Do Taste Factors Contribute to the Mediation of Voluntary Ethanol Consumption: An Investigation of Ethanol and Saccharin-Quinine Intake in Non-Selected Laboratory Rats

Frances L.W. Goodwin

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfilment of the Requirements
for the Degree of Master of Arts at
Concordia University
Montreal, Quebec

March 1995

ISBN 0-612-05100-5

Canada

ABSTRACT

Do Taste Factors Contribute to the Mediation of Voluntary Ethanol Consumption: An Investigation of Ethanol and Saccharin-Quinine Intake in Non-Selected Laboratory Rats

Frances Goodwin

Several recent studies have suggested that ethanol-preferring rodents may also have an affinity for sweet solutions (saccharin, sucrose), and conversely, that saccharin preference may predict ethanol preference. The purpose of the present investigation was to determine whether intake of ethanol and saccharin-quinine (SQ) solutions would be related in three non-selected strains of rats who differ in their ethanol preference: Lewis, Wistar Kyoto and Wistar. In the first phase of the experiment, all animals were presented with an ascending series of ethanol solutions (2-10%) in free choice with water, followed by a 10 day maintenance period of 10% ethanol with water. In the second phase, the same animals were presented with an ascending series of SQ solutions (saccharin: 0.4%, quinine: 0.001-0.04%) in free choice with water, followed by a 10 day maintenance period of 0.4% saccharin with 0.04% quinine and water. The results revealed an absence of a direct relationship between ethanol and saccharin-quinine consumption. The ethanol non-preferring Lewis rats showed a greater preference for the SQ solutions than Wistar Kyoto rats, while the ethanol-preferring Wistar Kyoto strain consistently consumed significantly less SQ. Wistar rats showed relatively stable consumption levels for both solutions which fell between those of the other two strains. These results suggested that the relationship between ethanol and SQ preference in rats was not a direct one and did not support the findings in the literature of a simple overall positive relationship between sweet and ethanol preference. These data do however provide further evidence for taste factors in the mediation of self-selection of ethanol in rats.

ACKNOWLEDGEMENTS

I would like to thank Zalman Amit for his much-needed guidance, his much-appreciated enthusiasm for science and life, and for performing the role of mentor with grace.

I would also like to thank Brian Smith for his always-valuable second opinion and for being a calming influence with respect to statistics and wordprocessing.

I thank my father, Harvey, for questioning my hypothesis and my mother, Elizabeth, for never questioning me.

And lastly, I thank Dennis for his armchair editing and never-failing support.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

The consumption of alcoholic beverages is a common behaviour in many cultures worldwide. In the United States, a large proportion of the population consumes alcoholic beverages, and most appear to do so without inflicting harm on themselves or others (Hunt, 1993). These socially-acceptable or "constructive" uses for alcohol include religious rituals, celebrations and social occasions such as cocktail parties (Rivers, 1994). One third of Americans over the age of 18 years are considered abstainers from alcohol while one third are categorized as light drinkers (Clark & Midnick, 1982), however the remaining portion of this population are considered moderate to heavy drinkers and display deviant drinking patterns which cause difficulties for themselves and those around them (Rivers, 1994). An estimated 9 to 12 million people in the United States are considered alcohol abusers (or alcoholics), and it is this group of drinkers that inflicts great economic costs on society as well as personal hardship for themselves and their families (Hunt, 1993). A recent estimate of these costs in the United States was $86 billion, including lost employment, health care and reduced productivity (Rice, Kelman, Miller & Dunmeyer, 1985).

It is well accepted today that people drink alcohol, whether occasionally or to excess, because of complex interactions between biological and environmental factors (Hunt, 1993). However, the manner in which these factors interact to promote drinking is still not clearly understood. Alcoholism in the mid-1970's was viewed as a "chronic, progressive, and potentially fatal disease" (Morse & Flavin, 1992, p. 1012), emphasizing the physiologic sequelae of alcohol use while failing to recognize the range of biopsychosocial factors (including genetic, psychological and environmental factors) that may influence the development of alcoholism and its manifestations. A revision of the definition of alcoholism, as developed by the National Council on Alcoholism and Drug Dependence and the American Society of Addiction Medicine in 1976 (National Council,

1976), has now acknowledged the importance of these factors while attempting to establish a more precise use for the term 'alcoholism', which has become vague and poorly understood if not "morally flavoured" by colloquial use (Morse & Flavin, 1992). The committee defined alcoholism as "a primary, chronic disease with genetic, psychosocial, and environmental factors influencing its development and manifestations" (Morse & Flavin, 1992, p. 1013). There is as yet no consensus among researchers and clinicians with regards to the causes of and classification systems for alcoholism: a 1980 study conducted by the National Institute on Drug Abuse reported 43 different theories on the development of drug abuse, representing biological, social developmental and personality perspectives (Lettieri, Sayers & Pearson, 1980). It was the hope of the revision committee that the revised definition would encourage earlier intervention in the course of alcoholism by professionals and the general population (Morse & Flavin, 1992). Research devoted to the elucidation of the role of biopsychosocial variables, as outlined in the definition, in harmful and excessive patterns of alcohol consumption in humans may provide important information as to the development of addiction itself.

Alcohol Consumption in Humans

Apart from alcohol's well-known behavioral effects, which can include social gregariousness and disinhibition at low levels of consumption, and uncoordination and unconsciousness at higher levels (Rivers, 1994), a person's decision to consume alcohol may also be regulated by other variables, including psychological, situational and sociocultural issues as well as the availability of alcohol in the environment (Madsen, 1974). When alcohol is consumed by humans, taste is generally acknowledged as a primary factor in determining choice of alcoholic beverage, although the rate of onset and intensity of pharmacologic effect or extent of hangover may also determine future beverage selection (York, 1981). The variety of alcoholic beverages available to humans is expansive, offering a wide range of flavours and alcoholic content (Rivers, 1994). For

example, beer, which is a generic term for all malt beverages, is produced by the fermentation of malting barley, corn or rice, and hops (Rivers, 1994). In this process, the aroma, flavour and general characteristics of the beer are determined. Beer may contain 0.5 to 7 percent alcohol by volume. Also, wine is produced as a result of the spontaneous fermentation of juice pressed from ripe grapes, which contain all of the necessary ingredients for the fermentation process: sugar, water and yeast (Rivers, 1994). The alcohol content in wines can range from 10 percent for table wines to 20 percent for fortified wines like vermouth and sherry. Distilled spirits all begin with the fermented alcohol solution from a fruit, grain or carbohydrate, which is then diluted with water and so carries the smell of the source material used (Rivers, 1994). Most commonly known among these in North America is whiskey, commonly produced as rye (distilled from rye grain), bourbon (distilled predominantly from corn), and Canadian whiskeys (distilled at higher proof or alcohol content and therefore have less carry-over of flavouring bodies). Other popular distilled spirits include brandy (distilled from fermented grape juice), rum (distilled from fermented molasses or sugar cane), gin (made from pure ethyl alcohol with added flavouring, usually juniper berry), and vodka (a mixture of alcohol and water without flavouring). Alcohol content of the distilled spirits can range from 40 to 95 percent alcohol by volume.

When 'learning' to consume alcohol, regardless of which type of alcoholic beverage, people exhibit unique patterns of drinking which are developed during early, generally socially-motivated, drinking experiences in adolescence and early adulthood (Jellinek, 1952, 1960). These drinking patterns eventually become more stable in adulthood (Jellinek, 1952, 1960). The familiar phrase "alcohol is an acquired taste" aptly describes this acquisition process, as younger children will commonly reject alcohol. However, teenagers, undergoing rapid growth and change in all facets of their lives, will begin to experiment with alcoholic beverages in many situations. Research has indicated that adolescents in fact tend to self-report higher rates of alcohol use than do adults

(Holland & Griffin, 1984). As adults, they may then arbitrarily become abstainers from alcohol, social drinkers (ranging from light to heavier consumers, yet remaining "intact" or stable in their everyday lives) or frequent heavy drinkers (consuming 5 or more drinks/episode, at least once per week) (Hilton, 1987). Environmental variables may play an important role in the development of stable alcohol consumption patterns in adulthood. One study has suggested that although adolescents may be introduced to their first alcoholic drink in any number of contexts, it is the manner in which they are introduced that seems to have long-term implications for the way they use alcohol as adults (Archambault, 1989). Archambault was examining adolescents and their drinking patterns and found that those teenagers who were introduced to their first drink with parental knowledge experienced fewer alcohol-related problems later in college years (Archambault, 1989). Therefore, although alcohol may seem unpalatable and offensive to children, adolescents will experiment with it in social settings regardless of beverage choice and will eventually develop taste preferences that may influence their drinking as adults.

With the development of more stable drinking patterns in adulthood, a 'range of rejection' for alcoholic beverages is developed which will vary according to taste preferences and consumption levels. Adult social drinkers display a wider range of rejection (or narrower range of preference) for alcoholic beverages than do adolescents, and the heavier social drinkers in turn show a greater "narrowing of the drinking repertoire", displaying a more selective beverage preference and consequent refusal to substitute for a favourite drink(s) (Pattison & Kaufman, 1982). The heaviest consumers, the "skid row" alcoholics, have gone beyond drinking for beverage or taste preference and undergo a recourse to "technical products", controlled only by the need to seek pure alcohol in any accessible fluid, including those which are considered poisonous and uningestible (such as lighter fluid or aftershave) (Jellinek, 1960).

Patterns of alcohol consumption vary dramatically in humans, the factors influencing drinking being too numerous to recount and rarely carrying over from subject

to subject. When considering the influence of environmental variables on the development of patterns of alcohol consumption in adulthood, it should be noted however that the term 'alcohol' refers to any number of beverages varying in alcohol content and taste, as outlined earlier. Beverage choice and taste factors should be recognized as a significant factor in the development of drinking patterns in humans.

Animal Models of Alcoholism

Researchers have long been using animals as subjects in the study of alcoholism (e.g., Cicero, 1980; Dole, 1986; Lester & Freed, 1973). The notion of an animal model of human alcoholism has been popular because it circumvents those difficulties involved in conducting human research, such as the ethical considerations in exposing human alcoholics and normal volunteers to alcohol, confounding nutritional and social variables, and stringent government-regulated guidelines which must be followed in conducting drug-related research (Cicero, 1980). Although it may be impossible to approximate in an animal all of the conditions which promote alcohol consumption in humans, the biological factors which initiate and maintain excessive alcohol consumption in animals may be the same or similar to those in humans (Cicero, 1980). It is not yet completely understood why humans begin abusing alcohol, but as with other self-administered drugs, alcohol is assumed to be primarily ingested for its pharmacological properties (Cicero, 1980). Therefore, its non-drug-related properties, such as taste, smell or caloric value, are assumed by some investigators to be of lesser importance in maintaining this behavior (Lester & Freed, 1973; Waller, McBride, Gatto, Lumeng and Li, 1984). In fact, one proposed critical requirement for an animal model of alcoholism was that the positive reinforcing feature of ethanol should stem primarily from the postabsorptive pharmacological actions of ethanol rather than from its taste and smell (Lester & Freed, 1973). But taste and smell are incontrovertibly tied to ethanol and therefore its consumption, and should not be disregarded in animal studies of alcoholism.

Consummatory Behaviours in Animals

The manner in which taste may guide an animal's consummatory activities is closely linked to the animal's ability to thrive, and should therefore be understood in its own evolutionary perspective before examining it more closely in relation to its specific role in influencing ethanol consumption (Garcia, Hankins & Rusiniak, 1974; Richter & Campbell, 1940b). The ingestion of food and water is perhaps the most important behavior an organism must undertake to ensure its survival (Garcia et al, 1974). The control of ingestion therefore requires "a system that takes into account both the internal state of the animal and the commodities available in the environment so that demands of the one are met by appropriate selections from the other" (Mook & Kenney, 1977, p.276). In coping with the external world, an animal must depend on its accurate interpretation of information via sensory modalities in order to survive (Garcia et al, 1974). It will use vision, audition and olfaction to seek out or avoid external stimuli, recording important time and space information to better orient itself toward or away from stimuli (Garcia et al, 1974). The animal will also respond to signals or demands from its internal sensory receptors in coping with its internal homeostatic environment (Garcia et al, 1974). For example, on the basis of previous experience, an animal must be able to use gustation to accept or reject a food substance before its ingestion may threaten survival. In fact, the ability of rats to make dietary selections seems to depend on the sense of taste more than on the postingestional effects (Richter, 1957; Richter & Campbell, 1940b). On the basis of this gustatory experience, animals will therefore acquire a preference for nutritive materials and an aversion to toxins (Garcia et al, 1974; Halpern & Tapper, 1971; Scott, 1974). In natural settings, sweet substances presumably have a high hedonic or pleasurable value because they usually signify carbohydrate nutrients, whereas bitter substances have low hedonic value because they often may signify alkaloid toxins (Garcia et al, 1974). Therefore, it would follow that animals selecting sweet and avoiding bitter foods would have a higher probability of survival and of passing on their food preferences to offspring (Garcia et al,

1974). Taste therefore provides two useful functions for an organism in the wild: discriminatory and motivational. It plays a discriminatory role which is directed at the outside world, such that an animal learns to associate stimuli with safety or danger (Garcia et al, 1974). In this external learning role, taste information is used in the same manner as any sensory information to direct future behaviors. Taste also plays a motivational role within the homeostatic processes, which is effected even across long delays and again may guide the animal's subsequent actions (Garcia et al, 1974).

Measurement of consumption in Animals: Intake Tests

In measuring the ingestion and rejection of food stimuli in laboratory animals, intake tests were the common method of study (Mook & Kenney, 1977). These tests, conducted both over short- and long-term time periods, involved measuring an animal's intake of the food or fluid of interest over the pre-determined time period. In the case of research on fluid consumption, the procedures often yielded 'preference-aversion functions', whereby the absolute intake of a fluid (or taste substance) was recorded in relation to simultaneous water consumption over the same time period (Schwartz & Grill, 1984). Another common name for this measure was the "preference ratio", which denoted the amount of taste substance consumed as a percentage of total fluid consumption (Schwartz & Grill, 1984). When the taste substance was consumed in smaller quantities than water, the response to the taste of the substance was presumed to be aversion. Ingestion of that taste substance would steadily decrease as a function of its increasing concentration (e.g., quinine hydrochloride dissolved in water) (Kahn & Stellar, 1960). In contrast, when the initial reaction to the taste substance was one of preference over water, ingestion commonly increased with stronger concentrations until a 'ceiling' was attained where further increases in concentration above this maximum concentration resulted in successive decreases in ingestion (e.g., sodium chloride, saccharin, or glucose dissolved in water) (Kahn & Stellar, 1960). Regardless of which response was made by the animal, the ability to detect flavour was measured by the taste threshold: the point at which an animal

first indicated that it could recognize a difference between water and the taste solution (Richter & Campbell, 1940b). When presented with a choice between water and a very dilute flavour solution, the animal may not differentiate between the two fluids immediately, but as the concentration of the flavour increased the preference for the taste solution would fall below or climb above 50 percent of the total fluid consumption (Richter & Campbell, 1940b). The taste threshold, therefore, served as an indication of the animal's taste sensitivity. When ethanol was used as the taste solution, Kahn and Stellar (1960) reported a taste threshold for rats at ethanol concentrations of 0.0039% to 0.0078% (100% ethyl alcohol v/v with tap water). Richter and Campbell (1940a) were the first to report on the preference-aversion curve for ethanol in rats: when presented with ascending concentrations of ethanol (0.01-15% v/v) in a free choice with water, the rats typically showed a greater preference for ethanol solutions up to 6%. Above this concentration, preference for ethanol slipped below that for water, with the greatest preference for ethanol falling between concentrations of 2.4-4.4% (Richter and Campbell, 1940a).

Genetics: Selective Breeding Procedures

The importance of genetic factors in contributing to the risk for the development of alcoholism in humans has received much attention in research and clinical circles (e.g., Peele, 1990; Schuckit, 1985). The data are conflicting regarding the existence of a pure inherited alcoholism, yet they do not rule out the possibility that an inherited predisposition for alcohol abuse may exist, which in turn may interact with environmental variables to augment an individuals' risk for alcohol-related problems (Rivers, 1994). In an attempt to better understand the genetic systems that may be involved in underlying human alcohol abuse, ethanol consumption has been studied in rodents selectively bred for their ethanol preference across several generations. Mardones and his colleagues (Mardones & Segovia-Riquelme, 1983) initially developed two strains of rats in the 1940's through selective inbreeding, which were differentiated by their preference for (UChB) or avoidance of (UChA) a 10% ethanol solution in a free choice situation with water. More currently in use

are three selectively-bred strains of albino rats: Alko, alcohol accepting (AA) and non-accepting rats (ANA) from Finland (Eriksson, 1968); alcohol preferring (P) and non-preferring rats (NP) (Lumeng, Hawkins & Li, 1977) and high (HAD) and low (LAD) alcohol drinking rats (Lumeng, Doolittle & Li, 1986) from the United States. While some researchers may contend that the only feasible way to study oral ethanol consumption in rodents is via these genetically selected strains of rats (e.g., Kalant, 1987; McBride, Murphy, Lumeng & Li, 1989), others have argued to the contrary, that this preoccupation with genetic 'models' of alcoholism may not be the most productive research strategy (Gill, 1989). Recent research has shown that rats bred for variables seemingly unrelated to ethanol intake (e.g., emotional reactivity: Maudsley Reactive; maze learning: Tryon Maze Bright) will exhibit as strong if not stronger affinities for ethanol as those strains selectively-bred for ethanol preference (Amit & Smith, 1992).

Frequently, the selection procedures followed in the rodent breeding programs were dependent upon preference for a single concentration of ethanol (Myers, 1968). For example, the selection criteria for the ethanol-preferring P rats was set at a daily absolute intake of 5.0 grams or more of ethanol/kg of body weight derived from a 10% ethanol solution (95% v/v with water) (Waller, McBride, Lumeng & Li, 1982). Ethanol preference for P rats was also required to be greater than 50% of total fluid consumed daily. The ethanol-non-preferring NP rats were required to consume 1.5 g/kg/day or less of the 10% ethanol solution with an ethanol preference of less than 20% of their total fluid intake (Waller et al, 1982). These selection criteria have been criticized as they rely heavily on an arbitrarily selected ethanol solution as a standard for reproducing the phenomenon of human alcohol dependence, and completely disregard basic pharmacological principles of dose-response analysis which may display differential patterns of preference or aversion at different concentrations of ethanol (Myers, 1968; Myers, 1978; Myers & Veale, 1972). At more dilute concentrations of ethanol, most rodents will prefer the ethanol solution to water, whereas at stronger concentrations they will tend to avoid it (Cicero & Myers,

1968). This criticism was also appropriate for issues of gustation since the taste of the ethanol solution becomes stronger and more aversive as the concentration of ethanol increases (Kahn & Stellar, 1960; Myers, 1961). When P and NP rats were presented with an ascending series of ethanol concentrations (3-30%), the P rats showed preference levels of 50% or higher for ethanol solutions ranging from 3-21.5% (their greatest daily ethanol intake was approximately 10.9 g/kg at 25%) (Lankford, Roscoe, Pennington & Myers, 1991). Ethanol preference in NP rats never rose above 12% of their total fluid intake, regardless of the concentration of ethanol presented (their average daily intake was approximately 2 g/kg/day at 30%) (Lankford et al, 1991). These results were deceiving, as measurement of ethanol at concentrations greater than 20% are highly susceptible to error due to evaporation and spillage. For example, it would only require a 350g rat to consume 4 ml of a 20% ethanol solution to achieve a moderately high absolute ethanol intake of 2 g/kg/day. These results do however support the contention that ethanol consumption can vary greatly across concentrations, perhaps due to an interaction between pharmacological effect and taste.

In order for a selective breeding program to succeed in principle, it was necessary to assume that ethanol preference (or aversion, as in the case of the NP rats) was a heritable trait and so could be bred across generations (Samson, Tolliver, Lumeng & Li, 1989). In studying the interaction between genetics and environmental variables in the regulation of ethanol intake in rats, examinations of the drinking patterns of the ethanol-nonpreferring NP rats were as revealing as studying the drinking patterns of the preferring P rats. In attempting to override the putative influence of lineage with external variables, researchers have tried unsuccessfully to initiate ethanol self-administration in rats selectively bred for low ethanol preference (e.g., George, 1987). In one study, change in ethanol intake was examined after pre-exposing NP rats to ethanol in two alternative acquisition procedures (Samson et al, 1989). In the commonly-used sucrose-substitution procedure, rats were trained to perform an operant for a 20% sucrose solution which was gradually adulterated

with increasing concentrations of ethanol, until eventually only 10% ethanol was available (Samson, 1986). The second procedure involved secondary conditioning whereby rats were required to lick at a drinking tube of 10% ethanol in order to gain access to a fluid dipper containing 10% sucrose (Grant & Samson, 1985). Following stable responding to 10% ethanol, the concentrations were increased in an ascending series to 40%. Over 80% of NP rats examined maintained responding for ethanol up to 40%, ingesting 0.6-0.8 g/kg in 30 minutes (50-150 responses/session) (Samson et al, 1989). Following both procedures, homecage intake and preference for 10% ethanol (in a free choice with water) were increased in both groups compared with pre-acquisition levels; preference rose from 10% to 40% of total fluid consumed (Samson et al, 1989). Although NP rats appeared to increase their ethanol acceptability following either acquisition procedure, their overall intake levels of 10% ethanol continued to be less than those recorded for P rats in general as well as for non-selected Long Evans (LE) rats (Li, Lumeng, McBride & Murphy, 1987; Samson, 1986). The authors concluded that while ethanol may be equally reinforcing for the three strains of rats (i.e. P, NP, LE), its ingestion may be limited in the nonpreferring strain because of additional factors such as taste sensitivity (Samson et al, 1989). The authors also suggested that genetic factors appeared to limit the extent to which ethanol ingestion increased and that perhaps the process of selective breeding for low ethanol preference may change not only ethanol intake values but also the ability of environmental factors such as taste to alter ethanol ingestion (Samson et al, 1989). Overall, these results seem to imply that the aforementioned breeding procedures may not select for ethanol preference or non-preference, but rather for other factors which regulate ethanol intake indirectly (such as taste sensitivity) but as yet are unknown (Amit & Smith, 1992).

"It's A Matter of Taste..."

