The role of taste in ethanol intake: Studies on taste preferences and neural systems in several rat strains

Frances L.W. Goodwin

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy at
Concordia University
Montreal, Quebec, Canada

ABSTRACT

The role of taste in ethanol intake:
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in several rat strains

Frances L.W. Goodwin, Ph.D.
Concordia University, 2002

High and low ethanol-drinking rodents show similar preferences for sweet solutions. Studies have suggested that sweet preference may be a reliable predictor of ethanol consumption in all rodents and, furthermore, that the sweet-ethanol relationship is mediated by a common biological mechanism. The present thesis investigated the role of taste preferences in ethanol intake in rats, to determine whether the relationship between ethanol and sweet intake was universal. Bitter and bittersweet solution intake were also compared with ethanol, based on research that rats find unflavoured ethanol to have a sweet and bitter taste. Fluid intake correlations among rat strains were examined following manipulation of the gabaergic, dopaminergic or opioidergic systems in order to reveal a common neural system mediating ethanol, sweet or bitter solution intake.

Experiments 1 and 2 showed that saccharin and ethanol intake were not related in four rat strains. However, ethanol intake was related to saccharin-quinine intake in those strains. Experiment 3 demonstrated that intake of dilute quinine differentiated Fawn-Hooded, Lewis and Wistar rats in a similar manner to ethanol intake, suggesting that taste preferences for bitter solutions, and not sweet solutions, were a more reliable predictor of ethanol intake.

Neither the $GABA_A$ agonist THIP, the dopamine D2/D3 antagonist raclopride nor the opioidergic antagonist naltrexone succeeded in altering ethanol, saccharin and quinine intake in a

consistent manner in Experiment 4. Naltrexone was shown to have no effect on the intake of five flavoured fluids during continuous access drinking, but did attenuate all but quinine intake during limited access drinking. It was unclear whether the effects achieved during limited access were due to a change in palatability or some nonspecific drug effect (e.g., attenuation of locomotor activity).

Overall, the findings from the current experiments challenged the notion that sweet solution preference is associated with ethanol intake in all rats, and that the opioid system is mediating this relationship. Results showed that while fluid intake interrelationships varied among the four strains tested, ethanol intake was most closely associated with the intake of a bitter and a sweet-bitter solution. In addition, the fluid intake relationships did not appear to be mediated by the gabaergic, dopaminergic or opioidergic systems.

ACKNOWLEDGMENTS

The point at which I decided that natural childbirth was easier than writing a thesis was a low one for me. Having done both twice, I felt like I knew what I was talking about. However, as one stumbles through the first weeks and months following the birth of a child, I stumbled through what remained to be written of my thesis. Early mornings, late nights, endless feedings and changings, but I finally managed to finish writing. I unofficially dedicate this thesis to all those who kept asking me: "aren't you done yet?". Yes, I'm done.

Without a doubt, I could not have accomplished all of this (the thesis part, that is) without the support and encouragement of my mentor and supervisor, Zalman Amit. In what was almost a comical play, while I was having babies and trying to finish courses, Zalman was taking sabbatical leave, moving away from Montreal and relocating in Nova Scotia and Israel. And yet he always stayed in touch. I'm still amazed at what we accomplished together. Zalman, you have also been through such a difficult time in your life while I wrote away, and I am truly sorry to have burdened you with what seemed to be comparatively trivial problems. I have learned from my courses, but I have also learned from your strength, your wisdom and your courage to face life's difficulties with great dignity and self-confidence.

I would like to acknowledge all of the students who have helped me along the way, whether for course credit or just for interest in science. Natasha Bergeron, Stephanie Fragman, Isabella Babinska, Michelangelo Campisi. My fellow lab colleagues, past and present, who I have missed since our lab has winded down in the last few years of my school experience: Brian Smith, Franc Rogan, Ricky Koechling, Alan Boyle, Wayne Bowers, Deena Davidson, Susan Rotzinger, Danny Kunin, Lana Pratt, Van Redila, Joanne Horan. I often remember my early days in graduate school and certain people who have touched me along the way: Kim Edelstein, Mark Legault, Sylvie Deschamps, Marilyn Rackover, Doug Funk. Too many to recall, but all part of the experience.

Where there are babies, there are babysitters, and I could not have completed this enormous task without their help: Pam Russell, Erin Harris, Dawn Hirsch, Jasmine Kastner, Stephanie Gobeil, Andrea and Derek my step-kids.

A few important people on the administrative end of my school career: Elizabeth Chau, Phyllis Webster, Gerry Kidney and Shirley Black, plus various other more-than-helpful secretaries and support staff who have saved me from over-anxious undergraduate students, man-eating photocopy machines and those troublesome registration deadlines (and I've signed for quite a few of them!). Thank you to my committee members: Dr. Barbara Woodside, Dr. Dave Mumby, Dr. Muriel Herrington, Dr. Stephen Kiefer, and Dr. Brian Smith. I also need to thank Dr. Anna-Beth Doyle, who had the unfortunate job of being Graduate Program Director for us Ph.D candidates while I was struggling along- whether she knows it or not, she kept me from drowning a few times.

And, last but not least, I want to acknowledge my family. I know that this has been a terribly stressful time for all those over the age of 5 years. Amazingly enough, while I was squirreled away with my computer, Andrea and Derek grew up into delightful and engaging young adults, and Victoria and Dylan grew from being babies to little people in their own rights, with impressive vocabularies, unique fashion senses and dry pants (most importantly!). My battle-weary husband, Dennis, who has tried to support me to the best of his ability all the way along (although I think I lost him about half way through) will be so glad to 'get my wife back'. My parents, Harvey and Liz, will notice a lighter spring to my step, and will no longer have to worry about mentioning the "Th" word around me anymore. Thank you all for waiting for me.

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The consumption of alcoholic beverages is a common behaviour in many cultures worldwide. In North America, a large proportion of the adult population consumes alcohol, and most appear to do so without inflicting harm on themselves or others (Hunt, 1993). In 1998, it was estimated that the average North American consumed 7.4 litres of absolute alcohol per year (World Drink Trend, 2000). When compared with the universally accepted level of risk for alcoholism of 10 litres of alcohol per capita (World Drink Trend, 2000), clearly North American alcohol consumption is well below what might be considered excessive. However, included within these statistics are heavy drinkers, numbering in excess of nine million people in the United States (Rivers, 1994) and 1.5 million in Canada (based on intake of 14 or more drinks per week, Statistics Canada 1998-1999). It is the aberrant drinking patterns displayed by this heterogeneous group of alcohol abusers that inflict great economic costs on society in the form of lost employment, increased burden on the health care system, reduced productivity and higher rates of crime (Busto, 2000; Rice, Kelman, Miller & Dunmeyer, 1985), as well as obvious personal hardships for the alcohol abusers and their families.

It is well known today that people drink alcohol, whether occasionally or to excess, because of complex interactions between biological and environmental factors (Cunningham, Fidler & Hill, 2000; 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" (National Council on Alcoholism/American Medical Society on Alcoholism Committee on Definitions, 1976), a definition which emphasized 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. The definition of alcoholism was revised in 1992 by a Joint Committee of the National Council on Alcoholism and Drug Dependence and the American Society of Addiction Medicine. This new definition now acknowledged the importance of these biological and environmental factors while attempting to establish a more precise use for the term 'alcoholism', which had 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: one study conducted by the National Institute on Drug Abuse reported 43 different theories on the development of drug abuse and alcoholism, representing biological, social developmental and personality perspectives (Lettieri, Sayers & Pearson, 1980). It was the hope of the revision committee that their 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 the development of harmful and excessive patterns of alcohol consumption in humans may provide important information about the development of alcohol dependence itself.

The role of taste factors in alcohol drinking

One factor implicated in the development and maintenance of alcohol drinking in both humans and experimental animals is taste, considered to be a genetic factor (i.e., taste sensitivities and preferences) which is further modified by environmental influence (e.g., flavouring) (Duffy & Bartoshuk, 2000). In North American culture, alcohol, unlike other drugs of abuse, is introduced into the body via the oral cavity, thereby stimulating the sensory system of taste. Taste is generally acknowledged as a primary factor in determining choice of alcoholic beverage, while the rate of onset and intensity of pharmacologic effect as well as the extent of hangover (withdrawal symptoms such as nausea, sweating, shakiness, and anxiety) are considered determinants of 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). The flavours of alcoholic beverages are produced from fermented grains (beer: alcohol content ranging from 0.5-7% alcohol by volume), fermented grapes (wine and fortified wine: 10-20% alcohol by volume) and fermented alcohol solutions derived from fruit, grain or carbohydrates (distilled spirits, e.g., whiskey, bourbon, gin: 40-95% alcohol by volume) (Rivers, 1994). Despite the variety of flavours available, in general humans prefer to add more flavouring to their alcoholic beverages (especially with respect to the distilled spirits, such as vodka with orange juice or rum with cola). When 'learning' to consume alcohol, regardless of the type of alcoholic beverage, it is a common practice for people to mix alcohol with sweet beverages such as fruit juices and soft drinks to increase palatability (Jellinek, 1952, 1960; Samson, Files & Brice, 1996; York, 1981; see also Samson, Maxwell & Doyle, 1989). As stable drinking patterns develop over time with

experience, individuals may undergo a narrowing of their drinking repertoire (i.e., a more limited range of preference for different alcohols) and hence flavour preferences, as well as a shift toward consuming alcohol that is unadulterated by other fluids (Pattison & Kaufman, 1982). Therefore, while flavouring is important when first learning about drinking, used to mask the unpleasant taste of undiluted alcohol, the flavour of the alcohol itself plays a role when drinking behaviors are well established, in acting to direct the drinker to their preferred beverage.

There is also psychophysical evidence of genetic variation in taste sensitivities among humans, in the form of sweet-liking or -disliking (Looy & Weingarten, 1992) and sensitivity to bitterness (Bartoshuk, 1991; Fox, 1931), which subsequently influence food intake and preferences in general (Duffy & Bartoshuk, 2000). It is thought that these taste preferences may also contribute to determining levels of alcohol consumption (Kampov-Polevoy, Garbutt & Janowsky, 1997; Pelchat and Danowski, 1992). For example, it has been demonstrated in humans in a laboratory setting that detection thresholds for bitterness are negatively associated with levels of beer ingestion (Mattes, 1994; Tanimura & Mattes, 1993). Therefore, a better understanding of the role of taste, both with respect to flavouring and taste sensitivities, in alcohol drinking is necessary in order to understand the development and maintenance of alcohol abuse in humans.

In the following sections, the use of animal models to study alcohol drinking behaviors will be discussed, as well as a justification for using rats as comparison for humans in studies of taste preferences. The experiments which reported that rats find alcohol to have a sweet and bitter taste will be described, as well as those suggesting that sweet-taste preference and alcohol intake are linked in rats. And finally, the notion that an

association between alcohol intake and taste preferences might be caused by common neural system is discussed in a selective summary of the literature focusing on the interaction of ethanol and sweetened solutions with opioidergic, dopaminergic and gabaergic neurotransmitter systems.

Using animals to study alcoholism

Researchers have long used animals to study various aspects of alcohol consumption (e.g., Cicero, 1980; Cunningham et al., 2000; Dole, 1986; Lester & Freed, 1973). The animal model for human alcoholism is still a popular research tool because it circumvents the difficulties involved in conducting human alcohol research, such as the ethical considerations in exposing human alcoholics and nonalcoholic volunteers to alcohol, confounding nutritional and social variables, and stringent government-regulated guidelines which must be followed in conducting drug-related research (Cicero, 1980). The assumption underlying the disputed success of the animal model of alcoholism was that the biological factors which initiated and maintained excessive alcohol consumption in animals may be the same or similar to those in humans (Cicero, 1980). Most investigators no longer view the animal model as an attempt to simulate 'human alcoholism', but use the variety of animal behavioural models primarily to characterize alcohol's motivational effects (e.g., increases in elation, alleviation of stress or anxiety, development of hangover or withdrawal) (Cunningham et al., 2000). While it is not yet completely understood why humans begin abusing alcohol, it is assumed to be primarily ingested for its pharmacological properties which result in both positive and/or negative motivational effects (Cicero, 1980). However, animal research shows that oral alcohol

self-administration is also influenced by many nonpharmacological variables, including taste, palatability and caloric value of the ingested alcohol (e.g., Kiefer, 1995; Stiglick & Woodworth, 1984; York, 1981). Therefore, it is possible that individual differences in intake, both in humans and animals, may also be related to variations in sensitivity to alcohol's orosensory effects, for example, as well as to its pharmacological effects (Cunningham et al., 2000).

Rodents drinking alcohol for taste

There is ample data showing that rodents, like humans, can detect the taste of alcohol and use this information to influence subsequent alcohol intake. For example, naive rats seldom drink high concentrations of laboratory ethanol upon first exposure (Cicero & Myers, 1968; Richter & Campbell, 1940a). The initial reluctance to drink high concentrations of ethanol is assumed to reflect the aversive taste properties of absolute ethanol diluted with tap water. Conversely, the majority of naive rats seem to prefer low concentrations of ethanol over water (Boyle, Smith & Amit, 1997; Gill, 1989; Kiefer, Lawrence & Metzler, 1987; Kulkosky, 1981). This observed preference would suggest that the taste properties of low concentrations of ethanol may be perceived as more palatable. Furthermore, rats will voluntarily consume pharmacologically-meaningful levels of ethanol only when: 1) exposed to relatively high concentrations of ethanol over an extended period of time (Daoust et al., 1987), 2) slowly introduced to high concentrations of ethanol following a period of acclimatization to lower and more palatable concentrations (Mendelson & Mello, 1964; Myers & Veale, 1972; Williams, Berry & Beerstecher, 1949), 3) presented with ethanol adulterated with a sweetened

solution (Samson, 1986), or 4) prevented from using taste and/or smell information to direct ethanol intake (Amit & Stern, 1969; Kahn and Stellar, 1960). Furthermore, the results from detailed examinations of rats' orofacial responses to ethanol, which are said to reflect palatability (Kiefer, 1995), supported the notion that rats find increasing concentrations of ethanol to taste more aversive (Kiefer & Dopp, 1989).

More recently, studies investigating ethanol and taste preferences have reported on a direct association between preference for sweet-tasting solutions and alcohol consumption in both humans and rodents (e.g., Kampov-Polevoy et al., 1997; Kampov-Polevoy, Kasheffskaya & Sinclair, 1990; Overstreet et al., 1993; Stewart, Russell, Lumeng, Li & Murphy, 1994). It has been suggested that sweet preference may act as a behavioral marker for future alcohol intake (Bell, Gosnell, Krahn & Meisch, 1994; Kampov-Polevoy et al., 1997; Kampov-Polevoy, Garbutt & Janowsky, 1999). The link may originate at the neural level, as it has been proposed that the reinforcement produced by both alcohol and sweetened solutions may have at least one neuronal system in common (Gosnell & Krahn, 1992; Overstreet et al., 1993; Sinclair, Kampov-Polevoy, Stewart & Li, 1992). Evidence for this notion comes from literature on the neurochemical systems underlying both saccharin and ethanol intakes, which have implicated both the opiate (e.g., Linseman, 1989; Touzani, Akarid & Velley, 1991) and dopaminergic systems (e.g., McBride, Murphy, Lumeng & Li, 1990; Muscat, Kyprianou, Osman, Phillips & Willner, 1991).

About the taste of ethanol in rats

When using an animal model to study a human behavior, it is necessary to establish commonalties between the two systems in order to successfully generalize from one to the other (see Cicero, 1980). The animal model for human alcoholism, and specifically for oral self-administration of alcohol, presupposes that humans and rodents find the taste of alcohol to be similar. This notion is supported by research in rats and hamsters showing that rodents and humans tend to categorize flavours in a like manner (Nowlis, Frank & Pfaffman, 1980).

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). The aversion generalization experimental paradigm has provided information on the process of taste categorization in animals (Nowlis et al., 1980). This technique requires that animals be conditioned to reject a fluid, the tastant, by pairing it with radiation or chemical poisoning (e.g., lithium chloride), as in the conditioned taste aversion paradigm (Bernstein, 1999; Hunt & Amit, 1987). Generalization of this aversion to other taste solutions is then tested, evaluated by measuring intake of these subsequent tastes. The solutions tested in this paradigm are assumed to be rejected (i.e., consumed to a lesser amount compared with an untreated control group) to the degree that their taste is qualitatively similar to the initially conditioned aversive tastant. The magnitude of rejection of the taste solution may be an indication of the degree of similarity in taste between the tastant and the taste solution (Tapper & Halpern, 1968).

In a simple experiment designed to compare the rat's 'taste world' with that of humans, rats were trained to avoid 1 of 27 test solutions (Nowlis et al. 1980). The rats were then tested for generalization of the aversion to solutions which corresponded with the four prototypical human taste categories. The sweet tastant was rejected by the rodents conditioned to avoid most of the solutions described as sweet by humans (e.g., sucrose, saccharin) with the exception of some of the artificial sweeteners (e.g., aspartame). For tastants described as predominantly salty or sour by humans, the rats generalized their aversions to the salty or sour test solutions. Two of the three tastants described as sour also showed signs of a generalized aversion to the bitter test solution. For most of the tastants described as having a predominantly bitter component by humans, the rodents generalized their aversion to the bitter test solution, and somewhat more weakly to sour test solution. The results demonstrated 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). Furthermore, they 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 sweet-bitter taste of ethanol

Data collected using the aversion generalization paradigm have greatly advanced understanding of the rat's conception of the taste of ethanol. In the first study reported, rats trained to reject one of three different ethanol solutions (of weak, medium or strong concentration) were then tested for generalization of the aversion to sweet, salty, sour and bitter solutions, as well as mixtures of sweet with bitter and salty with sour (Di Lorenzo,

Kiefer, Rice & Garcia, 1986). The only significant generalization to develop was from the medium- and strong-strength ethanol solutions to the sweet-bitter mixture, suggesting that both of these ethanol solutions tasted both sweet and bitter to the animals. In a second experiment, a second group of rats were trained to reject only a medium-strength ethanol solution in the same manner as the previous experiment, and were then tested with all paired combinations of the same four basic taste solutions (Di Lorenzo et al., 1986). A significant generalization was again observed between the ethanol tastant solution to the sweet-bitter mixture and more weakly to the sweet-sour solution.

These findings were significant because they were the first to describe the taste of unflavoured ethanol in rats in humans terms. As well, it was suggested that ethanol may be endowed with a complex set of gustatory qualities, one of which was related to sweetness. Further studies have supported these conclusions. For example, rats were shown to generalize aversions for sweet and bitter solutions to mid-range ethanol solutions and aversions for a sweet-bitter mixture carried over to both medium and strong ethanol solutions (Lawrence & Kiefer, 1987). These findings, along with those of Di Lorenzo et al. (1986), demonstrated that the relationship between ethanol and sweet-bitter tastes was reciprocal. Interestingly, in a subsequent experiment which examined sweet and sour taste combinations, no generalizations were found to carry over from sour or sweet-sour solutions to any of the ethanol solutions (Lawrence & Kiefer, 1987; see also Kiefer & Lawrence, 1988). That the sweet-sour aversion generalization reported previously (Di Lorenzo et al., 1986) was not replicated suggested that the sour component in ethanol may be weak (Lawrence & Kiefer, 1987).

The aversion generalizations observed from the sweet-bitter solution used previously (Lawrence & Kiefer, 1987) to ethanol seemed to be dependent on ethanol concentration, as no aversions were found when animals were tested with a dilute ethanol solution and only weak generalizations were noted to the most concentrated ethanol solution. The mid-range ethanol solution yielded the most consistent aversion generalizations, which may reflect the fact that this concentration had a significant taste yet was not so aversive as to reduce consumption (Lawrence & Kiefer, 1987). The investigators also noted that the inconsistent responses to the three ethanol solutions of differing concentrations indicated dissimilar gustatory qualities among them, further suggesting that a rat may perceive all three concentrations of ethanol solutions as different fluid categories rather than the same fluid at different concentrations (Lawrence & Kiefer, 1987).

Altogether, the results from these aversion generalization studies demonstrated that the taste of ethanol for rats is comprised of a combination of taste components: a general sweet component and a bitter component (Lawrence & Kiefer, 1987; Kiefer & Lawrence, 1988). Also, the mixture of the sweet and bitter tastes must produce a new complex taste not characterized by the simple sum of the individual components, because it was shown that ethanol generalizations did not reliably carry over to either sweet or bitter tastes alone (e.g., Di Lorenzo et al, 1986).

Predicting ethanol intake from taste preferences

Early studies reported that high and low ethanol-drinking rodent strains also showed corresponding levels of intake of sweet (Forgie, Beyerstein & Alexander, 1988; Ramirez & Sprott, 1978; Rodgers & McClearn, 1964) and bitter solutions (Le Magnen & Marfaing-Jallat, 1961). These findings are in line with the studies showing that rodents find the taste of unflavoured laboratory ethanol to be both sweet (Blizard & McClearn, 2000; Kiefer & Lawrence, 1988) and bitter (Lawrence & Kiefer, 1987). Therefore, it seemed feasible that preference for a basic taste such as sweet or bitter might act as a reliable behavioural marker for future ethanol intake. This issue has been researched and hotly debated since the early 1990's and is the focus of the present thesis.

The co-occurrence of increased ethanol and sweet solution intake

The first study examining taste preferences in rats that were selectively-bred for high and low ethanol intake reported corresponding differences in the consumption of sweet solutions (Sinclair et al., 1992). Two ethanol-preferring rat strains, alcohol preferring P rats (Lumeng, Hawkins & Li, 1977) and alcohol accepting AA rats (Eriksson, 1968), drank more of a sweet saccharin solution than their ethanol-nonpreferring counterparts, alcohol non-preferring NP rats and alcohol non-accepting ANA rats. In addition, the AA/ANA and P/NP line differences were still apparent in subsequent presentations of a series of solutions of ascending saccharin concentrations.

Sinclair and colleagues (1992) claimed that, because the two pairs of selectively-bred rat strains (P & NP, AA & ANA) were derived independently from different foundation stocks in two different breeding programs, the similarity of the variance in preference for ethanol and saccharin across strains was an indication that the consumption of the two fluids was somehow related, perhaps via the opioidergic neuronal system (Sinclair et al, 1992). The investigators suggested that the lower level of saccharin intake observed in the ethanol non-preferring strains was not mediated by innate taste sensitivity for ethanol because it had already been demonstrated that P and NP rats did not differ in first exposure to ethanol in the taste reactivity paradigm (Bice & Kiefer, 1990). However, a study conducted seven years later in AA and ANA rats would show that, unlike the P and NP strains, this pair did differ in their initial response to ethanol: AA rats showed more positive responses to ethanol as well as to sweet and bitter solutions when compared with ANA rats (Badia-Elder & Kiefer, 1999). These results suggested that AA rats may be genetically predisposed to prefer the taste of ethanol and other solutions which may in turn mediate their intake. Therefore, it was possible that the differences in ethanol and saccharin intake between AA and ANA rats may be a reflection of inborn taste sensitivities while the differences in P and NP rats may be due to the influence of another factor such as a learned association between the ethanol and sweetened fluids. In support of this notion, closer examination of the intake and preference curves for the ascending series of saccharin solutions generated in the Sinclair et al. study revealed that the two ethanol non-preferring strains, NP and ANA rats, responded differently. ANA rats decreased their intake of and preference for the saccharin solutions during the latter half of the ascending series, whereas NP rats showed increasing

intake of and preference for the saccharin solutions until the final two saccharin concentrations. Altogether, these findings suggested that the two strains of selectively-bred rats may show similar levels of fluid intake but for different underlying reasons.

A more detailed investigation of ethanol-preferring P and ethanol-nonpreferring NP rats was conducted comparing their intake of several concentrations of sweet sucrose solutions (Stewart et al., 1994). P rats consumed greater amounts of the sucrose solutions than NP rats but only for two of the eight concentrations presented (Stewart et al., 1994). Nonetheless, the investigators claimed that these strain differences in intake supported the results of Sinclair et al. (1992) for saccharin solutions, such that the differences in the consumption of sweet solutions observed between ethanol-preferring and -nonpreferring strains could be extended from saccharin to sucrose as well. However, both P and NP strains displayed high levels of sucrose intake and were not different in their consumption for most of the solutions presented. Altogether, these findings demonstrated that sucrose intake did not in fact differentiate the P and NP strains as reliably as their ethanol intake.

While a relationship between ethanol and sweet intake has been reported in other ethanol-preferring rat strains, such as the unselected, ethanol-preferring Fawn-Hooded strain (e.g., Kampov-Polevoy, Overstreet, Rezvani & Janowsky, 1995a,b) and the selectively-bred, ethanol-preferring Sardianian alcohol-preferring sP strain (e.g., Agabio et al., 2000), there have also been reports which have disputed the universal nature of the association in all strains. For example, the ethanol-nonpreferring counterparts to the sP strain, Sardianian alcohol-nonpreferring sNP rats, also show high intake of and preference for saccharin solutions and were not distinguishable from sP rats (Agabio et al., 2000).

Also, three strains of rats unselected for ethanol intake, but known to differ in their ethanol consumption (Lewis, Wistar Kyoto and Wistar), did not differ in their intake of a single saccharin solution (Goodwin & Amit, 1998). Taken together, these studies indicated that a simple sweet solution such as saccharin or sucrose may not be an appropriate taste comparison for ethanol for all rat strains, and subsequently that sweet solution intake does not always differentiate rat strains according to their preference for ethanol.

Testing the reciprocal nature of the saccharin/ethanol relationship

Further evidence to challenge the popularly-held notion that ethanol intake and sweet preference were linked in a predictable fashion came from studies which sought to demonstrate that this relationship was reciprocal, in that selection for saccharin intake would predict ethanol intake (Bell et al., 1994; Dess, Badia-Elder, Thiele, Kiefer & Blizard, 1998; Gahtan, Labounty, Wyvell & Carroll, 1996; Gosnell & Krahn, 1992). In the first experiment reported, rats were divided into three groups according to their mean limited access intake of a saccharin solution: high, medium and low saccharin drinkers (Gosnell & Krahn, 1992). Mean ethanol intake was then recorded for each group. Ethanol consumption differentiated the saccharin drinking groups in only 2 of 12 comparisons, where the high-drinking saccharin group drank more ethanol than the low-drinking saccharin group during the fourth presentation of a series of ethanol concentrations. These differences occurred only when food was continuously available, as no differences in ethanol intake between the saccharin groups were found when the rats were food deprived. Animals were re-tested for saccharin intake following ethanol intake testing,

and the relative standings of the saccharin subgroups remained stable. The findings from this study (Gosnell & Krahn, 1992) have been cited for a decade, by those supporting the view that there was a link between ethanol and sweet preference, as evidence for the reciprocal relationship between ethanol and sweet solution preference in unselected rats. However, careful scrutiny of the data, which showed inconsistent correlations between saccharin and ethanol intake, provided little support for this notion.

A second report also failed to demonstrate a link between saccharin selection and ethanol intake (Bell et al., 1994). Unselected rats, grouped according to their saccharin intake as per Gosnell and Krahn (1992), showed no differences in responding for ethanol reward in an operant paradigm across 32 separate comparisons, where both ethanol concentration and fixed ratio schedule were varied. And subsequently, a third study reported similarly unsupportive results in unselected rats working for ethanol reward in a lick-operated, operant drinking paradigm (Gahtan et al., 1996). The only significant correlation between saccharin intake and ethanol intake reported in this last study occurred when saccharin group membership was ignored and after one month of testing with ethanol (Gahtan et al., 1996). In addition, the high and low drinking differences in saccharin consumption in this same study, as established prior to ethanol exposure, were not stable and disappeared by the end of the experiment when all rats were re-tested for saccharin intake. The correlational analyses reported in this study may have been biased by post-hoc findings of differential ethanol intake among the supposed high- and low-drinking saccharin groups because the investigators chose to compare mean ethanol intake during the only ethanol exposure that produced group differences for the whole experiment. Therefore, biased analyses and the instability of the saccharin group

membership undermined the validity of the study's results with respect to predicting ethanol intake and consequently serious concerns were raised about the reliability of forming distinct groups based on a criteria such as saccharin intake.

The only successful demonstration of sweet selection predicting ethanol intake in rodents was reported in two strains of rats selectively-bred for saccharin preference. The strains, occidental High-Saccharin HiS and Low-Saccharin LoS, were developed in the mid-1990's, and were at the 17th generation of breeding by 2000 (Dess, 2000). Selection criteria for breeding was based on one unforced, 24-hour drinking test of a saccharin solution (Dess, 2000). Results have shown that the difference in saccharin intake between HiS and LoS lines has remained stable over time (Dess & Minor, 1996). With respect to ethanol drinking, HiS rats were shown to consume more ethanol than LoS rats across a range of ethanol concentrations in unforced, 24-hour intake conditions (Dess et al., 1998). These findings suggested that saccharin intake may be associated with ethanol intake but only after many generations of selective breeding for saccharin intake.

The relationship between ethanol and saccharin intake: correlational studies

The most promising evidence for the proponents of the relationship between ethanol and sweet intake came from statistical data generated by correlational analyses of ethanol and saccharin intake. The first report of a positive correlation between preference for ethanol and a sweet solution was published in 1990 (Kampov-Polevoy et al., 1990). In this study, rats unselected for ethanol intake were allowed continuous access to a bitter quinine solution, a sweet saccharin solution and an ethanol solution, all in free choice with water. Mean bitter and sweet solution intakes were both correlated with the intake of

ethanol (r = +.25 and +.33, respectively), but only with mean ethanol intake during initial exposure to the ethanol. Bitter and sweet solution intake were not correlated with ethanol intake when ethanol drinking behaviors were stabilized. The investigators concluded that the significant correlations obtained between bitter/ethanol and between sweet/ethanol, while not particularly strong, supported the notion that "individual gustatory differences influence initial ethanol acceptance" in rats (Kampov-Polevoy et al., 1990), or that taste plays a role when learning to drink ethanol.

Following this study, a more comprehensive experiment was conducted comparing the intakes of the same flavoured solutions in several selectively-bred rat strains (Overstreet et al., 1993). The strains selected were the ethanol-preferring P and -nonpreferring NP rats, as well as five rat strains bred for other phenotypic characteristics (e.g. emotional reactivity, cholinergic sensitivity) and with corresponding documented ethanol preferences: the ethanol-preferring strains were Fawn-Hooded (Rezvani, Overstreet & Janowski, 1991) and Maudsley Reactive rats (Adams, Shihabi & Blizard, 1991), and the ethanol-nonpreferring strains were Maudsley Nonreactive (Adams et al., 1991) and both Flinders Line rat strains, FSL and FRL (Overstreet, Rezvani & Janowski, 1992). All rats were presented with continuous access to bitter quinine, sweet saccharin, and ethanol solutions. The quinine solution was too concentrated for the rats and produced negligible intake across strains, making assessment of the relationship between ethanol intake and bitter preference unfeasible. Correlational analyses of ethanol and saccharin intakes performed on all rats combined revealed a significant positive relationship (r = +.61), which was higher when the analysis was performed on mean strain intake values (r = +.87). Ethanol and saccharin intake were also correlated (r =

+.65) in genetically heterogeneous F2 progeny from a cross between the ethanol-preferring Fawn-Hooded and ethanol-nonpreferring FRL rats, who displayed high ethanol and saccharin intake resembling that of the Fawn-Hooded parent stock. The findings from this study supported the notion of a link between saccharin and ethanol drinking first reported by Kampov-Polevoy and colleagues (Kampov-Polevoy et al., 1990) and provided a multi-strain analysis of the relationship between saccharin and ethanol intake. However, the investigators failed to provide data on correlational analyses within all of the individual strains. They did report that the correlation between saccharin and ethanol intake in the ethanol non-preferring Flinders Lines (FSL & FRL) was not significant (Overstreet et al., 1993). This finding implied that, because the sweet-ethanol relationship was not significant in these two rat strains, it may not represent a universal relationship among all rodents.

