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# An Examination of the Effects of the Catalase Inhibitor 3-Amino-1,2,4-triazole on Ethanol, Food and Water Intake.

Van Allan Redila

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# ABSTRACT

**An Examination of the Effects of the catalase Inhibitor 3-Amino-1,2,4-triazole on Ethanol, Food and Water Intake.**

**Van Allan Redila**

The effects of the catalase inhibitor 3-amino-1,2,4-triazole (AT) were examined on ethanol, food and water intakes to determine if it produced a decrease in ethanol consumption through the decrease in catalase produced acetaldehyde or to a general decrease in caloric intake. Experiment 1A demonstrated that a food containing a concentration of 0.6% w/w sodium saccharin was preferred over a neutral-flavoured food while a concentration of 0.15% w/w of quinine sulfate was less preferred. These two concentrations of saccharin and quinine were used in experiment 1B to study the effects of AT on taste reactivity. Results of experiment 1B showed that AT significantly decreased food intake but not ethanol, water or total fluid intakes. The saccharin, neutral and quinine food groups decreased their food intakes to the same extent suggesting that AT did not affect taste reactivity. Furthermore, quinine-fed rats significantly increased their ethanol consumption while decreasing their food and water intakes. Experiment 1C tested whether the quinine-induced changes in ethanol, food and water intakes were mediated through the 5-$HT_2$ receptor. The 5-$HT_2$ antagonist ritanserin failed to attenuate the quinine-induced ethanol consumption or to affect food or water intakes. Experiment 2 showed that AT significantly decreased ethanol consumption but did not significantly affect either food or water intake.

Taken together, the findings of experiments 1B and 2 demonstrated that AT does not produce a general decrease in the caloric requirement of the animal. The failure of AT to decrease ethanol consumption in experiment 1B is contradictory to previous

reports regarding the role of catalase produced acetaldehyde and its role in the mediation of ethanol intake. However, the decrease in ethanol intake observed in experiment 2 is consistent with the pattern of catalase inhibition suggesting that acetaldehyde may be involved in the mediation of voluntary ethanol consumption.

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# GENERAL INTRODUCTION

Research concerning the biological mechanism mediating ethanol consumption has provided a wealth of information and several theories about the role of certain neurotransmitters systems, enzymes, and brain structures in the mediation of ethanol intake. One branch of this research that has contributed to the understanding of ethanol consumption is on the role of acetaldehyde as a possible mediator of voluntary ethanol intake. Acetaldehyde is the primary metabolic product of ethanol degradation and has shown promise as a possible mediator of ethanol consumption. In fact, it has been proposed that many of the effects of alcohol are due to acetaldehyde and that alcoholism could be better referred to as acetaldehydism (Raskin, 1975). Several researchers have postulated that acetaldehyde, produced in the brain, possesses reinforcing properties and may in fact be responsible for the mediation of ethanol consumption as well as other ethanol-induced behaviours (for reviews see; Smith, Aragon and Amit, 1997; Hunt, 1996; Lindros, 1978; Truitt and Walsh, 1971).

Acetaldehyde is a chemically reactive compound that is more toxic than its parent compound ethanol and has been implicated in many of the toxicological and teratogenic effects of ethanol (for reviews see; Brien and Loomis, 1983; Streissguth, Landesman-Dwyer, Martin and Smith, 1980; Lindros, 1978). In fact, the aversive effects that peripheral acetaldehyde produce are the basis for a commonly used treatment of alcoholism (see Lindros, 1978). Artificial elevation of peripheral acetaldehyde by the inhibition of aldehyde dehydrogenase (ALDH), which inhibits the degradation of acetaldehyde, has been typically used in the treatment of excessive alcohol consumption. Disulfiram and calcium cyanamide are two ALDH inhibitors commonly used in

treatment of alcoholism (MacLeod, 1950). It has been shown that the administration of disulfiram, as well as calcium cyanamide, produced elevated blood acetaldehyde levels when ethanol was later presented (Schlesinger, Kakihana and Bennett, 1966). The consumption of moderate to high levels of alcohol during treatment with ALDH inhibitors leads to significantly elevated peripheral levels of acetaldehyde and typically produces a cluster of negative symptoms that include tachycardia, dizziness, nausea and vomiting (see Lindros, 1978; Asmussen, Hald and Larsen, 1948).

Although elevated acetaldehyde levels have been traditionally associated with negative or aversive consequences, there is evidence that acetaldehyde also possesses positive reinforcing properties (Brown, Amit, Smith, Sutherland and Selvaggi, 1983). Furthermore, it has been argued that the positive reinforcing effects of acetaldehyde are mediated centrally while the aversive effects are primarily due to peripheral acetaldehyde (Brown, Amit, Smith and Rockman, 1978). Acetaldehyde levels have been shown to be significantly and positively correlated with ethanol consumption (Myers, Ng and Singer, 1984; Myers, Ng, Marzuki, Myers and Singer, 1984; Brown, Amit and Smith, 1980) and with several ethanol-induced behaviours (Aragon, Spivak and Amit, 1985; Aragon, Spivak and Amit, 1989). In fact, it has been shown that human subjects who have been treated with the ALDH inhibitors disulfiram or calcium carbamide and then consumed low doses of alcohol report a state of euphoria and intoxication (Brown, Amit, Smith, Sutherland and Selvaggi, 1983).

The following sections of the present thesis will review topics on ethanol metabolism and the enzymes responsible for the production and degradation of acetaldehyde. The role of central acetaldehyde in the regulation and mediation of the

psychopharmacological and positively reinforcing properties of ethanol will also be discussed.

Ethanol metabolism

Ethanol, a fat-soluble molecule, is readily absorbed and distributed throughout the body after oral ingestion. It has been estimated that about 90% of orally ingested ethanol is rapidly metabolized into acetaldehyde in the liver (Kalant, 1971; Agarwal and Goedde, 1990). There are three known enzymatic routes for hepatic ethanol metabolism; alcohol dehydrogenase (ADH), the microsomal ethanol-oxidizing system (MEOS) and the catalase system (for review see Topel, 1985; Hawkins and Kalant, 1972).

Alcohol dehydrogenase can be found mainly in the cytosol of the hepatocytes. ADH metabolizes ethanol to acetaldehyde in a coupled reduction with nicotinamide-adenine dinucleotide (NAD) (Jacobsen, 1952; Erickson, 1979). ADH is the major pathway of ethanol metabolism in the liver (Matsumoto, Fujimiya, Fukui, 1994). Alcohol dehydrogenase has also been found in extrahepatic tissues including the kidney, pancreas (Buhler, Pestalozzi, Hess and von Wartburg, 1983) and in trace amounts in brain (Rout, 1992; Kerr, Maxwell and Crabb, 1989).

There are two non-ADH ethanol metabolizing systems; MEOS and catalase. The MEOS is an nicotinamide adenine dinucleotide phosphate (NADPH) dependent pathway that has been shown to have the capacity of metabolizing sufficient amounts of ethanol in vivo (Lieber and DeCarli, 1972). MEOS consists of the family of cytochrome P-450 enzymes (Koop, Morgan, Tarr and Coon, 1982), and includes an isozyme that is specific to the metabolism of ethanol, cytochrome P-450-2E1. Ethanol metabolism by MEOS has been estimated to be about 70% to 80% in a strain of alcohol dehydrogenase deficient

mice (Handler, Koop, Coon, Takei and Thurman, 1988). Although some have argued for a role of MEOS in ethanol metabolism (Lieber and Di Carli, 1972), others have argued that MEOS plays a relatively minor role in ethanol metabolism under normal biological conditions (Thurman and Handler, 1989).

The second non-ADH pathway for ethanol metabolism is the catalase system. The enzyme catalase can be found in tissues throughout the body such as liver peroxisomes and brain microperoxisomes in humans (Houdou, Kuruta, Hasegawa, Konomi, Takashima, Suzuki and Hashimoto, 1991) and rats (Gaunt and De Duve, 1976; Zimatkin and Lindros, 1996). Ethanol is metabolized to acetaldehyde by the peroxidatic activity of catalase (Oshino, Oshino and Chance, 1973). Catalase reacts with hydrogen peroxide ($H_2O_2$) to form a catalase-$H_2O_2$ complex referred to as compound I (Chance, 1947; Chance and Schonbaum, 1962). Compound I can then undergo two additional reactions; it can react with another peroxide molecule (the catalytic reaction) or with a hydrogen donor such as ethanol (the peroxidatic reaction).

The activity of the enzyme catalase is dependent on $H_2O_2$ availability. Hydrogen peroxide combines with catalase to produce compound I. It is thought that the ability of catalase to metabolize ethanol is dependent on the rate at which compound I is produced. That is, the amount of $H_2O_2$ available to react with catalase is a rate-limiting step in the metabolism of ethanol by catalase. It has been shown that the levels of $H_2O_2$ found in brain limits the peroxidatic activity of catalase (Oshino, Oshino and Chance, 1973). Several studies have reported that the addition of $H_2O_2$ or of a $H_2O_2$–generating system to tissue samples increased ethanol metabolism via the peroxidatic activity of catalase (Gill, Menez, Lucas and Deitrich, 1992; Oshino, Oshino and Chance, 1973). However, it

has also been argued that the amounts of endogenously produced $H_2O_2$ are sufficient to sustain ethanol metabolism via the catalase system (Aragon, Rogan and Amit, 1991; Handler and Thurman, 1988; Thurman and Handler, 1989). Although the activity of catalase can be increased by exogenous hydrogen peroxide, its endogenous levels have been found to support ethanol metabolism by the peroxidatic activity of catalase and thus is not a rate-limiting factor.

The production and elimination of acetaldehyde; ADH and ALDH

Two lines of research findings have helped to clarify a role for central acetaldehyde as a possible mechanism for mediating the reinforcing properties and pyschopharmacological effects of ethanol. The first research area involves studies on the relationship between voluntary ethanol consumption and the production and elimination of acetaldehyde. The production and elimination of acetaldehyde by the various ethanol-metabolizing systems are important in providing a source of acetaldehyde that may then act centrally. In fact, it is thought that the production and elimination of acetaldehyde may explain individual and ethnic differences in the propensity to consume alcohol in humans (Meier-Tackman, Leonhardt, Agarwal and Goedde, 1990; Mizoi, Adachi, Fukunaga, Kogame, Ueno, Nojo and Fujiwara, 1987; Goedde, Harada and Agarwal, 1979; Zeiner, Paredes and Dix Christensen, 1979). The second line of investigation involves studies which have shown that centrally produced acetaldehyde has reinforcing properties.

The production and elimination of acetaldehyde are critical factors in the determination of both central and peripheral acetaldehyde levels in the body following ethanol consumption. Alcohol dehydrogenase (ADH) is the major route for ethanol

metabolism and thus in itself an important determinant of acetaldehyde levels. Schlesinger, Kakihana and Bennett (1966) have shown that the levels of hepatic ADH are correlated with ethanol consumption. These researchers reported that a high drinking strain of mice, C57BL, had greater hepatic levels of ADH than the corresponding nondrinking strain of mice, DBA. In addition, a study comparing several strains of mice differing in ADH levels (Teichert-Kuliszewska, Israel and Cinader, 1988) showed that high drinking strains of mice had significantly higher hepatic ADH levels than the corresponding strain of nondrinking mice.

However, in contrast to the above reports, several studies have shown that high hepatic ADH levels are not correlated with greater ethanol consumption. Koivula, Koivusalo and Lindros (1975) have shown that a high drinking rat strain (AA) possessed lower hepatic ADH activity levels than the corresponding low ethanol drinking strain (ANA). Kovisto and Eriksson (1994) reconfirmed this finding after the revitalization of their selected rat lines.

The results of the above mentioned studies have produced contradictory and conflicting results regarding the role of ADH in the regulation of ethanol intake. Thus, it seems that the production of acetaldehyde through the action of ADH on ethanol may not be related to the propensity to drink ethanol. The evidence supporting a relationship between hepatic ethanol metabolism via ADH , and thus acetaldehyde production, and ethanol consumption is conflicting and weak at best. Therefore, since the production of acetaldehyde via ethanol metabolism by ADH is not well correlated with ethanol consumption, then the enzyme responsible for the degradation of acetaldehyde may prove

to be a better candidate as a regulatory mechanism in which acetaldehyde may exert its effects.

Acetaldehyde is metabolized into acetate by aldehyde dehydrogenase (Pettersson and Tottmar, 1982) and acetaldehyde's mediation of ethanol intake may in fact be related to the manner in which it is eliminated. Koivula et al (1975) and Koivisto and Eriksson (1994) have shown that the low drinking ANA strain of rats had significantly lower liver ALDH activity compared to the high drinking AA line. In addition to low hepatic activity, the same researchers reported that the ANA strain possessed higher hepatic ADH activity than the AA strain. Kovisto and Eriksson (1994) argued that low ALDH activity, coupled with high ADH activity may explain the aversion to ethanol that the ANA strain displays. That is, the aversion to ethanol may be the result of high peripheral levels of acetaldehyde. The increased levels of acetaldehyde in the periphery and the resultant negative consequences were thought to be the reason that the ANA line does not drink ethanol (Kovisto and Eriksson, 1994).

From the studies described above it seems that hepatic elimination of acetaldehyde may be of more importance to the regulation of ethanol consumption than its production through the enzyme ADH. It seems that high hepatic ALDH activity prevents the accumulation of elevated peripheral acetaldehyde levels, which would otherwise produce toxic effects in the body. The role of hepatic ALDH in the putative mediation of ethanol consumption by acetaldehyde provides support for acetaldehyde as a possible regulator of ethanol consumption. This position is even stronger when ethanol consumption is related to central ALDH activity.

Amir (1977) reported on a relationship between brain ALDH activity and ethanol consumption. Using male Wistar rats, he found a significant positive correlation between brain ALDH activity and ethanol consumption ($r = 0.5582$) and liver ALDH activity and ethanol consumption ($r = 0.3187$). Moreover, the higher demonstrated correlation between brain ALDH activity and ethanol consumption as compared to liver ALDH activity and ethanol consumption supports the assumption about the greater importance of central ALDH in regulating ethanol intake. The positive correlation between brain ALDH activity and ethanol consumption was also supported when the Long-Evans, Sprague-Dawley (Socaransky, Aragon, Amit and Blander, 1984) as well as Tyron Maze Bright and Tyron Maze Dull (Amir, 1978) strains of rats were used. Taken together, the above experiments show a positive relationship between brain ALDH and ethanol consumption and thus position this enzyme as a possible regulator of ethanol intake.

However, in contrast to Amir's findings (1977) Inoue, Rusi and Lindros (1981) measured brain aldehyde dehydrogenase activity in the AA and ANA strains of rats and found no differences in brain ALDH levels. Since there are no differences in brain ALDH activities between the two strains, brain ALDH activity cannot be used to explain the differences in ethanol consumption seen in this strain of rats. That is, these two strains of rats have different ethanol intake patterns that cannot be explained by differences in central ALDH activities.

Self-administration of acetaldehyde

The hypothesis that acetaldehyde may play a role in mediating some of the actions of ethanol can be inferred from self-administration studies of ethanol and acetaldehyde. Several studies have shown that ethanol can be self-administered using

various reinforcement schedules and weight restrictions (Meisch, 1976; Smith, Oei, Ng and Armstrong, 1980; Oei and Singer, 1978). In addition, rats have been shown to self-administer acetaldehyde intravenously (Myers, Ng and Singer, 1982; Myers, Ng, Marzuki, Myers and Singer, 1984) as well as intracerebroventricularly (Brown, Amit and Smith, 1980, Brown, Amit, Smith and Rockman, 1979). In fact, it has been shown that animals will readily self-administer acetaldehyde, but not ethanol, intraventricularly (Brown, Amit, Smith and Rockman, 1979). These authors have concluded that it is acetaldehyde rather than ethanol that may mediate the reinforcing effects of ethanol.

Central infusions (Brown, Amit and Smith, 1980; Myers and Veale, 1969) as well as intravenous self-injections of acetaldehyde (Myers, Ng and Singer, 1984; Myers, Ng, Marzuki, Myers and Singer, 1984) have been shown to increase the propensity to consume ethanol. Myers and Veale (1969) reported that animals that have had previously experienced intraventricular infusions of acetaldehyde showed an increase in ethanol consumption and preference. Brown, Amit and Smith (1980) have expanded on the previous experiment and have shown that rats who have learned to self-administer acetaldehyde showed a greater consumption and preference for ethanol. In fact, these researchers have shown a positive correlation between acetaldehyde self-administration rates and ethanol intake over a range of ethanol concentrations.

Peripheral acetaldehyde production

Support for the notion that acetaldehyde production and elimination are correlated with ethanol consumption, and the findings that central acetaldehyde may possess positive reinforcing properties have been presented. If the theory that acetaldehyde, in

brain, is a mediating factor in ethanol consumption is correct then acetaldehyde must either be transported to the brain from the periphery or be metabolized there.