Flavouring

Several studies have attempted to examine the effects of flavouring ethanol solutions on intake in selectively-bred ethanol preferring and non-preferring strains of rats (e.g., Murphy, Gatto, McBride, Lumeng & Li, 1989; Waller et al, 1982; York, 1981). It was hypothesized that if the pharmacological effects of ethanol were in fact aversive to those animals displaying consistently low preferences for ethanol, increasing the palatability of the solution should not markedly influence their ethanol consumption. York (1981) presented AA and ANA rats (Alko, alcohol accepting and non-accepting rats, respectively) with water and one of several ethanol solutions of differing flavours: a 10% ethanol solution (99% v/v with water), a 10% ethanol solution in Hawaiian punch (sugar content: 11%), red wine (ethanol content: 8.9% w/v; sugar content: 3%), and white wine (ethanol content: 9.6% w/v; sugar content: 1-2%). As would be expected, AA rats consumed more of the plain 10% ethanol diluted in tap water than ANA rats. However when ANA rats were offered white wine or the Hawaiian punch mixture, they markedly increased their ethanol consumption to approximately 80 g/kg/week and actually surpassed the intake of the AA rats, which was approximately 70/g/kg/week. Since it was very likely that some of the pharmacological effects of the ethanol were experienced by the ANA rats while drinking the sweetened solutions, it followed that the reason they were not willing to experience these effects when derived from solutions of ethanol mixed in tap water might be related to the flavour of these beverages (York, 1981). Taste factors were perhaps more salient to the seemingly non-preferring rats than other factors, such as pharmacological effects, in determining their absolute ethanol intake levels. It was of interest to note that when the author presented alcohol-preferring mice (C57BL/6J) and alcohol-nonpreferring mice (DBA/2J) with water and similar flavoured ethanol solutions, C57BL/6J mice consumed more of all alcoholic beverages as compared to DBA/2J mice (York, 1981). Although the presentation of the flavoured alcoholic beverages did not raise the ethanol

intake levels of the alcohol-nonpreferring mice above those of their alcohol-preferring counterparts, there was however a three-fold increase in the overall intake levels of the DBA/2J mice when compared to their baseline consumption of standard 10% ethanol in tap water solution.

In a study examining the effects of flavouring and weight reduction on the development of physical dependence on ethanol in P and NP rats, animals were presented with a 10% ethanol solution (v/v) flavoured with saccharin (0.125%) and sodium chloride (1.0%) (Waller et al, 1982). In conjunction with free access to water and food restriction (80% of the free-feeding weight), mean ethanol consumption in P rats increased from 7 to 14 g/kg/day and in NP rats from 1 to 12 g/kg/day. The results of this study were difficult to interpret as both flavouring and food restriction were manipulated together and it was difficult to determine a role for each of these factors in the dramatic increases in ethanol intake observed. However, the authors claimed that the enhanced consumption was, to a large extent, mediated by the reduced weight of the rats and their increased need for the calories in the ethanol, since it had been shown previously that flavouring alone increased ethanol consumption in the NP rats only to approximately 3 g/kg/day (Lumeng, Penn, Gaff, Hawkins & Li, 1978). Although these intake levels reported by Waller et al (1982) were exceptionally high and have not been replicated in other selectively-bred strains, the results did suggest that external manipulations such as food-restriction and flavouring of the ethanol solution may retain the capacity to elevate ethanol consumption above 'normal' or baseline levels in ethanol non-preferring as well as preferring rats, and therefore that environmental variables such as taste may play an important role in the enhancement of ethanol drinking.

More recent reports did not support the above contention that ethanol consumption in NP rats may be increased when the ethanol flavour was 'masked' (e.g., Murphy et al, 1989). In an attempt to determine whether different concentrations of ethanol other than the traditional 10% (v/v) would support the high and low ethanol preference levels of P and

NP rats, ethanol solutions were mixed with a flavour (banana or almond) to conceal the change in taste of the ethanol solutions as the concentration increased from 2-30% (v/v) (Murphy et al, 1989). Individual preferences for the banana or almond flavour were determined for each animal prior to testing. P rats were given ethanol mixed with their nonpreferred flavour and NP rats were in turn given ethanol mixed with their preferred flavour in order to avoid any possible bias toward an expected result (Murphy et al, 1989). Both groups had access to water ad libitum mixed with the other flavour: the preferred flavour for P rats and the nonpreferred flavour for NP rats. The P rats displayed a preference for ethanol over water at all concentrations, maintaining a constant high ethanol intake of 6 g/kg/day or greater (Murphy et al, 1989). The NP rats, however, preferred ethanol over water only at the 2 & 5% concentration levels, with a mean intake of 1-2 g/kg/day (Murphy et al, 1989). When 10% ethanol was mixed with the preferred flavour for NP rats in the same free-choice situation, they preferred water which was mixed with the nonpreferred flavour. These results were incompatible with previous findings which demonstrated that NP rats increased their ethanol consumption with the added flavouring (Lumeng et al, 1978; Waller et al, 1982). Interpretation of these data is also difficult as the authors provided no evidence that the taste of ethanol was masked by the flavouring, and therefore any conclusions about taste as a limiting factor in ethanol consumption in NP rats were not yet warranted (Murphy et al, 1989).

Olfaction vs. Gustation

In determining specific roles for gustatory and olfactory factors in ethanol consumption in animals, it must be considered that upon initial exposure, ethanol can be distinguished from other fluids by its distinctive taste and smell (Lester, Nachman & LeMagnen, 1970; Sherman, Rusiniak & Garcia, 1984). Naturally, postingestional effects do not develop before the actual consumption (Lester et al, 1970; Sherman et al, 1984). Therefore in an experimental paradigm, it would follow that if under proper experimental conditions the oral cavity could be bypassed in consumption and the digestive tract

accessed directly, an animal's response to ethanol may then be based solely on its reaction to the postingestional effects of the ethanol since any distinctive sensory cues such as taste and smell would be absent (Garcia et al, 1974). In demarcating functions for olfaction versus gustation in setting the hedonic tone of food in general, studies of anosmic animals (those lacking the sense of smell) have indicated that the olfactory system may play a lesser role in food selection than taste (e.g., Garcia et al, 1974; Kahn and Stellar, 1960). When anosmic rats were compared with normal controls for responses to a novel fluid (apple juice, which possesses both flavour and odor), they displayed a weak neophobic, or fearful, reaction (Garcia et al, 1974). When the apple juice was paired with foot shock, the anosmic rats did not acquire the motor avoidance response as compared to normals (Garcia et al, 1974). In contrast, when the apple juice was followed by experimentally-induced illness, the anosmic rats displayed a slightly stronger conditioned aversion than normals (Garcia et al, 1974). Other studies have also shown that normal rats have difficulty developing aversions to odors followed by illness in the absence of taste (Hankins, Garcia & Rusiniak, 1973). Therefore, these results seem to support the contention that taste, and not olfaction, may be the primary modality involved in the development of food aversions, and thus in food selection choices such as ethanol preference.

Kahn and Stellar (1960) were interested in determining whether the preference for ethanol at low concentrations may be mediated by olfaction. They presented rats with ascending concentrations of ethanol solutions and determined the mean maximal preference point (i.e., the highest concentration at which ethanol was preferred over water) and the mean taste threshold (i.e., the lowest concentration at which ethanol could be detected in the solution and preference for ethanol and water were no longer equal). These were found to be 5-6% and 0.0039-0.0078%, respectively. Olfactory bulbs were then surgically removed from these same rats. As a result, Kahn & Stellar (1960) found that the preference-aversion function for the anosmic rats shifted markedly toward the higher concentrations: the taste threshold rose to approximately 2% and the maximal preference

point rose to between 10-12%. The concentration at which preference for ethanol was greatest was 5%, an increase from the pre-surgical range of 0.0313%-3%. The anosmic rats therefore required stronger ethanol solutions to detect differences from water and to reject the ethanol, and they preferred stronger ethanol solutions overall. This study was the first reporting effects of anosmia on ethanol consumption in rats, and Kahn and Stellar (1960) suggested that in the absence of olfaction, ethanol's postingestional effects may have interacted with taste cues to reduce the normal aversion to stronger ethanol concentrations commonly observed in intact rats. These data were supported by later findings that olfactory bulbectomies in mice eliminated ethanol aversions to a 10% solution in alcohol non-preferring BALB/c mice, while they did not abolish the preference for ethanol in alcohol preferring C57BL mice (Nachman, Larue & Le Magnen, 1971). Olfaction, therefore, may influence ethanol consumption in rodents by mediating preference for ethanol at lower concentrations and inhibiting consumption at the higher concentrations (Kahn & Stellar, 1960).

Dicker (1958) was interested in eliminating gustatory sensations in rats by administering methylpentynol carbamate, a compound which acted to dull taste discrimination, and determining its effects on the normal aversion rats show for strong concentrations of ethanol. Rats were first exposed to ethanol in an ascending series of concentrations (4-16%, v/v) at which point no animals showed a preference for ethanol over water. The same animals were then presented with a 20% ethanol solution along with water. Six of the rats that had refused to drink the 20% ethanol solution were treated with oral methylpentynol carbamate (12.5 mg/100 g of body weight) for 5 days while water and 20% ethanol were continually available. Twenty-four hours following the first administration of the drug, the rats began to consume the 20% ethanol solution. Their preference for the ethanol increased to 50% of total fluid consumption, and persisted for several days following the end of drug treatment. The animals eventually reverted to their pre-treatment water intake levels with no ethanol consumption. Two rats that had consumed

the 20% solution during initial testing refused to drink it following the same drug treatment with methylpentynol carbamate. This rejection persisted following drug administration, and the author reported that these animals then rejected a 4% ethanol solution which they had preferred to water prior to drug treatment (Dicker, 1958). Although the methylpentynol carbamate was also found to decrease water intake in control rats allowed free access to food and water, water consumption returned to pre-treatment levels in all animals 4 days following treatment. Methylpentynol carbamate was found to have a generalized suppressant fluid effect as total fluid consumption decreased in both groups without a concurrent decrease in urine excretion, while food consumption remained unchanged. Although these results were difficult to interpret as the drug had an obvious hypodipsic effect, the increase in ethanol consumption in the non-drinking rats and the disappearance of any preference for ethanol in the drinking rats suggested that when taste was dulled the cues for ethanol were no longer available and the animals could perhaps no longer differentiate it from other odourless fluids such as water (Dicker, 1958).

Although the exact neural mechanisms underlying an animal's response to ethanol are unknown, the gustatory neocortex has been implicated in taste related behaviors such as ingestion or rejection of food stimuli (Braun, Lasiter & Kiefer, 1982). For example, previous studies have shown that rats who have had the gustatory neocortex removed (GN rats) had an increased rejection threshold for bitter substances (Benjamin, 1955a, 1955b) and that GN rats consumed highly concentrated solutions of sucrose and sodium chloride in larger amounts than controls (Braun et al, 1982). In a further examination of the effects of gustatory neocortex ablations on ethanol consumption, GN and control rats were presented with a series of ethanol solutions (0.5-12%, 95% ethanol v/v with distilled water) in ascending and descending orders in both restricted (10 or 20 minutes) and continuous access (24 hours) paradigms (Kiefer, Lawrence & Metzler, 1987). Lesion control animals received a lesion in the motor cortex dorsal to the gustatory neocortex, and surgery control animals received anaesthetic only. In the tests of restricted fluid access, GN

rats consumed more ethanol than control rats at certain random concentrations. The tests of continuous access failed to reveal any significant differences in consumption between GN rats and control rats. The overall pattern of ethanol consumption across concentrations by GN rats in all of the conditions resembled that of control rats, suggesting normal taste reactivity (i.e., a consistent reduction in ethanol consumption as the ethanol concentration exceeded 5-6%, greater consumption of ethanol overall in the ascending rather than the descending series). In all experiments, GN rats consumed similar amounts of ethanol but less fluid overall relative to controls (Kiefer et al, 1987). Previous reports have indicated that gustatory neocortex ablations eliminated neophobic reactions to ethanol seen in control rats (Kiefer, Metzler & Lawrence, 1985), and while having no effect on the acquisition of ethanol aversions, the removal of the gustatory neocortex was associated with their rapid extinction as compared to normals (Kiefer et al, 1985; Kiefer, Lawrence & Metzler, 1986). These data suggested therefore that the gustatory neocortex may not play a direct role in the detection of and preference/rejection responses to ethanol in rats, as the GN rats showed a normal responsiveness to ethanol as measured by consumption.

In attempting to separate the oral from the postingestional factors putatively involved in regulating ethanol consumption, experiments have been devised whereby a tasted substance was varied with an ingested substance in such a manner that the respective influence of each variable in regulating fluid intake could be more clearly elucidated (Mook, 1963). Researchers have surgically implanted rats with oesophageal fistulas, that allowed material swallowed to emerge from an opening in the throat, and gastric cannulas, through which solutions could be injected directly into the stomach for digestion and would bypass the mouth (Mook, 1963). In one study, rats implanted with oesophageal fistulas were presented with glucose, sucrose or saline to the mouth and preference-aversion functions were determined when the substance injected into the stomach was identical, different, or absent (i.e., nothing was injected) (Mook, 1963). Normal preference-aversion functions were obtained only when the same or a different substance of osmotically similar value

were both tasted and injected. However, preference for glucose and saline virtually disappeared when only water entered the stomach. These results suggested that fluid intake may be controlled by a complex interaction between oral and postingestional factors, and that the response to a taste may vary depending on what substance reached the stomach (Mook, 1963).

In determining a role for oropharyngeal sensations (i.e., sensations originating in the oral cavity and pharynx during ingestion) in the development of preference and/or aversion for sapid fluids in rats, intragastric self-injection of varying concentrations of saline, glucose, sucrose and saccharin was compared with oral ingestion (Borer, 1968). No preferences for the sweet or dilute salt solutions in relation to water were found as measured by lever-pressing or oral ingestion. Nor did rats receiving the intragastric infusions avoid hypertonic salt solutions as seen in oral consumption. Animals receiving intragastric infusions simply did not discriminate between the two levers, and responded to the fluids according to side preferences. In support of previous findings, these results also suggested that an interaction between oral and postingestional factors may be involved in regulating fluid intake (Mook, 1963). Taste may be required for substance discrimination while postingestional factors may play a role in satiety and hydration (Mook, 1963).

In determining whether taste and postingestional effects may interact in regulating voluntary ethanol consumption, rats were implanted with intragastric fistulas and presented with either water or 17% ethanol in an operant paradigm (Amit & Stern, 1969). One group of the animals were presented simultaneously with water orally and ethanol intragastrically while another group were presented with ethanol orally and water intragastrically. Animals receiving the ethanol intragastrically ingested more than those animals receiving ethanol orally. When the oral dispenser was inoperative for both groups, and lever responding activated only intragastric infusions, both groups infused equal amounts of their respective fluids until the final four days of the experimental period when responding by the group receiving ethanol was greater than that of the water group. However, this preference was

not maintained in home cage consumption (in free choice with water) subsequent to experimentation. These results suggested that taste may be a limiting factor in regulating ethanol consumption in rats, as the animals who did not taste the ethanol ingested significantly more (Amit & Stern, 1969). Therefore, taste may direct an animal toward consumption initially and in conjunction with postingestional effects may determine future consumption.

It has been suggested that the selectively-bred P and NP lines of rats may differ in their innate reactions to the taste of ethanol, thus resulting in their respective high and low ethanol preference levels (Sinclair, Kampov-Polevoy, Stewart & Li, 1992). P and NP rats were surgically implanted with transesophageal catheters for the intragastric delivery of ethanol (Waller et al, 1984). The rats learned to associate drinking an aqueous solution of one of two neutral flavours (almond or banana, 0.5 % per volume) with the intragastric infusion of ethanol (20% v/v), and to associate drinking the second flavour with the intragastric infusion of an equal volume of water alone (Waller et al, 1984). Results showed that P rats consistently self-infused greater volumes of ethanol than did NP rats (NP ethanol intake <1.0 g/kg; P ethanol intake: 3.0-9.4 g/kg). The volume of water self-infused was not different in either group. Blood-ethanol levels in P rats were considerably higher during intragastric self-administration than in free-choice drinking suggesting that the taste of ethanol might have been slightly aversive even to P animals and thereby limited oral consumption (Waller et al, 1984). When water was substituted for ethanol, the oral ingestion of the flavour originally associated with intragastric infusions of ethanol decreased dramatically in P rats (from 12 to 5 g/kg) (Waller et al, 1984). These results demonstrated that animals learned to associate arbitrary flavouring with the postingestional effects of ethanol in regulating their intake. P and NP rats maintained their respective high and low ethanol consumption levels in the absence of taste factors, ruling out the notion that they may differ according to innate taste sensitivities. The authors concluded that the postabsorptive, pharmacological effects of ethanol may be a more important factor

underlying ethanol preference in P rats and ethanol avoidance in NP rats, while innate taste sensitivities may play a lesser role (Waller et al, 1984.)

Taste Reactivity Studies

In both short- and long-term intake studies, where animals consumed a sapid fluid orally in free choice with water, ingestion was commonly measured by comparing the amount of the sapid fluid consumed to that of water (e.g., Richter & Campbell, 1940b). It was therefore presumed that any substance consumed in quantities smaller than water was aversive. However, intake studies did not directly determine aversion but rather the magnitude of intake as measured by volume. A test of taste reactivity was developed to examine in more detail the ingestive and aversive responses of rats elicited by intraoral infusions (i.e., into the mouth or oral cavity) of small volumes of taste stimuli (fluids) (Grill & Norgren, 1978). Orofacial reflexive responses were videotaped through a Plexiglas cage floor, and ingestive and aversive responses were coded and quantified in detail (Grill & Norgren, 1978). Ingestive responses, such as mouth movements and tongue protrusions, were considered consummatory as they functioned to move fluid to the rear of the mouth to be swallowed (Grill, 1985). Aversive responses, such as gapes and passive drips, generally served to move the fluid to the front of the mouth to be expelled or rejected (Grill, 1985). The taste reactivity test also did not require spontaneous ingestion of the fluid stimulus as it was delivered directly into the animal's mouth via cannula which was under experimenter control (Schwartz & Grill, 1984). As the fluids were continuously infused in pre-measured amounts, intake was measured at the same time as the taste reactivity responses were recorded, thereby providing more complete consumption data than simple intake measures (Schwartz & Grill, 1984). Therefore, this technique allowed for a more sensitive measure of the oral responses that accompanied fluid intake, such as the number, duration and pattern of ingestive and aversive response components.

In illustrating the variable relationship between data collected in intake and taste reactivity tests, Schwartz and Grill demonstrated that while hypertonic saline infused

intraorally elicited ingestive responses in rats (mouth movements, tongue protrusions and lateral tongue protrusions), short-term intake measures nevertheless revealed a greater preference for water overall, which would have suggested that the hypertonic saline was aversive to the rats (Schwartz & Grill, 1984). In contrast, sucrose and neutral saline (which are preferred over water by animals, as measured by consumption in traditional intake tests) elicited sequences of ingestive responses that persisted throughout the infusion period (1 ml/min), while quinine hydrochloride (generally consumed in smaller quantities relative to water in intake tests) evoked immediate sequences of purely aversive response components in taste reactivity testing: gapes, chin rubbing, head shakes, and forelimb flail (Schwartz & Grill, 1984). Sucrose, saline and quinine therefore elicited taste responses, both in type and magnitude, that were in agreement with results from intake tests, and the greater the concentration of the 'taste' in the fluid, the greater the number of responses, whether ingestive for sucrose and saline or aversive for quinine (Schwartz & Grill, 1984). Thus, the taste reactivity test provided a far more sensitive examination of the consumption behaviours of rats which revealed information that would not be evident from short-term intake tests.

The taste reactivity paradigm has been a useful tool in determining gustatory responses to ethanol. In one study, naive rats were orally infused with four solutions: 6% ethanol, mixtures of sucrose (0.1 M) with quinine hydrochloride (0.0001 M), hydrochloric acid (0.01 M), or sodium chloride (0.1 M) (Kiefer, Bice, Orr & Dopp, 1990). In comparing taste reactions to the four solutions, no significant difference in the number of ingestive responses was found. Furthermore, all solutions elicited more ingestive than aversive responses overall, although the 6% ethanol solution elicited three times as many aversive responses as the sugar solutions (mainly gapes, passive drips, and head shakes). All sucrose mixtures produced little or no aversive responding. Therefore, while ethanol elicited more aversive responses in naive rats when compared to responding to the three sucrose solutions, ingestive responding for the ethanol was still relatively high, suggesting

a pattern of responding to the ethanol that was more complex than that for the sweet solutions (Kiefer et al, 1990).

Taste reactivity in P and NP rats was also examined to determine whether there were differences which would correspond to their ethanol consumption patterns (Bice & Kiefer, 1990). Ethanol naive P and NP rats were infused intraorally with ethanol (in concentrations of 5, 10, 20, 30, 40%, v/v), sucrose (0.3 M) and quinine (0.0005 M) solutions, followed by a three week intake test of free access to 10% ethanol and water, and a taste reactivity retest. No differences were observed in the number of ingestive or aversive responses elicited by P and NP rats during the first taste reactivity test to any solution. However, P rats made more ingestive and less aversive responses to ethanol during the second taste reactivity test, even when the concentration of ethanol was raised to 40%. NP rats made significantly more mouth movements throughout the testing, a response evaluated as neutral (neither ingestive nor aversive) by the researchers (Bice & Kiefer, 1990). The data also indicated that there were no differences in responses to sucrose, a prototypical ingestive stimulus, or quinine, a prototypical aversive stimulus, between P and NP rats at either test (Bice & Kiefer, 1990). Mean ethanol consumption for the P rats during the two-bottle intake tests was higher than that of the NP rats on all test days. The increase in ingestive responses and decrease in aversive responses to ethanol in P rats at the second taste reactivity test suggested that the palatability of ethanol may have increased after their 3 week exposure to 10% ethanol. Taste reactivity to the ethanol remained unchanged in NP rats from the first to the second taste reactivity test. According to the authc s, the first taste reactivity test was designed to measure innate differences in taste reactivity between the strains, since the rats were naive to any fluids other than water. As no differences were observed in taste reactivity to ethanol in the two sub-strains, this suggested a lack of innate differences between P and NP rats in their taste response to ethanol. There were also no changes in the ingestive or aversive responses to the sucrose or quinine solutions at the second taste reactivity test for P and NP rats, indicating that the

change in taste reactivity in P rats was specific to ethanol. These findings implied that P rats do not show a genetic predisposition to prefer the taste of ethanol over NP rats, but do however demonstrate a tendency to find ethanol more palatable after exposure, as demonstrated in the taste reactivity paradigm by increased ingestive and decreased aversive responding (Bice & Kiefer, 1990).

