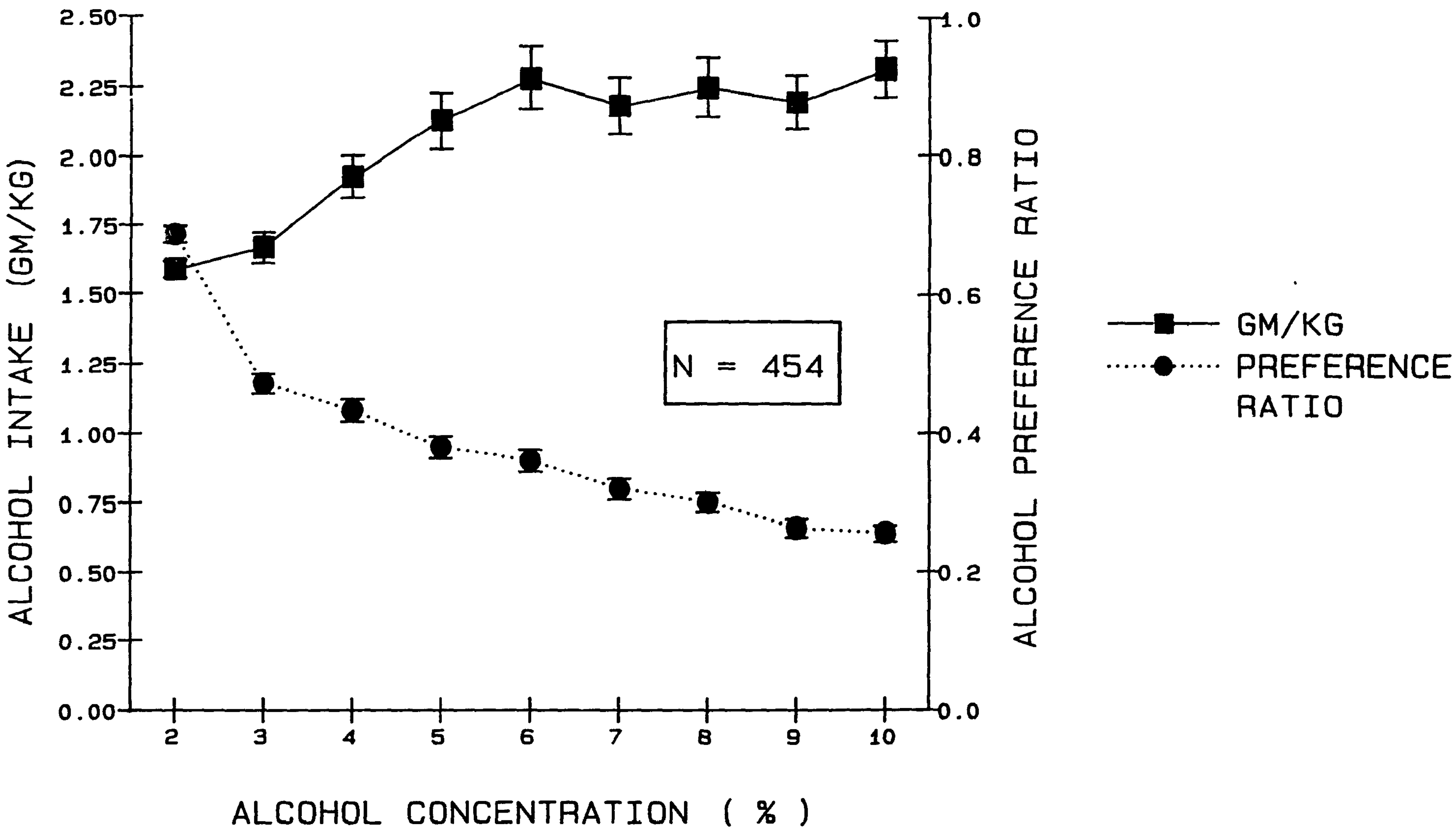


Figure 1. Alcohol intake (GM/KG) and the preference ratio across the acquisition curve from 2% to 10% alcohol.

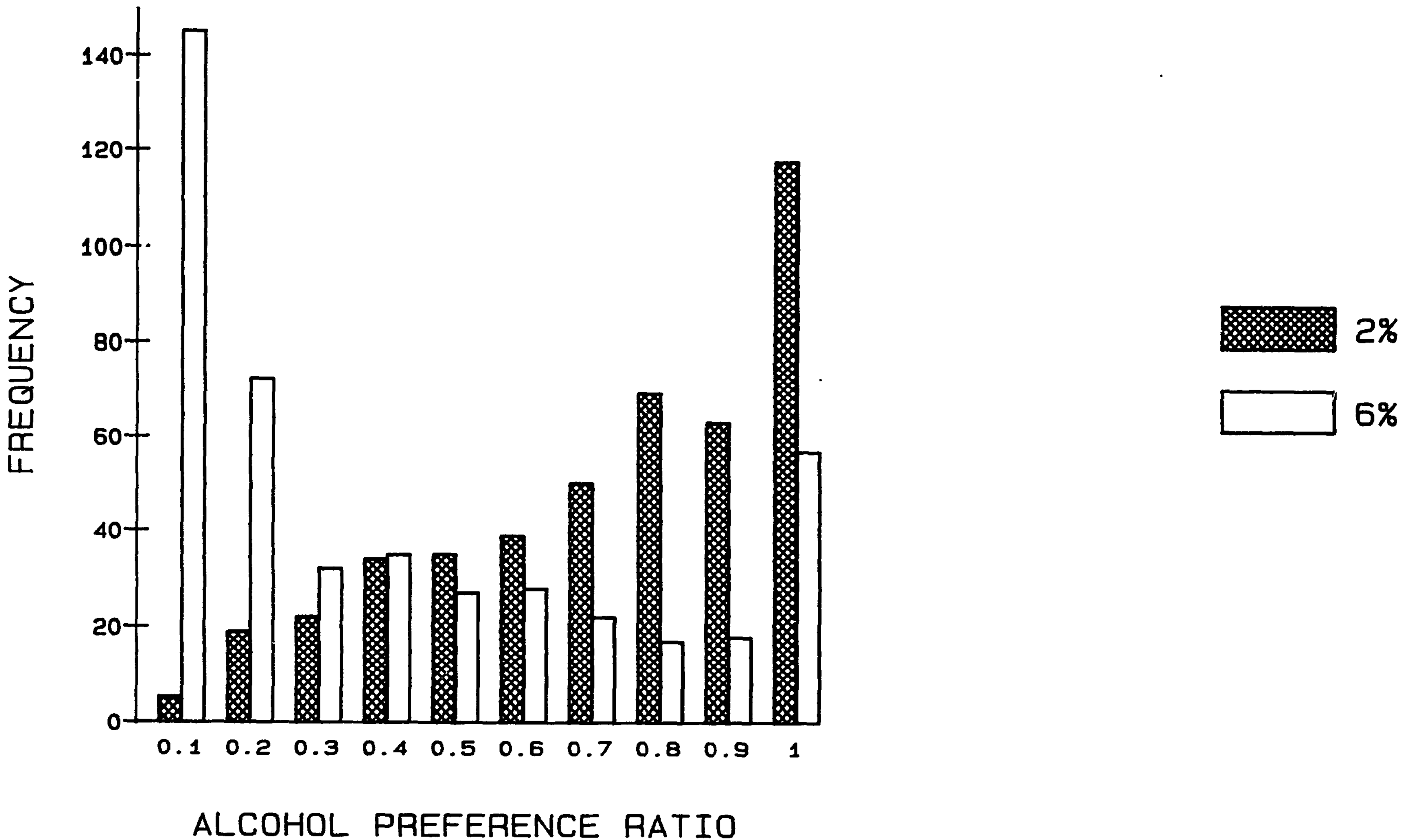


Figure 2. Frequency distribution of alcohol preference ratios at the 2% and 6% alcohol concentrations.

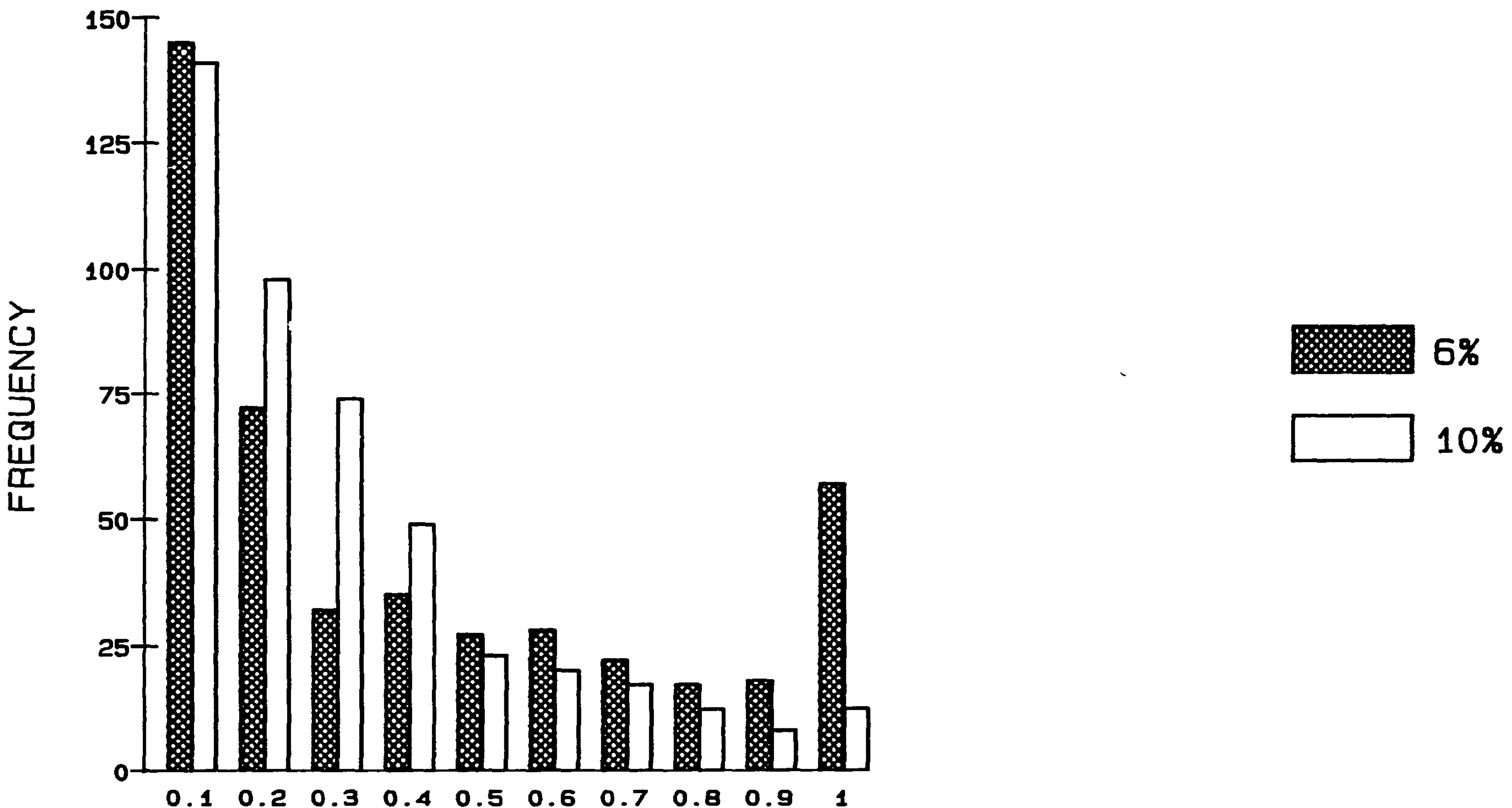


Figure 3. Frequency distribution of alcohol preference ratios at the 6% and 10% alcohol concentrations.

change in the distribution of preference ratios from the 6% to 10% alcohol concentrations as shown in Figure 3. Even though there was a continued decline in the mean preference ratio from 6 to 10% and a shift in the distribution, the absolute alcohol intake in GM/KG appeared to have reached a plateau at 6% alcohol as shown in Figure 1. Therefore, the net effect of increasing the alcohol concentration from 6% to 10% appeared to be a reduction in the preference ratio, with no change in absolute alcohol intake. The absolute alcohol intake (GM/KG) and alcohol preference ratios for the group of animals (n=182) maintained on 10% alcohol is presented in Figure 4. Both measures appeared to be very stable across days. This was confirmed by ANOVA with repeated measures, yielding nonsignificant changes for GM/KG [$F(4,474)=1.77$, $p=0.13$] and the preference ratio [$F(4,724)=0.26$, $p=0.91$] across days.

The overall mean alcohol intake displayed by this sample was 1.92 ± 0.05 gm/kg with a preference ratio of 0.23 ± 0.006. There was, however, considerable individual variability on both measures as shown in Figures 5 and 6. The mean alcohol consumption over the 10 day baseline period was computed for each animal and distributed into equal sized intervals. These values were plotted in Figure 5. Mean GM/KG ranged from 0 to 8 gm/kg/day. Similarly, the distribution of mean preference ratios yielded a range from 0 to 0.80.

DISCUSSION

The results from both the screening and maintenance phases of this study provided a description of the drinking behavior of a large sample of Long Evans rats. Overall there was a wide degree of variability across different alcohol concentrations in terms of the alcohol preference ratio and GM/KG, as well as a high degree of individual variability. The majority of animals preferred alcohol at the 2% concentration. It should be noted however, that a proportion of the

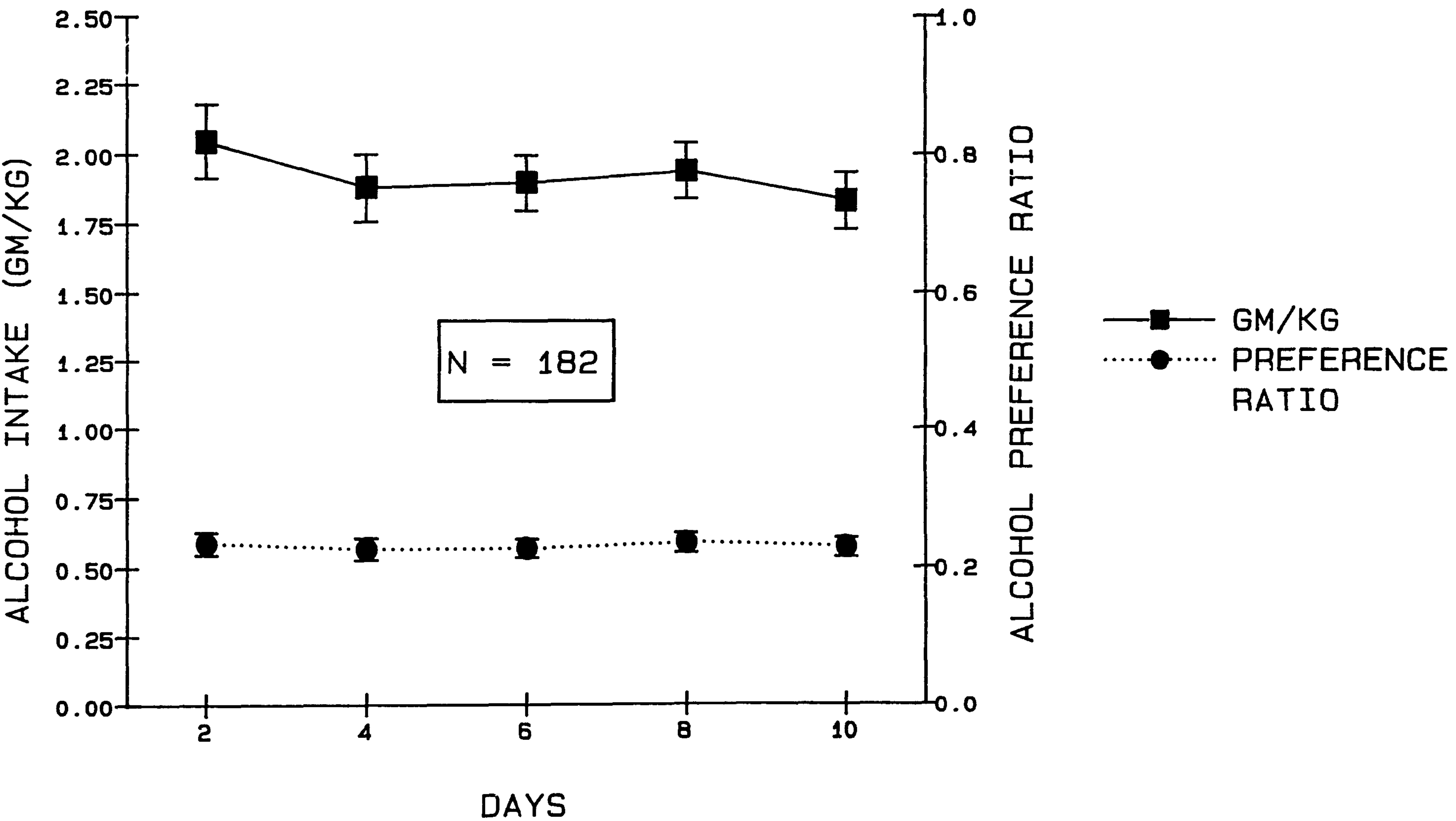


Figure 4. Alcohol intake (GM/KG) and the preference ratios during a 10 day maintenance period.

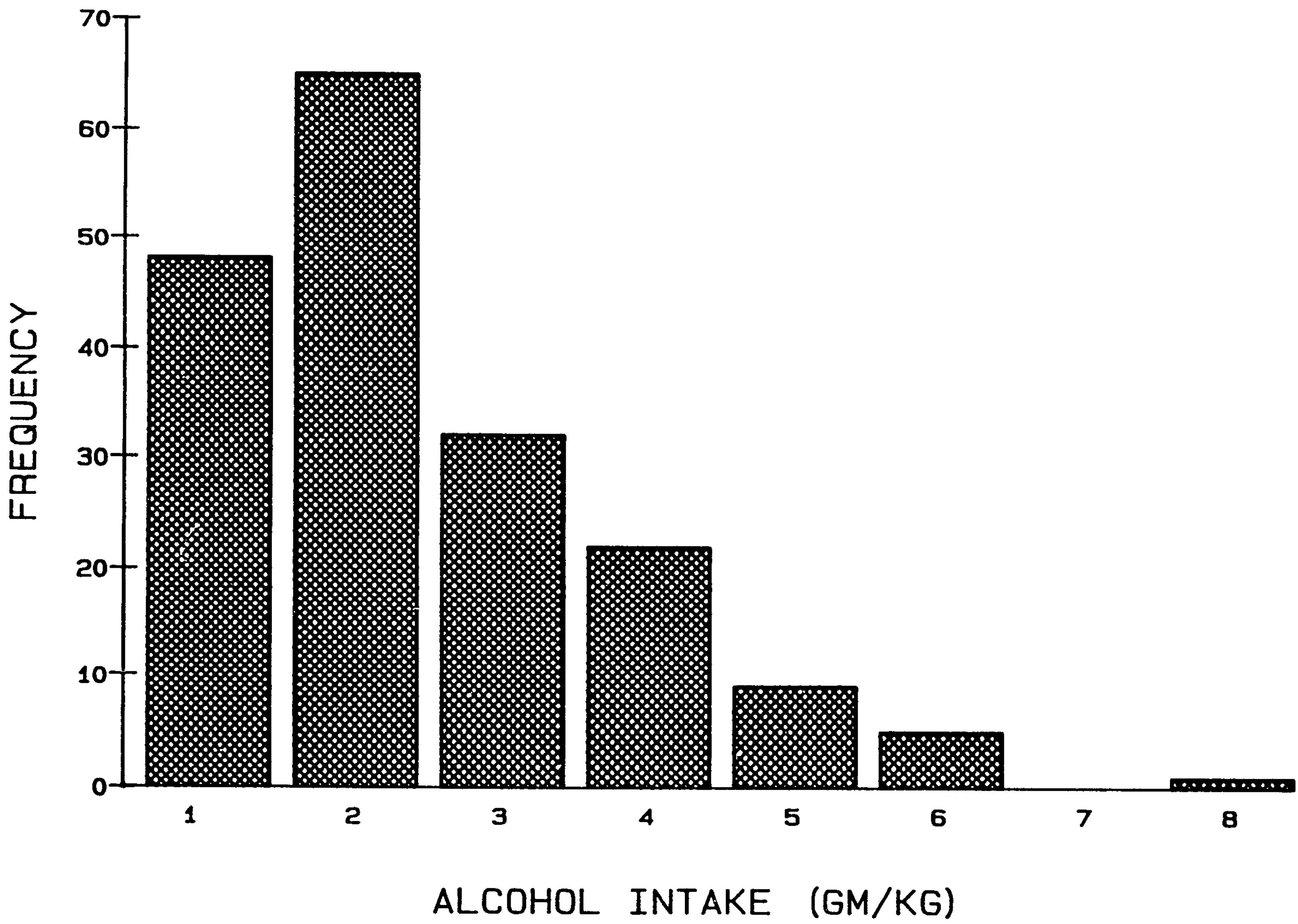


Figure 5. Frequency distribution of alcohol intake levels in the population of rats maintained on 10% alcohol.

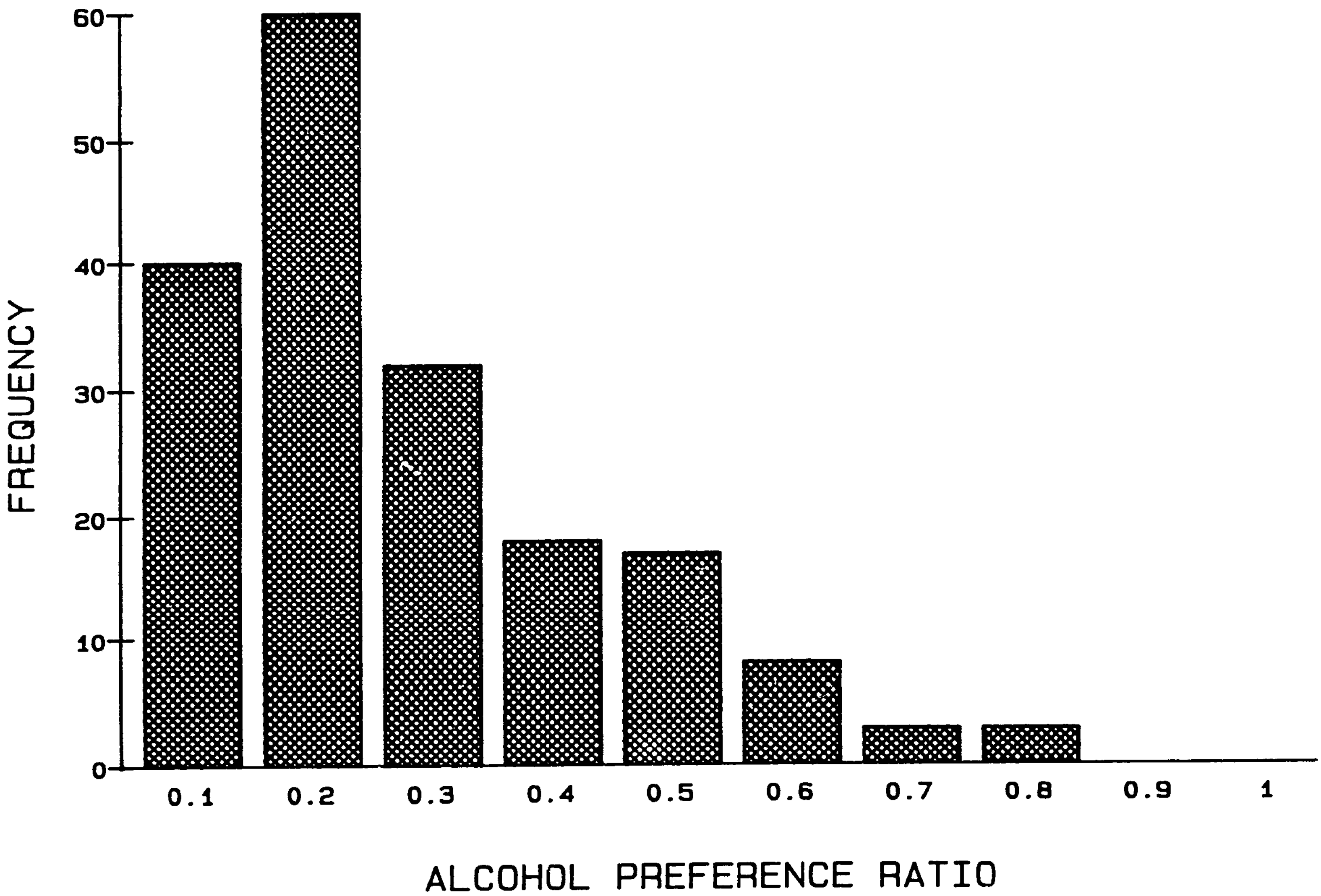


Figure 6. Frequency distribution of alcohol preference ratios in animals maintained on 10% alcohol.

sample (1.1%) completely rejected alcohol even at this low concentration. These animals continued to reject alcohol throughout the screening period. This initial rejection is clearly due to an extreme aversion to the smell and taste of even low concentrations of alcohol.

It would appear from Figure 1, that the preference ratio and the total amount of alcohol consumed in GM/KG were not always directly related. Animals can drink considerably greater amounts of alcohol on a daily basis at higher concentrations than at lower ones. Thus, GM/KG remained stable from the 6 to 10% alcohol concentrations, with corresponding decreases in the volume of alcohol consumed and in the alcohol preference ratio. This has some relevance to studies which include only one measure of alcohol intake. The preference ratio has been used in many studies, and interpreted as a measure of the motivation of the animal towards alcohol. It is possible that this measure could be very misleading without knowledge of changes in the total fluid consumption, water consumption and absolute alcohol intake. For example, the alcohol preference ratio could decrease due to an increase in water intake (in this case the absolute intake (GM/KG) would remain the same). Such a decrease in the preference ratio would be difficult to attribute to a change in the motivation to ingest alcohol in light of a constant absolute alcohol intake. It would appear that both measures of alcohol consumption should be reported and interpreted in the context of the total drinking behavior of the rat, including the water intake and total fluid intake.

In the maintenance phase of this study, stable consumption of alcohol ranged from 0 to 8 gm/kg/day. The range in drinking is similar to that displayed by the human population; some rats drink large quantities of alcohol and others abstain. Most researchers working with alcohol self-administration are struck by the large individual differences in alcohol intake within and across species

(Brown & Hutcheson, 1973; Grant & Johanson, 1988). This variability has been consistently observed in monkeys (Grant & Johanson, 1988; Woods & Winger, 1971), rats (Ellison & Potthoff, 1984; Mardones et al., 1988) and hamsters (DiBattista, 1986).

The possibility that inherent genetic factors controlled the individual differences in alcohol intake was first suggested by Williams, Berry and Beerstecher (1949). These researchers proposed that alcoholism was a "genetotrophic" disorder--caused by the joint action of genetic prediposition and nutritional deficiency. Since that early work, extensive evidence for the heritability of alcohol intake in animals has been established through work with inbred mice. Various inbred strains of mice (C57BL, BALB, DBA) have been shown to differ widely in their consumption of alcohol, as well as sensitivity to alcohol-induced narcosis and hypothermia (Kakihana, Brown, McClearn & Tabershaw, 1966; McClearn & Rodgers, 1959; Rodgers & McClearn, 1962), thus providing **prima facie** evidence for the heritability of various traits related to alcohol (McClearn, 1981b).

While the inbred animals have been used to prove the heritability of a given alcohol related trait, they are notoriously poor subjects for the study of the mechanisms underlying the trait (McClearn, 1981a). This is related to the fact that during the inbreeding process, many genes can become randomly fixed, potentially leading to the numerous fortuitous correlations between traits. Thus in inbred animals it is difficult to determine whether certain traits (e.g. enzymes activities, receptors numbers or affinity) are functionally related to others (e.g. level of alcohol intake), or merely fortuitous associations produced by the inbreeding process (McClearn, 1981b).

An alternative research strategy aimed at identifying the genetic basis for the observed variability in alcohol drinking has been selective breeding, as

discussed earlier (see Table 1). The basic assumption is that the genes producing a selected trait should segregate together during the breeding process, thus increasing the likelihood of identifying the genes through biochemical or neurophysiological correlates. In the genetic selection for high and low alcohol intake for example, animals have been tested for alcohol consumption using a standardized procedure and selected for breeding according to a specific criterion (see Table 1). Animals exhibiting the high and low drinking trait are mated in each generation, leading to the creation of two lines exhibiting divergent behavior with respect to the selection criterion. The proponents of this experimental strategy have viewed genetic selection as a potentially powerful technique aimed at obtaining "valuable information on the metabolic, behavioral and other biological factors that may be involved in the etiology of alcoholism" (Worsham et al., 1976).

It is interesting to note that the variability observed in the drinking of outbred strains of rats is most often considered to be an experimental nuisance; something to be overcome by various inducing procedures. However, if looked at in the light of its likely genetic basis, the range of alcohol drinking in Long Evans rats can be used as an experimental tool in the examination of potential biological factors underlying the drinking, in much the same way as the selected strains. In the following study the alcohol intake of Long Evans rats was further examined and compared with the selected P and NP lines developed at Indiana University.

EXPERIMENT 2

AN EXAMINATION OF THE ACQUISITION OF ALCOHOL DRINKING BEHAVIOR USING A COMPUTERIZED DRINKOMETER SYSTEM

In the traditional voluntary oral consumption paradigm used in Experiment 1, intake was measured once daily at the end of a 24 hour period. This method has clear disadvantages in that the pattern of alcohol intake is obscured. Lester (1966) noted many years ago, that without knowledge of the temporal distribution of water, food and alcohol consumption it would be impossible to determine what factors contributed to the limitation of alcohol intake. He speculated that there were several interacting forces; taste factors, an upper limit on total fluid intake related to the amount of food consumed, as well as a limit placed by the pharmacological effects of the alcohol.

Techniques for monitoring the temporal distribution of behavior are well developed in other areas of behavioral pharmacology. Within the field of research on feeding behavior for example, there has been a growing trend towards "microstructural" analyses of behavior. Blundell and Latham (1978, 1982) extensively studied free feeding using a paradigm in which food intake was monitored continuously, resulting in a description of the temporal patterns of meals, meal frequency and bout duration. Overall, this type of analysis appeared to provide a more detailed assessment of the behavior (Blundell & Latham, 1982; Fletcher & Burton, 1986).

Modelled on these types of "microstructural" analyses, a computer controlled system was designed for the purpose of examining alcohol drinking behavior in this study. Computerized systems have previously been described for the monitoring of a single behavior, either fluids (Dole, Ho & Gentry, 1983) or food (Blundell & Latham, 1982). In addition, commercially manufactured food and fluid monitoring systems are available. However, these are expensive and

generally limited in terms of the number of animals or behaviors that can be simultaneously monitored. The system described in this study was designed to monitor the consumption of water, alcohol and food, in order to examine the temporal distribution of intake i.e. time, frequency and duration of bouts and the interrelationship between the behaviors. Some previous work has been reported on the temporal distribution of alcohol consumption in C57BL mice (Dole & Gentry, 1984; Millard & Dole, 1983). However, there have been no detailed studies examining the microstructure of drinking in the preference paradigm, with simultaneous recording of food and fluid intake. Before describing the experimental protocol, a brief introduction to the P and NP lines of rats is presented.

THE ALCOHOL PREFERRING AND NON PREFERRING RATS

The selection of the P/NP lines began in 1971 from a foundation stock of Wistar rats consisting of 28 males and 50 females (Lumeng, Hawkins & Li., 1977). These animals were screened for alcohol intake by providing 10% alcohol as the sole fluid source for 4 days, followed by a 2-bottle preference test for 3 weeks. A single pair of high consuming animals and single pair of low consuming animals were mated thus starting the P and NP lines (Li, Lumeng, McBride & Waller, 1981). Initially, selective "inbreeding" was practiced by mating the males and females with the highest and lowest preference in each succeeding generation. In later generations sib mating was avoided in order to reduce the level of inbreeding. While the distribution of alcohol intake in later generations of P and NP rats has not been published, alcohol intake in a sample of the F26 generation was reported to be 6.0 ± 0.4 for the P rats and 0.098 ± .08 gm/kg/day for the NP line (Morzorati et al., 1988). The temporal distribution of licks on alcohol tubes, based on hourly measurements, was briefly described by Waller, McBride, Lumeng and Li, (1982). The paper did not

describe the patterns or amounts of alcohol intake in relation to water or food. Murphy, Gatto, Waller et al. (1986) observed bouts of drinking as high as 1.1-1.3 g/kg, consumed mainly during the dark cycle, in a sample of P rats.

The use of a single 10% alcohol solution as the basis for genetic selection has been severely criticized at various times in the literature (Myers, 1968). Myers maintained that the absolute intake of alcohol varied a great deal, depending on the concentration of the test solution and suggested that the practice of selecting solely on the basis of a single concentration would lead to inaccurate results. In an interesting study, York (1981) demonstrated that the ANA (alcohol non-accepting) strain drank considerable amounts of alcohol if the flavour or the concentration of alcohol was changed from the 10% laboratory grade alcohol used as the basis for selection. Li and coworkers did report that P rats of the F8 and F12 generations maintained a high alcohol preference across a broad range of alcohol concentrations, while the NP failed to drink alcohol even at concentrations as low as 2%. (Li, Lumeng, McBride, Waller & Hawkins, 1979). These data, however, were reported in a review paper without information on the procedure or information on whether the subjects were naive to alcohol prior to the experiment. In light of York's work it seemed possible that if offered different concentrations of alcohol, the P and NP rats would not show the same line differences observed with 10% alcohol. This was tested in the following experiment.

MATERIALS AND METHODS

Subjects: Forty male Long Evans rats weighing 175-200 grams were used in this study. The animals were obtained from the breeders (Charles River, Canada) in 4 batches of 10 animals. Twenty naive P and ten naive NP rats from the F28 and F29 generations were supplied by Dr T K. Li, Indiana University, Indianapolis. The animals were received in 3 batches, spaced over an 8 month

period. The P rats ranged from 5-7 weeks old and weighed between 150-200 grams when placed in the drinkometer chambers. The NP rats ranged from 7-9 weeks old and weighed 200-270 grams at the start of the experiment.

Description of the Computerized Drinkometer System: The components of the system included the rat chambers, a food delivery system, drinkometer circuits and an IBM XT-compatible computer equipped with a 20 MB hard disk and a dot-matrix printer. A Qua Tech PXB-721 Parallel Expansion Board (Qua Tech, Inc., Akron, Ohio) provided a 72 parallel I/O line interface between the computer and the cage drinkometer and food pellet delivery system.

The main feature of the system was two custom-designed computer programs for data collection and analysis. The first program (COLLECT) monitored the cages during the experiment and recorded the time and nature of all activity in a set of raw event files. In order to record all licks on the fluid spouts, the program was designed to poll the input lines from the cage circuity 18.2 times/ second, much faster that the observed licking rate of rats (Mundl & Malmo, 1979). The second program (REPORT) read the raw event files produced by the COLLECT program, accepted keyboard input of the volume of fluids consumed by each subject, computed bouts of behavior according to a user-defined criterion and produced a printed summary for each subject organized by behavior type (i.e. food, water, alcohol). Design specifications for the drinkometer hardware and software have previously been described in detail by Gill, Mundl, Cabilio and Amit, (1989).

Drinkometer Chambers: The chambers were custom made operant-type cages of size 30 cm x 30 cm x 27 cm, built of plexiglas and aluminium, with a metal grid floor. Each cage was equipped with a pellet dispenser and plexiglas food cups. The food cups were modified with the addition of a photoconductive

cell and light beam placed at the entrance to the food cup. The pellet dispensers were activated when an animal poked its nose into the food cup thus breaking the light beam, simultaneously providing a signal to the Qua Tech interface. Each time the animal poked its nose into the food cup it obtained one 45 mg food pellet.

Drinking tubes were attached to the front of the cages with metal clips. The spouts of the drinking tubes were recessed behind plexiglas covers to reduce inadvertent contact. Each drinking tube consisted of a plastic tube with a rubber stopper and steel double-ball bearing sipper tube. Each time the animal licked the metal spout, a pulse was generated and directed to the Qua Tech board.

Alcohol Screening Procedure: The animals had ad libitum access to a semi-purified diet in the form of Bioserve Precision food pellets (#F0021) and water for 7 days prior to start of the screening procedure. The animals were handled and weighed, and the cages cleaned every second day.