The notion that the sweet-ethanol relationship may not be common to all rat strains was subsequently supported by reports showing that differences in saccharin intake did not always mirror variability in ethanol intake. Bell and colleagues failed to show a significant correlation when rats were selected for saccharin intake (Bell et al., 1994), and Overstreet and colleagues reported non-significant ethanol-saccharin intake and preference associations prior to and following forced exposure to ethanol solutions (Kampov-Polevoy et al., 1995b). In addition, Overstreet and his colleagues later reported no significant correlation between voluntary ethanol and saccharin intake in a rat strain developed by crossing ethanol-preferring Fawn-Hooded rats with ethanol-nonpreferring ACI/N rats (Overstreet, Rezvani & Parsian, 1999). Based on the accumulated results from these studies, this latter group of investigators (Overstreet et al., 1999), who were one of

the first to propose that sweet intake was a predictor of ethanol intake in rats (Kampov-Polevoy et al., 1990), began to question the 'closeness' of the relationship between ethanol and saccharin intake.

Ethanol tastes both sweet and bitter to rats

There have been few studies which have directly compared the intake of bitter or sweet-bitter solutions with that of ethanol in animals, despite the fact that Di Lorenzo and colleagues had published their convincing data more than 10 years earlier showing that rats identified the taste of unflavoured laboratory ethanol as having both a sweet and bitter taste (Di Lorenzo et al., 1986). Sinclair et al. (1992) found no differences between AA and ANA rats in intake of a bitter quinine solution. Stewart et al. (1994) found no differences in the intake of a bitter sucrose octaacetate solution in P and NP rats. Goodwin and Amit (1998) compared ethanol and saccharin-quinine intake in three non-selected rat strains known to differ in their ethanol preference: Lewis (ethanol-preferrers; Suzuki, George & Meisch, 1988), Wistar Kyoto (ethanol-nonpreferrers; Cannon & Carrell, 1987; Spuhler & Deitrich, 1984) and Wistar rats, the strain from which Lewis and Wistar Kyoto rats were derived. In general, ethanol and saccharin-quinine intake were not related in Lewis and Wistar Kyoto rats. In fact, the relationship was inverse in nature: Lewis rats displayed low ethanol consumption but high intake of saccharin-quinine solutions while Wistar Kyoto rats showed the reverse pattern. Wistar rats displayed relatively stable consumption levels for both solutions, generally falling in between those of Lewis and Wistar Kyoto strains. These results reinforced the notion (Agabio et al., 2000; Bachmanov et al., 1996; Overstreet et al.,

1993) that taste preferences for a variety of flavoured solutions may be strain-specific and not common to all rat strains. However, the responses displayed by the Lewis and Wistar Kyoto strains did not appear to support the data showing that the taste of ethanol was most similar to a sweet-bitter taste in rats (Di Lorenzo et al., 1986), because their respective intakes of the ethanol and saccharin-quinine solutions were effectively negatively correlated.

The results of this study (Goodwin & Amit, 1998) may be more effectively interpreted as evidence of differential sensitivities for complex tastes across strains. Recently, Dess (2000) reported on the responses of her high and low saccharin-drinking rat strains, HiS and LoS rats, to a variety of flavoured solutions, including sweet and bitter tastants. These results were relevant to understanding ethanol intake based on the data showing that ethanol tastes bittersweet to rats (e.g., Di Lorenzo et al., 1986). The results of the intake tests revealed that avidity (defined as the change in total fluid intake relative to a measure of baseline water intake) for sucrose was higher among HiS rats compared with LoS rats (for sucrose concentrations 0.50-2.0%, w/v), there were no differences in intake of two bitter solutions (quinine, sucrose octaacetate), and the addition of quinine to a sucrose solution resulted in reduced avidity for the bitter-sweet solution in LoS rats (Dess, 2000). Dess postulated that the strain differences apparent in this study may have been a reflection of differential taste sensitivities: LoS rats were perhaps less responsive to sweet tastes, resulting in greater sensitivity to the bitter taste in bittersweet solutions such as saccharin (saccharin tastes both sweet and bitter for rats: Dess, 1993), and saccharin- or sucrose-quinine (Dess, 2000). Furthermore, this might explain why LoS rats show less preference than HiS rats for bittersweet tastants at lower

concentrations, and increased aversion to them at higher concentrations compared with HiS rats (Dess, 2000). As an extrapolation of this theory to ethanol drinking, Dess proposed that the finickiness of LoS rats toward bitterness in complex tastes (for example, a mixture of sweet and bitter tastes) may also contribute to the line difference seen previously in ethanol intake between LoS and HiS rats, where HiS rats drank greater amounts of ethanol compared to LoS rats (e.g., Dess et al., 1998). In other words, LoS rats were restricted in their ethanol intake due to their sensitivity to the aversive bitter taste of the ethanol solution. In addition, inconsistencies in the prediction of ethanol intake from the intake of bitter solutions in ethanol-preferring and -nonpreferring rodent lines (e.g., Bachmanov et al., 1996; Sinclair et al., 1992; Stewart et al., 1994) may have arisen as a result of presenting simple solutions (e.g., sucrose, saccharin, quinine, sucrose octaacetate) rather than mixtures, should a similar mechanism be responsible for fluid choices in rat strains selectively-bred for ethanol intake.

In summary, ethanol is a complex taste which includes a both a sweet and bitter component (e.g., Di Lorenzo et al., 1986). While there is evidence that ethanol intake and sweet solution intake may be related (e.g., Overstreet et al., 1993), there are also reports emerging to dispute this notion (e.g., Agabio et al., 2000; Overstreet et al., 1999). Little research has been conducted to date investigating the link between ethanol and bitterness, and less so of ethanol and sweet-bitterness. It is possible that sensitivity to bitterness within the complex sweet-bitter taste, and not bitterness alone, may more reliably distinguish the high- and low-ethanol drinking rodent lines (Dess, 2000) than sweet preference. This should also be true for all non-selected rodent strains, and may provide

more information about strain differences in taste preferences than intake of sweet solutions alone.

Taste preferences and a common neural system

Whether ethanol intake may be predicted by strain-specific taste preferences for sweetness or taste sensitivities for bitterness, several investigators have proposed that the association might be caused by some connection between the sweet solution and the postingestive effects of ethanol (e.g., Gosnell & Krahn, 1992; Sinclair et al., 1992). According to this notion, the rewarding effects experienced following the consumption of both ethanol and sweet solutions would be mediated by at least one neurotransmitter system in common, which in turn would result in their equivalent levels of intake. Most commonly cited is the opioidergic system, because it has been implicated in the reinforcement from saccharin intake (e.g., Cooper, 1982, 1983) and is also among the systems involved in the reinforcement from ethanol intake (e.g., Myers & Crichter, 1982; Sinclair, 1990). Other valid possibilities include the serotonergic (e.g., Gill & Amit, 1987; Myers & Veale, 1968; Rockman, Amit, Carr & Ogren, 1979a,b), dopaminergic (e.g., Dyr, McBride, Lumeng, Li & Murphy, 1993; Hodge, Samson & Chapelle, 1997; Weiss et al., 1990) and gabaergic neurotransmitter systems (e.g., Boyle, Segal, Smith & Amit, 1993; Boyle, Smith & Amit, 1992; Smith, Robidoux & Amit, 1992). Very little work has been conducted investigating the interaction of these neurotransmitter systems with bitter solution intake, therefore the following selective summary of the literature will focus on the interaction of ethanol and sweetened solutions with opioidergic, dopaminergic and gabaergic neurotransmitter systems as they pertain to the experiments of the present thesis.

There has been considerable research showing that endogenous opioid systems

exert some influence on a variety of consummatory behaviors, including food and water intake (Brown & Holtzman, 1979; Cooper, 1980; Frenk & Rosen, 1979). Opiate antagonists have also been shown to reduce intake of palatable foods in rats and humans (e.g., Apfelbaum & Mandenoff, 1981; Yeomans & Wright, 1991). With respect to the ethanol research field, opiate agonists such as morphine have been shown to both increase (Beaman, Hunter, Dunn & Reid, 1984; Hubbell et al., 1986; Reid & Hunter, 1984) and decrease ethanol consumption (e.g., Sinclair, Adkins & Walker, 1973), while opiate antagonists have consistently been shown to reduce ethanol intake in rats (e.g., Davidson & Amit, 1996, 1997a,b; Froehlich, Harts, Lumeng & Li, 1988, 1990; Marfaing-Jallat, Miceli & Le Magnen, 1983; Myers & Crichter, 1982; Sandi, Borrell & Guaza, 1988), and mice (Le, Poulos, Quan & Chow, 1993; Middaugh, Kelley, Cuison Jr. & Groseclose, 1999; Phillips, Wenger & Dorow, 1997). In humans, naltrexone has been reported to decrease alcohol intake and rate of relapse (O'Malley et al., 1992; Volpicelli, Alterman, Hayshida & O'Brien, 1992), decrease craving for alcohol (Davidson, Swift & Fitz, 1996), or to have no effect on these same measures of alcohol drinking (Krystal, Cramer, Krol, Kirk & Rosenheck, 2001). Opioid antagonism has also been shown to reduce consumption of sweetened ethanol (Gardell, Hubbell & Reid, 1996; Krishnan-Sarin et al., 1995), as well as of saccharin and sweet chocolate solutions in rats (Biggs & Myers, 1998). While there has been great debate as to the actual behavioral mechanism mediating the attenuation of ethanol intake following opioid antagonism, there have been suggestions that they may cause a shift in perceived palatability of a consumed substance (Biggs & Myers, 1998; Davidson & Amit, 1997; Hill & Kiefer, 1997; Levine, Murray, Kneip, Grace, & Morley, 1982; Parker, Maier, Rennie & Crebolder, 1992). One study

reported that low doses of naltrexone, a nonspecific opioid receptor antagonist, reduced ethanol intake by altering taste sensitivity while higher doses induced a conditioned taste aversion to the ethanol (Davidson & Amit, 1997). In addition, naltrexone was found to increase aversive responding and decrease ingestive responding for ethanol in the taste reactivity paradigm (Hill & Kiefer, 1997). These changes in taste reactivity responding occurred at the first presentation of ethanol, before the rats had experienced ethanol's post-ingestive effects. These latter investigators proposed that naltrexone was creating a shift in palatability or taste value of the ethanol solution.

The role that dopamine may play in regulating voluntary ethanol intake is less clear. In general, pharmacological studies have shown that dopamine antagonists have either attenuated (e.g., Fuchs, Burbes & Cooper, 1984; Pfeffer & Samson, 1985a) or had no effect on ethanol consumption (e.g., Brown, Gill, Abitbol & Amit, 1982; Goodwin, Koechling, Smith & Amit, 1996; Pfeffer & Samson, 1986; Silvestre, O'Neill, Fernandez & Palacios, 1996). Dopamine agonists and reuptake blockers have been reported to increase (e.g., Pfeffer & Samson, 1985b; Pothoff & Ellison, 1982), decrease (e.g., Dyr et al., 1993; Koob & Weiss, 1990; Samson, Tolliver & Schwartz-Stevens, 1990), and have no effect (e.g., Aalto & Kiianmaa, 1986; Linseman, 1990) on ethanol intake in animals. In contrast, dopamine antagonists have consistently been shown to reduce the intake of sweet solutions in rodents (Hsaio & Smith, 1995; Muscat et al., 1991; Muscat & Willner, 1989; Phillips, Willner & Muscat, 1991a,b; Smith & Schneider, 1988; Wise & Rompre, 1989), in a manner similar to that observed following a reduction in sweetness (Bailey, Hsiao & King, 1986; Geary & Smith, 1985; Towell, Muscat & Willner, 1987; Xenakis & Sclafani, 1981). This effect does not appear to be dependent on postingestive satiating

processes because it has been observed when postingestive effects were minimized or eliminated by sham feeding (Geary & Smith, 1985; Schneider, Davis, Watson & Smith, 1990). As well, this inhibitory effect can be obtained without evidence of motor impairments in licking behaviors (Hsaio & Smith, 1995; Schneider et al., 1990) or during taste reactivity testing (Kaczmarek & Kiefer, 2000). Interestingly, dopamine receptor antagonists seem to have the opposite effect when very sweet rewards are used, that of increasing intake. This has been observed in operant behavior maintained by a 95% sucrose pellet (Phillips et al., 1991b; Rowland & Engle, 1977) and in the consumption of very sweet sucrose solutions (Muscat & Willner, 1989; Phillips et al., 1991a,b; Willner, Papp, Phillips, Maleeh & Muscat, 1990). The mechanism underlying the action of dopamine antagonists on ethanol and sweet intake remains unclear. There has been speculation that the effects of dopamine antagonism are that of a shift to the right on the concentration/intake curve for sweet solutions (Phillips et al., 1991a). This is not the result of a change in the sensory properties of the sweet solutions, since discrimination of different concentrations of solutions remained unaffected (Phillips et al., 1991a). Many have concluded that the effects must be derived by a blunting of the rewarding properties of both solutions (Hsaio & Smith, 1995; Muscat et al., 1991; Phillips et al., 1991a,b).

Manipulations of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) have been shown to interact with ethanol intake in rats. The $GABA_A$ receptor agonist THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol) increased ethanol consumption in both continuous-access (Boyle et al., 1993; Boyle et al., 1992; Smith et al., 1992) and limited-access drinking paradigms (Tomkins & Fletcher, 1996). Muscimol, another $GABA_A$ receptor agonist, also enhanced ethanol intake when injected into the

dorsal raphe (Tomkins, Sellers & Fletcher, 1994a,b; Tomkins & Fletcher, 1996) and median raphe (Tomkins et al., 1994a,b), but was found to have no effect on ethanol intake when injected into the ventral tegmental area (VTA) (Hodge, Haraguchi, Chappelle & Samson, 1996; Nowak, McBride, Lumeng, Li & Murphy, 1998). Muscimol when injected into the median raphe also increased water consumption, suggesting that the effect on ethanol intake was non-selective (Tomkins et al., 1994a,b). When administered intraperitoneally, muscimol was reported to reduce responding for ethanol in an operant paradigm (Petry, 1997) but had no effect on voluntary ethanol intake (Daoust et al., 1987). The $GABA_B$ agonist baclofen has been shown to both increase (Smith, Boyle & Amit, 1999; Smith et al., 1992) and decrease (Colombo et al., 2000) voluntary ethanol intake in rats when administered systemically, and to increase ethanol-reinforced responding in an operant paradigm (Petry, 1997). Baclofen, when injected directly into the intra-dorsal raphe, did not produce any change in ethanol or water consumption (Tomkins & Fletcher, 1996). $GABA_B$ receptors have, as a result, been implicated in the development of sensitization to the locomotor stimulant effects of ethanol (Broadbent & Harless, 1999). In addition, administration of the functional $GABA_A$ antagonist picrotoxin, whether systemically or directly into neural structures such as the VTA, has been shown to attenuate both ethanol intake and preference while having no effect on overall fluid intake (Boyle et al., 1993; Nowak et al., 1998). Intra-VTA injections of bicuculline, another $GABA_A$ antagonist, reduced ethanol intake in rats (Nowak et al., 1998; Tomkins & Fletcher, 1996) but also reduced water intake (Tomkins & Fletcher, 1996). Based on these data, it has been proposed that $GABA_A$ receptors play an important

role in the acquisition and maintenance of ethanol drinking in rats (Boyle et al., 1993; Tomkins & Fletcher, 1996).

With respect to GABA functioning and sweet solution intake, the $GABA_A$ receptor agonist muscimol injected directly into the nucleus accumbens shell and the VTA was reported to elicit increases in the consumption of a sucrose solution and responding for sucrose rewards, while having no effect on saccharin solution intake (Hodge et al., 1996). The $GABA_B$ agonist baclofen had no effect on the intake of a glucose/saccharin mixture (Ward, Somerville & Clifton, 2000) but attenuated responding for a sucrose solution in an operant paradigm (Petry, 1997). In addition, the functional $GABA_A$ receptor-blocker picrotoxin was shown to attenuate responding for a sucrose-ethanol mixture (Petry, 1997), as well as attenuating saccharin intake when injected into the VTA, but only at 1 of 3 doses tested and under alternate day, limited access drinking conditions (Nowak et al., 1998).

The purpose of the above review was to demonstrate that the opioidergic, dopaminergic and gabaergic neurotransmitters, among others, each mediate the intake of ethanol and/or sweet solutions to some degree. Therefore, it is possible that one or more of these systems may be responsible for controlling any putative predictive relationship between ethanol and sweet or sweet-bitter solutions. It was originally postulated that selective breeding for ethanol consumption in rodent strains may have unintentionally resulted in the decreased efficacy of one or more of these neurotransmitter systems, such that the reinforcement obtained from the intake of ethanol and sweetened solutions was reduced (as in the ethanol-nonpreferring lines) (Overstreet et al., 1993; Sinclair et al., 1992). This in turn could have affected both the palatability as well as the post-ingestive

effects of the solution. Accordingly, it should follow that manipulation of the neurotransmitter of interest would result in equivalent alterations in the intake of both ethanol and sweetened solutions if they were both mediated by the same neural system. Confirmation of a common underlying neurochemical system mediating taste preferences for ethanol as well as for sweet, bitter or sweet-bitter solutions in such a manner would provide support for the notion that specific tastes preferences are related to ethanol intake in rats.

The present thesis

The purpose of the experiments, described below, was to further investigate the relationship between ethanol consumption and taste preferences in rats. Specifically, the experiments in this thesis were designed to test whether intake of sweet, bitter and sweet-bitter solutions correlate reliably with ethanol intake in rats, whether these putative relationships vary across rat strains, and whether taste preferences linking ethanol intake with sapid fluid intake are mediated by a common underlying neurochemical system.

The first experiment (Experiment #1) used a modification of a procedure previously described by Overstreet et al. (1993), but used three new rat strains and added a saccharin-quinine solution to the array of fluids presented. The rat strains chosen (Lewis, Wistar Kyoto and Wistar) were selected because they had previously been shown to differ in their ethanol and saccharin-quinine intake (Goodwin & Amit, 1998). The purpose of the modifications and additions to the Overstreet et al. (1993) procedure was to determine whether the positive correlations observed between ethanol and saccharin intake in their study were generalizable to other rat strains. In the second experiment (Experiment #2), the non-selected, high ethanol-drinking rat strain, Fawn-Hooded, was examined for ethanol and saccharin-quinine intake. A previous study (Goodwin & Amit, 1998), whose procedure was adopted for Experiment #2, was unable to show a relationship between the pattern of ethanol and saccharin-quinine consumption in three rat strains non-selected for ethanol intake. Thus it was of interest to replicate this experiment in a non-selected, high-drinking rat strain. The third experiment (Experiment #3) also used a modification of a methodology previously described in a classic study

carried out by Richter and Campbell (1940a) in which they examined changes in ethanol intake in rats over increasing concentrations. This study was extended to also investigate saccharin and quinine intake over increasing concentrations. Threshold and cut-off concentrations for all three solutions were compared in Fawn-Hooded, Lewis and Wistar rats. It was of interest to examine whether any relationship existed between the intakes of and taste thresholds for all three fluids in three rat strains known to differ in their levels of ethanol intake. In addition, changes in intake patterns for the sweet and bitter solutions among the strains may help to explain differences in ethanol intake over increasing concentrations.

The final two experiments of the thesis focused on the notion that there may be a common underlying neural system mediating the intake of ethanol and sweetened solutions such as sucrose and saccharin. The fourth experiment (Experiment #4) examined the effects of opiate and dopamine antagonists as well as a GABA agonist on the intake of ethanol, saccharin and quinine solutions in Lewis and Wistar rats. It was hypothesized that if ethanol intake was related to the intake of sweet or bitter solutions, manipulation of a neurotransmitter system which are known to alter ethanol intake should also affect the intake of the related flavoured fluid. The final experiment (Experiment #5) provided a more in-depth look at the effects of the opiate antagonist naltrexone on the intake of ethanol and sweetened ethanol, as well as of sweet, bitter and sweet/bitter solutions. It was of interest to pursue the study of the effects of naltrexone on fluid intake in greater detail for several reasons, not the least of which is that naltrexone is currently being used to treat alcohol dependence in humans. Naltrexone was approved by the United States Food and Drug Administration for the treatment of alcoholism in the late

1990's, to a large part based on research reporting that alcoholic volunteers experience described decreased craving for alcohol while undergoing naltrexone treatment (Volpicelli et al., 1992). Findings such as these raised the question of whether naltrexone might be acting to change the taste of ethanol, such that it was no longer a secondary reinforcer associated with alcohol's postingestive effects (Hill & Kiefer, 1997). Also, data from this laboratory has shown that low doses of naltrexone act to reduce ethanol intake via a modulation of the pre-ingestional components of ethanol self-administration, such as taste sensitivity, and not by some capacity to block ethanol reinforcement (Davidson & Amit, 1997b). This suggested that antagonism of opiate receptors may alter the intake of fluids through a shift in palatability. Therefore, naltrexone may have a generalized effect on the "taste value" (Hill & Kiefer, 1997) of all flavored fluids, causing decreases in intake, or more selective effects on certain related tastes, such as ethanol and sweet/bitter flavours.

Experiment 1

Differences in the consumption of ethanol and flavoured solutions in three strains of rats

One of the more influential studies in the field of ethanol and taste preferences was published by Overstreet and his colleagues in 1993. Along with reporting a strong positive correlation between ethanol and saccharin intake in rats, the significance of this study also lay in the comparison of seven strains of rats. At the time of publication, apart from one other report (Sinclair et al., 1992), there had been no other studies incorporating a multi-strain comparison in the study of taste preferences and ethanol intake. Overstreet et al. selected two rat strains bred for high and low ethanol preference, P and NP rats, and five rat strains bred for characteristics unrelated to ethanol intake (Fawn-Hooded, Maudsley Reactive and Nonreactive, Flinders Line FSL and FRL rats). The intake of ethanol and saccharin were significantly correlated when the data for all strains were considered together ($r = +0.61$), as well as when the data from certain high and low ethanol-drinking rat strains were combined (e.g., P vs. NP, $r = +0.58$; Fawn-Hooded vs. NP, $r = +0.88$). However, no report was made as to the comparison of ethanol and saccharin consumption in individual strains, information which would have revealed whether the global correlation between ethanol and saccharin intake found when all rats were combined also held for individual strains. For example, the investigators did report that ethanol and saccharin intakes were not related when the ethanol-nonpreferring Flinders Lines of rats (FSL & FRL) were combined ($r = -0.39$). This at least implied that the relationship between ethanol and saccharin was not generalizable to all rodent strains.

Experiment 1 was designed to test whether the ethanol-saccharin correlation found in different rat strains in Overstreet et al. (1993) was common to all strains. Three additional strains of rats were compared for ethanol, saccharin and quinine intake using a modified drinking procedure from Overstreet et al. The strains selected had previously been tested for ethanol intake in this laboratory and are known to differ in their ethanol intake: Lewis, Wistar Kyoto and Wistar strains (Goodwin & Amit, 1998). A saccharin-quinine solution, a sweet and bitter tasting solution, was added to the end of the fluid presentation schedule in order to compare the intake of ethanol with that of a sweet-bitter solution. In addition, fluid intake was measured under continuous fluid access drinking conditions, as per the Overstreet et al. (1993) procedure, as well as under limited fluid access drinking conditions, in order to determine whether exposure time to the flavoured fluids influenced their interrelationships.

Based on the findings of the Overstreet et al. (1993) study, it was expected that ethanol and saccharin intake would be related in these three rat strains. As well, according to several studies showing that rats find the taste of ethanol to have both sweet and bitter taste qualities (e.g., Di Lorenzo et al., 1986: Lawrence & Kiefer, 1987), it was also expected that ethanol intake would be positively correlated with both quinine and saccharin-quinine intake. The nature of the correlations of ethanol with all flavoured solutions was not expected to vary with respect to fluid presentation schedule.

Method

Subjects

Subjects for Phase 1 (continuous fluid access) were 10 naive, male Lewis rats, 10 naive, male Wistar rats, and 10 naive, male Wistar Kyoto rats (Charles River, Quebec). The animals weighed approximately 115g-212g (Lewis), 225g-235g (Wistar) and 227g-260g (Wistar Kyoto) at the start of the experiment. Subjects for Phase 2 (limited fluid access) were 8 of the 10 male Lewis rats used in Phase 1, 8 of the 10 male Wistar rats used in Phase I, and 8 of the 10 Wistar Kyoto rats used in Phase 1. These animals now weighed approximately 295g-370g (Lewis), 470g-560g (Wistar) and 450g-520g (Wistar Kyoto) at the start of Phase 2. Two Wistar Kyoto animals were removed following Phase 1 due to tooth malformations, and Lewis and Wistar groups were reduced accordingly by randomly removing two animals from each strain grouping from Phase 2.

All animals were housed individually in stainless steel cages, in a temperature and humidity controlled room. Animals were maintained in a 12 hour light/dark cycle (lights on at 0800 hr, lights off at 2000 hr). During Phase 1, drinking fluids were presented in two glass Richter-type tubes on the front of the cages. The position of the tubes was alternated daily so as to avoid side preference. During Phase 2, drinking fluids were presented in plastic tubes with steel ball-bearing spouts mounted on the front of the home cage during limited access testing. For the remainder of the 24-hour period during Phase 2, water was presented in glass bottles with steel spouts mounted on the front of the cages. Standard rat chow (Agway) and water were available ad libitum. In all experiments

presented in this thesis, the volume of fluids consumed (ml) was measured daily, and body weights (g) were recorded every 2-3 days.

The animals in all of the experiments of the present thesis were treated in accordance with guidelines from the Canadian Council on Animal Care, and the methods used were approved by the Concordia University Animal Care Committee.

Procedure

Phase 1: Continuous fluid access. Following a 3-week acclimatization period to the animal colony facilities, all rats were presented with a choice between tap water in one Richter tube and a flavoured solution in the other tube. Fluids were available for 23 hours every day (1 hour was reserved for measuring and weighing). Solutions were presented in the following order: 0.25% quinine (w/v) for 4 days, 0.1% saccharin (w/v) for 4 days, 10% ethanol (v/v) for 20 days and 0.4% saccharin (w/v)-0.04% quinine (w/v) solution for 16 days.

Phase 2: Limited fluid access. Following a 3-week washout period where water only was available, rats were exposed to a limited-access drinking training schedule. During all limited-access testing, only flavoured fluids were available and water was removed from the cages. The rats were provided with access to a 0.1% saccharin (w/v) solution during a daily 2-hr session for 1 week. Access to the saccharin solution was then further reduced to a daily 1-hr session for 1 week, followed by a daily ½-hr session for 1 week and finally to a daily 10-min session for 1 week. At this time, limited-access training was established and fluid presentations began. Flavoured solutions were presented as per Phase I. The solutions were available for 10 min per day, and water only

was available for the remainder of the 24-hour period. All limited-access testing took place during the lightened portion of the light/dark cycle (i.e., 0800 hr to 2000 hr).

Data Analysis

Daily fluid consumption data (ml) for water and the flavoured solution during Phase 1 were converted into: intake of the flavoured solution (milliliters of flavoured fluid consumed per kilogram of body weight per day, ml/kg/day), preference for the flavoured solution (calculated as a percentage of flavoured solution consumed to total fluid consumed, %), and total fluid consumption (total milliliters of all fluids consumed daily per kilogram of body weight per day, ml/kg/day). Consumption data (ml) for the flavoured solution during Phase 2 was converted into: intake of the flavoured solution (ml/kg/day). The traditional measure of absolute ethanol intake- grams of ethanol consumed per kilogram of body weight, g/kg - was foregone in the present thesis in favor of a volume measure- millilitres of ethanol intake per kilogram of body weight, ml/kg. This was done in order to compare intake measures of ethanol with all other flavoured fluids measured as ml/kg. Where necessary throughout the thesis, ethanol intake in g/kg will also be presented. All intake measures throughout the thesis were corrected for body weight at the time of measurement because of within-strain as well as between-strain variations in body size (see Analysis of Body Weights in corresponding appendices).

For each flavoured solution, consumption across the last four days of presentation was used for statistical analysis. Separate two-way Analyses of Variance (ANOVA) (strain x days) with repeated measures (Keppel, Saufley & Tokunaga, 1992) were conducted on the variables of intake, preference, and total fluid consumption for each flavoured fluid as well as weight, separately for Phases 1 and 2. Results are reported

concurrently for each flavoured fluid. Throughout the thesis, post-hoc Tukey tests are performed where appropriate for pairwise comparisons, $p < .05$, and Tests of Simple Effects were carried out following a significant interaction, $p < .05$. See Appendix A for a table and analysis of total fluid intake data. See Appendix C for analysis of body weights.

The mean intakes of the four solutions were further compared using rank-ordered Spearman correlation coefficients (Minium, King & Bear, 1993) in the following groupings: within each strain separately, collapsed across all strains, comparing high ethanol-drinking Wistar versus low ethanol-drinking Lewis strains, and comparing high ethanol-drinking Wistar versus low ethanol-drinking Wistar Kyoto strains. These correlations were conducted separately for Phases 1 and 2.

Results

Ethanol

Phase 1. Analysis of ethanol intake during the continuous fluid access phase revealed significant differences among Lewis, Wistar and Wistar Kyoto rats, $F(2, 27) = 25.33$, $p < .0001$ (see Table 1). The results of a Tukey test indicated that Wistar rats consumed significantly more ethanol than Lewis and Wistar Kyoto rats, $p < .01$, while Lewis and Wistar Kyoto rats did not differ from each other. Preference for ethanol was also significantly different among the strains, $F(2, 27) = 20.00$, $p < .0001$ (see Table 2). Wistar rats displayed the highest preference for ethanol, $p < .01$, and Lewis and Wistar Kyoto rats were not different from each other.

Table 1

Mean Fluid Intake (ml/kg) for All Strains during Phase 1: Continuous Fluid Access

	Fluids			
Strain	Ethanol	Saccharin	Saccharin-quinin	Quinine
Lewis	7.5 (0.7)	155.6 (23.2)	5.4 (0.6)	6.9 (1.0)
Wistar	32.3 (1.7)	204.5 (12.5)	11.7 (1.2)	5.0 (0.6)
Wistar Kyoto	11.0 (1.6)	144.1 (12.9)	8.1 (0.7)	6.1 (0.8)

Note. The values not enclosed in parentheses represent mean intake for the final 4 days of fluid presentation. Values enclosed in parentheses represent standard error of the mean (SEM).