Rats selectively bred for saccharin preference (Occidental High-Saccharin (HIS) and Low-Saccharin (LOS)) were examined for taste reactivity to 10% ethanol, sucrose, quinine and a sucrose/quinine mixture (Badia-Elder, Kiefer & Dess, 1994). After initial taste reactivity testing to all four fluids, the rats were presented with 10% ethanol in free choice with water in the homecage for 2 weeks, followed by a second taste reactivity test to 10% ethanol only. In the initial taste reactivity test, HIS and LOS rats did not differ in the number of ingestive and aversive responses to any of the fluids. Likewise, there were no significant differences between HIS and LOS rats during the second taste reactivity test to 10% ethanol, however all animals showed increased ingestive and decreased aversive responding for the ethanol. The substrains did not differ in voluntary ethanol intake during the two week exposure. Therefore, HIS and LOS rats selectively bred for saccharin preference displayed similar patterns of ethanol preference as measured by taste reactivity and intake tests, as well as taste reactivity responses to sucrose, quinine and sucrose/quinine (Badia-Elder et al, 1994). These results suggested that as with P rats (Bice & Kiefer, 1990), exposure to 10% ethanol increased palatability in HIS and LOS rats as evidenced by increased ingestive and decreased aversive responding in both strains. These results also suggested that as with humans, alcohol may be an 'acquired taste' for animals: after exposure to ethanol some rats will adapt to the flavour regardless of initial rejection.

Aversion Generalization Studies

In addition to studying intake and gustatory sensitivities to various flavours, researchers have also been interested in determining whether animals 'categorize' taste stimuli as humans do (Tapper & Halpern, 1968; Kiefer & Lawrence, 1988). It is generally agreed that in the human gustatory system, taste information may be divided and then stored in categories such as sweet, salty, sour and bitter sensations (McBurney & Gent, 1979). One experimental technique which may provide information on this process in animals is aversion generalization. This technique requires that animals be conditioned to reject a fluid by pairing it with radiation or chemical poisoning, as in the conditioned taste aversion paradigm (Kiefer & Lawrence, 1988). They are then tested for generalization of this aversion to other taste solutions. The solutions, or test chemicals, examined in this paradigm are assumed to be rejected to the degree that their taste is qualitatively similar to the initially conditioned aversive taste, known as the tastant, and the magnitude of rejection may be an indication of the degree of similarity in taste between the tastant and the test solution (Tapper & Halpern, 1968).

Drug discrimination theory would suggest that when the result of pairing one taste stimulus with poisoning establishes an aversion which in turn can be generalized to another taste stimulus, these two stimuli must share at least one common element (Mackintosh, 1974). This hypothetical common factor may be related to some common chemical structure between the two stimuli, which must nevertheless retain the capacity to elicit a common response component within the sensory system, i.e., a common sensation (Nowlis, Frank & Pfaffman, 1980). This common sensation may be a gustatory sensation, since it has been reported that conditioned aversions are disrupted by the removal of the gustatory neocortex but not by ablations of adjacent neocortical areas (Braun, Slick & Lorden, 1972; Lorden, 1976). Olfactory sensations may play some part in the acquisition of these aversions since animals will invariably smell, then taste the test stimuli before refusing to drink it further (Nowlis et al, 1980).

In attempting to determine a rodent's conception of the four prototypical human taste categories, one group of researchers trained animals to avoid 1 of 27 test solutions by pairing them with apomorphine hydrochloride poisoning (30 mg/kg i.p.) (Nowlis et al, 1980). The animals were then tested for generalization of the aversion to sweet (sucrose), salty (sodium chloride-NaCl), sour (hydrochloric acid-HCl), and bitter (quinine hydrochloride) solutions. Sucrose was rejected by the rodents conditioned to avoid most of the solutions described as sweet by humans: sucrose, fructose, sodium saccharin, glycine, D-phenylalanine, D- and L-alanine, glucose, and sodium cyclamate, with the exception of some of the artificial sweeteners (lead acetate and aspartame). With solutions described as predominantly salty or sour by humans (e.g., $NaNO_3$ and Na_2SO_4, citric and acetic acid, respectively), the animals generalized their aversions to NaCl or HCl. Two of the three solutions described as sour, including HCl, also generalized aversions to quinine. With most of the solutions described as having a predominantly bitter component by humans, the rodents generalized their aversion to quinine, and somewhat more weakly to HCl. These results indicated that there was considerable overlap between sets of stimuli that were capable of eliciting sweet, sour, salty and bitter sensations in rodents and in humans (Nowlis et al, 1980). The results also supported the use of the rodent gustatory system as an appropriate animal model for the physiological analysis of the gustatory nervous system (Nowlis et al, 1980).

The aversion generalization technique has been used to outline an animal's conception of the taste of ethanol. Rats trained to reject 3, 6 or 9% ethanol solutions (95% v/v) by pairing with lithium chloride (0.15 M LiCl, 3% of body weight, i.p.) were then tested for generalization of the aversion to sucrose (0.1 M), saline (0.1 M), hydrochloric acid (0.01 M) and quinine hydrochloride (0.0001 M) solutions, as well as mixtures of the sucrose with quinine, and the saline with hydrochloric acid (Di Lorenzo, Kiefer, Rice & Garcia, 1986). The only significant generalization observed was to the sucrose/quinine mixture from the 6 and 9% ethanol tastants. In a second experiment, another group of rats

were trained to reject only a 6% ethanol solution in the same manner as the previous experiment, and tested with all paired combinations of the above four basic taste solutions (Di Lorenzo et al, 1986). A significant generalization was again observed to the sucrose/quinine mixture and more weakly to the sucrose/hydrochloric acid solution from the 6% ethanol tastant solution.

The above study suggested that ethanol may be endowed with a complex combination of gustatory qualities, one of which may be related to sucrose. Lawrence & Kiefer (1987) examined whether the relationship between ethanol and sweet taste was reciprocal. Rats were trained to avoid either a sweet (sucrose, 0.1 M), bitter (quinine, 0.0001 M) or sucrose/quinine solution and were then tested for aversion generalization to 3, 6, or 9% ethanol solutions (95% v/v). Aversion was induced by intragastric intubation of lithium chloride (0.15 M LiCl, 3% of body weight). Results indicated that aversion to the sucrose and quinine solutions alone generalized only to the 6% ethanol mixture, while aversion to the sucrose/quinine mixture carried over to both the 6% and 9% ethanol solutions. In a second experiment, which examined sweet and sour taste combinations, rats were trained to reject a sweet (sucrose, 0.1 M), sour (hydrochloric acid, 0.01 M), or sucrose/hydrochloric acid solution and tested for generalization to the same ethanol solutions (Lawrence & Kiefer, 1987). No generalization was found to any of the ethanol solutions. Overall, these results supported the contention (Di Lorenzo et al, 1986) that ethanol may represent a sweet-bitter taste in animals. The sucrose/hydrochloric acid aversion generalization found in an earlier study (Di Lorenzo et al, 1986) was not replicated, suggesting that the sour component in ethanol may be weak (Lawrence & Kiefer, 1987). The aversion generalizations found also seemed to be dependent on ethanol concentrations, since no aversions were found when animals were tested with the 3% ethanol solution and only weak generalizations were noted to the 9% solution. The 6% ethanol solution yielded the most consistent aversion generalizations, which may reflect the fact that this concentration had a significant taste yet was not strong enough to reduce

consumption (Lawrence & Kiefer, 1987). The inconsistent responses to the three ethanol solutions may indicate different gustatory qualities of the 3, 6 and 9% ethanol, further suggesting that a rat may perceive all three concentrations as different fluid categories rather than the same fluid at different concentrations (Lawrence & Kiefer, 1987).

In questioning the notion that ethanol may have a general sweet taste that was not specific to sucrose, Kiefer & Lawrence (1988) trained rats to avoid a 5% ethanol solution (95%, v/v) by pairing with lithium chloride injections (LiCl 3% body wt of a 0.15 M solution, i.p.) and tested for generalization of the aversion to four sweet/quinine hydrochloride (0.0001 M) mixtures: sucrose (0.1 M), glucose (0.75 M), fructose (0.3 M), and sodium saccharin (0.0001 M). The rats generalized their aversions to all four mixtures, suggesting that the taste of the 5% ethanol solution had a general sweet component (with a bitter component) that was not specific to the taste of one sweetener (e.g., sucrose). When the experiment was repeated with hydrochloric acid (0.01 M) substituted for quinine, no generalizations were found to any of the sweet/sour solutions, suggesting that the bitter taste component in the 5% ethanol could not be replaced with a sour component (Kiefer & Lawrence, 1988). Rats were then trained to avoid a 6% ethanol solution by lithium chloride injection (0.15 M LiCl i.p., 3% of body weight) and tested for generalization to several sucrose/quinine hydrochloride mixtures of varying concentrations to determine whether the effect was concentration dependent (Kiefer & Lawrence, 1988). The rats showed significant aversions to all of the solutions tested, indicating that the generalization of learned aversions to sucrose/quinine was not dependent on the specific concentrations of sucrose or quinine hydrochloride. These results suggested that ethanol may have a taste which contains a combination of components: a general sweet component and a quinine-like component, which is generally bitter in nature (Kiefer & Lawrence, 1988). The mixture of the sweet and bitter taste components must yield a new taste not characterized by the simple sum of the individual components, based on the fact that ethanol generalizations do not carry over to either sweet or the bitter tastes alone (Di Lorenzo et al, 1986).

Although taste reactivity experiments demonstrated little similarity in aversive responding to a 6% ethanol solution when compared to a sucrose/quinine mixture (Kiefer et al, 1990), it was of interest to determine whether following ethanol aversion training, the taste reactivity pattern to several sucrose mixtures would correspond with that of the reactivity to ethanol itself. Rats were trained to avoid a 6% ethanol solution by lithium chloride intubation (0.15 M LiCl, 3% of body weight), and were then tested for taste reactivity to a 6% ethanol solution as well as to three sucrose (0.1 M) mixtures: quinine hydrochloride (0.0001 M), hydrochloric acid (0.01 M), or sodium chloride (0.1 M). Control animals received either sodium chloride by intubation (0.15 M NaCl, 3% of body weight) or lithium chloride intubation paired with distilled water. The aversion training resulted in fewer ingestive responses and more aversive responses to the 6 % ethanol solution as compared to control rats. Taste reactivity responses of the rats to the sucrose/quinine mixture were similar to their responses to ethanol: fewer ingestive responses and more aversive responses as compared to controls. The number of aversive responses elicited by the ethanol and sucrose/quinine solutions did not differ. Reactivity to the sucrose/hydrochloric acid and sucrose/sodium chloride solutions did not differ between aversion trained and control rats. Therefore, relative to the response of the control rats, these results suggested that a sucrose/quinine solution may have a perceived taste (as inferred from orofacial responses) similar to that of 6% ethanol when the latter was made highly 'unpalatable' through aversion conditioning (Kiefer et al, 1990). This conclusion was somewhat tentative as the control groups also showed a level of aversive responding to the sucrose/quinine solution which approximated that elicited by the ethanol (Kiefer et al, 1990). However, the authors concluded that the sucrose/quinine mixture was avoided by rats with ethanol aversions because the aversion training may have rendered the sucrose/quinine mixture as well as the ethanol solution unpalatable (Kiefer et al, 1990).

The Relationship between Ethanol and Sweet Preference

There was some question as to whether selective breeding for high and low oral

ethanol consumption may also result in different preferences for various flavoured, nonpharmacological solutions (Sinclair et al, 1992; Stewart, Russell, Lumeng, Li & Murphy, 1994). Although taste sensitivity did not appear to play a major role in determining the different levels of ethanol intake in the P and NP rats (Bice & Kiefer, 1990), there was evidence which suggested that selective breeding for ethanol preference may also have been associated with the development of preferences or aversions for certain nonpharmacological substances that possessed salient flavours. This was observed in selectively-bred mouse strains where ethanol-avoiding DBA/2J mice drank less of a saccharin solution than ethanol-preferring C57BL/6J mice (McClearn and Rogers, 1961; Forgie, Beyerstein & Alexander, 1988; Ramirez and Sprott, 1978). The AA and ANA, P and NP, and Wistar strains of rats were examined for their preferences for sweet (saccharin), salty (sodium chloride). bitter (quinine), and sour (citric acid) solutions (Sinclair et al, 1992). Both lines of rats developed for low ethanol consumption, the ANA and NP strains, drank much less saccharin when tested both with a single concentration (1 g/l) and with an ascending series (0.002-4.0 g/l), as compared with their respective sub-strains developed for high ethanol intake. ANA rats also drank less bitter, salty and sour solutions than the ethanol-preferring AA rats and non-selected Wistar rats. However, no such difference was found between NP and P rats. In the ascending series, saccharin consumption reached a maximum level of intake at approximately the same concentrations for AA, Wistar, NP and P lines but not for ANA rats, who discontinued their drinking at a lower concentration (Sinclair et al, 1992). Also, the preference threshold (i.e., the lowest concentration at which significantly more saccharin solution was consumed than water) was 4 mg/l for ANA rats, in contrast to 8 mg/l for Wistars, 16 mg/l for AAs (Sinclair et al, 1992) and a previously reported value of 37-55 mg/l for Sprague-Dawley rats (Touzani, Akarid & Velley, 1991). This suggested that ANA rats were more sensitive to the flavours (such as saccharin, sodium chloride, quinine and citric acid) compared to the other strains, and rejected them at more dilute concentrations in preference for water. NP rats only

showed a significant saccharin preference at the 256 mg/l concentration while the P rats began preferring the saccharin to water at a more dilute solution of 64 mg/l, suggesting that P rats were more sensitive to the taste of the saccharin in water and could identify it at weaker concentrations than NP rats (Sinclair et al, 1992). Because the two pairs of selectively-bred lines were derived independently from different foundation stocks in two different breeding programs, their similar variance in preference for ethanol and saccharin supported the contention that there may be an association in the consumption of the two fluids (Sinclair et al, 1992).

In further support of the above contention, P and NP rats were also found to differ in their consumption of a series of sucrose solutions (0.5-64.0 g/100 ml) when presented in free-choice with water over a 24 hour period (Stewart et al, 1994). P rats consumed greater amounts of the sucrose solutions than did NP rats. However, when presented with a series of salty solutions (NaCl 0.025-3.2 g/100 ml), NP rats consumed greater amounts than P rats, although this difference was not as great as that found with sucrose solutions. No differences were found between the substrains in consumption of sour (sucrose octaacetate 0.002-0.512 g/litre) or bitter (citric acid 0.008-2.048 g/litre) solutions. Closer examination of the ethanol and sucrose drinking behaviors established that despite the fact that P rats had a consistently greater preference for and intake of ethanol solutions compared with NP rats, both lines showed a preference for the sucrose solution over water. Therefore, although the higher preference for the sweet solution in P rats was deceiving as NP rats also preferred sweet solutions over water, P rats did consistently consume it in greater amounts (Stewart et al, 1994).

In determining whether there was a relationship between affinity for the taste of ethanol and for sweet or bitter tastes, Le Magnen and Marfaing-Jallat (1961) examined ethanol and quinine consumption in unselected high and low ethanol consuming rats. Intake of a 6% ethanol solution in free choice with water was recorded for all rats and they were divided into two groups based on mean ethanol preference: high drinkers= 39% of

total fluid consumption, non-drinkers= 3%. The rats were then presented with a series of quinine solutions (56×10^{-6} %, on an ascending scale in log units) and an ascending series of ethanol solutions (1-31.6 %, v/v, log unit increments), both in free choice with water. Every solution was presented for 4 consecutive days. The criteria for rejection was set at a preference for the quinine or ethanol of 40% or less of their total fluid intake. The non-drinkers were found to reject the quinine solutions at .00028% and the drinkers at .000687%. Thus, the non-drinkers appeared to be more sensitive to the taste of quinine in the water and rejected it at a weaker concentration than the drinkers. The non-drinkers also fell below the 40% preference cut-off criteria for ethanol at the 3.63% concentration, whereas the drinkers did so at 6.5%. When the criteria for rejection was raised to 20% or less of total fluid consumption, a significant correlation between quinine and ethanol consumption across both groups of animals was observed ($r = {}^{+}0.53$). The authors concluded that ethanol drinking may be affected by an innate, generalized "tendency to avoid" on the basis of gustatory sensitivity (Le Magnen & Marfaing-Jallat, 1961).

Another group of researchers were interested in pursuing the idea that selection for the consumption of bitter and sweet solutions may be related to subsequent ethanol preference (Kampov-Polevoy, Kasheffskaya & Sinclair, 1990). Non-selected, male albino rats were presented with a series of fluids in free choice with water: 0.0025% quinine (days 1-3), 0.1% saccharin (days 4-7), 15% ethanol (three weeks: days 8-28) solutions, and a mixture of the ethanol and saccharin solutions (days 29-32). As the ethanol consumption in the first week of exposure (days 8-14) was distributed bimodally among the rats according to preference for the ethanol solution over water (60% high preferring and 40% low preferring), the rats were then divided into high and low drinking groups for subsequent fluid preference analyses. The low drinkers consumed less quinine and saccharin overall compared to the high drinkers. The low drinkers also increased their ethanol intake over weeks 2 and 3 (days 15-28) of the exposure to the 15% ethanol solution such that they eventually matched the intake levels of the high drinking group. The intake levels of the

high drinkers remained unchanged during the 3 week exposure to the ethanol. Saccharin and quinine intakes were significantly correlated with mean ethanol consumption during the first week of exposure across all animals (saccharin/ethanol, r= +.33; quinine/ethanol, r= +.25) suggesting that individual gustatory differences may influence or at least be related to initial ethanol intake (Kampov-Polevoy et al, 1990). Ethanol consumption during weeks 2 and 3 was also significantly correlated (r= +.48), suggesting a stabilization of ethanol intake levels by the second week of exposure. Although saccharin and quinine consumption were not related to the stable levels of intake observed during the second and third weeks of ethanol consumption, it should be noted that the level of ethanol consumption in the high drinkers remained the same throughout the three week period while the low drinkers increased their consumption. Therefore, ethanol intake in the second and third weeks among high drinkers may have been correlated with their saccharin and quinine consumption while that of the low drinkers was not due to their increasing ethanol intake. However, the authors concluded that individual differences in tastes for saccharin and quinine were related only to the initial selection of 15% ethanol during the first week of access (Kampov-Polevoy et al, 1990).

In another study examining whether saccharin intake could predict ethanol intake in non-selected Wistar rats, animals were subdivided according to high, medium and low intake of a 0.1% saccharin solution (animals were presented with the saccharin and water for one hour daily over two weeks). Mean consumption for high drinking rats was 8.8 ml, mean medium consumption was 3.5 ml, and mean low consumption was 0.9 ml (Gosnell & Krahn, 1992). The rats were then presented with each of 2, 4, 6, 8% ethanol solutions for 1 hour across 7 days, in both ascending and descending orders after which they were retested for saccharin preference. The rats were food deprived overnight prior to each ethanol exposure. No differences in ethanol intake were observed between the three groups. During the second ascending presentation of ethanol solutions food was no longer restricted and ethanol consumption in the medium and high saccharin-preferring groups

was found to be significantly greater as compared to the low saccharin-preferring group. The retest of saccharin preferences revealed that all groups consumed significantly more saccharin overall: mean high consumption= 13.3 ml, mean medium consumption= 8.5 ml and mean low consumption= 5.8 ml. These results suggested, therefore, that rats screened for high levels of voluntary saccharin intake tended to consume more ethanol compared to rats selected for low levels of saccharin intake (Gosnell & Krahn, 1992). The authors speculated that this finding may have been due to a similarity in the taste qualities of ethanol and saccharin (Gosnell & Krahn, 1992). During the one hour access to the ethanol solutions, the rats may not have ingested enough ethanol to allow an association with its postingestive effects (8-12 ml/1 hour when food was restricted, 1-5 ml/1 hour when food was ad libitum), so the possibility that differences in ethanol intake were due to taste alone cannot be ruled out (Gosnell & Krahn, 1992). It is of interest to note that while levels of saccharin intake were increased during retest across all groups, the relative standings of the subgroups remained stable. This suggested that the group differences in saccharin intake were due to stable preferences for saccharin rather than to neophobic reactions to a novel taste or rates of adaptation to the testing procedure (Gosnell & Krahn, 1992).

The relationship between saccharin and ethanol intake was further examined in several ethanol preferring and non-preferring strains of rats (Overstreet, Kampov-Polevoy, Rezvani, Murrelle, Halikas & Janowski, 1993). Three strains of ethanol preferring rats were selected: P (alcohol-preferring), FH (Fawn Hooded) and MR (Maudsley Reactive). Four ethanol non-preferring strains were also used: NP (alcohol-nonpreferring), MNRA (Maudsley Nonreactive), and FSL/FRL (Flinders Line, selectively bred for cholinergic sensitivity and normal or depressive-like activity). All animals were presented with a series of fluids in free choice with water: 0.25% quinine (days 1-4), 0.1% saccharin (days 5-8), and 10% ethanol (days 9-28). The overall level of quinine intake in all groups was very low (1 ml or less) and no differences were observed between the groups. FH rats drank more saccharin than any other group, while P and MNRA rats drank more than the

remaining groups. Saccharin preference was high in all groups, ranging from 65-99% of total fluid intake. Mean ethanol consumption over the last 4 days of exposure revealed that FH rats drank significantly more than any other groups while P rats consumed more than the other groups. Ethanol preference as a function of total daily fluid consumption ranged from 4-60%. The correlations between ethanol and saccharin intakes across all animals was highly significant ($r = {}^{+}0.61$), and the correlation within the strains was higher ($r = {}^{+}0.87$). Ethanol and saccharin intakes were also significantly correlated when specific subgroups of drinking and nondrinking groups were compared: FH/NP, $r= {}^{+}0.88$; P/NP, $r= {}^{+}58$; MR/MNRA, $r= {}^{+}0.75$ (although unexpectedly in this case, the MNRA animals drank more of all fluids than the MR animals). These results supported the notion that there may be a relationship between ethanol and saccharin consumption in rats, perhaps due to taste factors.

The Present Experiment

Research to date on taste and ethanol intake in rats has yet to culminate in reliable theory or clinical application. Ethanol intake studies using rats selectively-bred for ethanol preference have indicated that the taste of ethanol may limit its intake in low alcohol-preferring rats, as flavouring of ethanol solutions was found to increase ethanol intake in the low alcohol-preferring ANA rats, NP rats, and DBA/2J mice (Lumeng et al, 1978; Waller et al, 1982; York, 1981). Intragastric delivery of ethanol, which largely eliminated taste as a factor in ethanol intake, resulted in higher ethanol intake and blood ethanol levels in P rats (Waller et al, 1984) than those found in P rats during free-choice drinking (Murphy, McBride, Lumeng & Li, 1983). Intragastric delivery also resulted in higher rates of responding to ethanol as compared to responding to intragastric water in non-selected hooded rats (Amit & Stern, 1969). These experiments suggested that the taste of ethanol might be aversive to all rats, thereby limiting its intake regardless of strain. Research on taste reactivity in naive P and NP rats revealed no innate differences in taste sensitivities to

ethanol, sucrose or quinine, while exposure to ethanol appeared to increase the palatability of ethanol only in P rats (Bice & Kiefer, 1990). Surgical interventions isolating olfactory or gustatory factors from fluid ingestion have shown that anosmic rats preferred, or showed less aversion to, stronger concentrations of ethanol than intact rats (Kahn & Stellar, 1960) and rats with gustatory neocortex ablations were not different in ethanol consumption from normals (Braun et al, 1982). Finally, aversion generalization studies have revealed that the taste of ethanol may be a complex combination of gustatory qualities: 6% ethanol was reliably found to have both sweet and bitter components (Di Lorenzo et al, 1986; Kiefer et al, 1990; Lawrence & Kiefer, 1987).