Following the acclimation period, an alcohol screening procedure was instituted as described in Experiment 1. The animals received an ascending series of alcohol concentrations, ranging from 2 to 10%, on an alternate day schedule. The drinking and feeding behavior of the animals was recorded on alcohol presentation days, for 23.5 hours. During the active recording period, the COLLECT program was stopped daily at 3 P.M., immediately prior to the start of the dark cycle. During a 30 minute shut-down period, fluids were measured and replaced and the REPORT program was invoked. The daily report for each animal was automatically transferred via the computer's serial port to a microVAX II computer for further analysis

Data processing and analysis: An example parts of a daily report for an individual animal is provided in Table 3 This animal was actively feeding or drinking throughout the recording period, for varying periods of time

TABLE 3

SESSION REPORT FOR AN INDIVIDUAL RAT

Session: 0243 20-JUL-1988 15:37:05 to 21-JUL-1988 15:11:00 Page 1

Box 1 FOOD — Pellet Distribution Vector (pellets in each minute)

Bout	Start	End	Duration	Total Pellets	Pellets/ Minute	1	2	3	4	5	6	7	8	9	10
1	1:40:18	1:43:25	00:03:07	52	16.36	9	13	28	2						
2	1:45:49	1:46:21	00:00:32	8	13.13	8									
3	3:05:19	3:07:28	00:02:09	50	22.79	28	20	2							
4	3:09:14	3:12:11	00:02:57	55	18.31	17	20	18							
5	3:19:30	3:19:30	00:00:00	1	****	1									
6	3:36:51	3:36:51	00:00:00	1	****	1									
7	4:27:38	4:27:38	00:00:00	1	****	1									
8	4:39:13	4:43:48	00:04:35	84	18.11	9	18	19	21	17					
9	5:06:46	5:06:46	00:00:00	1	****	1									
10	5:12:25	5:15:41	00:03:16	58	17.45	18	13	22	5						

Session: 0243 20-JUL-1988 15:37:05 to 21-JUL-1988 15:11:00 Page 2

Box 1 WATER (continued) — Lick Distribution Vector (licks in each minute)

Bout	Start	End	Duration	Total Licks	Licks/ Second	1	2	3	4	5	6	7	8	9	10	Volume (ml's)
19	4:55:21	4:57:42	00:02:21	173	1.22	169	0	4								0.8
20	5:17:04	5:17:07	00:00:03	3	0.67	3										0.0
21	5:19:03	5:20:04	00:01:01	5	0.07	2	3									0.0
22	8:50:55	8:50:58	00:00:03	4	1.00	4										0.0
23	8:52:05	8:52:05	00:00:00	1	****	1										0.0
24	9:07:54	9:08:48	00:00:54	306	5.65	306										1.5
25	9:30:02	9:30:38	00:00:36	145	4.00	145										0.7
26	9:57:10	9:58:12	00:01:02	322	5.18	313	9									1.5

Box 1 ETHANOL — Lick Distribution Vector (licks in each minute)

Bout	Start	End	Duration	Total Licks	Licks/ Second	1	2	3	4	5	6	7	8	9	10	Volume (ml's)
17	10:01:41	10:04:48	00:03:07	482	2.57	4	179	284	15							2.7
18	10:09:56	10:09:57	00:00:01	3	2.00	3										0.0
19	11:16:04	11:17:08	00:01:04	356	5.55	338	18									2.0
20	12:37:14	12:39:32	00:02:18	498	3.60	213	207	78								2.8
21	16:43:54	16:45:52	00:01:58	513	4.34	258	255									2.9
22	19:23:21	19:23:47	00:00:26	130	4.96	130										0.7
23	21:02:24	21:02:25	00:00:01	4	3.00	4										0.0

The duration of these "bouts" of behavior and the amounts consumed in each bout were calculated by the REPORT program. The end of a bout was defined when the animal stopped any given behavior for a period of 5 minutes, or if the animal switched to another behavior. These criteria were similar to those adopted in the feeding and drinking literature (Spector & Smith, 1984; Castonguay, Kaiser & Stern, 1986).

The amount of fluid consumed in each bout was determined by the REPORT program by calculating a volume to lick ratio (V/LRATIO) for each drinkometer. This procedure was based on the assumption that the volume per lick was constant for each animal. This assumption appeared to be warranted, based on the consistent relationship between the total volume consumed and the total number of licks across days (Gill et al., 1989).

The animals tended to make some inadvertent contact with the food cups (e.g see Table 3, food bouts 5-7) and drinking spouts during the recording period. This type of sporadic contact was easily distinguishable from a feeding or drinking bout. For the purposes of determining the mean bout frequency, duration and size, only bouts consisting of > 5 licks or pellets were considered in further analysis. Statistical analyses in the following sections were carried out using the BMDP statistical programs; 7D (2-way ANOVA), 4F (frequency distributions) and 2V (ANOVA with repeated measures).

RESULTS AND DISCUSSION

LONG EVANS: The mean alcohol intake and preference for the entire group of 40 Long Evans rats is shown in Figure 7. Although there appeared to be differences in the shape of the acquisition curve compared to those screened in the home cages on Agway food (see Figure 1), the final levels of intake at the 10% alcohol concentration, for both GM/KG and the preference ratio were similar. For the purposes of further analysis and comparison to the P/NP rats,

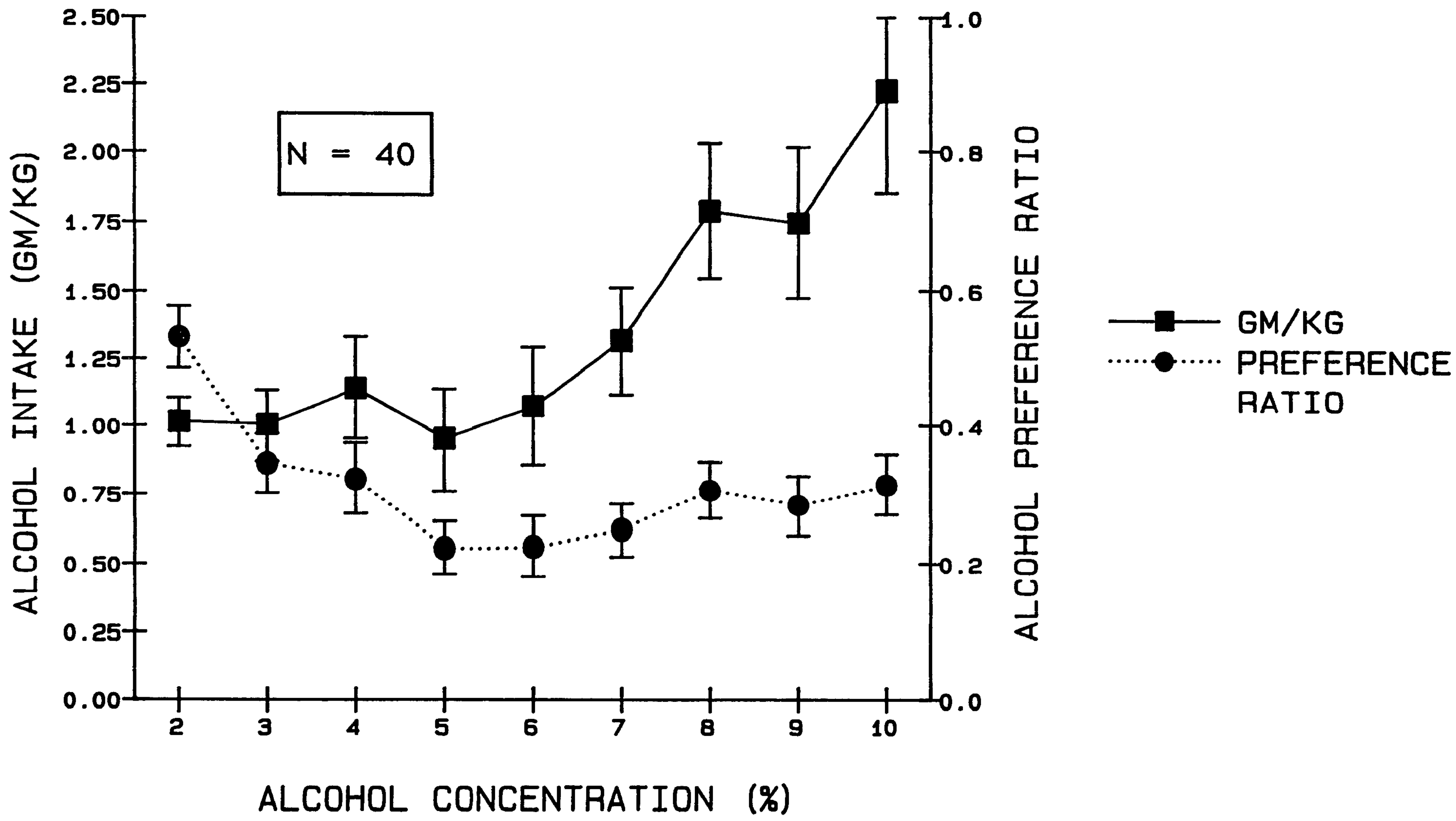
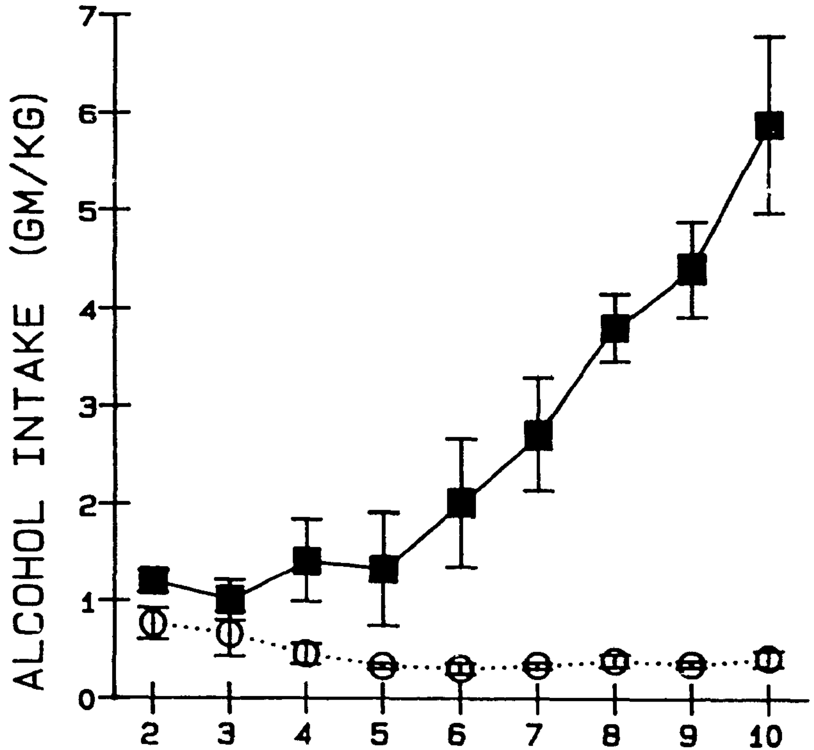


Figure 7. Alcohol intake (GM/KG) and preference ratios for the Long Evans rats screened in the drinkometer.

the animals were divided into two groups; Drinkers--those animals that acquired the alcohol drinking response, and Nondrinkers--those animals that failed to consume alcohol.

In genetic studies, criteria for determining "high" drinking animals have been established i.e P rats consuming greater that 5 gm/kg/day are selected for breeding (McBride et al., 1989). However, in work with outbred strains, selection has typically been based on arbitrary values of the preference ratio or GM/KG. In this study the animals were selected on the basis of the mean values of the sample as a whole. The mean alcohol preference ratio and GM/KG over the last 3 alcohol presentations was calculated for each animal. Those animals with mean values greater than 1 standard deviation above the grand mean for each alcohol consumption measure, were classified as Drinkers. This classification procedure resulted in a group of high drinking rats (n = 8) with a mean preference ratio of 0.74 ± 0.037. This group was compared to 8 animals that failed to consume any alcohol over the last 3 alcohol presentations. The Nondrinker group had a mean alcohol preference of 0.05 ± 0.005. Alcohol consumption (GM/KG) and preference ratios for these selected groups of animals are shown in Figure 8. Subsequently the groups were compared on measures of total food and fluid intake as well mean bout size and frequency by ANOVA with repeated measures. It should be noted here that the drinkometer system performed consistently across all measurements. There was no significant difference between the groups in terms of the calculated V/LRATIO [F(1,14)=2.11, p=0.16] and no changes in these ratios over days [F(8,112)=1.52, p=0.15].

The analysis of food intake indicated that there were no differences between the Drinkers and Nondrinkers on any measure related to food. Total daily intake [F(1,14)= 1.14, p=0.30], mean food bout size [F(1,14)=0.23, p=0.64] and



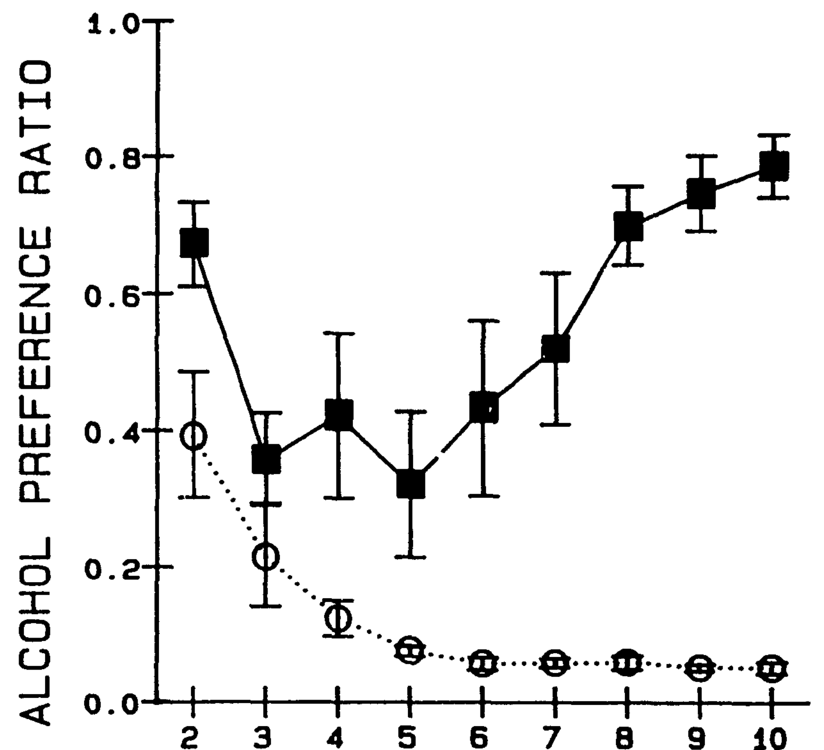


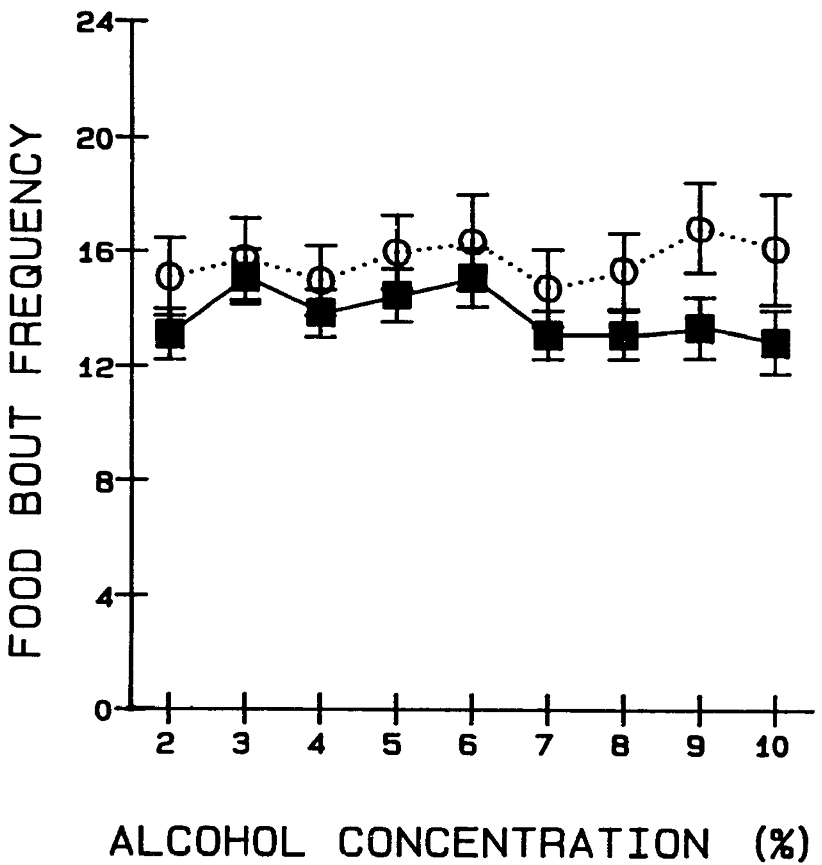
Figure 8. Alcohol intake (GM/KG) and preference ratios across the acquisition curve for the selected Long Evans rats.

mean food bout frequency [$F(1,14)=1.24$, $p=0.28$] were equivalent in the two groups. In addition, there were no changes in any of the parameters related to food, across the entire alcohol acquisition curve. Food bout frequency and size are shown in Figure 9.

Total fluid intake in the high alcohol Drinkers was significantly lower compared to the Nondrinkers as shown in Figure 10, [$F(1,14)=5.31$, $p=0.04$]. Total fluid intake in the Nondrinker group was accounted for exclusively by water, whereas, the Drinker group did consume varying amounts of water over the acquisition curve as indicated by the preference ratio in Figure 8. Water bout frequency was significantly lower in the Drinker group as expected, however, the mean size of water bouts was equivalent in the two groups [$F(1,14)=0.20$, $p=0.66$].

As verified by an analysis of the alcohol bout structure across the acquisition curve, there was surprisingly little change in the frequency of bouts over the different concentrations. The bout frequency and bout size for the preferred fluids, water in the Nondrinkers and alcohol in the Drinkers, are presented in Figure 11. There was a significant difference in mean bout frequency [$F(1,14)=6.11$, $p=0.026$], with water drinking animals consuming more bouts. The analysis of mean bout size yielded a significant group by days interaction [$F(8,112)=2.88$, $p=0.006$] that was accounted for by a fluctuations in the size of alcohol bouts in the Drinker group across the increasing alcohol concentrations.

In summary, there appeared to be significant differences in the overall pattern of alcohol consumption compared to water. Alcohol consuming rats took fewer bouts of fluid overall, and exhibited larger fluctuations in bout size.



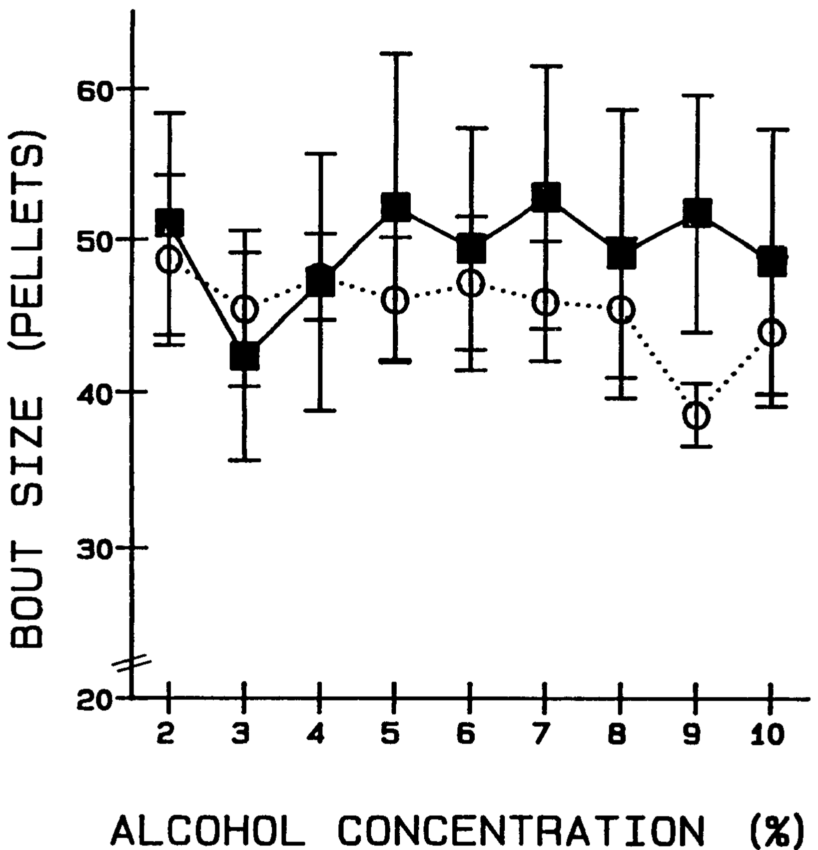


Figure 9. Mean food bout frequency and bout size for Long Evans Drinkers and Nondrinkers.

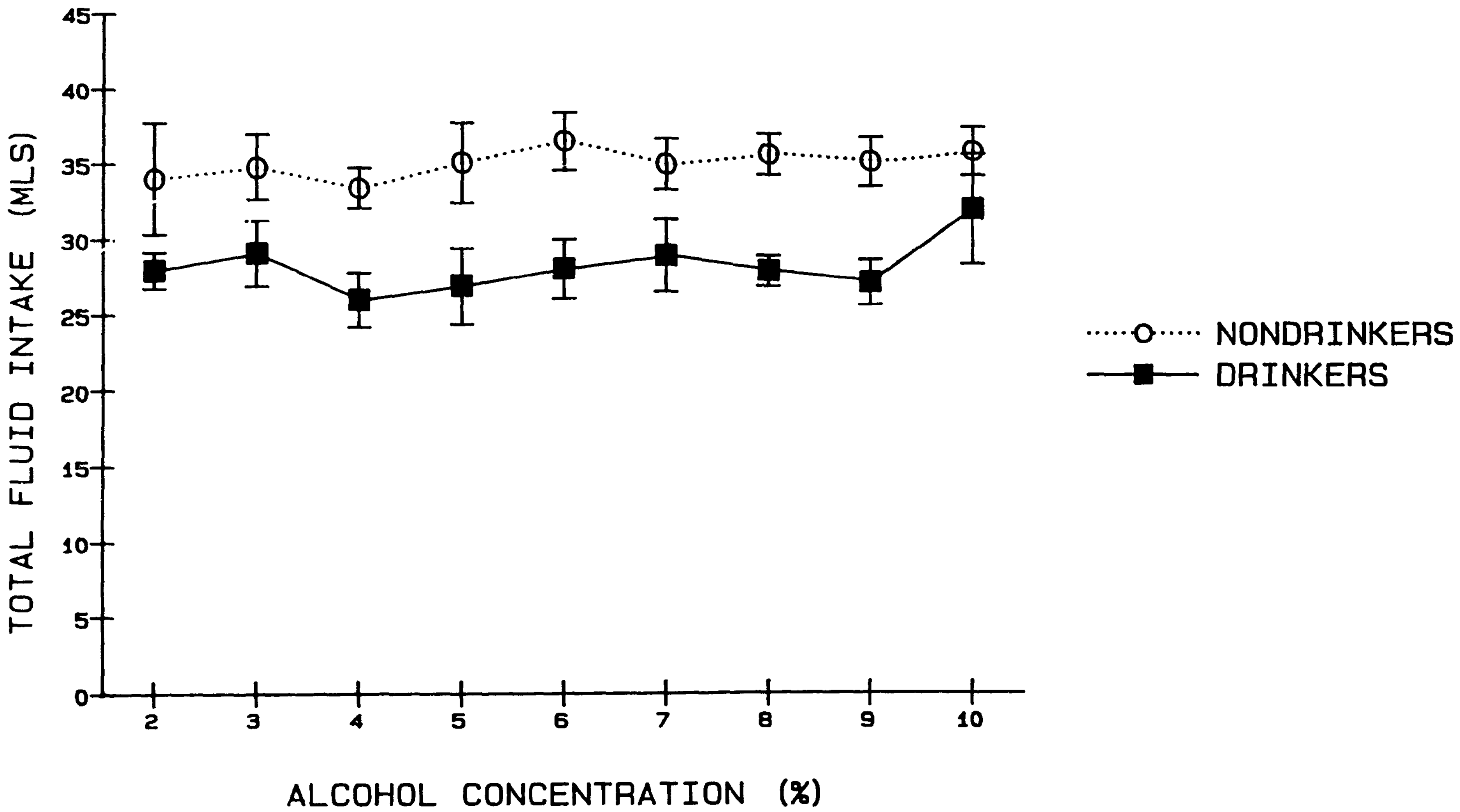
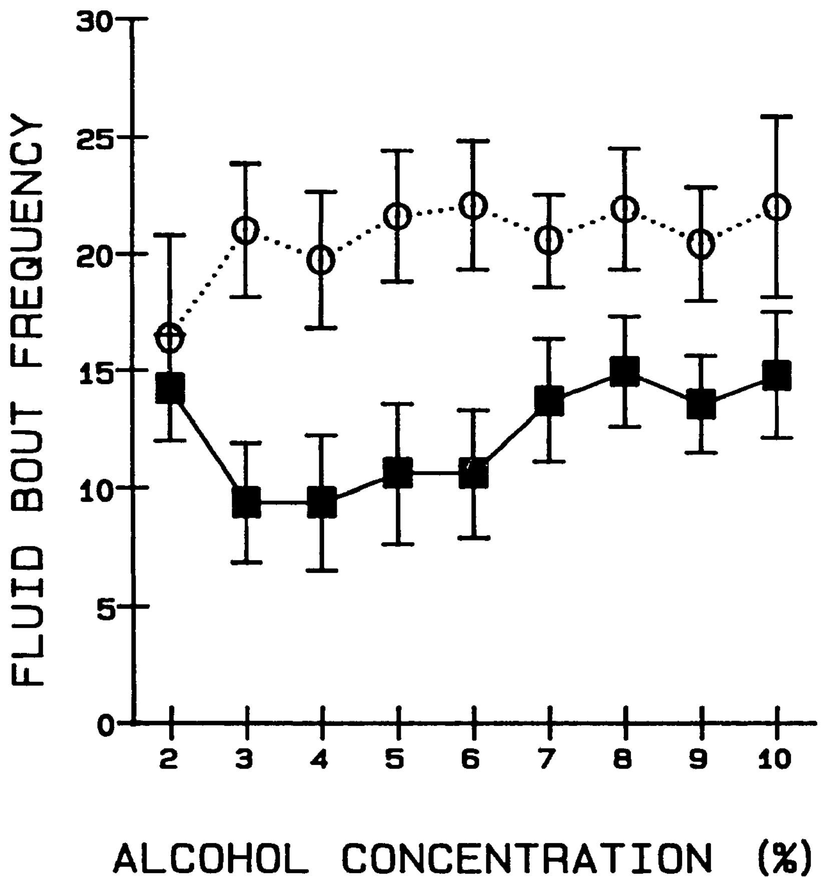


Figure 10. Total fluid intake (mls) across the acquisition curve for the selected groups of Long Evans rats.



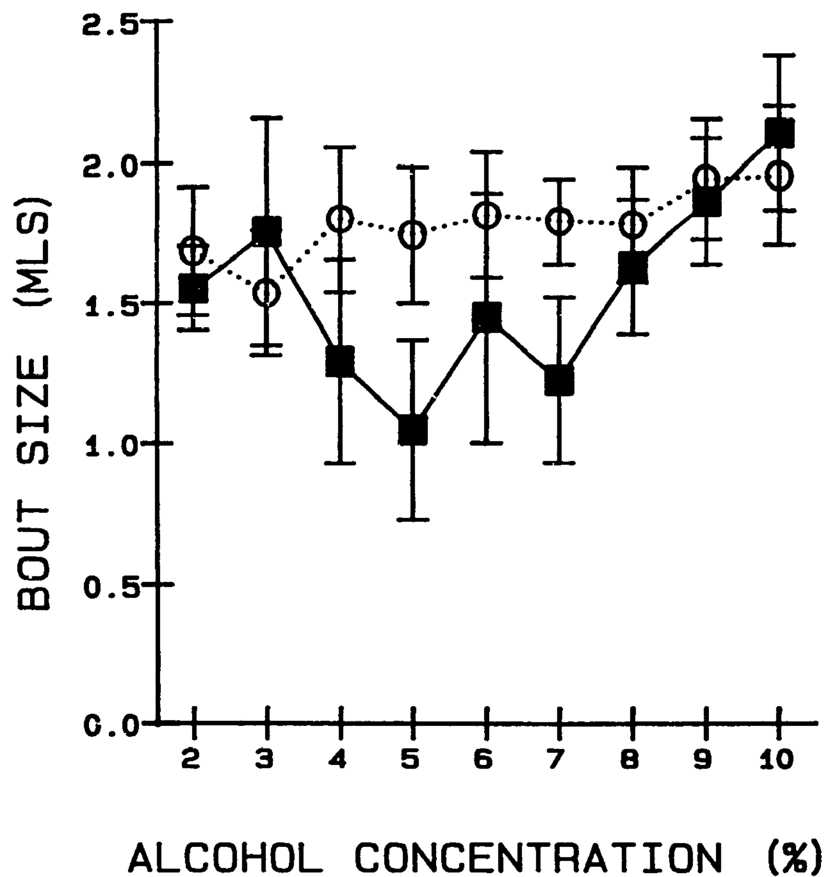


Figure 11. Mean bout frequency and bout size (mls) of preferred fluids in the Long Evans Drinkers and Nondrinkers.

There was a gradual increase in the size of bouts between the 5-10% concentrations. The net effect was an increase in the total alcohol intake and preference ratio over the acquisition curve. The Nondrinkers showed remarkable stability in the frequency, size and duration of bouts over days.

It was interesting to note that although there were significant differences in total fluid and alcohol intake between the groups, there were no corresponding differences in total food intake. This might suggest that during the acquisition period, there is little regulation or compensation for additional calories ingested in the form of alcohol.

P/NP RATS: The P rats were obtained in two shipments of 10 rats/each, at 4 month intervals. The mean alcohol intake and preference ratios for the two groups of P rats (n = 10/group) as well as the NP (n = 10) rats are presented in Figure 12. Surprisingly, the P rats exhibited lower alcohol intake and greater variability than is typically reported (Morzorati et al., 1988) for these animals. In fact approximately half the animals in each batch failed to consume alcohol at the higher concentrations. Intake across the acquisition curve for several of these nondrinker P rats is shown in Figure 13. There did not appear to be any particular pattern to the drinking in these animals, Rat 7 failed to consume alcohol at any concentration and the remainder dropped off at the higher concentrations. The animals all appeared to be in good health, eating normal amounts of food and gaining body weight.

Therefore, as in the analysis of the Long Evans strain above, high drinking P rats were selected out for further study. Those P rats that failed to consume alcohol (or consumed at the level of the NP rats) during the last 3 alcohol presentations were eliminated from the study. This selection process resulted in a group of P-Drinkers (n = 12) with a mean preference ratio of 0.78 ± 0.06. The 10 NP rats exhibited a mean alcohol preference of 0.10 ± 0.005.

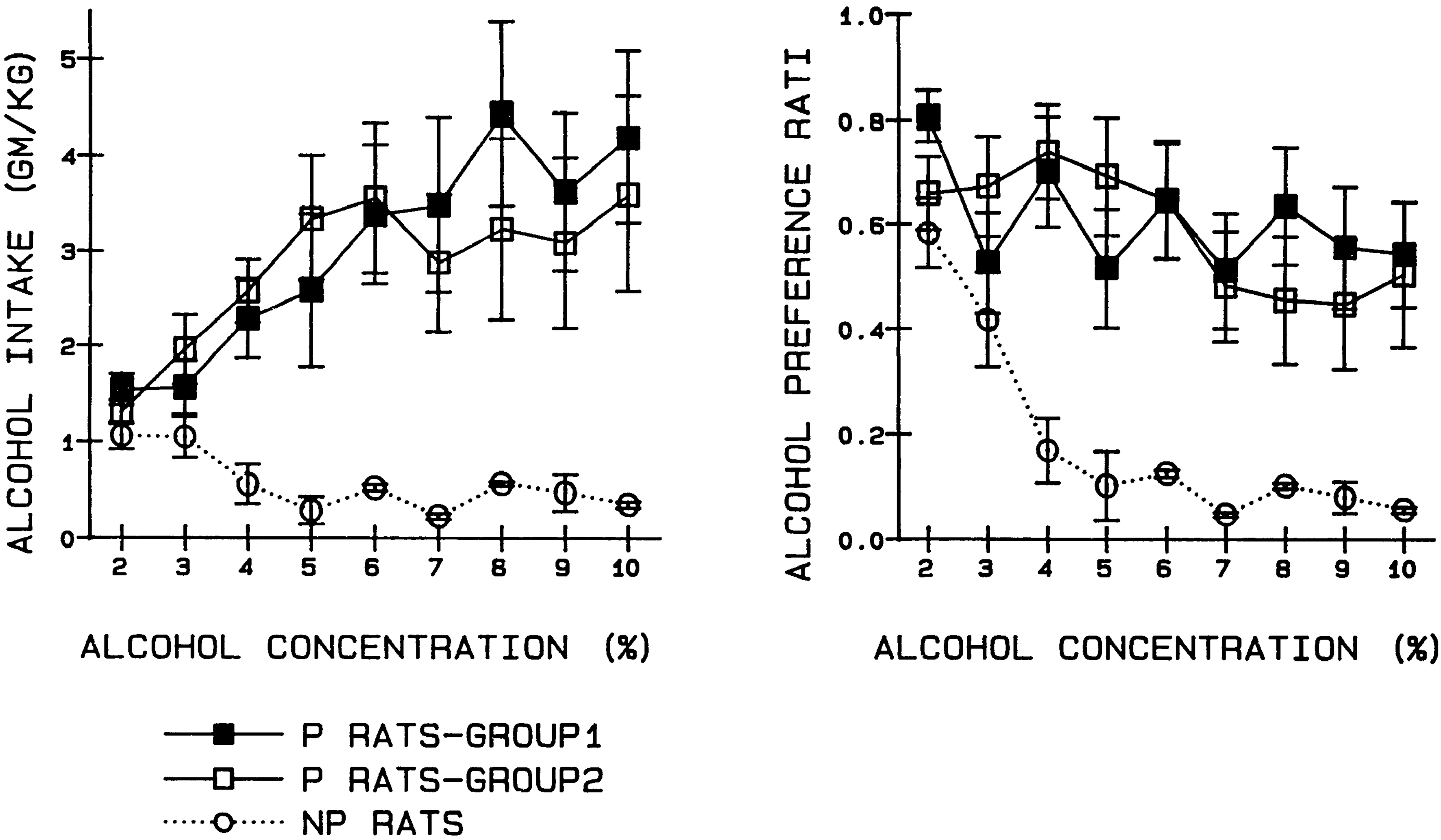


Figure 12. Alcohol intake (GM/KG) and preference ratios across the acquisition curve for P and NP rats.