Hepatic metabolism of ethanol can provide a source of acetaldehyde that may be transported to the brain in some fashion. Peripheral acetaldehyde has been thought of as one possible route through which hepatically produced acetaldehyde can travel to the brain and thus produce its effects. Blood bound acetaldehyde has been proposed as one of these possible routes (Hernandez-Munoz, Baraona, Blacksberg and Lieber, 1989; Baraona, Di Padova, Tabasco and Lieber, 1987a, 1987b). Baraona and coworkers (1987a, 1987b) have proposed that hepatically produced acetaldehyde can bind to the hemoglobin in red blood cells and together they would be able to pass through the blood brain barrier and act centrally. These researchers have shown that levels of acetaldehyde bound to red blood cells are much higher in alcoholics than in controls, and thus may provide a mechanism for the transport of acetaldehyde to the brain. However, the lack of a known mechanism for the uncoupling of acetaldehyde from the red blood cells once they have reached the brain may prevent this form of transportation from being a viable option for delivering acetaldehyde to the brain.

Increased acetaldehyde levels have been reported in peripheral blood after ethanol administration (Eriksson and Sipple, 1976; Pettersson and Kiessling, 1976; Keissling, 1962a). Several studies have shown high blood acetaldehyde levels in alcoholics (Lindros, Stowell, Pikkarainen and Salaspuro, 1980; Majchrowicz and Mendelson, 1970) when comparing alcoholics to nonalcoholic controls after ethanol administration (Korsten, Matsuzaki, Feinman and Lieber, 1975). It has also been shown that first-degree relatives of alcoholics have greater blood acetaldehyde levels after alcohol consumption

than matched controls suggesting a genetic component of acetaldehyde metabolism (Schuckit and Rayses, 1979).

Increased peripheral blood acetaldehyde levels found in alcoholics after alcohol consumption may be due to liver injury incurred by the chronic use of alcohol (Nuutinen, Lindros and Salaspuro, 1983; Pikkarainen, Gordon, Lesback and Lieber, 1981; Palmer, Jenkins and Sherlock, 1981; Koivula and Lindros, 1975). Nuutinen, Lindros and Salaspuro (1983) have shown that alcoholics have decreased hepatic ALDH activity compared to nonalcoholics. These researchers have postulated that the increased peripheral blood acetaldehyde levels seen in alcoholics may be the result of a decreased ability to metabolize acetaldehyde, thus leading to increased blood acetaldehyde levels. Contrary to these findings however, it has been shown that chronic ethanol consumption in rats did not lead to a decreased ALDH activity and in fact has been shown to increase it (Amir, 1978a; Horton, 1971; Dajani, Danielski and Orten, 1963). However, there has been some research showing no difference in acetaldehyde levels between alcoholics and controls after ethanol consumption (Eriksson and Peachey, 1980). Thus, it remains unclear as to the role of chronic alcohol consumption and its damage to hepatic functions in producing increased acetaldehyde levels.

The occurrence of high blood acetaldehyde levels has been questioned due to methodological problems that result in the production of artifactually high acetaldehyde levels (Lindros, 1982; Eriksson, 1980; Sipple, 1972, 1973). Recent refinement of methodological techniques has helped to minimize artifactual production of acetaldehyde in the measurement of blood and tissue acetaldehyde, but the possibility of minor artifactual acetaldehyde formation still exists (Eriksson and Fukunaga, 1993; Sipple,

1973). The improved techniques for the measurement of acetaldehyde in blood samples have led to substantially different results than those obtained in previous studies.

Studies performed using techniques aimed at reducing the impact of non-enzymatic production of acetaldehyde have for the most part found little or no acetaldehyde in peripheral blood after ethanol administration (Eriksson and Fukunaga, 1993; Eriksson and Peachy, 1980; Lindros, Stowell, Pikkarainen and Salaspuro, 1980). The fact that blood acetaldehyde levels were consistently lower than blood ethanol levels suggested that most of the acetaldehyde formed in the liver is metabolized there and that relatively little acetaldehyde escapes from the liver, even after ethanol administration (Eriksson and Sipple, 1976).

The use of cerebrospinal fluid (CSF) samples to obtain measures of acetaldehyde levels avoids the problems associated with using blood samples, specifically non-enzymatically produced acetaldehyde. Several researchers have shown that CSF levels of acetaldehyde while measurable are often lower than peripheral blood acetaldehyde levels (Westcott, Weiner, Shultz and Myers, 1980; Kiianmaa and Virtanen, 1978; Pettersson and Kiessling, 1977). Westcott and coworkers (1980) used a push-pull perfusion technique, a precursor of in-vivo microdialysis, which allowed in-vivo analysis of the interstitial fluid of the brain. These authors showed that acetaldehyde can be found in CSF at micromolar concentrations after intragastic infusions of ethanol. Although CSF acetaldehyde levels are typically lower than peripheral blood acetaldehyde there is evidence that shows that CSF levels of acetaldehyde are positively and significantly correlated with peripheral blood acetaldehyde levels (Kiianmaa and Virtanen, 1978; Pettersson and Kiessling, 1977). Thus, it appears that as blood acetaldehyde levels rise,

so do CSF levels of acetaldehyde. It has been argued that the discrepancy between acetaldehyde levels in the blood and CSF may reflect some type of aldehyde dehydrogenase system within the capillary walls of the blood brain barrier, thus preventing acetaldehyde from freely crossing into the brain (Westcott et al., 1980; Kiianmaa and Virtanen, 1978; Tabakoff, Anderson and Ritzman, 1976; Sipple, 1974).

Although the concentration of acetaldehyde in blood or CSF after ethanol administration varies greatly, blood acetaldehyde may not be necessary for the accumulation of acetaldehyde in the CNS. That is, the transport of hepatically produced acetaldehyde to the brain is not necessary for the hypothesis that acetaldehyde may mediate some of the reinforcing and psychopharmacological effects of ethanol. All that is needed for central accumulation of acetaldehyde in the CNS is an effective ethanol-metabolizing system to be present in the brain itself.

Ethanol metabolism in brain

The production of acetaldehyde in brain has been a controversial topic. Kiessling (1962a, 1962b) has shown that acetaldehyde can be measured in brain tissue. Keissling found increased levels of acetaldehyde in brain tissue of rats treated with ethanol compared to brain tissue of control rats. However, several researchers have found little or no acetaldehyde in brain tissue (Sipple, 1974; Eriksson and Sipple, 1976; Tabakoff, Anderson and Ritzman, 1976) or CSF (Lindros and Hillbom, 1979). Sipple (1974) failed to measure acetaldehyde in brain tissue of rats until the cerebral blood acetaldehyde concentrations were greater than 250 uM/ml. Lindros and Hillbom (1979) have shown a lack of acetaldehyde in the cerebral spinal fluid of a small sample of human alcoholics. Mukherji, Kashiki, Ohyanagi and Slovitter (1975) used [1-$^{14}$C]-ethanol and found no

incorporation of radioactive carbon in amino acids or $CO_2$ in brain tissue and concluded that the brain was incapable of metabolizing ethanol. This conclusion was later challenged when Cohen, Sinet and Heikkila (1980) demonstrated that ethanol can in fact be metabolized in brain. The finding that ethanol metabolism in brain is possible was of great theoretical importance because it provided a route for central acetaldehyde accumulation that does not depend on transport of peripheral acetaldehyde into the CNS. Now that ethanol metabolism was shown to occur in brain, the next step was to determine the mechanism responsible, and thereby identifying the putative process for mediating the psychopharmacological effects of ethanol. The finding that the brain contained the biological mechanisms necessary to metabolize ethanol, and thus to form acetaldehyde in the CNS was of central importance to the theory that acetaldehyde is crucial in mediating some of the effects of ethanol (Gill, Menez, Lucas and Deitrich, 1992; Aragon, Rogan and Amit, 1991, Aragon, Stotland and Amit, 1991, Cohen, Sinet and Heikkila, 1980).

The major enzyme responsible for ethanol metabolism in liver is ADH and may thus also play an important role in the metabolism of ethanol in brain. The presence of ADH in brain has been a controversial topic. Several studies have reported the presence of brain ADH in a limited amount using different methods (Kerr, Maxwell and Crabb, 1989; Buhler, Pestalozzi, Hess and Wartburg, 1983; Raskin and Sokoloff, 1970, 1972, 1968). Raskin and Sokoloff (1972, 1970, and 1968) detected low levels of ADH activity in whole brain homogenates. Kerr et al. (1989) used an immunohistochemical method and found that ADH was limited to the neuronal cytoplasm of only some neurons. However, this particular method cannot distinguish between an enzymatically active form of the ADH enzyme and an inactive form.

Several laboratories have shown that the only ADH enzyme found in the brain is one that is incapable of metabolizing ethanol (Rout, 1992; Giri, Linnoila, Neil and Goldman, 1989; Julia, Farres and Pares, 1987; Beisswenger, Holmquist and Vallee, 1985). Recently, six different alcohol dehydrogenase classes have been characterized according to their affinity towards ethanol (Jörnvall, and Höög, 1995). Class I ADH isozyme is capable of metabolizing short chain alcohols and has a high affinity for ethanol, while the class III ADH isozyme is incapable of metabolizing short chain alcohols, and thus incapable of metabolizing ethanol (Beisswenger et al., 1985). Beisswenger, Holmquist and Vallee (1985) have shown that the only ADH enzyme present in brain is the class III isozyme. It has also been confirmed that the class III ADH isozyme is the only ADH enzyme in brains of mice from different strains (Rout, 1992), rats (Julia, Farres and Pares, 1987) and humans (Giri, Linnoila, Neil and Goldman, 1989).

Furthermore, it has been demonstrated that the ADH enzyme is not involved in the metabolism of ethanol in brain. Gill, Menez, Lucas and Deitrich (1992) have shown that the administration of 4-methylpryazole, an ADH inhibitor, failed to decrease acetaldehyde levels in brain tissue. These researchers have concluded that ADH does not contribute to central acetaldehyde production. In conclusion, the lack of class I ADH and the predominance of class III ADH isozymes effectively prevents the metabolism of ethanol through the ADH pathway. In light of these findings, ADH seems an unlikely candidate for the metabolism of ethanol in brain.

The microsomal ethanol oxidizing system, and in particular, cytochrome P-450 2E1 has been localized in rat brain tissue (Anandatheerthavarasa, Shankar, Bhamre,

Boyd, Song and Ravindrananth, 1993; Hansson, Tindberg, Ingelman-Sundberg and Kohler, 1990). The presence of MEOS in brain may possibly provide a route for ethanol metabolism and thus central acetaldehyde production. However, Gill, Menez, Lucas and Deitrich (1992) have shown that the cytochrome P-450 inhibitor metyraprone did not decrease acetaldehyde production in brain homogenates and have thus discounted the role of MEOS in the central metabolism of ethanol.

Catalase has been found to be distributed in microperoxisomes in the brain (Gaunt and De Duve, 1976; Zimatkin and Lindros, 1996). As in the case of ADH and MEOS, the presence of an ethanol-metabolizing enzyme, in this case catalase, in the brain provides a possible route for the metabolism of ethanol centrally. Since the abilities of ADH and MEOS have been shown to be incapable of metabolizing ethanol in brain, catalase remains the most likely route for central ethanol metabolism. In fact, the role of brain catalase in ethanol metabolism has been shown in studies through the use of brain homogenates and several enzymatic inhibitors. For example, studies have shown that inhibition of the enzyme catalase with 3-amino-1,2,4-triazole (AT) resulted in a decrease in acetaldehyde production compared to saline treated animals (Cohen et al, 1980; Aragon, Rogan and Amit, 1991; Aragon, Stotland and Amit, 1991). Several other catalase inhibitors, sodium azide (Gill, Menez, Lucas and Deitrich; 1992), cyanamide (Gill, Menez, Lucas and Deitrich; 1992; Aragon, Stotland and Amit, 1991; Cederbaum and Dicker, 1985) and 4-hydroxypyrazole (Aragon, Stotland and Amit, 1991) have also been shown to decrease catalase activity and subsequently acetaldehyde accumulation. Thus, it seems that catalase can serve as a significant route for ethanol metabolism in brain, while the roles of ADH and MEOS has been virtually eliminated. Finally, ethanol

and methanol, which are competitive agents for catalase, have been shown to protect the activity of catalase from AT administration (Nelson, 1957; Gill et al., 1992).

The elimination of acetaldehyde in brain has been shown by Mukherji et al (1975). These researchers have showed that [1,2-$^{14}$C] acetaldehyde is metabolized in brain tissue suggesting that the brain contains an efficient aldehyde dehydrogenase system. Thus, during normal ethanol metabolism, the catalase system together with the aldehyde dehydrogenase system can maintain acetaldehyde concentrations in the low micromolar range (Aragon, Rogan and Amit, 1992; Gill, Menez, Lucas and Deitrich 1992, Aragon and Amit, 1985).

Micromolar concentrations of acetaldehyde have been shown to have significant biochemical consequences in the brain (Gill et al., 1992; Amir, Brown and Amit, 1980). Acetaldehyde has been shown to affect the metabolism of the biogenic amines (see Truitt and Walsh, 1971) and 5-hydroxytryptamine (Lahti and Majchrowicz, 1969, 1967). In addition, low micromolar concentrations of acetaldehyde has been shown to impair microtubule function leading to cell injury (Tuma, Smith and Sorrel, 1991) and to affect membrane potentials (Brown and Carpentier, 1990). Thus, it can be concluded that the concentration of acetaldehyde formed in brain after ethanol consumption is significantly within the range needed to produce biochemical changes.

Taken as a whole, these above studies implicate catalase as the major enzyme metabolizing ethanol in brain and that the biological mechanism for acetaldehyde production in the brain has been established. It has been proposed that the activities of brain catalase and of brain ALDH are important factors that mediate acetaldehyde concentrations in the brain (Gill, Amit and Smith, 1996; Aragon and Amit, 1985).

Correlation between catalase activity and ethanol intake

If the production of acetaldehyde in brain is thought to mediate the propensity of organisms to consume ethanol (Smith, Aragon and Amit, 1997), it would follow that there should be a correlation between the capacity of the brain to produce acetaldehyde and ethanol drinking. More specifically, if catalase is the primary enzyme responsible for the metabolism of ethanol in brain, then there should be a correlation between brain catalase activity and ethanol consumption. A study by Aragon, Sternklar and Amit (1985) demonstrated a positive correlation between brain catalase activity and ethanol drinking in rats. In this study, rats were maintained exclusively on either a 10% v/v concentration of ethanol or water for a period of 25 days. Rats from both groups were sacrificed either immediately after or 15 days after the end of the maintenance period. Brain catalase was measured and the results revealed a significant positive correlation, for the ethanol group, between ethanol intake and catalase activity in both the immediate and 15 day groups. Moreover, there was no difference in catalase activity between the ethanol group and the water group, suggesting that catalase was not induced by prolonged ethanol consumption, even when ethanol was the sole fluid available. The finding that catalase activity was not induced by prolonged exposure to ethanol rules out the possibility that ethanol consumption itself increases catalase activity, which may then lead to an increase in ethanol consumption.

In a subsequent study (Amit and Aragon, 1988) blood catalase was collected in young ethanol naive rats. These rats were then given access to and maintained on ethanol (10% v/v) and water in a free choice paradigm for 30 days. Animals were sacrificed 10 days after the last ethanol presentation. Blood samples and brains were removed from

each rat to determine catalase levels. A positive correlation between blood catalase activity and ethanol drinking was obtained supporting the previous finding that catalase activity and ethanol intake are related. In addition, brain catalase activity was positively correlated with blood catalase sampled before ethanol exposure and after ethanol exposure suggesting that blood catalase activity can also be used as an indicator of the catalase activity in brain.

Studies performed on rat strains selectively bred for differing ethanol consumptions levels have produced mixed results concerning catalase as the mediator of ethanol consumption. Gill, Amit and Smith (1996) measured brain catalase activity in the ethanol preferring (P) strain and the ethanol nonpreferring (NP) strain of rats. They found that the P rats had significantly higher brain catalase activity than the NP rats. In addition, Aragon and Amit (1993) have shown differences in the pattern of ethanol consumption in a strain of mice that differ in their catalase activity. The acatalasemic strain of mice (C3H-A) was shown to consume more ethanol than the normal strain of mice (C3H-N). This result is however contrary to previous findings that reduced catalase activity in rats produced a decrease in ethanol intake (Aragon and Amit, 1992; Tampier, Quintanilla and Mardones, 1995). Aragon et al. (1992) argued that this discrepancy may be explained by species differences. The important fact, however, is that catalase activity is related to a pattern in ethanol consumption, regardless of the direction.

Contrary results regarding catalase activity and ethanol consumption have been obtained in another line of rats selected for their preference to consume ethanol. Tampier, Quintanilla and Mardones (1995) have found no differences in brain catalase activity between their line of high drinking (UChB) and low drinking (UChA) strains of

rats. Thus, the authors argued that catalase activity could not be used as an explanation for the differences in voluntary ethanol consumption between the two strains of rats.

Koechling and Amit (1992) studied the relation between blood catalase activity and ethanol drinking in a human population. In a sample of 191 Caucasian adults, subjects were asked to complete several questionnaires about their alcohol consumption as well as to provide a blood sample. The questionnaires involved were the Michigan Alcoholism Screening Test (MAST), the MacAndrew Scale of the MMPI (MAC) and the Concordia Alcohol Screening Questionnaire (CASQ). The results of the catalase assay revealed that blood catalase activity was positively and significantly correlated to the typical alcohol intake (Q-Value) level of the subjects.