Early taste research revealed a strong relationship between ethanol and quinine preference in non-selected rats (Le Magnen & Marfaing-Jallat, 1961). More recent studies have been focusing on a putative association between the consumption of ethanol and sweetened fluids in rats. Ethanol and saccharin consumption in AA and ANA rats, P and NP rats (Kampov-Polevoy et al, 1990; Sinclair et al, 1992), and C57BL/6J and DBA/2J mice (McClearn & Rogers, 1961) was found to be positively correlated (i.e., higher ethanol-preferring strains were found to consume more saccharin than their low ethanol-preferring counterparts). P rats were also found to consume more sucrose solution than NP rats (Stewart et al, 1994). Saccharin consumption in non-selected rats was found to predict ethanol consumption (Gosnell & Krahn, 1992). These studies, therefore, supported the notion that ethanol intake may be related to the consumption of sweetened fluids in animals.

The present experiment was designed as a further examination of the relationship between ethanol intake and taste sensitivity for, or the consumption of, a novel, sweetened fluid. Three strains of albino rats were chosen according to their ethanol preference levels: Lewis (high ethanol-consuming (Suzuki, George & Meisch, 1988)), Wistar Kyoto (low ethanol-consuming (Cannon & Carrell, 1987; Spuhler & Deitrich, 1984)) and non-selected Wistar rats. All animals were exposed to ethanol during acquisition (2-10% alternate days) and maintenance (10% everyday) periods in the first phase of the experiment, and

saccharin/quinine (SQ) acquisition (0.4% sodium saccharin/ 0.001-0.04% quinine sulfate alternate days) and maintenance (0.4% sodium saccharin/0.04% quinine sulfate everyday) periods in the second phase. While other studies have used saccharin only as the alternate fluid to ethanol, it was felt that the gradual additions of the bitter quinine to the saccharin solution would equate the palatability of the two solutions and thus justify the comparison of ethanol with SQ intake across strains. Previous work in our laboratory using the same acquisition-maintenance paradigms has shown preference for ethanol and saccharin-quinine solutions (relative to water) to be approximately similar during the maintenance phase (Rotzinger, 1994). In the present experiment, ethanol and SQ were continuously available in free choice with water in the home cages, and fluid intakes were recorded daily. Of particular interest was whether preference for ethanol would be positively correlated with preference for SQ. Between group differences were examined comparing Lewis, Wistar Kyoto and Wistar rats in order to verify the presence of consistent variance in consumption attributable to strain.

METHOD

Subjects

The subjects in Phase 1 of the experiment were 28 Lewis, 30 Wistar Kyoto and 34 Wistar male rats weighing 221-295g, 220-296g and 267-394g respectively at the start of the experiment (Charles River Breeding Farms, QC). The subjects in Phase 2 of the experiment were the same as those in Phase 1. One Lewis and two Wistar rats were withdrawn at Phase 2 due to illness. Thus, at the start of Phase 2, there were 27 Lewis, 30 Wistar Kyoto and 32 Wistar rats weighing 350-421g, 316-410g and 417-540g respectively. All animals were housed individually in stainless steel cages in a humidity and temperature controlled animal colony maintained on a 12 hour light/dark cycle (lights on at 0800h, lights off at 2000h). Rats chow (Agway) was available ad lib, and all fluids were presented in two glass Richter type tubes mounted on the front of the cages.

Procedure

Phase 1. Following one week acclimatization to the colony, the rats were exposed to an ethanol acquisition schedule in which ethanol was given in an ascending series of concentrations in a free choice with water on alternate days. On intervening days, water only was available in both tubes. The first presentation was of a 2% (v/v) ethanol solution (acquisition day 1), prepared by mixing a 95% stock solution with tap water. On each subsequent ethanol presentation, the concentration was increased by 1% to a final concentration of 10% (acquisition day 9). Following the last water day, the animals were presented with 10% ethanol everyday in a free choice with water for a 10 day maintenance period. The position of the Richter tubes was alternated with each ethanol presentation to control for side preferences.

Phase 2. Immediately following the completion of Phase 1, the same rats as were subjects for Phase 1 were given water and food ad libitum for a one week wash-out period.

All rats were then exposed to a free choice schedule of sodium saccharin/quinine sulfate and water. On day 1 of the acquisition phase, the rats were offered 0.4% sodium saccharin solution in free choice with water, prepared by dissolving an aliquot of saccharin sodium (Mallinckrodt) with tap water. On subsequent presentations of the saccharin solution, quinine sulfate (Fisher Scientific) was added to the saccharin solution in increasing concentrations to approximate preference levels observed during the exposure to the ethanol acquisition schedule in Phase 1. The concentrations of quinine sulfate used were: 0.001% (acquisition day 2), 0.002% (day 3), 0.003% (day 4), 0.004% (day 5), 0.006% (day 6), 0.009% (day 7), 0.011% (day 8), 0.015% (day 9), 0.03% (day 10), 0.04% (day 11). Saccharin concentrations were held constant at 0.4% throughout. During the screening the saccharin-quinine solutions were presented on alternate days, with water only available in both tubes on intervening days. Following the last water day, the animals were presented with a 0.4% saccharin/0.04% quinine solution everyday in a free choice with water for a 10 day maintenance period. The position of the Richter tubes was alternated with each saccharin-quinine presentation to control for side preferences.

Throughout Phase 1 and Phase 2, fluid consumption (ml) was measured daily and body weights (g) were measured every two days.

RESULTS

Results are reported separately for Phase 1 and Phase 2. A two-way analysis of variance with repeated measures (fluid x days; strain x days) (Minium, King & Bear, 1993) was conducted on each daily measure of fluid intake (in ml, g/kg and ml/kg), fluid preference (% total fluid consumption), total fluid consumption (ml/kg) and weight (g) for Lewis, Wistar Kyoto and Wistar rats. Where noted, solution concentrations follow mention of specific days within experimental phases (e.g., acquisition day 1 (A1: 2% ethanol)). Analyses were conducted within strains as well as between strains (cross-strain comparison).

Phase I: Ethanol Acquisition and Maintenance

Within-strain Analyses:

1) Lewis: The top panel of Figure 1 represents data for mean ethanol and water consumption (ml) in Lewis rats during Phase 1 of the experiment. A two-way analysis of variance with repeated measures (fluid x days) yielded a significant difference in ethanol and water consumption during both the acquisition period [$F(1,54)=20.31$, $p<.0001$] and the maintenance period [$F(1.54)=524.70$, $p<.0001$]. There were also significant interactions of fluid type across days during the acquisition [$F(8,432)=57.01$, $p<.0001$] and maintenance phases [$F(9,486)=3.29$, $p<.001$]. Ethanol was consumed in greater quantities relative to water ($p<.01$) from acquisition day 1 (A1: 2% ethanol) until day 4 (A4: 5% ethanol) when there was no difference in ethanol and water consumption ($p>.05$). Water was then consumed in greater quantities than ethanol from acquisition day 5 (A5: 6% ethanol) ($p<.001$) through to the end of the maintenance phase (M10: 10% ethanol) ($p<.001$). Ethanol intake remained stable during the maintenance phase ($p>.05$) while water consumption varied across days ($p<.01$).

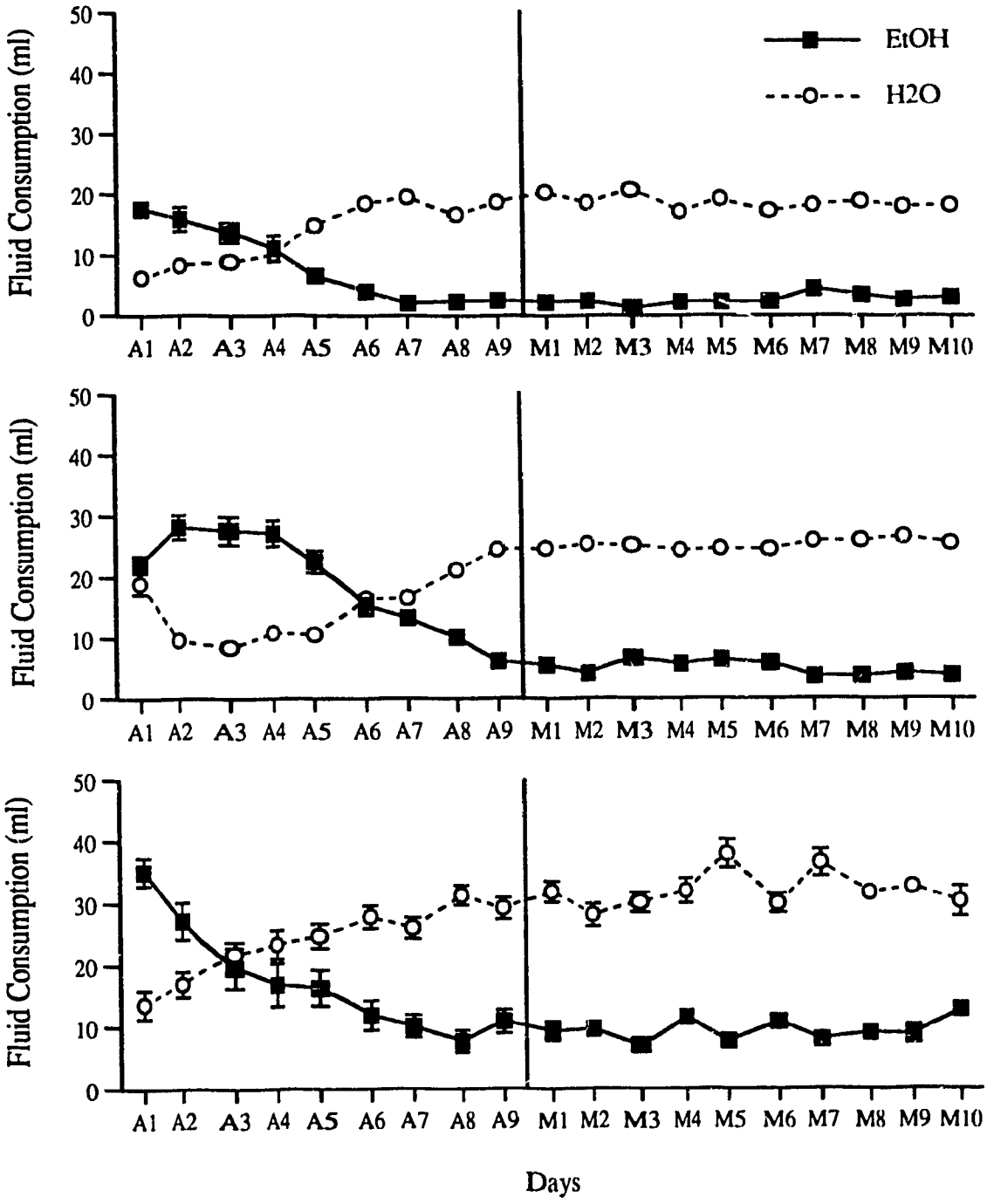

Figure 1. Mean (+/- SEM) ethanol (filled circles) and water (open circles) consumption in ml in Lewis rats (top panel), Wistar Kyoto rats (middle panel) and Wistar rats (bottom panel) during ethanol acquisition and maintenance periods.

2) Wistar Kyoto: Data for mean ethanol and water consumption in Wistar Kyoto rats across the ethanol acquisition and maintenance phases are presented in the middle panel of Figure 1. A two-way analysis of variance with repeated measures (fluid x days) yielded a significant difference in fluid consumption during the acquisition [$F(1,58)=6.97$, $p<.05$] and maintenance phases [$F(1,58)=433.90$, $p<.0001$]. The interaction of ethanol concentration across days was also significant during acquisition [$F(8,464)=68.95$, $p<.0001$] and maintenance [$F(9,522)=3.37$, $p<.001$]. There was no difference in ethanol and water consumption on acquisition day 1 (A1: 2% ethanol). Ethanol was consumed in greater quantities than water ($p<.001$) until acquisition days 6 and 7 (A6: 7% ethanol, A7: 8% ethanol) when there were no differences in ethanol and water intake. Water was then consumed in greater quantities than ethanol from acquisition day 8 (A8: 9% ethanol) ($p<.001$) through to the end of the maintenance phase (M10: 10% ethanol) ($p<.001$). Water consumption remained stable across the maintenance phase ($p>.05$) while ethanol intake was variable ($p<.01$).

3) Wistar: Data for mean ethanol and water consumption in Wistar rats during Phase 1 are presented in the bottom panel of Figure 1. A two-way analysis of variance with repeated measures (fluid x days) yielded significant differences in ethanol and water consumption during the acquisition [$F(1,66)=8.03$, $p<.0001$] and maintenance phases [$F(1,66)=146.15$, $p<.0001$]. There was also a significant interaction of fluid type across days during both acquisition [$F(8,528)=35.94$, $p<.0001$] and maintenance [$F(9,594)=9.06$, $p<.0001$]. Ethanol was consumed in greater quantities than water ($p<.001$) until acquisition day 3 (A3: 4% ethanol) when there was no difference in ethanol and water consumption ($p>.05$). Water was then consumed in greater quantities than ethanol from acquisition day 4 (A4: 5% ethanol) ($p<.05$) through to the end of the maintenance phase (M10: 10% ethanol) ($p<.001$). Ethanol and water consumption was not stable across days during the maintenance phase ($p>.05$).

Cross-strain Analyses:

1) Ethanol Acquisition: Mean ethanol intake (g/kg) for Lewis, Wistar Kyoto and Wistar rats during ethanol acquisition and maintenance phases are presented in Figure 2. A two-way analysis of variance with repeated measures (strain x days) yielded an overall difference in ethanol intake during the acquisition phase [$F(2,89)=20.71$, $p<.0001$]. Post hoc Tukey tests revealed that Wistar Kyoto rats consumed more ethanol during acquisition than Lewis and Wistar rats ($p<.01$). Lewis rats revealed the lowest ethanol intake pattern during acquisition ($p<.01$). A significant interaction of strain across days of the acquisition phase [$F(16,712)=7.25$, $p<.0001$] followed by tests of simple effects revealed that ethanol intake was significantly different between the strains on all days of acquisition except day 1 (A1: 2% ethanol). While ethanol intake in Lewis and Wistar Kyoto rats was variable across the acquisition phase as the concentration of ethanol increased ($p<.001$), intake by Wistar rats remained stable ($p>.05$).

Preference for ethanol over water was also different between strains during acquisition [$F(2,89)=8.69$, $p<.001$] (Fig. 3). Post hoc Tukey tests revealed that Wistar Kyoto rats showed a greater preference for ethanol compared to Lewis and Wistar rats ($p<.01$). Preference for ethanol was not different between Lewis and Wistar rats ($p>.05$), both displaying significantly lower preference levels than Wistar Kyoto rats ($p<.01$). A significant interaction of strain by ethanol concentration across days [$F(16,712)=8.54$, $p>.0001$] with tests of simple effects revealed that the strains had significantly different preference levels on all days ($p<.05$) except acquisition day 2 (A2: 3% ethanol) and day 9 (A9: 10% ethanol) ($p>.05$). Preference for ethanol relative to water decreased in all strains during the acquisition phase as the ethanol solution presented became more concentrated ($p<.001$)

Total fluid consumption was calculated by means of fluid consumed per unit of body weight (ml/kg) to account for a great disparity in size between the strains (see Fig. 5). Analysis of total fluid intake during the ethanol acquisition phase revealed significant strain

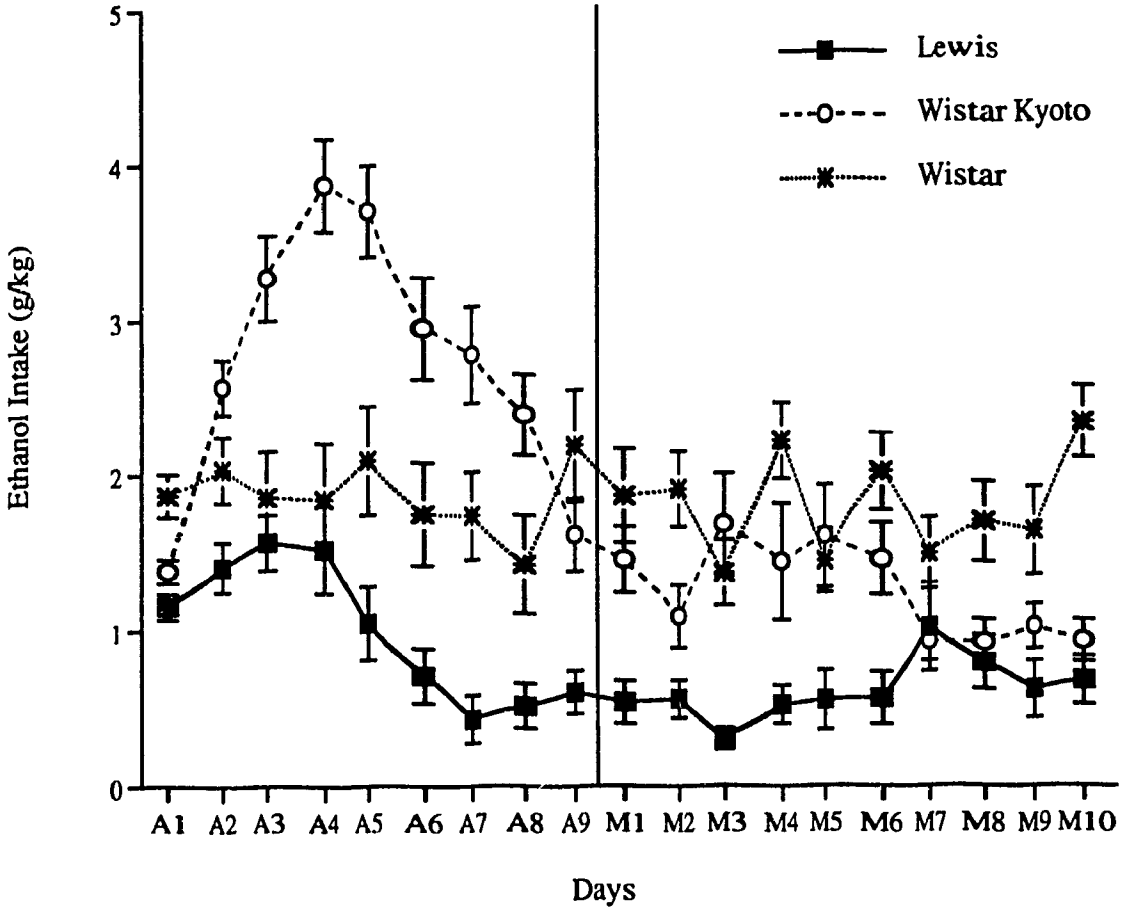

Figure 2. Mean (+/- SEM) ethanol intake in g/kg in Lewis, Wistar Kyoto and Wistar rats during ethanol acquisition and maintenance periods.

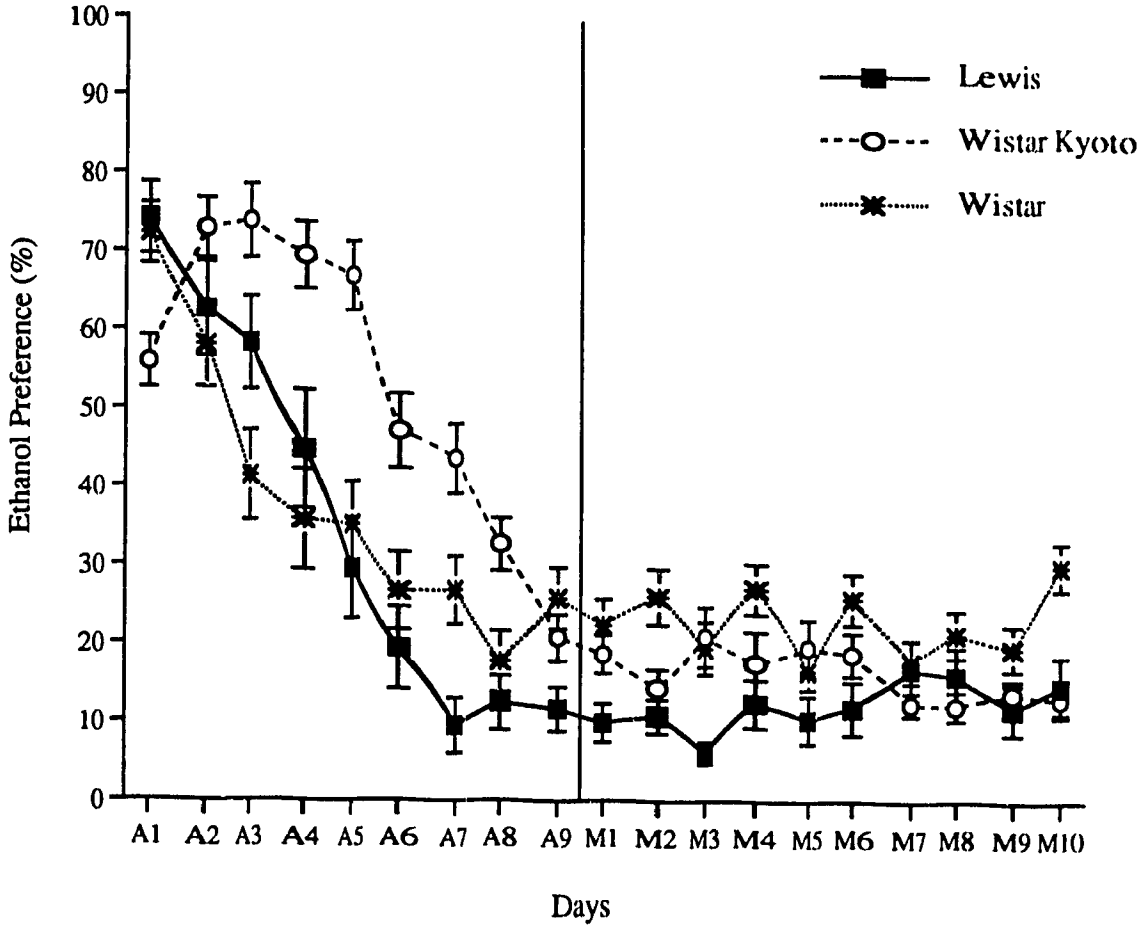

Figure 3. Mean (+/- SEM) ethanol preference in Lewis, Wistar Kyoto and Wistar rats during ethanol acquisition and maintenance periods.

differences [F(2.89)=76.67, p<.0001] (Fig. 4). Post hoc Tukey tests showed that Lewis rats displayed lower total fluid consumption than both Wistar Kyoto and Wistar rats (p<.01). The latter were not different from each other (p>.05). A significant strain by ethanol concentration interaction [F(16,712)=4.64, p<.0001] and test of simple effects revealed that all three strains showed variable consumption across all days of the acquisition phase (p<.001).