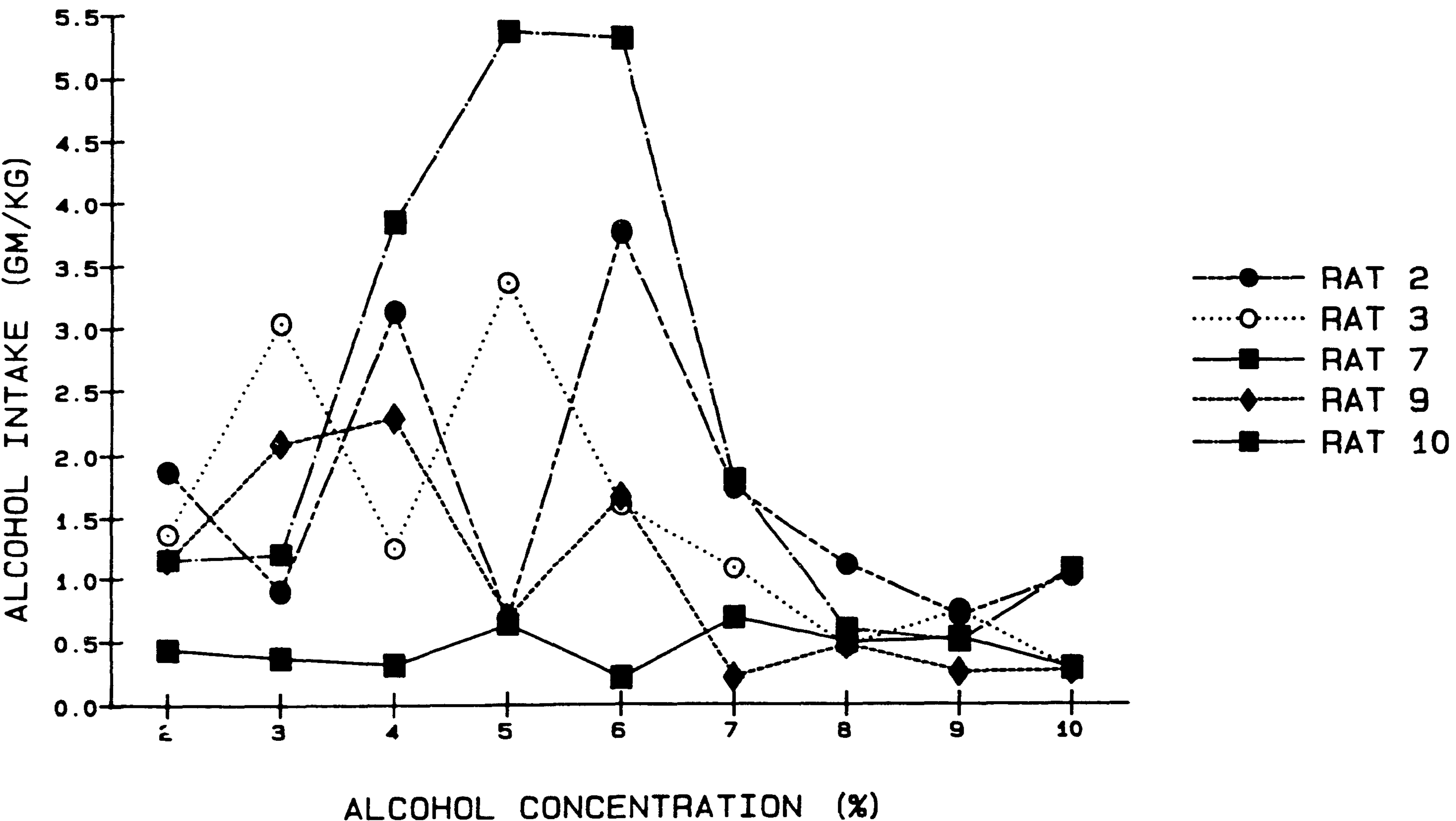


Figure 13. Alcohol intake (GM/KG) across the acquisition curve in a sample of the low drinking P rats.

Alcohol consumption in terms of GM/KG and preference ratios for these selected animals is shown in Figure 14. Like the Long Evans rats there were no significant differences between the groups in terms of total food intake [$F(1,20)=2.72$, $p=0.11$], food bout size [$F(1,20)=0.02$, $p=0.89$], or food bout frequency [$F(1,20)=0.39$, $p=0.53$]. However, unlike the Long Evans rats, there was a slight, though significant decline in total food intake over days in both groups [$F(8,160)=2.85$, $p=0.005$]). This was accounted for by a significant decrease in the frequency of food bouts [$F(8,160)=3.68$, $p=0.0006$] over days (see Figure 15).

Analysis of the total fluid yielded a significant group by days interaction [$F(8,160)=4.13$, $p=0.0002$] as shown in Figure 16. The P-Drinker rats display a slight increase in fluid intake over days. The pattern of alcohol drinking in the P-Drinker rats showed considerable change over days. As shown in Figure 17, there was a significant decrease in the frequency of alcohol bouts with a corresponding increase in the size of alcohol bouts over the different concentrations. The statistical analyses yielded a group by days interaction [$F(8,160)=9.33$, $p<0.00001$] for bout frequency as well as for bout size [$F(8,160)=23.69$, $p<0.0001$]. The P-Drinker rats therefore initially consumed very frequent small bouts of alcohol at the lower concentrations. The significant overall increase in alcohol intake (GM/KG) over the various concentrations of the acquisition curve was partially a function of an increase in the size of alcohol bouts.

COMPARISON OF THE P/NP AND LONG EVANS RATS

The NP and Long Evans Nondrinkers did consume some alcohol at the lower concentrations from 2-4%. The transition from drinking some alcohol, to completely avoiding the solution was very abrupt for all the rats. For example, all the NP's drank some alcohol at the 2 and 3% levels. However at 4%, 6 out

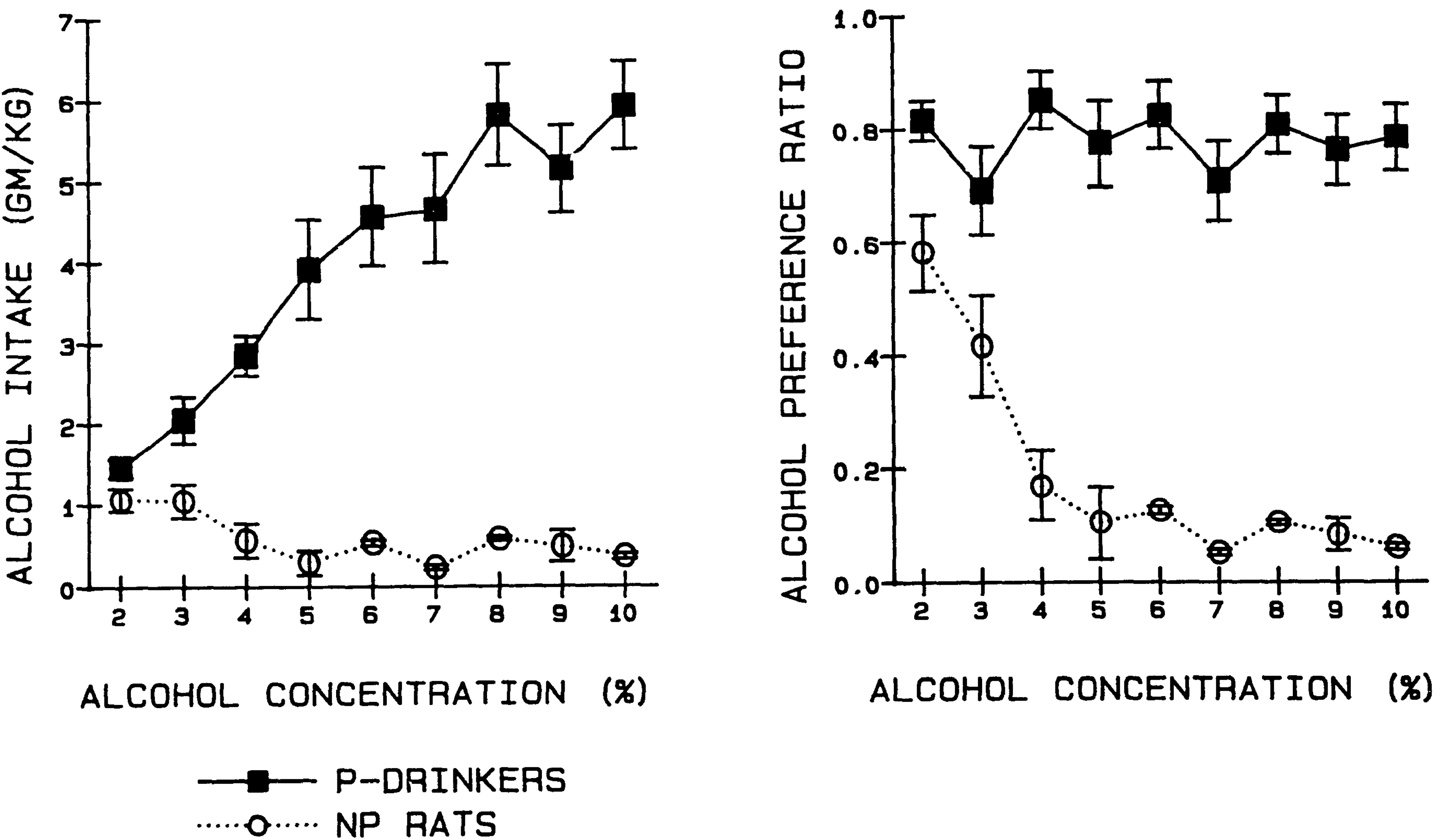
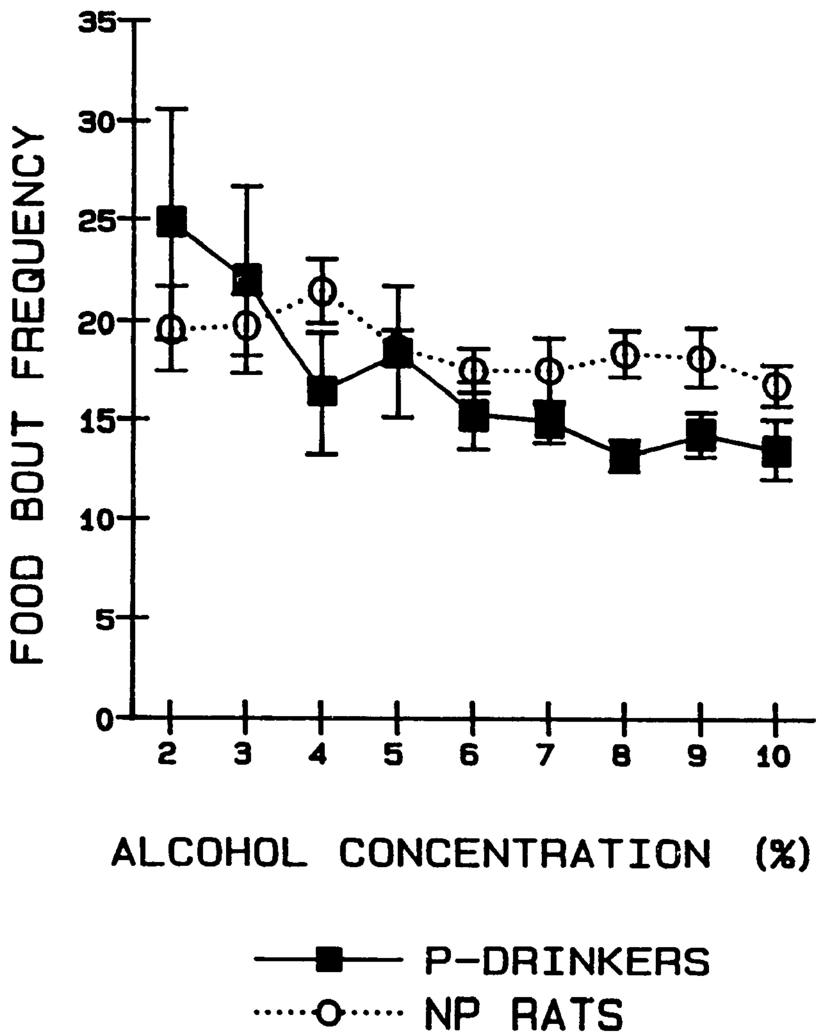


Figure 14. Alcohol intake (GM/KG) and preference ratios in the selected group of P-Drinkers and NP rats.



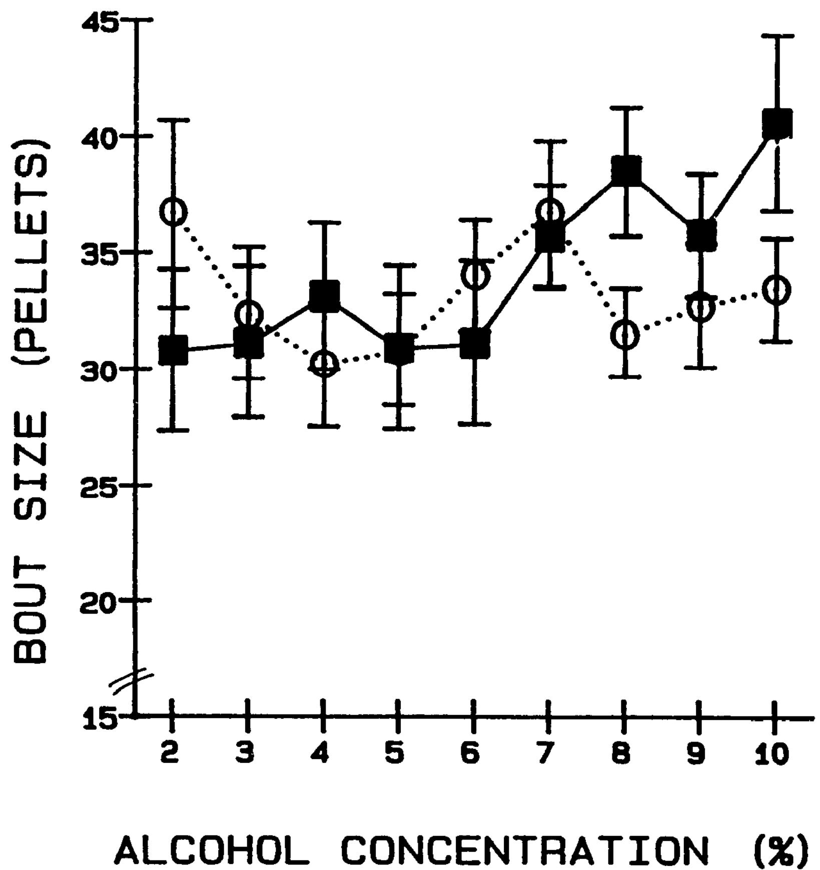


Figure 15. Mean food bout frequency and bout size for the groups of P-Drinker and NP rats.

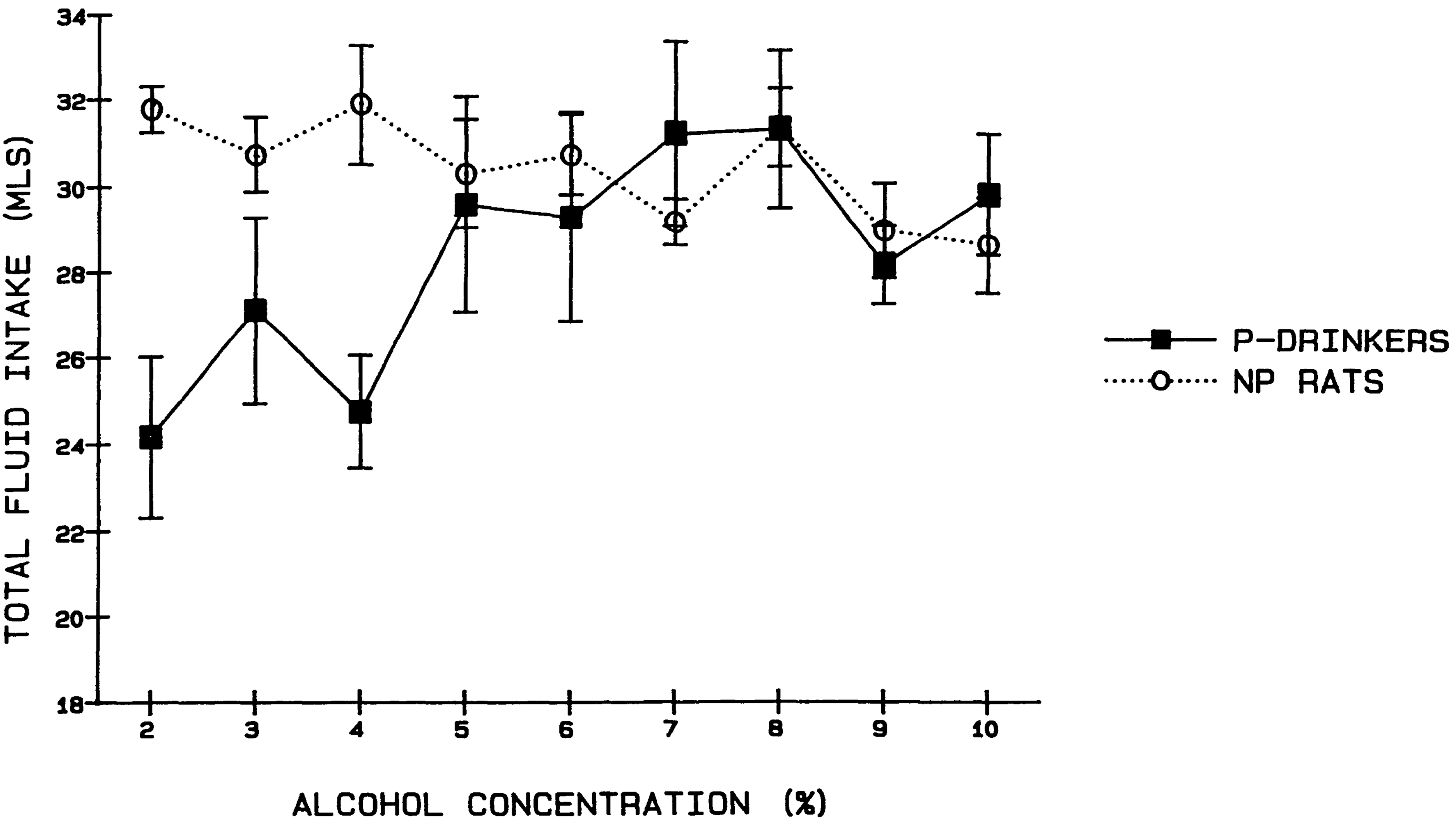


Figure 16. Total fluid intake (mls) over the acquisition curve for the P-Drinker and NP groups of rats.

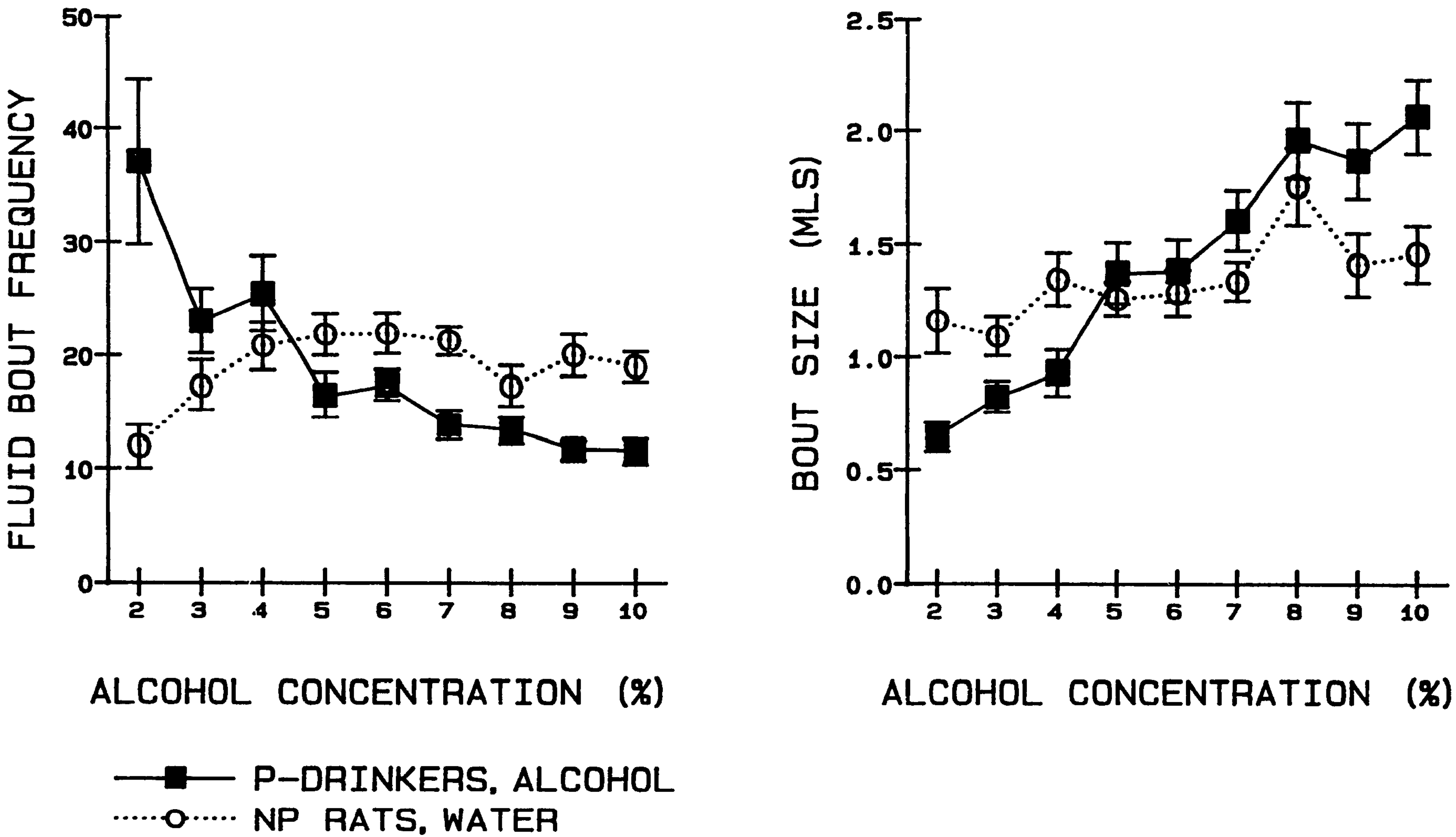


Figure 17. Mean bout frequency and bout size (mls) for preferred fluids in the P-Drinkers and NP rats.

of the 10 rats did not touch the alcohol tubes. This abrupt change in behavior was puzzling, suggesting perhaps that these rats were extremely sensitive to the olfactory properties of the alcohol solutions, rather than to the avoidance of the pharmacological effects of the alcohol. This possible interpretation is illustrated by the alcohol consumption records of NP Rat7 in Figures 18 and 19. At the 4% concentration this animal drank 21 mls of alcohol in 11 bouts spaced over the entire 24 hour recording period. The pattern of bouts at 4% did not suggest any development of aversion to the post-ingestional effects of alcohol. However, at 5% alcohol the animal made very few licks. NP rats have been induced to drink large quantities of alcohol (up to 12 g/kg/day) by flavouring the alcohol (saccharin and sodium chloride) and restricting food intake (Waller, McBride, Lumeng & Li, 1982). More recently, Murphy, Gatto, McBride, Lumeng and Li (1989) demonstrated that NP animals refused to drink 10% alcohol even when mixed with a preferred banana or almond flavour. However, these animals had previously been exposed to a period of forced alcohol intake and thus had a great deal of prior experience with the aversive taste of alcohol. On the basis of these observations it would be plausible to suggest that a proportion of low drinking NP and Long Evans are discouraged from drinking by an extreme aversion to the smell and taste of even low concentrations of alcohol.

A comparison between the P-Drinkers and the Long Evans-Drinkers yielded remarkably few differences. The selected P and Long Evans rats drank similar amounts of alcohol by the end of the acquisition period (approx 6 gm/kg/day) and exhibited a similar preference ratio (approx 0.80). Total fluid intake and mean alcohol bout frequency and size were the same at the 10% alcohol concentration. In general, high drinking animals increased the size of alcohol bouts over the course of the screening period. The average bout size of 2 mls

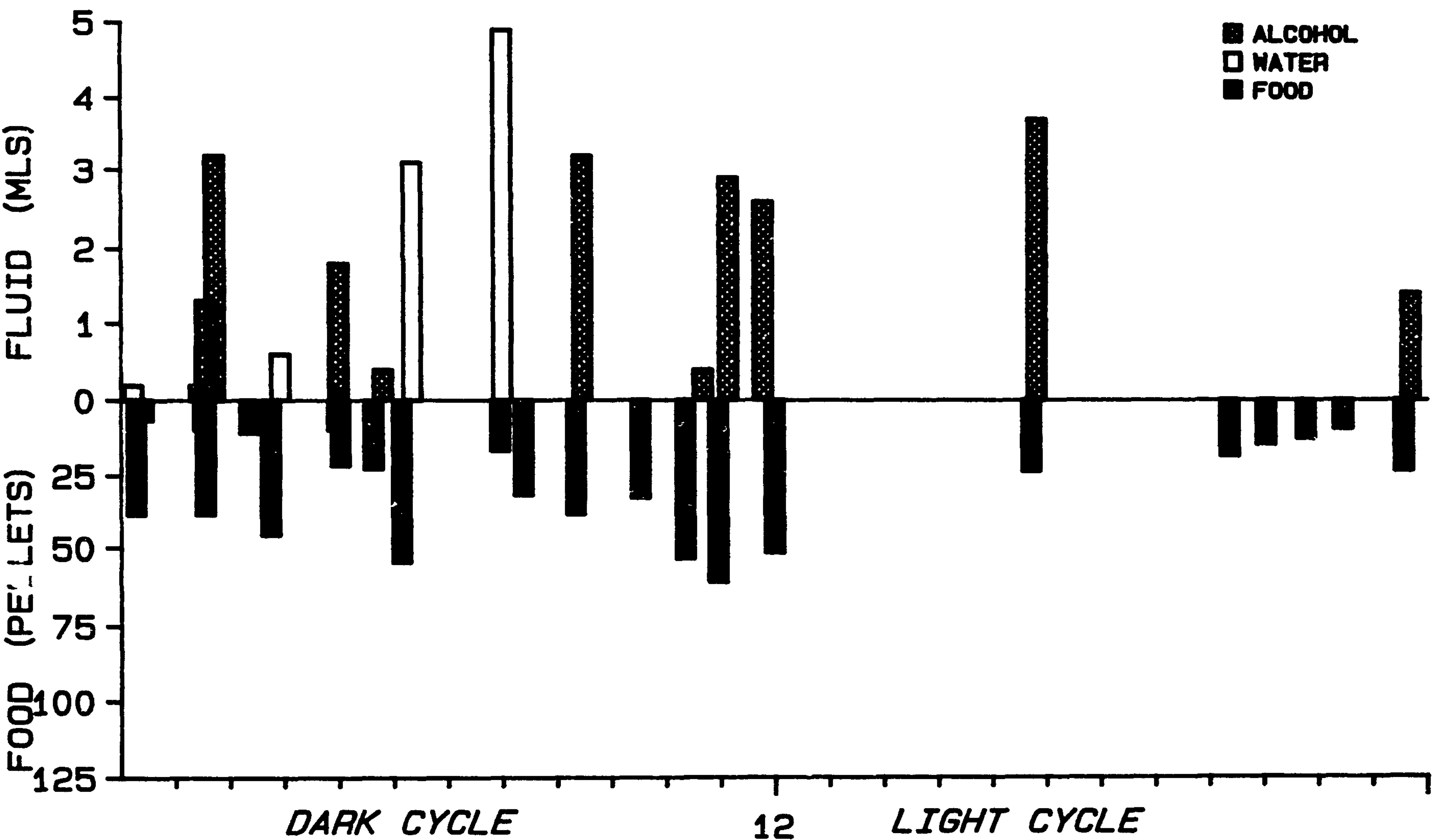


Figure 18. Food, water and alcohol consumption over a 24-hour period for NP Rat#7, consuming 4% alcohol.

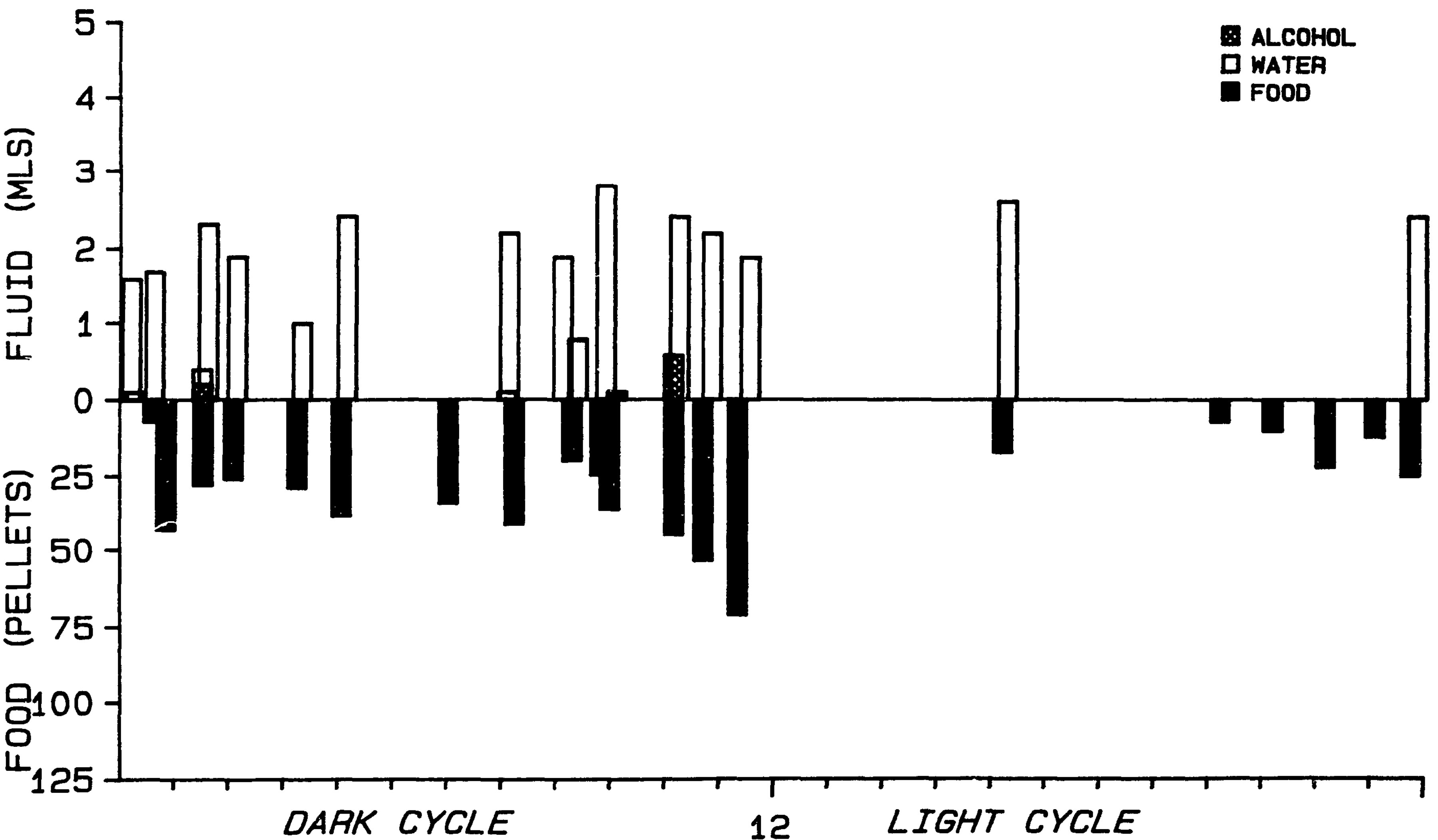


Figure 19. Food, water and alcohol consumption for NP Rat#7, consuming a 5% alcohol solution.

at the 10% concentration, led to a mean intake of approx 0.5 gm/kg/bout in both strains. This mean value does not reflect the large variability in bout sizes and consumption patterns among individual rats. Daily consumption records for individual P and Long Evans rats are displayed in Figures 20 to 23. Figure 20 shows a P-Drinker rat that consumed alcohol in more frequent small bouts averaging 1.5 mls, while the P rat in Figure 21 consumed an average of 2.9 mls/bout. This rat drank some large drafts of alcohol, notably two bouts of over 1.3 gm/kg/bout. Similarly, the same variability can be observed in the Long Evans Drinker rats. As displayed in Figure 22, this rat drank alcohol in bouts averaging 1.1 mls. The Long Evans rat shown in Figure 23 consumed, on average, 3.5 mls/bout along with some larger bouts of more than 1.2 gm/kg/bout.

Differences in total food intake between water and alcohol consuming animals have been observed following forced alcohol intake in rats (Richter, 1941). However, in both the Long Evans and P/NP strains of rats there were no differences between the drinkers and non-drinkers in terms of total food intake. This suggested that there is no immediate recognition of alcohol as a caloric source and no adjustments for differential total caloric intake between drinkers and non-drinkers. Moreover, this suggested that the initial motivation for ingesting the alcohol was not based on the caloric value of the alcohol. These issues will be further addressed in the following study on the maintenance of alcohol intake in Long Evans rats.

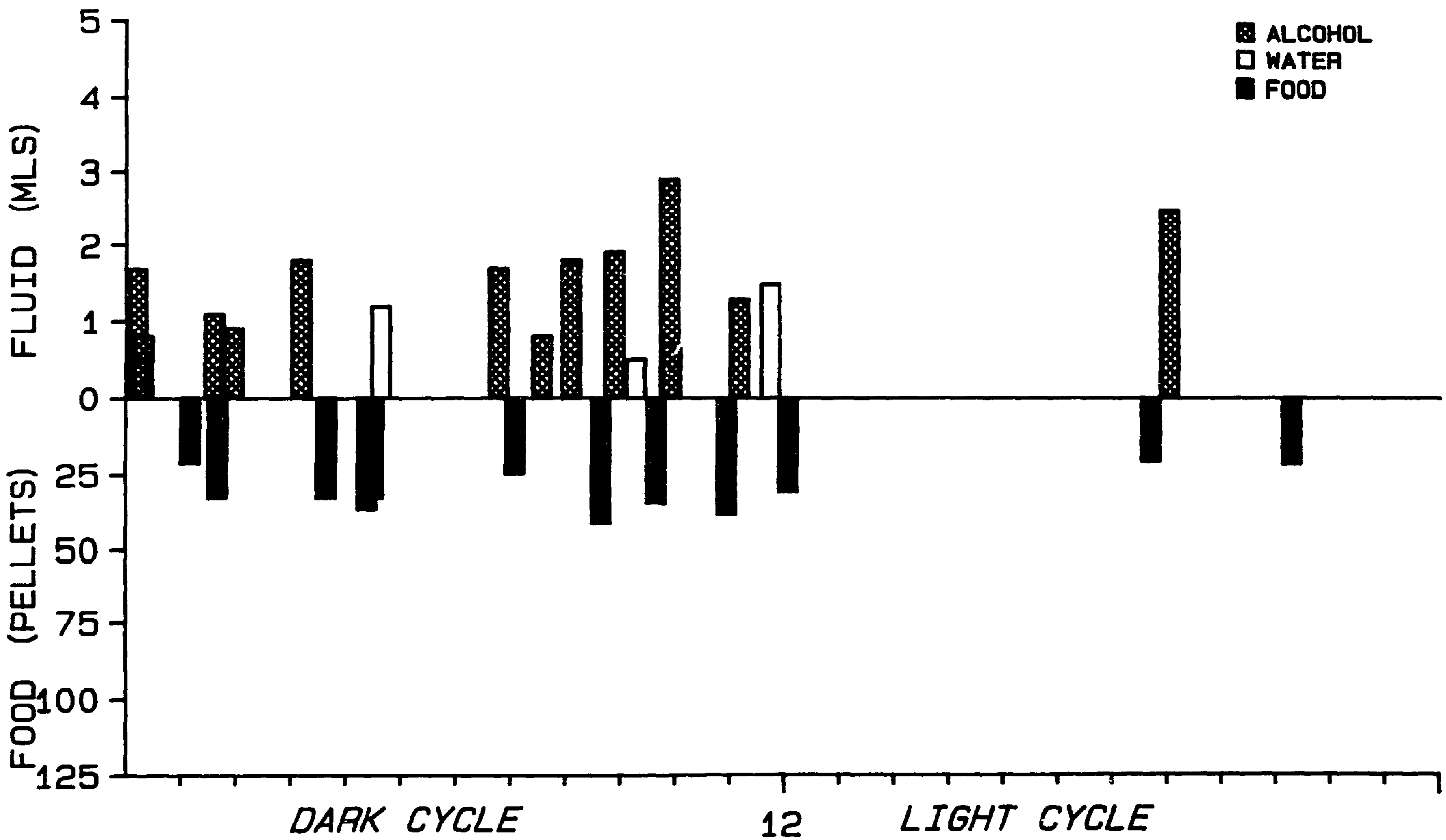


Figure 20. Food, water and alcohol consumption for P Rat#4, consuming a 10% alcohol solution.