In order to assess whether catalase activity is inheritable Koechling, Amit and Negrete (1995) studied the possible relation between familial history of alcoholism and catalase activity. A sample of 421 human subjects with either a family history of alcoholism (FH+) or without family history of alcoholism (FH-) completed the MAST, MAC, and CASQ and provided a blood sample. Results showed that FH+ subjects had significantly higher blood catalase activity and higher Q-Value means than FH- subjects.

The correlational studies (Koechling and Amit, 1992; Koechling et al., 1995; Aragon and Amit, 1988; Aragon et al., 1985) mentioned above provide evidence that support the hypothesis that catalase activity may play an important role in regulating ethanol consumption. Experimental studies on the manipulation of catalase activity provide further evidence that catalase activity may mediate many of the psychopharmacological effects associated with ethanol consumption.

3-Amino-1,2,4-triazole; Biochemistry and inhibition of catalase

The drug 3-amino-1, 2,4-triazole (AT) is a potent catalase inhibitor. AT has been shown to decrease catalase activity in rat liver and kidney (Heim, Appleman and Pyfrom, 1956; Tephly, Mannering and Parks, 1961) as well as in erythrocytes (Gaetani, Kirkman, Mangerini and Ferraris, 1994; Tephly, Mannering and Parks, 1961) after intraperitoneal and intravenous injections. AT inhibits the activity of catalase via an irreversible reaction with Compound I (Darr and Fridovich, 1986; Nicholls, 1962). Administration of AT results in a dose-dependent decrease in catalase activity in brain and liver homogenates (Aragon and Amit, 1992; Tephly, Mannering and parks, 1961). Catalase activity has been shown to be maximally inhibited from 1.5 to 5 hours after an injection of AT. Similarly, catalase activity in a strain of mice (ADH-negative deermouse) was inhibited by 87% 1.5 hours after an injection of AT and returned to control levels after 6 hours (Handler, Bradford, Glassman, Ladine and Thurman, 1986). Aragon, Rogan and Amit (1991) have shown that catalase activity is maximally inhibited 3 to 5 hours after a single IP injection of AT in rat brain while liver catalase activity was maximally inhibited between 1 to 3 hours after AT administration. In this study, liver catalase activity had not reached control levels until 24 hours after AT administration, while brain catalase activity remained below control levels after 48 hours.

Ethanol consumption and AT administration

Several researchers have shown that the inhibition of catalase, via AT administration, produced changes in the consumption of ethanol. Aragon and Amit (1992) showed that the administration of AT produced a dose dependent decrease in ethanol consumption in rats. A dose of AT as low as 0.625 g/kg produced an attenuation

of ethanol consumption, while the largest dose (1g/kg) produced the greatest decrease. Brain catalase activities of the AT treated animals were significantly lower than control animals. Aragon and Amit (1992) argued that catalase is likely to play a role in the regulation of ethanol consumption since a decrease in catalase activity resulted in a decrease in ethanol consumption. This notion is supported by a significant positive correlation between catalase inhibition and ethanol intake. That is, those animals that showed a greater amount of catalase inhibition also showed greater decreases in ethanol intake.

The administration of AT was also shown to decrease both ethanol consumption and preference in Swiss-Webster albino mice (Koechling and Amit, 1994) as well as to attenuate the acquisition of ethanol consumption in rats (Rotzinger, Smith and Amit, 1994). The effect of AT was argued to be specific to ethanol because there was no decrease in total fluid intake (Aragon and Amit, 1992; Koechling and Amit, 1994; Rotzinger, Smith and Amit, 1994). As well, it was argued that AT did not have an effect on caloric intake since there was no measurable differences in the weights of the AT injected group and the saline control group (Koechling and Amit, 1994).

Ethanol mediated behaviours and AT administration

Acetaldehyde has been implicated in mediating some of ethanol's psychopharmacological effects through its production in brain. Aragon, Spivak and Amit (1985) have shown that pretreatment with the catalase inhibitor AT attenuated an ethanol-induced conditioned taste aversion (CTA). Furthermore, the effects of AT were specific to an ethanol-induced CTA since AT pretreatment failed to attenuate either a morphine- or lithium-induced CTA (Aragon et al., 1985). Aragon, Spivak and Amit (1989) have

also demonstrated that pretreatment with AT attenuated ethanol-induced motor depression in rats.

Pretreatment with AT has also been shown to attenuate ethanol-induced narcosis and lethality (Aragon, Spivak and Amit, 1991; Tampier, Quintanilla, Letelier and Mardones, 1988). Tampier et al. (1988) administered AT 1-hour before an ethanol challenge and showed a reduction in ethanol-induced period of narcosis time in rats. However, administration of pyrazole, an alcohol dehydrogenase inhibitor, actually increased the length of narcosis following an ethanol challenge. Pretreatment with AT failed to shorten the narcosis period in a group of rats that were then injected with pyrazole followed by a challenge with ethanol. Thus it was argued that inhibition of acetaldehyde in brain and not in the periphery may mediate the narcotic effects of ethanol. In a subsequent study (Tampier and Quintanilla, 1991) it was shown that tolerance to the narcotic effects of ethanol was inhibited by pretreatment with AT while tolerance to the hypothermic effect of ethanol was not. In addition, pretreatment with AT has also shown to attenuate the lethal effects of ethanol (Aragon, Spivak and Amit, 1991; Tampier and Quintanilla, 1991).

Taken as a whole, these findings support the idea that centrally produced acetaldehyde may mediate some of the psychopharmacological effects of ethanol. The catalase inhibitor AT has been shown to effectively reduce catalase activity in brain and as a result attenuate several ethanol-induced behaviours. Acetaldehyde seems to play a putative role in the consumption of ethanol as well as the narcotic, lethal and motor effects of ethanol.

The attenuation of ethanol consumption by AT administration has been argued to occur through the decrease in acetaldehyde levels, which then attenuates the reinforcing properties of ethanol (Smith, Aragon and Amit, 1997; Hunt, 1995; Lindros, 1978; Truitt and Walsh, 1971). However, this notion has recently been questioned. Tampier, Quintanilla and Mardones (1995) have argued that the decrease seen in ethanol drinking as the result of the administration of AT is not due to the inhibition of catalase but to a decrease in the need for calories.

Mardones, Contreras and Segovia-Riquelme (1988) have developed a method for recognizing whether the effects of a particular manipulation, usually some type of drug, are specific to ethanol or the result of an effect that is specific to the consumption of calories. This method uses several measures of ethanol, food and water intakes. One of these measures is a ratio of the caloric intake from ethanol and food (Calorie Ratio (CR) = ethanol intake/food intake). It is assumed that the calories provided by ethanol and food remain constant throughout the experiment. That is, one ml of ethanol and one gram of food will always provide the same amount of calories respectively. With this in mind, if a drug treatment produces an effect that is specific to ethanol consumption, then CR should increase or decrease according to whether the drug manipulation increased or decreases ethanol consumption. If, however, the drug treatment produces an effect that produces a general effect on the need for calories, then CR should show no significant change. That is, the drug treatment produces an equal decrease, or increase, in both ethanol and food intakes.

Tampier et al. (1995) measured the effects of AT on ethanol, water and food intake. Administration of AT resulted in a decrease in ethanol intake in their strain of

high drinking rats (UChB) but not in the low drinking strain (UChA). Both strains had shown a decrease in food intake. More importantly, the CR of both strains did not significantly change following AT administration suggesting that the effect of AT is not specific to ethanol consumption but rather to a mechanism that regulates caloric intake. Thus, Tampier et al. (1995) have concluded that the decrease in ethanol consumption produced by AT is a secondary effect to the decrease in the need for calories.

Ethanol and caloric intake

Ethanol has been long thought to be a potential source of calories and therefore ethanol consumption could be regulated in the same manner as food (Forsander, 1994; Richter, 1953). The "caloric hypothesis" of ethanol intake assumes that ethanol consumption is mainly regulated through its caloric properties. This assumption stems from evidence reporting that the intake of both food and ethanol are tightly regulated. Animals will maintain a constant intake of calories despite dilution of food with water or noncaloric substances (Kissileff and Van Itallie, 1982). In the same manner, the consumption of ethanol is regulated so as to maintain a particular level of absolute ethanol intake despite the presentation of different concentrations of ethanol (e.g. Boyle, Smith , Spivak and Amit, 1994). Animals will decrease the amount of ethanol consumed as the concentration of the ethanol solution is increased in order to maintain a steady intake of absolute ethanol (Boyle, Smith , Spivak and Amit, 1994). In a similar fashion, when animals are administered ethanol intraperitoneally or via gastric intubation, the amount of orally consumed ethanol is subsequently decreased by the same amount of ethanol that was administered (Waller, McBride, Gatto, Lumeng and Li, 1984).

Forsander and Sinclair (1988) have shown that when ethanol is consumed, there is an equal and corresponding drop in food intake so that the overall caloric intake remains unchanged. These researchers have suggested that ethanol consumption may be regulated by a mechanism that controls overall caloric consumption. In addition, Stiglick and Woodworth (1984) have shown that food restricted rats consumed more ethanol than food satiated rats and have concluded that the caloric value of ethanol is an important determinant in ethanol consumption. Support for the caloric hypothesis has also been drawn from evidence showing that when food restriction is lifted, an increase in the amount of food consumed is accompanied by a corresponding reduction in ethanol intake (Richter, 1953; Westerfeld and Larrow, 1953).

The caloric hypothesis assumes that ethanol consumption may be regulated through an increase or decrease in the consumption of calories from other sources, particularly food. The caloric hypothesis assumes that decreases in food intake may result in an increase in ethanol intake so as to compensate for the loss of calories. Although it has been shown that a decrease in food intake resulted in an increase in ethanol consumption (Forsander and Sinclair, 1988; Stiglick and Woodworth; 1984), this is not always the case. Gill, Amit and Smith (1996b) have shown no significant correlation between ethanol intake and food intake. Using a microstructural paradigm these researchers have shown that the amount of food consumed after an ethanol bout was the same as the amount of food consumed after a water bout. If one were to expect a decrease in food intake in response to the extra calories provided by ethanol consumption, then food intake prandially associated with ethanol consumption should be smaller than food intake associated with water consumption. This was not the case and

thus in inconsistent with the idea that ethanol consumption is mediated by a mechanism regulating caloric intake.

Further evidence against a caloric regulation of ethanol consumption includes studies that have shown that a decrease in food intake did not result in a corresponding increase in ethanol intake (Linesman; 1989, 1987; Weisinger, Denton and Osborne, 1989). Westerfeld and Larrow (1953) have shown that ethanol consumption is increased during food restriction but that the increase in ethanol intake did not occur until the amount of food restriction was 50% of ad libitum levels. In addition, the amount of caloric intake from ethanol consumption did not completely replace the calories lost to food restriction. In other words, caloric compensation was incomplete.

Finally, food restriction itself has been shown to increase the intake of drugs that have no caloric value (Carroll and Meisch, 1981; Carroll, France and Meisch, 1981; Carroll and Meisch, 1979). The results of the above studies suggests that food restriction may lead to increased ethanol consumption via some mechanism that will also increase the intake of other noncaloric-containing drugs rather than through a mechanism that regulates caloric intake. For example, Koutsoukos, Harding and Grupp (1995) have postulated that the increase in ethanol consumption due to weight reduction or food restriction may possibly be due to changes in the renin-angiotensin system rather than a caloric mechanism.

The present investigation

The present set of experiments were designed to investigate whether the catalase inhibitor AT produced a decrease in caloric requirement that is primarily responsible for the decrease in ethanol consumption. Tampier et al. (1995) hypothesized that AT acted

in such a manner as to decrease the need for caloric intake and as a result produced an attenuation of ethanol consumption. These researchers have concluded that previous experiments reporting a decrease in ethanol consumption after the administration of AT (Koechling and Amit, 1994; Rotzinger, Smith and Amit, 1994, Aragon and Amit, 1992) and which have attributed this decrease to the attenuation of the reinforcing properties of ethanol are erroneous. Although Tampier et al. (1995) argue that AT produces a primary effect on the regulation of calories, they do not argue against the role of AT in attenuating other nonconsummatory ethanol-induced behaviours (Aragon, Spivak and Amit, 1989; Tampier, Quintanilla, Letelier and Mardones, 1988; Aragon, Spivak and Amit, 1985). Experiments 1B and 2 were designed to assess the effects of AT on ethanol, food and water intake. Specifically, these experiments were designed to test whether the decrease in ethanol intake usually seen with the administration of AT resulted from a decrease in caloric intake. In addition, the first experiment attempted to elucidate whether AT has an affect on the taste reactivity of the animals. The major focus in the present thesis is to measure the changes that may occur in fluids and food intake in response to AT. In thus doing so, it is hoped that the relationship between ethanol, food and water consumption, in response to catalase inhibition, and in particular the role of the catalase inhibitor AT can be further elucidated.

## EXPERIMENT 1A and 1B

Researchers have shown that the enzyme catalase is capable of metabolizing ethanol into acetaldehyde in brain homogenates (Aragon, Rogan and Amit, 1992; Gill, Menez, Lucas and Deitrich, 1992). The drug 3-amino-1, 2,4-traizole (AT) is a noncompetitive, irreversible catalase inhibitor that was used to assess catalase's, and thus acetaldehyde's, role in mediating the reinforcing effects of ethanol. Catalase inhibition, through AT administration, has been shown to decrease ethanol consumption in rats maintained on free choice between ethanol and water (Aragon and Amit, 1992) as well as in mice (Koechling and Amit, 1994). The administration of AT has also been shown to prevent the acquisition of ethanol consumption in rats (Rotzinger, Smith and Amit, 1994). AT has also been reported to attenuate many ethanol-induced behaviours such as conditioned taste aversions (Aragon, Spivak and Amit, 1985), locomotor depression (Aragon, Spivak and Amit, 1989) as well as narcosis and lethality (Aragon, Spivak and Amit, 1991; Tampier, Quintanilla, Letelier and Mardones, 1988). The inhibition of catalase by AT is thought to exert it's effects by inhibiting the production of acetaldehyde, and thus reducing the positive reinforcing properties of ethanol resulting in a decrease in ethanol consumption (Aragon and Amit, 1992). That is, the effect of AT on ethanol intake was assumed to be specific and due solely to the inhibition of catalase, and thus acetaldehyde (Aragon and Amit, 1992). However, this position has recently been questioned.

Tampier, Quintanilla and Mardones (1995) have argued that the decrease in ethanol intake seen following AT administration is secondary to AT's effect on the intake of calories. In their studies, AT decreased ethanol intake of the high drinking strain of

rats (UChB) but not in their low drinking strain (UChA). Furthermore, AT had decreased food intake in both strains of rats. The argument that Tampier et al. put forward was that AT had a primary effect of reducing the need for calories, as seen by the decrease in food intake in both strains of rats, and that a decrease in ethanol intake was simply a byproduct of this decreased need for calories. They have thus argued that AT does not produce its effects by blocking the reinforcing properties of ethanol but rather by reducing the need for calories.

The primary purpose of the present study was to investigate whether the catalase inhibitor AT produces its decrease of ethanol consumption by reducing the positive reinforcing effects of ethanol, or rather by producing a primary anorectic effect of which the decrease in ethanol is merely a byproduct. As well, there is reason to believe that AT may also alter the taste reactivity of rats leading to a decrease in the consumption of flavoured substances (Rotzinger, Smith and Amit, 1994). In their study Rotzinger et al. (1994) showed that AT administration decreased the aquisition of both ethanol and a saccharin-quinine solution. Since the saccharin-quinine solution does not have positive reinforcing properties, then AT must have produced some kind of nonspecific change in the way the rat perceived the taste of this solution. Therefore, a second objective of the present study was to test whether AT had differential effects on several flavoured food diets.

Experiment 1A was a preliminary study whose aim was to establish a concentration at which a saccharin-flavoured food would be preferred over an unflavoured (neutral) food as well to establish a concentration of quinine in food that

would be preferred less than the unflavoured (neutral) food, would be still consumed significantly by the animals.

## Method

Subjects

A total of 48 male Long-Evans rats (Charles River Canada Inc.) were used. Animals were housed individually in single or double hanging stainless steel cages in a temperature controlled colony room with a 12:12 hour light/dark cycle (lights on at 08:00).

Food

An unflavoured (neutral) food was prepared by mixing a powdered animal chow (Labdiet #5001) with tap water (80 mls water/100 g powdered food) to produce a thick, consistent wet mash. Flavoured foods were prepared by adulterating the neutral food with either sodium saccharin or quinine sulfate. Foods were prepared daily and presented to the animals, in small ceramic food cups that were attached to the front of the cage by metal straps. Small trays were placed underneath the cages to catch any food that was spilled.

Procedure

All animals were introduced to the mashed food diet for 12 days, of which the last 5 days were used as baseline days. Animals were then divided into 6 groups (n=5) with each group being given a different concentration of saccharin adulterated food. The concentrations of saccharin used were 0.7, 0.8, 0.9, 1.0, 1.5 and 2.0% (w/w) respectively. The saccharin-flavoured food was given for 5 days.