A two-way analysis of variance on body weight across days of the ethanol acquisition phase revealed that the strains were significantly different [F(2,89)= 156.98, p<.0001] (Fig. 5). Post hoc Tukey tests showed that the Lewis and Wistar Kyoto rats were not different from one another in body size (p>.05) while the Wistar animals were larger than both Lewis and Wistar Kyoto rats (p<.01). All strains gained weight across the acquisition phase [F(9,801)=440.84, p<.0001].

2) Ethanol Maintenance: Ethanol intake during the maintenance phase, which was comprised of everyday presentations of 10% ethanol and water, was significantly different among the three strains of rats [F(2,89)=11.34, p<.0001] (Fig. 2). Post hoc Tukey tests revealed that Lewis rats had a significantly lower level of ethanol intake compared to both Wistar Kyoto (p<.05) and Wistar rats (p<.01). Wistar Kyoto rats in turn had lower ethanol intake levels than Wistar rats (p<.05). A significant strain x days interaction [F(18,801)=4.39, p<.0001] and test of simple effects revealed that the strains had different ethanol intake levels on all days of maintenance (p<.05) except day 7 (M7: 10% ethanol). While Wistar Kyoto and Wistar rats displayed variable intake levels throughout the phase (p<.001), ethanol intake in Lewis rats was stable (p>.05). Mean ethanol intake levels during maintenance were: Lewis .62 g/kg/day, Wistar Kyoto 1.25 g/kg/day, Wistar 1.80 g/kg/day.

A two-way analysis of variance on ethanol preference data during the maintenance phase also revealed significant differences among the strains [F(2,89)=5.40, p<.01] (Fig. 3). Post hoc Tukey tests revealed that Wistar Kyoto rats no longer had the highest ethanol

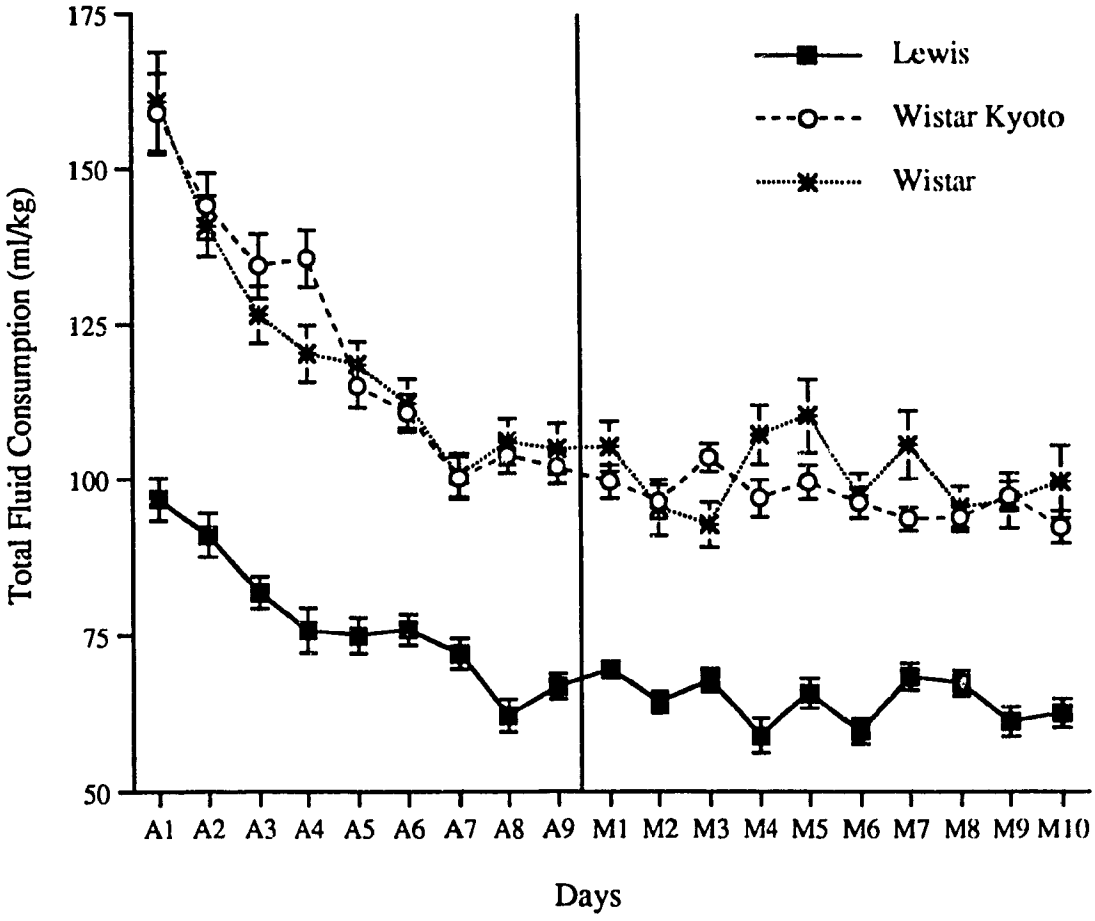

Figure 4. Mean (+/- SEM) total fluid consumption in ml/kg in Lewis, Wistar Kyoto and Wistar rats during ethanol acquisition and maintenance periods.

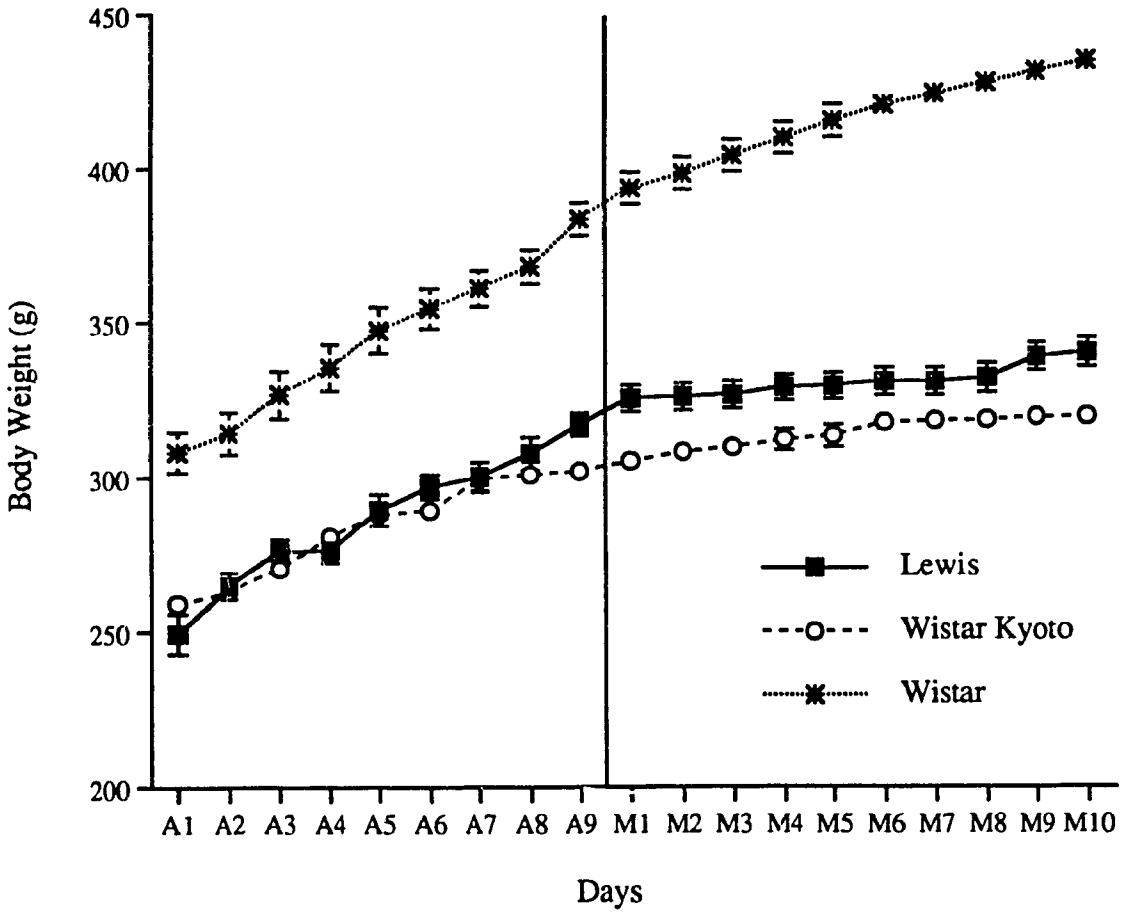

Figure 5. Mean (+/- SEM) body weight in g for Lewis, Wistar Kyoto and Wistar rats during ethanol acquisition and maintenance periods.

preference levels as during the acquisition phase, and were not different from the other strains on that measure ($p>.05$). Also, Wistar rats now showed greater preference for ethanol than Lewis rats ($p<.01$). A significant strain x days interaction [$F(18,801)=4.00$, $p<.0001$] and test of simple effects revealed that the strains showed significantly different preference levels for the 10% ethanol on all days of maintenance except days 5, 7, 8 and 9 (M5, 7-9: 10% ethanol). None of the strains showed stable preference levels for ethanol across the phase ($p<.05$). Mean maintenance preference levels were: Lewis 12.0%, Wistar Kyoto 16.1%, Wistar 22.6%.

Measures of total fluid consumption (ml/kg) during the maintenance phase yielded similar patterns to those observed during acquisition (Fig. 4). Significant differences between the strains [$F(2,89)=49.50$, $p<.0001$] revealed that while Wistar Kyoto and Wistar rats did not differ in their total fluid consumption ($p>.05$), both had higher levels of total fluid intake than Lewis rats ($p<.01$). A significant strain x days interaction during the maintenance phase [$F(18,801)=4.07$, $p<.0001$] indicated that total consumption levels were not stable among all strains during the maintenance period ($p<.05$). Mean consumption levels during maintenance were: Lewis 64.7 ml/kg, Wistar Kyoto 96.9 ml/kg, Wistar 100.6 ml/kg.

The strains also differed in body weight during the maintenance phase [$F(2,89)=46.52$, $p<.0001$] (Fig. 5). Post hoc Tukey tests revealed that the Lewis and Wistar Kyoto strains were not different in size ($p>.05$), while the Wistars were larger compared to both Wistar Kyoto and Lewis rats ($p<.01$). All groups gained weight across the maintenance phase [$F(8,712)=616.47$, $p<.0001$].

Phase II: Saccharin/quinine Acquisition and Maintenance

Within-strain Analyses:

1) Lewis: Mean fluid consumption data for Lewis rats during saccharin/quinine (SQ) acquisition and maintenance are presented in the top panel of Figure 6. Consumption of SQ and water was significantly different during the acquisition phase [$F(1,52)=265.08$, $p<.0001$]. A significant interaction of fluid type across days [$F(10,520)=78.32$, $p<.0001$] and test of simple effects revealed that Lewis rats consumed more SQ relative to water ($p<.001$) on all days of the acquisition phase except the last day (A11: saccharin (S) 0.4%, quinine (Q) 0.04%). Consumption of SQ and water was also different during the maintenance phase [$F(1,52)=198.88$, $p<.0001$]. A significant fluid type x days interaction [$F(9.968)=3.73$, $p<.001$] and test of simple effects revealed that Lewis rats consumed more water relative to SQ on all days. Water ($p<.05$) and SQ ($p<.01$) consumption were stable across the maintenance phase.

2) Wistar Kyoto: The middle panel of Figure 6 represents ethanol and water consumption in Wistar Kyoto rats during the SQ acquisition and maintenance phases. There was a significant difference in fluid consumption during the acquisition phase [$F(1,58)=74.90$, $p<.0001$] and the maintenance phase [$F(1,38)=1156.56$, $p<.0001$]. Significant fluid type x days interactions during the acquisition [$F(10,580)=227.60$, $p<.0001$] and maintenance phases [$F(9,342)=3.65$, $p<.001$], followed by tests of simple effects, revealed that Wistar Kyoto rats consumed significantly more SQ relative to water on days 1 and 2 of the acquisition period (A1: S 0.4%; A2: S 0.4%, Q 0.001%) ($p<.001$). Consumption of water exceeded that of SQ for the remainder of the acquisition phase and the maintenance phase ($p<.001$). While SQ consumption remained relatively stable during the maintenance phase ($p>.05$), water consumption was variable ($p<.001$).

3) Wistar: Data for mean fluid consumption in Wistar rats during SQ acquisition and maintenance are presented in the bottom panel of Figure 6. There was a significant difference in SQ and water consumption during acquisition [$F(1,62)=13.57$, $p<.0001$] and

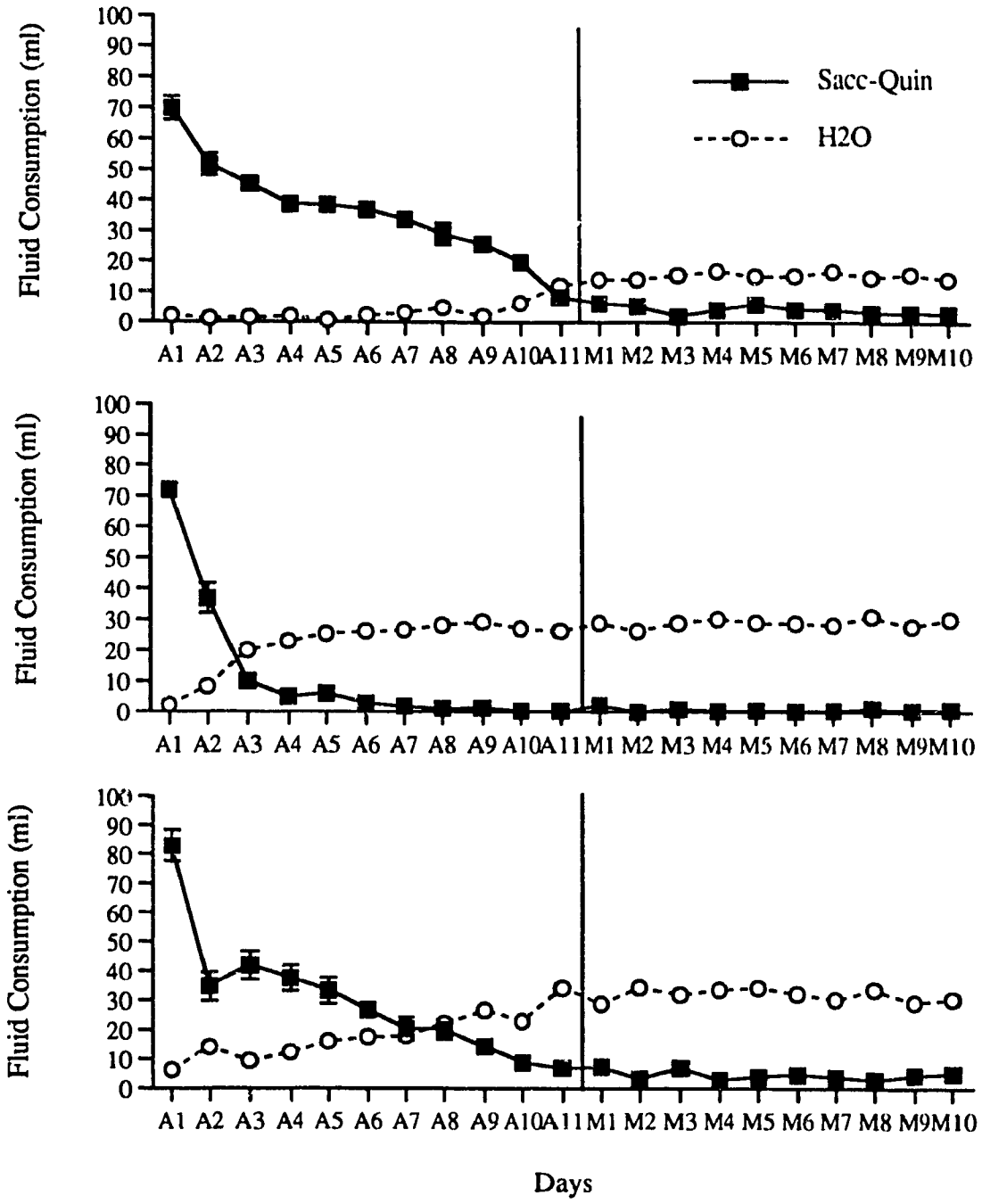

Figure 6. Mean (+/- SEM) saccharin-quinine (filled circles) and water (open circles) consumption in ml in Lewis rats (top panel), Wistar Kyoto rats (middle panel) and Wistar rats (bottom panel) during saccharin-quinine acquisition and maintenance periods.

maintenance [F(1,62)=457.27, p<.0001]. A significant interaction of fluid type x days during acquisition [F(10,620)=97.22, p<.001] and maintenance [F(9,558)=5.64, p<.0001] followed by tests of simple effects indicated that Wistar rats consumed significantly more SQ relative to water from acquisition day 1 to day 6 (A1: S 0.4%; A2-A6: S 0.4%, Q 0.001-0.006%) (p<.05). Water and SQ consumption was not different on acquisition days 7 and 8 (A5: S 0.4%, Q 0.009%; A6: S 0.4%, 0.011Q %), and water consumption exceeded SQ consumption from acquisition day 9 through to the end of the maintenance phase (A9: S 0.4%, Q 0.015%; A10-M10: S 0.4%, Q 0.04%). Water and SQ consumption did not stabilize throughout the maintenance phase (p<.01).

Cross-strain Analyses:

1) SQ Acquisition: Data for mean SQ intake (ml/kg) in Lewis, Wistar Kyoto and Wistar rats are presented in the top panel of Figure 7. A two-way analysis of variance revealed a significant strain difference in SQ intake during the acquisition phase [F(2,86)=31.46, p<.0001]. Post hoc Tukey tests showed Lewis rats to have the highest SQ intake levels above Wistar Kyoto (p<.01) and Wistar rats (p<.01). Wistar Kyoto rats were also found to have significantly lower SQ intake levels than both Lewis (p<.01) and Wistar rats (p<.01). A significant strain by days (quinine concentration) interaction [F(20,860)=15.27, p<.0001] and test of simple effects revealed that the strains had differential levels of intake on all days of the acquisition phase (p<.001) except the last day (A11: S 0.4%, Q 0.04%) (p>.05). All groups decreased their SQ intake consistently throughout the acquisition phase as the concentration of quinine in saccharin increased (p<.001).

Reflecting SQ intake data, strain difference in SQ preference [F(2,86)=132.49, p<.0001] showed Lewis rats to have higher SQ preference levels than both Wistar Kyoto (p<.01) and Wistar rats (p<.01) (Fig. 8). Similarly, Wistar Kyoto rats were found to have lower SQ preference levels than Lewis (p<.01) and Wistar rats (p<.01). A significant fluid

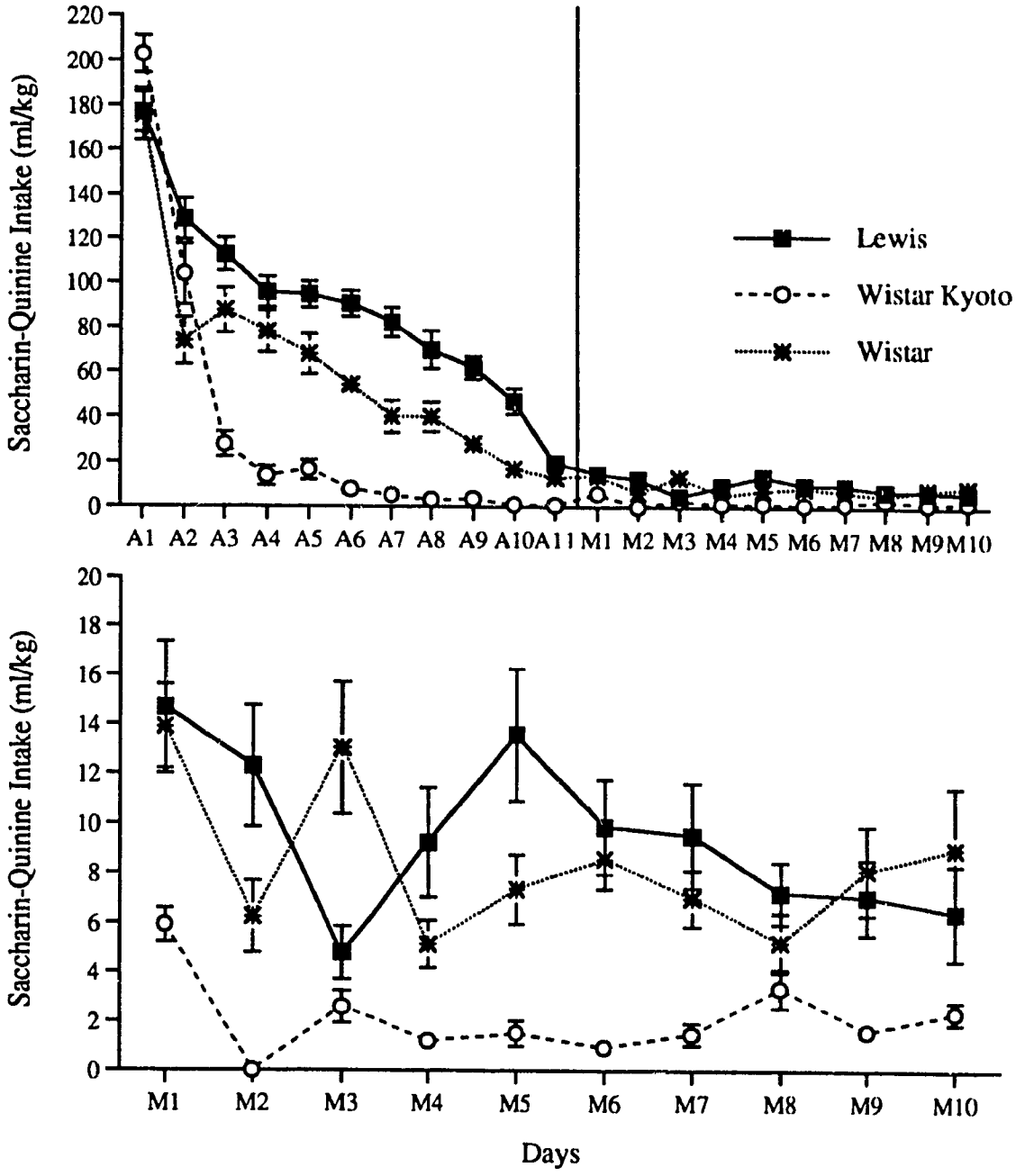

Figure 7. Mean (+/- SEM) saccharin-quinine intake in ml/kg in Lewis, Wistar Kyoto and Wistar rats during saccharin-quinine acquisition and maintenance periods (top panel), and during the maintenance phase alone (bottom panel).