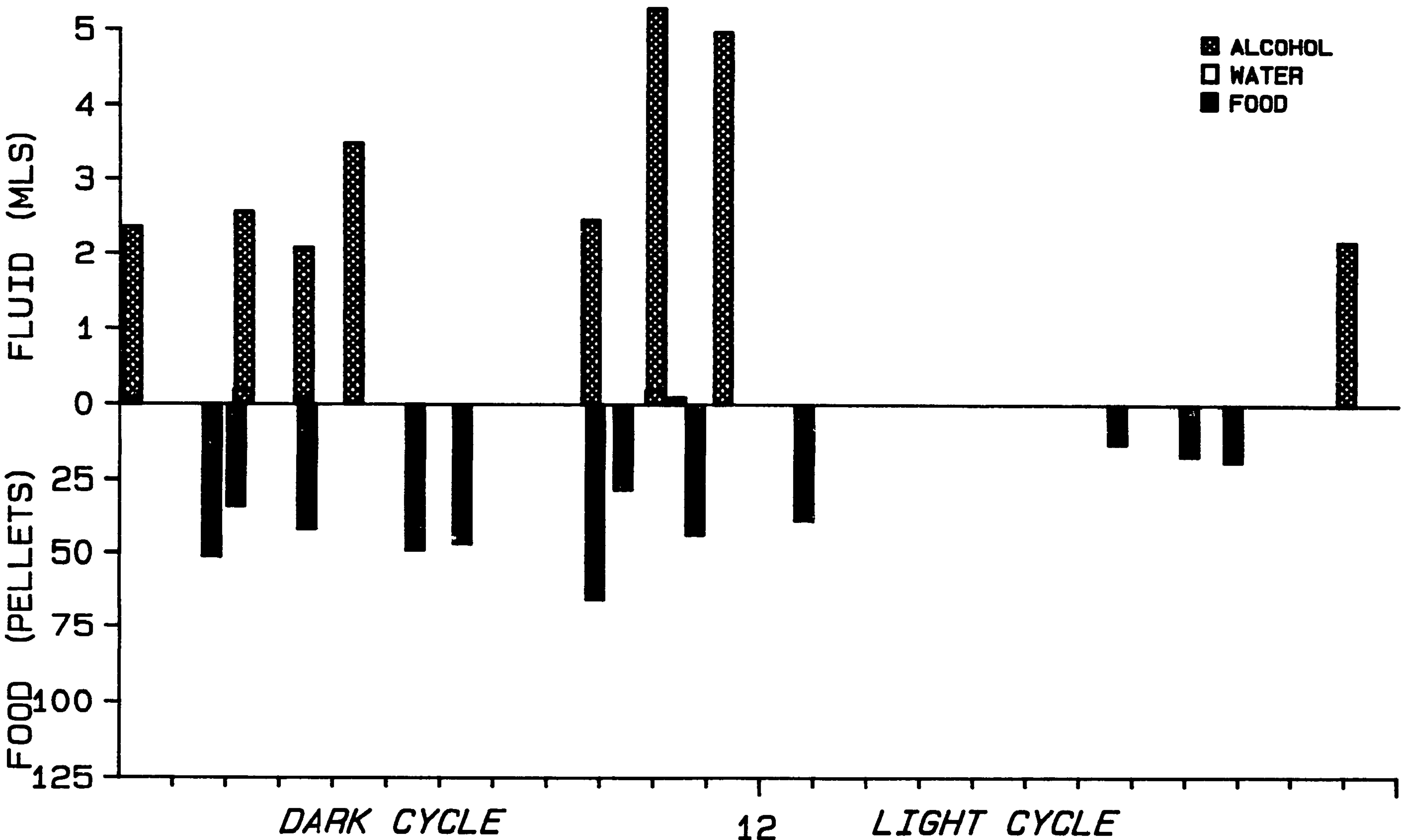


Figure 21. Food, water and alcohol consumption for P Rat#8, consuming a 10% alcohol solution.

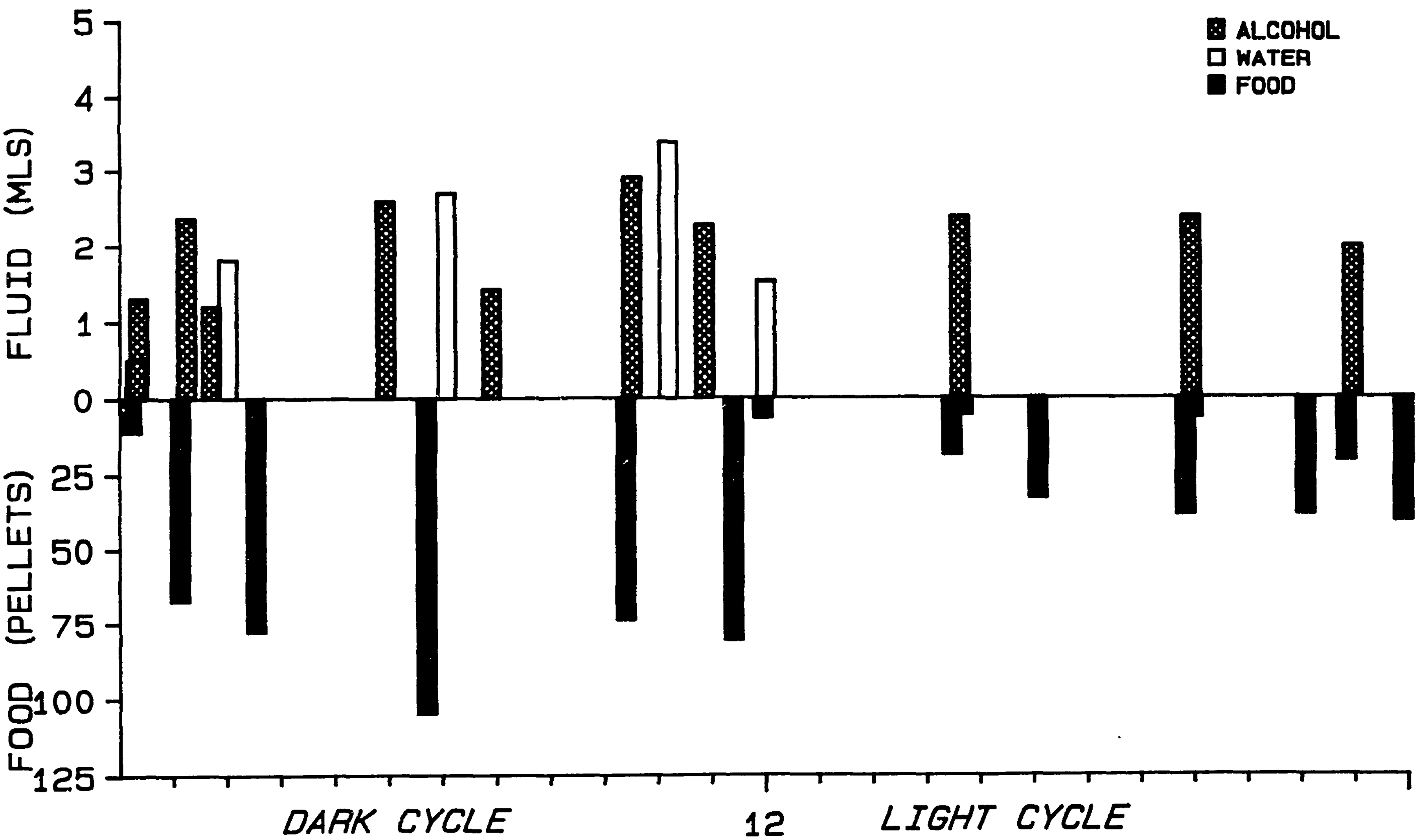


Figure 22. Food and fluid intake over the 24-hour period, for a Long Evans rat at 10% alcohol.

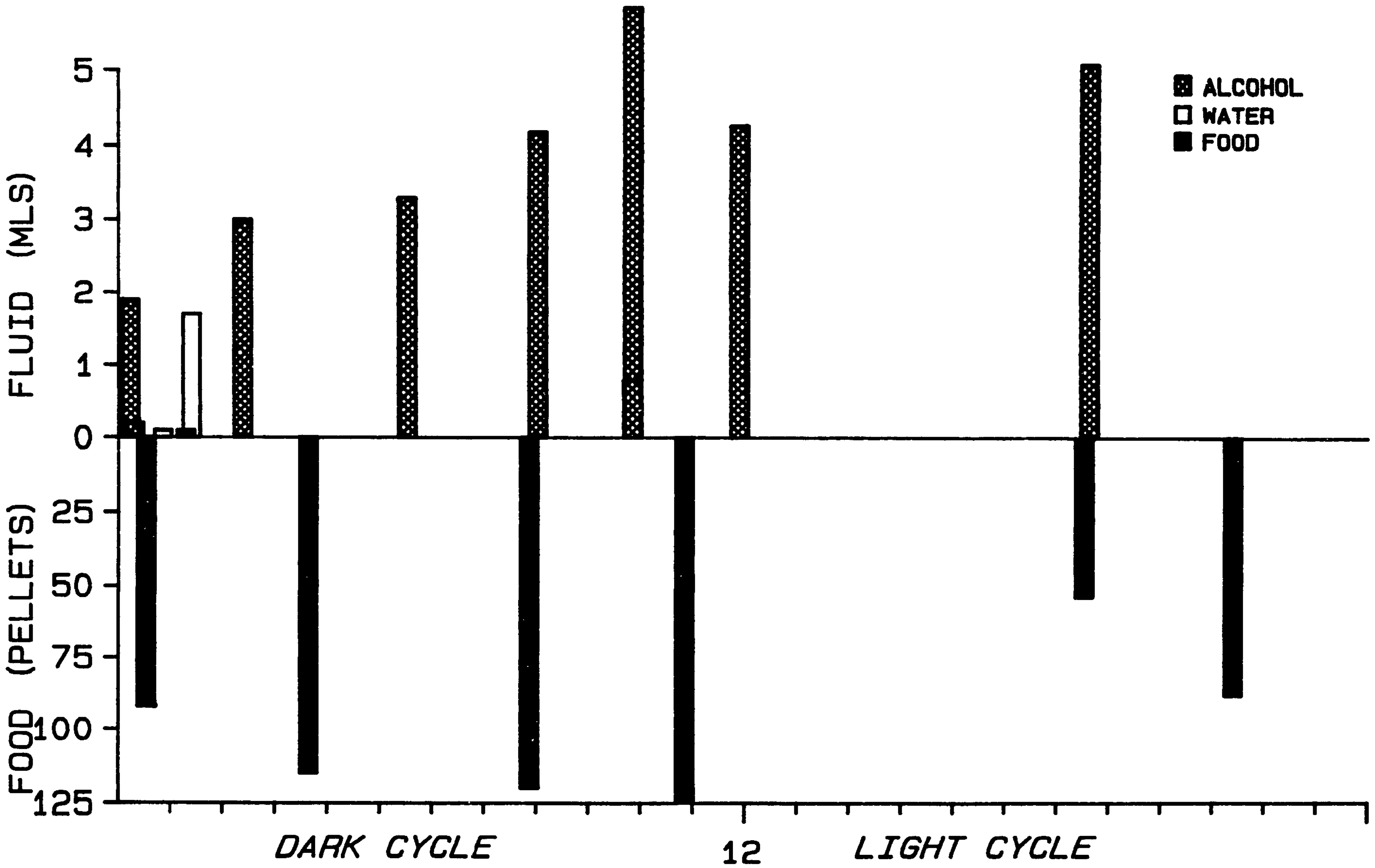


Figure 23. Food and fluid intake over the 24-hour period, for a Long Evans rat consuming 10% alcohol.

EXPERIMENT 3

AN EXAMINATION OF THE MAINTENANCE OF VOLUNTARY ALCOHOL INTAKE IN LONG EVANS RATS

Richter (1941) introduced the notion that alcohol was regulated by rodents in much the same way as food. Richter found that animals forced to consume 8, 16 and 24 % alcohol solutions ate less food, and that the total caloric intake from food plus alcohol closely approximated the previous total caloric intake from food alone. Richter (1941) concluded from these early studies that animals could replace a high proportion of their daily caloric intake with alcohol without any untoward effects.

It was an unfortunate point in the history of alcohol studies with animals--since from that point onward alcohol was marked as a caloric substance and the "appetite" for alcohol was considered in much the same way as the appetite for any food (e.g. Richter, 1953; Rodgers, McClearn, Bennett & Hebert, 1963). It was unfortunate because Richter did not consider alternate interpretations of his data. First, the forced ingestion of high concentrations of alcohol produced a severe restriction in the total water intake--as much as 50% reduction. In the group forced to consume 24% alcohol, 3 out of 8 animals died due to a persistent refusal to consume fluid. Water restriction has been shown to produce an immediate drop in food intake and lead to greater weight loss than comparable periods of food deprivation (Collier & Levitsky, 1967; Finger & Reid, 1952). Thus, it would be just as logical to conclude that the immediate reduction in food intake observed in Richter's animals, following the introduction of the alcohol, was due to the reduction in water, as to the increase in alcohol. At lower concentrations of alcohol (5-10%) Mendelson and Mello (1964) saw no evidence of caloric regulation in animals forced to consume alcohol.

Consideration of alcohol as a caloric source and a nutrient was further promoted by numerous studies showing that dietary manipulations such as vitamin and thiamin deficient diets or liver supplements could markedly increase or decrease alcohol intake (Williams et al., 1949; Mardones, 1951). Thus, as discussed previously, Williams et al. (1949) proposed that genetic makeup produced widely varying requirements for nutrients and vitamins, which if not met adequately by the diet resulted in craving for alcohol. Subsequent research in this area demonstrated that the most important factor responsible for the increased alcohol intake observed in vitamin deficient animals was a corresponding decrease in food intake (Westerfeld & Lawrow, 1953).

Food restriction, in some instances, increased alcohol intake; although it should be noted that compensatory increases in alcohol intake took several weeks to develop and did not occur unless there was more than a 25% reduction in daily caloric intake (Westerfeld & Lawrow, 1953). Recent work has also shown that decreases in food intake are not automatically followed by an increase in alcohol intake. Weisinger et al. (1989) showed that a significant decrease in food intake accompanied by a 17% drop in body weight did not result in an increase in the intake of 10% alcohol.

The most powerful evidence that can be marshalled against the simple interpretation of alcohol as a caloric source is the work of Marilyn Carroll and colleagues (Carroll & Boe, 1982; Carroll & Meisch, 1984; Carroll, Pederson & Harrison, 1986). These researchers observed that food deprivation increased oral and intravenous self-administration of a number reinforcers, including drugs that do not contain calories (etonitazene, cocaine, amphetamine). Similar results have been obtained with cocaine and amphetamine in other laboratories, (Glick, Hinds & Carlson, 1987). These results established that increased alcohol intake following food deprivation is not likely to be primarily

related to the caloric properties of the alcohol. It should be noted that food deprivation did not increase the consumption of non self-administered drugs, bitter tasting substances, or water. Thus, the food deprivation effect appears to be specific to the consumption of reinforcing substances (Carroll & Meisch, 1984).

However, despite this evidence, the view that alcohol is ingested as a source of calories has persisted in the literature (Dole, 1986; Kulkosky, 1985). Kulkosky (1985) has claimed that "virtually all successful animal models... employ some degree of food restriction...", concluding that free choice alcohol intake represented an attempt to maximize caloric repletion while at the same time avoiding the aversive post-ingestional consequences of intoxication. There have been numerous reports however, that animals will perform an operant and drink alcohol when food satiated (e.g. Henningfeld & Meisch, 1981; Iso, 1986). In addition, animals have been shown to learn to bar press for alcohol solutions, without prior food or fluid deprivation (Sinclair, 1974; Hyytia & Sinclair, 1989).

In this study, the maintenance of alcohol drinking behavior was examined in the computerized drinkometer chambers using a group of animals with alcohol drinking experience. These animals were screened for intake in the home cages and placed in the drinkometer following a maintenance period of not less than two weeks. Since a great deal of emphasis has been placed on the notion of alcohol as a caloric source, the analysis was focussed primarily on the relationship between food and alcohol in terms of total intake and at the level of individual bouts.

MATERIALS AND METHODS

Subjects: Male Long Evans rats weighing 350-500 grams were selected from the sample of rats that had been screened for alcohol intake using plastic tubes and ball bearing spouts in Experiment 1. These animals were housed in the animal colony in a humidity and temperature controlled environment with a 12 hour dark: 12 hour light cycle. Following alcohol screening, these animals were maintained in their home cages (standard stainless steel hanging cages) with free access to Agway 4070 rat chow, water and a 10% alcohol solution, for a minimum of 10 days. The animals were handled and weighed every 2-3 days.

Following the screening and baseline periods the Agway rat chow was replaced with Bioserve Precision Pellets #F0021 (Bioserve, Frenchtown, N.J.). The food pellets were placed inside the home cage, in a glass jar fixed to an aluminium holder. The animals had free access to Bioserve food, water and 10% alcohol in the home cage for a minimum 5 day period prior to being moved to the drinkometer chambers. The animals were run through the drinkometer procedure in 7 batches of 10 rats each (n = 70).

Drinkometer Procedure: The animals were housed in the drinkometer chambers with ad libitum access Bioserve food, water and a 10% alcohol solution, for a minimum of 10 days prior to the active recording of feeding and drinking behavior. The animals were handled and weighed, and the cages cleaned every second day.

During the active recording period, the COLLECT program was stopped daily at 3 P.M., immediately prior to the start of the dark cycle. During a 30 minute shut-down period, fluids were measured and replaced and the REPORT program was invoked. The daily report for each animal was automatically transferred via the computer's serial port to a microVAX II computer for further analysis.

RESULTS

An Overview of the Patterns of Voluntary Alcohol Intake

Table 4 lists the total amounts of food and fluid consumed, as well as mean bout frequency, size and duration for the last 24-hour baseline recording period, in a sample the rats run in this study. The rats selected for inclusion in this table were considered to be representative of the large range of alcohol consumption and preference exhibited by Long Evans rats. Thus, rats drinking in the range of 0.74 to 5.0 gm/kg/day, exhibiting preference ratios from 0.03 to 0.91 were listed.

The temporal pattern of intake of Rat 10 is illustrated in Figure 24. Differences between dark and light cycle food and fluid intake have been extensively reported in the literature (Aalto, 1986; Castonguay et al., 1986; Dole & Gentry, 1984; Spector & Smith, 1984). Therefore, it was not unexpected that consummatory behavior in this rat closely reflected circadian activity cycles. This rat displayed the highest levels of feeding and drinking activity during the dark cycle with few bouts during the light cycle. A total alcohol intake of 2.87 gm/kg was distributed throughout the 24 hour period into 11 discrete bouts. The most notable aspect of this pattern of alcohol intake was the rapid rate of ingestion of the alcohol. This rat consumed a mean of 0.26 g/kg/bout during period of 2.46 minutes.

The temporal pattern of alcohol and food intake in the remaining animals in Table 4 was similar, although the degree of variability between animals with regards to the pattern and size of bouts was remarkable. To illustrate this point, individual daily records of consumption for other animals listed in Table 4 were plotted in Figures 25 to 29. The rats shown in Figures 24 and 25 displayed the same total alcohol intake in terms of GM/KG and preference ratio, however, the alcohol was distributed in very different patterns.

TABLE 4

FOOD, WATER AND ALCOHOL BOUT STATISTICS

RAT	FOOD TOTAL PELLETS	#	DUR (MIN)	SIZE	WATER TOTAL VOLUME	#	DUR (MIN)	SIZE (MLS)	ALCOHOL TOTAL VOLUME	#	DUR (MIN)	SIZE (MLS)	GM/KG/ DAY	GM/KG/ BOUT
1	652	15	4.7	43.5	14.4	9	0.98	1.60	6.5	5	0.92	1.30	0.74	0.15
2	447	12	4.4	37.3	17.0	6	2.58	2.83	8.0	4	1.59	2.00	1.03	0.26
3	400	8	9.5	50.0	18.3	6	1.35	3.05	9.0	8	0.71	1.13	1.50	0.19
4	612	6	8.9	102	11.1	6	1.22	1.85	11.5	4	2.42	2.88	1.53	0.38
5	613	20	5.5	30.6	7.6	8	1.08	0.95	12.1	7	1.88	1.73	1.63	0.23
6	598	7	8.8	85.4	17.8	8	2.06	2.22	14.5	11	0.94	1.32	2.00	0.18
7	544	16	4.7	34.0	20.9	9	2.17	2.32	11.1	6	1.25	1.85	2.20	0.36
8	489	8	7.4	61.1	6.9	7	1.66	0.98	14.9	6	2.58	2.48	2.55	0.42
9	677	11	5.8	61.5	13.1	8	0.51	1.64	17.9	5	1.41	3.58	2.85	0.57
10	543	11	3.9	49.4	11.4	5	2.89	2.28	14.0	11	2.46	1.27	2.87	0.26
11	660	11	6.2	60.0	6.9	8	1.12	0.86	19.7	21	1.09	0.94	3.33	0.16
12	379	8	4.4	47.4	1.9	1	1.58	1.90	21.4	6	4.27	3.57	3.44	0.57
13	474	9	3.0	52.6	24.0	12	1.65	2.00	21.0	7	1.82	3.00	4.10	0.58
14	508	6	7.0	84.6	3.0	5	0.82	0.60	21.0	6	1.87	3.50	4.36	0.73
15	488	14	3.8	34.8	11.9	12	1.65	0.99	19.9	12	1.86	1.66	5.00	0.42

 # & Dur (Min)--Frequency and duration (minutes) of food, water or alcohol bouts.

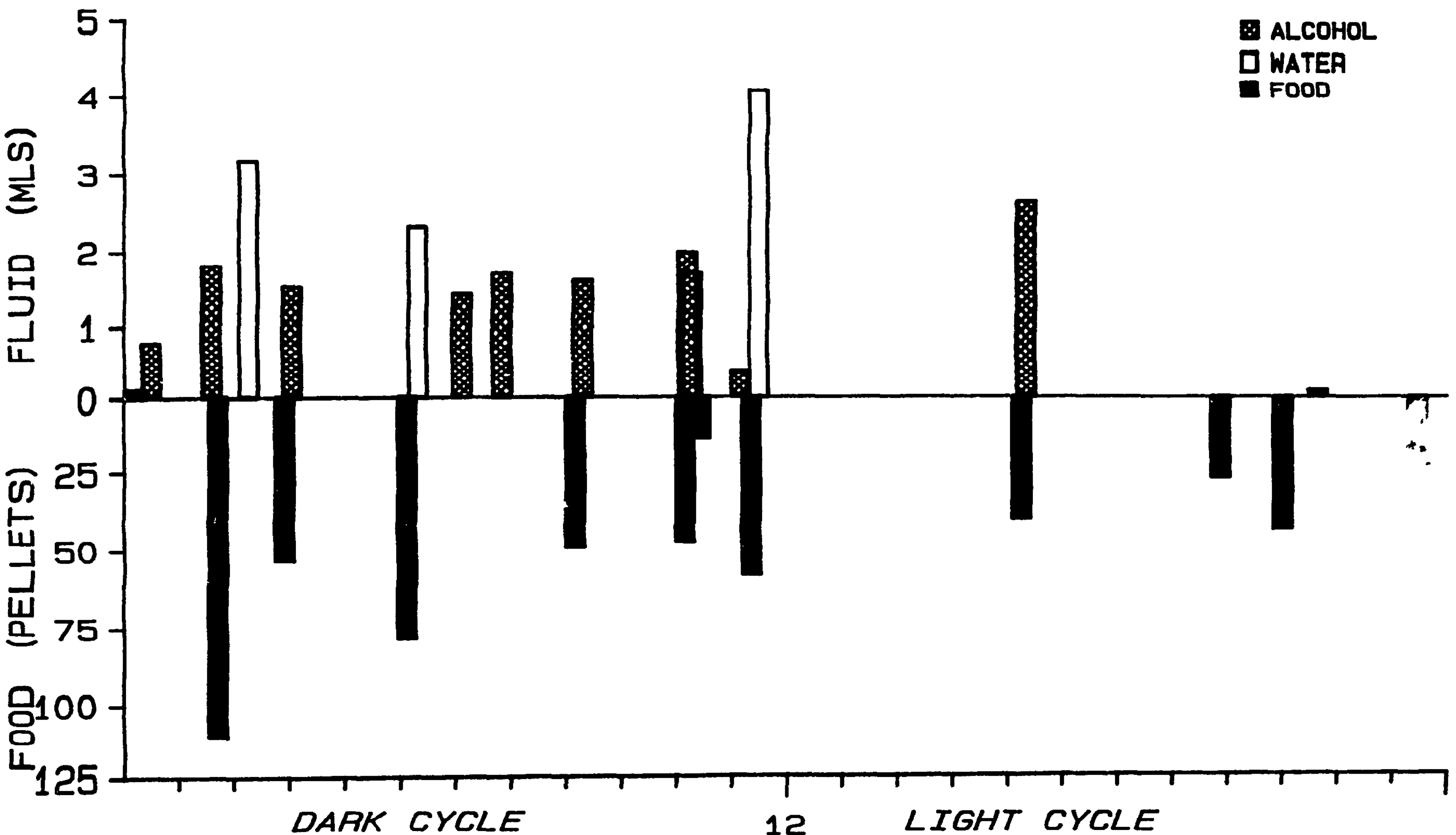


Figure 24. Food and fluid intake for Rat 10, Table 4.
GM/KG= 2.87, Preference ratio= 0.55

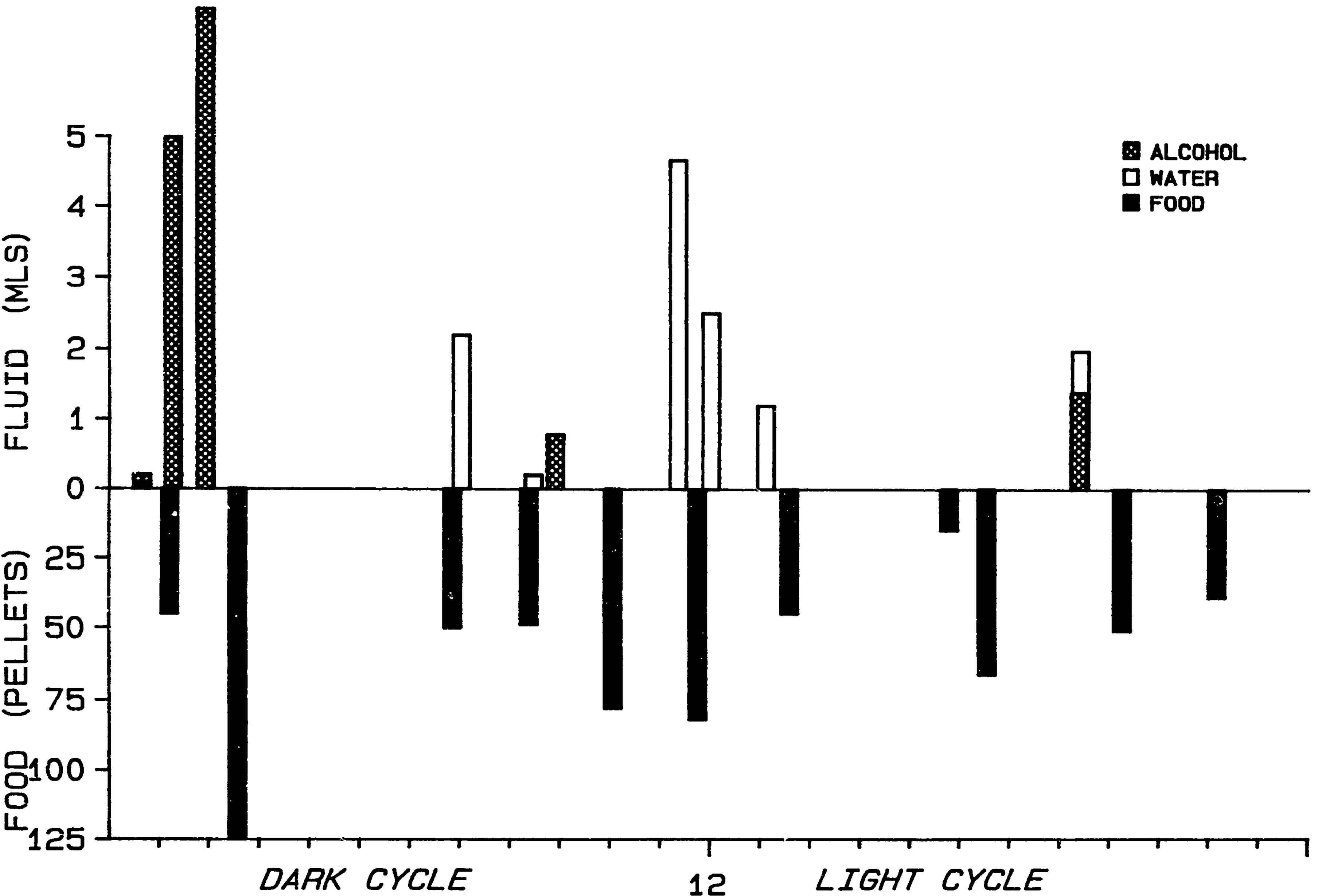


Figure 25. Food and fluid intake for Rat 9, Table 4.
GM/KG= 2.85, Preference ratio= 0.58

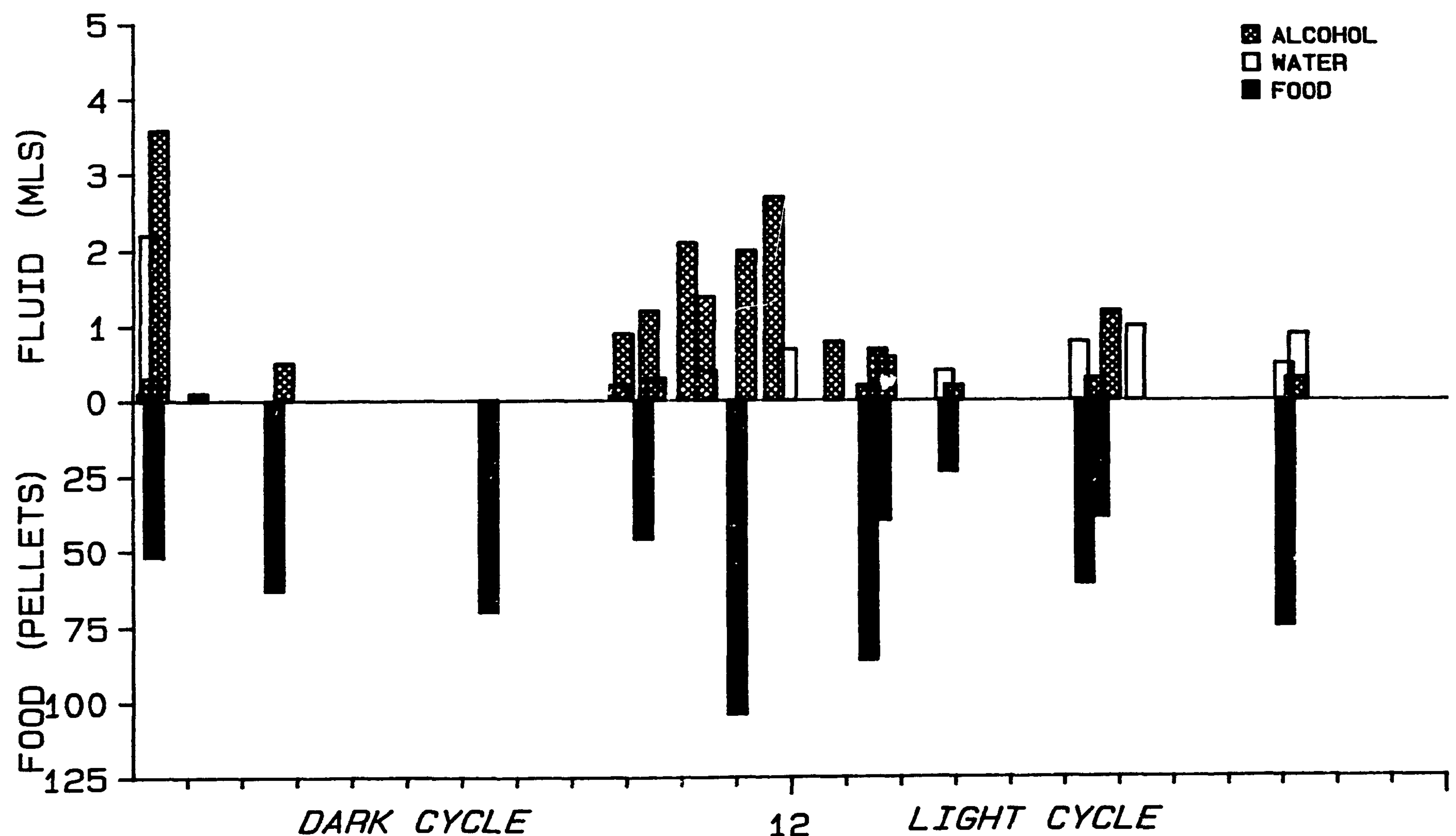


Figure 26. Food and fluid intake for Rat 11. Table 4.
GM/KG= 3.33. Preference ratio= 0.74

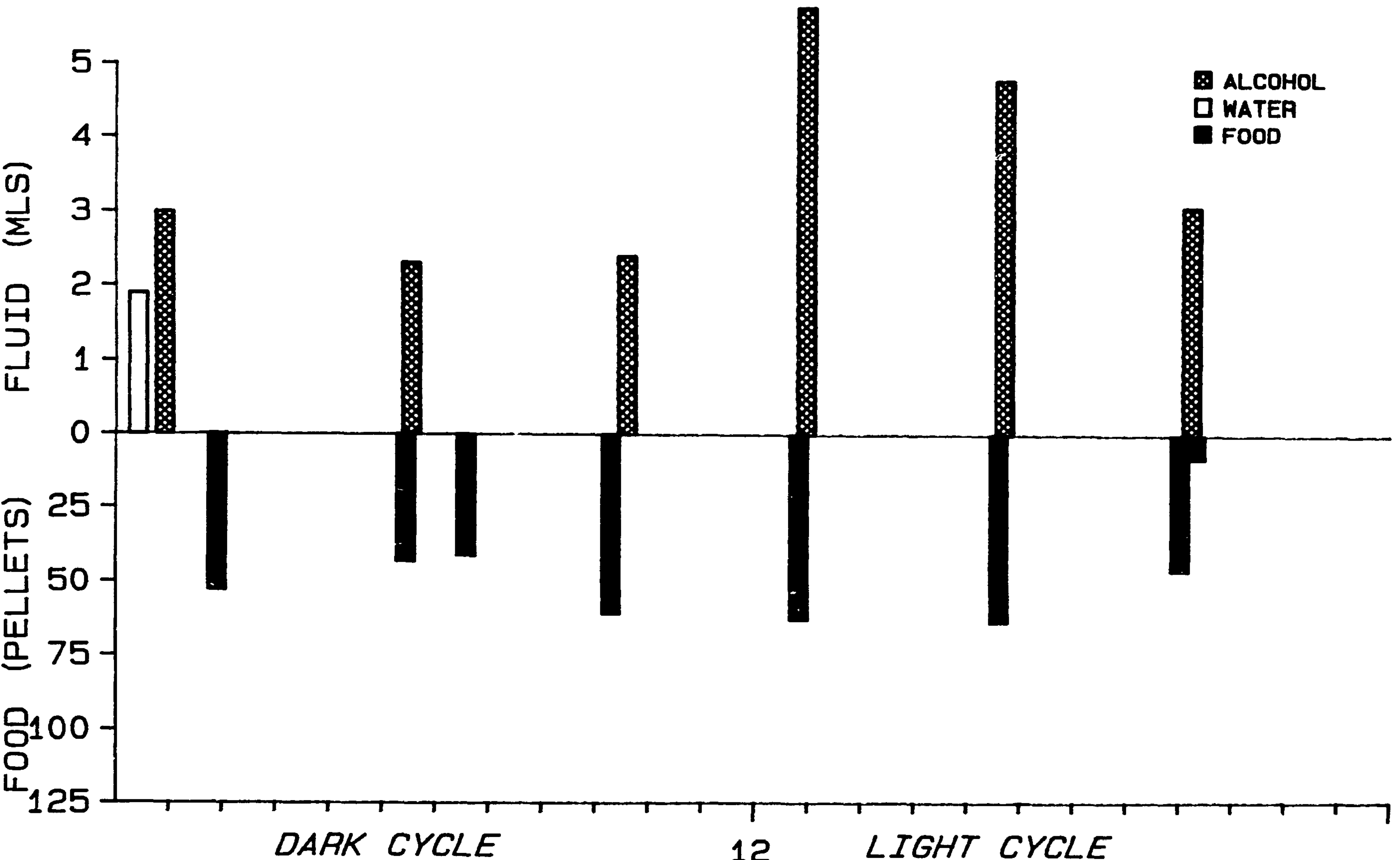


Figure 27. Food and fluid intake for Rat 12, Table 4.
GM/KG= 3.44, Preference ratio= 0.91