Table 2

Mean Fluid Preference (%) for All Strains during Phase 1: Continuous Fluid Access

	Fluids			
Strain	Ethanol	Saccharin	Saccharin-quinin	Quinine
Lewis	7.3 (0.6)	65.4 (6.3)	5.7 (0.5)	5.2 (0.8)
Wistar	31.0 (0.6)	81.5 (2.3)	12.2 (1.2)	4.0 (0.5)
Wistar Kyoto	15.0 (1.6)	75.1 (2.4)	13.3 (0.7)	5.8 (0.8)

Note. The values not enclosed in parentheses represent mean preference for the final 4 days of fluid presentation. Values enclosed in parentheses represent standard error of the mean (SEM).

Phase 2. Ethanol intake during the limited fluid access phase was not significantly different among the strains, $F(2, 21) = 2.99$ (see Table 3).

Saccharin

Phase 1. All rats drank large volumes of the saccharin solution during the continuous fluid access phase (see Table 1). Analysis revealed no overall significant differences in intake among Lewis, Wistar, and Wistar Kyoto strains, $F(2, 27) = 1.17$. Preference for the saccharin solution relative to water was also very high for all rats (see Table 2) and there were no significant differences among the strains, $F(2, 27) = 0.83$.

Saccharin intake was not significantly correlated with ethanol intake during Phase 1 in any of the rat strains or when all three strains were considered together (see Appendix B for Spearman correlation coefficient tables).

Phase 2. Saccharin intake during the limited fluid access phase did not differentiate the three strains, $F(2, 21) = 2.51$ (see Table 3). However, saccharin intake was significantly correlated with ethanol intake in Lewis rats, $r_s = +0.905$, $p < .01$, and when the Lewis and Wistar strains were combined for analysis, $r_s = +0.544$, $p < .05$ (see Appendix B).

Saccharin-quinine

Phase 1. Saccharin-quinine intake during the continuous fluid access phase was not significantly different among Lewis, Wistar, and Wistar Kyoto rats, $F(2, 27) = 3.22$, $p = .056$ (see Table 1). However, there were significant differences among the strains in their

Table 3

Mean Fluid Intake (ml/kg) for All Strains during Phase 2: Limited Fluid Access

	Fluids			
Strain	Ethanol	Saccharin	Saccharin-quinin	Quinine
Lewis	1.27 (0.33)	4.72 (0.45)	0.47 (0.23)	0.08 (0.08)
Wistar	2.93 (0.28)	5.19 (0.27)	0.32 (0.13)	0.11 (0.11)
Wistar Kyoto	1.84 (0.46)	7.49 (0.95)	1.06 (0.17)	0.13 (0.07)

Note. The values not enclosed in parentheses represent mean intake for the final 4 days of fluid presentation. Values enclosed in parentheses represent standard error of the mean (SEM).

preference for the saccharin-quinine solution, $F(2, 27) = 3.88$, $p < .05$ (see Table 2). Mean preference for saccharin-quinine in Lewis rats was significantly lower than that of Wistar Kyoto rats ($p < .05$) but not of Wistar rats. Wistar and Wistar Kyoto rats did not differ from each other in saccharin-quinine preference.

Saccharin-quinine intake and ethanol intake were significantly correlated when all three rat strains were combined, $r_s = +0.660$, $p < .01$, and when the Lewis and Wistar strains were combined, $r_s = +0.762$, $p < .01$. When the strains were considered separately, only Lewis rats showed a significant correlation between ethanol and saccharin-quinine intakes, $r_s = +0.964$, $p < .01$. Saccharin-quinine intake was also related to quinine intake in Lewis rats, $r_s = +0.915$, $p < .01$, and to saccharin intake in Wistar rats, $r_s = +0.806$, $p < .01$, and when Wistar rats were combined with Wistar Kyoto rats, $r_s = +0.633$, $p < .01$ (see Appendix B).

Phase 2. Saccharin-quinine intake during the limited fluid access phase differed significantly among the strains, $F(2, 21) = 4.32$, $p < .05$. Results from a Tukey test revealed that Wistar Kyoto rats consumed more saccharin-quinine than Wistar rats ($p < .05$) and Lewis rats did not differ from either strain (see Table 3). There were no significant correlations between saccharin-quinine intake and the intake any of the other flavoured fluids during Phase 2.

Quinine

Phase 1. There were no significant differences among Lewis, Wistar, and Wistar Kyoto rats in quinine intake during the continuous fluid access phase, $F(2, 27) = 0.37$ (see

Table 1). There were also no differences among the strains in their preference for the quinine solution, $F(2, 27) = 0.47$ (see Table 2).

Quinine intake was significantly correlated with ethanol intake only in Lewis rats. $r_s = +0.830$, $p < .01$ (see Appendix B).

Phase 2. Quinine intake during the limited fluid access phase did not differentiate the strains, $F(2, 21) = 0.05$ (see Table 3). Additionally, quinine intake was not correlated with the intake of any of the flavoured fluids during this phase.

Discussion

There was no overall correlation between ethanol and saccharin intake across Lewis, Wistar Kyoto and Wistar rats together. This finding was in contrast to the results of Overstreet et al. (1993) who reported a positive correlation between ethanol and saccharin intake in seven strains of rats in the same drinking paradigm. Within individual strains, ethanol and saccharin intake were related only in Lewis rats and when Lewis rats were combined with Wistar rats during limited access conditions. It was interesting to note that while differences existed in ethanol intake between the three strains, supporting a previous report of their respective mean ethanol intake levels (Goodwin & Amit, 1998), there were no strain differences in saccharin intake or preference regardless of fluid exposure schedule. Although the values for mean saccharin intake appeared to indicate a difference, within-strain variability was large for each strain (e.g., ranging from 5 to 278 ml/kg in Lewis rats) and thus eliminating between-strain differences. All rats showed a high level of preference for the saccharin solution over water (greater than 60% of total

fluid intake), which is commonly reported in many studies irrespective of rat strain (e.g., Agabio et al., 2000; Overstreet et al., 1993; Sinclair et al., 1992). Therefore, these results showed that the ethanol-saccharin correlation is not common to all rat strains, as was originally proposed by Overstreet et al. (1993), because saccharin consumption does not differentiate strains in a similar fashion to ethanol.

Saccharin-quinine intake was positively correlated with ethanol intake during the continuous fluid access phase when all strains were combined. The strength of this correlation ($r = +0.66$) was comparable to that reported in the Overstreet et al. (1993) study for seven rat strains drinking ethanol and saccharin ($r = + 0.61$). This finding, while supporting previous research suggesting that the taste of ethanol for rats was most similar to a sweet-bitter taste (Di Lorenzo et al., 1986), was in contrast to the results of an earlier study which showed an inverse pattern of intake of ethanol and saccharin-quinine solutions in Lewis and Wistar Kyoto rats (Goodwin & Amit, 1998). These contradictory results may be due to the different schedules of fluid presentation used in each study. In the current study, rats were presented with a single concentration of both ethanol and saccharin-quinine while the Goodwin and Amit (1998) study examined intake following an acclimatization schedule of gradually increasing concentrations. Ethanol and saccharin-quinine consumption were also related in the Lewis strain alone, and when Lewis rats were compared to Wistar rats but only during continuous fluid access.

There were no differences in quinine intake or preference among the three strains in either phase of the experiment. However, this was not a meaningful result because the amounts of quinine solution consumed were negligible in each strain. Overstreet et al. (1993) also reported no differences in quinine intake in their seven rat strains. While it

was clear that the concentration of quinine chosen (0.25%) was overly aversive to the rats, it was necessary in the present study to use the same solutions in order to control for any carry-over effects which may influence the perceived palatability of each solution and, consequently, its intake.

The significant relationships in intake described above were not similar in all three rat strains. In fact, the three strains displayed very different interrelationships among the flavoured solutions and across fluid access phases. For example, while quinine intake was correlated to both ethanol and saccharin-quinine intake in Lewis rats during continuous-access drinking, Wistar rats consumed saccharin in a manner similar to saccharin-quinine. Wistar Kyoto rats did not reveal any interrelationships in fluid intake in either drinking phase. This lack of consistency in the correlational results provides evidence of the disparity in rat strains for taste preferences, as measured by intake in the present study, and furthermore that taste preferences are able to be modified by environmental variables such as fluid exposure schedule and perhaps water availability.

The rat strains used in the present study were considered comparatively low ethanol-drinkers relative to the high ethanol-drinking rat strains used in the Overstreet et al. study (1993) (mean ethanol intake of 2.6 g/kg/day for Wistar rats in the present study, versus 3.2 g/kg/day for P rats and 4.9 g/kg/day for Fawn-Hooded rats). It is possible that the failure to replicate the ethanol-saccharin correlation from Overstreet et al. in Experiment 1 was due to the low levels of ethanol-drinking in the rat strains used, and that the ethanol-saccharin correlation is somehow exclusive to high ethanol-drinking rats. Therefore, in order to characterize the relationship between ethanol and both sweet and bittersweet solutions in more detail, Experiment 2 was designed to compare the intakes of

both ethanol, saccharin and saccharin-quinine solutions in the non-selected, high ethanol-drinking Fawn-Hooded rats. A wider range of ethanol and saccharin-quinine concentrations than those used in Experiment 1 were presented.

Experiment 2

Ethanol and saccharin-quinine intake in ethanol-preferring Fawn-Hooded rats

The Fawn-Hooded rat strain originated in Michigan, derived by cross-breeding German-Brown, Long Evans and Lashley Albino rats by E.L. Walker (Harrington, 1981; Overstreet & Rezvani, 1996; Provoost & DeKeijzer, 1993). The strain was then dispersed within the United States and Europe, resulting in at least four inbred strains of Fawn-Hooded rats (Overstreet & Rezvani, 1996). It has been subsequently demonstrated that these four strains of Fawn-Hooded rats display dissimilar behavioral and physiological characteristics, including differences in the manifestation of hypercortisolemia, proteinuria and hypertension (Lahmame, Gomez & Armario, 1996; Provoost & DeKeijzer, 1993; Raymond & Dodds, 1975) as well as immobility in the forced swim test, a model of depression (Lahmame et al., 1996; Overstreet et al., 1992; Rezvani, Overstreet & Janowsky, 1990). The strain used in the present experiment (known as FH/Wjd) was maintained by Jean Dodds of the New York State Department of Health, and subsequently transferred to the University of North Carolina School of Medicine, where it has been maintained and inbred for the past decade (Rezvani et al., 1990, 1991).

This particular strain of Fawn-Hooded rats has been of interest to ethanol researchers because they display elevated levels of both ethanol and saccharin intake (Kampov-Polevoy et al., 1995a,b; Kampov-Polevoy et al., 1996; Rezvani et al., 1990; Overstreet et al., 1993; Overstreet et al., 1997) as well as a subsensitivity to serotonergic agonists (Rezvani et al., 1991). Studies in Fawn-Hooded rats in general (i.e., specific

sub-strain unknown) have shown that they possess impaired peripheral and central nervous system serotonergic functioning (Arora, Tong, Jackman, Stoff & Meltzer, 1983; Dumbrille-Ross & Tang, 1981; Rezvani et al., 1990; Wang, Aulakh, Hill & Murphy, 1988). This may be related to findings in heterogeneous rats that pharmacological manipulations that enhance cerebral serotonin neurotransmission, whether by precursors, agonists or reuptake blockers, reduce voluntary ethanol intake (e.g., Amit, Sutherland, Gill & Ogren, 1984; Geller, 1973; Gill, Filion & Amit, 1988; McBride, Murphy, Lumeng & Li, 1989; Rockman et al., 1979a,b). Similarly, it has been postulated that the high levels of ethanol intake observed in P rats may be the result of lower brain levels of serotonin and/or its metabolites, in several brain loci including nucleus accumbens, hippocampus and frontal cortex, compared to low ethanol-drinking NP rats (Murphy, McBride, Gatto, Lumeng & Li, 1988). Therefore, the high levels of ethanol intake observed in Fawn-Hooded rats were presumed to be a behavioral manifestation of their diminished serotonergic functioning (Rezvani et al., 1990).

Experiment 2 was designed to determine whether ethanol intake was related to sweet solution intake in a high ethanol-drinking rat strain. Overstreet et al. (1993) used Fawn-Hooded rats combined with six other rats strains of varying ethanol preference to show that ethanol and saccharin intakes were positively correlated. However, Experiment 1 did not show the same significant findings in three other non-selected, low ethanol-drinking strains of rats. Therefore, the purpose of Experiment 2 was to compare ethanol drinking in Fawn-Hooded rats with both saccharin and saccharin-quinine drinking in order to determine whether sweet preference and/or sweet-bitter preference might be predictive of ethanol intake in this ethanol-preferring rat strain. The present investigation

adopted the methodology of a previously published study which reported on the intake of these fluids in three non-selected rat strains (Lewis, Wistar Kyoto and Wistar) in which a wide range of concentrations were presented (Goodwin & Amit, 1998). No direct relationship between ethanol and saccharin-quinine consumption was revealed in these strains, and so it was of interest to determine whether such a relationship might exist in a rat strain who showed high ethanol preference and yet were not selectively-bred for ethanol consumption.

The strain of Fawn-Hooded rats used in the present experiment, the same as that used in Overstreet et al. (1993), are a high ethanol-drinking rat strain, and it is expected that this will be confirmed in the present study. It is also expected that their corresponding intake of the saccharin-quinine solutions will also be high, given the results of Experiment 1 where ethanol intake and saccharin-quinine intake were positively correlated in three unselected rat strains.

Method

Subjects

Subjects for Phase 1 of the experiment (ethanol) were 24 naive, male Fawn-Hooded rats, weighing 245-361g at the start of the experiment (University of North Carolina, Skipper Bowles Center for Alcohol Studies). The subjects in Phase 2 of the experiment (saccharin-quinine) were the same as those in Phase 1, now weighing 300-455g. Housing conditions were the same as described in Experiment 1. Drinking fluids were presented in two glass Richter-type tubes on the front of the cages. The

position of the tubes was alternated daily so as to avoid side preference. Standard rat chow (LAB DIET'S Rodent Diet; Checkers 5001 from PMI) and water were available ad libitum throughout the experiment.

Procedure

Phase 1: Ethanol. Following a 2-week acclimatization period to the animal colony facilities, all rats were exposed to an ethanol acquisition schedule in which ethanol was presented 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). 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 every day in a free choice with water for a 10-day maintenance period.

Phase 2: Saccharin-quinine. Immediately following the completion of Phase 1, the rats were given water and food ad libitum for a 1 week wash-out period. All rats were then exposed to a free choice schedule of sodium saccharin-quinine sulfate solutions and water. On day 1 of the acquisition period, the rats were offered 0.4% sodium saccharin (Mallinckrodt) (w/v) in free choice with water. On subsequent presentations of the saccharin solutions, quinine sulfate (Fisher Scientific) was added to the saccharin solution in increasing concentrations. The concentrations of quinine sulfate (w/v) 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), and 0.04% (day 11). Saccharin concentrations were held constant at 0.4% throughout. During the acquisition schedule, 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 every day in a free choice with water for a 10-day maintenance period.

Data Analysis

Ethanol intake (ml) and saccharin-quinine intake (ml) were converted as per Experiment 1 of the present thesis: intake (ml/kg/day), preference (%), and total fluid intake (ml/kg/day).

A two-way ANOVA (fluid x concentration) with repeated measures was performed on the ethanol and water intake data (ml), as well as the saccharin-quinine and water intake data (ml), to determine the point at which flavoured fluid consumption was less than water consumption (see Appendix D for graphs and analyses of water and flavoured fluid intake data (ml) for both phases). Separate one-way ANOVAs (concentration) with repeated measures were performed on all remaining measures of ethanol and saccharin-quinine intake, preference, total fluid consumption and weight to assess change in consumption patterns and body weight. See Appendix E for graphs and analyses of total fluid intake data during ethanol and saccharin-quinine phases. See Appendix F for analysis of body weights for both phases.

Peak ranges of intake and preference for both solutions were determined by first identifying the concentrations of ethanol and quinine in saccharin for which intake and preference variables were the highest during the respective phases of the experiment. The minimum and maximum concentrations of the peak ranges were then defined as the first concentration for which there was no significant difference ($p > .05$) when compared with the most preferred concentration, both above and below the value of the most preferred

concentration. Separate one-way ANOVAs (concentration) with repeated measures were performed on all peak ranges over the course of the experiment.

Pearson product-moment correlations (Minium et al., 1993) were performed on ethanol and saccharin-quinine intake and preference data to determine whether there were any predictive relationships in the consumption of the two fluids. A mean value for each rat was calculated from their final 5 days of intake during the maintenance period of Phases 1 and 2. As well, Pearson correlations were performed on mean baseline ethanol intake and preference (calculated as above) with saccharin intake and preference (day 1 of the saccharin-quinine acquisition schedule).

Results

Ethanol intake and saccharin-quinine intake in Fawn-Hooded rats (Maintenance days 6-10) were significantly correlated, $r = +0.45$, $p < .05$. Similarly, ethanol preference and saccharin-quinine preference during the same time period were also correlated, $r = +0.44$, $p < .05$. However, ethanol intake was not correlated with intake of the 0.1% saccharin solution (day 1 of the saccharin-quinine acquisition period), $r = +0.32$, $p > .05$. Similarly, ethanol preference was not correlated with saccharin preference (day 1: saccharin-quinine acquisition period), $r = -0.21$, $p > .05$.

Phase I: Ethanol

Fawn-Hooded rats displayed high levels of ethanol intake in this acquisition-maintenance drinking paradigm. Analysis of ethanol consumption (ml/kg)

showed that Fawn-Hooded rats gradually decreased their intake as the ethanol solutions presented became more concentrated, $F(18, 414) = 41.13$, $p < .0001$ (see Figure 1, top panels). Intake on acquisition days 1 to 5 was significantly higher than intake during the final eight days of the maintenance period, $p < .01$. The highest mean level of ethanol intake attained was 165 ml/kg on acquisition day 2 (3% ethanol), and the highest range of intake occurred from the acquisition days 1-5 (2-6% ethanol). Interestingly, their highest absolute ethanol intake was 9 g/kg on acquisition day 10 (10% ethanol) when the ethanol solutions presented were at their maximal concentration. Also, their corresponding highest range of absolute intake was more selective than for the volume intake measure, occurring between acquisition day 8 and maintenance day 2 (8-10% ethanol), suggesting a preference for stronger ethanol concentrations. Intake was unchanged from maintenance day 3 through to the end of the experiment.

Preference for ethanol was also high in Fawn-Hooded rats, remaining high throughout the acquisition period and decreasing only during the maintenance period, $F(18, 414) = 25.97$, $p < .0001$ (see Figure 1, bottom panels). The highest preference range for ethanol was from acquisition day 2 to maintenance day 1 (2-10% ethanol), and the highest mean preference level for ethanol was 92.4% on acquisition day 2 (ethanol 3%). Preference levels stabilized from maintenance day 3 until the end of the experiment. Preference for ethanol remained above 50% throughout the experiment.

Phase 2: Saccharin-quinine

Saccharin-quinine intake in Fawn-Hooded rats was also high, decreasing steadily

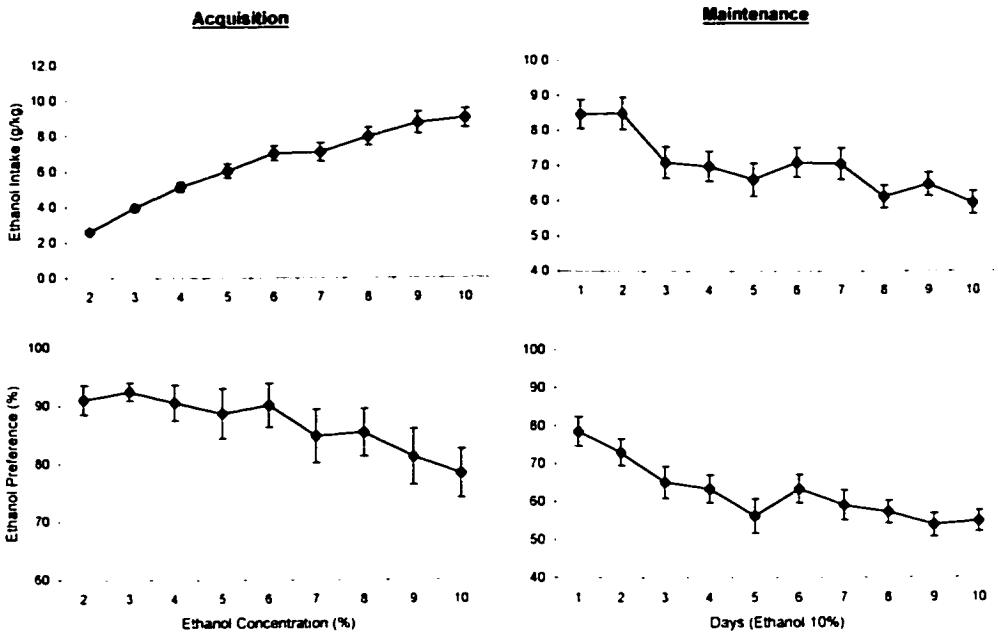

Figure 1. Mean (+/- SEM) ethanol intake (ml/kg) (top panels) and ethanol preference (%) (bottom panels) in Fawn-Hooded rats during the acquisition period (left column) and maintenance period (right column) of Phase 1.

throughout the acquisition period as quinine concentrations increased, $F(20, 440) = 85.81$, $p < .0001$ (see Figure 2, top left panel). The highest mean intake occurred on acquisition day 2 (saccharin 0.4%, quinine 0.001%) and was 230.0 ml/kg. The highest range of intake covered the days of acquisition 1 to 7 (saccharin 0.4%, quinine 0-0.009%). Intake then stabilized from acquisition day 10 (saccharin 0.4%, quinine 0.03%) through to the end of the maintenance period (see Figure 2, top right panel).

Saccharin-quinine preference in Fawn-Hooded rats showed a similar pattern to saccharin-quinine intake (see Figure 2, bottom left panel). Their highest preference range for saccharin-quinine was from acquisition days 1 to 7 (saccharin 0.4%, quinine 0-0.009%), and the highest mean preference level was 93.2% for the simple saccharin solution (0.4%) on acquisition day 1 (the highest mean preference level for a saccharin-quinine solution was 91.4% on acquisition day 2: saccharin 0.4%, quinine 0.001%). Preference gradually dropped as the quinine in the saccharin solution became more concentrated across the acquisition period, $F(20, 440) = 74.65$, $p < .0001$, and became stable from acquisition day 10 (saccharin 0.4%, quinine 0.03%) through to the end of the maintenance period (see Figure 2, bottom right panel).

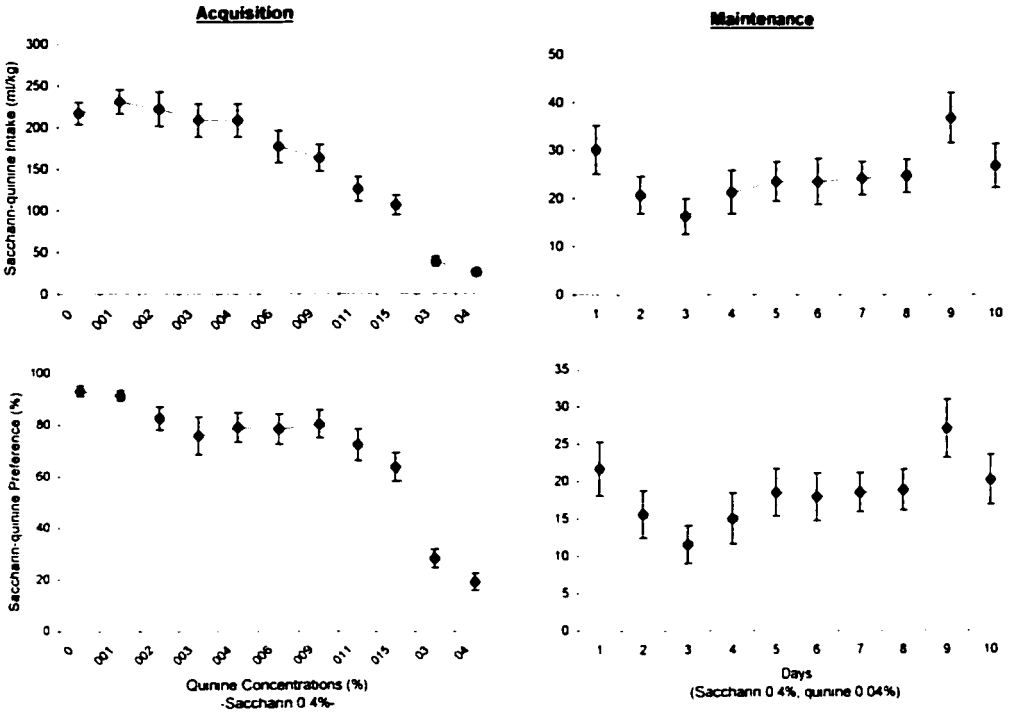

Figure 2. Mean (+/- SEM) saccharin-quinine intake (ml/kg) (top panels) and saccharin-quinine preference (%) (bottom panels) in Fawn-Hooded rats during the acquisition period (left column) and maintenance period (right column) of Phase 2.

Discussion

Correlational analysis of ethanol and saccharin-quinine drinking behaviors during the last days of the maintenance period revealed that they were positively correlated in Fawn-Hooded rats. When ethanol drinking was compared with intake of the saccharin solution presented on the first day of the saccharin-quinine acquisition period, the correlation was not significant. These findings supported the results of Experiment 1, which showed that ethanol drinking was not related to saccharin drinking but to saccharin-quinine drinking in low ethanol-drinking unselected rat strains. However, the lack of significance of the ethanol-saccharin correlation in Fawn-Hooded rats does not support the findings of Overstreet et al. (1993), who showed a significant correlation between ethanol and saccharin intakes in a large group of rat strains including Fawn-Hooded rats. There are no studies reporting a link between ethanol and saccharin intake in the Fawn-Hooded strain alone, however there is data showing that ethanol intake was not related to either saccharin intake or preference in Fawn-Hooded rats both before and after forced-choice ethanol exposure (Kampov-Polevoy et al., 1995b). On the basis of these findings and the results of the present experiment, it is suggested that Fawn-hooded rats find the taste of 10% ethanol to be most similar to a sweet-bitter complex taste rather than sweet alone, as has been suggested previously in other unselected rat strains (Kiefer et al., 1990; Kiefer & Lawrence, 1988; Lawrence & Kiefer, 1987) and was demonstrated by the significant ethanol and saccharin-quinine correlation in the present experiment. Moreover, the data provided more support for the notion that intake of a saccharin solution is not an appropriate comparison solution for ethanol intake

in Fawn-Hooded rats because it did not differentiate them in accordance with their ethanol intake.

Fawn-Hooded rats displayed high levels of ethanol intake during Phase 1 of the present experiment, peaking at 9 g/kg/day (112 ml/kg/day) on the last day of the acquisition period. As a comparison, rats selectively-bred for ethanol preference tend to drink in the range of 6-8 g/kg of ethanol per day (e.g., AA: Badia-Elder & Kiefer, 1999; UChA: Contrera & Mardones, 1988; P: Kampov-Polevoy et al., 1996; sP: Lobina et al., 1997). The present results therefore confirmed previous findings of very high ethanol intake levels in Fawn-Hooded rats (Overstreet et al., 1993; Rezvani et al., 1990). Fawn-Hooded rats also showed correspondingly high intake of the saccharin-quinine solutions, and at higher quinine concentrations, when compared with Lewis, Wistar Kyoto and Wistar strains presented in the same drinking paradigm (Goodwin & Amit, 1998). Ethanol intake levels in Fawn-Hooded rats were also higher than those observed for Lewis, Wistar Kyoto and Wistar rats in the same drinking paradigm (Goodwin & Amit, 1998). Fawn-Hooded rats preferred ethanol to water throughout Phase 1 of Experiment 2 (concentration range of 2-10% ethanol), unlike Lewis, Wistar Kyoto and Wistar rats whose preference levels for ethanol fell below 50% at concentrations ranging from 6-8% ethanol (Goodwin & Amit, 1998). This finding supported previous research reporting preference ratios for 10% ethanol in Fawn-Hooded rats of greater than 50% of daily fluid intake (Overstreet et al., 1997; Overstreet et al., 1993; Rezvani et al., 1990). Ethanol consumption, calculated as either absolute ethanol intake (i.e., g/kg/day) or as a volume measure of intake (ml/kg), varied as the ethanol solutions presented became more concentrated, ranging from 2.5-9.0 g/kg or 74-165 ml/kg of ethanol. Both ethanol intake

(ml/kg) and overall total fluid intake was stable for the first half of the ethanol acquisition period (ethanol 2-6.7%), and then dropped to a lower level for the remainder of the experiment. There is previous work showing that ethanol intake in rats for concentrations ranging from 2-6% ethanol, when ethanol is perceived as 'sweet' tasting (Bice et al., 1992; Di Lorenzo et al., 1986; Kiefer et al., 1990), may be regulated by factors other than its pharmacological properties, such as taste (Boyle, Smith & Amit, 1997). Intake of greater than 6% ethanol, when the taste and smell of ethanol become more aversive and the taste becomes both 'sweet' and 'bitter' (Bice, Kiefer & Elder, 1992; Di Lorenzo et al., 1986; Kiefer, Bice, Orr & Dopp, 1990), is more likely mediated by pharmacological effect (Boyle et al., 1997). Therefore, the change in volume intake patterns observed in Fawn-Hooded rats at around 6% ethanol may be a reflection of the change in factors influencing ethanol intake.

Fawn-Hooded rats also showed correspondingly high intake of the saccharin-quinine solutions, and at higher quinine concentrations, when compared with Lewis, Wistar Kyoto and Wistar strains presented in the same drinking paradigm (Goodwin & Amit, 1998). This would suggest that this ethanol-preferring rat strain did not find the increasing concentrations of quinine to be so aversive as to reduce intake levels. Interestingly, Fawn-Hooded rats most resembled the low ethanol-preferring Lewis strain during the saccharin-quinine drinking phase because both of these strains continued to prefer the saccharin-quinine solutions over water until the end of the acquisition period (Goodwin & Amit, 1998). There have been no other descriptions in the literature of Fawn-Hooded rats drinking saccharin-quinine, therefore it was not possible to compare these findings for reliability.

Examination of drinking behaviors on the first day of the saccharin-quinine acquisition period, when rats were presented with a 0.1% saccharin solution, showed that Fawn-Hooded rats as well as the three other rat strains from Goodwin and Amit (1998), displayed high intake of and preference for the saccharin solution (Fawn-Hooded: 216.5 ml/kg & 93.5%, respectively; Wistar Kyoto: 199.1 ml/kg & 95.8%, Lewis: 176.8 ml/kg & 96.4%, Wistar: 175.8 ml/kg & 90.9%, Goodwin & Amit, 1998). These findings contradicted the notion that saccharin intake differentiates rat strains with respect to their ethanol intake (e.g., Overstreet et al, 1993; Sinclair et al., 1992; Stewart et al., 1994), because these four strains exhibited ethanol preference levels ranging from approximately 10-60% of daily fluid intake yet they drank copious amounts of the simple saccharin solution.