Following the above procedure it was found that additional trials were needed to establish if the rats could actually differentiate a saccharin-flavoured food from the neutral-flavoured food. Thus, rats were tested on their preference for the neutral-flavoured food or the saccharin-flavoured food in a two-cup free-choice situation. In this test, animals were housed individually in double stainless hanging cages that were fitted with two food cups. Neutral-flavoured food was given in both cups for a period of 5 days to ensure that steady levels of intake were attained. After this period, the neutral-flavoured food and a particular concentration of the saccharin-flavoured food was presented to the animals for 4 consecutive days. One food cup contained the neutral-flavoured food while the other contained the saccharin-flavoured food. The position of the food cup containing the saccharin-flavoured food was switched so as to prevent the acquisition of a side bias. The concentrations of saccharin used in this experiment were 0.5, 0.6, 0.7, and 0.75% (w/w). Rats were assigned to one of the 4 saccharin concentration groups.

Some of the same animals were then tested on several different concentrations of a quinine-flavoured food. During this procedure, rats were given the neutral-flavoured food for 4 days and then were switched to a quinine-flavoured food that had a concentration of 0.01, 0.05, 0.1 or 0.15% w/w. A second group of rats were run using quinine concentrations of 0.2 and 0.3% (w/w).

## Results

The analysis of the first saccharin test revealed a significant interaction of saccharin concentration by food flavour [$F(5,20)=7.14$, $p<0.01$]. Simple effects holding the saccharin concentration variable constant showed that the neutral flavoured food was

consumed significantly greater than the saccharin flavoured food at the 0.9% [$F(1,4)= 15.37$, $p<0.017$], 1.5% [$F(1,4)= 123.09$, $p<0.001$] and 2.0% [$F(1,4)= 12.73$, $p<0.025$] w/w concentrations suggesting that the rats did not prefer the saccharin flavoured food at the higher concentrations.

In order to test whether a saccharin flavoured food could be differentiated from the neutral flavoured food a second study was conducted. Analysis of the second saccharin test revealed a significant concentration by Food interaction [$F(3,24)= 4.22$, $p<0.0156$). Simple effects showed that rats consumed more of the saccharin flavoured food than the neutral-flavoured food at the 0.6% w/w concentration [$F(1,12)= 11.46$, $p<0.005$] suggesting that rats could differentiate and prefer a saccharin flavoured food..

The results of the quinine food tests showed that the quinine-flavoured food was consumed significantly less than the neutral-flavoured food at the 0.15% concentration [$F(1,9)= 16.58$, $p<0.002$]. At the concentrations of 0.01, 0.05 and 0.1% w/w, the quinine-flavoured food was not different than the neutral flavoured food. The additional quinine test using 0.2 and 0.3% (w/w) concentrations of quinine showed a significant main effect for the food group [$F(1,4)= 5.55$, $p<0.01$]. The quinine-flavoured food was consumed significantly less than the neutral-flavoured food at the 0.2% w/w ($p<0.05$) and 0.3%w/w ($p<0.05$) concentrations.

## Discussion

The results of the first saccharin test showed that rats consumed significantly less of the saccharin-flavoured food than the neutral-flavoured food at the 0.9, 1.5 and 2.0% w/w concentrations. Thus, at these concentrations the neutral-flavoured food was preferred over the saccharin-flavoured food. There were no significant differences in

food consumption between the saccharin-flavoured food and the neutral-flavoured foods at the 0.7, 0.8 and 1.0% w/w saccharin concentrations. At the concentrations used in this test (0.9-2.0% w/w) it was impossible to tell whether the rats perceived the saccharin-flavoured food as different from the neutral-flavoured food (figure 1; top graph). Thus, to test whether the rats could differentiate a saccharin-flavoured food from the neutral-flavoured food a two-cup free-choice test was implemented. The results of this test showed that rats could differentiate a saccharin-flavoured food from the neutral-flavoured food at a concentration of 0.6% w/w (figure 1; middle graph).

The results of the analysis of the first quinine food test showed that the quinine-flavoured food, at a concentration of 0.15% w/w, was consumed significantly less than the neutral-flavoured food [$F(2,18)= 21.88$, $p<0.001$]. There was no difference in the amount of food consumed between the quinine-flavoured food (0.01, 0.05 or 0.1% w/w) and the neutral-flavoured food. The additional quinine food test showed that at concentrations of 0.2 and 0.3% w/w the quinine-flavoured food was consumed significantly less than the neutral-flavoured food [$F(1,4)= 5.55$, $p<0.01$].

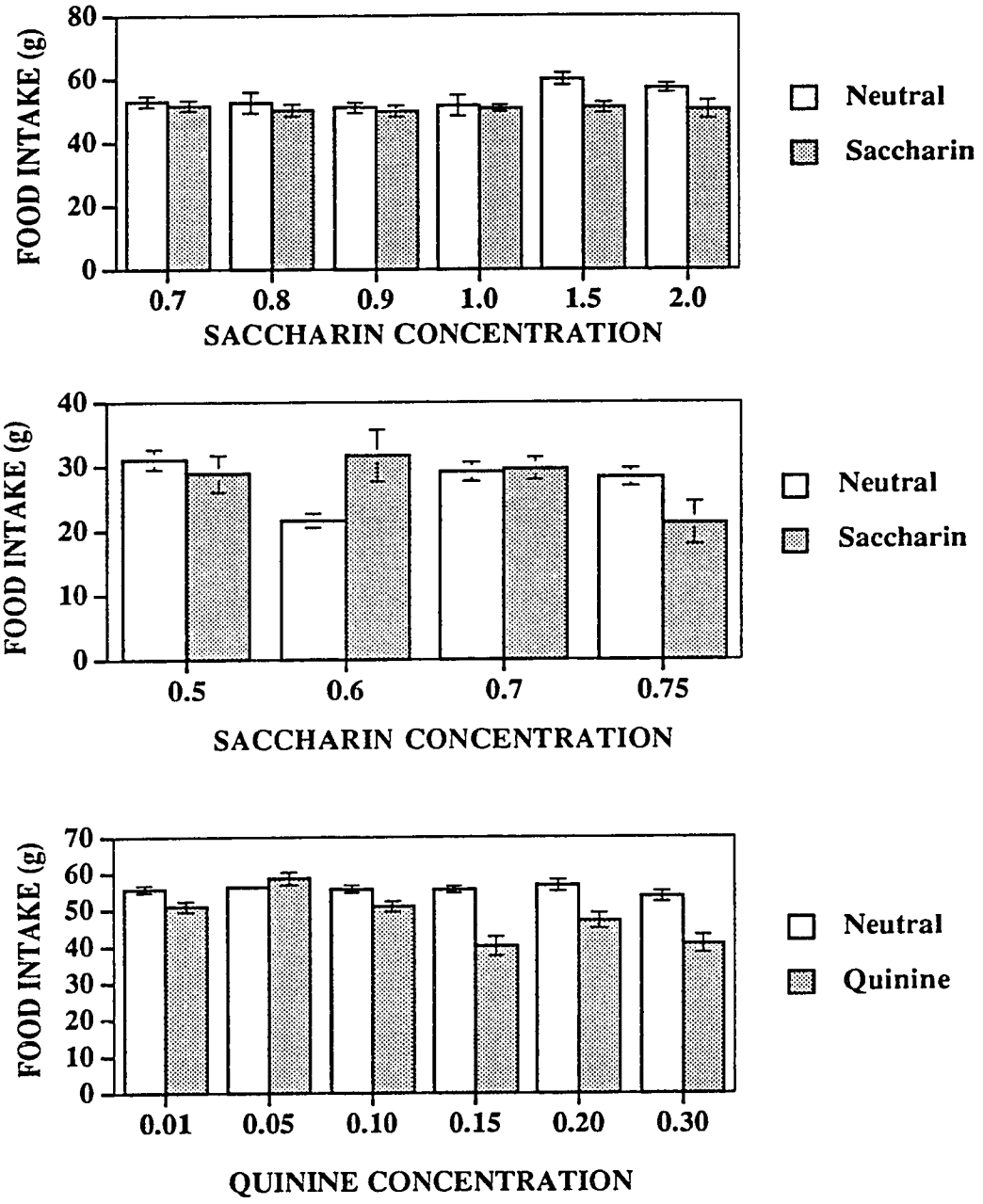


Figure 1. Mean food intake (g) for differing saccharin concentrations (top and middle graphs) and quinine concentrations (lower graph). Vertical lines represent S.E.M.

## Experiment 1B

The primary purpose of the present experiment was to assess whether the catalase inhibitor 3-amino-1,2,4-triazole (AT) produces an attenuation of ethanol consumption due to a decrease in the production of central acetaldehyde or to some nonspecific effect which acts to decrease the caloric requirement of the animal. In addition, a secondary purpose of the present study was to test whether AT has an effect on taste reactivity. The results of the previous experiment revealed that rats will prefer a saccharin flavoured food at a concentration of 0.6% w/w while avoiding a quinine flavoured food at a concentration of 0.15% relative to a neutral flavoured food. These concentrations of saccharin and quinine were used to assess whether AT produces a change in the taste reactivity of the animal. Rotzinger et al. (1994) reported that AT produced a decrease in the acquisition of a saccharin-quinine solution and suggested that AT may have altered the taste of this solution making it less palatable. Thus, it hypothesized that if AT does somehow alter that taste reactivity of the rat, that the quinine-flavoured food may be affected more than a neutral- or saccharin-flavoured food.

### Method

Subjects

Subjects were 63 male Long-Evans rats (Charles River Canada Inc.) each weighing 150 -175 grams before the start of the experiment. Animals were housed individually in hanging stainless steel cages in a temperature controlled colony room maintained on a 12:12 hour light/dark cycle (lights on at 08:00). Animals initially had free access to laboratory food pellets and water. Fluids were presented in two glass Richter tubes attached to the front of each home cage.

Drugs

3-amino-1, 2,4-triazole (AT) (Sigma co.) was dissolved in 0.9 % saline (1g/2ml/kg). A 10% (v/v) ethanol solution was prepared by diluting 95% laboratory ethanol with tap water. Sodium saccharin (BDH Fine Chemicals) and Quinine sulfate (Fisher Scientific) were used as noncaloric food flavouring.

Food

An unflavoured (neutral) food was prepared by mixing a powdered animal chow (Labdiet #5001) with tap water (80 mls water/100 g powdered food) to produce a thick, consistent wet mash. Flavoured foods were prepared by adulterating the neutral food with either sodium saccharin (0.6% w/w) or quinine sulfate (0.15% w/w). Foods were prepared daily and presented to the animals, in small ceramic food cups, in the late afternoon. Food and fluids were then measured and recorded in the late afternoon of the next day.

Procedure

During the 7 days of habituation to the housing conditions, all animals were first fed the unflavoured mashed diet. All animals had continuous access to and were maintained on this mashed diet for the remainder of the experiment. Animals were then exposed to 10% (v/v) ethanol solution through a forced choice procedure. For four consecutive days ethanol was the only fluid made available to the animals. On the fifth day water only was presented. From this point forward, and until the end of the experiment, EtOH was made available on an alternate day schedule, in a free choice with water. Water only was presented on the intervening days. The position of the Richter

tube containing the ethanol was alternated so as to avoid the acquisition of a position bias.

Animals were maintained on this procedure for a period of 20 days (10 EtOH presentations). Following this maintenance phase nondrinkers were eliminated from the study. A nondrinker was defined as an animal that, during the last 5 EtOH presentations of the maintenance phase, consumed less than 1.0 g/kg of EtOH.

Animals were then assigned to one of three food conditions. One group remained on the neutral food (n=16), a second group now received the saccharin-flavoured food (n=17), while the last group received the quinine-flavoured food (n=16). Animals would remain in their respective food condition throughout the remainder of the study.

A period of 10 days (5 EtOH presentations) was allowed for the animals to attain steady levels of ethanol, food and water intakes following the introduction of the flavoured foods (introduction phase). The introduction phase was followed by the baseline, drug and postdrug phases each consisting of 10 days (5 EtOH presentations). After the baseline phase animals in each food condition were assigned to either the drug or control condition. During the drug phase, injections of AT (1 g/kg) or saline were given intraperitoneally 2 and 2.5 hours before the presentation of food and fluids. Injections were given once per day during the five EtOH/water presentation days of the drug phase.

## Results

Statistical analyses were performed using a four factor mixed design repeated measures ANOVA with Food condition and Drug group as the between factors and Treatment phase and Days as the within factors. Significant interactions were further

analyzed by conducting simple effects tests (Kepple, 1991). Post hoc Tukey HSD tests were used to test significant differences between means. An alpha level of 0.05 was used to determine significant results.

Absolute ethanol intake, g/kg, is presented for the saccharin-, neutral- and quinine-fed groups in figure 2. The administration of 3-amino-1,2,4-triazole (AT) did not produce a significant decrease in absolute ethanol intake (g/kg) [$F(2,82)= 0.25$, $p<0.7796$] or preference ratios [$F(2,82)= 1.31$, $p<0.2752$] (figure 3) as indicated by nonsignificant Drug group by Treatment phase interactions. Water intake [$F(2,82)= 0.0.43$, $p<0.6505$] (figure 4) and total fluid intake [$F(2,82)= 0.42$, $p<0.6611$] also yielded nonsignificant Drug group by Treatment phase interactions suggesting that AT does not produce a general effect of fluid consumption.

Results showed that there was a significant interaction between Drug group and Treatment phase for food intake. Administration of AT produced a significant decrease in food intake whether measured in grams [$F(2,82)=5.38$, $p<0.0064$] or grams per kilogram of body weight [$F(2,82)=5.51$, $p<0.0057$] (figure 5). That is, the AT group significantly decreased food intake during the drug phase ($p<0.05$) compared to the saline controls. The food groups did not differ in the degree of attenuation in food intake demonstrated by a nonsignificant Food group by Drug group by Treatment phase interaction [$F(4,82)= 0.82$, $p<0.5131$] suggesting that AT did not affect taste reactivity.

Caloric intake from food was calculated using the metabolizable energy of the powdered Labdiet, which was listed as 3.04 Kcal/gram of food (PMI feeds). Caloric intake of ethanol was calculated using a value of 7.01 Kcal/gram (Agarwal and Goedde, 1990). Analysis of the data revealed a significant Drug group by Treatment phase

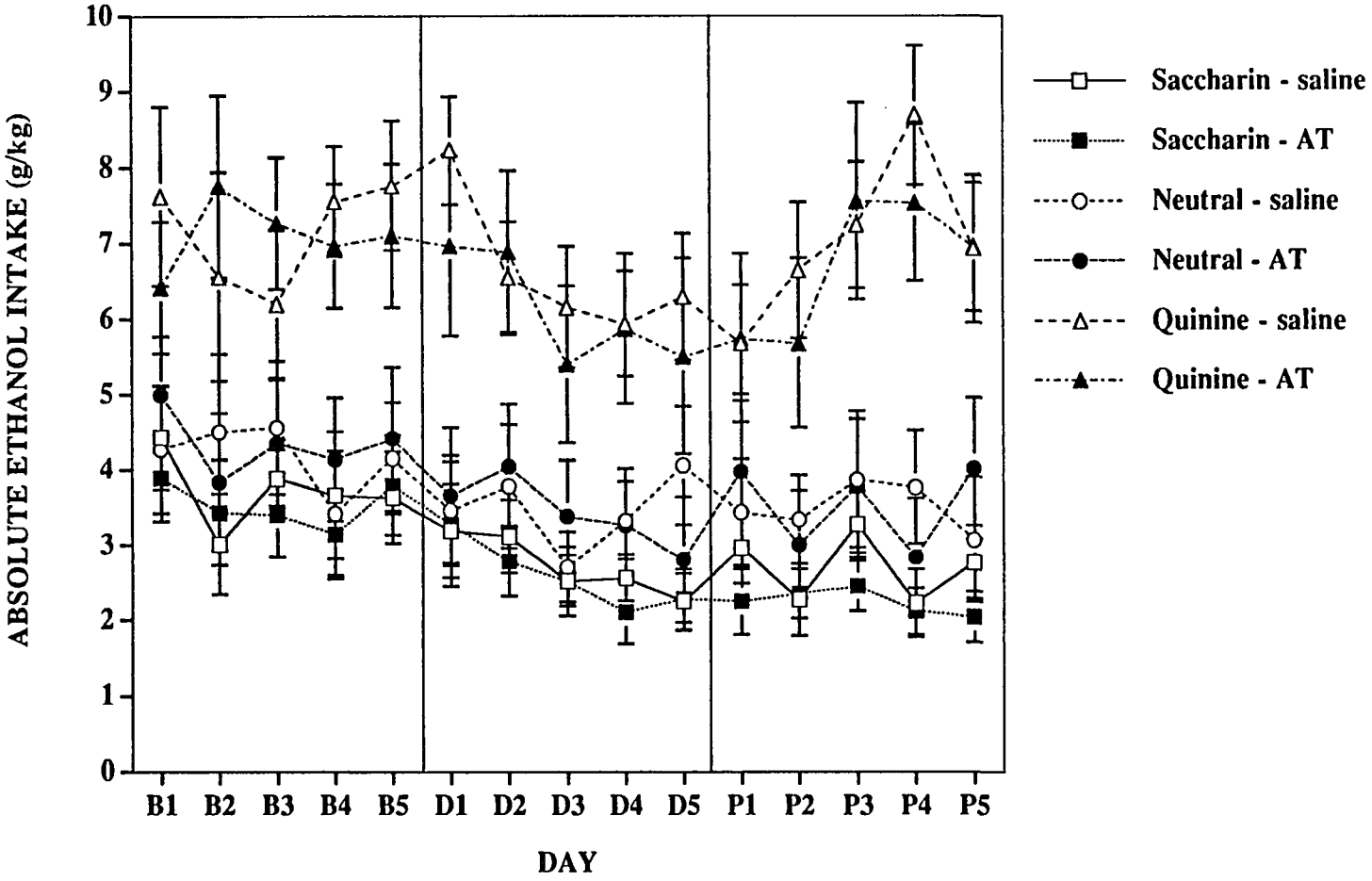


Figure 2. Mean absolute ethanol intake (g/kg) across baseline, drug and postdrug phases for the saccharin-, neutral- and quinine-fed groups. Vertical lines represent S.E.M.