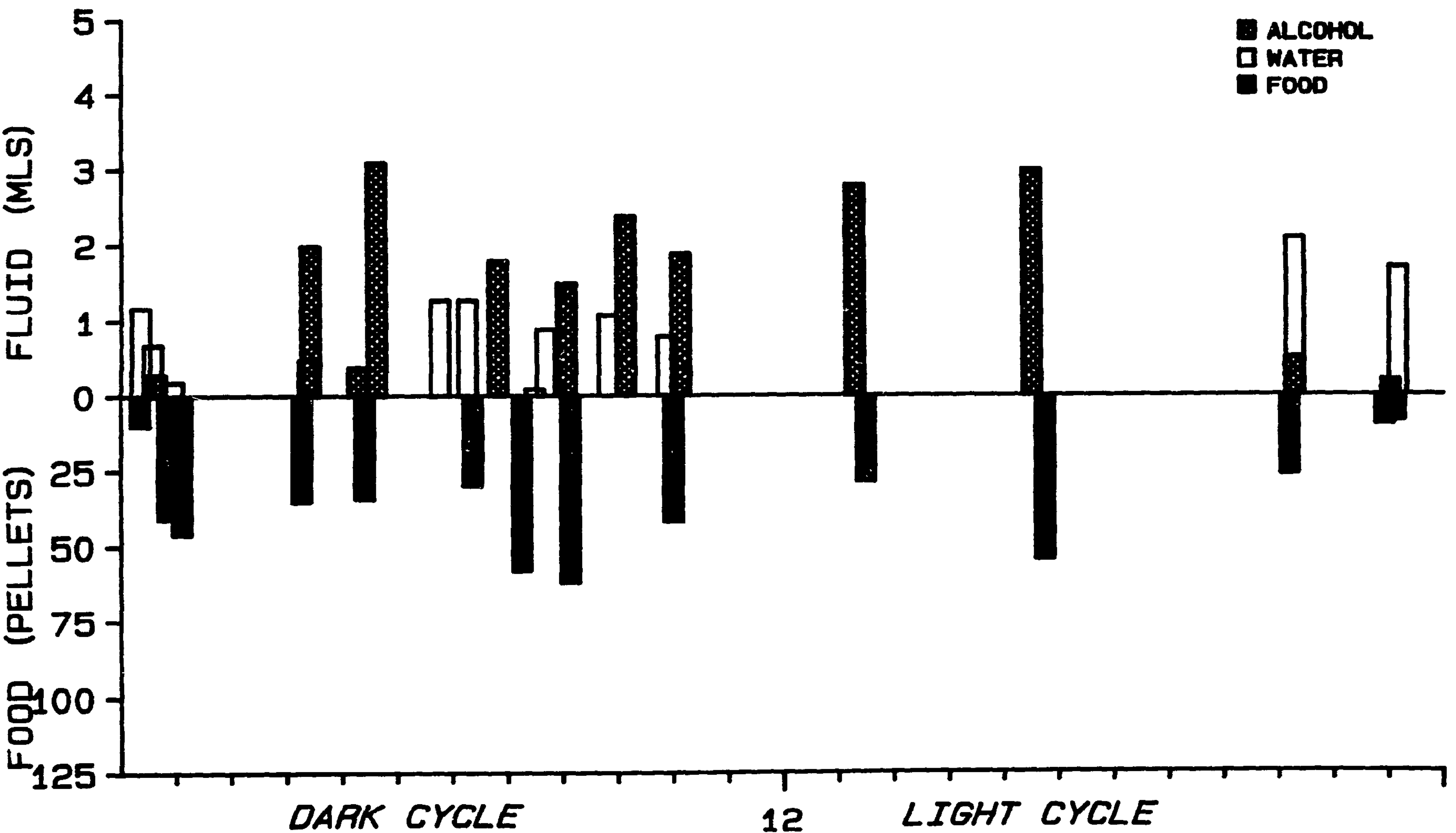


Figure 28. Food and fluid intake for Rat 15, Table 4. GM/KG= 5.0, Preference ratio= 0.62

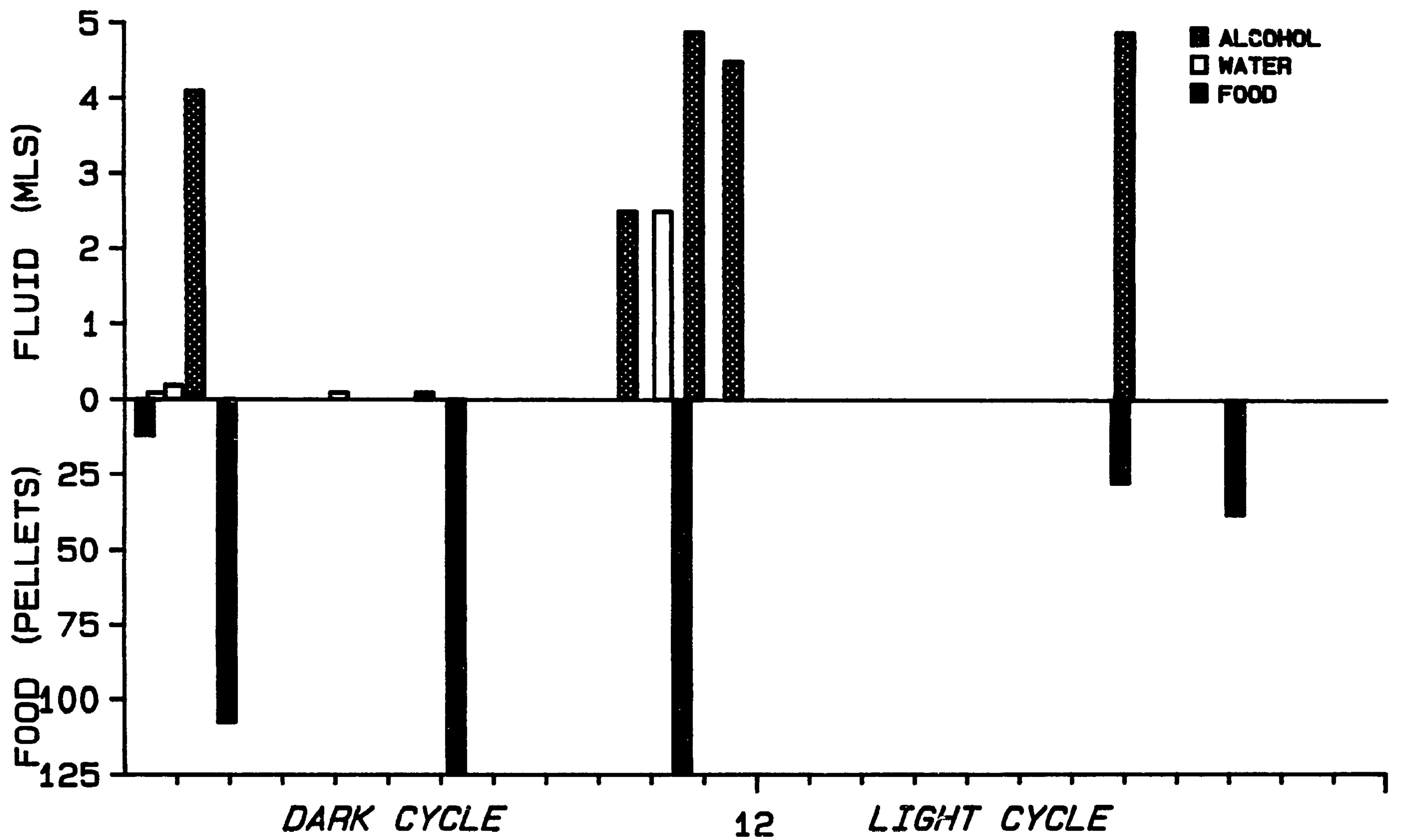


Figure 29. Food and fluid intake for Rat 14, Table 4.
GM/KG= 4.36, Preference ratio= 0.87

The rat in Figure 25 drank two very large alcohol bouts at the start of the dark cycle, with very little intake thereafter. The variability in alcohol bout distribution was similarly demonstrated by the rats in Figures 26 and 27. Rat 11 in Figure 26 distributed alcohol intake of 3.33 gm/kg/day into 21 bouts, averaging 0.16 gm/kg/bout. In a markedly different pattern, Rat 12 consumed a total daily intake of 3.44 gm/kg in 6 large, well spaced bouts averaging 0.57 gm/kg/bout (Figure 27). As shown in Figure 28, Rat 15 consumed 5.0 gm/kg/day in 12 bouts, with an alcohol preference ratio of 0.62, indicating that the animal also consumed considerable water. The mean alcohol bout size in this animal was 0.42 gm/kg/bout. Rat 14 on the other hand, distributed its alcohol intake into fewer, larger bouts averaging 0.73 gm/kg/bout. Thus, although the rats in Figures 27 and 29 consumed less alcohol than the animal in Figure 28 in terms of GM/KG, they displayed high alcohol preference ratios, and consumed considerable volumes of alcohol in each bout.

A Detailed Analysis of the Relationship between Food and Fluid Intake in Alcohol Consuming Rats

In this analysis of the relationship between food and alcohol intake, animals were selected for study based on a composite index of the preference ratio and GM/KG. Of the original 70 animals run in this study 4 were eliminated due to missing data and fluid spillage. For the remaining 66 animals the mean GM/KG and preference ratio across the last five days of recording were calculated. Animals that showed a preference ratio and/or GM/KG greater than 1 standard deviation above their respective grand means were selected for subsequent analysis. Thus, animals exhibiting a preference ratio of greater than 0.51 and/or a value for GM/KG greater than 2.3 gm/kg were selected out. The mean daily GM/KG and preference ratio for this selected group of rats (n = 19) across the last 5 days of recording is presented in Figure 30.

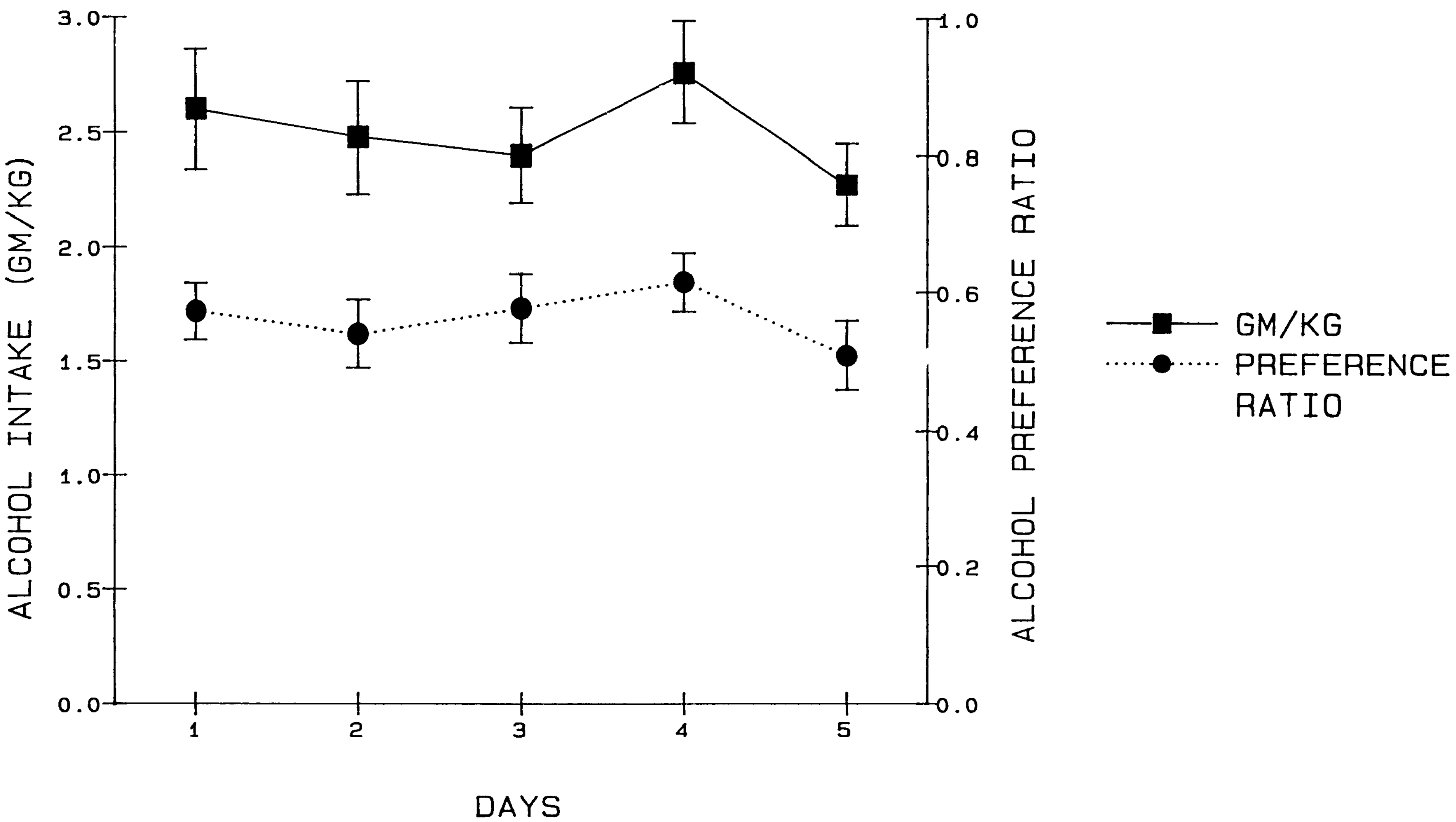


Figure 30. Alcohol intake (GM/KG) and the alcohol preference ratio across 5 days of baseline.

Statistical analyses in the following sections were carried out using the BMDP statistical programs; 7D (2-way ANOVA), 4F (frequency distributions) and 2V (ANOVA with repeated measures).

Stability of Feeding and Drinking Behavior Across Days: The mean bout frequencies for food and fluid intake across the 5 day maintenance period is shown in Figure 31. There were no significant fluctuations in food [$F(4,72)=1.43$, $p=0.23$], water [$F(4,72)=0.68$, $p=0.61$] or alcohol [$F(4,72)=0.34$, $p=0.85$] bout frequencies across the days. Similarly, there were no significant changes in food and fluid mean bout size or duration over days, thus confirming the stability of the pattern of feeding and drinking in these alcohol consuming animals. As a measure of the accuracy of the drinkometer system, there were no significant changes in the calculated V/LRATIO for either water [$F(4,72)=0.91$, $p=0.46$] or alcohol [$F(4,72)=1.21$, $p=0.31$] over days. Therefore, over a number of variables, it would appear that the baseline behavior of animals was very consistent and stable.

Food Intake vs Fluid Intake: As discussed in Experiment 2, there were no differences between high and low consuming alcohol groups on any measure related to food. The same picture has emerged in this study. Overall, in this selected sample of animals there appeared to be little association between total food intake and total alcohol intake as shown in the scatterplot in Figure 32 ($r= 0.07$, $p>0.05$). This scatterplot was constructed by plotting total food intake vs total alcohol intake, for each animal on each of the 5 days of recording. There was a wide range in daily alcohol intake between rats. Despite this range, there was no apparent compensation for the extra calories ingested via a reduction in food intake.

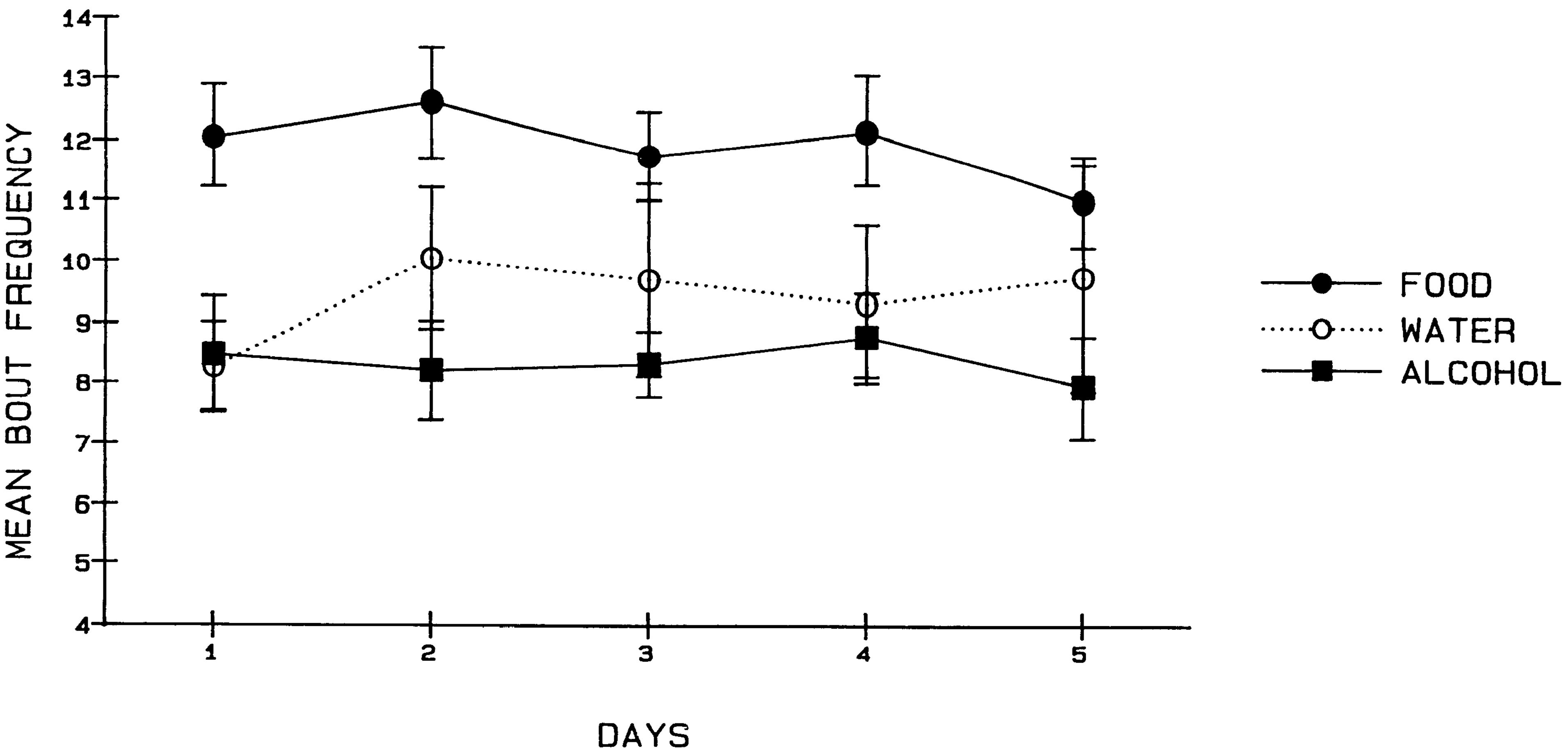


Figure 31. Mean frequency of food, water and alcohol bouts over baseline period.

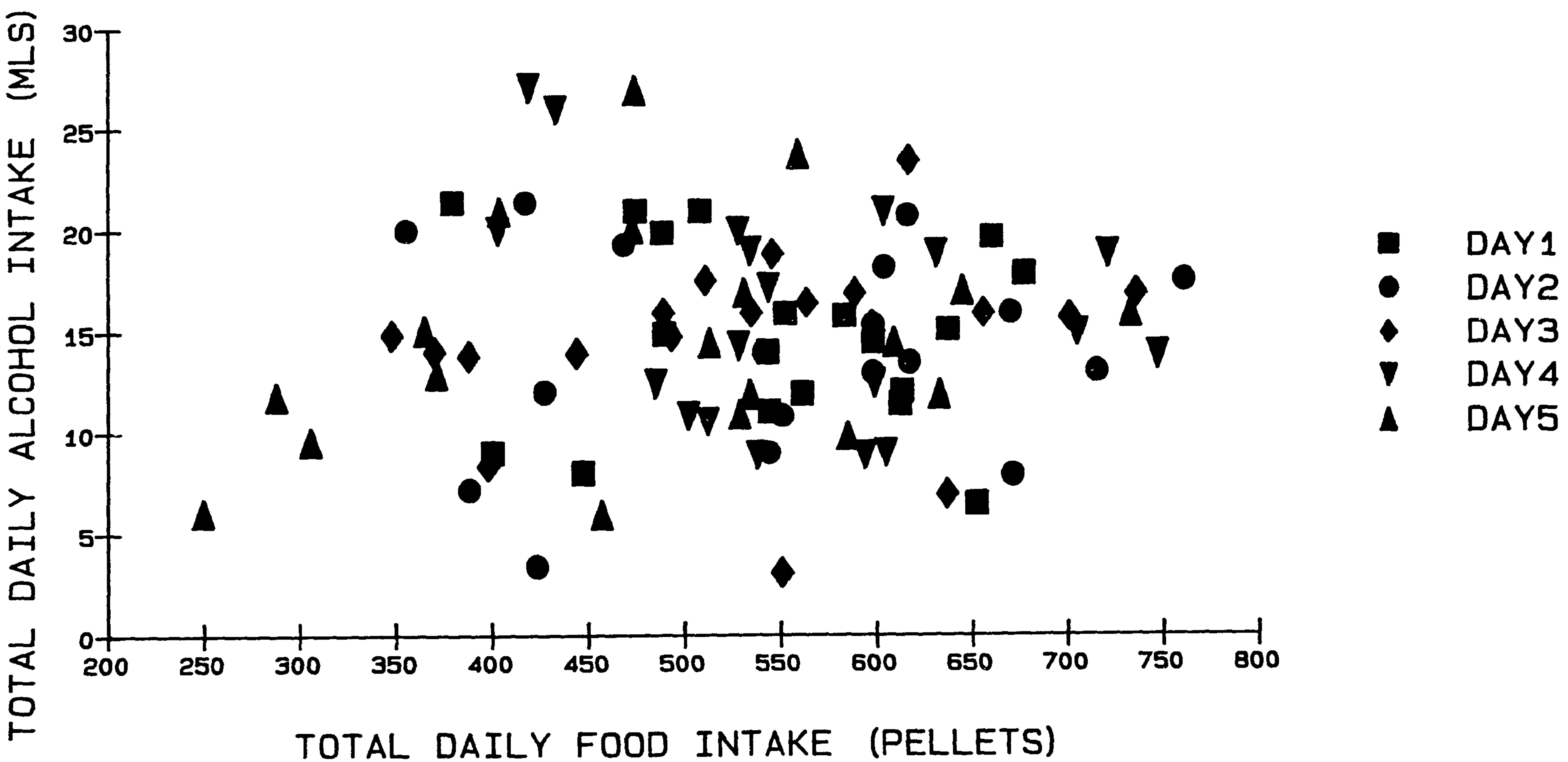


Figure 32. Scatterplot comparing total food intake with total daily alcohol intake, r= 0.07

Water Intake versus Alcohol Intake: Averaging the individual preference ratios over the 5 days for the entire sample yielded a value of 0.58. This indicated that the sample as a whole consumed a considerable amount of water in addition to alcohol. When comparing water and alcohol there were no significant differences in bout frequency [$F(1,18)= 0.80$, $p= 0.38$], duration [$F(1,18)=1.58$, $p=0.22$] or volume/lick ratio [$(F1,18)=1.28$, $p=0.27$] for the two fluids. The only factor that distinguished these two fluids was mean bout size. As shown in Figure 33, mean alcohol bouts were significantly larger than mean water bouts [$F(1,18)=5.94$, $p=0.025$]. This difference between water and alcohol bout sizes was explored further in the following section.

Comparison of Prandial and Nonprandial Alcohol and Water Bouts

Early work on the drinking behavior of rats convincingly demonstrated that rats drink water in excess of their daily metabolic needs, and that drinking is largely a learned, anticipatory response based on the animals' experience with the normal fluid requirements associated with eating a particular diet (Fitzsimons & LeMagnen, 1969; Gawley, Timberlake & Lucas, 1988; Kraly, 1984). It has been reported that approximately 70% of the total daily water is taken in association with meals, either shortly prior to or following a meal (Fitzsimons & LeMagnen, 1969; Kissileff, 1969; Kraly, 1984). Kissileff (1969) observed precise temporal relationships between feeding and drinking behavior and quantified the size of water bouts typically associated with meals. These bouts ranged in size from 0.5 to 2.5 mls, in animals consuming 45 mg food pellets similar to those used in the present studies.

Given this well described association between feeding and water drinking bouts it was of some interest to examine the prandial relationship between water, alcohol and food bouts. It was possible that in alcohol drinking rats,

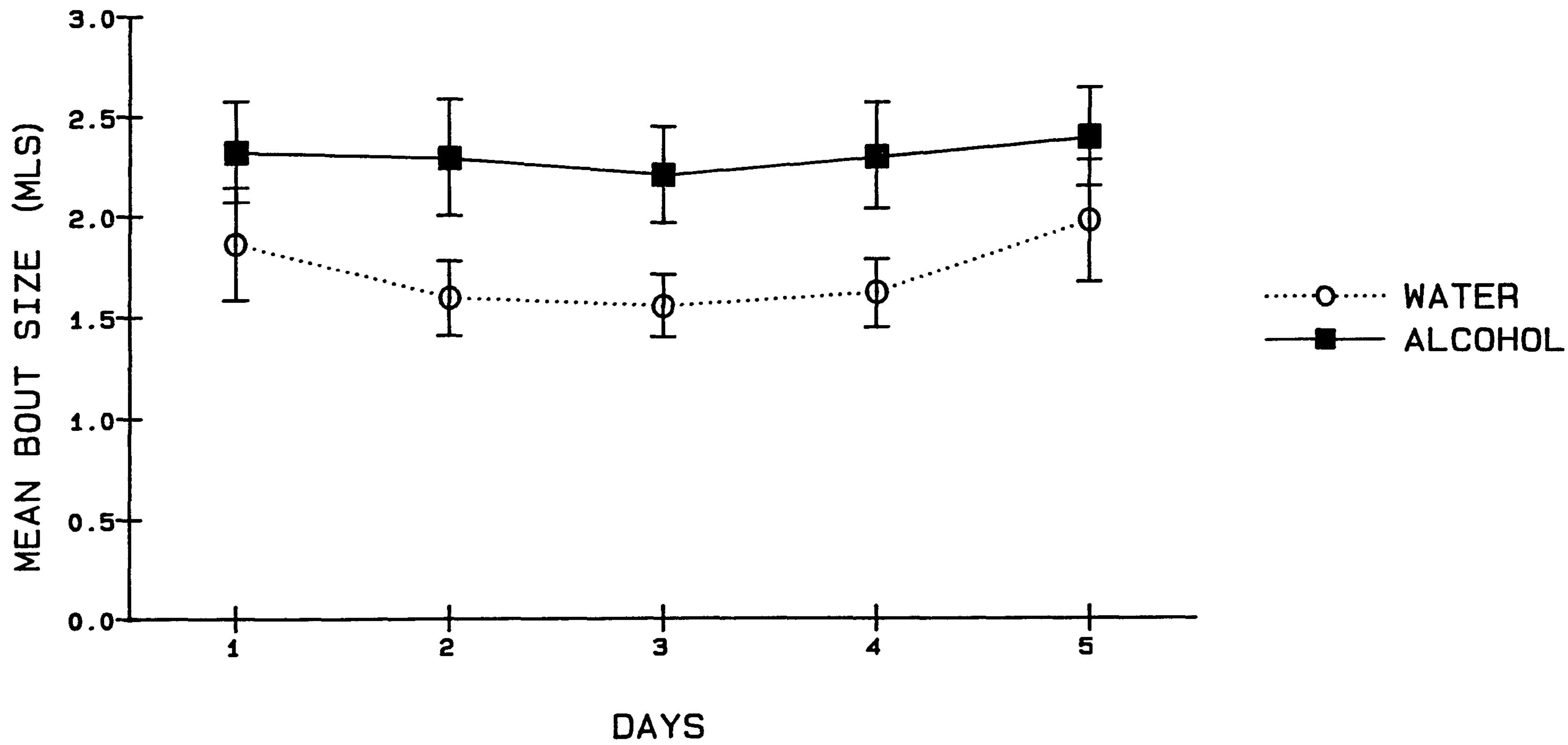


Figure 33. Mean size of water and alcohol bouts over baseline period.

wat - would be primarily associated with meals, and alcohol would be predominantly non-prandial.

In order to carry out this analysis, the daily 24 hour records of water and alcohol bouts (shown in Experiment 2, Table 3) were examined and scored in terms of their temporal relationship to food. The criteria for scoring the bouts were as follows: a fluid bout was considered to be "prandial" if it occurred 10 minutes prior (preprandial) or up to 30 minutes following a food bout (postprandial) (Fitzsimons & LeMagnen, 1969). All remaining bouts were scored as nonprandial. For each of the 19 rats, 2 of the 5 baseline days were randomly selected for scoring. This resulted in a sample of 38 days of data with total sample of 586 fluid bouts. Once the bouts were tabulated, a number of questions were asked about the relationship between food and fluid:

1) Were water bouts associated with food (pre and postprandial) more often than alcohol bouts? The distribution of pre, post and nonprandial bouts for both fluids is displayed in Table 5. Overall, 57% (333 out of 586 bouts) of all the fluid bouts were associated with food, regardless of fluid type. Alcohol and water bouts were distributed in exactly the same pattern. There was no significant difference between the two fluids in terms of the distribution of non, pre and postprandial bouts [$X^2(2)=3.65$, $p=0.16$].

2) Was there any difference in the size of food bouts associated with water vs alcohol? A 2-way ANOVA comparing the size of food bouts associated with the two fluids (water vs alcohol) and different bout types (pre, post) yielded no significant main effect for fluid type [$F(1,329)=1.23$, $p=0.11$] or bout type [$F(1,329)=1.83$, $p=0.17$]. These data suggested therefore that alcohol and water are equally likely to be associated with food, and that the food bouts were the same size regardless of whether they were accompanied by water or alcohol. See Table 6.

TABLE 5

DISTRIBUTION OF NONPRANDIAL, PREPRANDIAL AND POSTPRANDIAL BOUTS FOR WATER VS ALCOHOL

	NONPRANDIAL	PREPRANDIAL	POSTPRANDIAL	TOTAL
WATER	127	29	112	268
ALCOHOL	126	37	155	318
TOTAL	253	66	267	586

TABLE 6

A COMPARISON OF WATER AND ALCOHOL PRANDIAL BOUTS

	WATER			ALCOHOL		
	NON	PRE	POST	NON	PRE	POST
FOOD BOUTS (#PELLETS)		53.7 (±4.2)	47.3 (±2.2)		56.8 (±6.4)	52.8 (±2.3)
FLUID BOUT SIZE (MLS)	1.23 (±0.10)	1.02 (±0.15)	1.59 (±0.12)	1.92 (±0.15)	1.98 (±0.39)	2.40** (±0.12)
% TOTAL DAILY FLUID	0.44 (±0.05)	0.11 (±0.03)	0.42 (±0.05)	0.35 (±0.03)	0.10 (±0.03)	0.55 (±0.03)

Values are expressed as group mean (± S.E.M).

** Significantly different than all water bouts, $p < 0.05$.

3) Were the sizes of the three different types of fluid bouts different for water vs alcohol? A 2-way ANOVA comparing the size of bouts for the two fluids (water vs alcohol) and different bout types (non, pre, post) yielded significant main effects for fluid [$F(1,580)=29.3$, $p<0.0001$], and bout type [$F(2,580)=6.31$, $p<0.002$]. In post-hoc pairwise comparisons, alcohol post prandial bouts were found to be significantly larger than all types of water bouts ($p<0.001$, Student's t-tests with Bonferroni correction). The three types of water bouts did not differ significantly from one another in size. The distribution of water vs alcohol bouts sizes (all bout types combined) is presented in Figure 34 . Alcohol and water bouts ranged from < 0.5 mls to > 5.5 mls. There was a significant difference in the distribution of bout sizes, with more alcohol bouts at the high end of the distribution ($X^2(11) = 46.06$, $p<0.0001$). Only 24% of the water bouts were > 2.5 mls, compared to 48.4% of the alcohol bouts. The proportion of the total daily water or alcohol associated with each bout type was calculated and displayed in Table 6. An ANOVA with repeated measures yielded no significant main effect for fluid type [$F(1,37)=1.0$, $p=0.32$], and an expected difference for bout type [$F(2,74)=29.10$, $p< 0.0001$].

DISCUSSION

In general it appeared that rats distribute their alcohol intake into several discrete, short bouts which occur mainly during the dark cycle. There were considerable individual differences in total alcohol consumption (GM/KG), alcohol preference ratio, alcohol bout frequency, duration and size, as well as the prandial distribution of bouts. All of these variables together determine the "pattern" of alcohol intake in individual animals.

It was apparent from Figures 24 to 29 that the traditional measures of alcohol intake, GM/KG and the preference ratio, did not necessarily provide the same information about drinking. As shown in Figure 28, animals could exhibit high

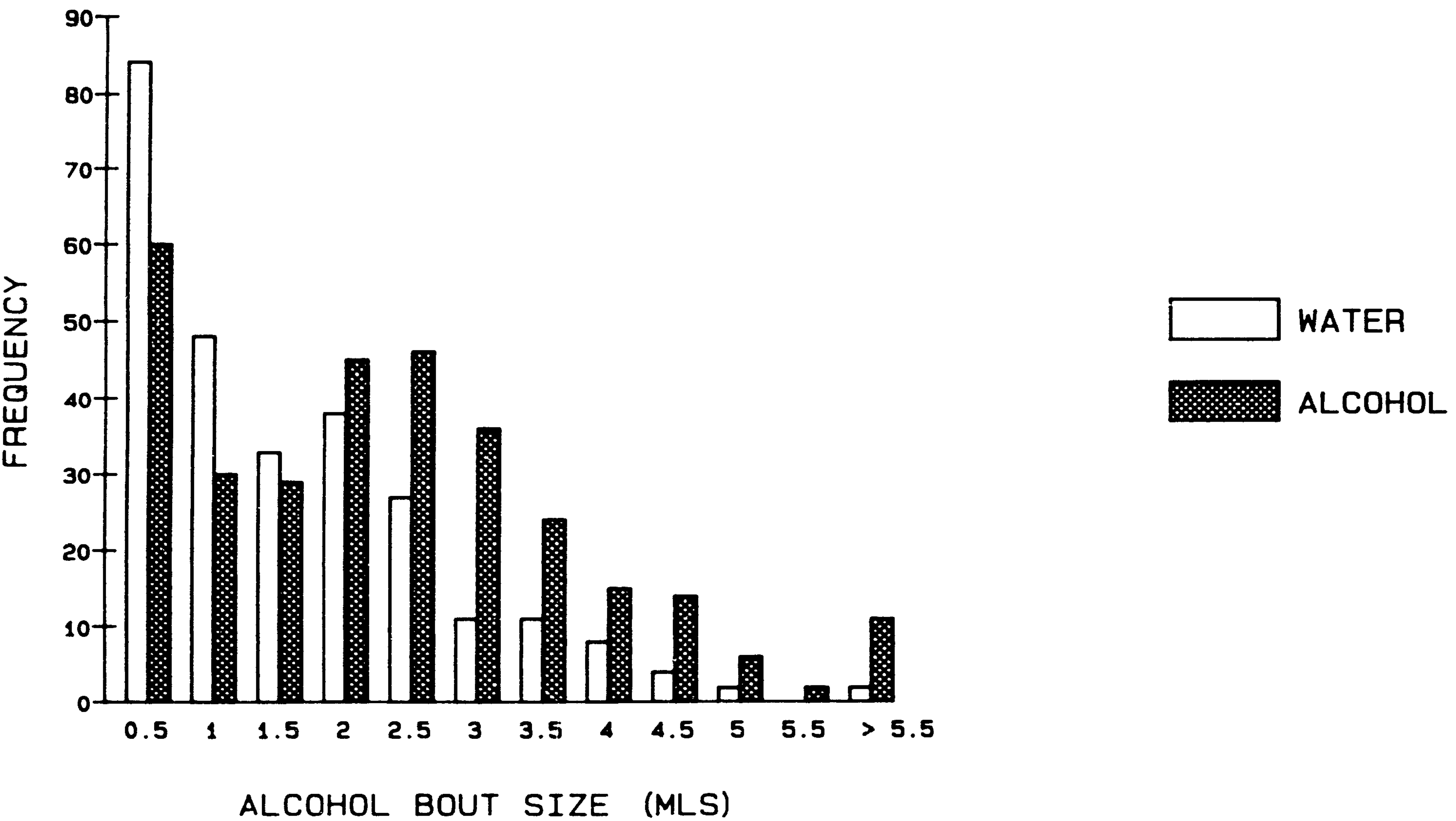


Figure 34. Frequency distribution of Non-, Pre- and Post-Prandial bouts for water vs. alcohol.