Experiment 3 was designed to compare the patterns of intake of ethanol and sweet and bitter solutions in three rat strains used previously in Experiments 1 and 2: Lewis, Wistar and Fawn-Hooded rats. All rats were presented with a wide range of concentrations of each solution. It was of interest to examine differences in taste preferences, as measured by intake patterns across increasing solution concentrations, in greater detail than had previously been reported here and elsewhere in the literature. Traditionally, very few concentrations of flavoured solutions are offered in animal drinking experiments, thereby restricting generalization of intake behaviors to a small range of solutions. It has been noted that rats display inconsistent responses to ethanol solutions of varying concentrations in the taste reactivity paradigm, suggesting that they may perceive these as different fluid categories altogether rather than the same fluid at different concentrations (Lawrence & Kiefer, 1987). Therefore, it is possible that subtle

changes in drinking behavior patterns, especially for bitter solutions which tend to be highly aversive, may be revealed, thus allowing for more rigorous comparison of fluid intake between ethanol and sweet or ethanol and bitter tastes.

Experiment #3

Relative taste thresholds for ethanol, saccharin and quinine solutions in three strains of rats non-selected for ethanol consumption: A comparative study

This project evolved from an early, classical study conducted by Richter and Campbell (1940a) initially designed to investigate rodent regulatory activities. They devised a simple technique to determine the taste thresholds of rats for salts (Richter, 1939), sugars (Richter & Campbell, 1940b) and ethanol (Richter & Campbell, 1940a). In this paradigm, animals were presented with gradually increasing concentrations of a flavoured solution in free choice with water in a continuous access drinking paradigm. From the data collected, they were able to determine an individual rat's taste threshold for the flavoured solution, defined as "[t]he point at which the rats first indicated that they recognized a difference between distilled water and the [flavoured] solutions..." (Richter & Campbell, 1940b). As well, they were able to gather information about the range of preference and most preferred concentration of the flavoured solution in question.

In the present study, Fawn-Hooded (high ethanol-drinking: Overstreet et al., 1993), Lewis (low ethanol-drinking: Goodwin & Amit, 1998) and Wistar rats were presented with the complete series of ethanol solutions as set out by Richter and Campbell (1940a). In addition, comparable saccharin and quinine taste threshold paradigms were designed to examine sweet and bitter taste preferences in relation to ethanol intake. Thus, the purpose of Experiment 3 was to provide a detailed description of how three strains of rats, known to differ in their preference for ethanol, vary in their taste sensitivities for ethanol as well as the two component flavours said to most resemble

the taste of ethanol when combined (e.g., Di Lorenzo et al., 1986; Lawrence & Kiefer, 1987).

It is expected that the three stains will vary in their levels of ethanol intake, showing differences in threshold concentrations as well as cut-off concentrations: Fawn-Hooded rats are expected to continue consuming ethanol at higher concentrations and Lewis rats to discontinue consumption at more dilute concentrations. It is also expected that the three strains will show comparable drinking patterns for the saccharin solution, as it has been previously shown that there is little variability in saccharin preference among rat strains (e.g., Goodwin & Amit, 1998; Sinclair et al., 1992). It is expected that the quinine solutions, the most aversive-tasting of the three presented, will differentiate the three rat strains in a similar manner to ethanol.

Method

Subjects

Subjects were 24 male Fawn-Hooded rats (Skipper Bowles Center for Alcohol Studies, UNC Chapel Hill), 30 male Lewis rats, and 28 male Wistar rats (both Charles River, Quebec). All animals had been used previously in different ethanol intake studies involving continuous or limited-access exposure to ethanol, with a minimum 1 month wash-out period between experiments. The animals weighed approximately 360g-495g (Fawn-Hooded), 376g-503g (Lewis) and 465g-680g (Wistar) at the start of the experiment. Housing conditions and food were the same as described in Experiment 1. Drinking fluids (water and flavoured fluid) were presented as per Experiment 2.

All three strains were randomly divided into groups according to the flavoured fluid presented (ethanol, saccharin or quinine). Any animal that never developed a preference for the flavoured solution over water (i.e., preference for the flavoured solution of less than 50% of the daily total fluid) was excluded from the study: 1 Lewis rat in the ethanol group and 1 Lewis rat in the quinine group, 1 Wistar rat in the ethanol group and 3 Wistar rats in the quinine group. Also, 6 Fawn-Hooded rats were unable to complete the experiment due to illness: 5 Fawn-Hooded rats in the saccharin group and 1 Fawn-Hooded rat in the quinine group. These data were not included in the experiment. For the final sample size totals, see Appendix G.

Procedure

Animals were presented with water in both Richter tubes for 8 days. The rats were then presented with free access to a flavoured solution in one drinking tube, and water in the other tube on an everyday schedule. The ethanol solutions (v/v) were presented over 50 days according to the following daily increases in concentration: 0.01%, 0.02%, 0.02-0.1% by 0.2% increments, 0.1-1.0 by 0.1% increments, 1.2-5.0% by 0.2% increments, 5.0-10.0% by 0.5% increments, 10-15% by 1% increments (Richter & Campbell, 1940a). Comparable schedules for saccharin and quinine solutions were designed. The saccharin solutions (w/v) were presented over 51 days in the following daily concentration increases: 0.002-0.02% by .001% increments, 0.02-0.1% by .005% increments, 0.1-0.5% by .05% increments, 0.5-2% by 0.5% increments, 3%. The quinine solutions (w/v) were presented over 31 days in the following daily concentration

increases: 0.0001-0.0025% by 0.0001% increments, 0.0025-0.0055% by 0.0005% increments.

Data Analysis

Daily fluid consumption data (ml) for water and the flavoured solutions were converted as per Experiment 1: intake (ml/kg/day and g/kg/day), preference (%), and total fluid intake (ml/kg/day). Separate two-way ANOVAs (strain x concentration) with repeated measures were conducted on these measures as per Experiment 1. See Appendix H for graphs and analyses of total fluid intake data during ethanol, saccharin and quinine presentations. See Appendix I for analysis of body weights for both phases.

Taste threshold and cut-off concentrations were identified for each rat for the purpose of conducting strain comparisons. In order to calculate taste thresholds, the daily fluid consumption data (ml) for each animal was converted into a two-day mean preference value to correct for side preferences. The taste threshold was operationally defined as the first concentration at which preference for the flavoured solution rose above 50% for three consecutive days. The cut-off concentration was defined as the first concentration at which preference for the flavoured solution fell below 50% for three consecutive days. A maximal preference range was determined for each rat as the first concentration at which preference for the flavoured solution rose above 50% (minimum concentration) to the last concentration before which preference for the flavoured solution fell below 50% (maximum concentration). Mean minimum and maximum concentrations were calculated for each fluid within each strain. Separate one-way ANOVAs (strain) were performed on the taste threshold and cut-off concentration data for each flavoured solution.

Results

Ethanol

There were differences in ethanol intake when Fawn-Hooded, Lewis and Wistar strains were compared, strain x concentration $F(98, 1078) = 5.37$, $p < .0001$ (see Figure 3, top panel). Fawn-Hooded rats consumed more ethanol than Lewis rats until the ethanol solution presented reached a concentration of 12%, $p < .001$, and more ethanol than Wistar rats until the ethanol solution presented reached a concentration of 11%, $p < .05$. Wistar rats displayed higher consumption levels compared with Lewis rats only when the concentrations of ethanol were 2.6-6.5%, $p < .05$. At all other concentrations, these two strains were not different in their intake of ethanol.

Ethanol intake was converted into grams of ethanol consumed per kilogram of body weight. The three strains also showed differences in their absolute ethanol intake, $F(98, 1078) = 9.54$, $p < .0001$ (see Figure 3, middle panel). The strains began to differ significantly when the concentration of ethanol was 1.6%, $p < .01$. Fawn-Hooded rats consumed more ethanol than Lewis rats for the remainder of the experiment (ethanol 1.6-15%), $p < .001$. Fawn-Hooded rats also displayed greater ethanol intake than Wistar rats for ethanol concentrations ranging from 2.4-12%, $p < .05$. And Wistar rats had higher ethanol intake than Lewis rats for ethanol concentrations of 3.4-14%, $p < .01$.

Measures of ethanol preference revealed significant differences from the first ethanol presentation until the concentration of ethanol reached 11%, strain x concentration $F(98, 1078) = 2.38$, $p < .0001$ (see Figure 3, bottom panel). Fawn-Hooded rats maintained

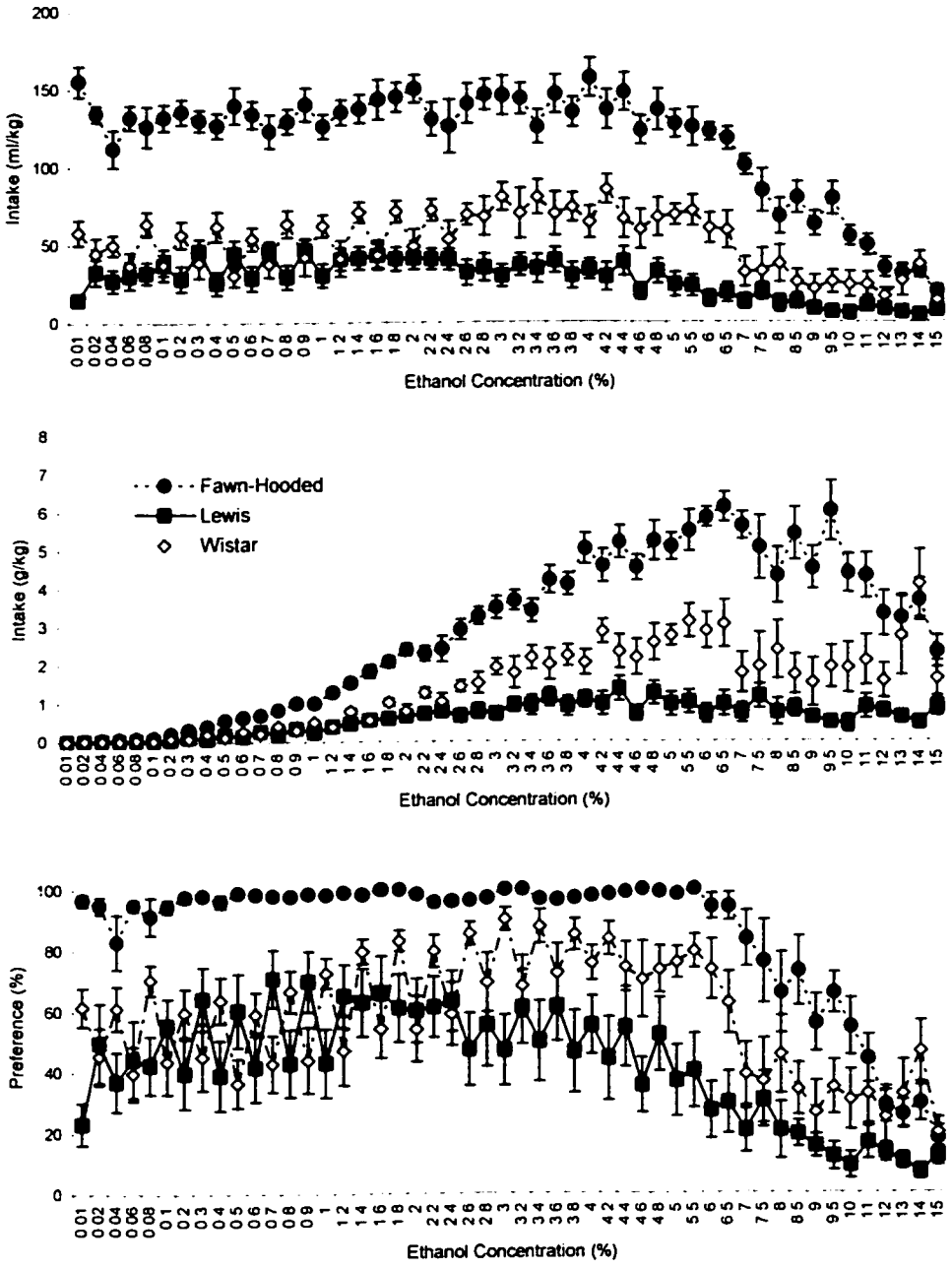

Figure 3: Mean (+/- SEM) ethanol intake (top: ml/kg and middle: g/kg) and ethanol preference (%) (bottom) in Fawn-Hooded, Lewis and Wistar rats.

a mean preference level for ethanol of greater than 50% during this period, which was significantly higher than both Wistar and Lewis strains, $p < .05$. Lewis and Wistar rats were not consistently different in their preference for ethanol.

When comparing mean threshold concentrations for ethanol across strains, there were significant group differences, $F(2, 22) = 6.31$, $p < .01$ (see Table 4). Fawn-Hooded rats had a lower threshold point for ethanol than Wistar rats, $p < .01$, but were not different from Lewis rats. Similarly, there were group differences in mean ethanol cut-off concentrations, $F(2, 20) = 6.23$, $p < .01$ (see Table 4). Fawn-Hooded rats had higher cut-off concentrations than Lewis rats, $p < .01$, but were not different from Wistar rats. Wistar and Lewis rats were not different from each other on either measure.

Saccharin

The three rat strains differed significantly in their saccharin intake, strain x concentration $F(100,1050) = 11.99$, $p < .0001$ (see Figure 4, top panel). Fawn-Hooded rats displayed the highest saccharin intake from the first day saccharin was presented (0.002% saccharin), $p < .01$, to a concentration of 1.5%, $p < .05$. Wistar rats consumed more saccharin than Lewis rats when the saccharin concentration rose above 0.01%, $p < .001$, until it reached 1.5% saccharin, $p < .05$.

The differences between the strains were less pronounced in saccharin preference, strain x concentration $F(100,1050) = 4.13$, $p < .0001$ (see Figure 4, bottom panel). While Fawn-Hooded rats maintained a higher preference for the saccharin solutions compared to Wistar rats at concentrations lower than 0.008%, $p < .01$, Lewis rats displayed lower

Table 4

Mean threshold, cut-off and preference range concentrations (%) for Fawn-Hooded, Lewis and Wistar rats drinking ethanol solutions

	Threshold			Cut-off			Preference Range				
Strain	M	SD	n	M	SD	n	Min	SD	Max	SD	n
Fawn-Hooded	<0.01	0	8	10.06	2.2	8	0.02	0	7.88	2	8
Wistar	1.18	1.1	9	7.83	2.7	9	2.01	1.5	6.81	2.6	9
Lewis	0.28	0.5	8	5.58	2.2	8	0.64	0.7	4.8	2.2	5[a]

[a]Only 5 of 8 LEW rats showed a consistent enough preference for the ethanol solutions to determine a preference range.

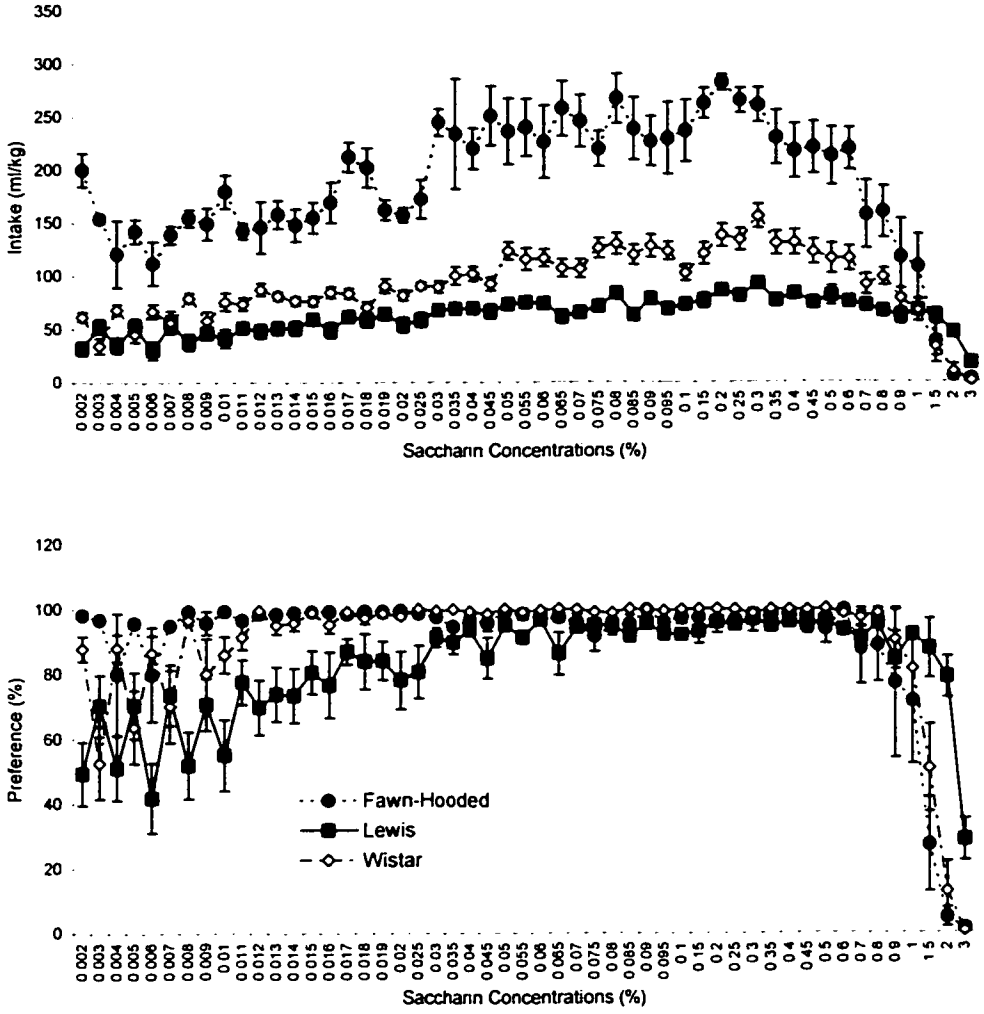

Figure 4: Mean (+/- SEM) saccharin intake (ml/kg) (top) and saccharin preference (%) (bottom) in Fawn-Hooded, Lewis and Wistar rats.

preference values compared to both Fawn-Hooded and Wistar rats until saccharin reached a concentration of 0.016%, $p < .05$. From this concentration until the saccharin solutions reached 1.5%, there were no differences between the strains in saccharin preference.

There were no strain differences in mean threshold concentrations, $F(2, 21) = 3.07$, or in mean cut-off concentrations, $F(2, 21) = 0.11$, for the saccharin solutions (see Table 5).

Quinine

Quinine intake was significantly higher in Fawn-Hooded rats when compared with Lewis and Wistar rats, strain x concentration $F(60, 540) = 8.25$, $p < .0001$ (see Figure 5, top panel). While Wistar rats consumed more quinine than Lewis rats only until the quinine solution reached a concentration of 0.0008%, $p < .05$, Fawn-Hooded rats had higher intake levels than both strains until the concentration of the quinine solution reached 0.0015%, $p < .05$. Lewis rats displayed consistently low consumption of the quinine solutions throughout the experiment.

Similar group differences were also apparent in quinine preference, strain x concentration $F(60, 540) = 3.66$, $p < .0001$ (see Figure 5, bottom panel). Fawn-Hooded rats had greater preference for the quinine solutions when compared with Wistar and Lewis rats until the quinine solution presented reached a concentration of 0.0013%, $p < .01$. Wistar rats had greater quinine preference than Lewis rats until the quinine solutions reached 0.0014%, $p < 01$.

It was not possible to calculate mean quinine threshold concentrations for Lewis and Wistar rats based on the operational definition for the threshold calculation (see

Table 5

Mean threshold, cut-off and preference range concentrations (%) for Fawn-Hooded, Lewis and Wistar rats drinking saccharin solutions

	Threshold			Cut-off			Preference Range			
Strain	M	SD	n	M	SD	n	Min	SD	Max	SD
Fawn-Hooded	<0.002	0	4	1.6	0.5	4	0.0033	0.003	1.08	0.3
Wistar	0.0022	0.001	10	1.74	0.6	10	0.0046	0.003	1.22	0.4
Lewis	0.0064	0	10	2.79	0.9	10	0.0108	0.008	2.03	0.6

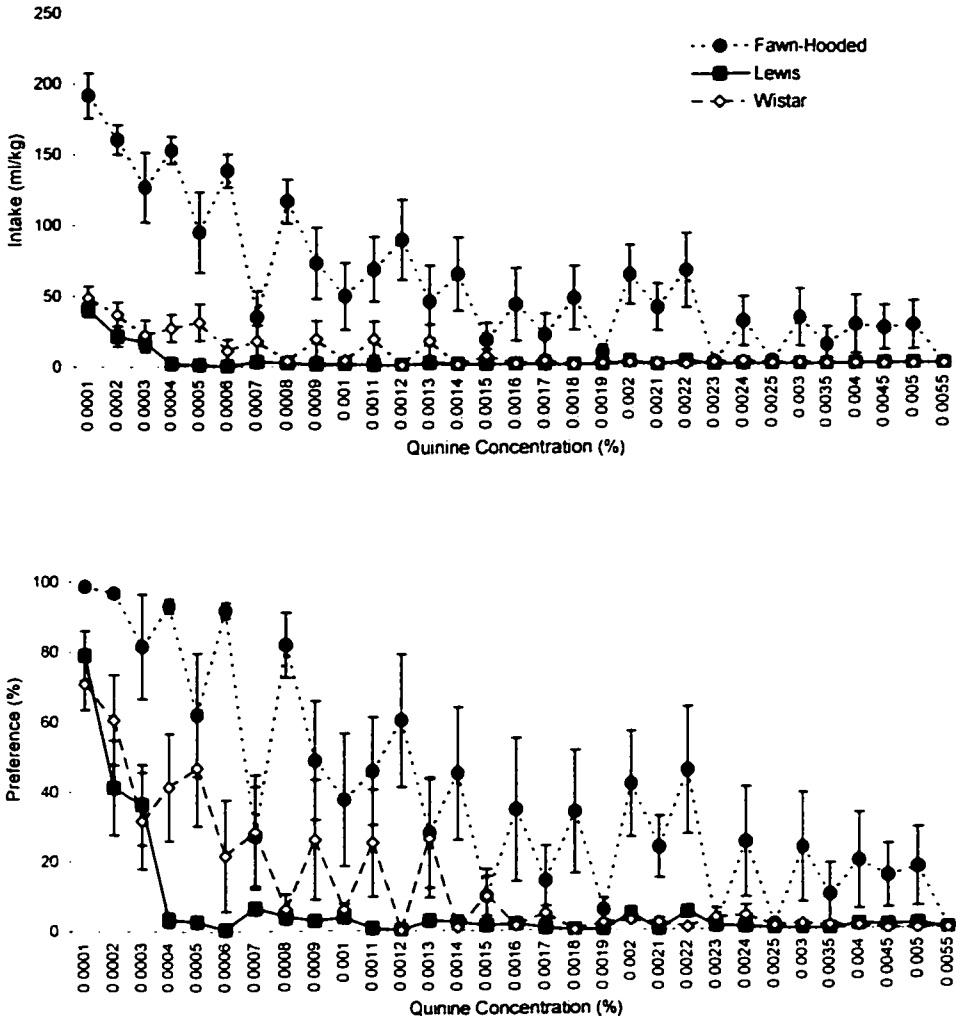

Figure 5. Mean (+/- SEM) quinine intake (ml/kg) (top) and quinine preference (%) (bottom) in Fawn-Hooded, Lewis and Wistar rats.

Method Section: Data Analysis) because there were rats in each group who never displayed a preference for the quinine solutions. Therefore, no statistics comparing mean quinine threshold concentrations among the strains were conducted. Fawn-Hooded rats had significantly higher mean cut-off concentrations for the quinine solutions, $F(2, 18) = 6.61$, $p < .01$, than both Lewis, $p < .01$, and Wistar rats, $p < .05$ (see Table 6).

Discussion

The three strains of rats used in this study, all unselected for ethanol intake, showed very different patterns of fluid intake for all three fluids presented. Fawn-Hooded rats, known to be high ethanol-drinkers (e.g., Overstreet et al., 1993; Rezvani et al., 1990), consumed the greatest amounts of ethanol. Interestingly, Fawn-Hooded rats also consumed the greatest amounts of the saccharin and quinine solutions. Conversely, Lewis rats, previously shown to be low ethanol-drinkers (e.g., Goodwin & Amit, 1998), consumed the least amount of ethanol as well as of the saccharin and quinine solutions. Wistar rats were consistently positioned between the two other strains and consumed moderate amounts of the ethanol, saccharin and quinine solutions.

Fawn-Hooded rats preferred all three flavoured solutions over water from the lowest concentrations presented. Furthermore, their range of preferred concentrations for each fluid was larger when compared with Lewis and Wistar rats, as they tended to drink more of the flavoured solutions compared with water at more dilute (ethanol, saccharin)as well as at stronger concentrations (ethanol, quinine). Fawn-Hooded rats exhibited a lower mean threshold concentration for the detection of ethanol in water compared with

Table 6

Mean threshold, cut-off and preference range concentrations (%) for Fawn-Hooded, Lewis and Wistar rats drinking quinine solutions

	Cut-off			Preference Range			
Strain	M	SD	n	Min	SD	Max	SD
Fawn-Hooded	0.00127	0.0012	6	0.0001	0	0.00072	0.0004
Wistar	0.00042	0.0002	6	0.00015	0.0001	0.00032	0.0002
Lewis	0.00029	0.0002	9	0.00011	0	0.00019	0

[a]Only 5 of 6 Fawn-Hooded rats showed a consistent enough preference for the quinine solutions to determine a preference range.

Wistar rats but not Lewis rats. This would suggest that while Fawn-Hooded rats used this information to guide their increased ethanol intake and Lewis rats used it to avoid drinking the ethanol solutions, both strains were comparably sensitive to the increasing ethanol concentrations. Fawn-Hooded rats also drank ethanol at higher concentrations when compared with Lewis rats and drank quinine at higher concentrations when compared with both Wistar and Lewis rats. These findings may indicate that Fawn-Hooded rats were more tolerant of the aversive taste properties of both flavoured solutions when compared with the Lewis and Wistar strains.

There were no differences between the strains in mean threshold or cut-off concentrations for the saccharin solutions. There were also no differences in preference ratios among the strains, as distinct from amount consumed, for the majority of the saccharin schedule (for saccharin concentrations ranging from 0.016% to 1.5%). When compared with ethanol preference, which showed differences between the strains particularly across ethanol concentrations most commonly used in research (i.e., 2-10%), it was clear that these particular measures of saccharin and ethanol intake did not differentiate the strains in a similar fashion. Interestingly, the volume measure of saccharin intake did show a consistent pattern of consumption quite similar to that of ethanol intake, in that Fawn-Hooded rats consumed the greatest amounts throughout most of both fluid presentation schedules, followed by Wistar and Lewis rats. This may have resulted from introducing rats to a long period of acclimatization to the flavoured solutions at very gradual concentration increments. Saccharin has been described as having aversive taste properties for rats at concentrations as low as 0.1% or 0.004 M (Dess, 1993), unlike sucrose which is considered to have a purely sweet flavour. As well,

concentrations of ethanol below 6% are reported to be 'sweet tasting' in nature (Bice et al., 1992; Di Lorenzo et al., 1986; Kiefer et al., 1990), and it is possible that the comparable patterns of intake were a reflection of the 'sweetness' of the ethanol solutions below 6%. Nonetheless, the concentrations of ethanol and saccharin most commonly compared in the literature (10% ethanol, 0.1% saccharin) appeared to show clear similarities in the present drinking paradigm, at least between Fawn-Hooded and Wistar rats. However, the comparison is not so obvious when absolute ethanol intake (g/kg) is compared with saccharin intake (ml/kg). It should be noted that the sweet-ethanol correlations reported in the literature have been with absolute ethanol intake (e.g., Overstreet et al., 1993; Rezvani et al., 1990).

The present results provided evidence in support of the notion that responses to a series of quinine solutions may also be relevant in predicting the affinity of a rat strain for ethanol intake. In the present experiment, differences in quinine intake and cut-off concentrations at the lower end of the quinine presentation schedule allowed for a clearer differentiation between the intake patterns of the strains compared to data collected about saccharin preference. While we and other laboratories have previously attempted to investigate possible ethanol-quinine relationships in rats (e.g., Overstreet et al., 1993), the failure of those experiments to obtain supportive data may have been the result of presenting animals with quinine concentrations which were too strong (e.g., quinine concentration of 0.25%), and therefore highly unpalatable and not consumed with any reliability by the animals. The present experiment, using the Richter and Campbell (1940a) procedure to investigate quinine intake, allowed for the preliminary description of data about differential taste sensitivities for bitter flavours among strains.

While the results of this experiment were derived in animals who had had prior experience with ethanol, both in continuous and limited fluid access drinking experiments, this was considered to have little effect on innate taste thresholds. However, it is possible that group differences observed in fluid intake among these three strains may have been in part the result of an interaction between prior experience with ethanol and the current experimental manipulations. In order to confirm the present results, these experiments should be replicated in naive animals.

Altogether, the current data provided another instance where sweet preference, in particular, did not appear to be related to ethanol preference in rats. However, saccharin intake over the extended fluid presentation schedule did appear to more successfully mirror ethanol intake at concentrations more dilute than those most commonly used in research. The notion that bitter preference was a potentially reliable behavioral marker for ethanol preference was further suggested. The experiments to follow, Experiments 4 and 5, sought to extend the previous research elucidating fluid intake interrelationships across rat strains by investigating whether these associations were mediated by a common neurotransmitter. For example, it has been speculated that there may be a genetic influence on saccharin and ethanol intakes that is operating directly on one or more of the neurochemical systems underlying the reinforcing effects of these agents, such as the opiate system (Overstreet et al., 1993; Sinclair et al., 1992). Such positive findings would provide support, and perhaps clarity, for the data derived from traditional intake studies and may show that strain differences in fluid intake and their concomitant associations are related to differences in one or more of the neurotransmitter systems.

Experiment #4

The effects of THIP, naltrexone and raclopride on the voluntary intake of ethanol, saccharin, and quinine, in two strains of rats

The purpose of the present study was to attempt to determine whether a common neurochemical system may mediate taste preferences for ethanol and the putatively related sweet and bitter solutions across two rat strains. The three neurotransmitter systems chosen for investigation have previously been shown to play a role in both ethanol and sweet solution intake in rats. Specifically, there have been reports that antagonism of the opioidergic and dopaminergic systems in rats will attenuate ethanol intake (e.g., Davidson & Amit, 1997b; Pfeffer & Samson, 1985a; Sinclair, 1990) as well as intake of sweetened ethanol (e.g., Gardell et al., 1996) and sweet-tasting solutions (e.g., Biggs & Myers, 1998; Hsaio & Smith, 1995; Muscat et al., 1991), while gabaergic agonists have been shown to increase both ethanol (e.g., Boyle et al., 1993; Tomkins & Fletcher, 1996) and sucrose intake (Basso & Kelley, 1999).