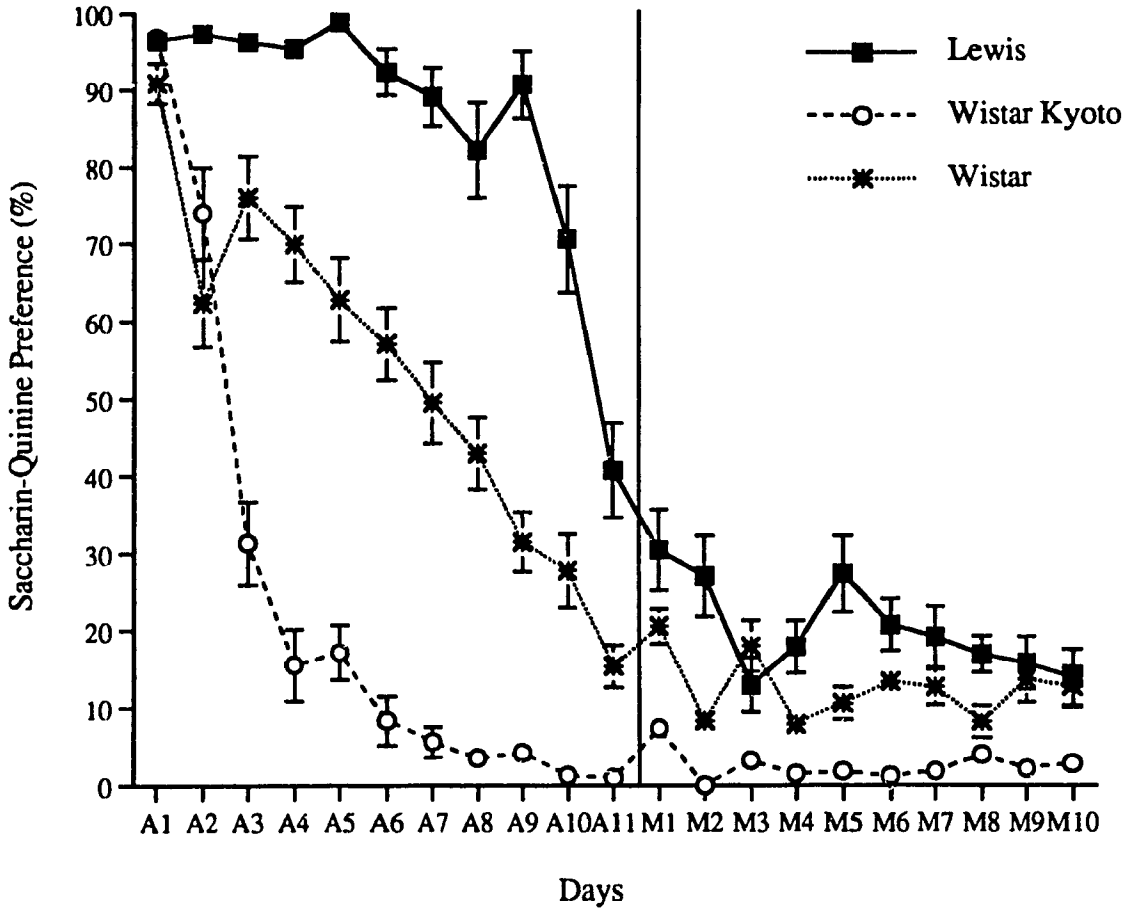

Figure 8. Mean (+/- SEM) saccharin-quinine preference in Lewis, Wistar Kyoto and Wistar rats during saccharin-quinine acquisition and maintenance periods.

x days (quinine concentration) interaction [F(20,860)=26.56, p<.0001] and test of simple effects revealed that preference among the strains was different on all acquisition days (p<.001) except day 1, when saccharin 0.4% was presented without quinine (p>.05). Preference for the SQ solution decreased in all strains as the concentration of quinine increased across the acquisition phase (p<.001).

Analysis of total fluid consumption (ml/kg) revealed no overall strain differences during the acquisition phase [F(2,86)=.11, p>.05] (Fig. 9). However, a significant fluid x days (quinine concentration) interaction [F(20,860)=7.15, p<.0001] and tests of simple effects indicated that there was some variability in consumption levels, reflective of decreases in SQ intake: at A1, A3 and A4, Wistar Kyoto rats had higher consumption levels than both Lewis and Wistar rats (p<.01); at A2, Wistar rats had higher consumption levels than both Lewis and Wistar Kyoto rats (p<.01); and at A11, Lewis rats had higher consumption levels than both Wistar Kyoto and Wistar rats (p<.01). Total fluid consumption decreased in all three strain across the acquisition phase (p<.001), again reflective of decreases in SQ intake.

The strains differed significantly in body weight during the SQ acquisition phase [F(2,86)=183.94, p<.0001] (Fig. 10). Post hoc Tukey tests revealed that Wistar rats were larger than both Lewis (p<.01) and Wistar Kyoto rats (p<.01), and that Lewis rats were now larger that Wistar Kyoto rats (p<.01). All strains gained weight throughout the SQ acquisition phase [F(10,860)=282.41, p<.0001].

2) SQ Maintenance: Analysis of SQ intake (ml/kg) in Lewis, Wistar Kyoto and Wistar rats during the maintenance phase revealed significant differences [F(2,76)=13.06, p<.0001] (Fig. 7, bottom panel). Wistar Kyoto rats displayed lower intake levels than both Lewis (p<.01) and Wistar rats (p<.01). Intake was not different between Lewis and Wistar rats (p>.05). A significant fluid x days interaction [F(18,684)=2.94, p<.0001] and tests of simple effects revealed that the strains were different on all days of the maintenance phase (p<.05) except day 8 (M8: S 0.4%, Q 0.011%) (p>.05). Lewis and Wistar SQ intake was

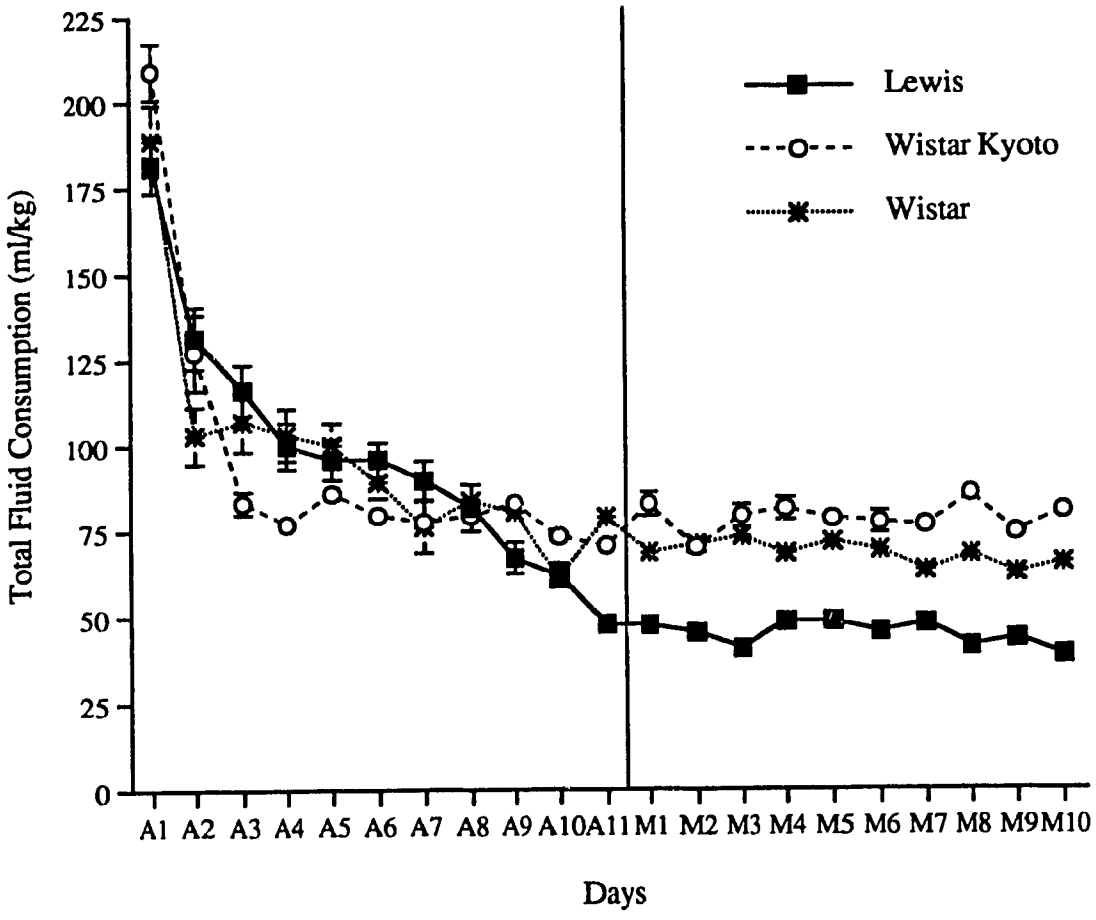

Figure 9 Mean (+/- SEM) total fluid consumption in ml/kg in Lewis, Wistar Kyoto and Wistar rats during saccharin-quinine acquisition and maintenance periods.

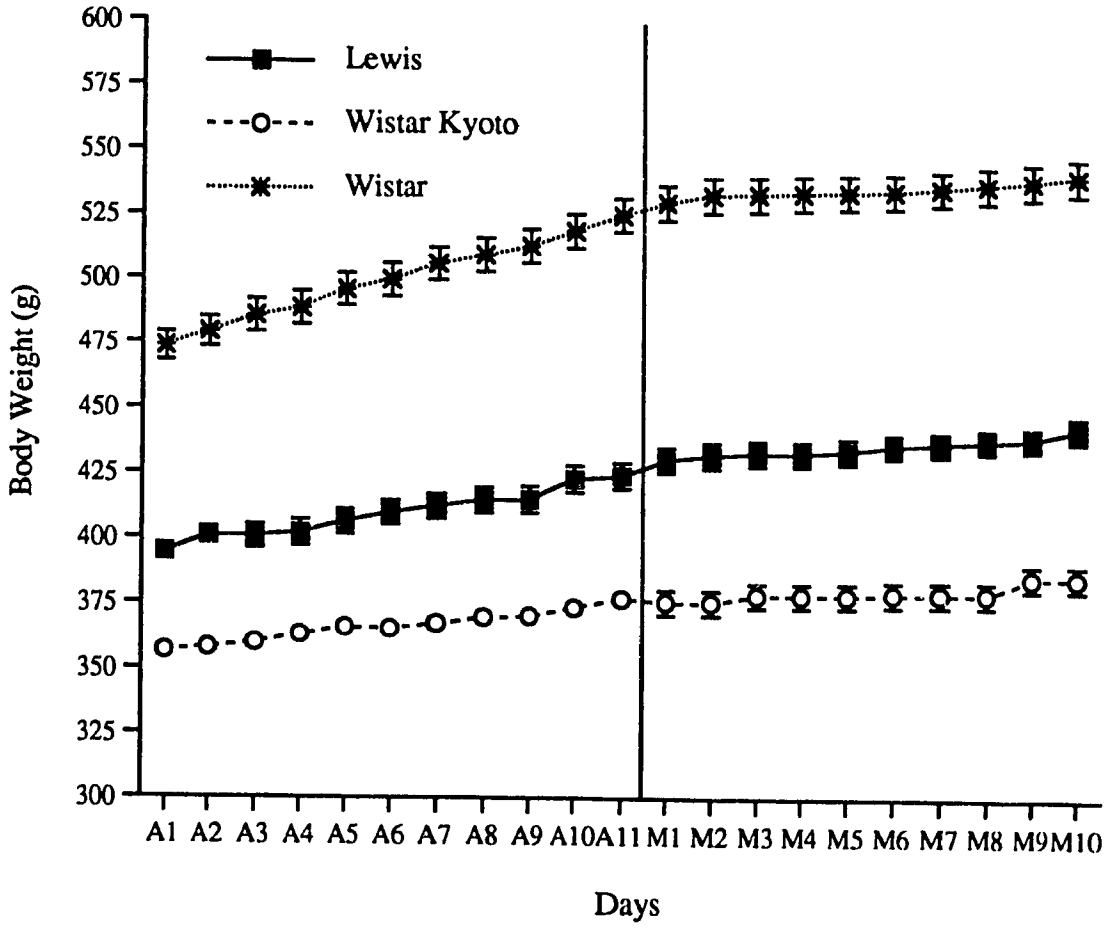

Figure 10. Mean (+/- SEM) body weight in g for Lewis, Wistar Kyoto and Wistar rats during saccharin-quinine acquisition and maintenance periods.

variable throughout the maintenance period ($p<.001$) while Wistar Kyoto rats displayed little change in intake ($p>.05$). Mean SQ intake levels during the maintenance phase were: Lewis 9.47 ml/kg, Wistar Kyoto 2.08 ml/kg, Wistar 8.35 ml/kg.

Significant differences in SQ preference during the maintenance phase [$F(2,76)=22.54$, $p<.0001$] revealed that Wistar Kyoto rats had lower preference levels for SQ than both Lewis ($p<.01$) and Wistar rats ($p<.01$) (Fig. 8). Lewis rats also displayed greater overall preference for SQ than Wistar rats ($p<.05$). A significant fluid x days interaction [$F(18,684)=2.73$, $p<.001$] and tests of simple effects revealed that the strains were different on all days of the maintenance phase ($p<.05$). As was revealed in SQ intake data, Lewis and Wistar preference for SQ was variable throughout the maintenance period ($p<.001$) while Wistar Kyoto rats exhibited little change in preference for SQ ($p>.05$). Mean preference levels for the maintenance period were: Lewis 20.15%, Wistar Kyoto 2.56%, Wistar 12.54%.

Total fluid consumption (ml/kg) during the SQ maintenance period was different among strains [$F(2,76)=79.59$, $p<.0001$] (Fig. 9). Post hoc Tukey tests revealed that Wistar Kyoto rats had significantly higher total fluid consumption than both Lewis ($p<.01$) and Wistar rats ($p<.01$). Lewis rats also displayed lower fluid consumption levels than Wistar rats ($p<.01$). A significant strain x days interaction [$F(18,684)=4.79$, $p<.0001$] followed by tests of simple effects revealed that the strains were different on all days of the maintenance phase ($p<.001$) and that all strains displayed variable consumption levels across the phase ($p<.001$). Mean total fluid consumption during the maintenance phase was: Lewis 44.62, Wistar Kyoto 78.36, Wistar 67.95.

Body weight among the strains differed significantly during the maintenance phase [$F(2,76)=180.92$, $p<.0001$] (Fig. 10). Post hoc Tukey tests revealed that as for the acquisition phase Wistar rats were significantly larger than both Lewis ($p<.01$) and Wistar Kyoto rats ($p<.01$), and that Wistar Kyoto rats were smaller than Lewis rats ($p<.01$). All strains gained weight during the SQ maintenance phase [$F(9,684)=81.32$, $p<.0001$].

Correlational Analysis

Spearman Rank order correlations (Minium et al, 1993) were performed within each strain to further determine any relationship between ethanol and SQ intake. Mean ethanol and SQ intake levels were calculated for each animal across the final 5 days of the maintenance phase and consumption levels were compared. Results revealed no significant correlations between ethanol and SQ consumption within any strain: Lewis, $r = 0.188$, Wistar Kyoto, $r = 0.030$, Wistar, $r = -0.061$.

Summary

During the ethanol acquisition phase, as the ethanol solutions presented became more concentrated, Wistar Kyoto rats displayed the highest ethanol intake and preference levels while Lewis rats exhibited the lowest intake values. When ethanol was presented everyday at a constant concentration of 10% (v/v) during the maintenance phase, ethanol intake in Wistar Kyoto rats decreased and was no longer significantly different from Wistar rats. Lewis rats again displayed the lowest ethanol intake levels throughout the maintenance phase.

Consumption of SQ solutions during the acquisition phase revealed that Lewis rats had significantly higher levels of SQ intake and preference than both Wistar Kyoto and Wistar rats. Wistar Kyoto rats displayed very low levels of SQ intake and preference throughout the acquisition phase. During the SQ maintenance phase, as the presentations of SQ were at a constant concentration on an everyday basis, Wistar Kyoto showed unchanged SQ intake and preference levels from the acquisition phase, while Lewis rats decreased their SQ intake levels and were no longer different from Wistar rats. Both Lewis and Wistar rats consumed significantly more SQ during the maintenance phase than Wistar Kyoto rats.

DISCUSSION

The purpose of the present thesis was to expand current knowledge on the role of taste factors in ethanol consumption. As part of the examination, the daily consumption of ethanol and saccharin-quinine solutions was measured in two inbred strains of rats (Lewis and Wistar Kyoto) and one outbred strain of rat (Wistar), each known to differ in their preference for ethanol (Spuhler & Deitrich, 1984; Suzuki et al, 1988). It was of interest to determine whether preference for (or aversion to) ethanol was regulated by innate taste sensitivities for fluids in general, and more specifically whether preference for ethanol may predict saccharin-quinine intake in these three different strains of rat. There is a growing body of evidence suggesting that the ingestion of ethanol and sucrose or saccharin solutions may in fact be positively correlated in rats (Gosnell & Krahn, 1992; Kampov-Polevoy et al, 1990; Overstreet et al, 1993; Sinclair et al, 1992). Furthermore, saccharin intake has been found to be a good predictor of subsequent ethanol intake in several strains of rats with varying preference for ethanol (Gosnell & Krahn, 1992; Overstreet et al, 1993). As well, rats selected for high ethanol intake were found to drink more saccharin than rats initially selected for low ethanol intake (Sinclair et al, 1992). These data suggested a common mechanism mediating ethanol and saccharin intake by the rodents, whether neurochemical or behavioral. It also served as the basis of the present investigation. Results however did not support a relationship between ethanol and saccharin-quinine consumption in Lewis, Wistar Kyoto and Wistar rats and yet did reveal definite strain preferences in taste.

Discussion of the Results

During phase I of the experiment, rats were exposed to an ascending series of increasingly concentrated ethanol solutions on alternate days followed by everyday presentations of a 10% ethanol solution. The three strains of rats exhibited great differences in their preference for the ethanol solutions. All three strains began the acquisition period

preferring the more dilute ethanol solutions to water. Both Lewis and Wistar strains decreased their ethanol consumption below that of water around the presentation of a 6% ethanol solution. This change in fluid preference appears to be a common response made by rats to 6% ethanol and was first documented by Richter and Campbell (1940a). Their early work demonstrated that when rats were presented with an ascending series of ethanol solutions in free choice with water, they generally preferred ethanol to water only up to a 6% ethanol solution, after which they continuously preferred water to ethanol (Richter and Campbell, 1940a). In contrast, Wistar Kyoto rats did not show a significant preference for water until the presentation of 9% ethanol. These findings implied that the Wistar Kyoto rats found the ethanol solutions less aversive than did the Lewis or Wistar rats and thus were able to consume it at stronger concentrations. The absolute ethanol intake and preference data for the three strains during the acquisition phase suggested that the Wistar Kyoto strain was an ethanol-preferring strain compared to Lewis and Wistar rats, as their ethanol intake was well elevated above that of Lewis and Wistar strains. Lewis rats were very low ethanol consumers compared with Wistar Kyoto and Wistar rats. It should be noted that the Wistar Kyoto and Lewis rats were much smaller in size than the Wistar rats throughout this phase. However, despite their smaller size, Wistar Kyoto rats in particular consumed equivalent amounts of total daily fluid as compared to the heavier Wistar rats. This suggested that the preference for ethanol demonstrated by Wistar Kyoto rats during the acquisition phase was strong enough to raise their level of total daily fluid intake to those of a heavier strain like the Wistars whose daily fluid requirements were naturally greater due to their larger size (the effect of inflated daily fluid intake is often seen with the presentation of preferred solutions such as saccharin or sucrose, see Phase II and Kampov-Polevoy et al, 1990; Sinclair et al, 1992).

The ethanol maintenance period may be considered a better indicator of ethanol preference since the same ethanol solution (10%) was presented everyday and thus resulted in more stable levels of ethanol intake than those seen during the acquisition phase. During

maintenance, the Lewis rats remained the lowest ethanol consumers as in the acquisition phase. Wistar Kyoto rats, however, who were the highest ethanol consumers of the three strains during the acquisition phase, decreased their ethanol intake and were now no longer different from Wistar rats. Their ethanol intake was still greater than Lewis rats although their ethanol preference was no longer different from either Lewis or Wistar rats. The decreases in ethanol intake without comparable changes in total fluid consumption, seen in Wistar Kyoto rats, were the primary reason for the decrease in their ethanol preference values. By the end of the maintenance phase, ethanol intake in Wistar Kyoto animals had decreased to such an extent that they were no longer higher than Lewis rats. The intake of Wistar rats on the other hand increased above both groups. Due to time limitations imposed on the maintenance phase, it is impossible to extrapolate as to whether this descending pattern would have continued further or whether Wistar Kyoto rats would have regained previous intake levels, as previous studies have shown that rats develop a progressive increase in ethanol consumption over prolonged periods of exposure (Rick & Wilson, 1964).

Overall, data from both the ethanol acquisition and maintenance phases revealed that Wistar Kyoto rats were higher ethanol-preferrers than Lewis rats. This high preference for ethanol seen in Wistar Kyoto rats was specific and not a function of increased fluid requirements due to body size (they were not the largest strain). This difference in ethanol preference was also stable throughout Phase I until the end of maintenance.

These results are however difficult to reconcile with the literature documenting ethanol preference in Wistar Kyoto and Lewis rats. The Wistar Kyoto strain, inbred from Wistar stock as a normotensive control strain for Spontaneously Hypertensive Rats (SHR) (Baker, Lindsey, & Weisbroth, 1979), has been shown to have actually lower levels of daily ethanol intake and preference ratios relative to the ethanol-preferring Marshall (M520) strain (Li & Lumeng, 1984). As well, the Wistar Kyoto strain was found to have a longer sleep time following a 3.5 g/kg dose of ethanol (Spuhler & Deitrich, 1984), slower ethanol

metabolism (Lester et al, 1970) and higher blood-ethanol levels following a 3.0 g/kg dose of ethanol (Spuhler & Deitrich, 1984) relative to M520 rats. Cannon and Carrell (1987) showed that when compared again to M520 rats, Wistar Kyoto rats acquired an aversion to ethanol during ethanol self-administration whereas M520 rats did not. As well, the Wistar Kyoto rats required a lower dose of ethanol to acquire a conditioned taste aversion to saccharin. The authors concluded that ethanol served as a more effective unconditioned stimulus for Wistar Kyoto rats than for M520 rats (Cannon & Carrell, 1987).