GM/KG along with a low preference ratio. In addition, animals consuming similar amounts of alcohol in terms of GM/KG and the alcohol preference ratio displayed remarkable differences in the size and temporal distribution of the alcohol bouts (Figures 24 and 25). Thus, a great deal more information about alcohol drinking could be obtained through the use of the computerized drinkometer system, compared to the traditional measure (GM/KG) obtained from a once daily recording of total alcohol intake. It would appear that High drinkers can be more accurately identified by alcohol bout frequency, duration and size, rather than by the total GM/KG consumed.

In terms of the prandial associations of alcohol drinking bouts, the results obtained were contrary to those expected at the outset of this research. On average alcohol bouts were distributed in much the same way as water bouts in terms of their relationship to food. In this group of animals, 55% of the total daily alcohol intake was consumed following a food bout. It is interesting to note that the postprandial alcohol bouts were significantly larger than all types of water bouts. In a parallel study, Henningfield and Meisch (1981) found that contrary to expectations presession feeding produced an increase in subsequent alcohol intake--with no effect on water intake--in Rhesus monkeys. They suggested that monkeys regulated alcohol intake via pharmacological feedback from the ingested alcohol, and were thus attempting to compensate for the slower rate of alcohol absorption produced by food in the gastrointestinal tract. Thus it would appear possible to speculate that a similar mechanism was at work here, producing a compensatory increase in post-prandial alcohol bouts in rats.

Similar to the observations reported here, Dole (1986) noted that food and alcohol drinking "matched like replicate prints from the same finger." Based on observations from a series of studies in C57BL mice, Dole and Gentry (1984)

concluded that the C57BL drinks alcohol for its taste and caloric properties, not for its pharmacological effects. Dole, Ho and Gentry (1985) suggested that an adequate animal model should demonstrate that alcohol is consumed at abnormal times of the day, temporally dissociated from food intake. In practice, it is very unlikely that such a pattern could be induced in animals.

First, like the other criteria established for adequate models of alcoholism, this additional criterion is based on a very limited notion of human alcohol intake. There is little reason to assume that alcohol intake in humans is dissociated from normal feeding and activity cycles. In fact, surveys of the general U.S population have shown that there was a strong temporal pattern to alcohol intake, with daily peaks of drinking in the early evening (6 to 9 p.m.), troughs in the early morning (3 to 6 a m.) and increased drinking on weekends (Arfken, 1988) Second, feeding and drinking behaviors appear to be highly entrained and occur in close temporal association in most animal species including humans. In laboratory studies of human subjects, approximately 68% of all drinking occurred in association with meals (Engell, 1988). This has been corroborated by work by DeCastro (1988) who found that in humans 80% of total daily fluid intake was taken in association with meals Food associated drinking has been linked to a number of factors, including the requirement for fluid to facilitate chewing and swallowing (Kissileff, 1969)

The existence of a circadian rhythm to most behaviors and physiological functions is well recognized Feeding and macronutrient selection, drinking and locomotor activity (i.e wheel running) are closely linking to circadian cycles (e g. Tempel, Shor-Posner, Dwyer & Leibowitz, 1989) Similarly, many physiological measures (e g body temperature, hormone release, neurotransmitter synthesis) exhibit diurnal cycles (e g Brick, Pohorecky, Faulkner & Adams, 1984, Kittrell & Satinoff, 1988) Most importantly, given that

many physiological parameters affecting alcohol-induced behaviors (e.g. blood alcohol elimination rate, activities of the enzymes alcohol and aldehyde dehydrogenase in liver and brain) have been shown to follow circadian variations (Brick et al., 1984; Sturtevant & Garber, 1981; Yamazaki, Nishiguchi, Miyamoto & Nakanishi, 1986), one might expect that alcohol drinking would show the same diurnal pattern.

In conclusion, alcohol drinking in rats follows normal circadian activity cycles, with approximately 55% consumed following food bouts. Whether an individual rat drinks sufficient quantities of alcohol to become repeatedly intoxicated during the night cycle most likely depends on the pattern of intake, as defined by the size and frequency of individual drinking bouts and their temporal relationship with food. The possibility that bouts of alcohol drinking have behavioral effects on these animals was examined in the following study.

EXPERIMENT 4

AN EXAMININATION OF BLOOD/BRAIN ALCOHOL LEVELS AND BEHAVIOR FOLLOWING VOLUNTARY ORAL ALCOHOL INTAKE

As discussed in the introduction, Cicero (1980) stated that evidence for intoxication following drinking could be provided by measuring blood alcohol levels and evaluating whether these levels were sufficient to produce a pharmacological effect. In the previous experiments alcohol was ingested in bouts averaging from 0.14 gm/kg/bout to over 0.73 gm/kg/bout (see Table 4). In addition, some animals took much larger bouts as shown in Figures 27 and 29. It has not been established whether these levels of oral alcohol intake have any effect on behavior.

The behavioral changes associated with the state of intoxication in humans have been measured in various tests of affect as well as cognitive and social functioning (Avery, Overall, Calil & Hollister, 1982, Moss,Yao & Maddock, 1989) However for rodents there are no analogous measures of intoxication Similarly, a great many of the sensitive techniques that have been developed to measure the psychophysiological and psychomotor effects of alcohol in humans (McCaul, Turrkan & Stitzer, 1989) have no parallel measures in rodents The most typical measures of intoxication used for rodents are loss of righting reflex or performance on a tilting plane, moving belt or rotorod These types of performance tests are, in general, only sensitive to relatively large doses of alcohol. Moving belt, tilt plane and rotorod performance appeared to be insensitive to alcohol doses below 1 gm/kg in rats and mice (Alkana & Malcolm, 1980) The importance of measuring low dose effects in rats is clear from the preceding studies

P and NP rats have been compared on a number of alcohol induced measures (i.e sleep time, hypothermia, conditioned taste aversion), at doses

much higher than those that would typically be encountered during alcohol drinking. However, in an examination of the locomotor effects of 0.12 - 1.5 gm/kg doses of alcohol, P rats were shown to be activated at doses of 0.12 and 0 25, with little effect at the higher doses. (Waller, Murphy, McBride, Lumeng & Li, 1986). In general, however, rats appear to be insensitive to the locomotor activating effects of alcohol (Frye & Breese, 1981; Masur, DeSouza & Zwicker, 1986).

A search of the literature yielded two paradigms that have successfully measured low dose activating effects of alcohol. File and Wardhill (1975a,1975b) reported that a hole board apparatus was a reliable measure of locomotor activity and demonstrated that low doses of alcohol (0.4 or 0.8 gm/kg) increased the frequency and duration of the head dipping response. In addition, in a one-trail step-through passive avoidance task the administration of low doses of alcohol prior to training has been shown to decrease the step-through latency on training day and to decrease the retention of the task (Bammer & Chesher, 1982).

Therefore, in the following study, locomotor activity in a hole board apparatus, as well as performance on the passive avoidance task were used to determine whether the voluntary oral consumption of alcohol produced behavioral signs of intoxication. Animals were placed on a schedule where alcohol was limited to a short time period daily, in order to simulate a single alcohol drinking bout. Bouts of drinking were simulated in order to gain experimental control over the timing of bouts; allowing the determination of blood and brain alcohol levels as well as behavior at specific time intervals post drinking.

MATERIALS AND METHODS

Subjects: Male Long Evans rats (Charles River, Canada) initially weighing 150-175g were housed individually in suspended stainless steel cages. The animal colony was controlled for temperature and humidity and was maintained on a 12 hour dark/light cycle. Agway rat chow (Charles River, Canada) was available ad libitum in the home cages. Fluids were presented in glass Richter tubes mounted on the front of the cages.

Alcohol Screening Procedure: Following a 5-day acclimation period, an alcohol screening procedure was initiated for all rats used in the subsequent studies Rats received alcohol solutions presented in an ascending series of concentrations on alternate days in a free choice with water. Initially, rats were given a 2% (v/v, 95% ethanol mixed with tap water) alcohol solution. On each subsequent alcohol presentation the concentration was increased by 1%. Once a final concentration of 8% alcohol was reached, the schedule was switched to everyday alcohol presentation and baseline consumption was monitored for at least one week prior to experimentation On alcohol presentation days during the screening and baseline periods, animals had free access to alcohol and water tubes, 24 hours/day

Limited Access Procedure: Following stabilization of baseline alcohol consumption in the free access paradigm (i e 24 hour alcohol availability), a group of animals (n = 59) were placed on a limited access schedule where alcohol was presented for a short time period each day Initially, animals received access to a tube containing 8% alcohol for a two hour period The access time was reduced on each subsequent week until animals received access to alcohol for a 10 minute period daily Two additional control groups were run on an identical limited access schedule These animals were obtained from the same batch and at the same weight as those screening for

alcohol consumption. During the alcohol screening procedure these animals were maintained on water bottles. When the limited access schedule was initiated these animals received access to water (n = 29) or saccharin (0.05%, w/v, n = 10) for the same length of time and under the same schedule as the alcohol-consuming animals. Alcohol, water and saccharin were presented in rubber stoppered plastic tubes fitted with double, steel ball-bearing sipper spouts. Consumption was measured daily. Food was available ad libitum. Water tubes were continuously available except during the presentation of their respective solutions during the limited access period.

Animals were maintained on this schedule for a minimum of five weeks prior to experimentation. One group of alcohol-consuming animals was sacrificed immediately following the 10-min drinking period for the determination of brain and blood alcohol levels as described below. The remaining animals were tested post-drinking in either a passive avoidance task or for locomotor activity in the hole board apparatus as described below.

Blood and Brain Alcohol Determinations: Immediately following the termination of the 10-min period of access to alcohol, animals (n = 12) were removed from their home cages, and carried to a separate laboratory where they were sacrificed by decapitation. Trunk blood was collected into heparinized tubes. Aliquots of blood were mixed with 4 volumes of buffered isotonic semicarbazide reagent and were processed according to the methods of Stowell (1979). Brains were quickly removed, washed in ice-cold saline, blotted dry, and homogenized in 5 volumes of perchloric acid-thiourea mixture as described by Tabakoff, Anderson and Ritzmann (1976). Following centrifugation at 10,000 x g, at 4 ^{o}C for 10 minutes, 0.5 ml of the clear supernatant was placed in a vial, stoppered and stored at -85 ^{o}C until analysis. Blood and brain alcohol levels were determined in duplicate by comparison to

external standards by head space gas chromatography. The Varian 1400 gas chromatograph was fitted with a glass column (6', 2mm i.d.), packed with Chromosorb 101 (mesh size 80/100) and a flame ionization detector. The column was maintained at 150 ^{o}C, injector 165 ^{o}C and the detector at 195 ^{o}C. Values were corrected for blank activity using blood and brain tissue obtained from control animals.

Passive Avoidance: The passive avoidance box was a modified two-compartment shuttlebox (Lafeyette Instrument Co.). Both compartments were of equal size and were constructed of stainless steel with a grid floor. The brightly illuminated starting compartment was separated from the darkened goal box by a sliding metal door. On training day, animals were placed in the start box facing away from the hole leading into the dark chamber. The step-through latency was measured using a stop watch Following entry into the dark chamber, the door between the compartments was automatically closed and a 5-sec footshock (1 mA) was delivered using a Grason-Stadler constant current shock generator with scrambler. Animals were tested 24 hours later using the same procedure except that no shock was delivered If an animal did not step through into the dark compartment within 180 seconds, the trial was terminated and the latency to step through was recorded as 180 sec

Initially, one group of animals consuming alcohol (E1, n = 11) and one group consuming water (W1, n = 9), under the limited access schedule, were trained and tested using this procedure Both groups were trained immediately following the 10 minute fluid access period On test day, 24 hours later, both the alcohol and water groups were tested prior to receiving their daily access to alcohol or water

An additional two groups were subsequently tested on the passive avoidance task In this experiment, one group of alcohol-consuming animals

(E2, n = 11) and one water-consuming group (W2, n = 10) were trained and tested immediately following the limited fluid access period. These two groups were run in order to determine whether alcohol consumption prior to testing on day 2 would reduce the step-through latency (note that E1 was tested drug-free).

Locomotor Activity: The hole board was constructed according to the specifications outlined by File and Wardhill (1975a). It was a wooden box with a floor 66 x 56 cm and walls 47 cm high. Four equally spaced holes were placed in the floor. Objects (an aluminum box, tissue paper, soap and a glass jar containing paper chips) were placed 3 cm beneath the holes. The testing procedure consisted of placing the animal in the center of the board facing away from the observers. Each animal was observed for a 10 minute period by two observers. A general activity measure for each animal was determined by counting the total number of head dips, rearings and crossings. A head dip was scored when the animal's eyes disappeared beneath one of the holes. Crossings were determined by dividing the box into four quadrants; a crossing was scored when all four paws were placed in the next quadrant. The duration of head dips was recorded using a stop watch. The hole board was washed and dried between trials.

Initially, two groups of animals were tested for hole board activity. The first group (E1, n = 12) received alcohol under the limited access schedule. Immediately after the 10-min drinking period, animals were placed in the hole board apparatus and activity was monitored for 10 minutes. The control group received 10 minutes access to water (W, n=10) under the identical procedure as described for the limited access alcohol group.

A second experiment was carried out in which two additional groups, one consuming alcohol (E2, n = 13) and the other consuming saccharin (S, n = 10),

were tested for hole board activity immediately following a 10 minute limited access drinking period. These additional groups were tested in order to rule out the possibility that the restricted access schedule per se was responsible for locomotor activation apparent in the E1 group.

RESULTS

Animals maintained on a limited alcohol access schedule consumed during the 10 minute period quantities of alcohol which were comparable to that of a single bout in animals given free access to alcohol. Typically, these rats drank immediately upon tube placement for a 2-4 minute period. As shown in Figure 35, the group of animals sacrificed immediately post-drinking exhibited a range of consumption from 0.69 - 1.6 gm/kg. Detectable levels of brain and blood alcohol were observed in these animals. Significant correlations between the quantities consumed (gm/kg) and blood ($r = 0.71$, $p< 0.01$) or brain ($r = 0.82$, $p< 0.001$) alcohol levels were found.

Passive avoidance training and test day step-through latencies for all groups tested are presented in Table 7. In the initial experiment, the alcohol-consuming group (E1), trained immediately following access to alcohol showed a reduced latency to enter the dark chamber compared to the water-consuming controls ($t(18)= -2.11$, $p<0.05$) The E1 group consumed 0 72 ± 0.09 gm/kg of alcohol on training day Due to the use of a cutoff latency of 180 sec, test day data were analyzed using a nonparametric Mann-Whitney U test This test revealed that there was no significant difference between the E1 or W1 groups on test day, indicating that both groups retained memory for the task to a similar degree

In the second experiment the E2 and W2 groups were trained **and** tested immediately following the 10 min fluid access period As shown in Table 7, there were no significant differences between these groups on training or test

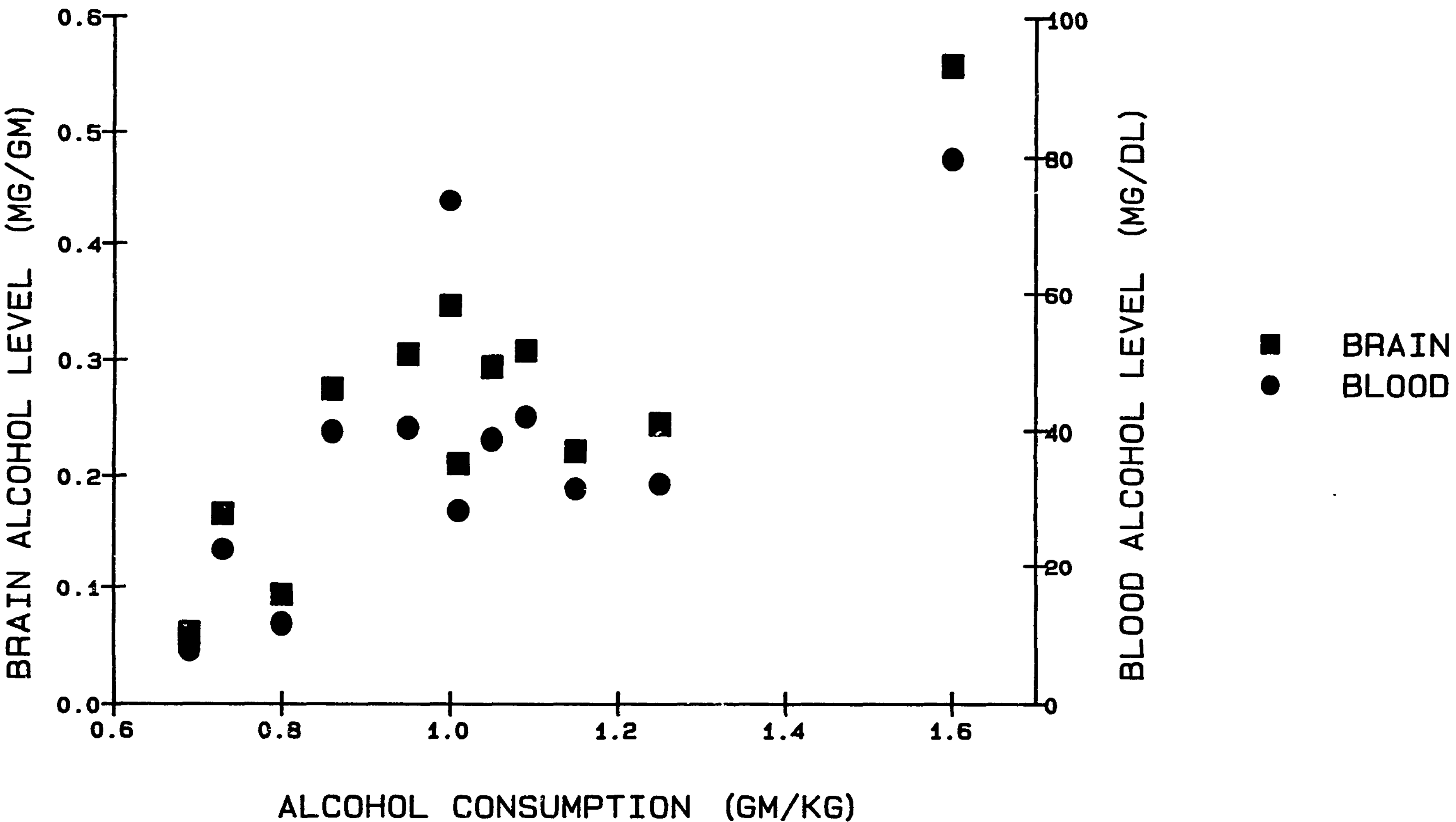


Figure 35. Comparison of alcohol consumption to brain ($r = 0.82$) and blood ($r = 0.71$) alcohol levels.

TABLE 7
PASSIVE AVOIDANCE

GROUP	TRAINING DAY LATENCY (Seconds)	TEST DAY LATENCY (Seconds)
E1	16.33 ± 2.62 **	130.80 ± 21.62
W1	37.58 ± 10.70	170.00 ± 10
E2	27.46 ± 9.12	180.00 ± 0
W2	25.60 ± 7.41	174.50 ± 5.5

Passive avoidance training and test day step-through latencies for groups of animals drinking alcohol (E1, E2) or water (W1,W2) prior to training.

** Significantly different from W1 group by two-tailed Student's t-test, ($p < 0.05$).

days. The failure to observe a reduced step-through latency in the E2 group, similar to that reported above for the E1 group cannot be readily explained, but may be due to the fact that the E2 group consumed a slightly smaller quantity of alcohol (0.63 ± 0.07 gm/kg) compared to that of the E1 group (0.72 ± 0.09 gm/kg). This type of variation between experiments is particularly difficult to control, due to the fact that the alcohol dose was self-administered. It would appear that the effects of alcohol in the passive avoidance task are not particularly robust and that they may be manifest only above a certain dose of alcohol.

General activity levels as well as duration of head dipping for all groups is presented in Table 8. Comparisons of the E1 and W groups (Student's t tests) indicated that the E1 group scored significantly higher on the measure of general activity ($t(20)= 3.17$, $p <0.01$) and spent a greater amount of time head dipping ($t(20)= 2.24$, $p <0.05$). The E1 group consumed 0.70 ± 0.07 gm/kg of alcohol on test day.

Comparison of the E2 and S groups confirmed that the alcohol consuming group scored higher on the measure of general activity ($t(21)= 2.24$, $p <0.05$). However, there was no significant difference between the groups in terms of duration of head dipping. The E2 group drank a mean of 0.61 ± 0.05 gm/kg of alcohol on test day.

TABLE 8

HOLE BOARD ACTIVITY

GROUP	DURATION OF HEAD DIPPING (Seconds)	GENERAL ACTIVITY (Counts)
E1	39.42 ± 11.39 *	112.1 ± 8.29 *
W	10.73 ± 2.82	69.3 ± 10.93
E2	35.10 ± 8.6	115.6 ± 10.14**
S	25.30 ± 5.9	86.4 ± 6.82

Hole board activity for groups of animals drinking alcohol (E1, E2), water (W1) or saccharin (S) immediately prior to testing.

* Significantly different from W group by two-tailed Student's t-test, $p < 0.05$.

** Significantly different from S group, $p < 0.05$

GENERAL DISCUSSION--PHASE 1

Various critics (Cicero, 1980; Lester & Freed, 1973; Dole & Gentry, 1984) have maintained that voluntary access or preference paradigms do not adequately satisfy criteria for an animal model of alcoholism. A primary criticism has been the lack of demonstrable blood alcohol levels and intoxication following drinking. The results presented here, however, suggested that animals are motivated to ingest "pharmacologically meaningful" or "intoxicating" levels of alcohol.

Simulation of a single drinking bout using a limited access schedule resulted in readily detectable brain and blood alcohol. The bouts consumed were within the range of those taken by animals in Experiments 2 and 3, given free access to alcohol. Therefore, it is likely that animals in a free drinking paradigm would experience repeated elevations in blood alcohol throughout the dark cycle. As discussed in Experiment 3, a large proportion of the alcohol drinking bouts are associated with food in free drinking animals. However, postprandial alcohol bouts were significantly larger than postprandial water bouts, perhaps due to compensation for slower absorption of alcohol.

The passive avoidance and hole board paradigms demonstrated that the oral ingestion of alcohol resulted in significant behavioral changes in these organisms. Animals displayed enhanced exploratory and/or locomotor activity as measured in a paradigm previously shown to be sensitive to the effects of low doses of alcohol. Both the E1 and E2 groups scored higher than their respective controls, suggesting that the enhanced locomotor activity exhibited in the E groups was a function of the pharmacological effects of the ingested alcohol per se, rather than a nonspecific effect of the restricted access schedule itself. While the S group readily and rapidly consumed saccharin on the limited access schedule, these animals did not display the same level of general

activity as the E2 group. There are few reports in the literature on the behavioral effects of self-administered alcohol. Typically, behavioral studies are conducted following experimenter administered alcohol and locomotor activation has rarely been observed in rats under these conditions. The results of this study have been published (Gill, France & Amit, 1986).

Over the past few years, various types of limited access paradigms have been developed. Stewart and Grupp (1984) used a procedure where alcohol drinking was induced by pairing alcohol with food during daily 1 hour sessions. The animals were reduced to 80% of free-feeding body weight and moved to separate drinking cages during training. Alcohol consumption levels and blood alcohol levels were within the range of values reported in the present study. Grant and Samson (1985) trained rats to lick a drinking tube containing 5% alcohol in order to gain access to a dipper cup containing 20% sucrose. Following extensive training, including some periods of water deprivation, animals were shaped to lever press to receive alcohol in the dipper cups, in place of the sucrose. Repeated daily training over a 2 month period led to intakes averaging 0.38 gm/kg in 30 minute sessions when 10% alcohol was available. Marcucella and Munro (1986) offered animals 20 minute access periods to alcohol every 3 hours over a 50 day period. In free-feeding animals the sizes of alcohol bouts reached a stable asymptote and ranged from 0.25 to 0.75 gm/kg/bout. Finally, Linseman (1987, 1989b) observed intakes of 12% alcohol ranging from 0.5 to 1.0 gm/kg during 1 hour test sessions using a limited access paradigm in which animals were neither food or water deprived. The training procedure for this paradigm was lengthy and labour intensive; animals were transferred to separate drinking cages for one hour on a daily basis. In one experiment Linseman (1989b) reported a mean intake of 0.87 gm/kg with blood alcohol levels measured 30 minutes post drinking (from tail blood

samples) were similar to those reported in the present study (averaging 44 mg%)

These procedures resulted in typical consumption levels of 0.38 - 1.0 gm/kg in short time periods, resulting in the same range of blood alcohol levels reported here. It is interesting to note that individual bouts of drinking across a wide range of conditions were similar. The similarity in drinking levels and blood alcohol levels achieved lead to two important conclusions about drinking and drinking paradigms.

The first point point relates to the way in which the criteria for an animal model have dominated the alcohol literature, as discussed in the introduction to this thesis. The extensive procedures employed by these experimenters pointed to their preconceptions about alcohol consumption in rats--as the introduction to each of their papers revealed. Each researcher discussed the fact that animals do not voluntarily consume alcohol, that animals do not drink sufficient alcohol to become intoxicated or to induce physical dependence (e.g. Grant & Samson, 1985; Linseman, 1987; Marcucella, 1989; Stewart & Grupp, 1984). Thus rats had to be "induced" to drink alcohol through extensive training methods involving food and/or water deprivation, prandial-induced drinking etc. All of the above procedures required additional equipment and/or operant testing boxes as well as extensive training procedures that were labour intensive. It is clear from the results of the present series of experiments that alcohol intake did not have to be "induced". Stable voluntary drinking at "high" levels was displayed in a proportion of the animals screened in each of the preceding studies. Wide individual variability was the norm, but selected "high" drinking Long Evans rats displayed a similar drinking pattern to P rats genetically selected for high alcohol intake. In addition, the simple presentation of a tube of alcohol on a limited access schedule resulted in alcohol

consumption at the level typically observed in a single bout in animals with free access on a 24 hour basis.

The second point relates to the similarly of bouts of alcohol drinking over a wide range of conditions. Overall, these results suggest that animals may limit their intake by pharmacological feedback from the ingested alcohol. As shown in experiment 2, the mean size of alcohol bouts of P and Long Evans rats were similarly shown to be 0.5 gm/kg/bout, although individual bouts did range as high as 1.3 gm/kg/bout. Similarly, Murphy, Gatto, Waller, McBride and Li (1986) reported that in a selected group of P rats scheduling access to short 1 hour periods each day resulted in bout sizes of approximately 1.1 gm/kg with blood alcohol levels reaching 62 mg%. In one group of P rats, the alcohol concentration was systematically altered over days from 10 to 20%. Regardless of the concentration these animals consumed an average of 1.3 gm/kg/bout with blood alcohol levels ranging from 40 to 67 mg%. Excitatory locomotor effects in the P rats were observed only when BAC's ranged between 16 to 75 mg% (Waller, Murphy, McBride, Lumeng & Li, 1986). In Experiment 4, mean alcohol consumption ranged from 0.63 to 1.0 gm/kg/bout, with BAC's ranging from 8 to 80 mg%--a dose range that similarly produced locomotor activation. Overall, the similarly across many studies suggests that animals are regulating intake according to blood (or brain) alcohol levels. Blood alcohol levels following voluntary alcohol intake in rats, typically remains below 100 mg%, regardless of experimental paradigm.

In summary, the results of the present investigation provided evidence for the statement that voluntary oral intake of alcohol exerts a significant pharmacological influence on the behavior of rats. It is very likely that animals perceive the pharmacological effects of and are affected by the alcohol they consume. Animals display persistent and stable intake of alcohol over long

periods of time, both in the drinkometer paradigm, as well as during the limited access schedule. It is apparent from an examination of the patterns of alcohol consumption that alcohol is consumed in periodic, discrete, short bouts in similar patterns in both the Long Evans and the genetically selected P rats. It would appear that there is some mechanism that controls the initiation of alcohol drinking and that limits the size and duration of individual drinking bouts. This invites the question of what potential biological (and genetic) mechanisms regulate alcohol intake. Biological factors involved in the regulation of alcohol intake are discussed in Phase II.

INTRODUCTION--PHASE II

THE REGULATION OF ALCOHOL INTAKE: AN EXAMINATION OF BIOLOGICAL FACTORS PREDISPOSING TO HIGH ALCOHOL INTAKE

In 1947, when the first genetic selection study in animals started at the University of Chile (Mardones & Segovia-Riquelme, 1983), little was known about the genetics of alcoholism in humans. Since that time a considerable body of evidence has been gathered from family, twin, and adoption studies indicating that alcoholism may have an inherited component. This statement is supported by data showing an increased incidence of alcoholism in the families of alcoholics (Volicer, Volicer & D'Angelo, 1985) and a higher concordance for alcoholism in monozygotic (60%) compared to dizygotic (30%) twins (Schuckit, 1985). Adoption studies have provided some of the strongest evidence for the contribution of genetic factors to the development of alcoholism. Adopted sons of alcoholic biological parents showed a four fold higher rate of alcoholism than adoptees with non-alcoholic biological parents (Bohman, Sigvardsson, Cloninger & Von Knorring, 1987; Cloninger, Bohman, Sigvardsson, 1981). There appears to be sex differences in the heritability of alcoholism, with two types of genetic transmission identified thus far (1) male-limited: a severe, early onset form in males, related to alcoholism in biological fathers and (2) milieu-limited: a milder, late onset form in both sexes, associated with alcoholism in either parent (Bohman et al., 1987).

What is inherited in alcoholism? It has been generally agreed that alcoholism is a polygenic, multifactorial disorder, involving a complex gene-environment interaction, in which an individual inherits genes that confer a "predisposition" to develop alcoholism (Bohman et al., 1987). However, what

this predisposition consists of, from the biological or behavioral point of view, is unidentified at this time.

The products of genes are proteins: proteins such as enzymes or receptor subunits that interact in vast biochemical and behavioral networks to produce phenotypes. In concrete terms, researchers are looking for markers of genetic risk by examining endocrine and neurochemical systems that regulate ethanol metabolism, the sensitivity to intoxication, the development of tolerance and physical dependence, the severity of withdrawal as well as ethanol consumption (Begleiter & Porjesz, 1988; Cloninger, 1987; Schuckit, 1988). Many of these factors have been shown to be influenced by genetics. For example, in work with human twins, genetic factors were shown to contribute to variations in alcohol drinking in terms of the frequency and quantity of consumption (Kaprio et al., 1987).

THE BIOLOGICAL BASIS OF ALCOHOL DRINKING

From the earliest work with the alcohol preference paradigm, experimenters have been attempting to discover the biological mechanisms governing drinking (e.g. Forsander,1966; Rodgers et al., 1963). Much of this work was carried out in inbred mice as well as outbred strains of rats. In addition, as discussed in the introduction to Phase I of this thesis, the genetic selection of animal strains has been viewed as a powerful research strategy with the same aims. Several rat strains have been bred on the basis of alcohol consumption as shown in Table 1.

One of the first and most obvious biological factors considered to play a likely role in the regulation of alcohol intake was the rate of alcohol metabolism. Individual differences in the rate of alcohol elimination were shown to be almost entirely governed by genetic factors in human twins (Vesell, Page, Passananti, 1971) and highly heritable in rats (Thurman, 1980). Alcohol metabolism is a

complex topic, involving consideration of multiple enzymatic pathways in the liver as well as the brain (Crow & Hardman, 1989). The terms alcohol and ethanol are used interchangeably throughout this section.

ALCOHOL METABOLISM IN THE LIVER AND BRAIN

Following ethanol ingestion the drug is rapidly absorbed and distributed to all tissues of the body in proportion to aqueous content. Ethanol is rapidly oxidized to acetaldehyde and to acetate in a two-step process involving the enzymes alcohol dehydrogenase (ADH) (Alcohol:NAD oxidoreductase; E.C. 1.1.1.1) and aldehyde dehydrogenase (ALDH) (Aldehyde: NAD oxidoreductase; E.C. 1.2.1.3). The liver ADH pathway is considered to be the primary pathway of ethanol metabolism, oxidizing 90-95% of the total ethanol ingested in the presence of the cofactor nicotinamide adenine dinucleotide (NAD) (Crow & Hardman, 1989). Other enzymes have been shown to contribute to ethanol metabolism, including the microsomal ethanol oxidizing system (MEOS) and catalase. The MEOS system consists of a group of cytochrome P-450 proteins, that have been shown to oxidize alcohols at high substrate concentrations (Koop, 1989). Catalase (Hydrogen peroxide: hydrogen peroxide oxidoreductase, E.C. 1.11.1.6), can oxidize ethanol to acetaldehye with the concurrent conversion of hydrogen peroxide to water (Koop, 1989). The availability of hydrogen peroxide appears to be the limiting condition in the oxidation of ethanol by catalase (Koop, 1989). Under normal physiological conditions these two systems are not considered to have a major role in ethanol oxidation in the liver; however this topic is highly controversial and has not been resolved to date (Koop, 1989; Norsten et al., 1989).

All these enzymatic systems have been shown to be widely distributed in mammalian extra-hepatic tissues, including brain (Raskin, 1983; Von Wartburg & Buhler, 1984). Von Wartburg and Buhler (1984) suggested that although the

activity of ADH, for example, was low in various tissues, the local oxidation of ethanol could yield significant, biologically reactive concentrations of acetaldehyde. Evidence that acetaldehyde could be produced in brain by the peroxidative activity of catalase has been obtained by a number of investigators through a variety of methods (Aragon, Rogan & Amit, 1989; Cohen, Sinet & Heikkila, 1980, 1983; Tampier, Quintanilla, Letelier & Mardones, 1988). Brain homogenates incubated in the presence of ethanol and inhibitors of cytochrome P-450 and ADH were shown to produce acetaldehyde. Inhibitors of catalase (3-amino-1,2,4-triazole (AT), methanol), on the other hand, reduced the production of acetaldehyde (Aragon et al., 1989; Tampier et al., 1988). In addition, the brain has been shown to produce levels of peroxide sufficient to activate catalase (Sinet, Heikkila & Cohen, 1980).

In summary, while the role of minor oxidative enzymatic pathways in liver and brain are currently controversial issues, the weight of the evidence points to the likely production of acetaldehyde in brain tissue, through the activity of the enzyme catalase. A reduction in this oxidative capacity through the use of AT has been shown to modify alcohol-induced behaviors. This will be discussed in a later section of this introduction.

Acetaldehyde is oxidized by ALDH, an enzyme widely distributed throughout the body, and localized in the mitochondrial, cytosolic and microsomal fractions of the cell. Liver and brain contain several molecular forms of ALDH exhibiting wide substrate affinity (Km). Broadly, there are two classes of ALDH: Low Km activity with a Km for acetaldehyde in the micromolar range and a high Km activity with a Km in the millimolar range (Tipton, Henehan & Harrington, 1989). Acetaldehyde is rapidly oxidized to acetate due to the presence of the low Km form of ALDH with very high affinity for acetaldehye; therefore, below saturating

conditions little acetaldehyde escapes to peripheral circulation (Tipton et al., 1989).

Wei and Singh (1988) noted that the steady state level of acetaldehyde is regulated by a balance between the enzymatic systems controlling the rate of production and that controlling the rate of elimination. Thus it is important to note that brain and liver ADH and ALDH activities have been shown to be inducible upon alcohol exposure (Deitrich, Bludeau, Roper & Schmuck, 1978; Wei & Singh, 1988) and affected by nutritional status (Lakshman, Chambers, Chirtel & Ekarohita, 1988; Lumeng, Bosron & Li, 1977) and chronic forced ingestion (Koivula & Lindros, 1975). In addition, Wei and Singh (1988) demonstrated that following exposure to alcohol in four strains of mice, liver ALDH was significantly induced in some strains and repressed in others. Thus, in studies of the relationship between alcohol metabolism, alcohol metabolizing enzymes and drinking behavior, care must be taken to distinguish predisposing "inherent" factors that might influence alcohol drinking from the results of the alcohol exposure itself.

In addition, the actual rate of alcohol elimination from the blood is a function of a number of enzymatic systems in addition to ADH and ALDH (i.e. enzymes involved in the recycling of the cofactor NAD) and should therefore be considered as being distinct from the measurement of the activities of ADH and ALDH.