The working hypothesis underlying this study was that changes in intake due to neurotransmitter manipulation may reflect a shift in palatability of ethanol and other related flavoured fluids which, in turn, may vary across strains. The gabaergic $GABA_A$ agonist THIP, the dopaminergic D2/D3 antagonist raclopride or the opioidergic antagonist naltrexone were administered to Wistar and Lewis rats. The dose levels for each drug were chosen on the basis of previous research which has shown them to be effective on either saccharin or ethanol intake (Davidson & Amit, 1997b; Muscat et al., 1991; Smith et al., 1992). Intake of ethanol, sweet (saccharin) and bitter (quinine)

solutions were subsequently measured. As previous research has demonstrated that ethanol has a sweet/bitter taste (Di Lorenzo et al., 1986; Kiefer et al., 1990; Kiefer & Lawrence, 1988; Kiefer & Mahadevan, 1993; Lawrence & Kiefer, 1987), it was expected that any changes in ethanol intake due to neurotransmitter manipulation accompanied by changes in the consumption of the sweet or bitter solutions may support the notion of a possible common underlying neural system.

Method

Subjects

Subjects were 40 naive, male Lewis rats and 40 naive, male Wistar rats (Charles River, Quebec). The animals weighed approximately 190-315g (Lewis) and 200-330g (Wistar) at the start of the experiment. [Fawn-Hooded rats, used in both Experiments 2 & 3 of the present thesis, were no longer available to be used a subjects for the present experiment due to old age and concomitant illnesses.] Housing conditions and food were the same as described in Experiment 1. Drinking fluids (water and flavoured fluid) were presented as per Experiment 2.

Both strains were randomly divided into groups according to the drug received (THIP, naltrexone, raclopride or saline). There were 10 animals from each strain in each drug group. All animals received all flavoured fluids (quinine, saccharin and ethanol).

Drugs

THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol) (Research Biochemicals International), naltrexone hydrochloride (Research Biochemicals International) and

raclopride (Astra) were administered intraperitoneally at a volume of 1 ml/kg. The dose levels were: THIP 16 mg/kg, naltrexone 10 mg/kg and raclopride 0.4 mg/kg. A control group received 1 ml/kg physiological saline. These injections were given 1 hour prior to lights out.

Procedure

Following a 2-week acclimatization period to the animal colony facilities, all animals were presented with water and flavoured fluids. The solutions were presented sequentially in the following order: 0.04% (w/v) quinine for 5 days, 0.1% (w/v) saccharin for 5 days, and 10% (v/v) ethanol for 20 days. The number of exposure days for each solution were chosen based on a previous study (Overstreet et al., 1993) as well as Experiment 1 of the present thesis. Animals received drug injections on days 4-5 of the quinine drinking period, days 4-5 of the saccharin drinking period and days 16-20 of the ethanol drinking period.

Data Analysis

Daily fluid consumption data (ml) for water and the flavoured solution were converted as per Experiment 1: intake (ml/kg/day), preference (%), and total fluid intake (ml/kg/day), including a measure of water intake (milliliters of water consumed per kilogram of body weight per day, ml/kg/day).

For each flavoured solution, the mean intake measures during a baseline phase (quinine days 2-3, saccharin days 2-3, ethanol days 11-15), and the treatment phase (quinine days 4-5, saccharin days 4-5, ethanol days 16-20) were compared. Separate two-way ANOVAs (strain x phase) with repeated measures were conducted on all intake variables for each drug group. See Appendix J for a data table and statistical analysis of

water intake. See Appendix K for a data table and statistical analysis of total fluid intake. See Appendix L for statistical analysis of body weights.

The mean baseline intake and preference values from all drug groups were collapsed across three flavoured solutions for further comparison using rank-ordered Spearman correlation coefficients (Minium et al., 1993) in order to determine within-strain relationships in fluid intake and preference prior to drug treatment.

Results

Correlational analyses of intake and preference data revealed that ethanol intake was significantly correlated with quinine intake in Lewis rats, $r_s = +0.793$, $p < .01$. Ethanol preference was also correlated with quinine preference only in Lewis rats, $r_s = +0.743$, $p < .01$. Within Wistar rats, ethanol intake was correlated with saccharin intake, $r_s = +0.374$, $p < .05$. There were no significant correlations in the Wistar preference data (see Appendix M for complete table of correlations).

Ethanol

THIP. There were significant interactions in both ethanol intake, strain x phase $F(1, 18) = 5.06$, $p < .05$ (see Table 7), and ethanol preference, strain x phase $F(1, 18) = 4.49$, $p < .05$ (see Table 8). Lewis and Wistar rats were not different at baseline, however Wistar rats increased their intake of ethanol by 37%, $p < .01$, and their preference for ethanol by 52%, $p < .001$, from baseline to treatment phases.

Table 7

Mean fluid intake (ml/kg) across drug groups for Lewis and Wistar rats during baseline and treatment phases

	Ethanol		Saccharin		Quinine	
Phase	B	T	B	T	B	T
	Lewis					
THIP	8.5 (1.0)	7.8 (1.4)	102.9 (19.8)	92.0 (12.7)	8.4 (1.8)	6.4 (1.6)
Naltrexone	7.3 (1.1)	6.6 (1.2)	109.1 (17.4)	77.1 (14.8)	8.2 (1.5)	6.8 (1.1)
Raclopride	4.5 (1.3)	3.7 (1.1)	85.7 (19.9)	74.8 (14.2)	4.6 (1.4)	3.8 (1.5)
Saline	8.4 (1.5)	7.8 (1.6)	119.5 (14.0)	127.3 (6.9)	8.6 (2.2)	8.7 (2.1)
	Wistar					
THIP	19.9 (6.6)	27.2 (7.4)	219.8 (3.3)	183.9 (7.9)	5.0 (1.6)	3.3 (0.5)
Naltrexone	27.5 (6.7)	30.1 (7.2)	232.3 (10.9)	182.0 (12.4)	8.3 (1.2)	6.5 (0.5)
Raclopride	33.8 (6.2)	39.5 (7.0)	231.4 (6.0)	194.9 (11.7)	8.0 (1.2)	6.1 (0.5)
Saline	17.4 (4.4)	23.6 (5.0)	215.1 (2.8)	203.1 (6.0)	5.7 (1.1)	3.6 (0.1)

Note. Values enclosed in parentheses represent standard error of the mean (SEM). B = Baseline Phase; T = Treatment Phase.

Table 8

Mean fluid preference (%) across drug groups for Lewis and Wistar rats during baseline and treatment phases

	Ethanol		Saccharin		Quinine	
Phase	B	T	B	T	B	T
	Lewis					
THIP	100.6 (1.9)	95.6 (2.9)	144.0 (0.3)	127.6 (1.7)	123.6 (2.2)	113.9 (2.7)
Naltrexone	102.2 (1.9)	94.0 (2.0)	158.1(2.5)	122.8 (3.7)	128.3 (3.0)	112.8 (0.3)
Raclopride	93.6 (1.2)	92.4 (1.2)	144.0 (9.7)	113.7 (1.2)	114.4 (0.5)	106.4 (1.0)
Saline	101.7 (2.0)	97.0 (1.8)	150.4 (3.6)	142.2 (3.8)	128.1 (1.6)	122.0 (1.5)
	Wistar					
THIP	15.7 (1.6)	23.9 (1.5)	75.3 (6.3)	80.1 (0.7)	3.0 (0.9)	2.3 (0.4)
Naltrexone	21.1 (2.2)	26.1 (3.0)	76.5 (0.7)	78.5 (0.8)	4.4 (0.6)	4.0 (0.3)
Raclopride	24.9 (1.9)	30.4 (1.1)	82.8 (3.0)	84.2 (2.8)	4.8 (0.6)	4.1 (0.3)
Saline	14.1 (1.1)	20.2 (0.7)	76.7 (2.4)	85.5 (0.1)	3.4 (0.8)	2.5 (0.2)

Note. Values enclosed in parentheses represent standard error of the mean (SEM). B = Baseline Phase; T = Treatment Phase.

Naltrexone. Wistar rats drank more and had higher preference for the ethanol solution when compared with Lewis rats, Fs(1, 18) = 11.82 (see Table 7) and 9.92 (see Table 8), respectively, ps< .01. There was no effect of naltrexone treatment on ethanol intake or preference across phases, Fs(1, 18) = 0.10 and 0.87, respectively.

Raclopride. Significant interactions in ethanol intake and preference indicated that Wistar rats consumed more ethanol, strain x phase F(1, 18) = 5.13, p< .05 (see Table 7), and had a higher preference for the ethanol solution, strain x phase F(1, 18) = 11.96, p< .01 (see Table 8), when compared with Lewis rats, during both baseline and treatment (ps< .001) phases. Wistar rats also increased their ethanol intake by 17% (p< .05) and their ethanol preference by 22% (p< .001) during the treatment phase compared with baseline values. Ethanol intake and preference remained unchanged in Lewis rats following raclopride treatment.

Saline. Significant interactions in both ethanol intake and preference data, strain x phase F(1, 18) = 6.21 (see Table 7) and 6.12 (see Table 8) respectively, ps< .05, revealed that Wistar rats had greater intake of and preference for the ethanol solution during the treatment phase when compared with Lewis rats, ps< .01. Also, Wistar rats increased their ethanol intake by 36% and preference by 43%, ps< .01, from baseline to treatment phases.

Saccharin

THIP. Analysis of saccharin intake showed that Wistar rats consumed significantly more saccharin than Lewis rats, F(1, 18) = 7.66, p < .05 (see Table 7). There was no effect of THIP treatment on saccharin intake in either strain, F(1, 18) = 3.78.

There were no differences in saccharin preference between the strains, $F(1, 18) = .33$, or due to drug treatment, $F(1, 18) = 3.56$ (see Table 8).

Naltrexone. Wistar rats consumed significantly more of the saccharin solution than Lewis rats, $F(1, 18) = 8.14$, $p < .05$ (see Table 7), however there was no difference in their saccharin preference, $F(1, 18) = 1.34$ (see Table 8). Both strains reduced their saccharin intake by 24% following naltrexone treatment, $F(1, 18) = 9.67$, $p < .01$. There were no changes in saccharin preference across the phases of the experiment, $F(1, 18) = 0.05$.

Raclopride. Wistar rats consumed more saccharin overall compared with Lewis rats, $F(1, 18) = 23.98$, $p < .001$ (see Table 7). There were no differences in preference for the saccharin solutions between the strains, $F(1, 18) = 2.94$ (see Table 8). And there was also no effect of raclopride treatment on saccharin intake, $F(1, 18) = 3.02$ or preference, $F(1, 18) = 1.52$.

Saline. Wistar rats consumed significantly more saccharin solution than Lewis rats, $F(1, 18) = 5.95$, $p < .05$ (see Table 7). However, there were no differences in saccharin preference, $F(1, 18) = 0.03$ (see Table 8). There was no effect of saline treatment on saccharin intake, $F(1, 18) = 0.04$, and saccharin preference increased by 15% from baseline to treatment phases, $F(1, 18) = 18.96$, $p < .001$.

Quinine

THIP. Quinine intake was not different between Lewis and Wistar rat strains, $F(1, 18) = 3.01$ (see Table 7). The strains showed an overall decrease in quinine intake of 27% following THIP treatment, $F(1, 18) = 11.43$, $p < .01$. While Lewis rats preferred the

quinine solution more than Wistar rats, $F(1, 18) = 6.90$, $p < .05$, both strains also reduced their preference for quinine by 19% during the treatment phase, $F(1, 18) = 7.19$, $p < .05$ (see Table 8).

Naltrexone. There were no significant differences between Lewis and Wistar rats in quinine intake, $F(1, 18) = 0.00$ (see Table 7). Quinine intake in both strains decreased by 19% following naltrexone treatment, $F(1, 18) = 6.39$, $p < .05$. There were no differences in quinine preference between the strains or across experimental phases, $Fs(1, 18) = 1.77$ and 0.80, respectively (see Table 8).

Raclopride. There were no differences between Lewis and Wistar rats in quinine intake, $F(1, 18) = 1.48$ (see Table 7), or quinine preference, $F(1, 18) = 0.27$ (see Table 8). Both strains showed a 21% decrease in intake following drug treatment, $F(1, 18) = 7.55$, $p < .05$. There was no change in quinine preference across the phases, $F(1, 18) = 2.02$.

Saline. A significant interaction in the quinine intake data, strain x phase $F(1, 18) = 4.72$, $p < .05$, indicated that Lewis rats consumed more quinine than Wistar rats, $p < .05$, and that Wistar rats reduced their quinine intake by 36% from baseline to treatment phases, $p < .05$ (see Table 7). Similarly, a significant interaction in the preference data, strain x phase $F(1, 18) = 6.10$, $p < .05$, indicated that Lewis rats preferred the quinine solution more than Wistar rats, $p < .05$, and Wistar rats reduced their quinine preference by 26 % from baseline to treatment phases, $p < .05$ (see Table 8).

Discussion

The present investigation found little evidence to support the notion that the gabaergic, opioidergic or dopaminergic neurotransmitter systems may play a role in mediating the relationship between taste and ethanol intake, specifically with respect to sweet and bitter tastes. And this concluded despite the fact that ethanol drinking was significantly correlated with sweet or bitter solution intake in Lewis and Wistar rats.

The $GABA_A$ receptor agonist THIP attenuated quinine intake and preference in Lewis and Wistar rats. However, water intake was also reduced. Saline-treated Wistar rats showed a similar decrease in quinine intake and preference and water intake following THIP treatment, a finding which suggested that the attenuation of quinine intake measures following THIP treatment may have been the result of a non drug-related generalized reduction in fluid intake. THIP treatment also had no effect on saccharin intake or preference in either Wistar or Lewis rats. While there have been no previous reports of the effects of THIP on sweet solution intake, two studies reported similar findings of a lack of effect of muscimol ($GABA_A$ agonist) or baclofen ($GABA_B$ agonist) on saccharin and glucose/saccharin intake (Basso & Kelley, 1999; Ward et al., 2000). THIP treatment was associated with an increase in ethanol intake and preference only in Wistar rats, supporting previous research showing that administration of THIP at similar dose levels caused increases in ethanol consumption in rats in 24-hour (Boyle et al., 1992, 1993; Smith et al., 1992) and limited-access drinking paradigms (Tomkins & Fletcher, 1996). These effects appeared to be specific to ethanol because total fluid intake remained unchanged following THIP treatment, a finding which also supported previous research (Boyle et al., 1992, 1993; Smith et al., 1992; Tomkins & Fletcher, 1996). However, there were comparable increases in ethanol intake and preference in the saline-treated Wistar

rats, indicating that the significant increases in intake in both groups of Wistar rats may have been the result of a presumed familiarity with the solutions, which can act to increase preference over time (Veale & Myers, 1969). This effect, known as the alcohol acclimation effect, was first noted in rats following repeated exposure to several series of increasing concentrations of ethanol over a prolonged period of time (Veale & Myers, 1969).

Naltrexone administration resulted in decreases in both quinine and saccharin intakes in both rat strains, while having no effect on preference for either fluid. Previous studies on the effects of opioid receptor blockade on quinine intake found no effect on intake (Levine et al., 1982) or taste reactivity (Parker et al., 1992). On the other hand, there have been ample number of studies demonstrating an attenuation of saccharin intake following administration of naltrexone or naloxone (Chow, Sellers & Tomkins, 1997; Cooper 1982, 1983; Lynch & Libby, 1983, Touzani et al., 1991). Interestingly, naltrexone had no effect on ethanol intake or preference in either rat strain, a finding which was at odds with studies showing an attenuation of ethanol consumption following naltrexone administration in rats (Davidson & Amit, 1997a,b; Hill & Kiefer, 1997; Myers & Crichter, 1982; Sinclair, 1990).

There were also decreases in water intake following naltrexone administration in both strains drinking quinine and ethanol, however there were decreases in water intake in the respective saline-treated rats as well. Previous research has reported water suppression following naltrexone treatment (e.g., Gardell et al., 1996; Reid, 1985), and some have posited that this generalized fluid effect may signal an antidipsogenic

side-effect of opioid antagonists. However, in the present experiment, this notion was not supported because the saline groups also showed an attenuation of water intake.

The dopamine D2/D3 antagonist raclopride suppressed quinine intake in both strains while having no effect on quinine preference, a result of concurrent reduction in water intake. Previous research reported no effect of a smaller dose of raclopride on quinine intake in rats (Phillips et al., 1991a), however this was during limited fluid access and the present experiment used a 24-hour fluid exposure paradigm. Saccharin intake and preference were unaffected by raclopride treatment in the present experiment, a finding which was at odds with a previous study which found an attenuation of the intake of 0.02% and 0.2% saccharin solutions following the same dose of raclopride during 24-hour access (Muscat et al., 1991). Raclopride has consistently been shown to reduce the intake of another sweet solution, sucrose, at low concentrations (e.g., Hodge, Samson, Tolliver & Haraguchi, 1994; Muscat et al., 1991; Phillips et al., 1991a; Schneider et al., 1990). Additionally, raclopride had no effect on ethanol intake or preference in Lewis rats, but was associated with a significant increase in ethanol intake and preference in Wistar rats. This has not been found in any previous studies, one of which reported no effect of raclopride at higher doses in 24-hour drinking (Sylvestre et al., 1996) and yet another which showed attenuation of ethanol drinking during limited access sessions (Kaczmarek & Kiefer, 2000).

Decreases were observed in water intake following raclopride treatment in both strains drinking quinine solutions and in Wistar rats drinking ethanol. A previous study showed reductions in water intake following treatment with 0.1 and 1.0 ml/kg raclopride (Silvestre et al., 1996), and these investigators suggested that the nature of raclopride's

effect was that of a nonspecific attenuation of consummatory behaviors. In the present experiment, it was not possible to support these conclusions because there were also reductions in water intake following saline treatment in both strains. The attenuation of water consumption observed in the present experiment was more likely the result of a nonspecific reduction in fluid intake.

Notwithstanding these results, Experiment 5 was designed to examine the effects of naltrexone on the intake of five flavoured fluids, including ethanol and sweetened-ethanol, in greater detail. The reasons for pursuing the effects of opioid manipulation via naltrexone administration despite the negative findings of Experiment 4 were two-fold. First, naltrexone is commonly used to treat alcoholism in humans, with some reports of reduced cravings for alcohol, number of drinking days and rates of relapse (e.g., O'Malley et al., 1992; Volpicelli et al., 1992), while others report no effect of naltrexone on these same measurements of human alcohol abuse (Krystal et al., 2001). Naltrexone's mechanism of action in humans is unknown, but several investigators have proposed that it may involve a shift in taste sensitivity for alcohol (Biggs & Myers, 1998; Davidson & Amit, 1997b; Hill & Kiefer, 1997; Levine et al., 1982; Parker et al., 1992). Secondly, changes in taste reactivity responding for ethanol following naltrexone treatment in rats showed increased aversive responding, providing support for the notion that naltrexone acts to cause a shift in the palatability of ethanol in rats (Hill & Kiefer, 1997). Together, the findings from these separate fields of research suggest a possible role for the opioid system in mediating taste preferences.

Experiment #5

Effects of naltrexone on the intake of ethanol and flavoured solutions in rats

Experiment 5 was designed to investigate the effects of the opioid antagonist naltrexone on the intake of five flavoured solutions differing in their sweet, bitter, or ethanol components. The specific dose of naltrexone administered was the same as that used in Experiment 4, and had previously been shown to attenuate the acquisition of voluntary ethanol consumption in rats in a 24-hour exposure paradigm (Davidson & Amit, 1997b). Saccharin, ethanol, and saccharin-ethanol solutions were used, in line with previous research (Gardell et al., 1996; Overstreet et al., 1993). Also, a quinine solution was introduced to examine the effects of naltrexone on bitter taste, and a saccharin-quinine mixture was also introduced to examine the effects of naltrexone on the intake of a sweet-bitter solution said to reflect the taste of ethanol more accurately than a saccharin solution alone (Kiefer & Mahadevan, 1993). Two strains of rats, Long-Evans and Wistar, served as subjects for the present experiment in order to highlight any strain differences in the effects of naltrexone. In addition, fluid intake was measured in two drinking schedules: 24-hour access and limited-access. Thus the focus of the experiment was an attempt to determine whether naltrexone would have a generalized effect on the "taste value" (Hill & Kiefer, 1997) of all five flavoured fluids regardless of strain or fluid presentation schedule, causing decreases in intake in all solutions in an equivalent manner, or more selective effects on certain tastes, namely the sweet or bitter flavors.

Method

Subjects

Subjects for Phase 1 (continuous fluid access) were 31 naive, male Long-Evans rats and 31 naive, male Wistar rats (Charles River, Quebec). The animals weighed approximately 160-220 g (Long-Evans) or 150-190 g (Wistar) at the start of the experiment. Subjects for Phase 2 (limited fluid access) were 31 naive, male Long-Evans rats and 31 naive, male Wistar rats (Charles River, Quebec). These animals weighed approximately 320-390 g (Long-Evans) or 335-420 g (Wistar) at the start of Phase 2 (after limited-access training). Housing conditions and food were the same as described in Experiment 1; however during Phase 2, animals were maintained in a 12 hour reversed light/dark cycle (lights on at 2000h, lights off at 0800h).

During both phases of the experiment, animals of each strain were randomly divided into the following experimental groups according to the flavoured fluid received: quinine (n = 6), saccharin (n = 6), ethanol (n = 7), saccharin-quinine (n = 6), and saccharin-ethanol (n = 6).

Drugs

Naltrexone hydrochloride (SIGMA-Research Biochemicals Incorporated) was dissolved in saline and administered to all rats intraperitoneally in a volume of 1 ml/kg. The dose level chosen was 10 mg/kg, based on previous research showing this dose to be effective on either ethanol or saccharin intake (Davidson & Amit, 1997b). Naltrexone was administered 1 hour before lights out during Phase 1, and 10 minutes prior to the limited-access drinking period during Phase 2.

Procedure

Phase 1: Continuous fluid access. After a period of 1-week acclimatization to the animal colony facilities, all rats were presented with water and flavoured fluids as per Experiment 1, Phase 1. Each experimental group received one of the following flavoured solutions: 0.0006% quinine (w/v), 0.1% saccharin (w/v), 10% ethanol (v/v), 0.4% saccharin (w/v)+ 0.04% quinine (w/v), or 0.4% saccharin (w/v)+ 10% ethanol (v/v). The flavoured solutions were presented for 16 days before a 5-day baseline intake measure was recorded (baseline period). After baseline measurements were taken, rats received daily injections of naltrexone for a period of 5 treatment days (treatment period). After this period, fluid intake was recorded for a final 5 days (after-treatment period).

Phase 2: Limited fluid access. After a 2-week period of acclimatization to the reversed light cycle in the animal colony, rats were exposed to a limited-access drinking training schedule. Water bottles were removed from the cages, and flavoured fluids were presented to the rats in plastic tubes with steel ball-bearing spouts. The experimental groups were randomly assigned to receive one of the same five flavoured solutions as per Phase 1. Rats were provided with access to their respective flavoured solution during a daily 2-hour session for 1 week. Access to the solution was then further reduced to a daily 1-hour session for 1 week, and then access was reduced to 30 minutes daily. At this time, limited-access training was considered to be complete. The flavoured solutions were then presented for 30 minutes per day for 16 days to establish stable intake patterns. Baseline, treatment, and after-treatment measures were recorded as per Phase 1. All testing occurred halfway (6 hours) into the dark cycle, and food was available during testing.

Data analysis

Daily fluid consumption data (ml) for water and the flavoured solutions during Phases 1 and 2 were converted as per Experiment 1 and 4: intake (ml/kg/day), preference (%), and water intake (ml/kg/day).

For each flavoured solution in both phases of the experiment, the mean intake measures during baseline (5 days), treatment (5 days), and after-treatment (5 days) consumption were compared (treatment period). Separate two-way ANOVAs (strain x treatment period) with repeated measures were conducted by comparing the two strains of rats on the variables of flavoured fluid intake and preference for Phase 1, and flavoured fluid intake only for Phase 2. Results were reported separately for each flavoured fluid. See Appendix N for figures and statistical analysis of water intake data. See Appendix O for statistical analysis of body weights.

Results

Phase 1: Continuous fluid access

Ethanol. Analysis of ethanol intake during Phase 1 revealed that although Long-Evans and Wistar rats did not differ in mean ethanol intake levels, $F(1, 10) = 2.13$, both strains exhibited a significant change in intake across treatment periods, $F(2, 20) = 6.85$, $p < .01$ (see Figure 6, top panel). Ethanol intake increased by 45% from baseline to after-treatment periods in both strains, $p < .01$. Preference for ethanol was also significantly different across the treatment periods, $F(2, 20) = 9.77$, $p < .01$ (see Figure 6, bottom panel). Preference for ethanol in both strains rose from baseline to treatment

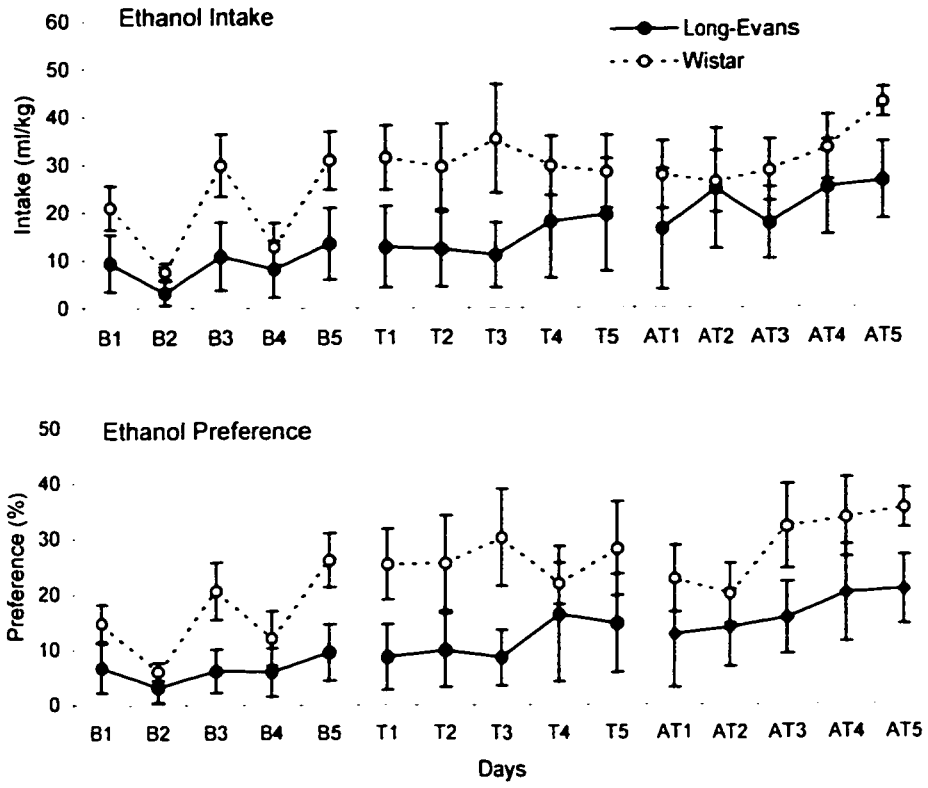

Figure 6. Mean (+/- S.E.M.) 10% ethanol intake (top) and preference (bottom) in Long-Evans and Wistar rats across the days of baseline (B), naltrexone treatment (T), and after naltrexone treatment (AT) during Phase 1: continuous fluid access.

periods by 70%, $p < .05$, and after-treatment levels were 104% higher than baseline levels, $p < .01$. There were no differences in overall mean ethanol preference between the strains, $F(1, 10) = 3.13$, which was 17% for Long-Evans rats and 29% for Wistar rats during the after-treatment period.

Saccharin. There were no differences in mean saccharin intake between the two rat strains during Phase 1, $F(1, 10) = 2.82$ (see Figure 7, top panel). There was also no effect of naltrexone treatment on saccharin intake, $F(2, 20) = .41$. Whereas mean preference for the saccharin solution across all treatment periods was 91% in Wistar rats and 71% in Long-Evans rats, there were no significant differences in their mean preference levels, $F(1, 10) = 1.77$ (see Figure 7, bottom panel). Saccharin preference was also unaffected by drug treatment, $F(2, 20) = 1.71$.

Saccharin-ethanol. Long-Evans rats consumed significantly more saccharin-ethanol than did Wistar rats, $F(1, 10) = 24.48$, $p < .001$ (see Figure 8, top panel). There was no effect of naltrexone treatment on saccharin-ethanol intake in either strain, $F(2, 20) = 2.24$. Long-Evans rats also displayed a higher preference for the saccharin-ethanol solutions overall, $F(1, 10) = 13.03$, $p < .01$. In both strains, preference increased from baseline to after-treatment by 36%, $F(2, 20) = 5.58$, $p < .05$ (see Figure 12, middle panel). During the after-treatment period, mean preference for saccharin-ethanol in Long-Evans rats was 47%, and in Wistar rats was 25%.

Saccharin-quinine. Saccharin-quinine intake did not differ between Long-Evans and Wistar rats during the continuous fluid access phase, $F(1, 10) = .05$, (see Figure 9, top panel). Both strains increased their intake of saccharin-quinine over the treatment periods,

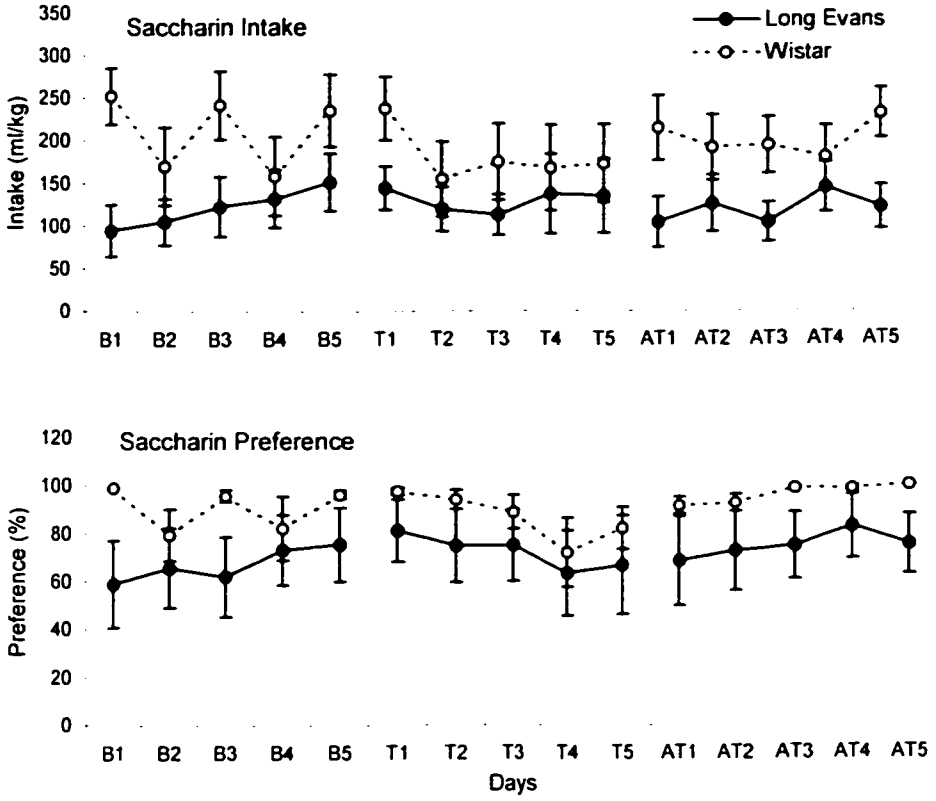

Figure 7. Mean (+/- S.E.M.) 0.1% saccharin intake (top) and preference (bottom) in Long-Evans and Wistar rats across the days of baseline (B), naltrexone treatment (T), and after naltrexone treatment (AT) during Phase 1: continuous fluid access.