The Lewis rats, on the other hand, derived from Wistar stock as the inbred partner for a number of congenic strains at the major histocompatibility complex (Baker et al, 1979), have been shown to be an ethanol-preferring strain. When compared with the Fischer 344 (F344) strain, Suzuki and colleagues have shown that Lewis animals will maintain substantially higher response rates for ethanol across both concentrations and fixed-ratio schedules (Suzuki et al, 1988). As well, the synthetic opioid etonitazene was shown to be an effective reinforcer for Lewis rats but not for F344 rats, as measured by operant responding and intake values (Suzuki, George & Meisch, 1992). In comparison to other dependence-producing drugs, Lewis rats have been shown to exhibit a greater preference for morphine and codeine than do F344 rats (Suzuki, Otani, Koike & Misawa, 1988). While no direct comparisons between Wistar Kyoto and Lewis strains have been reported in the literature, F344 rats were found to have higher daily intake and preference for ethanol than Wistar Kyoto rats, as well as higher ethanol metabolism following a 2.5 g/kg ethanol dose (Spuhler & Deitrich, 1984). Therefore, according to the literature and by association, it may be concluded that Lewis rats are a higher ethanol-preferring strain than Wistar Kyoto rats. The results of the present thesis, however, contradict this conclusion, in that the Lewis strain was found to consume significantly less ethanol than the Wistar Kyoto strain during both the ethanol acquisition and maintenance phases. Given that the ethanol acquisition and maintenance periods were conducted over 28 days and the variance in consumption between the strains was relatively stable throughout, these results are deemed

to reflect reliable strain differences. The Wistar rats were initially chosen to act as controls for the measurement of ethanol intake in Lewis and Wistar Kyoto rats because they were the stock from which the Lewis and Wistar Kyoto strains were derived and are also the most commonly used rat strain in ethanol intake studies. In the present study, they showed stable levels of ethanol intake throughout acquisition and maintenance (generally falling between those of the Lewis and Wistar Kyoto rats), thus supporting their use as appropriate controls for the two inbred strains.

During Phase II of the experiment, the same animals used in Phase I were exposed to a saccharin solution which was mixed on alternate days with quinine in increasing concentrations (SQ). All three strains preferred the saccharin alone to water on the first acquisition day, consuming more than 60 ml of saccharin alone over 24 hours and inflating total fluid intake values. In similar fashion to Phase I, differences in fluid preference between the strains were apparent as the concentration of quinine in saccharin increased across the days of acquisition. While Lewis rats greatly preferred the SQ solution to water until the end of the acquisition phase, Wistar Kyoto rats markedly decreased their SQ consumption below water very early in the acquisition phase. In this phase, Wistar rats again displayed similar 'taste' responses to Lewis animals as both strains preferred SQ to water until the ninth day of acquisition. These data suggested that Wistar Kyoto rats disliked the quinine in the mixture and so began to reduce their consumption of it early in the acquisition phase while the concentrations of quinine were still weaker. Lewis and Wistar rats who preferred the SQ solutions to water until the latter part of the acquisition phase, appeared less sensitive to the aversive taste of the quinine.

When comparing the three strains on measures of SQ intake and preference during the acquisition phase, Lewis rats displayed higher levels than both Wistar Kyoto and Wistar rats. Wistar Kyoto rats had the lowest preference for and intake of SQ during this phase. Total fluid consumption, unlike what was observed during Phase I, did not differ among the groups . This suggested that regardless of differing preferences for SQ, the

three strains were all combining and adjusting their water and SQ intake such that equivalent amounts of total fluid were consumed daily.

During the SQ maintenance phase, while the concentrations of saccharin and quinine presented remained constant, Lewis rats were no longer the highest consumers of SQ. Their intake decreased such that they were not different from Wistar rats. Both groups consumed more SQ than Wistar Kyoto rats, whose mean daily SQ intake was now less than 2 ml per day. Such low levels of consumption observed in the Wistar Kyoto rats are not reliable measures of consumption since they could be the result of spillage and/or other measurement errors. It is actually possible that Wistar Kyoto rats may not have been consuming any SQ at all. Nonetheless, whether Wistar Kyoto rats did or did not reject the SQ solution entirely, they appeared to be endowed with a lower rejection threshold for the quinine in the SQ solution compared to the other two strains. While Lewis rats were found to have a lower daily fluid intake during maintenance compared with the other strains, their preference for SQ remained higher than that of Wistar Kyoto rats. This suggested that while their preference for SQ decreased when presented everyday, as observed also in the two other strains, the Lewis rats actively sought to consume a greater proportion of their daily fluid needs from SQ rather than water. This further supported the notion that the preference of Lewis rats for SQ was specific and not the result of increased fluid needs due to size.

GENERAL DISCUSSION

Alcohol is perhaps the most behaviourally active drug legally available in North America that "is purchased primarily because of its intoxicating properties" (Winger, Young and Woods, 1983). As a result, moderate use of alcohol is more socially acceptable than similar levels of use of other drugs (Winger et al, 1983). In order to examine the reinforcing properties of alcohol in humans more closely, researchers have attempted to model alcoholism in animals. The necessary assumption for the success of an experimental research program on alcoholism is that the property of ethanol that makes it a reinforcer in 'non-human animals', whether by intravenous, intragastric or oral routes, will not be different from the property that makes it a reinforcer orally in humans (Winger et al, 1983).

The Animal Studies

There is little disagreement that ethanol's primary route of administration in humans is virtually exclusively restricted to the oral route (Meisch, 1977). As a result, orosensory factors are necessarily implicated in its consumption. The revised definition for alcoholism (Morse & Flavin, 1992) has now acknowledged that it is a 'heterogeneous disease'. This concept implicates both biopsychosocial factors and genetic vulnerability in the causes, signs and symptoms, as well as complications and treatment of alcoholism (Morse & Flavin, 1992). These factors may also include innate taste and smell sensitivities which act as moderators of alcohol intake. Animal research has shown repeatedly that orosensory factors are involved in the regulation of ethanol intake in animals. Some researchers have even asserted that orosensory stimuli accompanying ethanol solutions are the primary determinants of the amount and concentration of ethanol ingested (Lester, 1966; Myers & Veale, 1972; Marfaing-Jallat, Pruvost & Le Magnen, 1974). These sensitivities may even regulate intake so that systemic concentrations of ethanol do not reach toxic levels (Lester et al, 1970). The results of studies supporting the contention that taste and smell factors potentially play a role in mediating ethanol intake are diverse and span several decades. For

example, Samson and colleagues reported that while attempting to initiate high levels of ethanol self-administration in rats selectively-bred for low ethanol preference, only marginal increases in their overall ethanol intake were attained (Samson et al, 1989). These authors concluded that while ethanol appeared to be equally reinforcing for ethanol-preferring and non-preferring rats (as determined by rates of operant responses), it appeared that ethanol ingestion may have been limited in the non-preferring rats by taste factors as they consumed equivalent amounts of ethanol as the higher ethanol-consuming, non-selected Long Evans rats when it was mixed with sucrose. Previously, Amit and Stern (1969) were the first to report a 'facilitation' of ethanol intake when intragastric infusion was the route of ethanol administration. This procedure therefore elegantly bypassed the oropharyngeal cavity and isolated ethanol's postingestional effects from taste and smell influences. These results were supported by a later study showing that the ethanol-preferring P rats also self-infused greater volumes of ethanol during intragastric self-administration compared to free-choice drinking (Waller et al, 1984).

The findings from several studies that alcohol may in fact be 'an acquired taste' support the contention that taste does play a role in ethanol intake in animals. Bice and Kiefer (1990) showed that ethanol-preferring P rats will increase their ingestive responding and decrease their aversive responding to ethanol, as measured by orofacial responses in a taste reactivity paradigm, after a three week period of free access to ethanol. Badia-Elder and colleagues also reported increased ingestive and decreased aversive responding to ethanol after only two weeks of exposure to ethanol in the home cage (Badia-Elder et al, 1994). Similarly, Kampov-Polevoy and colleagues found that initially low ethanol-preferring non-selected rats increased their ethanol intake levels during a three week exposure to ethanol such that their intake matched that of the initial high ethanol-preferring rats (Kampov-Polevoy et al, 1990). These results, while not the main body of evidence in these studies, indicated nevertheless that animals will adapt to the taste of ethanol over time as evidenced by their increased intake levels.

Data from several studies has also shown on several occasions that flavouring ethanol solutions will induce higher levels of ethanol intake in rats. For example, one common method of initiating ethanol intake in rats (sucrose-substitution) requires animals initially to perform an operant response for a sucrose solution (Samson, 1986). Once the operant behavior has been learned, the sucrose solution is gradually adulterated with increasing concentrations of ethanol until the rats are responding solely for ethanol (Samson, 1986). This procedure is as efficacious as any for inducing ethanol self-administration in rats, but relies on the rats' innate preference for sweet tastes as the vehicle for induction. York (1981) effectively showed that flavoured ethanol solutions, such as wine or alcoholic punch, were much more appealing even to ethanol non-preferring strains of rats and mice. Ethanol intake in the ethanol non-accepting ANA rats surpassed that of their alcohol-accepting counterparts (AA), therefore compelling them to experience the pharmacological effects they are purported to actively avoid (York, 1981).

It is important to remember that animals rely on their sense of taste and smell differentially from humans in that in rodents these senses are undeniably linked with food selection and therefore survival (Garcia et al, 1974). When a rat is presented with a novel substance, its only method for evaluating the safety of that substance is to sniff and taste it. There are evolutionary and experiential 'rules' animals will follow to determine whether they should 'accept or reject' a substance: sweet usually signifies carbohydrate nutrients, bitter usually signifies alkaloid toxins (Garcia et al, 1974). The animals will therefore select sweet and reject bitter foods for the sake of survival. Unlike rats in the wild, laboratory rats used in the majority of experiments today are bred in large colonies and so are naive to any substances other than water and rat chow. Therefore, their taste-related food selections are presumably based on inherited food preferences rather than prior experiences. Modern aversion generalization studies conducted in Kiefer's laboratory have shown that in the case of ethanol, rats may categorize its taste as both sweet and bitter (Di Lorenzo et al, 1986; Kiefer et al, 1990; Kiefer & Lawrence, 1988; Lawrence and Kiefer, 1987). If this is indeed

the case, a rat's initial reaction to ethanol may be somewhat confused: sweet means accept, but bitter means reject (Garcia et al, 1974). Kiefer and his colleagues found that generalizations of lithium-induced aversions to many sweetened fluids were specific only to 6% ethanol, suggesting that this concentration of ethanol has a predominantly sweet taste. Therefore, as the concentration of ethanol in water changes, the taste of the ethanol solution overall changes (perhaps from sweet to bitter) and the animals' reaction to the solution will change from accept to reject. This may also explain why rats decrease their preference for ethanol with increasing concentrations (e.g., Richter & Campbell, 1940b).

The research examining taste preferences in rats is comprehensive, particularly when one considers the fact that the bulk of it was conducted in the 1990's (Overstreet et al, 1993; Sinclair et al, 1992; Stewart et al, 1994). This research seems to follow a recent trend in experimental research on alcoholism that breaks away from the more traditional search for biochemical substrates of alcoholism and is returning to a more behaviourally-oriented approach. The more recent studies on taste factors have employed traditional intake tests in correlating fluid consumption of various inbred and outbred strains of rats. In summary, studies using rats selectively-bred for ethanol preference have shown that ethanol-preferring P and AA strains consumed more of a saccharin solution than the ethanol non-preferring NP and ANA rats (Sinclair et al, 1992) and that P rats consumed more of a sucrose solution than NP rats (Stewart et al, 1994). Also, when 7 strains of rats of varying preference for ethanol were presented with saccharin, quinine and ethanol solutions, two of the three ethanol-preferring strains (including P rats) consumed significantly more saccharin and ethanol than the ethanol-nonpreferring strains (including NP rats) (Overstreet et al, 1993). Altogether these data are very convincing and strongly suggest some link between sweet and ethanol preference. In the context of these data, the results of the present study are therefore difficult to interpret.

Data collected for the present thesis has shown that ethanol and saccharin [illegible] consumption were not related in two inbred non-selected strains, Lewis and Wistar Kyoto.

In fact, these two strains showed a negative relationship in ethanol and SQ preference, in preferring one solution and rejecting the other and vice versa. Low within strain variability in intake of and preference for either solution suggested however that observed strain differences reflected innate sensitivities to the solutions. Of additional interest was the fact that the standard laboratory Wistar rats, used much more frequently in studies of alcohol consumption compared to both Lewis or Wistar Kyoto rats, maintained consumption levels for the two fluids that fell in both cases between those of the Lewis and Wistar Kyoto strains. This consistency may indicate a more stable taste reactivity to the two fluids in Wistar rats.

The responses of the Lewis and Wistar Kyoto rats to the ethanol and SQ solutions are unexplainable within the boundaries of this experiment and equally difficult to interpret with respect to the literature. There is however a very important difference between the present experiment and all others conducted previously concerning the relationship between the consumption of ethanol and sapid fluids. The fluid used in the present study to contrast with ethanol was not pure saccharin as is the case in most studies in this area but rather a mixture of quinine and saccharin. Comparisons with other studies reported in the literature may therefore not be possible. Despite this known disadvantage, a saccharin-quinine solution was selected because aversion generalization studies have suggested that ethanol may represent a dual sweet-bitter taste for rats (Di Lorenzo et al, 1986; Lawrence & Kiefer, 1987; Kiefer & Lawrence, 1988; Kiefer et al, 1990). It is therefore the contention of this author that comparing sucrose and/or saccharin consumption to ethanol consumption in rats is inappropriate because sweetened fluids have a high hedonic value for rats (Garcia et al, 1974) and will be consumed to the exclusion of water in much greater quantities than any concentration of ethanol (see Sinclair et al, 1992). Subsequently, studies comparing sucrose or saccharin intake to ethanol intake may in fact be misleading and should be considered cautiously. The results of the present investigation suggested that since there was no positive relationship between the consumption of ethanol and the saccharin-quinine

combination (i.e., two solutions of more similar taste properties than ethanol and saccharin alone) the correlations reported in the literature between the preference for ethanol and purely sweetened fluids may be artifactual. In fact, the inverse relationship in preference for the two fluids displayed by the Lewis and Wistar Kyoto rats implied the existence of innate and specific taste sensitivities that do not uphold a theory of a positive unidirectional relationship between ethanol and sweet substances.

Related Human Studies

The issue concerning a possible relationship between innate taste sensitivity and alcoholism has not been exclusive to the field of animal research. Early human work searching for trait differences between alcoholic and non-alcoholic individuals alluded to metabolic patterns, including taste sensitivities, which might distinguish the above mentioned groups (Beerstecher et al, 1950). Those early results suggested an increased taste sensitivity to sodium chloride and potassium chloride in alcoholics (Beerstecher et al, 1950). Taste thresholds for alcohol have also been examined in alcoholics (Settle, 1978). This author found that alcoholics had a higher taste threshold for alcohol compared to control patients. Those alcoholics whose last drinking episode occurred within 21 days of testing reported less aversion to 6.0, 8.25, 12.0 and 17.0 % alcohol solutions (according to a nine-point hedonic category scale) than controls or alcoholics whose last episode occurred more than 21 days before testing (Settle, 1978). All subjects, both alcoholic and non-alcoholic, showed aversions to stronger concentrations of alcohol but did not differ in their preference ratings for water. These results supported the notion that differences in taste perception of certain substances may exist among alcoholic and non-alcoholic individuals.

Another line of research examining taste sensitivity in alcoholics has used gustatory reaction to the compound phenylthiocarbamide (PTC), which forms a bitter-tasting aqueous solution with a slight odour, in distinguishing between alcoholics and nonalcoholics (Swinson, 1983). In one study which asked subjects to discriminate between water and a PTC solution, no overall differences were found between the percentage of nontasters (i.e.,

subjects who could not discriminate between water and ascending concentrations of PTC solutions) in alcoholic and control groups (Swinson, 1973). Closer examination of taste responses across concentrations of PTC in water revealed less taste sensitivity to the most dilute solutions among the alcoholics (Swinson, 1973). It could not be determined whether this was due to innate taste reactivity or a loss of taste sensitivity as a result of excessive alcohol consumption and smoking in the alcoholics (Swinson, 1973). Another study which required subjects to taste PTC on filter paper found no differences between alcoholics and controls (Peeples, 1962). However, the method of tasting filter paper was deemed highly unreliable because the concentration of PTC was weaker than the accepted concentration for differentiating populations, therefore these results were questionable (Swinson, 1983).

In an attempt to side-step the possibility that subjects may be detecting the slight odour rather than the taste of the PTC solution, researchers began using the odourless, bitter-tasting compound 6-*n*-propyl-2-thiouracil (PROP) (Swinson, 1983). One study found a significant increase in the number of nontasters of PROP among alcoholics as compared with controls (Spiegel, 1972). Later studies have shown that there was also a larger percentage of nontasters of PROP in children of alcoholics (Pelchat & Danowski, 1992). These data suggested that earlier findings associating nontasters of PROP and alcoholism were not simply the result of chronic alcohol abuse (Overstreet et al, 1993).

Overall, the PTC and PROP studies imply a connection between taste sensitivity and alcoholism. These studies were based on the notion that alcoholics may have inherently duller taste sensitivities thus allowing them to consume more alcohol than normals, and conversely that non-alcoholics do not consume alcohol excessively because of taste. One factor which may potentially confound interpretation of these studies as mentioned by Swinson (1973) is that alcoholics may have dulled taste sensitivities due to many years of drinking and smoking and not genetics at all.

In another vein, clinicians who treat alcoholics have reported, while anecdotal in many cases, that many of their newly sober patients develop a carbohydrate appetite or

'sweet tooth' (Yung, Gordis & Holt, 1983). They may begin consuming large amounts of cake, ice cream, chocolate or candies which they had not liked before (Yung et al, 1983). Alcoholics Anonymous traditionally advises new members to carry candies with them to help suppress the urge to drink (Yung et al, 1983), suggesting that the intake of sweets will dampen 'cravings' for alcohol. One study examining the influence of dietary composition on sobriety found that subjects who stayed sober for longer than 30 days chose diets that contained more carbohydrates, including three times more sugar per cup of beverage (Yung et al, 1983). This difference disappeared when comparing sobriety at 50 days, indicating that higher carbohydrate intake contributed to sobriety and wasn't merely a result (Yung et al, 1983). When comparing sweet preferences with PROP tasting, one group of researchers found a greater proportion of nontasters of PROP among subjects with a greater hedonic response to sucrose (Looy & Weingarten, 1992). Therefore, as both sweet-preferrers, alcoholics and their children are less likely to be able to taste bitter substances, perhaps due to decreased taste sensitivities, it may be reasonable to predict that a greater percentage of people with a sweet tooth will be found in the alcoholic population (Overstreet et al, 1993).

Finally, a wide range of behavioral treatment methods for alcoholism using taste as a conditioned stimulus have been developed (Nathan, 1985). These methods have largely been confined to aversive conditioning therapies employing either electric shock or nausea-inducing drugs to induce aversion (Nathan, 1985). While chemical aversion therapies have been more successful than electrical (it has been suggested that nausea is "biologically appropriate" to alcohol consumption while electric shock is not; Garcia et al, 1974), both rely on the taste and smell of alcohol for treatment success (Nathan, 1985). In standard electrical aversion sessions, electric shock was administered to the subject when a sip of alcohol was taken and terminated when it was spit out, constituting aversion relief (Nathan, 1985). Apart from conceptual and ethical concerns about the treatment procedures, electrical aversion therapy has not reliably been shown to attenuate drinking in alcoholics

following treatment (Miller & Hersen, 1972; Wilson & Nathan, 1975; Wilson, 1978). Chemical aversion therapy for alcoholics has however been shown to have at least 50% success rates in several studies (Lemere & Voegtlin, 1950; Neubuerger, Matarazzo, Schmitz & Pratt, 1980; Thimann, 1949; Wiens, Montague, Manaugh & English, 1976). In this treatment paradigm, an emetine-pilocarpine-ephedrine mixture was administered to subjects intravenously, producing nausea within 2 to 8 minutes (Voegtlin, 1940). Immediately prior to the first signs of nausea, the subjects were given a drink of their favourite alcoholic beverage to smell, then to taste (Voegtlin, 1940). Additional drinks were provided over a 30-60 minute period as the signs of nausea intensified (vomiting, sweating, increased respiration; Voegtlin, 1940). Abstinence rates at one year follow-up ranged from 52% to 63% (Lemere & Voegtlin, 1950; Neubuerger et al, 1980; Thimann, 1949; Wiens et al, 1976). However, no control groups were reported in any of these studies and the patients entering chemical aversion programs and participating in the research were a homogeneous group of financially stable, well-educated, highly motivated, intact alcoholics (Nathan, 1985). While these behavioral treatment methods do not deal with why these patients became alcoholic in the first place, they do demonstrate that a conditioned taste aversion paradigm linking the taste of alcohol with nausea may be a useful tool in the treatment of alcoholism in conjunction with other therapies such as coping strategies.

CONCLUSIONS

The results of the present study challenge the contention about a simple, unidirectional relationship between ethanol and sweet preference in rats. While it was expected that preference for ethanol would be echoed by preference for the saccharin-quinine solution, thus revealing the existence of generalized, innate taste sensitivities which may predict ethanol preference, results indicated that in fact the ethanol-preferring strain rejected the SQ while the ethanol-nonpreferring strain showed very high preference levels for SQ. The animal literature to date strongly suggests that there is a positive correlation between ethanol and sweet preference (Gosnell & Krahn, 1992; Kampov-Polevoy et al, 1990; Overstreet et al, 1993; Sinclair et al, 1992), and the human literature supports this notion in describing alcoholics as sweet-preferrers with elevated desires for sugars (carbohydrates) during abstinence periods (Yung et al, 1983). Whereas there can be no argument that taste and olfaction play a role in guiding ingestion particularly in animals, caution should be exercised in attempting to create a theory of innate taste sensitivities and alcoholism. While in disagreement with the literature, the results of the present study are nevertheless of value in revealing the existence of ostensibly inherited, specific taste sensitivities which governed the ingestive behaviors of the three different rat strains. This data has now provided the basis for more thorough investigations of the relationship in rats between preference for ethanol and other sapid fluids of comparable flavours.

REFERENCES

Amit, Z., & Smith, B.R. (1992). Differential ethanol intake in Tryon maze-bright and Tryon maze-dull rats: Implications for the validity of the animal model of selectively bred rats for high ethanol consumption. Psychopharmacology, 108, 136-140.

Amit, Z., & Stern, M.H. (1969). Alcohol ingestion without oropharungeal sensations. Psychonomic Science, 15(3), 162-3.