ALCOHOL METABOLIZING ENZYMES AND ALCOHOL CONSUMPTION

The rapid inducibility or repression of enzymes, as well as the effects of nutritional status, were not taken into consideration in early work on the role of enzymes in drinking. Rodgers et al. (1963) found a perfect rank order correlation between liver alcohol dehydrogenase activity and preference for a

10% alcohol solution using inbred strains of food-deprived mice. Kulkosky (1985) demonstrated that daily alcohol consumption correlated highly with total metabolic capacity in rats. However, in this work animals were given access to alcohol prior to the measurements of ADH, thus the correlations may have been due to the induction of ADH activity in high drinking animals.

Recently, it has been shown that, in mice, liver alcohol dehydrogenase was not a major determinant of alcohol preference (Teichert-Kuliszewska, Israel & Cinader, 1988). Comparing C57BL/6 mice with MRL/Mp mice it was shown that significant differences in ADH were not related to the strain differences in alcohol intake. The authors suggested that differences in alcohol dehydrogenase were responsible for only a minor fraction of the interstrain variability in alcohol intake.

The overall rate of alcohol elimination--a factor potentially determining the amount of alcohol consumed--has also failed to consistently correspond to line differences in alcohol intake. High drinking C57BL mice did not exhibit significantly different alcohol elimination rates compared to low drinking DBA or BALB mice (Rodgers, 1972). In early studies on the UChA/B rats no strain differences in rate of alcohol metabolism were found following 60 days of voluntary alcohol consumption (Segovia-Riquelme, Vitale, Regsted & Mardones, 1956). Sex differences in the rate of alcohol elimination were observed in the AA/ANA rats, however only minor (8%) differences were observed between females of the high and low drinking lines. There were no line differences between male AA vs male ANA rats (Eriksson, 1973). Alcohol elimination rates and liver activities of ADH and ALDH have been shown to be equivalent in the P/NP selected lines (Li & Lumeng, 1977).

In summary, there are few consistent data that support the notion that the differences in alcohol intake exhibited by inbred mice, outbred rats and

genetically selected rats are regulated through the rate of alcohol elimination or the levels of liver ADH. Liver and brain ALDH on the other hand, have consistently been shown to be related to alcohol intake in a number of strains.

BRAIN AND LIVER ALDEHYDE DEHYDROGENASE ACTIVITY, ACETALDEHYDE LEVELS AND ALCOHOL CONSUMPTION

Sheppard, Albersheim and McClearn (1968, 1970) found that liver ALDH activity in the alcohol drinking C57BL mouse was three times higher than in the low consuming DBA. Confirming the functional significance of the ALDH differences, injections of alcohol gave rise to higher circulating acetaldehyde levels in the DBA mouse (Schlesinger, Kakihana & Bennett, 1966; Sheppard et al., 1970). Similarly, the F17 and F43 generations of the ANA strain have been found to have higher blood acetaldehyde levels following an alcohol challenge, due to lower liver mitochondrial low Km aldehyde dehydrogenase activity (Hilakivi, Eriksson, Sarviharju & Sinclair, 1984; Koivula & Lindros, 1975; Koivula, Koivusalo & Lindros, 1975). Overall these results established that high ALDH activity was associated with high levels of alcohol consumption, while lower acetaldehyde clearance rates were associated with low spontaneous alcohol drinking. Several investigators suggested therefore that low ALDH activity was a significant factor producing alcohol-avoidance in some strains of animals (Rodgers, 1972, Sheppard et al., 1970).

This interpretation was supported by the work of Amir (1977, 1978a) who demonstrated that brain ALDH was related to drinking levels. In Wistar rats given voluntary access to alcohol (5% or 10%) over a 50 day period, brain ALDH activity was positively correlated to alcohol intake (r= 0.558) (Amir, 1977). There were no significant differences in liver or brain ALDH between water and alcohol exposed animals indicating that at the levels of intake encountered during voluntary consumption, no induction of ALDH occurred (Amir, 1977).

Amir (1978a) went on to demonstrate that alcohol consumption in various strains of rats (Wistar, Tryon Maze-dull and Tryon Maze-bright) correlated to brain ALDH activities The overall correlation between brain ALDH and alcohol intake over the three strains was reported at r= 0.59. Amir (1977) suggested that differences in the disposition of acetaldehyde in brain tissue could alter the biochemical consequences of acetaldehyde, thus altering the behavioral response to alcohol.

Inoue, Rusi and Lindros (1980), however, failed to find a relationship between brain ALDH and alcohol intake in the AA/ANA line of rats. In alcohol-naive rats there were no significant strain or sex differences in ALDH activities. In animals exposed to 10% alcohol for approximately 6 weeks, high alcohol consuming AA male rats were found to have lower ALDH compared to the low drinking ANA; in female rats this relationship was reversed. Thus, in the selected AA/ANA strains no consistent correlation with alcohol intake was observed. Tampier and Quintanilla (1983) demonstrated that in the high drinking UChB rats the rate of acetaldehyde oxidation in cortex-diencephalon homogenates was significantly higher than that of the UChA rats. Control groups included in the study demonstrated that acetaldehyde oxidizing capacity was not induced by alcohol exposure. Positive correlations between alcohol intake and brain ALDH activity were confirmed by Socaransky, Aragon, Amit and Blander (1984). In this study, correlations ranging from 0.7 to 0.84 were obtained between brain ALDH and alcohol consumption in Long Evans, Sprague-Dawley and Wistar rats.

Along with the evidence that a high rate of acetaldehyde clearance in brain and liver was associated with high drinking are the numerous reports that ALDH inhibitors such as cyanamide, disulfiram, coprine, reduced alcohol consumption and alcohol preference in mice and rats (Lamboeuf & De Saint Blanquat, 1980;

Schlesinger et al., 1966; Sinclair, Lindros & Terho, 1980; Spivak, 1987a). High blood acetaldehyde levels have been shown to be aversive in humans, as demonstrated by the use of ALDH inhibitors in the treatment of alcoholism (Peachey, Annis, Bornstein, Sykora, Maglana & Shamai, 1989). Alcohol ingestion following administration of these compounds results in the development of a syndrome characterized by facial flushing, headache, tachycardia, sweating and nausea, due to the accumulation of acetaldehyde following inhibition of liver ALDH (Peachey et al., 1989). A naturally occurring form of the acetaldehyde-induced syndrome can be observed in individuals of oriental descent following alcohol ingestion. This innate alcohol intolerance, termed the "oriental flushing response" has been shown to be due to the deficiency of one low Km isozyme of liver mitochondrial ALDH (Mizoi, Fukunaga & Adachi, 1989). The enzyme abnormality appears in approximately 40% of the Japanese population. It has been proposed that the high levels of acetaldehyde produced are aversive, thus discouraging alcohol intake and protecting against the development of alcoholism in this population (Harada, Agarwal, Goedde & Ishikawa, 1983).

It is interesting to note, however, that ALDH deficient subjects exhibiting the typical flushing response reported more euphoria shortly following a low dose of alcohol (0.4 g/kg), compared to normal subjects (Mizoi et al., 1989).

This latter point is of particular importance to the topic of the research in this phase of the thesis. A key to this puzzle has been suggested to involve a biphasic behavioral action of acetaldehyde where low brain levels of acetaldehyde are reinforcing and actively sought by organisms, while high peripheral levels are aversive (Amit & Smith, 1989). Furthermore, the local production of acetaldehyde in the brain via oxidation of alcohol by catalase has

been posited as being of central importance in the psychopharmacological actions of alcohol (Amit, Smith & Aragon, 1986).

THE REINFORCING PROPERTIES OF ACETALDEHYDE

Reports of enhanced euphoria following ALDH inhibition with drugs such as disulfiram and calcium carbimide (CC) have appeared extensively in the clinical literature. As reported by Chevens (1953) in a paper entitled "Antabuse Addiction" some patients receiving antabuse (disulfiram) enjoyed the combination of ALDH inhibition and alcohol, claiming that it enhanced the euphoric properties of alcohol. Similarly, in a study of human volunteers in a laboratory setting, the prior administration of disulfiram or CC potentiated the mood altering effects of low doses of alcohol (Brown, Amit, Smith, Sutherland & Selvaggi, 1983).

In reviewing much of the recent literature in this area of research, Amit and Smith (1989) have proposed that acetaldehyde is involved in mediating the "euphorogenic" or positively reinforcing properties of alcohol. Support for this notion has been obtained from extensive behavioral studies in rats, demonstrating that animals will self-administer acetaldehyde through the intracerebroventricular route (Brown, Amit & Rockman, 1979; Brown, Amit & Smith, 1980) as well as intravenously (Myers, Ng & Singer, 1982). Thus, a substance that has been considered aversive has also been shown to be self administered. Smith, Amit and Splawinsky (1984) also demonstrated that acetaldehyde was capable of inducing a conditioned place preference.

THE LOCAL PRODUCTION OF ACETALDEHYDE IN BRAIN TISSUE

Catalase in liver and brain can be irreversibly inhibited, by the compound, 3-amino-1,2,4-triazole (AT) (Koop, 1989). Ethanol oxidation in rat brain "in vivo" was confirmed by the demonstration that the prior administration of alcohol, competitively protected catalase from deactivation by AT (Cohen, Sinet &

Heikkila, 1980; 1983). The ability of alcohol to protect catalase against AT provided indirect evidence that alcohol was oxidized in brain tissue.

In a series of studies, Mardones and colleagues demonstrated that prior treatment with AT (1.0 gm/kg) produced a 63% reduction in cerebral catalase activity and significantly decreased the duration of the loss of righting reflex following a narcotic dose of alcohol. AT had no effect on the hypnotic actions of paraldehyde or pentobarbital (Tampier & Mardones, 1983; Tampier, Quintanilla & Mardones, 1979; Tampier et al., 1988; Quintanilla, Tampier & Mardones, 1980). These researchers suggested that these data supported the conclusion that the hypnotic actions of alcohol were mediated through the central production of acetaldehyde by catalase. Further behavioral experiments supported this conclusion. Administration of AT (1.0 gm/kg) blocked an alcohol-induced conditioned taste aversion (Aragon, Spivak & Amit, 1985) and locomotor depression produced by 2.0 gm/kg alcohol (Aragon, Spivak & Amit, 1989) in rats. No alterations in the peripheral levels of alcohol have been noted following treatment with AT (Aragon et al., 1989; Tampier & Mardones, 1987). Thus, a reduction in catalase-mediated production of acetaldehyde in the brain produced a decrease in a variety of alcohol-induced behaviors.

In summary, over the last decade a great number of data have accumulated that support the notion that acetaldehyde is produced in the brain, that it has reinforcing properties, and that interference with its production or elimination has significant effects on behavior, in both rats and humans (Amit & Smith, 1989; Von Wartburg, 1987). The following study was conducted in order to further examine the role of brain alcohol metabolizing enzymes, specifically in the consumption of alcohol.

EXPERIMENT 5

THE RELATIONSHIP BETWEEN BRAIN CATALASE AND ALDEHYDE DEHYDROGENASE ACTIVITY AND ALCOHOL CONSUMPTION IN RATS

The first report on the relationship between voluntary alcohol intake and brain catalase activity appeared in 1985. In this study, groups of Long Evans rats were given water only, 10% alcohol only, or a free choice between 10% alcohol and water for a 25 day period (Aragon, Sternklar & Amit, 1985). Brain catalase activity was measured in groups of animals sacrificed immediately (Group A) or 15 days (Group B) following the withdrawal of alcohol. Significant correlations between brain catalase activity and voluntary alcohol intake were obtained in Group A (r= 0.69) as well as Group B (r= 0.54). There were no differences in brain catalase activity between water-exposed and alcohol-exposed animals, indicating that brain catalase was not induced by exposure to alcohol. In a second experiment, a significant correlation (r= 0.82) between blood catalase activity measured in naive rats and subsequent voluntary alcohol intake was obtained in Long Evans rats (Amit & Aragon, 1988). These data confirmed that the relationship between catalase and voluntary intake observed in the previous study (Aragon et al., 1985) was most likely due to inherent individual differences in brain catalase, existing prior to the first exposure to alcohol.

Based on these data, as well as the previously reported relationship between voluntary intake and ALDH (i.e. Amir, 1977), Amit and Aragon (1988) proposed that the enzymatic system involved in the formation and elimination of acetaldehyde may play a critical role in regulating alcohol intake, through modulation of brain acetaldehyde levels. The relationship between brain levels

of catalase, ALDH and alcohol consumption was examined in the following study.

A random sample of animals from Experiments 1, 2 and 3 was sacrificed following the termination of the experiments. The brain activities of catalase and ALDH were determined, and compared to the levels of voluntary alcohol intake. Considering that different conditions were in effect in each study (i.e. different strains of rats, housing conditions and diets) the robustness of the phenomenon could be evaluated. In addition, given the disparate alcohol intakes of P vs NP rats, it was of considerable interest to determine whether there were any differences in the brain levels of catalase and ALDH between the strains.

MATERIALS AND METHODS

Subjects:

Experiment 1: This group of rats has been designated as the MAINTENANCE-HOME CAGE group. The animals were sampled from 4 different batches of the Long Evans rats screened and maintained in Experiment 1 (n = 30). Subsequent to the 10 day maintenance period described in Experiment 1, these animals were used in an experiment unrelated to the present investigation. During this period they were maintained in the animal colony and received continuous access to water, plain or flavoured alcohol and food. Following the termination of the study, all animals were maintained on water and Agway 4070 chow for 3 weeks prior to sacrifice.

Experiment 2: Twenty P rats, 10 NP rats and 20 Long Evans rats, screened as described in Experiment 2 were used. These animals have been designated as the SCREENING-DRINKOMETER group. The rats were used in another experiment following the alcohol screening, however they were given continuous access to food water and a 10% alcohol solution during the entire

period. Following the termination of the experiment, the animals were maintained on water and Bioserve food pellets for 3 weeks prior to sacrifice.

Experiment 3: Forty-five Long Evans rats from Experiment 3 were used. This group was designated as the MAINTENANCE-DRINKOMETER group. These animals were randomly sampled from the 7 batches of rats maintained in the drinkometer cages. Following the termination of the baseline recording period, as described in Experiment 3, these animals were used in another experiment, where they had continuous access to alcohol, Bioserve food pellets and water. They were maintained on water and Bioserve food pellets for 3 weeks prior to sacrifice.

Preparation of tissues and homogenates: The animals were sacrificed by exsanguination under ether anesthesia as described by DeMaster, Redfern, Shirota and Nagasawa (1986). The brains were perfused in situ by whole body perfusion using 400-500 ml of heparinized (1000 units/liter) isotonic saline. Following the perfusion, brains were quickly excised, rinsed in saline and blotted dry. Brains were stored at - 85 ^{o}C until assay.

On the day of the assay, the brains were weighed and 10% homogenates prepared in ice-cold 0.25M sucrose containing 0.1mM EDTA. The samples were homogenized using a Polytron homogenizer with a glass mortar and teflon pestle. All samples were stored on ice during preparation and processing. Following homogenization the sample was divided in two; one half was processed for the ALDH assay and the other for the catalase assay.

Aldehyde Dehydrogenase Assay: Triton X-100 was added to the brain homogenate to a final concentration of 1%. The samples were thoroughly mixed, capped and stored at 0 ^{o}C for 20 minutes and then centrifuged at 100,000 x g, at 4 ^{o}C for 60 minutes, and the clear supernatant was used in the enzyme assay. ALDH activity was determined by following the production of

NADH at 340nM using an LKB Ultrospec II spectrophotometer (Pettersson & Tottmar, 1982). The spectrophotometer was equipped with a thermostatted 4-cell turret, connected to a water bath maintained at 25 ^{o}C. The entire system was controlled by an Apple IIe computer, running LKB Enzyme Kinetics software.

The assay mixture contained 50mM sodium pyrophosphate (pH 8.8), 1mM NAD, 2uM rotenone, 0.2mM 4-methylpyrazole and 1mM magnesium chloride. Rotenone was dissolved in methanol and added in a final volume of 0.2% (Pettersson & Tottmar, 1982). The assay mixture (1.2 ml) was incubated with 0.2 ml enzyme supernatant in a 25 ^{o}C water bath for 20 minutes. The reaction was started by the addition of 0.1 ml of the substrate, acetaldehyde. Total ALDH activity was determined using an acetaldehyde concentration of 5mM, while low Km activity was determined using a substrate concentration of 50uM. Each reaction was run in duplicate and followed over a 5 minute period. Blank activity in the presence of enzyme supernatant was determined for each sample in duplicate. Protein levels were determined by the Lowry method using bovine serum albumin as the standard (Lowry, Rosebrough, Farr & Randall, 1951). Brain ALDH activity was expressed as nMoles NADH produced/min/mg protein.

Catalase Assay: Triton X-100 was added to the homogenate to a final concentration of 0.1%. The samples were mixed and stored at 0 ^{o}C for approximately 30 minutes. Catalase activity was measured using a Yellow Springs Biological Oxygen monitor (Model 53) equipped with a Clark type oxygen electrode as described by DeMaster et al. (1986). The reaction cell, maintained at 25 ^{o}C by a circulating water bath, contained 0.01mM potassium phosphate buffer (pH 7.0) that had previously been deoxygenated with a stream of nitrogen. Hydrogen peroxide (7.6 or 38 uMoles in 10 ul) was added to the

cell at zero time, and the baseline O_2 formation was recorded. Following stabilization of the baseline, 25 ul aliquots of brain homogenate were added and the production of O_2 was followed for 3 minutes. The samples were assayed in duplicate or triplicate. Brain samples were diluted by 5 volumes and protein content determined by the Lowry method using bovine serum albumin as the standard (Lowry et al., 1951). Brain catalase activity was expressed as nMoles O_2 formed/min/ug protein. In this assay, the highest substrate concentration (38 uMoles) was run only as a test of the reliability and consistency of the assay. The average values are reported below, as well as the correlations between the activity of catalase measured at the two substrate concentrations. However, the activity measured at this substrate concentration was not used in determining the correlations to drinking behavior.

NAD, rotenone and 4-methylpyrazole were purchased from Sigma Biochemial Co. (St. Louis, MO). Potassium phosphate, sodium pyrophosphate, hydrogen peroxide and magnesium chloride were obtained from Fisher Scientific, Montreal. Acetaldehyde, purchased from Aldrich, was redistilled and stored at - 20 ^{o}C. Fresh solutions were mixed on each assay day.

RESULTS

Total ALDH activity, low Km ALDH activity and 7.6uM catalase activity were tabulated and compared to various measures of alcohol consumption. These included the baseline alcohol intake (GM/KG) and alcohol bout size and frequency. No differences in brain weights, or brain homogenate protein levels were observed between high and low consuming animals within the three groups. Therefore, these data are not reported. Given that the animals had such varying experimental histories, the data for each group was analysed separately. Statistical analyses were performed using the BMDP programs 7D

(ANOVA), 2V (ANOVA with repeated measures), and 6R (multivariate regression).

<u>MAINTENANCE-HOME CAGE</u>: The mean alcohol intake across the 10 day baseline period, immediately after screening, was calculated for each animal and compared to enzyme activities. The mean alcohol intake exhibited by the entire group was 3.49 gm/kg/day with a range from 1.69 - 5.33 gm/kg/day. Mean values for enzyme activities were as follows: total ALDH 2.86 ± 0.07 nMoles NADH/min/mg protein, low Km ALDH 0.29 ± 0.01 nMoles NADH/min/mg protein, 7.6uM catalase 0.60 ± 0.02 nMoles O_2/min/ug protein, 38uM catalase 2.2 ± 0.036 nMoles O_2/min/ug protein. The two measures of catalase activity correlated highly ($r= 0.90$) as expected.

The highest correlations between mean GM/KG and enzyme activities were obtained for the low Km ALDH activity ($r= 0.40$) and 7.6uM catalase activity ($r= 0.29$). Both variables considered together in a multiple regression yielded a significant correlation of $R= 0.49$ [$F(2,27)= 4.3$, $p= 0.02$] with mean GM/KG. When total ALDH activity was also entered into the the multiple regression, a significant multiple correlation of $R= 0.51$ [$F(3,26)= 2.99$, $p= 0.049$] was obtained. Overall, the three enzyme activities accounted for roughly 26% of the variability in drinking.

The individual correlations between GM/KG and low Km ALDH or 7.6uM catalase were positive i.e. higher enzyme activities were associated with higher drinking levels. It was interesting to note that there was no correlation ($r= 0.036$), between Low Km ALDH and 7.6uM catalase enzyme activities themselves. This suggested that some animals had high catalase activity and low ALDH or vice versa. It was of some interest to explore the potential distribution of subgroups within the sample; thus animals displaying high catalase as well as high ALDH were compared to animals with other

combinations of enzyme activities. Therefore, each animal was ranked above or below the mean for both 7.6uM catalase and low Km ALDH activities, forming four more homogeneous groups: HH- high catalase, high ALDH (n= 7), HL- high catalase, low ALDH (n= 8), LH- low catalase, high ALDH (n= 4), LL- low catalase, low ALDH (n= 11). The mean alcohol consumption for the four groups is presented in Figure 36. A two-way ANOVA for baseline alcohol intake (GM/KG) in these groups, with a factors for catalase activity and ALDH activity, yielded a significant main effect only for catalase activity [$F(1,26) = 5.45$, $p = 0.027$]. Post-hoc comparison using Student's t-tests with a Bonferroni correction, indicated that the HH group drank significantly more alcohol than the LL group ($t(26) = 4.0$, $p < 0.01$). The frequency of LL animals (n= 11) was greater than the other combinations, as would be predicted on the basis of the greater frequency of low drinkers in the overall population.

Based on this analysis, there appeared to be a significant positive relationship between brain enzyme activities (both catalase and ALDH) and alcohol drinking behavior in Long Evans rats in this study. All enzyme activities considered together, predicted a significant proportion of the variance in drinking levels.

SCREENING-DRINKOMETER: A preliminary analysis established that the P rats had significantly higher catalase levels than NP and Long Evans rats, as shown in Table 9. The catalase activity was higher at both substrate concentrations (7.6uM [$F(2,47)=15.32$, $p= 0.00001$]; 38 uM [$F(2,47)=8.37$, $p= 0.0008$]. The correlation between the two measures of catalase activity over all three strains of rats, was $r= 0.69$. There were no differences between strains on the measure of low Km ALDH, however P rats had significantly higher total ALDH activity compared to the Long Evans rats [$F(2,47)=6.75$, $p=0.003$].

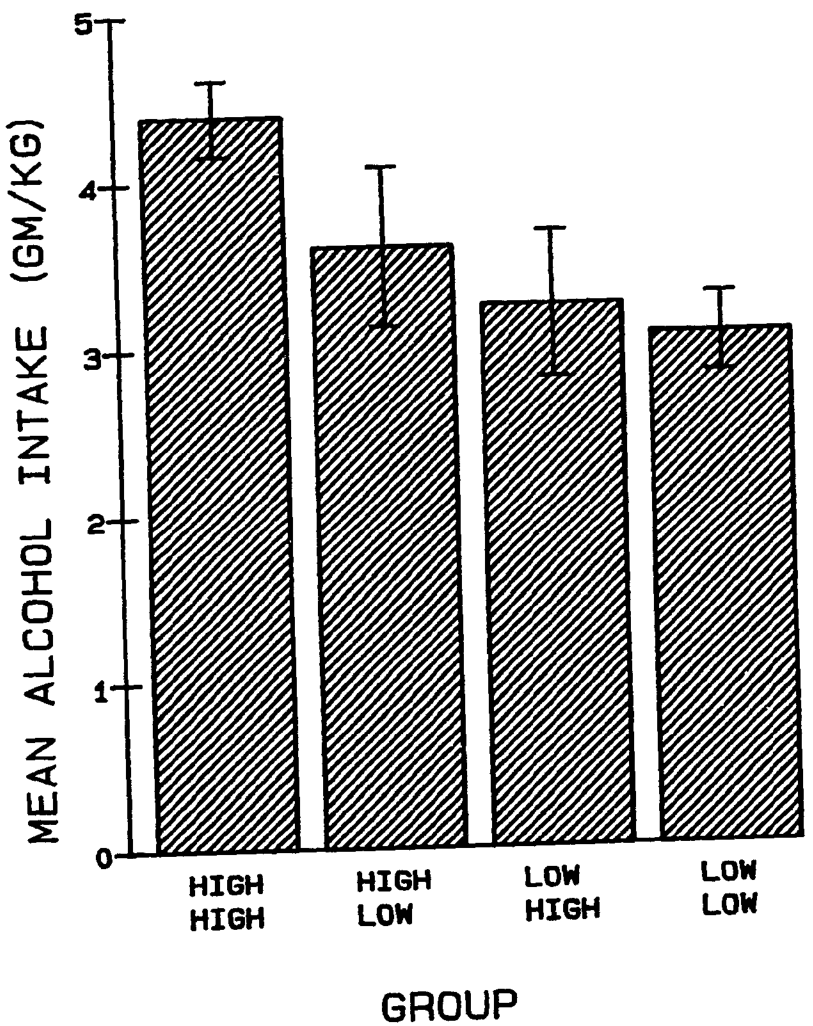


Figure 36. Alcohol intake (GM/KG) in MAINTENANCE-HOME CAGE groups formed on basis of Catalase and ALDH.

TABLE 9

COMPARISON OF ENZYME ACTIVITIES FOR SCREENING-DRINKOMETER GROUPS

	P RATS (n=20)	LONG EVANS RATS (n=20)	NP RATS (n=10)
Total ALDH	2.84 * (±0.13)	2.26 (±0.10)	2.66 (±0.13)
Low Km ALDH	0.305 (±0.02)	0.235 (±0.018)	0.295 (±0.036)
7.6uM Catalase	0.72 ** (±0.019)	0.59 (±0.019)	0.56 (±0.02)
38uM Catalase	2.54 ** (±0.07)	2.15 (±0.06)	2.19 (±0.11)
Mean GM/KG (2% to 10%)	2.92 (±0.39)	1.38 (±0.27)	0.56 (±0.06)

The data are listed as the group mean (± S.E.M.).
ALDH activity is expressed as nMoles NADH produced/min/mg protein.
Catalase activity is expressed as nMoles O_2 produced/min/ug protein.

* Significantly different from Long Evans group; ** Significantly different from NP and Long Evans groups, ($p < 0.05$) by Student's t-tests with Bonferroni corrections.

In order to compare enzyme activities to drinking levels, the mean GM/KG for each animal was calculated by collapsing across the entire acquisition curve. This measure was chosen since it represented the overall consumption of each animal, including the amounts consumed at the lower concentrations. Mean alcohol consumption in the P rats, collapsed over the acquisition curve was 2.92 gm/kg, with animals displaying a wide range of intake from 0.43 - 6.27 gm/kg. Since the NP rats did not drink alcohol at the higher concentrations, overall intake was very low at 0.56 gm/kg with a range from 0.35 - 0.95 gm/kg. For the Long Evans rats the mean GM/KG was 1.38, with intakes ranging from 0.29 - 4.05 gm/kg.

In the sample of rats analysed in Experiment 2, the Long Evans Drinker group was indistinguishable from the P-Drinker rats across a number of measures. In addition, since the environmental and assay conditions were held constant for all groups it appeared appropriate to combine the P, NP and Long Evans into one group for the analysis. With 7.6uM catalase, low kM ALDH and total ALDH enzyme activities entered into the computation, a multiple correlation between enzyme activity and alcohol intake of $R= 0.42$ [$F(3,46)=3.36$, $p= 0.026$] was obtained. Other measures of alcohol intake were considered in the analysis. Mean alcohol bout size (gm/kg/bout) and mean alcohol bout frequency for each animal were calculated by averaging over the acquisition curve. There was no significant relationship between mean bout size and enzyme activities [$R= 0.36$, $F(3,46)=2.28$, $p=0.09$]. However, there was a strong relationship between the frequency of alcohol drinking bouts and the activities of catalase and ALDH [$R= 0.68$, $F(3,46)=13.55$, $p=0.00001$].

As described in the analysis of the MAINTENANCE-HOME CAGE group, the animals were divided into four groups HH (n=15), HL (n= 11), LH (n=7), LL (n=17) on the basis of the 7.6uM catalase and low Km ALDH activities. All of

the NP rats fell into the LL group. However, none of the low drinking P rats, described in Experiment 2, found their way into this group. These rats all had high catalase levels, and like their high drinking counterparts fell into the HH or HL groups. Thus, the low drinking observed in some of the P rats could not be accounted for by low levels of catalase activity. These data also suggested that high catalase activity exhibited by the P-Drinker group were not due to exposure to alcohol itself, and were therefore most likely genetically determined differences that existed prior to the alcohol screening.

A two-way ANOVA comparing alcohol consumption across the HH, HL, LH and LL groups yielded only a main effect for the catalase activity [$F(1,46)=4.78$, $p= 0.03$]. There was no main effect for ALDH activity or an interaction. The results of this analysis were plotted in Figure 37. Thus, it would appear that the most significant factor predicting alcohol intake in these groups, as in the previous analysis of the MAINTENANCE-HOME CAGE group, was brain catalase activity. The correlation for catalase (7.6uM) alone and mean GM/KG, for the P, NP and Long Evans rats is presented in Figure 38. A correlation of $r= 0.37$ ($p <0.05$) was observed. The relationship between catalase activity and mean alcohol bout frequency yielded a correlation of $r= 0.63$ ($p<0.01$). Thus, catalase activity accounted for the largest portion of the variability in drinking, in terms of mean GM/KG as well as mean alcohol bout frequency, with low Km ALDH and total ALDH contributing smaller amounts to the explained variance.

MAINTENANCE-DRINKOMETER: This group of animals was screened in the home cage environment on Agway food and then moved to the drinkometer chambers following a maintenance period. Alcohol drinking in this group of rats showed a less consistent relationship to the alcohol metabolizing enzymes. A number of measures of alcohol intake were considered in the

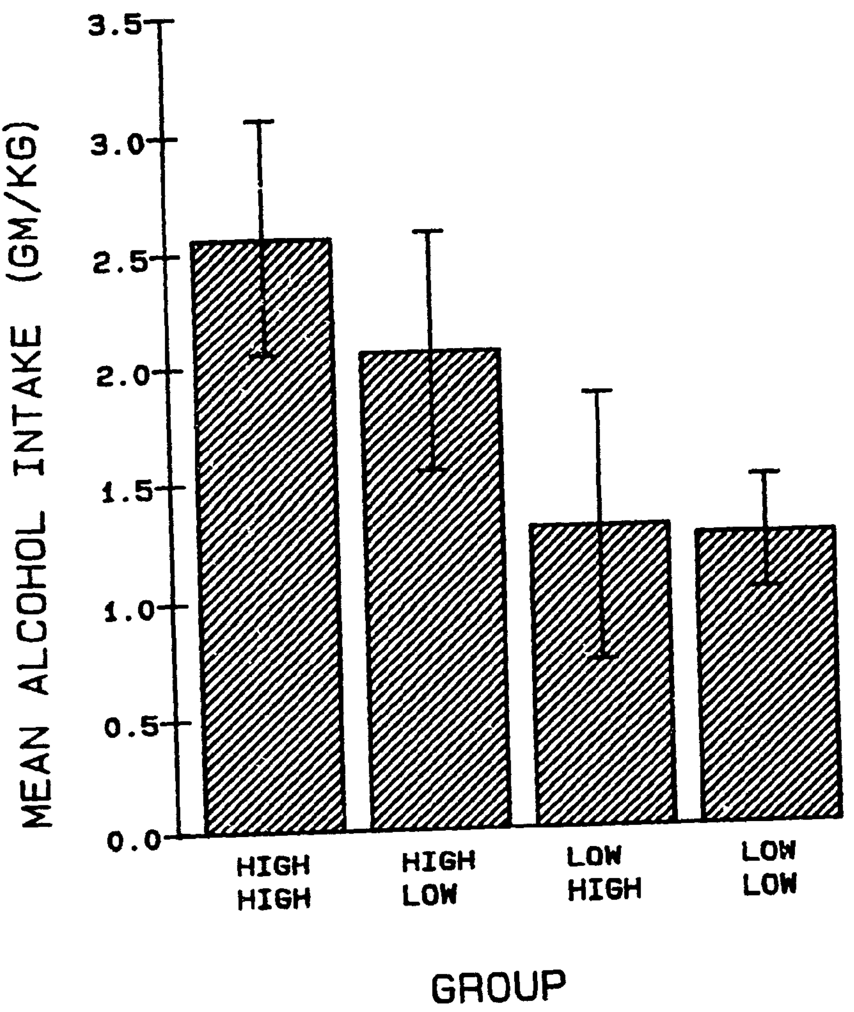


Figure 37. Alcohol intake (GM/KG) in SCREENING-DRINKOMETER groups formed on basis of Catalase and ALDH.

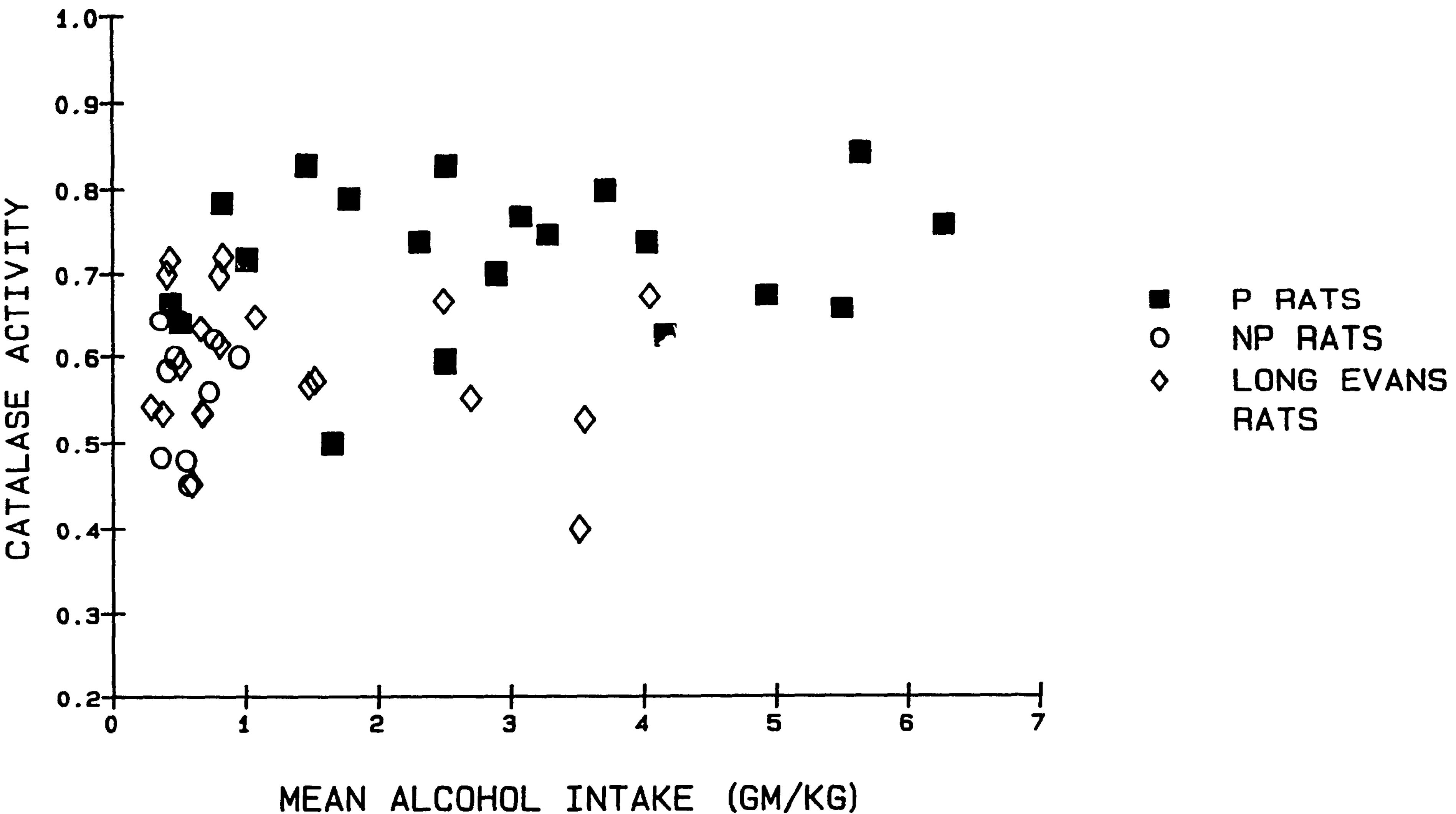


Figure 38. Correlation between alcohol intake and Catalase activity. $r = 0.36$ ($p<0.05$).

analyses. First, alcohol intake over the 10 day home cage maintenance period following screening, when animals were consuming Agway food was compared to subsequent enzyme activities. A multiple correlation computed between mean GM/KG and the activities of the low Km ALDH and 7.6uM catalase, yielded a significant correlation of R= 0.39 [F(4,42)= 3.85, p= 0.029]. Adding total ALDH to the regression equation did not improve the correlation, R= 0.40 [F(3,41)=2.61, p=0.064].