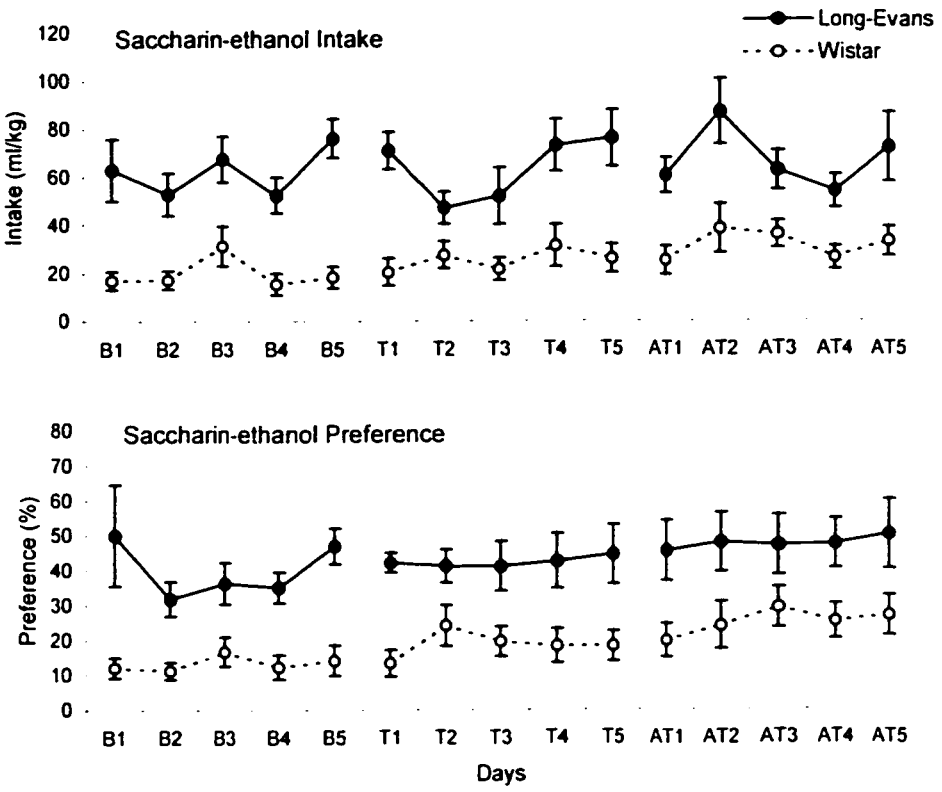

Figure 8. Mean (+/- S.E.M.) 0.4% saccharin-10% ethanol (top) and preference (bottom) in Long-Evans and Wistar rats across the days of baseline (B), naltrexone treatment (T), and after naltrexone treatment (AT) during Phase 1: continuous fluid access.

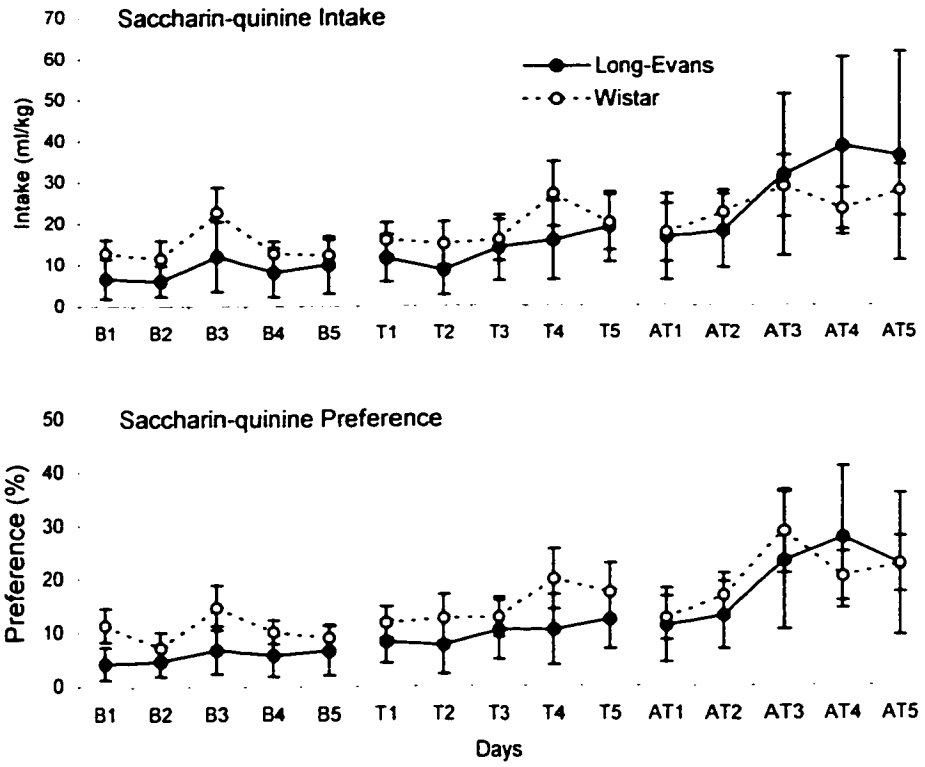

Figure 9. Mean (+/- S.E.M.) 0.4% saccharin-0.04% quinine intake (top) and preference (bottom) in Long-Evans and Wistar rats across the days of baseline (B), naltrexone treatment (T), and after naltrexone treatment (AT) during Phase 1: continuous fluid access.

$F(2, 10) = 5.01$, $p < .05$. Saccharin-quinine intake during the after-treatment period for both strains was 128% higher compared with baseline intake levels, $p < .05$. Measures of saccharin-quinine preference also did not differentiate the strains, $F(1, 10) = .25$, and increased from baseline to after-treatment by 147%, $F(2, 20) = 8.13$, $p < .01$ (see Figure 9, bottom panel). Mean preference during the after-treatment period for both Long-Evans and Wistar rats were 20%.

Quinine. When quinine was continuously available, there were no significant differences in mean quinine intake between Long-Evans and Wistar rats, $F(1, 10) = .05$, or across treatment periods, $F(2, 20) = 1.62$ (see Figure 10, top panel). There were also no differences in preference for the quinine solution among the strains, $F(1, 10) = .14$, and across the periods of Phase 1, $F(2, 20) = 2.11$ (see Figure 8, bottom panel). Mean preference for the quinine solution across all periods was 12.9% for Long-Evans rats and 10.1% for Wistar rats.

Phase 2: Limited fluid access

Ethanol. There were no differences in ethanol intake during Phase 2 between Long-Evans and Wistar rats, $F(1, 10) = 4.24$ (see Figure 11, top panel). There was a significant effect of treatment periods in both strains, $F(2, 20) = 12.35$, $p < .001$, as ethanol intake dropped 39% from baseline to treatment periods, $p < .01$. Intake during the after-treatment period increased by 67%, $p < .01$, and was not different from mean baseline values.

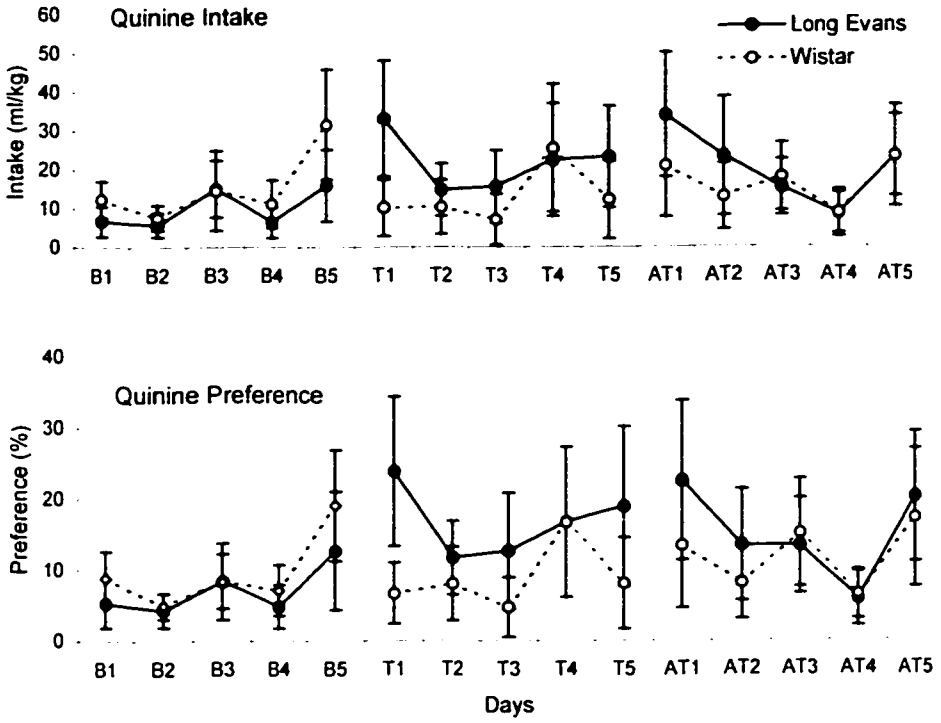

Figure 10. Mean (+/- S.E.M.) 0.0006% quinine intake (top) and preference (bottom) in Long-Evans and Wistar rats across the days of baseline (B), naltrexone treatment (T), and after naltrexone treatment (AT) during Phase 1: continuous fluid access.

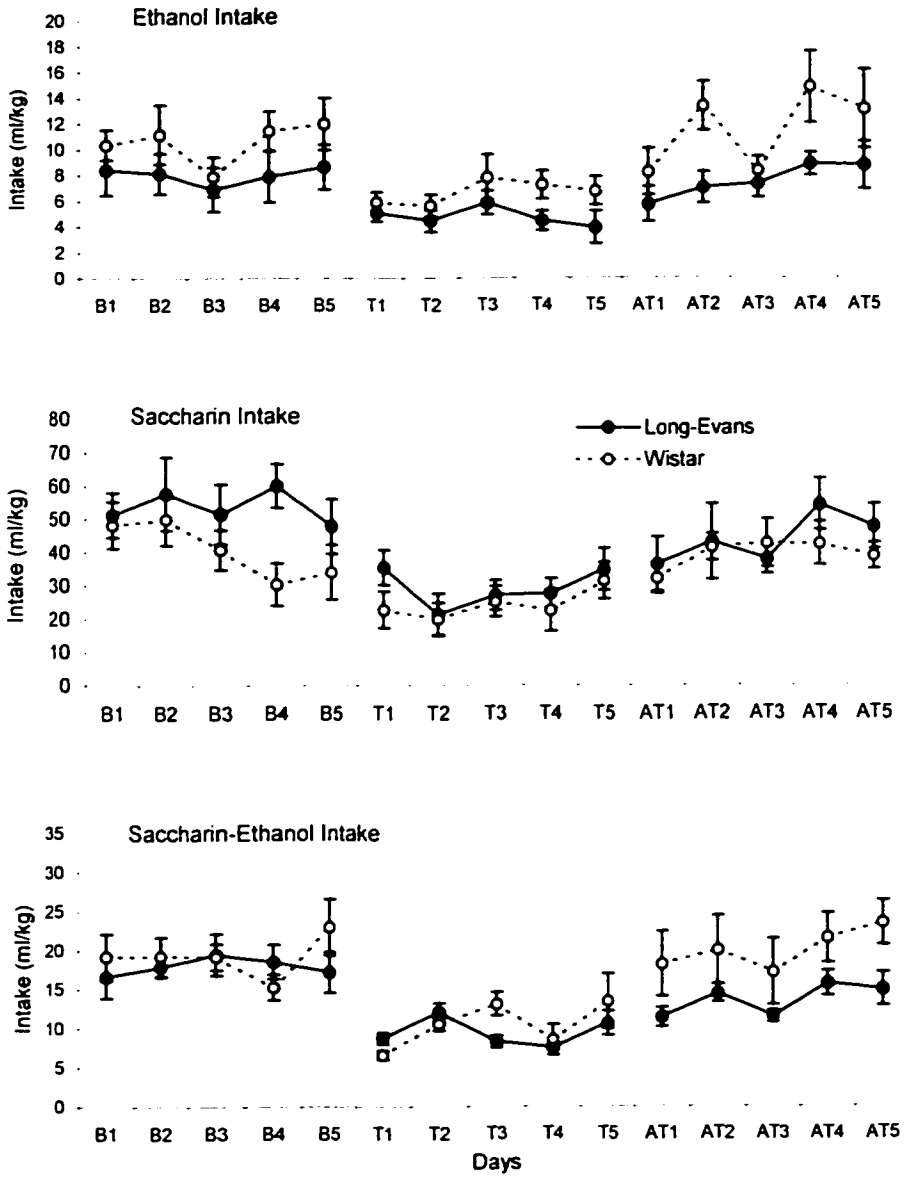

Figure 11. Mean (+/- S.E.M.) 10% ethanol (top), 0.1% saccharin (middle), and 0.4% saccharin-10% ethanol (bottom) intake in Long-Evans and Wistar rats across the days of baseline (B), naltrexone treatment (T) and after naltrexone treatment (AT) during Phase 2: limited fluid access.

Saccharin. When the saccharin solution was presented under limited-access conditions, there were no differences in intake between Long-Evans and Wistar rats, $F(1, 10) = 1.16$ (see Figure 11, middle panel). When the strains were combined, there was a significant change in intake across the treatment periods, $F(2, 20) = 22.28$, $p < .0001$. Saccharin intake dropped 43% from the baseline to the treatment periods, $p < .01$, and recovered during the after-treatment period, $p < .01$, an increase in intake of 55%.

Saccharin-ethanol. During limited-access drinking, there were no differences in intake between the strains, $F(1, 10) = 1.82$ (see Figure 11, bottom panel). However, both strains showed changes in intake across the periods of treatment, $F(2, 20) = 23.07$, $p < .0001$, as consumption fell 47% from baseline to treatment periods , $p < .01$, and rose again to baseline levels during the after-treatment period by 69%, $p < .01$.

Saccharin-quinine. Saccharin-quinine intake during limited-access presentations did not differ between Long-Evans and Wistar rats, $F(1, 10) = .18$ (see Figure 12, top panel). There was a change in intake across the periods of treatment, $F(2, 20) = 15.41$, $p < .001$. Intake in both strains decreased by 59% from baseline to treatment periods, $p < .01$, and rose again during the after-treatment period by 101%, $p < .01$. Intake during the after-treatment period did not differ from baseline intake.

Quinine. Quinine intake during limited-access presentations did not differ between Long-Evans and Wistar rats, $F(1,10) = 1.24$, or across periods of treatment, $F(2,20) = 2.84$, as a result of naltrexone treatment (see Figure 12, bottom panel).

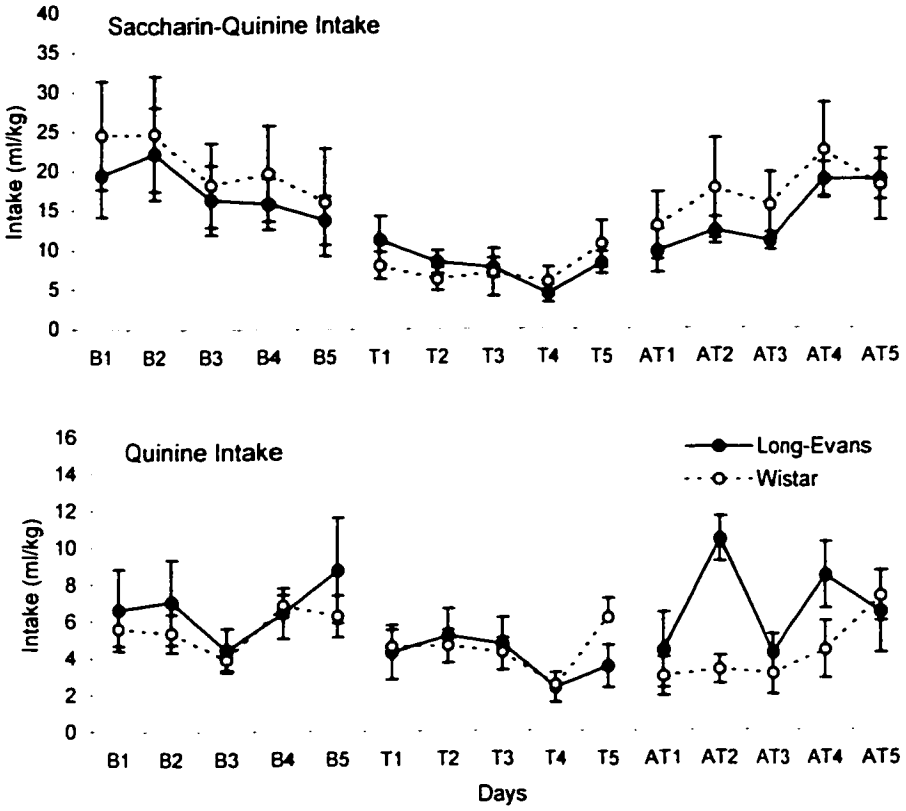

Figure 12. Mean (+/- S.E.M.) 0.4% saccharin-0.04% quinine (top) and 0.0006% quinine (bottom) intake in Long-Evans and Wistar rats across the days of baseline (B), naltrexone treatment (T), and after naltrexone treatment (AT) during Phase 2: limited fluid access.

Discussion

In the present study, administration of naltrexone did not affect the consumption of all flavoured fluids equally under all circumstances. For example, naltrexone did not attenuate intake of any flavoured fluid during the continuous fluid access phase. In fact, those animals drinking ethanol and saccharin-quinine actually increased their fluid intake over time, rather than showing decreases due to drug administration. These significant increases in intake may have been the result of a presumed familiarity with the gustatory and olfactory properties of the solutions, which can act to increase preference over time (e.g., Veale & Myers, 1969). It is also worth noting that in all groups except those drinking saccharin, water consumption decreased over the course of the experiment. Although previous research has shown that opiate antagonists can attenuate water intake when access to water is restricted (Akkok, Manha, Czirr & Reid, 1988; Frenk & Rosen, 1979; Levine et al., 1982) and when it is offered as the sole source of fluid (Froehlich et al., 1990), the reductions in water intake seen in the present experiment were more likely brought about by increases in flavoured fluid intake, as no recovery was evident when naltrexone treatment ended. Water intake during saccharin presentations may have remained unaffected because it was consumed in such low volume compared with the sweet solution.

When flavoured fluids were presented for 30-minute periods during Phase 2, all groups showed decrements in fluid intake following naltrexone treatment except those drinking quinine. The lack of drug effect on quinine intake may have been due to a floor

effect, as consumption of the quinine solution in both strains was very low before drug treatment.

The results of the present study were not consistent with the findings of Experiment 4 concerning a suppressant effect of naltrexone on quinine and saccharin intake during continuous-access drinking. All fluids in Experiment 5 were presented for 16 days before baseline measures were recorded, whereas in Experiment 4, there was no pre-exposure period to the flavoured fluids prior to testing. It was felt that a longer period of exposure to the flavoured fluids would result in more stable levels of drinking during Experiment 5. This, along with the more dilute quinine solution selected for Experiment 5, may have resulted in higher intake and preference levels in the Long-Evans and Wistar rats of Experiment 5 when compared with the Lewis and Wistar rats of Experiment 4. It is likely that drinking patterns were more solidly established in Experiment 5 following longer exposure to the flavoured fluids, thus making the intake of quinine and saccharin less susceptible to the transient effects of naltrexone in the 24-hour access paradigm.

The results obtained in the limited-access experiment may support conclusions from previous research which suggested that opiate antagonists such as naltrexone affected fluid intake by altering palatability. Results of an early study showed that flavouring enhanced the antidipsogenic properties of naloxone in rats (Levine et al., 1982). The addition of sweet, salty, and sour flavoring to water resulted in increased suppression of water intake after treatment with 1-20 mg/kg of naloxone (Levine et al., 1982). These investigators concluded that the opiate antagonist may have caused a decreased taste acuity for the sweet and salty flavors while enhancing the perception of the sour taste, thus resulting in lower fluid intake levels (Levine et al., 1982).

Interestingly, naloxone had no effect on quinine intake in this study (Levine et al., 1982), as was found in the present experiment. Results from a more recent report examining the effects of naltrexone on ethanol taste reactivity showed that a dose as low as 1 mg/kg resulted in increased levels of aversive responding for ethanol (Hill & Kiefer, 1997). In this same study, it was reported that 3 mg/kg naltrexone also served to attenuate ingestive responding and reduce oral intake of ethanol (Hill & Kiefer, 1997). The investigators concluded that because the alterations in taste reactivity responding occurred on the first day of testing, before the animals were able to associate the taste of ethanol with its postingestive effects, the opiate antagonist must have produced the change in responding via a reduction in palatability, or hedonic value, of the ethanol solution (Hill & Kiefer, 1997). If naltrexone had produced the palatability shift by blocking ethanol's rewarding postingestive effects, the changes in taste reactivity responding and intake would not have been evident on the first treatment (Hill & Kiefer, 1997). On the basis of these findings, it is possible that, in the present experiment, naltrexone may have acted on the taste of the flavoured solutions, or their palatability, to cause the attenuation in intake seen during the limited-access drinking phase.

However, the decreases in fluid consumption seen during the limited-access experiment may also have been the result of an attenuation of locomotion due to naltrexone treatment. Studies investigating the effects of opiate antagonists on motor activity in rodents have shown decrements in motor activity following administration of both naltrexone and naloxone (e.g., Amir, Galina, Blair, Brown & Amit, 1980; DeRossett & Holtzman, 1982; Jones & Holtzman, 1992; Leventhal, Cole & Bodnar, 1996), as well as attenuation of amphetamine-induced motor behaviors (e.g., Balcells-Olivero & Vezina,

1997; Ng Cheong Ton, Blair, Holmes & Amit, 1983). This would suggest that administration of opiate antagonists such as naltrexone, in the doses used in the present experiment, could have decreased motor activity in rats and thus attenuated their ability to approach the drinking tube and/or lick at the spout. The resultant outcome would be a non-discriminant decrease in flavoured fluid intake.

The lack of effect of naltrexone during continuous-access exposure may have been due to the animals' engaging in compensatory drinking to fulfill daily fluid requirements once the suppressant effects of naltrexone, whether on taste or locomotion, had worn off. In effect, naltrexone may have attenuated fluid consumption closer to the time of administration, but then the animals drank more fluid during the remainder of the 24 hour cycle as naltrexone was metabolized to make up for the earlier decrease in intake. For example, Davidson and Amit (1997b) found that although ethanol intake was suppressed at 4 hours after a single injection of 10 mg/kg of naltrexone prior to the onset of the dark cycle, there was no difference in ethanol intake at 24 hours compared with control animals. As well, Overstreet and colleagues (Overstreet et al., 1999) have shown that the reductive effects of naloxone on ethanol intake (in doses ranging from 2.5 to 20 mg/kg) were stronger during the first 2 hours after injection and less so when ethanol consumption was measured at 24 hours after injection. Furthermore, there were no differences in the amount of suppression of ethanol intake when naloxone was administered by means of surgically-implanted minipumps (releasing naloxone at a rate of 1.1 mg/kg/h) or by chronic injections (13 mg/kg naloxone twice daily), in both limited- and continuous-access drinking paradigms (Overstreet et al., 1999). These latter investigators concluded that evaluation of the suppressant effects of opiate antagonists on

ethanol consumption will vary according to when ethanol is available relative to the introduction of the antagonist into the animal's system. For this reason, the lack of effect of opiate antagonists administered only once in 24-hour drinking paradigms, particularly of short-acting antagonists such as naloxone, may be due to their lower potency as the drug is being eliminated from the system (Overstreet et al., 1999). However, research in humans has shown that the occupation of mu-opiate receptor sites after administration of naltrexone can last for as long as 72-108 hours (Lee et al., 1988) and that naltrexone can prevent opiate action for up to 12 hours after a single administration (Martin, Jasinski & Mansky, 1973; Resnick, Volavka, Freedman & Thomas, 1974). As well, micro-structural analysis of ethanol drinking patterns in rats over 24-hour periods has shown that they consume only negligible amounts of ethanol during the course of the 12 hour light cycle (Gill, 1989). Rats in the present investigation were administered naltrexone 1 hour before the onset of the 12 hour dark cycle so that naltrexone would be maximally effective while the animals were actively consuming fluids and food (naltrexone has a half-life of 8 to 10 hours in rats: Gilman, Goodman, Rall & Murad, 1985; Misra, Bloch, Vardy, Mule & Verebely, 1976). It is unlikely that the rats in this experiment would have compensated for any suppression in ethanol intake during the remaining light hours of the experiment when the drug had been eliminated from the system. These data, therefore, challenge the argument for compensation during the continuous-access experiment as an explanation for the lack of effect of naltrexone on the intake of flavoured fluids.

The results of the present experiment question the role of the opiate system in mediating both ethanol and sapid fluid intake in rats. Naltrexone had no effect on fluid intake in the 24-hour drinking paradigm, and yet may have had a non-specific attenuative

effect on the intake of all fluids except quinine during restricted access. As a result, the present findings do not provide reliable support for the notion that the ethanol/sweet relationship observed in other standard volume intake studies (e.g., Overstreet et al., 1993; Sinclair et al., 1992; Stewart et al., 1994) are mediated by the opioid system. On the contrary, these findings seem to indicate that naltrexone's effects on the intake of flavoured fluids may be volatile in nature, conditional upon factors such as experimental paradigm and strain of rat. These conclusions are also in line with a recent study carried out in chronic alcoholic men, showing no differences following treatment with naltrexone versus a placebo in such measures of alcohol intake as number of days to relapse, percentage of days on which drinking occurred and number of drinks per drinking day (Krystal et al., 2001).

General Discussion

The present series of experiments were conducted to determine whether the intake of sweet, bitter or bittersweet solutions reliably predicted ethanol consumption in rats. This was done by comparing the intake of ethanol with that of saccharin, quinine and saccharin-quinine solutions in several rat strains and under a series of drinking conditions. Furthermore, it was of interest to determine whether taste preferences which putatively linked ethanol intake with sapid fluid intake were mediated by a common underlying neurochemical substrate. This was accomplished by examining the effects of three centrally-acting agonists and antagonists on the intake of ethanol, sweet and bitter solutions in three rat strains.

Ethanol and saccharin intake are not related in all rat strains

The results of Experiments 1 and 2 revealed no evidence for a relationship between ethanol and saccharin intake in all rat strains. In particular, the results of Experiment 1, which sought to replicate a previous study reporting a positive association between ethanol and saccharin intake across seven rat strains (Overstreet et al., 1993), showed that ethanol and saccharin intakes were not correlated in Lewis, Wistar Kyoto and Wistar rats. Examination of the intake data from Experiment 1 revealed that there were differences in the levels of mean ethanol intake among the three strains but there were no comparable differences in their saccharin intake. This comparison demonstrated that saccharin intake did not characterize these three rat strains in the same way as their ethanol intake. In Experiment 2, ethanol intake in the non-selected, high ethanol-drinking

Fawn-Hooded rats was also unrelated to saccharin intake. In addition, mean saccharin intake in Fawn-Hooded rats was not different from that of three ethanol-nonpreferring rat strains previously tested in the same drinking paradigm (Lewis, Wistar Kyoto and Wistar; Goodwin & Amit, 1998), again showing that saccharin intake is not a suitable comparison for ethanol intake.

Moreover, there was evidence from Experiment 1 that the relationship between various fluid intakes in general, such as a correlation between ethanol and saccharin intake, may be vulnerable to the influence of extraneous factors such as strain and fluid exposure paradigms. In this experiment, saccharin and ethanol intake were significantly correlated during limited access drinking in Lewis rats and when the data for Lewis and Wistar rats were grouped for the purpose of comparison. There were no significant saccharin-ethanol intake correlations reported for the other strains or strain groupings during limited access fluid exposure, or during the continuous fluid access phase. These findings clearly demonstrated that external variables such as fluid presentation schedule affect fluid intake relationships within strains.

When ethanol and saccharin solutions were presented over a long period of time (50-53 days) and in gradually increasing concentrations, the measures of volume intake (ml) appeared to differentiate the three rat strains of Experiment 3 in a like manner. Specifically, Fawn-Hooded rats consumed the greatest amounts of both solutions, followed by Wistar rats, and Lewis rats consumed the least amounts of both ethanol and saccharin solutions. Animals find the taste of low concentrations of ethanol solutions to be sweet-tasting (Di Lorenzo et al., 1986; Kiefer et al., 1990), and therefore it has been proposed that intake of ethanol solutions below a 6% concentration may be primarily

motivated by its taste rather than pharmacological factors (Boyle et al., 1997). The majority of the ethanol concentrations presented in Experiment 3 were lower than 6%. Comparatively, it has also been demonstrated that rats find saccharin solutions of concentrations less than 0.1% to be sweeter and more palatable compared with stronger saccharin concentrations (i.e., greater than 0.3%), which have an aversive bitter taste (Dess, 1993). Therefore, it is possible that the rat strains used in Experiment 3 displayed the same rank ordering in ethanol and saccharin intake across increasing concentrations because they nondiscriminantly evaluated both solutions as 'sweet' tasting and drank each fluid as if it were the same. This was reflected in their levels of ethanol intake for concentrations ranging from 2.6-6.5% and of saccharin intake for concentrations ranging from 0.01-1.5%. When the concentration of ethanol presented was greater than 7% and when saccharin concentrations were greater than 2%, the aforementioned ranking of strain differences in fluid intake disappeared, perhaps because the tastes of both solutions changed and were no longer exclusively sweet but included a bitter component as well. As a comparison, the reports of a positive correlation between ethanol and saccharin intake in the literature have generally been for concentrations of ethanol ranging from 8-15% with a 0.1% saccharin solution (e.g., Bell et al., 1994; Kampov-Polevoy et al., 1990; Overstreet et al., 1993). Therefore, the similarities in the intakes of ethanol and saccharin solutions observed among Fawn-Hooded, Lewis, and Wistar rats in Experiment 3 did not support those studies showing a relationship between ethanol and sweet preference because the range of concentrations of both solutions where these similarities were apparent fell below those most commonly used in experimental studies.

Altogether, the results from the present experiments, which examined ethanol and saccharin intake in several rat strains, demonstrated that saccharin intake does not reliably predict ethanol intake. The predictive relationship between ethanol and saccharin intake was not found in all rat strains and moreover, was influenced by fluid exposure paradigm. These findings, along with reports from other laboratories (Agabio et al., 2000; Bell et al., 1994; Kampov-Polevoy et al., 1995; Overstreet et al., 1999), strongly question the association between saccharin and voluntary ethanol intake.