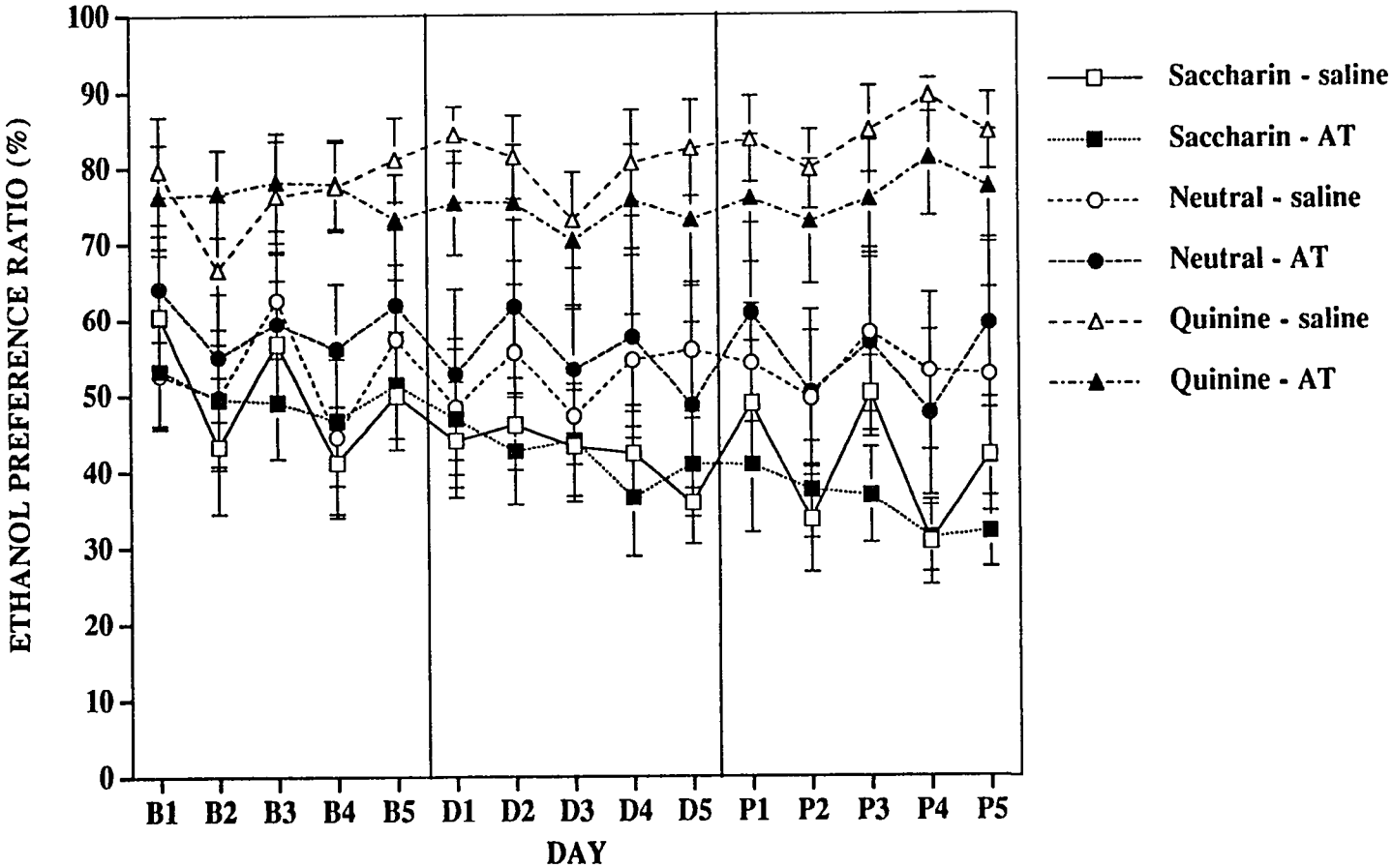


Figure 3. Effect of AT on mean ethanol preference ratio (ethanol mls/total mls) across baseline, drug and postdrug phases for the saccharin-, neutral- and quinine-fed groups. Vertical lines represent S.E.M.

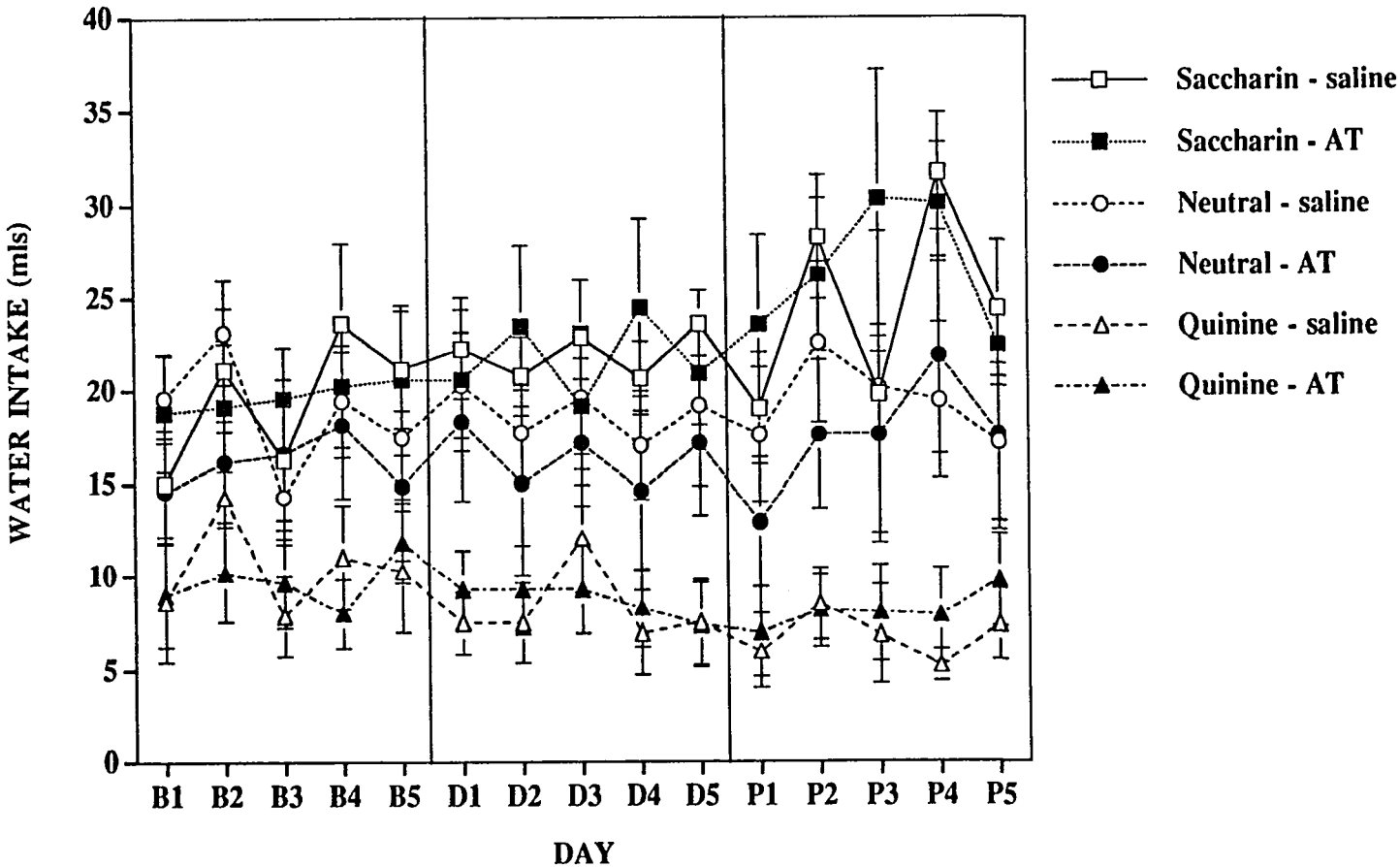


Figure 4. Effect of AT on mean water intake (mls) across baseline, drug and postdrug phases for the saccharin-, neutral- and quinine-fed groups. Vertical lines represent S.E.M.

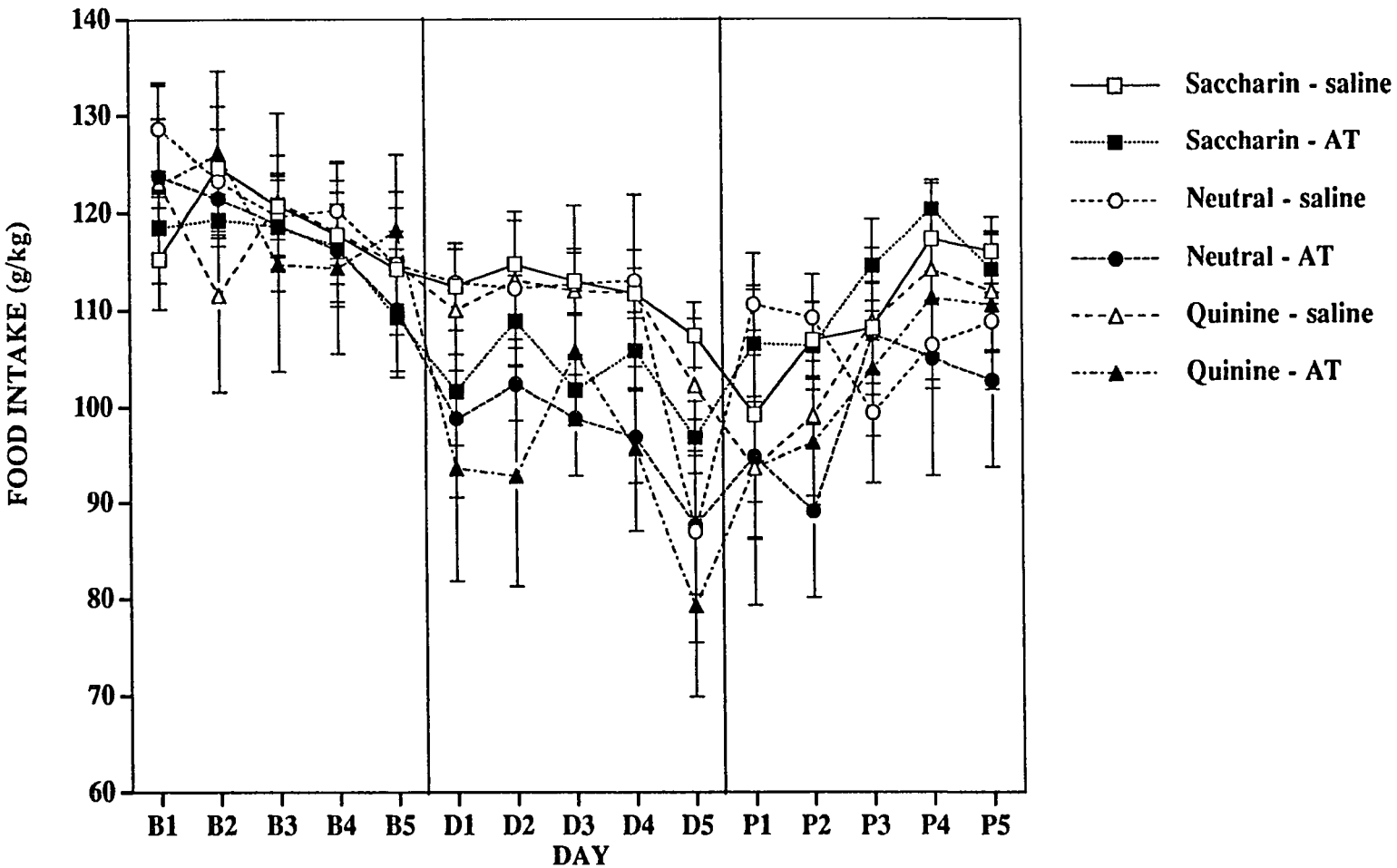


Figure 5. Effect of AT on mean food intake (g/kg) across baseline, drug and postdrug phases for the saccharin-, neutral- and quinine-fed groups. Vertical lines represent S.E.M.

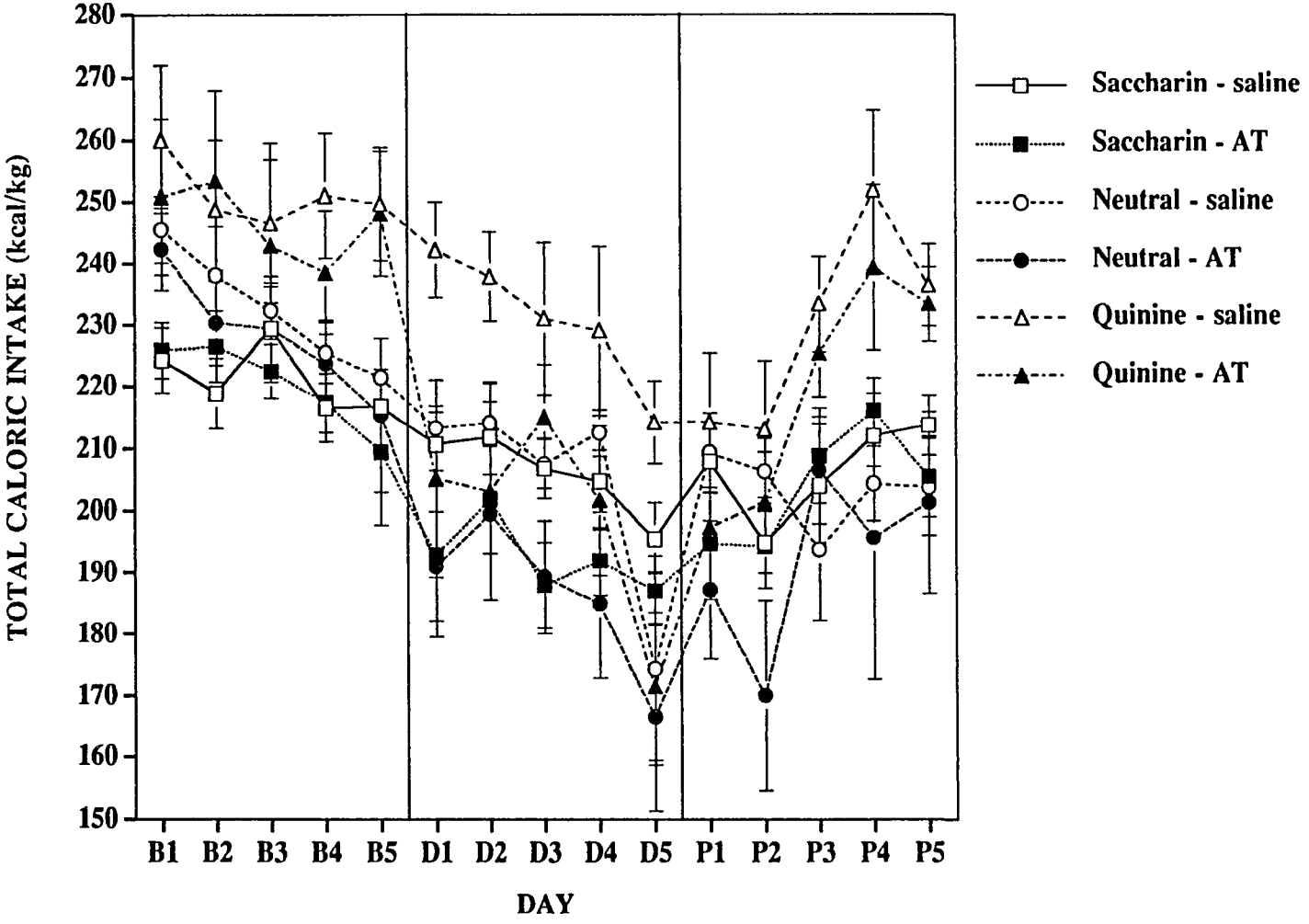


Figure 6. Effect of AT on total caloric intake (kcal/kg) across baseline, drug and postdrug phases for the saccharin-, neutral- and quinine-fed groups. Vertical lines represent S.E.M.

interaction for total caloric intake (Kcal/kg) [$F(2,82)=7.25$, $p<0.0013$] (figure 6) and caloric intake from food [$F(2,82)= 5.38$, $p<0.0064$] but not for caloric intake from ethanol [$F(2,82)= 0.23$, $p<0.7937$] reflecting the finding that AT had decreased total caloric intake and caloric intake obtained from food during AT administration compared to the saline controls.

Analysis of the maintenance, introduction and baseline phases.