Archambault, D. (1989). Adolescence: A physiological, cultural and psychological no man's land. In G. Lawson and A. Lawson (Eds.), Alcoholism and substance abuse in special populations (pp. 223-245). Rockville, MD: Aspen Systems.

Badia-Elder, N.E., Kiefer, S.W., & Dess, N.K. (1994). Alcohol preference in rats selectively bred for saccharin consumption. Society for Neuroscience Meeting, November 1994, Abstract 658-V58.

Baker, H.J., Lindsey, J.R., & Weisbroth, S.H. (1979). The Laboratory rats, Volume 1: Biology and diseases. Orlando, Florida: Academic Press Inc.

Beerstecher, Jr., E., Sutton, H.E., Berry, H.K., Brown, W.D., Reed, J., Rich, G.B., Berry, L.J., & Williams, R.J. (1950). Biochemical individuality. V. Explorations with respect to the metabolic patterns of compulsive drinkers. Archives of Biochemistry, 29, 27-40.

Benjamin, R.M. (1955a). Cortical taste mechanisms studied by two different test procedures. Journal of Comparitive and Physiological Psychology, 48, 119-122.

Benjamin, R M. (1955b). The effect of fluid deprivation on taste deficits following cortical lesions. Journal of Comparitive and Physiological Psychology, 48, 502-505.

Bice, P.J., & Kiefer, S.W. (1990). Taste reactivity in alcohol preferring and nonpreferring rats. Alcoholism: Clinical and Experimental Research, 14(5), 721-7.

Borer K.T. (1968). Disappearance of preferences and aversions for sapid solutions in rats ingesting untasted fluids. Journal of Comparative and Physiological Psychology, 65(2), 213-221.

Braun, J.J., Lasiter, P.S., & Kiefer, S.W. (1982). The gustatory neocortex of the rat. Physiological Psychology, 10, 13-45.

Braun, J.J., Slick, T.B., & Lorden, J.F. (1972). Involvement of gustatory neocortex in the learning of taste aversions. Physiology and Behavior, 9, 637-641.

Cannon, D.S., & Carrell, L.E. (1987). Rat strain differences in ethanol self-administration and taste aversion learning. Pharmacology, Biochemistry and Behavior, 28, 57-63.

Cicero, T.J. (1980). Animal models of Alcoholism? In K. Eriksson, J.D. Sinclair, & K. Kiianmaa (Eds.), Animals Models in Alcohol Research. London: Academic Press

Inc..

Cicero, T.J., & Myers, R.D. (1968). Selection of a single ethanol test solution in free-choice studies with animals. Quarterly Journal of Studies on Alcohol, 29, 446-448.

Clark, W., & Midnick, L. (1982). Alcohol use and alcohol problems among U.S. adults: Results of the 1979 national survey. In National Institute on Alcohol Abuse and Alcoholism, Alcohol consumption and related problems (pp.3-32). (DHHS Publication No. ADM 82-1190). Washington, DC: U.S. Government Printing Office.

Dicker, S.E. (1958). The effects of methylpentynol on ethanol drinking and on water metabolism in rats. Journal of Physiology, 144, 138-147.

Di Lorenzo, P.M., Kiefer, S.W., Rice, A.G., & Garcia, J. (1986). Neural and behavioral responsivity to ethyl alcohol as a tastant. Alcohol, 3, 55-61.

Dole, V.P. (1986). On the relevance of animal models to alcoholism in humans. Alcoholism: Clinical and Experimental Research, 10, 361-363.

Eriksson, K. (1968). Genetic selection for voluntary alcohol consumption in the albino rat. Science, 159, 739-741.

Forgie, M.L., Beyerstein, B.L., & Alexander, B.K. (1988). Contributions to taste factors and gender preference in C57BL and DBA mice. Psychopharmacology (Berlin), 95, 237-244.

Garcia, J., Hankins, W.G., & Rusiniak, K.W. (1974). Behavioral regulation of the milieu interne in man and rat. Science, 185, 824-831.

George, F.R. (1987). Genetic and environmental factors in ethanol self-administration. Pharmacology, Biochemistry and Behavior, 27, 379-384.

Gill, J.G. (1989). A critical evaluation of the use of animal models in alcohol research: An examination of voluntary alcohol consumption in rodents. Unpublished Doctoral Thesis, Concordia University, Montreal, Qc., Canada.

Gosnell, B.A., & Krahn, D.D. (1992). The relationship between saccharin and alcohol intake in rats. Alcohol, 9, 203-206.

Grant, K.A., & Samson, H.H. (1985). Induction and maintenance of ethanol self-administration without food deprivation in the rat. Psychopharmacology, 86, 475-479.

Grill, H.J. (1985). Introduction: Physiological mechanisms in conditioned taste aversions. Annals of the New York Academy of Sciences, 443, 67-88.

Grill, H.J., & Norgren, R. (1978). The taste reactivity test: I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Research, 143, 263-279.

Halpern & Tapper (1971). Taste stimuli: Quality coding time. Science, 171, 1256-1258.

Hankins, W.G., Garcia, J., & Rusiniak K.W. (1973). Dissociation of odor and taste in baitshyness. Behavioral Biology, 8, 407.

Hilton, M.E. (1987) Drinking patterns and drinking problems in 1984: Results from a general population survey. Alcoholism: Clinical and Experimental Research, 11, 167-175.

Holland, S., & Griffin, A. (1984). Adolescent and adult drug treatment clients: Patterns and consequences of use. Journal of Psychoactive Drugs, 7, 79-89.

Hunt, W.A. (1993). Neuroscience research: How has it contributed to our understanding of alcohol abuse and alcoholism? A review. Alcoholism: Clinical and Experimental Research, 17(5), 1055-1065.

Jellinek, E.M. (1952). Phases of alcohol addiction. Quarterly Journal of Studies on Alcohol, 13, 673-684.

Jellinek, E.M. (1960). The disease concept of alcoholism. New Haven, CT: Hillhouse Press.

Kahn, M., & Stellar, E. (1960). Alcohol preference in normal and anosmic rats. Journal of Comparitive and Physiological Psychology, 53(6), 571-575.

Kalant, H. (1987). Current trends in biomedical research on alcohol. Alcohol & Alcoholism, Supplement 1, 1-12.

Kampov-Polevoy, A.B., Kasheffskaya, O.P., & Sinclair, J.D. (1990). Initial acceptance of ethanol: Gustatory factors and patterns of alcohol drinking. Alcohol, 7, 83-85.

Kiefer S.W., Bice, P.J., Orr, M.R., & Dopp, J.M. (1990). Similarity of taste reactivity responses to alcohol and sucrose mixtures in rats. Alcohol, 7, 115-120.

Kiefer, S.W., & Lawrence, G.J. (1988). The sweet-bitter taste of alcohol: aversion generalization to various sweet-quinine mixtures in the rats. Chemical Senses, 13(4), 633-641.

Kiefer, S.W., Lawrence, G.J., & Metzler, C.W. (1986). Learned alcohol aversions in rats: Gustatory and olfactory components. Alcohol, 3, 27-31.

Kiefer, S.W., Lawrence, G.J., & Metzler, C.W. (1987). Alcohol preference in rats lacking gustatory neocortex. Alcohol, 4, 37-43.

Kiefer, S.W., Metzler, C.W., & Lawrence, G.J. (1985). Neocortical involvement in the acquisition and retention of learned alcohol aversions in rats. Alcohol, 2, 597-601.

Lankford, M.F., Roscoe, A.K., Pennington, S.N., & Myers, R.D. (1991). Drinking of high concentrations of ethanol versus palatable fluids in alcohol-preferring (P) rats: Valid animal model of alcoholism. Alcohol, 8, 293-299.

Lawrence, G.J., & Kiefer, S.W. (1987). Generalization of specific taste aversions to alcohol in the rat. Chemical Senses, 12(4), 591-599.

Le Magnen, J., & Marfaing-Jallat, P. (1961). Le ro_le des afferences buccales dans le determinisme des consommations spontanees d'alcool chez le Rat. Journal de Physiologie, Paris, 53, 407-408.

Lemere, F. & Voetglin W.L. (1950). An evaluation of the aversion treatment of alcoholism. Quarterly Journal of Studies on Alcohol, 11, 199-204.

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

Lester, D., Nachman, M., & LeMagnen, J. (1970). Aversive conditioning by ethanol in the rat. Quarterly Journal of Studies on Alcohol, 31, 578-586.

Lettieri, D.J., Sayers, M., & Pearson, H.W. (Eds.) (1980). Theories on Drug Abuse, Selected Contemporary Perspectives. National Institute of Drug Abuse Research Monograph 30. Rockville, Maryland: Department of Health and Human Services.

Li, T.K., & Lumeng, L. (1984). Alcohol preference and voluntary alcohol intakes of inbred strains and the National Institutes of Health heterogeneous stock of rats. Alcoholism: Clinical and Experimental Research, 8(5), 485-486.

Li, T.K., Lumeng, L., McBride, W.J., & Murphy, J.M. (1987). Rodent lines selected for factors affecting alcohol consumption. Alcohol and Alcoholism (Supplement), 1, 91-96.

Looy, H, & Weingarten, H.P. (1992). Facial expressions and genetic sensitivity to 6-*n*-propylthiouracil predict hedonic response to sweet. Physiology and Behaviour, 52, 75-82.

Lorden, J. (1976). Effects of lesions of the gustatory neocortex on taste aversion learning in the rat. Journal of Comparative and Physiological Psychology, 90, 665-679.

Lumeng, L., Doolittle, D.P., & Li, T.K. (1986). New duplicate lines of rats that differ in voluntary alcohol consumption. Alcohol & Alcoholism, 21, A37.

Lumeng, L., Hawkins, T.D., & Li, T.K. (1977). New strains of rats with alcohol preference and nonpreference. In R.G.Thurman, J.R.Williamson, H.R.Drott & B.Chance (Eds.), Alcohol and Aldehyde Metabolizing Systems: Volume III (pp. 537-544). New York: Academic Press, Inc.

Lumeng, L., Penn, P.E., Gaff, T.M., Hawkins, T.D., & Li, T.-K. (1978). New strains of rats with alcohol preference and non-preference. In R.G. Thurman, J.R. Williamson, H. Drott & B. Chance (Eds.), Alcohol and aldehyde metabolizing systems, vol. 3. New York: Academic Press.

Mackintosh, N.J. (1974). The psychology of animal learning. London: Academic Press.

Madsen, W. (1974). The american alcoholic: The nature-nurture controversies in alcoholic research and therapy. Springfield, Illinois: Charles C. Thomas.

Mardones, J., & Segovia-Riquelme, N. (1983) Thirty-two years of selection of rats by ethanol preference: UChA and UChB strains. Neurobehaviour, Toxicology and Teratology, 5, 171-178.

Marfaing-Jallat, P., Pruvost, & Le Magnen, J. (1974). La consommation d'ethanol par auto-administration intragastrique chez le rat. Journale de Physiologie (Paris), 68, 81-95.

McBride, W.J., Murphy, J.M., Lumeng, L., & Li, T.-K. (1989). Serotonin and ethanol preference. In M. Galanter (Ed.), Recent Developments in Alcoholism, Volume 7 (pp. 187-209). New York: Plenum Press.

McBurney, D.H. & Gent, J.F. (1979). On the nature of taste qualities. Psychological Bulletin, 86, 151-167.

McClearn, G.E., & Rodgers, D.A. (1961). Genetic factors in alcohol preference of laboratory mice. Journal of Comparative and Physiological Psychology, 54, 116-119.

Meisch, R.A. (1977). Ethanol self-administration: Infrahuman studies. In T. Thompson and P. Dews (Eds.), Advances in Behavioral Pharmacology, Volume 1 (pp. 35-84). New York: Academic Press, Inc.

Miller, P.M. & Hersen, M. (1972). Quantitative changes in alcohol consumption as a function of electrical aversive conditioning. Journal of Clinical Psychology, 28, 590-593.

Minium, E.W., King, B.M., & Bear, G. (1993). Statistical reasoning in psychology and education (3rd ed.). New York: John Wiley & Sons, Inc.

Mook, D.G (1963). Oral and postingestional determinants of the intake of various solutions in rats with esophageal fistulas. Journal of Comparative and Physiological Psychology, 56(4), 645-659.

Mook, D.G., & Kenney, R. (1977). Taste modulation of fluid intake. In J.A.W.M. Weijnen and J. Mendelson (Eds.), Drinking behavior, oral stimulation, reinforcement and preference (pp. 275-315). New York: Plenum Press.

Morse, R.M., & Flavin, D.K. (1992). The definition of alcoholism. JAMA: The Journal of the American Medical Association, 268(8), 1012-1014.

Murphy, J.M., Gatto, G.J., McBride, W.J., Lumeng, L., & Li, T.-K. (1989). Operant responding for oral ethanol in the alcohol-preferring P and alcohol-nonpreferring NP lines of rats. Alcohol, 6, 127-131.

Murphy, J.M., McBride, W.J., Lumeng, L., & Li, T.-K. (1983). Monoamine and metabolite levels in CNS regions of the P line of alcohol-preferring rats after acute and chronic ethanol treatment. Pharmacology, Biochemistry and Behaviour, 19(5),

849-856.

Myers, R.D. (1961). Changes in learning, extinction, and fluid preferences as a function of chronic alcohol consumption in rats. Journal of Comparitive and Physiological Psychology, 54(5), 510-516.

Myers, R.D. (1968). Ethyl alcohol consumption: Valid measurement in albino rats. Science, 161, 76-77.

Myers, R.D. (1978). Psychopharmacology of alcohol. Annual Review of Pharmacology and Toxicology, 18, 125-144.

Myers, R.D., & Veale, W.L. (1972). The determinants of alcohol preference in animals. In H. Kissin & H. Begleiter (Eds.), Biology of Alcoholism, Volume 2 (pp. 131-168). New York: Plenum Press.

Nachman, M., Larue C., & Le Magnen, J. (1971). The role of olfactory and orosensory factors in the alcohol preference of inbred strains of mice. Physiology and Behavior, 6, 53-59.

Nathan, P.E. (1985). Aversion therapy in the treatment of alcoholism: Success and failure. Annals of the New York Academy of Sciences, 443, 357-364.

National Council on Alcoholism/America Medical Society for Alcoholism Committee on Definitions (1976). Definition of Alcoholism. Annals of Internal Medecine, 85, 764.

Neubuerger, O.W., Matarazzo, J.D., Schmitz, R.E. & Pratt, H.H. (1980). One year follow-up of total abstinence in chronic alcoholic patients following emetic counterconditioning. Alcoholism: Clinical and Experimental Research, 4, 306-312.

Nowlis, G.H., Frank, M.E., & Pfaffman, C. (1980). Specificity of acquired aversions to taste qualities in hamsters and rats. Journal of Comparative and Physiological Psychology, 94(5), 932-942.

Overstreet, D.H., Kampov-Polevoy, A.B., Rezvani, A.H, Murelle, L., Halikas, J.A., & Janowsky, D.S. (1993). Sacharin intake predicts ethanol intake in genetically heterogeneous rats as well as different rat strains. Alcoholism: Clinical and Experimental Research, 17(2), 366-369.

Pattison, E.M., & Kaufman, E. (1982). The alcoholism syndrome: Definitions and models. In E.M. Pattison and E. Kaufman (Eds.), Encyclopedic handbook of alcoholism (pp. 3-23). New York: Gardner Press.

Peele, S. (1990) Second thoughts about a gene for alcoholism. The Atlantic Monthly, August, 52-58.

Peeples, E.E. (1962). Taste sensitivity to phenylthiocarbamide in alcoholics. Unpublished Master's thesis, Stetson University, Deland, FL.

Pelchat, M.L., & Danowski, S. (1992). A possible genetic association between PROP-tasting and alcoholism. Physiology and Behavior, 51, 1261-1266.

Ramirez, I., & Spiott, R.L. (1978). Genetic mechanisms of drinking and feeding. Neuroscience and Biobehavioral Revues, 2, 15-26.

Rice, D.P., Kelman, S., Miller, L.S., & Dunmeyer, S. (1985). The economic cost to society of alcohol and drug abuse and mental illness. DHHS Publication No. ADM 90-1694. Washington, DC: Supt. of Docs, U.S. Government Printing Office, 1990.

Richter, C.P. (1957). Production and control of alcoholic craving in rats. In H.A. Abramson (Ed.), Neuropharmacology: Transactions of the third conference, 1956, Princeton, NJ (pp. 39-146). New York: Josiah Macy, Jr. Foundation.

Richter, C.P., & Campbell, K.H. (1940a). Alcohol taste thresholds and concentrations of solution preferred by rats. Science, 91, 507-508.

Richter, C.P., & Campbell, K.H. (1940b). Taste thresholds and taste preferences of rats for five common sugars. Journal of Nutrition, 20, 31-46.

Rick, J.T., & Wilson, C.W.M. (1964). Alcohol preference in the rat: Its relationship to total fluid consumption. Quarterly Journal of Studies on Alcohol, 27, 447-458.

Rivers, P.C. (1994). Alcohol and human behavior: Theory, research, and practice. New Jersey: Prentice Hall.

Rotzinger, S. (1994). Catalase inhibition attenuates the acquisition and maintenance of ethanol and saccharin-quinine consumption in laboratory rats. Master's Thesis, Concordia University, Montreal, Quebec, Canada.

Samson, H.H. (1986). Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. Alcoholism: Clinical and Experimental Research, 10, 436-442.

Samson, H.H., Tolliver, G.A., Lumeng, L., & Li, T.-K. (1989). Ethanol reinforcement in the alcohol nonpreferring rats: Initiation using behavioral techniques without food restriction. Alcoholism: Clinical and Experimental Research, 13(3), 378-385.

Schwartz, G.J., & Grill, H.J. (1984). Relationships between taste reactivity and intake in the neurologically intact rat. Chemical Senses, 9(3), 249-272.

Schuckit, M.A. (1985) Genetics and the risk for alcoholism. JAMA: The Journal of the American Medical Association, 253, 2614-2617.

Scott, T.R. (1974). Behavioral support for a neural taste theory. Physiology and Behavior, 12, 413-417.

Settle, R.G. (1978). The alcoholics' taste perception of alcohol: Preliminary findings. In M. Galanter (Ed.), Currents in Alcoholism, V, biomedical I and clinical effects of

alcoholism (pp.257-267). New York: Grune and Stratton.

Sherman, J., Rusiniak, K.W., & Garcia, J. (1984). Alcohol ingestive habits: The role of flavor and effect. In M. Galanter (Ed.), Recent Developments in Alcoholism, Vol. 2. New York: Plenum.

Sinclair, J.D., Kampov-Polevoy, A., Stewart, R., & Li, T.-K. (1992). Taste preferences in rat lines selected for low and high alcohol consumption. Alcohol, 9, 155-160.

Spiegel, J.A. (1972). Taste sensitivity as a possible genetic marker for inheritance of alcoholism. Unpublished Honors thesis, St. Louis.

Spuhler, K., & Deitrich, R.A. (1984). Correlative analysis of ethanol-related phenotypes in rats inbred strains. Alcoholism: Clinical and Experimental Research, 8(5), 480-484.

Stewart, R.B., Russell, R.N., Lumeng, L., Li, T.-K., & Murphy, J.M. (1994). Consumption of sweet, salty, sour, and bitter solutions by selectively bred alcohol-preferring and alcohol-nonpreferring lines of rats. Alcoholism: Clinical and Experimental Research, 18(2), 375-381.

Suzuki, T., George, F.R., & Meisch, R.A. (1988). Differential establishment and maintenance of oral ethanol reinforced behavior in Lewis and Fischer 344 inbred rats strains. The Journal of Pharmacology and Experimental Therapeutics, 245(1), 164-170.

Suzuki, T., George, F.R., & Meisch, R.A. (1992). Etonitazene delivered orally serves as a reinforcer for Lewis but not Fischer 344 rats. Pharmacology, Biochemistry and Behavior, 42, 579-586.

Suzuki, T., Otani, K., Koike, Y., & Misawa, M. (1988). Genetic differences in preferences for morphine and codeine in Lewis and Fischer 344 inbred rat strains. Japanese Journal of Pharmacology, 47, 425-431.

Swinson, R.P. (1973). Phenylthiocarbamide taste sensitivity in alcoholism. British Journal of Addiction, 68, 33-36.

Swinson, R.P. (1983). Genetic markers and alcoholism. Recent Developments in Alcoholism, 1, 9-24.

Tapper, D.N., & Halpern, B.P. (1968). Taste stimuli: A behavioral categorization. Science, 161, 708-709.

Thiman, J. (1949). Conditioned reflex treatment of alcoholism. II. The risk of its application, its indications, contraindications and psychotherapeutic aspects. New England Journal of Medicine, 241, 408-410.

Touzani, K., Akarid, K., & Velley, L. (1991). Modulation of saccharin preference by morphine and naloxone: Inversion of drug effects as a function of saccharin

concentration. Pharmacology, Biochemistry and Behavior, 38, 37-41.

Voegtlin, W.L. (1940). The treatment of alcoholism by establishing a conditioned reflex. American Journal of Medical Science, 199, 802-809.

Waller, M.B., McBride, W.J., Lumeng, L., & Li, T.-K. (1982). Induction of dependence on ethanol by free-choice drinking in alcohol-preferring rats. Pharmacology, Biochemistry & Behavior, 16, 501-507.

Waller, M.B., McBride, W.J., Gatto, G.J., Lumeng, L., & Li, T.-K. (1984). Intragastric self-infusion of ethanol by ethanol-preferring and -nonpreferring lines of rats. Science, 225, 78-80.

Wiens, A.N., Montague, J.R., Manaugh, T.S. & English, C.J. (1976). Pharmacological aversive counterconditioning to alcohol in a private hospital: One year follow-up. Journal of Studies on Alcoholism, 37, 1320-1324.

Wilson, G.T. & Nathan, P.E. (1975). The aversive control of excessive drinking by chronic alcoholics in the laboratory setting. Journal of Applied and Behavioral Analysis, 8, 13-26.

Wilson, G.T. (1978) Booze, beliefs and behavior: Cognitive processes in alcohol use and abuse. In P.E. Nathan & G.A. Marlatt (Eds.), Alcoholism: New Directions in Behavioral Research and Treatment. New York: Plenum Press.

Winger, G., Young, A.M., & Woods, J.H. (1983). Ethanol as a reinforcer: Comparison with other drugs. In B. Kissin and H. Begleiter (Eds.), The pathogenesis of alcoholism (pp.107-131). New York: Plenum Press.

York J.L. (1981). Consumption of intoxicating beverages by rats and mice exhibiting high and low preferences for ethanol. Pharmacology, Biochemistry & Behavior, 15, 207-214.

Yung, L., Gordis, E. & Holt, J. (1983). Dietary choices and likelihood of abstinence among alcoholic patients in an outpatient clinic. Drug and Alcohol Dependence, 12, 355-362.