Bitter taste sensitivity is a better predictor of ethanol intake

Several of the experiments in the present thesis examined whether intake of bitter solutions, namely quinine solutions, can be predictive of ethanol intake. This was only found in one rat strain but in two different experiments. Lewis rats showed a positive correlation between quinine intake (both at 0.25% quinine, Experiment 1, and 0.04% quinine, Experiment 4) and ethanol intake, a reflection of their low intake levels of both solutions. The results of Experiment 3, where quinine consumption was measured over a longer schedule of increasing concentrations, demonstrated more conclusively that rat strains could be differentiated according to their levels of quinine intake. In this experiment, Lewis rats also consumed the least amounts of both ethanol and quinine solutions compared with Wistar and Fawn-Hooded rats, however the high ethanol-drinking Fawn-Hooded rats consumed more of the quinine solutions. Wistar rats displayed higher intake of the first 7 quinine concentrations presented (of a total 31 concentrations) compared with Lewis rats. Therefore, the pattern of quinine intake among these three particular strains (Fawn-Hooded > Wistar > Lewis) was the same as that

observed for ethanol intake and, while not assessed by means of correlational analysis, was a clear demonstration of a similarity in taste preferences for both solutions.

The notion that bitter preference may reflect ethanol intake in rats has been proposed previously in the literature. One early experiment showed that, in a similar manner to Experiment 3 of the present thesis, unselected low ethanol-drinking rats rejected a quinine solution at a weaker concentration when compared with ethanol-drinkers (0.00028% vs. 0.000687% quinine, respectively) (Le Magnen & Marfaing-Jallat, 1961). The non-drinking rats also fell below a 40% preference cut-off criteria for ethanol at 3.6%, whereas the high ethanol-drinking rats did so at 6.5% ethanol (Le Magnen & Marfaing-Jallat, 1961). These investigators concluded that low ethanol-drinking may have been mediated by an innate, generalized "tendency to avoid" on the basis of gustatory sensitivity, which in turn was reflected in mean levels of quinine intake (Le Magnen & Marfaing-Jallat, 1961). In addition, another study reported that the intake of 0.0025% quinine was positively correlated with intake of 15% ethanol in unselected rats (Kampov-Polevoy et al., 1990). However, this relationship was only significant during the first of three weeks of access to the ethanol solution, and not to mean ethanol intake in the final two weeks when stability of drinking patterns were established (Kampov-Polevoy et al., 1990). On the basis of these findings, it is suggested that gustatory factors such as sensitivity to bitterness play a role in initial ethanol acceptance, before the taste of the ethanol solution has acquired a value as a conditioned reinforcer by association with ethanol's postingestive effects.

The difficulty in determining the nature of a quinine-ethanol intake relationship in rats is that quinine itself is excessively aversive and therefore not generally consumed

willingly or in large quantities by animals. This was demonstrated clearly in Experiment 3, where some rats in the Lewis and Wistar strains appeared to detect the quinine in water from the first concentration presented (0.0001%) and, moreover, never developed a preference for the quinine solutions. It has been speculated that a sensitivity to and dislike of bitterness has provided an important adaptive safeguard throughout both animal (Garcia, Hankins & Rusiniak, 1974) and human evolution by reducing the intake of potentially dangerous foods (Fischer & Griffin, 1964; Garcia & Hankins, 1975; Scott & Mark, 1987), because virtually all naturally occurring poisons taste bitter (Brieskorn, 1990; Brower, 1984; Garcia & Hankins, 1975). Consequently, it is not surprising that preference for a bitter solution, such as quinine or ethanol, is difficult to induce in most rats, with the exception of the selectively-bred, ethanol-preferring rat strains who display a preference for ethanol solutions from the first taste. Therefore, for most rat strains, it would be necessary to begin quinine presentations at a much more dilute concentration in order to reliably determine taste thresholds and be able to examine intake patterns over a longer presentation schedule, such as those used for ethanol and saccharin in Experiment 3.

Nonetheless, the differences in quinine intake displayed in Experiment 3 among three rat strains of varying ethanol preference provided evidence that intake of a bitter solution, such as quinine, was a better predictor of ethanol intake than saccharin intake. In fact, it was surprising to find differences between the strains in the consumption of the bitter solutions at all, because bitter substances are generally avoided by all rats in the wild (Garcia et al., 1974). That the high ethanol-drinking Fawn-Hooded strain was able to tolerate the more concentrated quinine solutions suggested that this strain was also less

sensitive to the aversive, bitter taste of unflavoured ethanol, particularly at higher concentrations. And, conversely, that some rats in both the Lewis and Wistar strains never developed a preference for the quinine solutions demonstrated that for some animals, the bitter flavour may have been so overwhelming as to inhibit the intake of quinine as well as of any other bitter-tasting solution, including ethanol. These latter observations lend support to the notion that low ethanol-drinking in rodents may be mediated by a 'tendency to avoid' on the basis of taste sensitivity (Le Magnen & Marfaing-Jallat, 1961). For these animals, the aversive bitter taste of the ethanol solution would forewarn the animals to reject that fluid before ingestion in order to avoid distress in the internal milieu (Garcia et al., 1974). On the other hand, high ethanol-drinking rodents might use the bitter taste of ethanol as a cue to drink more in order to experience its pharmacological effects. In either case, whether for high or low ethanol-drinking strains, sensitivity to bitterness appears to be a more appropriate approximation of the manner in which an animal will respond to unflavoured ethanol.

The relationship between ethanol and saccharin-quinine intake

In line with the notion that sensitivity to bitterness is a valid predictor for ethanol intake, the consumption of saccharin-quinine, a solution that has both a bitter and sweet taste, was found to be related to ethanol intake in the experiments of the present thesis. In both Experiments 1 and 2, ethanol and saccharin-quinine intake were positively correlated, whether presented as a single concentration to three rat strains (Experiment 1) or following exposure to a series of gradually increasing saccharin-quinine concentrations in one strain (Experiment 2). However, a positive correlation between ethanol and

saccharin-quinine intake was not found in all individual rat strains tested: only in Lewis rats in Experiment 1 and in Fawn-Hooded rats in Experiment 2. The lack of significant ethanol/saccharin-quinine associations within all individual strains in Experiment 1 indicated that the relationship between the intake of ethanol and a sweet-bitter solution, as with the relationship between ethanol and sweet intake, was not universal among all rat strains.

The results from Experiments 1 and 2 provided new evidence to support the data reported in both aversion generalization (Di Lorenzo et al., 1986; Lawrence & Kiefer, 1987) and taste reactivity studies (Kiefer et al., 1990) which have consistently demonstrated that unflavoured laboratory ethanol, although at weaker concentrations (5-6% ethanol), has a complex sweet and bitter taste for rats. It is possible that the inconsistent findings from Experiments 1 and 2 concerning the significance of the ethanol and saccharin-quinine correlations among individual strains was due to differential sensitivities to the bitter, quinine component in the saccharin-quinine mixture. It was demonstrated in Experiment 3 that rat strains will vary in their responses to increasing quinine solutions. While the bitter taste of quinine in the saccharin-quinine solutions may have been somewhat masked by the sweetness of the saccharin component, there is nonetheless a bitter taste component to saccharin-quinine. Its corresponding intake may therefore be considered a measure of bitter taste sensitivity, and hence a better approximation of ethanol intake than the intake of a sweet solution alone.

Sweet preference and bitter sensitivity in rodents and humans

The results from several experiments in the present thesis demonstrated that rat strains vary according to their sensitivity to bitterness, which in turn may mediate their respective preferences for unflavoured ethanol. And while intake of a sweet-bitter solution was also significanlty correlated with ethanol intake, preference for a simple sweet solution provided little information relative to patterns of ethanol consumption. Sensitivity to bitterness has also been reported to vary within the human population, and also to correlate with alcohol drinking. Research has shown that humans differ quite reliably in our taste sensitivity for solutions of phenothioureas (Fischer, 1971; Fox, 1931, 1932; Lawless, 1980), a class of 40 compounds which all contain a characteristic molecular grouping (H-N-C=S) (Fischer, 1967). Two examples are phenylthiocarbamide (PTC) and 6-*n*-propyl-2-thiouracil (PROP). It is estimated that in North America, 25% of the population are non-tasters of PTC/PROP, 50% are medium tasters and perceive these compounds as only moderately bitter, and 25% are supertasters who perceive PTC/PROP as extremely bitter (Bartoshuk, Duffy & Miller, 1994). Family studies have led to the conclusion that PTC non-tasting is a mendelian recessive characteristic, i.e., individuals with two recessive alleles were non-tasters and individuals with two dominant alleles were supertasters (Blakeslee & Fox, 1932). It is suspected that medium tasters have one dominant allele and one recessive allele (Bartoshuk, 2000). A connection between PTC/PROP-tasting status and alcoholism has been found, in that there are generally more nontasters in the alcoholic population (e.g., Bartoshuk et al., 1993; Intranuovo & Powers, 1998; Peeples, 1962) and there is a higher proportion of nontasters of PROP among children of alcoholics compared with children of non-alcoholics (DiCarlo & Powers, 1998; Pelchat and Danowski, 1992). On the basis of these findings it is suggested that,

perhaps related to genetic differences in the ability to taste PTC/PROP, alcoholic individuals may have inherently duller taste sensitivities thus allowing them to consume more alcohol than non-alcoholic individuals, and conversely that non-alcoholics do not consume alcohol excessively because they are limited by its bitter taste.

Along the same lines, Dess (2000) proposed that the variability observed in ethanol intake among rodent strains, as well as in the intake of sweet-bitter solutions such as saccharin-quinine, could be explained as differential sensitivities to bitterness. She postulated that fluid intake levels were a reflection of strain-specific responses to the sweet or bitter taste components of solutions. Some rodent strains may be less responsive to sweetness and more sensitive to the bitterness in sweet-bitter solutions, while others are able to tolerate aversive bitterness because they are responding to the more pleasurable sweet taste. In the experiments of the present thesis, Lewis rats consistently displayed sensitivity to bitterness by consuming small amounts of quinine, ethanol, and saccharin-quinine solutions. Wistar rats may have been more responsive to the sweet component in the flavoured solutions, as evidenced by positive correlations between the intake of saccharin, ethanol, and saccharin-quinine solutions. And Fawn-Hooded rats, who tolerated the greatest amounts of bitterness in quinine solutions compared with the other strains of Experiment 3, drank large amounts of ethanol, quinine, and saccharin-quinine solutions.

Therefore, it seems very plausible that, based on the data gathered in the present thesis in several rat strains, rodent strains possess unique sensitivities to bitterness which in turn mediate their consumption of fluids such as ethanol, quinine and

saccharin-quinine. And consequently, that quinine and saccharin-quinine intake are good predictors of ethanol intake.

Lack of evidence for a common neural system mediating fluid intake relationships

Experiments 4 and 5 sought to determine whether there was a common neural system mediating the fluid intake associations observed in rats drinking ethanol, sweet, and bitter solutions. The hypothesis being tested in these studies was that any changes in ethanol intake following neurotransmitter manipulation which were accompanied by similar changes in the consumption of the saccharin or quinine solutions would support the notion of a possible common underlying neural system. The results of Experiment 4 showed that neither the opioidergic, dopaminergic nor gabaergic neurotransmitter systems were responsible for contributing to any relationship in the intake of ethanol, bitter, or sweet solutions in Lewis or Wistar rats.

The $GABA_A$ receptor agonist THIP was related to increases in ethanol intake in one rat strain (Wistar) but not the other (Lewis) in Experiment 4. In addition, THIP had no effect on saccharin intake in either strain but caused a decrease in quinine intake and preference in both strains. However, the effects of THIP on both ethanol and quinine intake measures may have been the result of a nonspecific fluid effect, because saline-treated controls also showed comparable changes in fluid intake. Therefore, THIP treatment at the dose tested did not appear to have a unidirectional effect on the intakes of ethanol, saccharin, and quinine intake.

Naltrexone treatment served to attenuate the intake of both quinine and saccharin solutions in Lewis and Wistar rats. There was no comparable decrease in ethanol drinking

following naltrexone treatment in either strain. This latter finding was at odds with results from previous studies reporting an attenuative effect of naltrexone on ethanol intake in rats (Davidson & Amit, 1997a,b; Hill & Kiefer, 1997; Myers & Crichter, 1982; Sinclair, 1990). However, the majority of these studies showed an effect of naltrexone in limited-access drinking paradigms, whereas Experiment 4 examined the effects of naltrexone on fluid intake over 24-hour periods (Davidson & Amit, 1997a; Hill & Kiefer, 1997; Myers & Crichter, 1982; Sinclair, 1990). In directly comparing the effects of naltrexone in a limited versus a continuous-access (i.e., over 24 hours) drinking paradigm, one study reported a decrease in ethanol consumption 4 hours after naltrexone administration (i.e., after limited exposure to ethanol) but not at 24 hours following treatment (Davidson & Amit, 1997b). On the basis of these findings, it was suggested that animals compensated for an earlier attenuation of ethanol intake, observed at 4 hours after treatment, by drinking more ethanol when naltrexone was fully eliminated. The net effect was no observable decrease in ethanol consumption when measured at 24 hours after drug administration. However, this explanation for the lack of effect of naltrexone on ethanol intake in Experiment 4 is challenged because naltrexone did have an attenuative effect on both quinine and saccharin intake during the same time period. Furthemore, these findings demonstrated that naltrexone did not have a generalized attenuative effect on the consumption of all three solutions.

Raclopride, a dopamine D2/D3 antagonist, appeared to suppress quinine intake in both rat strains while having no effect on quinine preference, a reflection of the concurrent decrease in water intake. Raclopride had no effect on saccharin intake or preference in either strain. Raclopride treatment was also associated with an increase in

ethanol intake but only in Wistar rats. As with THIP-treated animals, ethanol intake and preference in saline-treated Wistar rats also increased over the course of the experiment, suggesting that the rise in intake observed in raclopride-treated animals was not a specific drug effect. Therefore, these results also seemed to indicated that, like the effects of THIP and naltrexone, raclopride did not have a common influence on the intake of all three flavoured solutions.

The two rat strains in Experiment 4 showed differences in their fluid intake correlations, such that the intake of ethanol and quinine solutions were correlated in Lewis rats while the intake of ethanol and saccharin solutions were correlated in Wistar rats. Therefore, in light of the results showing that none of the drugs tested had a unidirectional effect on the intake of any of the flavoured solutions, it was evident that the strain-specific correlations of fluid intake observed in Lewis and Wistar rats were not mediated through a common neural system.

The results of Experiment 5, a more in-depth examination of the effects of opioid antagonism on ethanol and flavoured fluid consumption, also did not provide any additional evidence for the involvement of the opioid neurotransmitter system in the mediation of the intake of different solutions. There were no attenuative effects of naltrexone on the intake of any of the solutions presented (ethanol, saccharin, quinine, saccharin-quinine, saccharin-ethanol) during continuous access drinking in Long-Evans or Wistar rats. However, when these flavoured fluids were presented for a limited-access period, naltrexone produced a decrease in the intake of all solutions except quinine in both strains.

More detailed examination of the fluid consumption patterns in rats following naltrexone treatment in a micro-structural drinkometer-type apparatus (e.g., Gill, Mundl, Cabilio & Amit, 1989) would be more effective in providing answers regarding the discrepant results in the two fluid exposure paradigms. Data gathered from a drinkometer would provide detailed information about changes in the size, duration and frequency of food and fluid bouts as well as eating and drinking rates (Gill et al., 1989). In the present instance, one might be able to ascertain whether the lack of drug effect on fluid intake observed during continuous access drinking was, in essence, the consequence of mathematical averaging, which was insensitive to changes in intake over the course of the 24-hour measurement period as naltrexone was metabolized. Also, it would be important to determine whether the attenuative effects on fluid intake seen during the limited fluid access phase were a true effect of naltrexone treatment, whether on reinforcement or taste sensitivity, or a nonspecific effect such as diminished motor ability. For example, it has previously been proposed that naltrexone acts to attenuate ethanol intake by inducing a conditioned taste aversion, and several studies have reported conditioned taste aversions in animals administered high doses of opiate antagonists (e.g., Davidson & Amit, 1996; Van Der Kooy & Phillips, 1977).

Altogether, the findings of Experiments 4 and 5 demonstrated that THIP, raclopride and naltrexone, at the doses tested, did not mediate fluid intake relationships for ethanol, bitter, and sweet solutions. On the basis of these results, it is suggested that the mechanism underlying fluid intake interrelationships in rodents is not controlled at a neural level, as was previously proposed (e.g., Gosnell & Krahn, 1992; Overstreet et al.,

1993; Sinclair et al., 1992), but may lie within the pre-ingestional components of oral ethanol self-administration at the level of taste sensitivity.

Conclusion

The data presented in this thesis challenge two popularly-held notions in the field of ethanol intake and taste preferences in rats: 1) that preference for sweet solutions is predictive of ethanol preference in all rats (e.g., Gosnell & Krahn, 1992; Overstreet et al. 1993), and 2) that this relationship is mediated by a common underlying neurotransmitter system (e.g., Overstreet et al. 1993; Sinclair et al., 1992). While the intake of ethanol and saccharin were not correlated in all rat strains examined in the present thesis, there was evidence to suggest that saccharin-quinine intake, and even quinine intake alone, may be more predictive of ethanol intake. Furthermore, these fluid intake relationships were not mediated by the dopaminergic, opioidergic, or gabaergic neurotransmitter systems, as demonstrated in Experiments 4 and 5.

The field of taste preferences and ethanol intake is a developing one, and the techniques used to examine behaviors related to taste (i.e., intake, licking) in rodents are consequently primitive. For example, the use of fluid intake tests as a measure of taste, such as the type used in the experiments of this thesis, present some interpretive difficulties. Fluid intake in animals is affected by many factors other than taste, such as the nutritional and gastrointestinal status of the animal as well as their prior experience with the fluid (Grill, Spector, Schwartz, Kaplan & Flynn, 1987). As a result, volume intake may not accurately approximate taste preferences. Furthermore, the orofacial responses elicited by rodents in the taste reactivity test are unrelated to fluid consumption (Kiefer, 1995). This disparity is unfortunate and needs to be clarified before further interpretation of ingestive and aversive responses are proposed.

Nonetheless, the studies to date, including intake (e.g., Bachmanov et al., 1996; Overstreet et al., 1993; Sinclair et al., 1992; Stewart et al., 1994) and taste reactivity studies (e.g., Bice & Kiefer, 1990; Kiefer & Dopp, 1989), have provided the field of ethanol research with essential information about taste and ethanol drinking in rodents. It is well known that ethanol's aversive taste limits its intake (e.g., Amit & Stern, 1969, York, 1981), that rats ascribe the taste of unadulterated laboratory ethanol with both sweet and bitter taste qualities (e.g., Di Lorenzo et al., 1986; Lawrence & Kiefer, 1987), and that there is variability across strains in the consumption of, and subsequent relationships between, ethanol and flavoured solutions (e.g., Bachmanov et al., 1996; Stewart et al., 1994). What remains to be determined more conclusively is whether these strain-specific taste sensitivities are innate, unchangeable or elastic, and whether they are vulnerable to environmental pressures. For example, animal and human research suggests that we are hardwired to reject bitter foods because virtually all naturally occurring poisons taste bitter (Brieskorn, 1990; Brower, 1984; Garcia & Hankins, 1975). Notwithstanding this innate tendency, some foods and beverages with pronounced bitter tastes, such as coffee and alcohol, are widely consumed by certain segments of the human population (Diamant, Funakoshi, Strom & Zotterman, 1963; Mela, Mattes, Tanimura, & Garcia-Medina, 1992). It is possible that the positive post-ingestive effects of the caffeine and alcohol may override the aversiveness of the bitter flavouring, thus allowing for consumption despite the displeasing taste. In support of this notion, high ethanol-drinking rodents were found to consume corresponding amounts of bitter quinine solutions in the present thesis. Additionally, it would be important to establish whether taste preferences were reliably 'strain-specific', and not just an artifact of experimental paradigms or

specific laboratory techniques (i.e., limited versus continuous fluid access exposure schedules, operant chamber versus homecage drinking). Finally, it is necessary to determine the manner in which taste preferences are linked to post-ingestive effects, particularly in the case of ethanol, and whether this association in turn varies across rodent strains. For example, while Fawn-Hooded and Lewis rats were equally sensitive to the addition of very dilute concentrations of ethanol in water (Experiment 3 of the present thesis), Fawn-Hooded rats appeared to use this cue to drink more ethanol while Lewis rats used it to avoid ethanol.

It is becoming apparent from the accumulated research (e.g., Agabio et al., 2000; Bell et al., 1994; Kampov-Polevoy et al., 1995; Overstreet et al., 1999) that while ethanol-preferring strains tend to consume greater amounts of sweet solutions than their ethanol-nonpreferring counterparts, using sweet preference as a marker for ethanol intake is not reliable. This is because there is generally very little variability in sweet solution preference, unlike in the intake of ethanol, which is characterized by large variability (e.g., Amit & Smith, 1992; Li & Lumeng, 1984). Further investigation of the patterns of intake of bittersweet solutions as well as of bitter solutions may provide clear information about the taste preferences that are most reliably predictive of ethanol consumption. However, as was demonstrated in the present thesis, and elsewhere (e.g., Agabio et al., 2000; Goodwin & Amit, 1998; Overstreet et al., 1999), the sweet-ethanol relationship is not a universal phenomenon among rodent strains, and it is also possible that the same will be true for the relationship between the intake of ethanol and bittersweet solutions. This would be a reflection of specific strain differences. As was proposed by Dess (2000),

rodent strains may differ in their relative sensitivities to sweet and bitter tastes, resulting in dissimilar levels of intake.

There has been no direct evidence reported in the literature to show that the interrelationships between ethanol and flavoured fluid intake are mediated by a common neurotransmitter. And the experiments of the present thesis, which examined the effects of gabaergic, dopaminergic, or opioidergic neurotransmitter manipulation on ethanol, sweet, and bitter fluid intake, found no new evidence of specific neurotransmitter involvement. The involvement of a common neural system cannot be ruled out entirely based on the experiments of the present thesis, and the notion warrants further investigation into other systems known to affect both ethanol and sweetened solution intake, such as the serotonergic (e.g., Amit, Smith & Gill, 1991) and noradrenergic neurotransmitter systems (Amit, Brown, Levitan & Ogren, 1977; Matthews, Gibson & Booth, 1985). However, it is possible that research may show that fluid intake interrelationships are not centrally-mediated, but directed by pre-absorptive mechanisms such as taste sensitivity (see Davidson, 1994).

In conclusion, the experiments of the present thesis demonstrated that sweet preference and ethanol preference are not reliably related in all rat strains. The data indicated that patterns of intake of bitter-tasting solutions, whether alone or in combination with a sweetener, were more relevant as predictors of ethanol intake. However, these relationships did not appear to be mediated by the gabaergic, dopaminergic, or opioidergic systems. Future research into the nature of the association between ethanol and bitter preference may provide information about the variability of

ethanol drinking in rodent strains, and, in a like manner, about the development of harmful and excessive patterns of alcohol consumption in humans.

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Appendix A

Mean Total Fluid Consumption (ml/kg) for All Strains during Phase 1 of Experiment 1: Continuous Fluid Access

	Fluids			
Strain	Quinine[a]	Saccharin[b]	Ethanol[c]	Saccharin-quinine[d]
Lewis	131.1 (1.4)	204.3 (15.9)	108.9 (0.5)	103.0 (2.2)
Wistar	134.5 (3.4)	237.6 (15.2)	106.3 (2.7)	91.4 (0.9)
Wistar Kyoto	109.1 (3.5)	186.2 (10.6)	84.7 (4.3)	72.6 (1.9)

Note. The values not enclosed in parentheses represent mean total fluid consumption for the final 4 days of fluid presentation. Values enclosed in parentheses represent standard error of the mean (SEM).

[a] No strain differences in total fluid intake, $F(2, 27) = 1.06$, $p > .05$.

[b] No strain differences in total fluid intake, $F(2, 27) = 1.13$, $p > .05$.

[c] No strain differences in total fluid intake, $F(2, 27) = 1.78$, $p > .05$.

[d] Strain differences in total fluid intake, $F(2, 27) = 3.47$; Lewis > Wistar Kyoto ($p < .05$).

Appendix B

Spearman Correlation Coefficient Tables for Experiment 1

Table B1

Spearman Correlation Coefficients for Mean Fluid Intake in Different Strains/Groupings during Phase 1: Continuous Fluid Access

	Strain/Grouping					
Fluid Comparison	Lewis	Wistar	Wistar Kyoto	All rats	Lewis vs. Wistar	Wistar Kyoto vs Wistar
ETOH vs. Quin	0.830**	0.227	0.103	0.117	0.099	-0.011
ETOH vs. Sacc	-0.212	-0.079	-0.067	0.092	0.084	0.247
ETOH vs. SQ	0.964**	0.285	0.091	0.660**	0.762**	0.371
Sacc vs. SQ	-0.152	0.806**	0.321	0.322	0.323	0.633**
Sacc vs. Quin	0.127	0.018	0.139	0.072	0.05	0.043
Quin vs. SQ	0.915**	0.092	0.285	0.305	0.343	0.116

Note. ETOH= ethanol; Sacc= saccharin; Quin= quinine; SQ= saccharin-quinine.

* $p < .05$, ** $p < .01$.

Table B2

Spearman Correlation Coefficients for Mean Fluid Intake in Different Strains/Groupings during Phase 2: Limited Fluid Access

	Strain/Grouping					
Fluid Comparison	Lewis	Wistar	Wistar Kyoto	All rats	Lewis vs. Wistar	Wistar Kyoto vs. Wistar
ETOH vs. Quin	-0.412	0.234	0.412	0.237	0.186	0.38
ETOH vs. Sacc	0.905**	0.357	-0.429	0.283	0.544*	-0.029
ETOH vs. SQ	-0.262	0.238	0.643	0.237	0.124	0.171
Sacc vs. SQ	-0.381	-0.095	0.167	0.169	-0.268	0.368
Sacc vs. Quin	-0.412	-0.514	-0.247	-0.311	-0.41	-0.345
Quin vs. SQ	0.412	0.156	0.247	0.193	0.22	0.091

Note. ETOH= ethanol; Sacc= saccharin; Quin= quinine; SQ= saccharin-quinine.

* $p < .05$, ** $p < .01$.

Appendix C

Analysis of body weights for Lewis Wistar Kyoto and Wistar rats in Experiment 1

During the 24-hour drinking phase, analysis of body weights indicated that there were significant differences among the three strains during each fluid phase: quinine phase $F(2, 27) = 10.45$, $p < .001$, saccharin phase $F(2, 27) = 15.61$, $p < .0001$, ethanol phase $F(2, 27) = 13.95$, $p < .001$, saccharin-quinine phase $F(2, 27) = 29.86$, $p < .0001$. Specifically, Wistar and Wistar Kyoto rats were significantly larger than Lewis rats ($p < .01$) while Wistar and Wistar Kyoto rats did not differ in weight.

During limited-access drinking, these group differences in weight persisted: quinine phase $F(2, 21) = 71.67$, $p < .0001$, saccharin phase $F(2, 21) = 66.99$, $p < .0001$, ethanol phase $F(2, 2) = 67.52$, $p < .0001$, saccharin-quinine phase $F(2, 2) = 69.55$, $p < .0001$. Lewis rats remained significantly smaller than both Wistar and Wistar Kyoto rats throughout ($p < .01$), and the mean weight for Wistar Kyoto rats fell below that of Wistar rats only during the quinine and saccharin phases ($p < .05$).

Appendix D

Ethanol and saccharin-quinine intake (ml) figures for Experiment 2

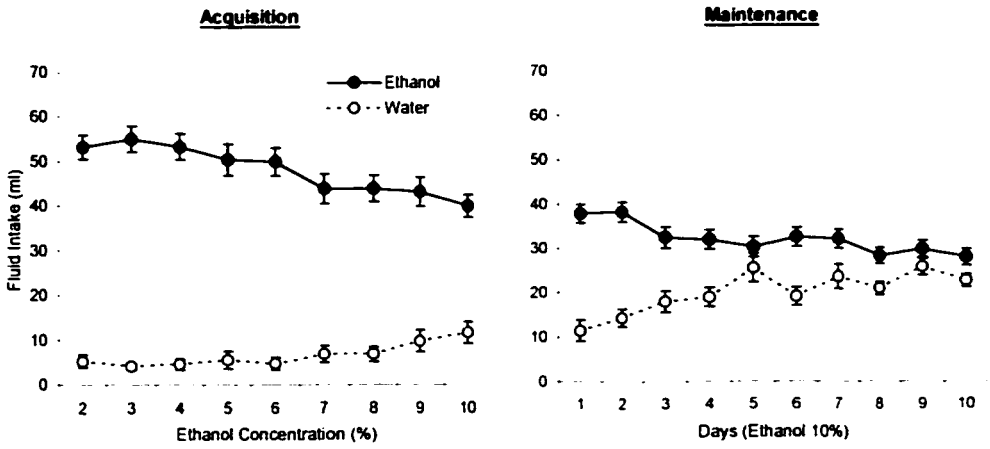

Figure 1D. Mean (+/- SEM) ethanol and water consumption (ml) in Fawn-Hooded rats during the acquisition period (left panel) and maintenance period (right panel) of Phase 1. Fawn-Hooded rats drank more ethanol than water throughout the acquisition period and most of the maintenance period, fluid x concentration $F(18, 828) = 53.05$, $p < .0001$. The results of a Test of Simple Effects revealed that, during the maintenance period, intake of 10% ethanol and water did not differ on days 5, 9 and 10, $ps > .05$.

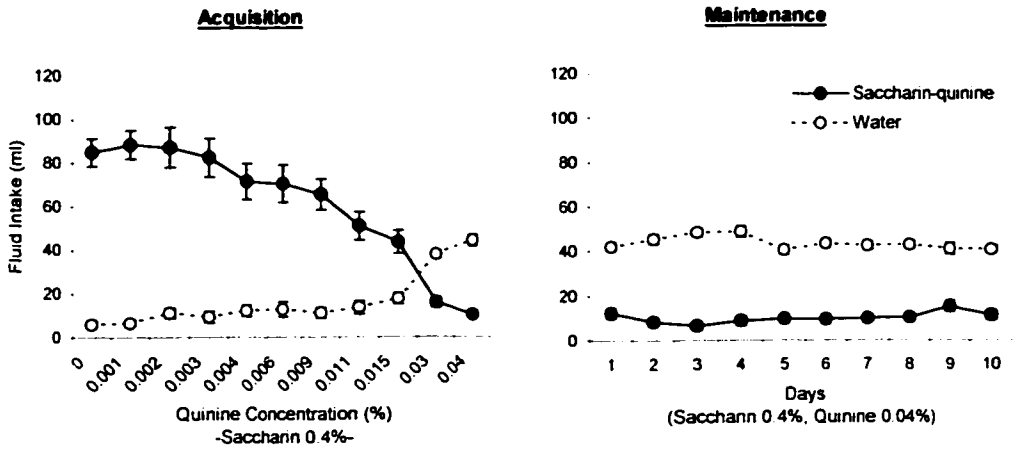

Figure 2D. Mean (+/- SEM) Saccharin-quinine and water consumption (ml) in Fawn-Hooded rats during the acquisition period (left panel) and maintenance period (right panel) of Phase 2. Fawn-Hooded rats consumed more saccharin-quinine than water throughout most of the acquisition period, fluid x concentration $F(20, 880) = 136.95$, $p < .0001$ (left panel). Intake of saccharin-quinine dropped below that of water on acquisition day 10 (saccharin 0.4%, 0.03%), and remained lower than water intake through to the end of the maintenance period (right panel).