Data analysis revealed a consistent pattern in consummatory behaviours between the food groups. The quinine group tended to differ from both the saccharin and neutral group on many of the food and fluid intake measures. Due to this fact analyses were conducted on data collected through the maintenance, introduction and baseline phases. Analyses were performed using three way mixed design repeated ANOVAs with Food group as the between factor and Treatment phase and Days as the within factors. The Treatment phase factor was divided into 4 blocks of 5 days each. Days 1 to 5 and 6 to 10 of the maintenance phase were designated as Maintenance 1 and Maintenance 2 blocks respectively. Days 1 to 5 of the introduction phase and baseline phase were designated as the Introduction and Baseline blocks respectively.

The introduction of the quinine-flavoured food during the Introduction phase significantly increased absolute ethanol intake (g/kg) [$F(6,132)=14.51$, $p<0.0001$] (figure 7) and preference for ethanol [$F(6.132)=12.59$, $p<0.0001$] compared to both the saccharin-fed and neutral-fed groups.

Food intake (g) for the quinine-fed group significantly decreased during the Introduction phase compared to the saccharin and neutral groups [$F(6,132)= 2.242$, $p<0.043$] (figure 8). Similarly, water intake significantly decreased for the quinine

group, compared to the saccharin and neutral groups, following the addition of quinine to the diet [$F(6,132)=10.771$, $p<0.0001$].

Figure 9 shows mean water intake across treatment phases. Results showed a significant Drug by Treatment phase interaction [$F(6,132)= 10.771$, $p<0.0001$]. Simple effects revealed that the quinine-fed group drank significantly less water during the Introduction ($p<0.05$) and Baseline ($p<0.05$) phases compared to the saccharin- and neutral-fed groups.

The total caloric intake of the quinine-fed animals were significantly greater [$F(6,132)= 4.767$, $p<0.0002$] (figure 10) than both the saccharin-fed and neutral-fed groups during the Introduction ($p<0.05$) and Baseline ($p<0.05$) blocks. The greater total caloric intake of the quinine-fed group can be related to their increased intake of calories from ethanol [$F(6,132)= 15.568$, $p<0.0001$]. Analysis of caloric intake from food failed to show a significant difference between the food groups [$F(6,132)= 0.71$, $p<0.6452$]. Thus, unlike the results from food intake (g), there was no difference between the food groups and how much calories they obtained from food. The quinine group did not differ in the amount of calories obtained from food (Kcal/kg) because they weighed significantly less than either the saccharin-fed or neutral-fed groups [$F(2,41)=12.26$, $p<0.0001$].

## Discussion

The results of the present experiment revealed that aminotriazole (AT) did not have a significant effect on ethanol, water or total fluid intakes. The analyses for ethanol, water and total fluid intakes all failed to show significant Drug x Treatment phase interactions. However, analysis of food intake revealed that AT had significantly

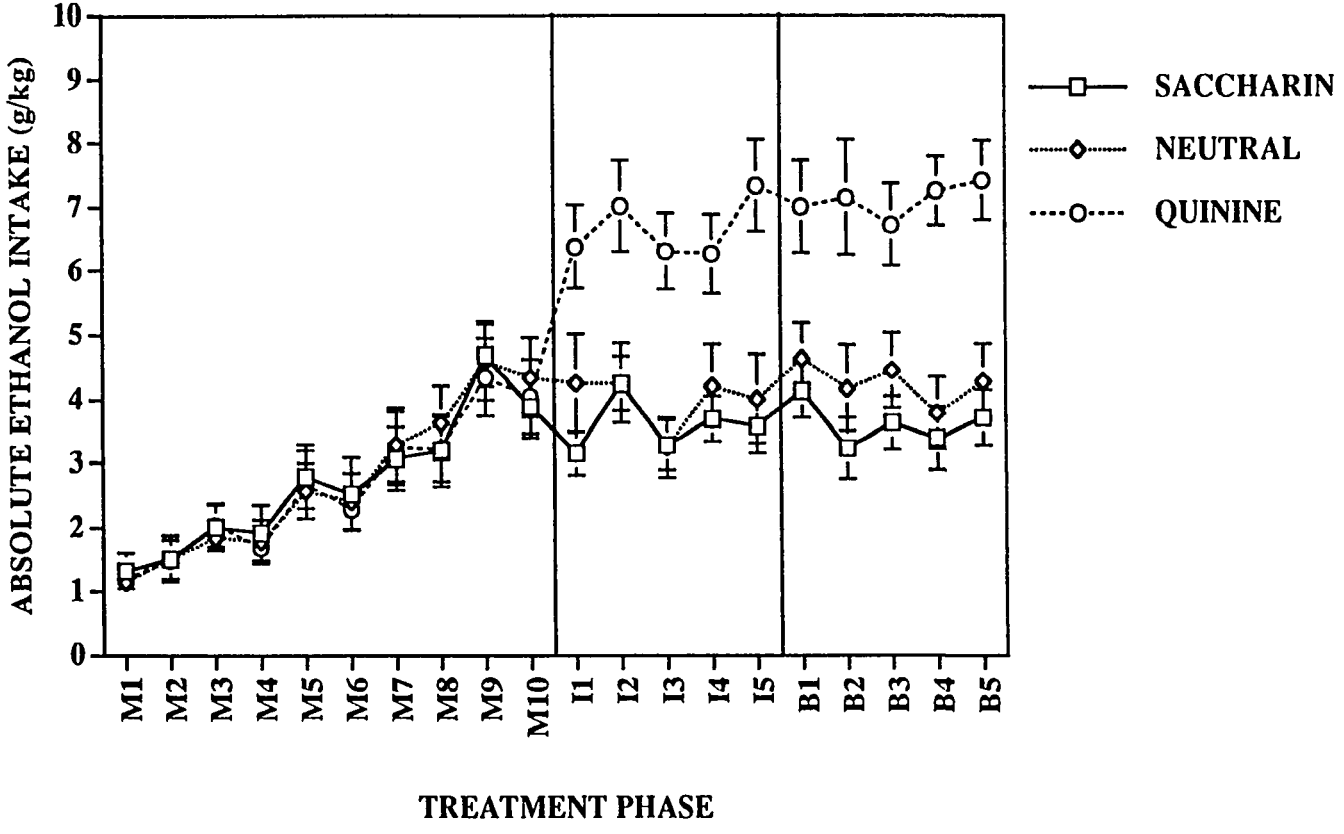


Figure 7. Mean absolute ethanol intake (g/kg) across the maintenance (M1-M10), food introduction (I1-I5) and baseline (B1-B5) phases for the saccharin-, neutral- and quinine-fed groups. Vertical lines represent S.E.M.

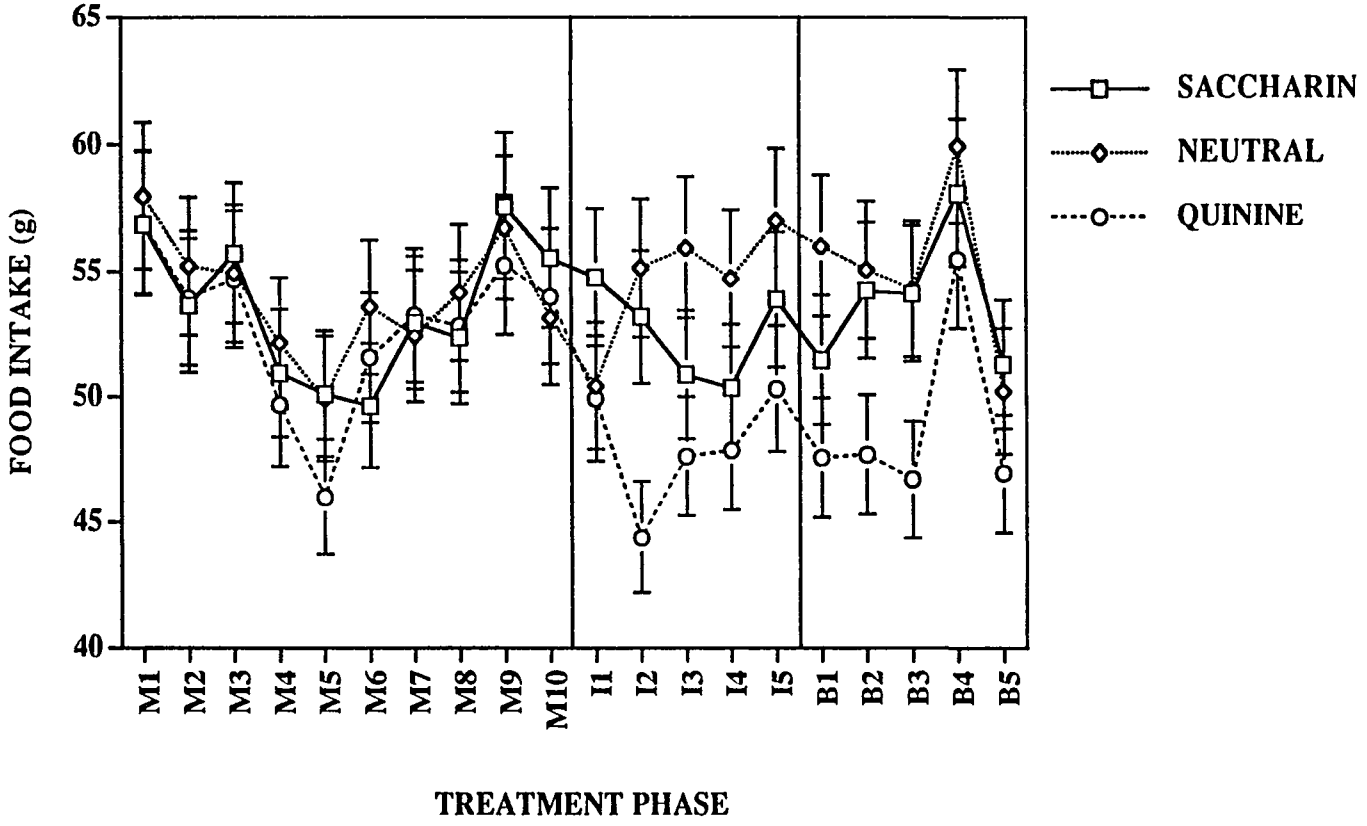


Figure 8. Mean food intake (g) across the maintenance (M1-M10), food introduction (I1-I5) and baseline (B1-B5) phases for the saccharin-, neutral- and quinin-fed groups. Vertical lines represent S.E.M.

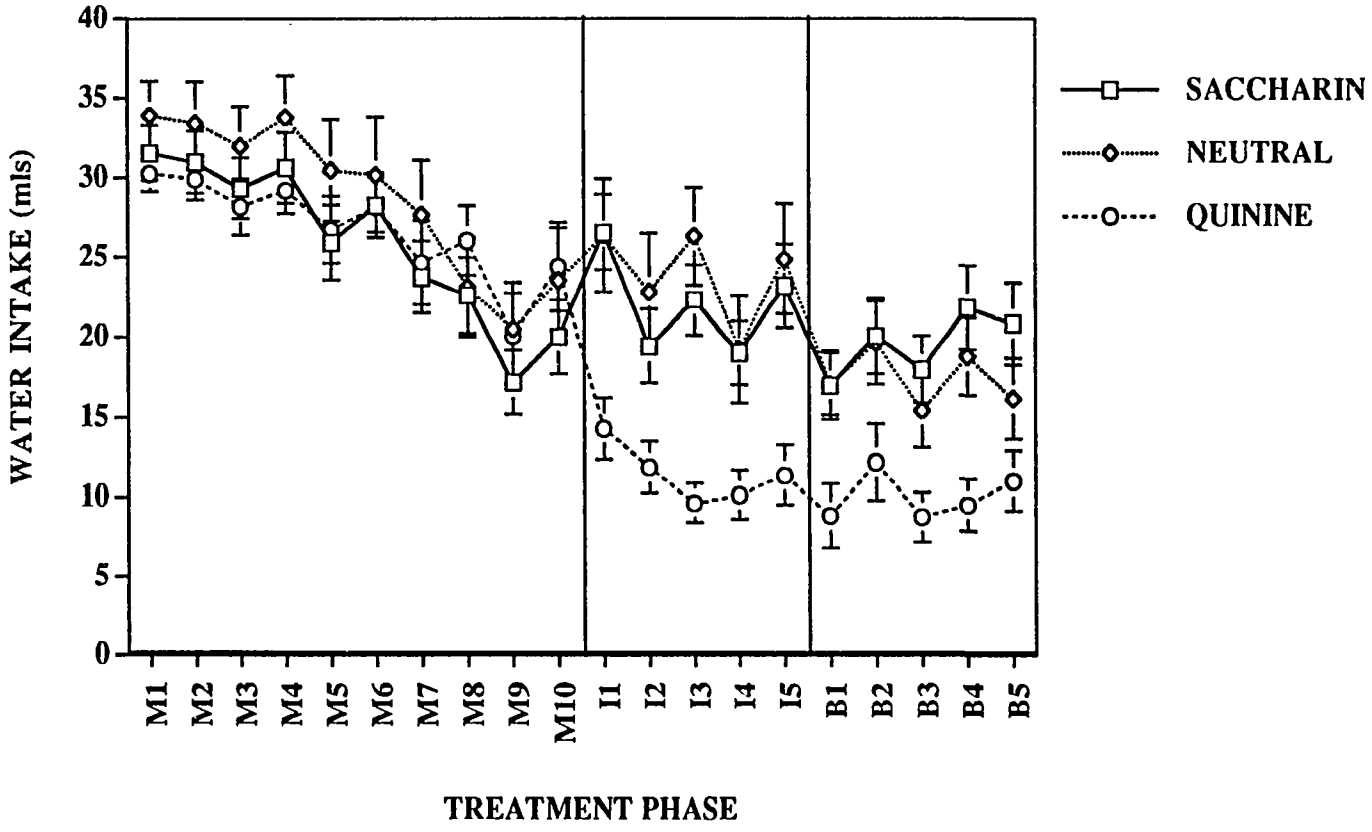


Figure 9. Mean water intake (mls) across the maintenance (M1-M10), food introduction (I1-I5) and baseline (B1-B5) phases for the saccharin-, neutral- and quinine-fed groups. Vertical lines represent S.E.M.

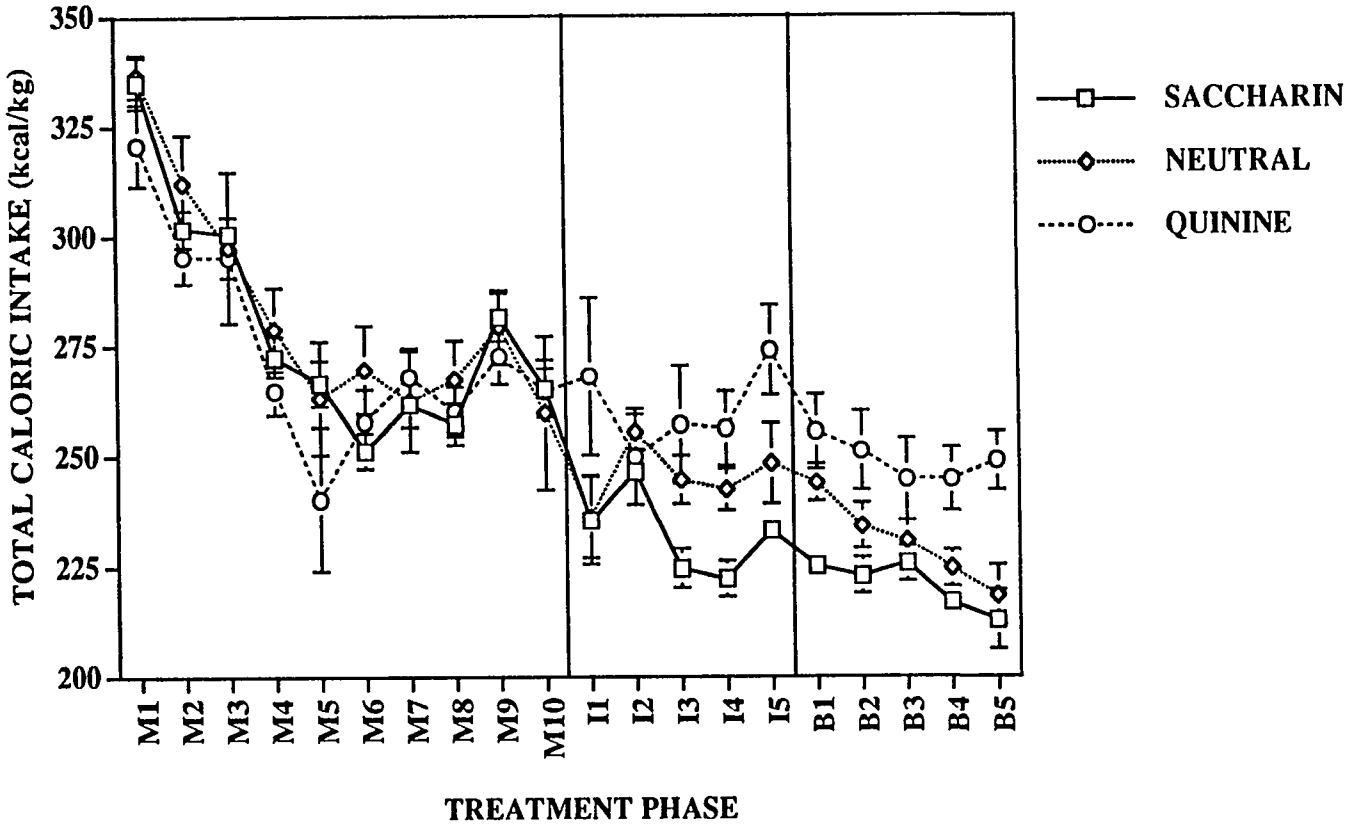


Figure 10. Mean total caloric intake (kcal/kg) across the maintenance (M1-M10), food introduction (I1-I5) and baseline (B1-B5) phases for the saccharin-, neutral- and quinine-fed groups. Vertical lines represent S.E.M.

attenuated food consumption. The AT group decreased the amount of food it consumed during the drug phase when compared to the saline control group.