The second measure of alcohol intake was obtained in the drinkometer chambers, as described in Experiment 3. Mean alcohol bout frequency and bout size (gm/kg/bout), as well as mean GM/KG over the 5 day baseline recording period were computed for each animal. The relationship between mean GM/KG and the activities of low Km ALDH, total ALDH and 7.6uM catalase yielded a multiple R= 0.27 [F(3,41)=1.05, p=0.38]. In the same analysis, the size of alcohol bouts (gm/kg/bout) yielded an R= 0.31 [F(3,41)= 1.42, p= 0.25]. Thus, the total amount of alcohol consumed as well as the average amount of alcohol consumed within individual bouts did not appear to be related in any way to the activities of the alcohol metabolizing enzymes. Alcohol bout frequency however, showed a significant relationship, R= 0.46 [F(3,41)=3.73, p= 0.018].

The distribution of animals into groups based on catalase and low Km ALDH activities yielded the following distribution: HH (n= 7), HL (n= 14), LH (n= 3) and LL (n= 21). There were no significant differences between the groups in terms of home cage or drinkometer baseline GM/KG. Thus, unlike the MAINTENANCE-HOME CAGE and SCREENING-DRINKOMETER groups, alcohol drinking in this group failed to show a consistent relationship to alcohol metabolizing enzymes. The home cage alcohol intake measured prior to the period in the drinkometer chambers showed a significant multiple correlation

with low Km ALDH and 7.6uM catalase (R= 0.39). However, alcohol intake as measured in the drinkometer failed to yield positive results. The significant relationship between alcohol bout frequency measured in the drinkometer, and enzyme activities is difficult to interpret considering that all other measures of alcohol intake failed to demonstrate the same relationship.

DISCUSSION

The correlations between brain alcohol metabolizing capacity and alcohol intake obtained in this study, were lower than those obtained in previous published work (i.e. Amir, 1977; Aragon et al., 1985; Socaransky et al., 1984) and they were not consistently observed in all groups of animals tested. Aragon and Amit (1985) reported a multiple correlation between catalase activity, Low Km ALDH and alcohol drinking of R= 0.80 in a group of Long Evans animals (n= 18) screened and maintained in a similar fashion to the MAINTENANCE- HOME CAGE group described here. Overall, there were considerable differences in methodology compared to the present study. Catalase activities in all previous reports (Amit & Aragon, 1988; Aragon et al., 1985; Aragon & Amit, 1985) were measured using a colorimetric method, rather than the oxygen electrode method described here. ALDH activities in most previous work (Amir, 1977, 1978a; Socaransky et al., 1984) were measured with a fluorometric method using indole-3 acetaldehyde as the enzyme substrate rather than acetaldehyde. Additional factors that may have contributed to variable results included differences in alcohol screening procedures, housing conditions and diets. The combination of the differences in animal strains, diets and assay procedures, however, did not obscure the relationship between enzymes and drinking altogether.

In the SCREENING-DRINKOMETER and MAINTENANCE-HOME CAGE groups, catalase activity and ALDH activity together, accounted for 18 to 26

percent of the variability in drinking. When animals were distributed into groups based on both catalase and ALDH, HH animals were shown to consume significantly more alcohol than LL animals. In addition, as a group, the P rats were found to have significantly higher catalase activity, and to drink more alcohol than either the LE or NP rats. Thus, while the correlations were lower than previously reported, there is a consistent, significant relationship between higher alcohol metabolizing capacity and alcohol drinking over three strains of rats (P, Long Evans, NP), different diets (Agway vs Bioserve food) and two different housing conditions (home cage vs drinkometer cage).

Chronic alcohol exposure has been shown to have a variable effect on enzyme activity in different tissues. DeMaster, Kaplan and Chesler (1981) demonstrated that forced alcohol intake via a liquid diet increased the activity of heart catalase, but had no effect in liver, erythrocyte or skeletal muscle. These data were confirmed by Aragon et al. (1985), who also demonstrated that chronic forced choice alcohol intake failed to induce catalase. It should be noted that the failure to induce central ALDH following voluntary alcohol consumption, has also been reported (Amir, 1977; Socaransky et al., 1984). Thus, in general the induction of enzyme activity following the voluntary intake of alcohol has not been observed. There is little reason to assume that high alcohol intake induced the corresponding high levels of catalase activity in the present study.

Alcohol intake in MAINTENANCE-DRINKOMETER group failed to display the same relationship to alcohol metabolizing enzymes. There are a number of differences between this group of rats and the SCREENING-DRINKOMETER and MAINTENANCE-HOME CAGE groups. These animals were screened in the home cage on Agway food, and moved to the drinkometer boxes following a maintenance period. Between the two measurements of alcohol intake (i.e.

home cage maintenance vs drinkometer maintenance) the animals underwent two changes in environmental conditions (i.e. first a change in diet, followed by a change in housing conditions). In some animals the change in diet, from Agway to Bioserve food pellets induced as much as 50% reduction in alcohol intake and in others, the change in housing had as great an influence.

In some preliminary studies, (data not reported in this thesis), the Agway and Bioserve diets were compared for their effects enzyme activities as well as alcohol elimination rates. Groups of alcohol naive animals fed either the Agway or Bioserve diets were sacrificed and brain and liver catalase and ALDH activities were determined. In additional groups of rats, blood alcohol levels were determined from tail blood samples obtained at various time periods following an intraperitoneal injection of alcohol (1.0 gm/kg). No significant differences between animals fed Agway vs Bioserve food were observed on any of these measures. Thus, the change in diets between the home cage and drinkometer conditions did not induce any changes in the disposition of alcohol. It would appear from this preliminary work, that "change" per se (diets or housing conditions) had a major influence on drinking.

Thus, it would appear that in the MAINTENANCE-DRINKOMETER group, changes in the environmental conditions may have obscured the relationship between drinking and alcohol metabolizing enzymes. These data point to the importance of environmental influences on drinking behavior and as well dictate that it is impossible to examine potential genetic factors regulating alcohol intake when environmental conditions are changed drastically within a study.

GENERAL DISCUSSION

> Falk and Tang (1988) noted that "it would facilitate the task of constructing an animal model if there were some general agreement as to what unequivocally constitutes the definition of a case of human alcoholism."

> In his overview of research presented at the International Conference on Animal Models in Alcohol Research, Forsander (1980) commented that it might be more profitable to ask "... why most people and animals do not drink alcohol rather than ask why a few misuse it."

Both these statements have important implications for researchers attempting to develop animal models of alcoholism. Based on an evaluation of the clinical literature, a clear-cut definition of human "alcoholism" is not likely to be forthcoming. In addition, Forsander's comment implied the existence of factors that normally serve to regulate alcohol intake in the majority of individuals and animals. The majority do not become "alcoholic" following exposure to the drug, thus the long search for an animal preparation that will spontaneously display the characteristics of a very small proportion of the human alcoholic population is unlikely to be realizable. More than one observer has noted this fact. Holloway, Rundell, Kegg, Gregory and Stanitis (1984) in discussing the continued controversy over animal models, suggested that "excessive concern about developing the perfect or ideal replica model can be nonproductive." Similarly, McClearn (1988) noted the tendency of researchers to demand absolute comprehensiveness in animals models; "disdaining partial models while searching for an all-embracing animal model of human alcoholism may be idealistic...but not very realistic."

It seems plausible to suggest therefore, that studying the initiation and maintenance of the oral intake of alcohol in rodents--including the behavioral and neurobiological regulation of this behavior--in isolation from the consequences of long term chronic use and physical dependence, would seem to be an important and potentially useful experimental strategy for understanding the development of alcoholism. In so far as biological factors are involved in the regulation and limitation of alcohol intake in humans, animal models of alcohol "drinking" may provide useful experimental tools for elucidating these factors. The data presented in Phase I of this thesis provided evidence that alcohol consumption in rodents has many features in common with human alcohol intake.

SIMILARITIES BETWEEN ALCOHOL INTAKE IN HUMANS AND RODENTS

In humans, alcohol is consumed in the form of flavoured, sweetened, cold beverages aimed at masking the aversive flavour of alcohol. Humans display wide variability in the consumption of alcohol with the classification of individuals into social or heavy drinking categories based on the number of weekly drinks as well as the pattern of intake (i.e. daily vs weekend drinking) (Hilton, 1987). In Experiment 1, rats given plain laboratory grade alcohol, displayed a similar wide range of alcohol intake (from 0 to 8 gm/kg/day). Following a maintenance period the rats exhibited stable intake over time.

Humans display a temporal pattern of alcohol intake with the heaviest intake ocurring in the evening (Arfken, 1988). In general, humans consume the majority of their daily fluid intake in association with meals (DeCastro, 1988). Even in alcoholics the assumption that alcohol consumption is dissociated from normal patterns of activity and meals appears unwarranted. The neglect of food intake is not an invariant feature of alcoholism; depending on culture, drinking

practices, the type of alcohol consumed as well as social variables (i.e. marital status and living arrangements)(Price, Kerr & Williams, 1989).

The patterns of alcohol intake in rodents were examined in Experiments 2 and 3. Rats drank alcohol in discrete, short bouts, mainly during the dark cycle. Some of the animals consumed large bouts of alcohol (> 1.0 gm/kg/bout) that were sufficient to achieve intoxicating levels of blood alcohol, as measured in Experiment 4.

The limited access paradigm described in Experiment 4 has parallels to human alcohol consumption patterns. Alcohol is rarely available ad libitum to human drinkers. Societal constraints are placed such that drinking for most of the population is restricted to certain time periods or occasions. The lunch hour cocktail, happy hour after work, and the Friday night binge, are examples of restricted access. Such occasions are often marked by the rapid intake of alcohol. Intermittent schedules of alcohol access have been shown to increase the daily amount of alcohol consumed. Marcucella (1989) has described an extensive series of studies showing that free access alcohol consumption increased dramatically in rats following repeated periods of restricted access to alcohol. Similar effects following intermittent exposure (Wayner et al., 1972; Wise 1973) or longer deprivation periods have been reported (Sinclair & Senter, 1968). The significance of these observations have yet to be determined, however frequent limited access conditions are encountered by humans during the early stages of their drinking history.

VALIDATION OF ANIMAL MODELS OF ALCOHOL DRINKING

Logically, nothing can be inferred from the observation of blood alcohol levels following alcohol intake, about why an animal consumes alcohol. Behavioral scientists have continually grappled with the question of why animals consume alcohol, or drugs of any type. Ultimately one can only

measure the behavior of the animals, and the inferences one draws tend to be circular. The difficulties inherent in interpreting drug-seeking behavior are similar to attempts to infer from measures of food intake why animals eat. No doubt, many factors, including taste, caloric value and nutritive content of the food, reinforce eating behavior. With alcohol the problem is more complex; alcohol is a fluid, it has a strong taste and smell, it has caloric content and also possesses a pharmacological impact. Dissociating these factors has not been an easy task. No doubt many factors reinforce alcohol drinking behavior.

One way of circumventing this problem has been through cross-validation employing multiple measures of alcohol intake. That some rats will voluntarily consume large quantities of alcohol has been amply demonstrated in a variety of studies (Amit & Stern, 1971; Gill, Amit & Ogren, 1984; Sinclair & Senter, 1968) as well as in the present investigation (Experiments 1 to 4). A number of investigators have demonstrated that rats will also learn to perform an operant response in order to receive alcohol through the oral route (Meisch & Beardsley, 1975; Sinclair, 1974). The work in this area was recently reviewed by Samson, Pfeffer and Tolliver (1988). It was interesting to note that George (1988) demonstrated that there was a significant rank correlation between oral alcohol consumption as measured in "preference" paradigms and that obtained from operant alcohol self-administration studies. Thus a variety of experimental methods have confirmed that animals drink alcohol in amounts that are likely to be intoxicating.

Therefore, it would appear that in terms of population distribution, temporal distribution and pattern of alcohol intake, rats are comparable to humans. Parsimony would suggest therefore, that similar mechanisms may be involved in the regulation of intake.

Johanson, Woolverton and Schuster (1987) suggested that animal models are evaluated by assessing reliability of the results obtained (both intersubject and intrasubject), the generality of the results across species and different experimental contexts, as well as the predictive validity in comparing results from animals to humans. Predictive validity has most frequently been cited as the only means that experimenters have to ultimately validate their experimental tools. The genetic contribution to alcohol consumption as measured in the "**preference**" paradigm, in outbred, inbred and genetically selected animals provided predictive validity to the human population. It remains to be seen whether the biological factors underlying the genetic contribution to alcohol drinking are similar in rodents and man. As simply stated by McClearn (1988) "the only way to establish the relevance of an animal model to the human situation is to continue its development until hypotheses emerge that are ethically and practically testable on human subjects...and then test them."

THE DEVELOPMENT OF MORE SOPHISTICATED MEASURES OF ALCOHOL INTAKE

The job of developing animal models would be greatly facilitated by an end of the debate over "adequate" animal models of "alcoholism", with a refocussing of energy on development of an adequate technology to study alcohol drinking in animals. The development of the computerized drinkometer was viewed as one step in that direction. The computerized drinkometer system described in Experiment 2, has been running reliably for two years. The rat chambers, drinkometer circuitry and computer system have performed consistently throughout 455 sessions to date.

A detailed microstructural analysis of drug taking behavior has several advantages over the "traditional" voluntary oral consumption paradigm, where typically intake is measured once daily, at the end of a 24 hour period. With the

traditional preference paradigm, the effects of pharmacological or behavioral manipulations can only be determined by changes in total intake, the preference ratio, as well as changes in body weight. Pharmacological manipulations of the catecholaminergic and serotonergic neuronal systems have previously been shown to affect alcohol consumption in rats (e.g. Brown & Amit, 1977; Corcoran, Lewis & Fibiger, 1983; Davis, Smith & Werner, 1978; Gill et al., 1984; Gill & Amit, 1987). There are a number of discrepancies in this literature however, in terms of the specificity for alcohol, as well as the direction and magnitude of effects on drinking behavior. It is clear from the analysis presented in Experiment 2, that the drinkometer system is capable of providing a much more detailed analysis of changes in food and fluid bout size, duration and frequency, as well as information on eating and drinking rates. In addition the time course of any drug effects can be determined. Such a detailed analysis would be invaluable when examining the effects of pharmacological agents (i.e. neurotoxins, uptake inhibitors, receptor agonists and antagonists) on alcohol consumption. The use of the drinkometer paradigm would be very useful for understanding of the types of changes induced by these agents, and to aid in the detection of nonspecific effects on food or water consumption.

Apart from the importance of using a more sophisticated paradigm in pharmacological studies of drinking behavior, the drinkometer has a fundamental usefulness in studying the patterns of intake of individual animals, with the aim of understanding some of the underlying biological correlates of drug taking behavior. As discussed in Experiments 3 and 4, the discrete nature of alcohol drinking bouts suggested that there were some mechanisms that limited the size and duration of individual bouts. The work discussed in Phase II and Experiment 5 were viewed as an examination of one of these potential regulatory factors.

THE REGULATION OF ALCOHOL CONSUMPTION

Extensive biochemical and behavioral evidence has now been provided by a number of laboratories demonstrating that acetaldehyde can be produced in brain by the enzyme catalase, and that modification of this enzyme by AT affects a diverse array of alcohol-induced behaviors (Aragon et al., 1985, 1989; Cohen et al., 1980; Tampier et al., 1988). In addition, a variety of researchers have demonstrated that both catalase and ALDH activities are related to the voluntary selection of alcohol by rodents (Amir, 1977; Aragon et al., 1985). It should be noted however, that, to date, the largest portion of research related to the role of alcohol metabolism in the regulation of alcohol effects has focussed on liver enzymes; as yet little scientific curiosity has been directed at the potential role of brain enzymes in determining the response to alcohol.

The data presented in Experiment 5 added further weight to the evidence that the central alcohol metabolizing enzymes, catalase and ALDH, are related to alcohol intake. In addition, there are currently few known biochemical factors that can account for the variations in drinking between the P and NP strains. The demonstration that differences in alcohol drinking between P and NP rats can be partially accounted for by differences in the level of brain catalase is a significant finding. That the same relationship can be observed in the outbred strain of Long Evans rats suggested that the differences were not spurious. It is not implausible, therefore, to suggest that the activities of catalase and ALDH are involved in the regulation of alcohol intake, possibly through control of acetaldehyde production and elimination in brain.

Support for the importance of centrally produced acetaldehyde in maintaining drinking behavior has been provided by the work of Sinclair and Lindros (1981). In their work, the administration of the ALDH inhibitor cyanamide was shown to decrease alcohol intake in Long Evans and AA/ANA

rats. When the high peripheral accumulation of acetaldehyde--assumed to be responsible for the decrease in drinking behavior--was prevented through the concurrent administration of 4-methylpyrazole (4MP) in the drinking water, alcohol intake was still reduced (Sinclair & Lindros, 1981). Thus, a reduction in peripheral acetaldehyde by 4MP did not block the decrease in alcohol intake produced by cyanamide. These data suggested that peripheral acetaldehyde was not responsible for the decrease in alcohol intake, and that the suppressant effect of cyanamide must be related to direct effects on brain ALDH or some other unknown action of the compound. More recent research has provided a tentative explanation for their data. DeMaster et al. (1986) have shown that cyanamide in fact is also a very potent inhibitor of catalase. Therefore, in cyanamide treated animals, cerebral catalase activity would be inhibited, leading to a reduction in the production of central acetaldehyde, and potentially a reduction in the reinforcing impact of the ingested alcohol. Cyanamide treated animals, with catalase and ALDH inhibited, would be analogous to the LL (low catalase, low ALDH) groups in Experiment 5. The LL groups displayed lower alcohol intake than HH or HL animals.

THE MECHANISM OF ACETALDEHYDE ACTION

As suggested by Amir (1977), potential individual differences in the disposition of acetaldehyde in brain tissue could alter the behavioral response to alcohol. At the present time however, little is known about the actions of acetaldehyde in brain. It is impossible to specify where acetaldehye accumulates in the brain during alcohol metabolism, how it produces its effects, and how it might regulate subsequent alcohol intake; however there are some interesting findings that allow speculation.

Catalase activity is not homogeneously distributed in rat brain. Histochemical analyses have shown that catalase is contained in

microperoxisomes, located in close proximity to mitochondria, in high density in neurons of the hypothalamus, substantia nigra and locus coeruleus (Brannan, Maker & Raes, 1981; McKenna, Arnold & Holtzman, 1976). The high levels of catalase in catecholamine (CA) rich areas may be significant for a number of reasons. First, peroxide, necessary for the activation of catalase, is produced in these cells from several sources; mitochondrial oxidation of various substrates, the breakdown of CA's by monoamine oxidase (MAO), as well as through the metabolism of ascorbate (Patole, Swaroop & Ramasarma, 1986; McKenna et al., 1976). Ascorbate has been shown to enhance the metabolism of alcohol to acetaldehyde, via a catalase-peroxide dependent system (Susick & Zannoni, 1984). Thus, it would appear that CA rich areas of the brain possess high levels of catalase, along with the metabolic machinery necessary to activate the enzyme.

Second, acetaldehyde has been shown to interact with CA release, turnover and metabolism. Acetaldehyde competitively inhibited the metabolism of biogenic aldehydes produced during the metabolism of catecholamines and serotonin, shifting metabolism from the normal oxidative pathway to a reductive pathway (Beck, Eriksson, Kiianmaa & Lundman, 1986; Walsh, Truitt & Davis, 1970). For example, alcohol administration produced an increase in the production of 5-hydroxytryptophol, and a corresponding reduction in 5-hydroxyindoleacetic acid, the normal product of serotonin oxidation (Beck et al., 1986). In addition, activities of the biogenic amine synthetic enzymes (i.e. tryptophan hydroxylase) have been shown to be inhibited by aldehydes, producing a reduction in endogenous neurotransmitter levels (Nilsson & Tottmar, 1987). While the mechanism of this inhibition is not known, it is clear that perturbations of normal levels of biogenic aldehydes in brain tissue may

have far reaching metabolic and behavioral consequences (Nilsson & Tottmar, 1987; Von Wartburg, 1987).

An interesting development in this area of research is the increasing evidence that aldehydes bind with cellular proteins (albumin, hemoglobin) and phospholipids, forming stable and unstable adducts (Helander & Tottmar, 1989). Ethanol adminstration has been shown to produce changes in the functioning of several receptors and receptor-effector coupling systems such as the GABA-benzodiazepine chloride ionophore (Littleton, 1989), as well as several enzymes (e.g. adenylate cyclase, MAO, Na^{+} K^{+}-ATPase) (Tabakoff, Hoffman & Liljequist, 1987). It has not been determined whether acetaldehyde binding can account for these molecular level changes in the functioning of various cellular proteins. Unfortunately, most of the research in this area is directed at the functional implications of acetaldehyde binding to serum and liver proteins, and their potential role in inducing liver injury (e.g. Wickramasinghe, Marjot, Rosalki & Fink, 1989), rather than to the potential biochemical and behavioral effects of acetaldehyde-protein binding in the brain.

At present, therefore, it is only possible to speculate that genetically determined differences in the activities of the enzymes catalase and ALDH, resulting in altered disposition of acetaldehyde in brain, may produce far reaching effects on brain metabolism and functioning.

A COMPARISON OF RESEARCH STRATEGIES IN THE ELUCIDATION OF THE BIOLOGICAL AND GENETIC FACTORS REGULATING ALCOHOL INTAKE

A great deal of emphasis has been placed throughout the research presented in this thesis on a comparison between genetically selected animals and the genetically heterogeneous outbred Long Evans strain. As stated in the introduction, there is a developing consensus that only genetically selected rats

strains will be useful for studying the neurobiological determinants of alcohol consumption (McBride et al., 1989). On the other hand, there is a great deal of support for the contention that genetic selection--as currently practiced--in fact has not been, and will not be, a productive research strategy in this area of research. This will be discussed in the following section.

A COMPARISON OF GENETICALLY SELECTED STRAINS

It should be noted at the outset of this discussion, that selective breeding has not primarily been aimed at determining the heritability of alcohol-related traits. First, as stated by McClearn (1981a) there are less costly ways to establish the heritability of alcohol related traits than through the use of selective breeding--i.e. through the use of already existing populations of inbred mice. Genetic selection was primarily aimed at obtaining information on the "mechanisms" underlying the trait. The directors of the two major alcohol drinking selection programs--AA/ANA (Eriksson, 1972) and the P/NP (Lumeng et al., 1977)--both emphasized that the objective of genetic selection was the determination of the physiological, biochemical and behavioral determinants of drinking.

As shown in Table 10, high and low drinking strains have been compared on numerous variables: levels of neurochemicals, sensitivity to alcohol, development of tolerance etc. Despite similar selection criteria (see Table 1) and similar alcohol consumption levels (see Table 10), to date no variable has been found that consistently accounts for differential alcohol consumption between the high and low drinking animals in each selected strain. Most often, the various selected strains have yielded opposite results in many of the studies examining biochemical and behavioral correlates of drinking behavior. These discrepancies are most obvious when examining the results of an extensive series of studies aimed at determining the neurochemical correlates of drinking in the AA/ANA and P/NP lines (note section in Table 10). At the present time it

TABLE 10

COMPARISON OF SELECTED RAT STRAINS

ALCOHOL INTAKE (GM/KG/DAY)

F63 generation UChA 1.16- 1.3 UChB 5.3- 6.4
(Contreras & Mardones, 1988).

F48 generation ANA 0.33 AA 6.5
(Korpi et al., 1988).

F26 generation NP 0.098 P 6.0
(Morzorati et al., 1988).

NEUROCHEMICAL DIFFERENCES

ANA > AA AA rats have lower levels of NE in cortex.
AA > ANA AA rats have higher 5HT levels in whole brain, hypothalamus, midbrain and cortex than the ANA strain. AA rats have higher DA levels in whole brain, limbic regions and the striatum. AA rats have higher levels of NE in the hypothalamus compared to the ANA rats (Ahtee & Eriksson, 1972, 1973; Ahtee, Attila & Kiianmaa, 1980; Korpi et al., 1988).

P > NP P rats have higher levels of NE in the cortex.
NP > P P rats have lower 5HT levels in the hypothalamus, striatum, nucleus accumbens, thalamus, hippocampus and hypothalamus and cortex than the NP line. P rats have lower DA, DOPAC AND HVA levels in the nucleus accumbens (Murphy, McBride, Lumeng & Li, 1982; 1987).

TABLE 10........continued

ALCOHOL SENSITIVITY- SLEEP TIME

UChB > UChA UChA had a shorter sleep time and regained righting reflex at a higher BAC following a dose of 90 mmole/kg (Quintanilla & Tampier, 1982).

ANA = AA No differences following a dose of 3.5 gm/kg in the F49 (Hilakivi et al., 1984; Le & Kiianmaa, 1988).

NP > P P rats had a shorter sleep time and regained righting reflex at a higher BAC than NP. No differences in BAC at loss of righting reflex (Li et al., 1981).

ALCOHOL SENSITIVITY- MOTOR IMPAIRMENT

ANA = AA No significant differences on tilting plane task in the F43 or F49 generations following 2.0 -2.5 gm/kg. No significant differences in open field following dose of 1.0 gm/kg (Hilakivi et al., 1984; Le & Kiianmaa, 1988).

NP > P NP significantly more impaired by alcohol in jump task following doses of 1.5 - 2.0 gm/kg. NP show more locomotor depression in open field following doses of 1.0- 1.5 gm/kg (Lumeng, Waller, McBride & Li, 1982).

ALCOHOL SENSITIVITY- HYPOTHERMIA

UChB > UChA High drinkers developed greater hypothermia following a 2 gm/kg dose (Tampier, Urrutia & Quintanilla, 1988; Tampier et al., 1984).

ANA = AA Same hypothermic response following 2.5 gm/kg alcohol (Hilakivi et al., 1984; Le & Kiianmaa, 1988).

NP > P NP significantly greater hypothermia, 2-3 hours following a dose of 2.5 gm/kg (Li et al., 1981).

is impossible to comment on whether any of the neurotransmitters examined play a role in mediating the genetic differences between selected lines.

Considerable energy was invested in the notion that the variation in alcohol intake was related to innate neurosensitivity to alcohol (Le & Kiianmaa, 1988; McClearn & Anderson, 1979; Tabakoff & Hoffman, 1988). Many researchers have attempted to demonstrate that alcohol preferring animals were less sensitive to the acute effects of alcohol (George, 1988). However, as shown in Table 10, there is no consistent relationship between alcohol preference and sensitivity on the sleep time, hypothermia or locomotor depression tests. Thus, while it has been agreed that selective breeding should provide a "more powerful" means of differentiating potentially important biochemical and physiological genetic markers, thus far no consistent marker has been found within or between selected strains.

In 1980, many years after the initial selection for the UChA/B, AA/ANA and P/NP strains, Eriksson alluded to problems with the various genetic selection programs. He suggested that the original base populations were not large and he erogenic enough, that the maintenance populations were too small, that environmental agents had been left uncontrolled and that "the breeders do not always have reasonable genetic or alcohol metabolic competence" (Eriksson, 1980). It was clear by that time that the genetic selection studies were producing a great deal of data, but few findings. Costly animal selection studies were not providing the scientific pay-off that was predicted.

VALIDITY OF SELECTION CRITERIA AND CONCEPTUAL ISSUES RELATED SELECTION

An examination of the literature on genetic selection allows no easy answer to the question of why the technique has not lived up to its promise. There have been numerous questions about the validity of the selection criterion itself

(Myers, 1968). As discussed in several sections of this thesis, drinking behavior can vary in rodents, depending on the method of screening, length of exposure, concentration of alcohol used and whether animals are given intermittent or continuous access to alcohol. In some cases, selection produced animals that show marked line differences, only at the selection criterion (i.e. consumption of a 10% alcohol solution, locomotor depression on one type of task, etc). An example of potential selection specificity concerns AA/ANA rats. York (1981) found the typical line difference on 10% alcohol, however, when the alcohol was contained in white wine or punch, the ANA markedly increased their alcohol intake, consuming more than the AA rats. Significant alcohol self-administration, resulting in high blood alcohol levels has also been observed in the NP strain of rats following a sucrose-fading procedure and the presentation of varying concentrations of alcohol (Samson, Tolliver, Lumeng & Li, 1989). In Experiment 2 of the present investigation, the NP rats were shown to drink alcohol when the concentration was below 5%.

Most recently the ALKO group in Finland has suggested that selecting on one criterion (consumption of 10% alcohol) was inadequate and that a more comprehensive measure should include absolute alcohol intake, alcohol preference ratio and the alcohol energy preference ratio (Hyytia et al., 1987). This was based on the observation that the AA/ANA selection criterion actually selected for alcohol intake as well as body weight, (i.e. due to the selection on the basis of gms absolute alcohol/kilogram body wt), thus resulting in a high drinking AA line with a lower body weight and different energy and total fluid requirements.

There has been a recent advocacy for the use of multivariate techniques to create selection indices based on a composite of various aspects of the trait. Principal components analysis was used to create an index of withdrawai

severity used in the selection of the SEW/MEW mice (Wilson, Erwin, DeFries, Petersen & Cole-Harding, 1984). In the selection for high alcohol drinking rats one could imagine an index based on several measures described in Experiments 2 and 3, including alcohol intake across the different alcohol concentrations as well as the mean alcohol bout size, and alcohol bout frequency. As discussed extensively in Experiment 3, the selection of animals based on the pattern of alcohol intake may provide a more accurate identification of high drinking animals.

THE PROBLEM OF INBREEDING: THE NEED FOR CONTROL AND REPLICATE LINES

As pointed out by Eriksson (1980), a number of factors relating to technical aspects of selection (maintenance of an adequate breeding population, degree of inbreeding, amount of random genetic drift) may have contributed to differences between the various strains selected for alcohol intake. Inbreeding was practiced at some point in all the major alcohol drinking selection studies (Eriksson, 1972; Lumeng et al., 1977; Mardones & Segovia-Riquelme, 1983). Thus differences in the heterogeneity of the foundation stock, combined with different practices with regards to inbreeding was bound to result in very different gene segregation in the various lines, despite similar selection criteria.

None of the selection programs for alcohol intake have maintained a colony of unselected control animals (except the HAD/LAD line). The control line provides a baseline from which the response to selection can be determined and a control for environmental factors that may fluctuate from generation to generation (Crabbe, 1989; DeFries, 1981). As discussed above, it was very difficult to sort out the relationship between neurotransmitter levels (the correlated response) and drinking in the AA/ANA and P/NP lines. This might have been possible, had there been control lines of heterogeneous stock

available for comparison (Murphy, McBride, Lumeng & Li, 1982). In this case, if the control animals had exhibited the same negative correlation between brain serotonin levels and drinking as displayed by the P/NP stock, then the notion that serotonin was involved in alcohol preference would have been confirmed. However, without the control line it was impossible to determine whether in fact the differences in serotonin levels were merely spurious--unrelated to the differences in drinking. Thus it is clear that heterogeneous stock are indispensable tools in the elucidation of the biochemical determinants of alcohol drinking behavior. However, as Roberts (1981) pointed out, the establishment of a control line is only a partial answer to errors that can be introduced in selection studies.

Random genetic drift refers to the changes in frequencies of particular alleles, due to random sampling errors. If only a few parents are chosen to begin a new generation, particular alleles may be lost due to random sampling, leading to random changes in phenotype, unrelated to the selection process (Sinclair, Viitamaa & Hyytia, 1987). The problem that this creates in the interpretation of selection studies is enormous, due to the increased potential for spurious correlations with the selected response (Roberts, 1981; De Fries, 1981). Recently, Sinclair et al. (1987) noted that a very high rate of random genetic drift had occurred during the selection of the AT/ANT rats on the basis of sensitivity to alcohol. The drift was noted in the coat colour of F27 AT/ANT lines; the ANT had lost the albino allele while the AT had lost the agouti allele. Sinclair et al. (1987) concluded that "maintaining selected lines is not the optimal method for finding genetically-based factors related to alcohol sensitivity or other characteristics...." Sinclair et al. (1987) recommended that selection experiments should be abandoned after approximately 10

generations, before the problem of random drift became too serious, and new selection experiments started.

CONCEPTUAL ISSUES RELATED TO SELECTION

The discussion thus far has centered on the technical difficulties of carrying out an adequate selection study. It is clear that very large breeding populations must be maintained, that control lines, (as well as replicates of the high, low and control lines) (Crabbe, 1989; De Fries, 1981; Lynch, 1981) should be maintained, resulting in an enormous economic expenditure.

However, there is a very important conceptual issue related to the basic strategy employed in a genetic selection experiments that should be addressed. As discussed above, the aim of selection is to determine the underlying basis for the differences in the selected lines. The strategy can best be described as a post hoc attempt to understand why the selection worked i.e. why are the lines different? Alcohol is known to produce a myriad of biochemical and physiological actions in the brain--affecting cell membranes, neurotransmitter turnover, calcium uptake and channel function (e.g. Kalant, 1987)-- none of which have been specifically identified with alcohol preference. It is very clear that questions about what determines line differences are being directed at virtually all the processes that alcohol is known to affect. The overall picture is that of a fishing expedition, with every major neurochemical and/or receptor system being measured as potential markers for the differences between lines--an astronomical undertaking. It is plausible to suggest therefore, that this strategy of searching for the biochemical determinant following selection should be abandoned. An alternative strategy might well be to turn the process around and work from biochemistry to behavior.

In such a selection, a biochemical trait of theoretical interest--developed from less costly studies in outbred strains--could be used as the selection criterion,

and animals bred accordingly. For example, activities of catalase and ALDH measured in blood samples could provide a multivariate selection criterion. Such a selection experiment would be a good test of the hypothesis concerning the role of catalase and ALDH in determining the levels of alcohol drinking behavior. As discussed previously, numerous factors have been linked to the regulation of voluntary alcohol intake (catalase, ALDH, serotonin, NE, GABA), through correlational methods as described in Experiment 5, or through the use of pharmacological agents that affect the system of interest. The problems associated with the use of pharmacological agents (uptake inhibitors, receptor antagonists) have been discussed, particularly their specificity of action. Selection on the basis of biochemical traits could be a useful hypothesis testing tool, that would avoid problems associated with correlational and pharmacological approaches. There are a number of breeding strategies that could be employed in the cases where it would be necessary to sacrifice the animals in order to determine the biochemical trait of interest (e.g. retrospective selection where animals are mated and offspring selected retrospectively on the basis of the phenotype of the parents, or sibling selection) (Lynch, 1981).

SUMMARY AND CONCLUSIONS

This thesis has explored voluntary alcohol intake in rodents. The notion that rodents do not drink sufficient alcohol to serve as adequate animal models in alcohol research, is pervasive within the alcohol field. The data presented in this thesis demonstrate that animals drink considerable quantities of alcohol in stable patterns. The amount of alcohol consumed can be determined by the pattern of intake, in terms of the alcohol bout frequency and size, as well as the preference ratio. A comparison of drinking in the outbred Long Evans and genetically selected P/NP rats indicated that high drinking animals of both the Long Evans and P strains consumed alcohol in a similar pattern. Animals

consume sufficient alcohol, in single bouts, to produce measureable blood alcohol levels and to produce effects on locomotor behavior. Taken together, these data suggest that the voluntary preference paradigm, the drinkometer paradigm as well as the limited access paradigm can serve as useful tools in the elucidation of the behavioral and underlying biological determinants of drinking behavior with potential for understanding the factors involved in the development of alcoholism.

Although it is clear that selective breeding for particular phenotypes has been accomplished, the objectives of selective breeding have not. Thus 40 years of endeavor has yielded little information concerning the genetic basis for these traits or any insights into the genetics of alcoholism. The technique has been subject to little critical scrutiny on the part of the scientific community. As currently practiced, the technique of genetic selection suffers from serious methodological and conceptual issues that have rendered many of the results of the current selection experiments uninterpretable. Considering the enormous costs of these types of research programs it is possible that the usefulness of the technique should be questioned and alternate research strategies employed.

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