Appendix E

Total fluid intake graphs for ethanol and saccharin-quinine phases of Experiment 2

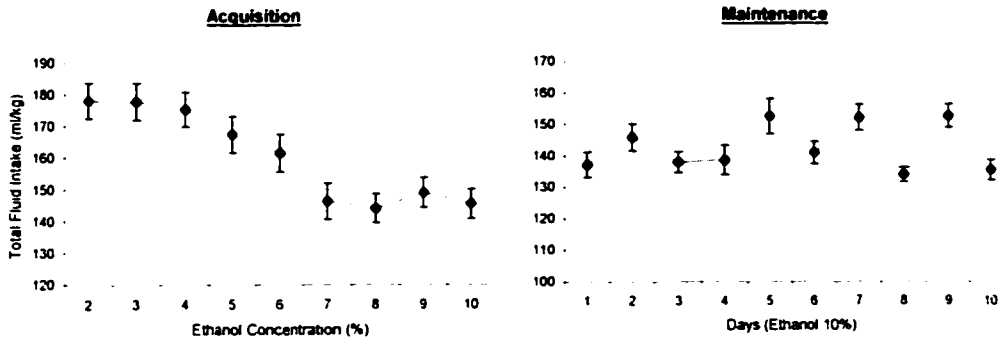

Figure 1E. Mean (+/- SEM) total fluid intake (ml/kg) in Fawn-Hooded rats drinking ethanol and water during the acquisition period (left column) and maintenance period (right column) of Phase 1. There was a decrease in fluid consumption over the days of the experiment, $F(18, 414) = 14.40$, $p < .0001$. Total fluid consumption remained unchanged until acquisition day 6 (7% ethanol) when it dropped, $p < .01$, and remained unchanged across the remaining days of the maintenance period.

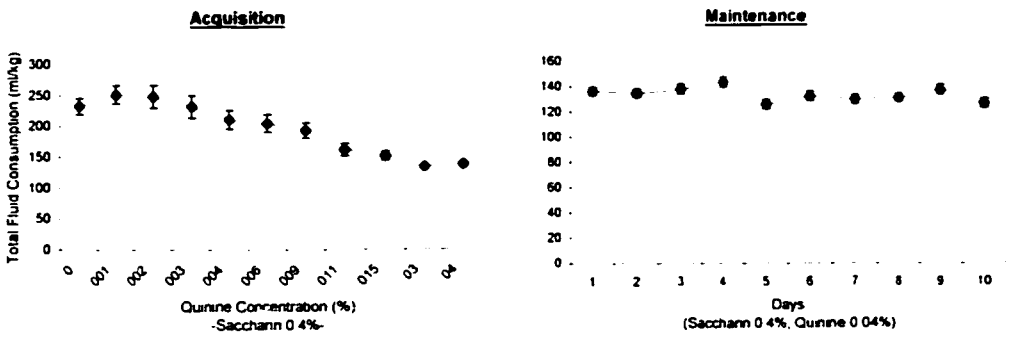

Figure 2E. Mean (+/- SEM) total fluid intake (ml/kg) in Fawn-Hooded rats drinking saccharin-quinine and water during the acquisition period (left column) and maintenance period (right column) of Phase 2. There was a gradual decrease in total fluid intake over the course of Phase 2, $F(20, 440) = 29.16$, $p < .0001$ (see Figure 4, bottom panels). Fluid consumption was highest for the first 6 days of the acquisition period (saccharin 0.4%, quinine 0-0.006%), and then decreased until acquisition day 10 (saccharin 0.4%, quinine 0.03%), where it remained unchanged until the end of the maintenance period.

Appendix F

Analysis of body weights for Fawn-Hooded rats in Experiment 2

Fawn-Hooded rats consistently gained weight across the days of Phase 1 (Ethanol acquisition and maintenance) of Experiment 2, $F(18, 414) = 113.17$, $p < .0001$.

Analysis of body weights showed that Fawn-Hooded rats continued to gain weight throughout the Phase 2 (Saccharin-quinine acquisition and maintenance) of Experiment 2, $F(20, 440) = 45.19$, $p < .0001$.

Appendix G

Final sample sizes for Experiment 3

	Sample Size		
Strain	Ethanol	Saccharin	Quinine
Fawn-Hooded	8	4	6
Wistar	8	10	6
Lewis	9	10	9

Appendix H

Total fluid intake graphs and analysis for Experiment 3

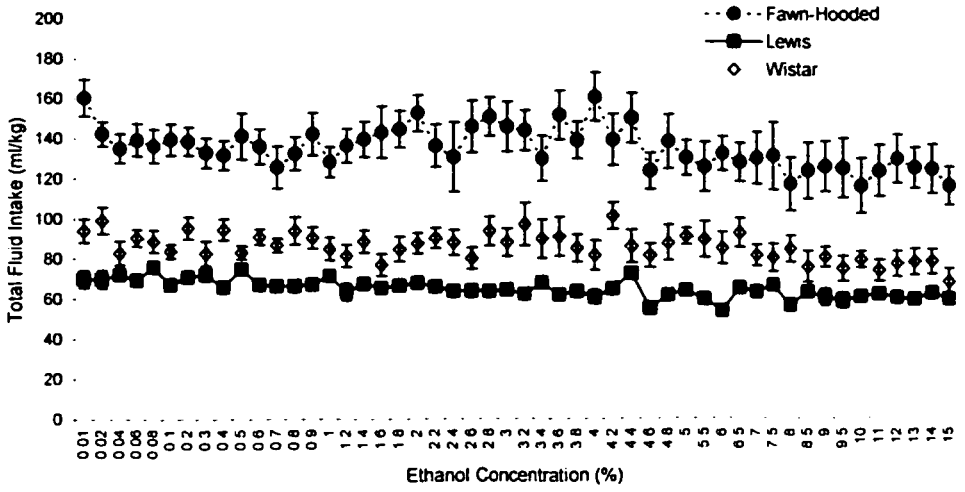

Figure 1H. Total fluid intake (ml/kg) for Fawn-Hooded, Lewis and Wistar rats drinking ethanol and water in Experiment 3. Total daily fluid consumption was different across the strains, strain x concentration $F(98, 1078) = 2.50$, $p < .0001$, as Fawn-Hooded rats displayed the highest levels throughout the experiment, followed by Wistar and Lewis rats, $p < .001$.

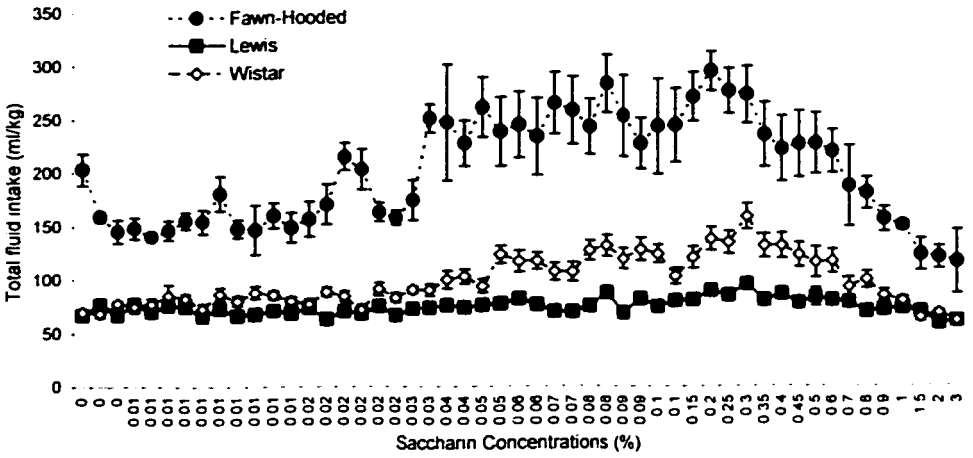

Figure 2H. Total fluid intake (ml/kg) for Fawn-Hooded, Lewis and Wistar rats drinking saccharin and water in Experiment 3. Fawn-Hooded rats also displayed very high total fluid intake values throughout the saccharin presentation schedule compared with Lewis and Wistar rats, strain x concentration $F(100, 1050) = 10.90$, $p < .0001$. In addition, Wistar rats had consistently greater total fluid intake values than Lewis rats when the saccharin solutions presented were between 0.019% and 0.7%, $p < .05$.

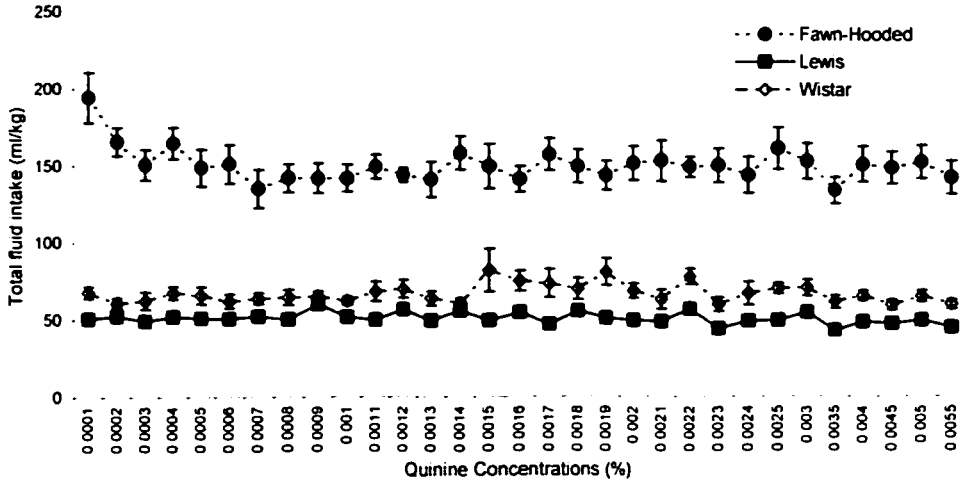

Figure 3H. Total fluid intake (ml/kg) for Fawn-Hooded, Lewis and Wistar rats drinking quinine and water in Experiment 3. Fawn-Hooded rats displayed higher total fluid consumption levels compared with Wistar and Lewis rats, strain x concentration $F(60, 660) = 3.64$, $p < .0001$. Wistar rats consumed more fluids overall compared with Lewis rats, $p < .001$.

Appendix I

Analysis of body weights for Fawn-Hooded, Lewis and Wistar rats in Experiment 3

Within all three drinking groups, analysis of body weights indicated that there were significant differences among the three strains: ethanol $F(2, 22) = 41.40$, $p < .0001$, saccharin $F(2, 21) = 66.92$, $p < .0001$, quinine $F(2, 22) = 40.80$, $p < .0001$. In the ethanol and quinine groups, Wistar rats were larger than both Lewis and Fawn-Hooded rats, $p < .01$, and Lewis rats were larger than Fawn-Hooded rats, $p < .05$. In the saccharin group, Wistar rats were larger than both Lewis and Fawn-Hooded rats, $p < .01$, and there were no differences between Lewis and Fawn-Hooded rats.

Appendix J

Water intake table and analysis for Experiment 4

Mean water intake (ml/kg) across drug groups for Lewis and Wistar rats during baseline and treatment phases of Experiment 4

	Ethanol		Saccharin		Quinine	
Phase	B	T	B	T	B	T
	Lewis					
THIP[a]	92.1 (4.3)	87.8 (3.8)	41.1 (13.1)	35.7 (11.3)	115.2 (5.3)	107.4 (6.0)
Naltrexone[b]	94.9 (3.0)	87.3 (3.0)	49.0 (12.2)	45.7 (12.4)	120.1 (4.6)	106.0 (6.2)
Raclopride[c]	89.1 (3.6)	88.7 (3.4)	58.3 (23.1)	38.8 (14.3)	109.8 (4.0)	102.6 (6.5)
Saline[d]	93.3 (3.7)	89.2 (2.4)	30.9 (8.3)	15.0 (2.1)	119.4 (4.9)	113.3 (5.8)
	Wistar					
THIP[a]	102.8 (6.7)	86.9 (7.8)	46.9 (16.4)	31.5 (15.1)	164.8 (7.5)	148.0 (7.2)
Naltrexone[b]	103.2 (8.2)	85.7 (6.8)	42.3 (16.1)	35.0 (13.8)	174.8 (6.3)	150.6 (5.3)
Raclopride[c]	102.7 (6.9)	86.2 (5.1)	37.8 (11.7)	31.9 (12.1)	168.7 (11.2)	155.1 (5.6)
Saline[d]	104.6 (5.3)	96.0 (6.3)	37.4 (15.5)	21.5 (10.5)	167.7 (6.2)	146.5 (4.1)

Note. Values enclosed in parentheses represent standard error of the mean (SEM). B = Baseline Phase; T = Treatment Phase.

[a]Ethanol: phase $F(1, 18) = 12.54$, $p < .01$, reduction of 10% in both strains; Saccharin: no differences; Quinine: strain $Fs(1, 18) = 27.10$, $ps < .001$ Wistar > Lewis, phase $Fs(1, 18) = 13.67$, $p < .01$, reduction of 9% in both strains.

[b]Ethanol: phase $F(1, 18) = 22.80$, $p < .001$, reduction of 13% in both strains; Saccharin: no differences; Quinine: strain x phase $F(1, 18) = 6.60$, $p < .05$, Wistar > Lewis reduction in intake $p < .001$.

[c]Ethanol: strain x phase $F(1, 18) = 19.83$, $p < .001$, reduction in Wistar rats of 16%; Saccharin: no differences; Quinine: strains $F(1, 18) = 23.85$, $p < .001$, Wistar > Lewis, phase $F(1, 18) = 9.97$, $p < .01$, reduction of 7% in both strains.

[d]Ethanol: phase $F(1, 18) = 13.28$, $p < .01$, reduction of 6% in both strains; Saccharin: phase $F(1, 18) = 10.57$, $p < .01$, reduction of 47% in both strains; Quinine: strain x phase $F(1, 18) = 11.29$, $p < .01$, Wistar > Lewis during baseline ($p < .001$) and treatment phases ($p < .01$), reduction in Wistar of 10% ($p < .001$).

Appendix K

Total fluid intake table and analysis for Experiment 4

Total fluid intake (ml/kg) across drug groups for Lewis and Wistar rats during baseline and treatment phases of Experiment 4

	Ethanol		Saccharin		Quinine	
Phase	B	T	B	T	B	T
	Lewis					
THIP[a]	100.6 (1.9)	95.6 (2.9)	144.0 (0.3)	127.6 (1.7)	123.6 (2.2)	113.9 (2.7)
Naltrexone[b]	102.2 (1.9)	94.0 (2.0)	158.1(2.5)	122.8 (3.7)	128.3 (3.0)	112.8 (0.3)
Raclopride[c]	93.6 (1.2)	92.4 (1.2)	144.0 (9.7)	113.7 (1.2)	114.4 (0.5)	106.4 (1.0)
Saline[d]	101.7 (2.0)	97.0 (1.8)	150.4 (3.6)	142.2 (3.8)	128.1 (1.6)	122.0 (1.5)
	Wistar					
THIP[a]	122.7 (2.6)	114.1 (3.3)	266.6 (17.9)	215.4 (9.1)	169.7 (0.8)	151.4 (7.6)
Naltrexone[b]	130.8 (2.1)	114.6 (3.2)	274.6 (14.7)	217.0 (15.9)	183.1 (2.1)	157.1 (0.7)
Raclopride[c]	136.5 (3.4)	125.7 (1.1)	269.2 (13.7)	226.8 (5.7)	176.7 (2.7)	160.6 (0.1)
Saline[d]	122.0 (2.7)	119.6 (1.4)	252.5 (6.2)	224.6 (7.3)	173.3 (1.7)	150.1 (5.7)

Note. Values enclosed in parentheses represent standard error of the mean (SEM). B = Baseline Phase; T = Treatment Phase.

[a]Ethanol: strain $F(1, 18) = 10.36$, $p < .01$, Wistar > Lewis; Saccharin: strain $F(1, 18) = 17.18$, $p < .001$, Wistar > Lewis, phase $F(1, 18) = 15.52$, $p < .01$, reduction of 16% in both strains; Quinine: strain $F(1, 18) = 20.12$, $p < .001$, Wistar > Lewis, phase $F(1, 18) = 19.30$, $p < .001$, reduction of 10% in both strains.

[b]Ethanol: strain x phase $F(1, 18) = 6.12$, $p < .05$, Wistar > Lewis during baseline ($p < .001$) and treatment ($p < .01$) phases, reduction of 12% in Wistar rats ($p < .001$) and 8% in Lewis rats ($p < .01$); Saccharin: strain $F(1, 18) = 15.63$, $p < .001$, Wistar > Lewis, phase $F(1, 18) = 16.04$, $p < .001$, reduction of 21% in both strains; Quinine: strain x phase $F(1, 18) = 6.44$, $p < .05$, Wistar > Lewis, reduction of 17% in Wistar rats and 14% in Lewis rats, $ps < .001$.

[c]Ethanol: strain x phase $F(1, 18) = 5.13$, $p < .05$, Wistar > Lewis, reduction in Wistar rats of 8%, $ps < .001$; Saccharin: strain $F(1, 18) = 37.44$, $p < .001$, Wistar > Lewis, phase $F(1, 18) = 15.12$, $p < .01$, reduction of 18% in both strains; Quinine: strain $F(1, 18) = 28.83$, $p < .001$, Wistar > Lewis, phase $F(1, 18) = 12.14$, $p < .01$, reduction of 8% in both strains.

[d]Ethanol: strain $F(1, 18) = 11.42$, $p < .01$, Wistar > Lewis, phase $F(1, 18) = 4.98$, $p < .05$, reduction of 3% in both strains; Saccharin: strain $F(1, 18) = 14.06$, $p < .01$, Wistar > Lewis phase $F(1, 18) = 7.67$, $p < .05$, reduction of 9% in both strains; Quinine: strain x phase $F(1, 18) = 11.57$, $p < .01$, Wistar > Lewis, reduction in Wistar rats of 10%, $p < .001$.

Appendix L

Analysis of body weights for Lewis and Wistar rats in Experiment 4

During quinine drinking, there were no differences in body weights between the strains regardless of drug group, and both strains increased in weight over the course of the baseline and treatment phases: THIP strain x phase $F(1, 18) = 6.53$, $p < .05$, naltrexone strain x phase $F(1, 18) = 9.09$, $p < .01$, raclopride strain x phase $F(1, 18) = 15.99$, $p < .001$, saline strain x phase $F(1, 18) = 5.72$, $p < .05$.

The strains were not different in weight during saccharin drinking, and both strains generally increased in weight: THIP strain x phase $F(1, 18) = 7.03$, $p < .05$, naltrexone $F(1, 18) = 2.38$, ns., raclopride strain x phase $F(1, 18) = 7.28$, $p < .05$, saline $F(1, 18) = .69$, ns.

During the ethanol drinking period, their body weights did not change over the course of the baseline and treatment phases in any drug group, and Wistar rats were larger than Lewis rats in all drug groups: THIP $F(1, 18) = 19.31$, $p < .01$, naltrexone $F(1, 18) = 23.24$, $p < .01$, raclopride $F(1, 18) = 25.14$, $p < .01$, saline $F(1, 18) = 15.99$, $p < .01$.

Appendix M

Spearman correlation coefficients for mean fluid intake and preference in Lewis and Wistar rats during baseline: Experiment 4

	Strain			
	Lewis		Wistar	
Fluids	Intake	Preference	Intake	Preference
Quinine/Saccharin	0.180	-0.027	0.190	0.101
Quinine/Ethanol	0.793**	0.743**	0.224	0.136
Saccharin/Ethanol	0.197	0.026	0.374*	0.071

* $p < .05$, ** $p < .01$.

Appendix N

Water intake figures and analysis for Experiment 5

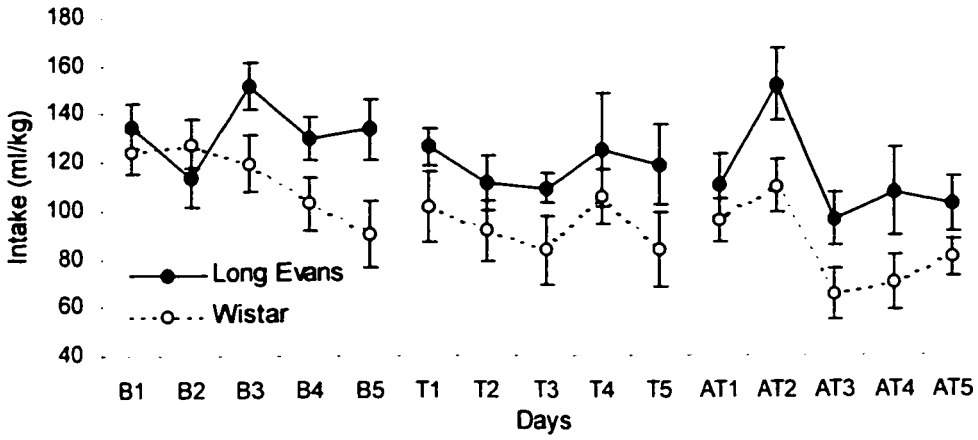

Figure 1N. Mean (+/- S.E.M.) water intake in Long-Evans and Wistar rats during ethanol presentations across the days of baseline (B), naltrexone treatment (T), and after naltrexone treatment (AT) during Phase 1 (continuous fluid access) of Experiment 5. Water intake was not different between the strains, $F(1, 10) = 4.09$, and decreased from baseline to the after-treatment period by 19% in both strains, $F(2, 20) = 14.43$, $p < .001$.

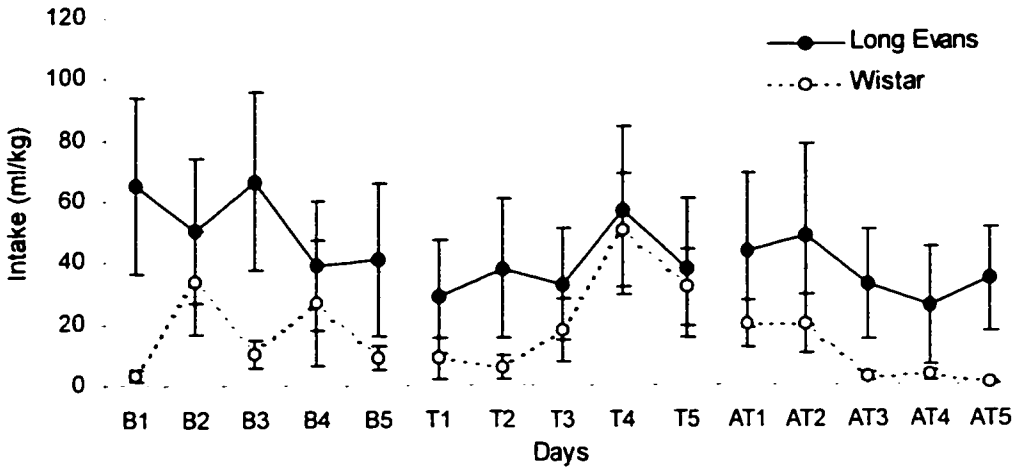

Figure 2N. Mean (+/- S.E.M.) water intake during saccharin presentations in Long-Evans and Wistar rats across the days of baseline (B), naltrexone treatment (T), and after naltrexone treatment (AT) during Phase 1 (continuous fluid access) of Experiment 5. There were no differences between the strains in water intake, $F(1, 10) = 1.5$, and no changes in water intake following naltrexone treatment, $F(2, 20) = 1.82$.

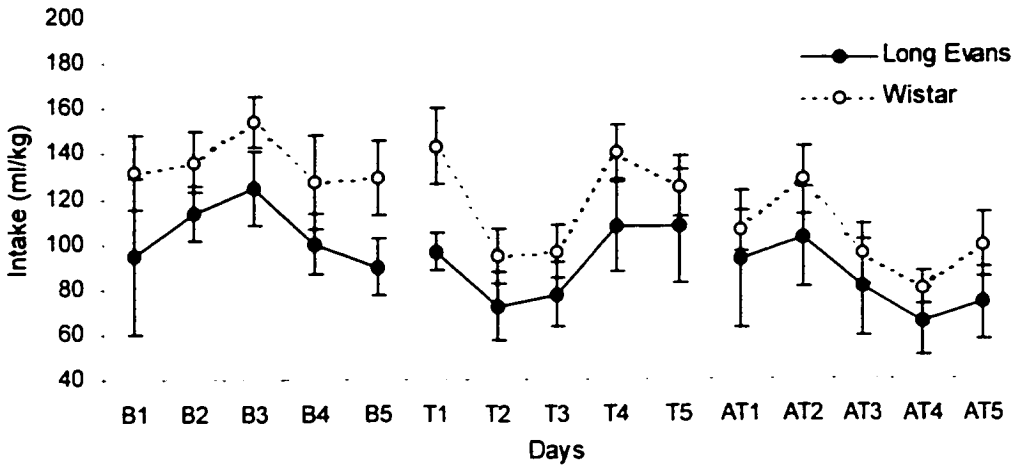

Figure 3N. Mean (+/- S.E.M.) water intake during saccharin-ethanol presentations in Long-Evans and Wistar rats across the days of baseline (B), naltrexone treatment (T), and after naltrexone treatment (AT) during Phase 1 (continuous fluid access) of Experiment 5. Water intake was not different between the two strains, $F(1, 10) = 1.74$, and decreased over the course of the experiment, $F(2, 20) = 10.95$, $p < .001$. Water intake decreased 23% from baseline through the after-treatment period in both strains, $p < .01$.

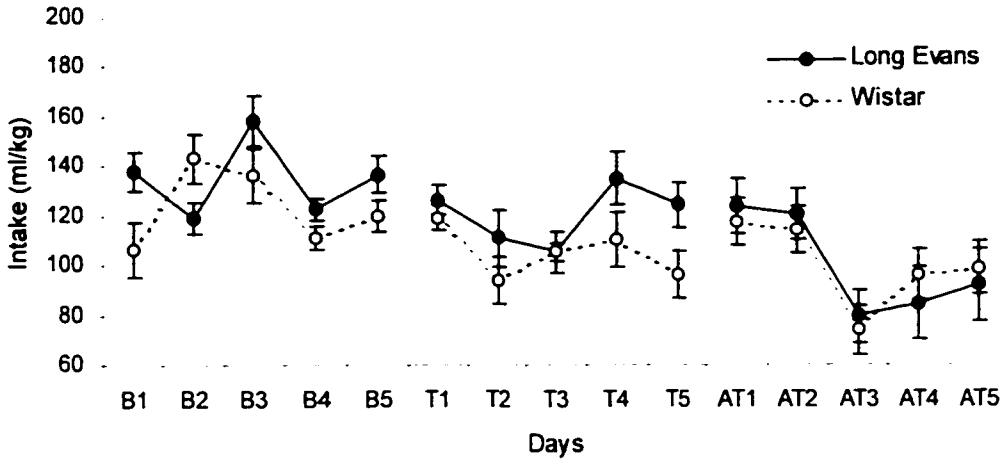

Figure 4N. Mean (+/- S.E.M.) water intake during saccharin-quinine presentations in Long-Evans and Wistar rats across the days of baseline (B), naltrexone treatment (T), and after naltrexone treatment (AT) during Phase 1 (continuous fluid access) of Experiment 5. There were no strain differences in water intake, $F(1, 10) = .76$. There was a significant decrease in water consumption in both strains, $F(2, 20) = 23.61$, $p < .0001$, as water intake was reduced by 13% from the baseline to the treatment periods, $p < .01$, and by a further 11% at the after-treatment period, $p < .05$.

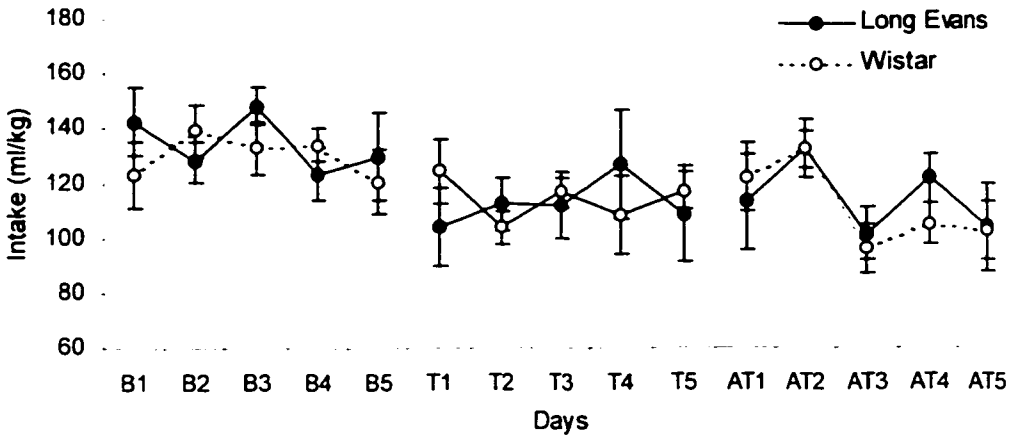

Figure 5N. Mean (+/- S.E.M.) water intake during quinine presentations in Long-Evans and Wistar rats across the days of baseline (B), naltrexone treatment (T), and after naltrexone treatment (AT) during Phase 1 (continuous fluid access) of Experiment 5. There were no strain differences in water intake, $F(1, 10) = 0.03$, and both strains reduced intake by 14% from baseline to treatment periods, $F(2, 20) = 14.64$, $p < .0001$, and remained at this lower level through the after-treatment period, $p < .01$.

Appendix O

Analysis of body weights for Long-Evans and Wistar rats in Experiment 5

During Phase 1 (continuous fluid access), there were no differences in body weights between the Long-Evans and Wistar rats except in those groups drinking saccharin-ethanol, where Long-Evans rats were significantly larger than Wistar rats: quinine $F(1, 10) = 0.47$, saccharin $F(1, 10) = 0.33$, ethanol $F(1, 10) = 0.01$, saccharin-quinine $F(1, 10) = 0.07$, saccharin-ethanol $F(1, 10) = 7.55$, $p < .05$.

During Phase 2 (limited fluid access), Wistar rats were significantly larger than Long-Evans rats in all groups: quinine $F(1, 10) = 11.24$, $p < .01$, saccharin $F(1, 10) = 9.23$, $p < .05$, ethanol $F(1, 10) = 46.18$, $p < .01$, saccharin-quinine $F(1, 10) = 16.12$, $p < .01$, saccharin-ethanol $F(1, 10) = 11.25$, $p < .01$.