The results of the experiment 1B showed that AT reduced food intake and that this decrease is not mediated through a change in taste reactivity. The saccharin, neutral and quinine food groups decreased their food intake to the same extent suggesting that AT had a generalized effect on feeding behaviour and that food consumption was attenuated regardless of the taste of the diet.

The presentation of the flavoured foods had a significant effect on ethanol, food and water consumption. The quinine-fed group behaved strikingly different from both the saccharin- and neutral-fed groups immediately following the presentation of the flavoured diet. The quinine group significantly increased their consumption of and preference for ethanol, while at the same time they decreased their intakes of food and water.

In conclusion, the findings of experiment 1B do not support the hypothesis that AT produces a primary and general reduction in the intake of calories. If one were to expect that AT produced a general reduction in caloric intake, then all sources of calories should be equally decreased. The results of this experiment showed a decrease in food intake but no decrease in ethanol intake. On the other hand, AT did not attenuate ethanol consumption as expected. Thus acetaldehyde, within the framework of the present experiment may not have exerted its previously reported effects in the mediation of ethanol consumption. The lack of a significant decrease in ethanol consumption by AT administration in this study is contrary to previous reports (Koechling and Amit, 1994; Rotzinger, Smith and Amit, 1994; Aragon and Amit, 1992).

# EXPERIMENT 1C

The results of the previous experiment have shown that quinine-fed animals behaved differently from either neutral- or saccharin-fed animals. The difference in ethanol, food and water intakes seen during exposure to the quinine-flavoured food suggested that quinine itself might have produced some change in consummatory behaviour. Quinine has traditionally been used as a noncaloric agent to alter the taste of fluid or food (e.g. Rotzinger, Smith and Amit, 1994). However, there has been some evidence suggesting that quinine itself produced changes in behaviour (Jerram and Greenhalgh, 1988; Verghese, 1988).

Quinine has been shown to block calcium-activated potassium channels (Cherubini, North, Surprenant, 1984). Potassium channel blockers, like quinine, have been shown to increase neurotransmitter release (Kumamoto and Kuba, 1985). Taken together, these studies suggest that quinine can act to enhance the release of neurotransmitters.

Wang and Grahame-Smith (1992) and Green (1984) have listed a set of behaviours as being primarily mediated through the serotonin system and have referred to this set of behaviours as 5-HT-mediated behaviours. These behaviours include locomotor activity, headweaving, forepaw treading, hindlimb abduction and headtwiching among others (see Green, 1984 for a complete list). Wang and Grahame-Smith (1992) have shown that rubidium, caesium and quinine, all potassium-channel blockers, produced changes in 5-HT-mediated behaviours in rats and mice. Pretreatment with quinine was shown to potentiate the 5-HT behavioural syndrome in rats and 5-$HT_2$ mediated behaviours in mice when several 5-HT agonists were later administered (Wang and Grahame-Smith, 1992). However, pretreatment with quinine failed to alter a 5-$HT_{1A}$ induced hypothermia in mice. In the same study, it was shown that several 5-HT antagonists inhibited the 5-HT behavioural syndrome produced by quinine (Wang and

Grahame-Smith, 1992). Since quinine had been shown to potentiate 5-$HT_2$ behaviours in rats and mice, and that quinine did not affect hypothermia induced by a the 5-$HT_{1A}$ agonist 8-OH-DPAT, it may be concluded that quinine might exert its effect through the 5-$HT_2$ receptor. Thus, it is conceivable that the quinine-fed animals in the experiment 1B had increased levels of 5-HT that had affected their consummatory behaviours.

The present experiment was conducted to test the hypothesis that the changes in consummatory behaviours seen in animals fed a quinine-flavoured diet are the result of an increase in the activity of 5-$HT_2$ receptors. The 5-$HT_2$ receptor antagonist ritanserin was used to assess whether quinine increased serotonin activity and whether or not the increase in ethanol consumption maybe related to an increase in serotonin activity.

On the other hand, the results of experiment 1B showed that the quinine-fed group had reduced their intake of the quinine-flavoured food by approximately 10% when compared to their intake of a neutral-flavoured food. The possibility thus exists that the changes in ethanol and water intake observed in quinine-fed animals may have been due to a decrease in food intake rather than to changes in the serotonin system caused by quinine. In order to assess whether a decrease in food intake is responsible for the increase in ethanol intake seen after the introduction of quinine to the food, a restricted food group was included in this experiment. The restricted food group was fed the neutral-flavoured food at 90% of their baseline levels.

## Method

Subjects

Forty male Long-Evans rats (Charles River Canada Inc.) each weighing 150-175 grams before the experiment were used. Animals were housed in stainless steel hanging cages in a temperature controlled facility that was maintained on a 12:12 hour day/night cycle (lights on at 08:00). Animals initially had free access to laboratory chow and

water. Fluids were presented in two glass Richter tubes which were placed on the front of each cage.

Drugs

Ritanserin (1mg/kg) was diluted in a 1: 2,000 dilution of 1.0 normal acetic acid to a pH of 3.8-4.0. A 10% (v/v) ethanol solution was prepared by diluting 95% laboratory ethanol with tap water. Quinine sulfate (Fisher Scientific) was used as noncaloric food flavouring.

Food

An unflavoured (neutral) food was prepared by mixing a powdered animal chow (Labdiet #5001) with tap water (80 mls water/100 g powdered food) to produce a thick, consistent wet mash. Flavoured foods were prepared by adulterating the neutral food with quinine sulfate (0.15% w/w). Foods were prepared daily and presented to the animals, in small ceramic food cups, in the late afternoon. Food and fluids were then measured the next day.

Procedure

Animals were allowed seven days of habituation to their new environment before the experiment started. During this time all animals were fed the new mashed diet and were handled by the experimenters. After the habituation period, animals were introduced to a 10% v/v ethanol solution through a forced choice procedure. For 4 consecutive days ethanol (10% v/v) was the only fluid available. On the fifth day water was presented in both Richter tubes. From this point forward, and until the end of the experiment, animals were given a free choice between ethanol (10% v/v) and water on an alternate day schedule. Water only was presented in both Richter tubes on the intervening days. The position of the tube that contained the ethanol solution was alternated so as to prevent the acquisition of a position bias. Animals were maintained on this procedure for 20 days (10 EtOH/water presentations). At the end of this maintenance period those animals that were considered nondrinkers were excluded from the study. A

nondrinker was defined as a rat that consumed less than 1.0 g/kg of absolute ethanol intake during the last 10 days of the maintenance phase.

After the maintenance period, the remaining animals were assigned to one of three food conditions; a quinine-flavoured food group (n= 14), a neutral food flavoured group (n= 7) and a restricted food group (neutral-flavoured food, n= 6). Animals in the quinine food group were assigned to either the ritanserin (1mg/kg) drug group (Q-R, n= 7) or the vehicle control group (Q-V, n= 7). The restricted food (RF) group received 90% of their respective food intake during the last 10 maintenance days. The neutral food group (N-V) received vehicle injections while the restricted food group received no injections at all. During the drug phase injections of ritanserin or vehicle were given subcutaneously daily for 20 consecutive days 2 hours before the presentation of EtOH. The postdrug phase consisted of 5 EtOH presentation days during which injections of ritanserin or vehicle were discontinued. During the postdrug phase the restricted food group was taken off food restriction and now received ad lib amounts of the neutral-flavoured food. After the postdrug phase was completed, the quinine food groups received the neutral-flavoured food for an additional 5 EtOH presentations (days N1-N5).

## Results

The experimental design of this study involved an incomplete design, thus statistical analysis with a single mixed design repeated measures ANOVA was inappropriate. Instead, separate mixed designed ANOVAs were used to analyze the data. Three assumptions were tested in this experiment. First, does the addition of quinine to the diet result in an increase in ethanol consumption? Second, if a quinine-flavoured food does induce ethanol consumption, will ritanserin attenuate the quinine induced increase in ethanol intake. Third, as shown by the previous experiment, animals fed a

quinine-flavoured food tended to decrease their food consumption. Thus, it is possible that a decrease in food intake may have caused an increase in ethanol consumption. A restricted food group was added to this experiment in order to observe the effects of a decrease in food intake on ethanol and water intakes. Post hoc Tukey HSD tests were performed to establish significant differences between means. All tests used a significant alpha level of 0.05.

3-way mixed repeated measures design used either food group or drug group as the between variable depending on which groups were being tested. The food variable was used as the between factor when comparing the Q-V group to the N-V group and when comparing the Q-V group to the RF group. The drug variable was used as the between factor when comparing the Q-V group to the Q-R group. The within variables consisted of treatment phases and days. Treatment phases were blocked into 5 separate groups consisting of 5 days each. The 10 days of the maintenance phase were divided into 2 blocks of 5 days (Maintenance 1 and Maintenance 2). The 10 days of the introduction phase were also divided into 2 blocks of 5 days (Introduction 1 and Introduction 2). The postdrug phase was the last block of 5 days.

Quinine-Vehicle vs. Neutral-Vehicle

Results of the present study replicated the findings obtained in experiment 1B during the maintenance and introduction phases. The results showed that absolute ethanol intake (g/kg) was significantly increased after the introduction of a quinine-flavoured food [$F(4,44)=3.358$, $p<0.0175$] (figure 11) compared to the N-V group. The Q-V group had significantly higher ethanol intake (g/kg) than the N-V group at the Maintenance 1 ($p<0.05$) and Postdrug phases ($p<0.05$).

Food intake yielded a significant Food group by Treatment phase interaction [$F(4,44)= 4.441$, $p<0.0042$] (figure 12). Post hoc analysis revealed that the Q-V group had significantly decreased their food intake during the Introduction 1, Introduction 2 and Postdrug blocks when compared to its Maintenance 1 and Maintenance 2 phases ($p<0.05$).

The Q-V group showed a significant decrease in their water intake after the quinine-flavoured food was introduced [$F(4,44)=6.967$, $p<0.0002$] (figure 13). The Q-V group significantly decreased their water intake during the Introduction 1, Introduction 2 and Postdrug phases compared to their Maintenance 1 and Maintenance 2 phases ($p<0.05$).

Quinine-Vehicle vs. Quinine-Ritanserin

The results of the comparison between the Q-V and Q-R groups failed to show significant differences between the two groups on ethanol (g/kg) [$F(4,48)= 1.633$, $p<0.1813$] (figure 11), food [$F(4,48)= 0.254$, $p<0.9056$] (figure 12) or water consumption {$F(4,48)= 0.474$, $p<0.7545$] (figure 13). Thus, the administration of the 5-$HT_2$ receptor antagonist ritanserin failed to attenuate quinine induced changes in ethanol, food and water intakes. That is, the Q-V group did not differ from the Q-R group on any measure of ethanol, food or water consumption.

The analysis of ethanol, food and water intakes showed the same pattern as that seen in experiment 1B for the quinine-fed animals. The analysis showed significant main effect of Treatment phase for ethanol [$F(4,44)= 35.737$, $p<0.0001$], food [$F(4,44)= 37.787$, $p<0.0001$] and water [$F(4,44)=85.228$, $p<0.0001$] intakes. Post hoc test revealed that ethanol intake significantly increased, while food and water intakes significantly

decreased during the Introduction 1 ($p<0.05$), Introduction 2 ($p<0.05$) and Postdrug ($p<0.05$) treatment phases.

Quinine-Vehicle vs. Neutral-Restricted Food

Analysis of absolute ethanol intake yielded a significant main effect of Food group [$F(4,44)= 19.936$, $p<0.0001$] (figure 11). Simple effects showed that the Q-V group significantly increased its ethanol intake across Treatment phases [$F(4,44)= 3.874$, $p<0.009$] while the RF did not [$F(4,44)= 0.839$, $p<0.508$] suggesting that restricting food intake by 10% did not produce a significant increase in ethanol intake.

The decrease in food intake during the Introduction phase for the restricted food group (10%) was not significantly lower when compared to the food intake during the Maintenance phase [$F(4,44)= 1.006$, $p<0.413$] (figure 12). However, the decrease in food intake in the Q-V group was significantly lower during the introduction and postdrug phases than during the maintenance phase [$F(4,44)= 4.633$, $p<0.003$]. Water intake was significantly decreased during the introduction and postdrug phases for both the restricted food group and the quinine-fed group [$F(4,44)= 5.88$, $p<0.0011$] (figure 13).

## Discussion

The results of the present experiment showed that the addition of quinine sulfate to an unflavoured mash diet resulted in an increase in ethanol consumption as well as a decrease in food and water intakes when compared to either a neutral-fed or a restricted food group. The administration of ritanserin, a 5-$HT_2$ antagonist, failed to attenuate the effects of quinine on ethanol, food or water consumptions. That is, there were no

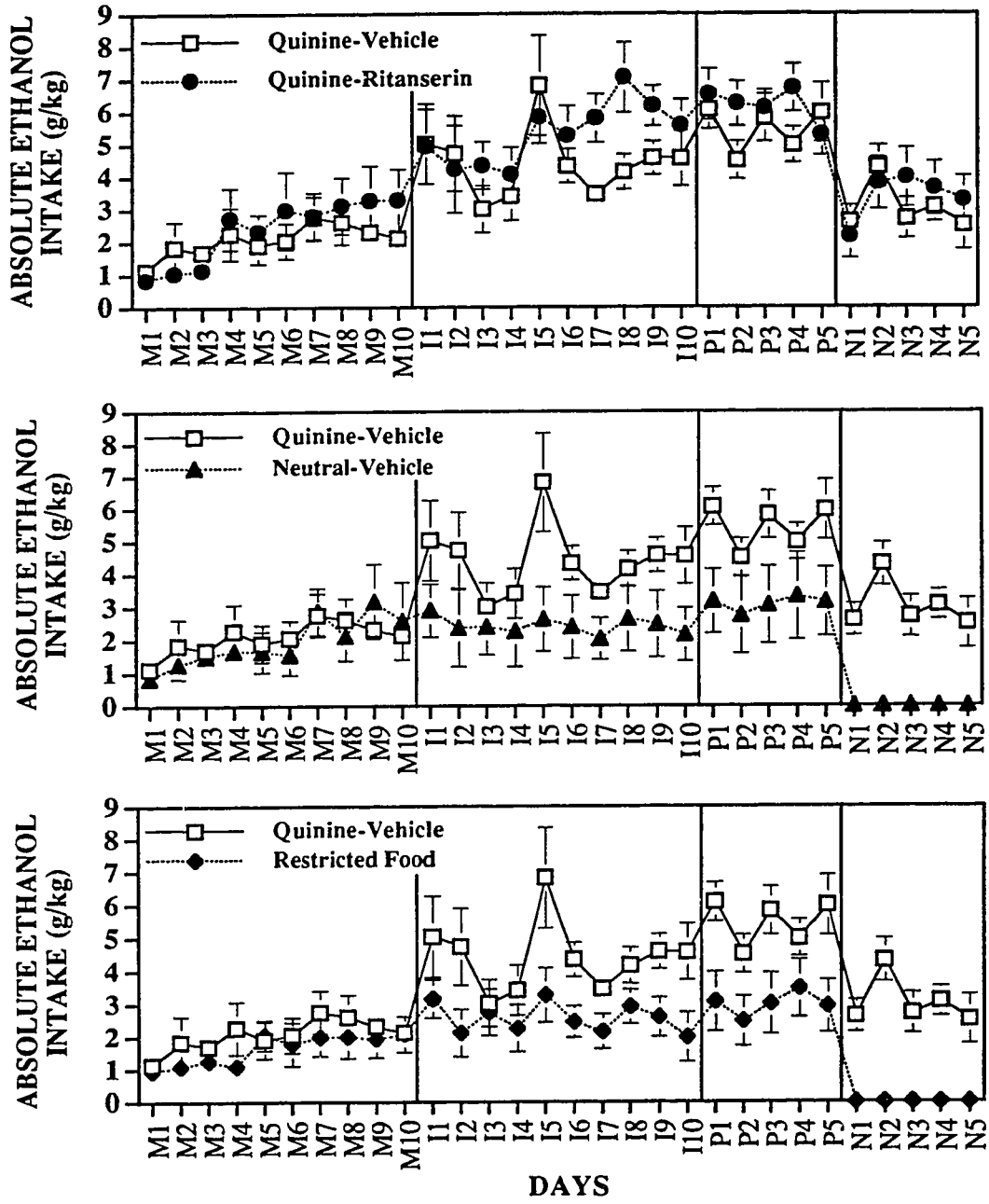


Figure 11. Mean absolute ethanol intake (g/kg) across maintenance (M1-M10), food introduction (I1-I10), postdrug (P1-P5) and reintroduction of neutral food (N1-N5). Comparison between the quinine-vehicle and quinine-ritanserin groups (top graph), quinine-vehicle and neutral-vehicle groups (middle graph) and quinine-vehicle and food restricted groups (lower graph). Vertical lines represent S.E.M.

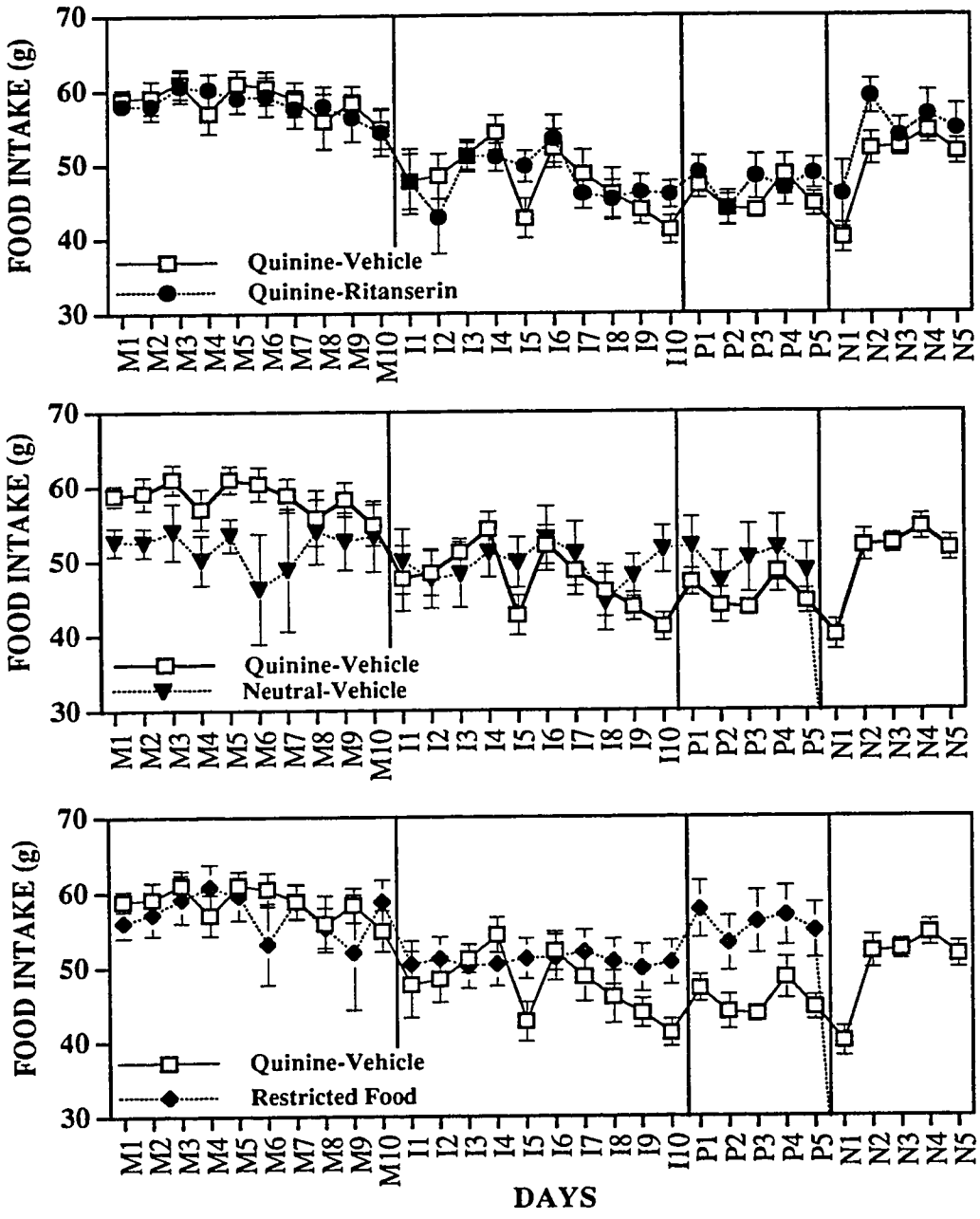


Figure 12. Mean food intake (g) across maintenance (M1-M10), food introduction (I1-I10), postdrug (P1-P5) and reintroduction of neutral food (N1-N5) phases. Comparisons between the quinine-vehicle and quinine-ritanserin groups (top graph), quinine-vehicle and neutral-vehicle groups (middle graph) and quinine-vehicle and food restricted groups (lower graph). Vertical lines represent S.E.M.

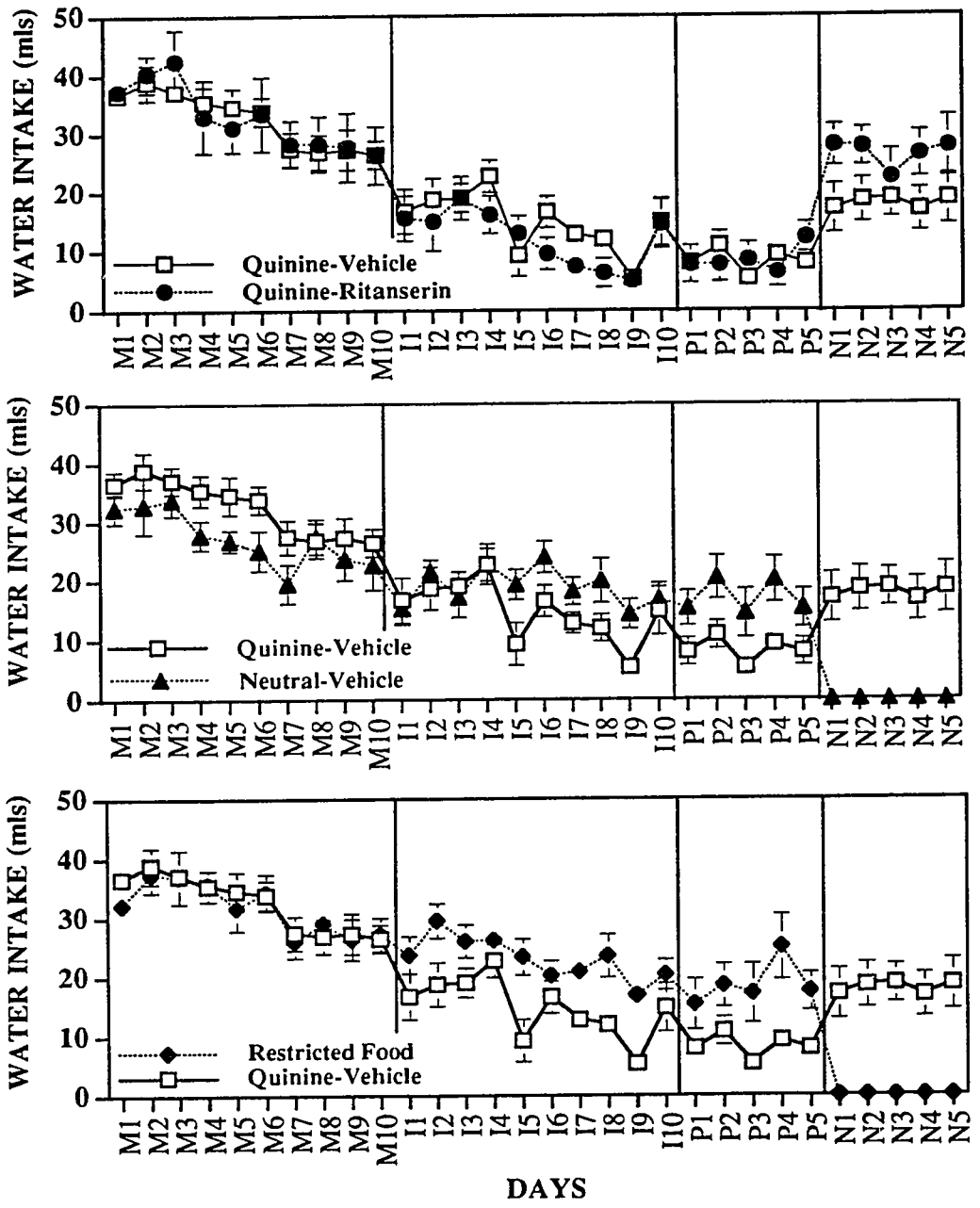


Figure 13. Mean water intake (mls) across maintenance (M1-M10), food introduction (I1-I10), postdrug (P1-P5) and reintroduction of neutral food (N1-N5) phases. Comparisons between the quinine-vehicle and quinine-ritanserin groups (top graph), quinine-vehicle and neutral-vehicle groups (middle graph) and quinine-vehicle and food restricted groups (lower graph). Vertical lines represent S.E.M.

significant differences in ethanol, food or water intakes between the quinine-ritanserin (Q-R) group and the quinine-vehicle (Q-V) control group.

It was initially hypothesized that the quinine-induced changes in ethanol, food and water intakes may be the result of an increase in the activity of the serotonin system. To that end, it was thought that antagonism of the serotonin system might result in an attenuation of the quinine-induced behaviours. Inhibition of the 5-$HT_2$ receptor by ritanserin failed to attenuate ethanol consumption and it can thus be concluded that the quinine related changes in ethanol may not be mediated by the 5-$HT_2$ receptor.

Ritanserin, a 5-$HT_2$ receptor antagonist, has been shown to decrease ethanol consumption (Panocka, Ciccoioppo, Polidori and Massi, 1993). However, the decrease in ethanol consumption was reported while using a relatively weak ethanol concentration, 3% v/v, and these results may not be meaningful to any examination of the reinforcing properties of ethanol intake. On the other hand, several studies have shown that administration of ritanserin had failed to decrease ethanol consumption (Myers and Lankford, 1993; Svensson, Fahlke, Hard and Engel, 1993) or to affect food intake (Massi and Marini, 1987). However, the above mentioned studies were performed on aminals with normal levels of serotonin while in the present study it was hypothesized that quinine had increased serotonin levels above normal levels. Thus, the resulting increase in serotonin activity may be the mechanism driving the increase ethanol consumption as well as the decrease in food and water intakes. The results of the present experiment showed a failure of ritanserin to reduce ethanol consumption or increase food or water intakes. Thus, these present findings are consistent with reports showing that ritanserin does not significantly affect ethanol or food intakes.

The possibility existed that the increase in ethanol intake seen after the introduction of quinine to the food may have been due to a decrease in the amount of food that the quinine-fed groups consumed compared to their maintenance phase levels. This decrease in food intake may have induced ethanol consumption as a form of compensation for lost calories. In order to rule out this possibility, a restricted food group was added to the study to assess the effect of a decrease in food intake on ethanol consumption. The restricted food group received 10% less food relative to its maintenance phase levels. A 10% reduction in food availability was chosen because it was shown in experiment 1B that the quinine-fed group decreased their food intake by 10% during the introduction of quinine to their diet. The results of the present experiment showed food restriction did not result in a significant increase in ethanol intake.

Reinstatement of the neutral-flavoured food (days N1 to N5) to both of the quinine-fed groups resulted in an increase in food intake with a concomitant decrease in ethanol intake. This pattern of food and ethanol intake suggests that at least part of the increase in ethanol consumption after the introduction of quinine might be mediated through a compensation of calories. Support for the notion of a compensation for lost calories can be inferred because the total caloric intake of the quinine-fed group remained virtually unchanged between the quinine-fed phase and the reintroduction of the neutral food.

The specific mechanism involved in the increase in ethanol intake as the result of the addition of quinine to the food diet is unknown at the present time. Food restriction itself, and thus a caloric deficit, did not produce an increase in ethanol intake and thus it

is unlikely that the decrease in food or caloric intake seen in the quinine-fed groups could be the cause for their increase in ethanol intake. However, the fact that reinstatement of the neutral-flavoured food to the quinine-fed groups resulted in some kind of caloric regulation suggests that some form of interaction between quinine and calories maybe involved.

# EXPERIMENT 2

Studies within the area of feeding behaviour have typically made use of systems capable of recording the microstructural pattern of both food and fluid intakes (Fletcher and Burton, 1986; Burton, Copper and Popplewell, 1981; Fitzsimmons and Le Magnen, 1969; Kisseleff, 1969). This type of microstructural approach allowed researchers to measure feeding and drinking behaviours in much greater detail than typical oral paradigms using only gross measures of food and fluid intakes. A microstructural paradigm is capable of providing additional information on the patterns of food and fluid consumption not available otherwise. Bout frequency, bout duration and bout size are parameters that have given microstructural paradigms a greater power of resolution in comparison to absolute or overall consummatory measurement paradigms. In this context, researchers in the area of feeding behaviour have made use of a microstructural paradigm to study the effects of drug manipulations on both food and water intake (Burton, Copper and Popplewell, 1981). As well, researchers in the alcohol field have followed suit and have begun using systems that look not only at ethanol intake, but also at food and water intakes concurrently (Higgins, Tompkins, Fletcher and Sellers, 1991; Gill, Mundl, Cabilio and Amit, 1988). The microstructural paradigm has been adapted to track the effects of drugs on ethanol, food and water intake (Boyle, Smith and Amit, 1997; Boyle, Smith and Amit, 1992; Gill, Fillon and Amit, 1988; Gill, France and Amit, 1986).

As mentioned previously, several researchers have argued that the primary metabolite of ethanol, acetaldehyde, may be responsible for mediating the reinforcing effects of ethanol (Gill, Amit and Smith, 1996a; Aragon and Amit, 1985; Lindros, 1978). The enzyme catalase has been implicated in the production of central acetaldehyde (Gill, Menez, Lucas and Deitrich, 1992; Aragon, Rogan and Amit, 1992; Cohen, 1980). It has been reported that ethanol consumption has been decreased following the administration

of the catalase inhibitor 3-amino-1,2,4-triazole (AT) because levels of acetaldehyde production in brain was inhibited, thus leading to a putative inhibition of the positive reinforcing properties of ethanol (Koechling and Amit, 1994; Aragon and Amit, 1992). However, this position has recently been questioned.

A study by Tampier, Quintanilla and Mardones (1995) has shown that administration of AT resulted in a decrease of both ethanol and food consumption in a strain of rats selectively bred for high ethanol drinking (UChB). They reported that food intake only was decreased in their strain of low ethanol drinking rats (UChA). These authors have argued that AT produces a decrease in the need for caloric intake. Thus, the decrease in ethanol intake seen following AT administration, in the UChB strain, can be considered the result of a decrease in the need for calories and not because of a decrease in the reinforcing properties of ethanol. The purpose of the present study was to measure the changes that occur in ethanol, food and water intakes following administration of the catalase inhibitor 3-amino-1, 2,4-triazole within a microstructural paradigm.

## Method

Subjects

Twenty male Long-Evans rats each weighing 150-175 grams at the start of the experiment were used. Animals were housed individually in Plexiglas operant chambers. The experimental room was kept at a constant temperature and on a 12:12 hour light/dark cycle (lights off at 13:30). The experimental room consisted of ten operant chambers. Two groups of 10 rats each were tested.

Drugs

3-amino-1,2,4-triazole (AT) (Sigma Co.) was prepared in 0.9% saline (1g/2ml/kg). A 10% (v/v) ethanol solution was prepared by diluting 95% stock laboratory ethanol with tap water.

Apparatus

The drinkometer system consisted of Plexiglas operant chambers (Grason-Stadler) connected to a microcomputer. Food pellets (45 mg Precision pellets) were dispensed whenever the photo beam inside the food hopper was interrupted. Fluids were made available through two plastic tubes, each with metal ball bearing spouts, attached to the front of the operant chambers.

Bout Parameters

The drinkometer system uses a program that records individual food, EtOH and water events for a 23-hour period per day. The computer then collects and packages these data into particular bout measures. For fluid intake, in the form of EtOH or water, there are five measures that are recorded by the system. These are bout frequency (number of bouts per day), bout size (amount consumed per bout), bout duration (duration of each bout in seconds), total bout duration (duration for all bouts in a 23 hour period in seconds) and total licks. There are five bout measures taken for food: total intake of pellets per day, bout frequency, bout size, bout duration and total bout duration.

The start of a bout is defined as the activation of either the food dispenser or one of the fluid spouts. The end of a bout is defined by a 5-minute interbout interval. That is, a particular bout has ended when 5 minutes has elapsed since the last activation of the food dispenser or spouts. A bout is also deemed to have ended when the animal has switched from one consummatory source to another (e.g. switching from food to fluid, fluid to food or from one fluid to the other). To avoid the inclusion of inadvertent activations of the food dispenser or spouts, only responses that comprised of a minimum of 5 consecutive food dispenser or fluid spout activations were categorized as a bout.

Procedure

Animals were allowed 7 days to habituate to their new surroundings before the start of the experiment. After the habituation period animals were introduced to 10 % v/v EtOH through a forced choice procedure. For four consecutive days EtOH was the sole

fluid available. On the fifth day, water was the only fluid presented. From this point onward, and for the remainder of the experiment, EtOH and water were presented on an alternate day schedule, with water only in both tubes presented on the intervening days. The positions of the tubes containing the EtOH and water were alternated so as to prevent the acquisition of a position bias. The drinkometer system was activated for 23 hours per day. During the one hour shut down period EtOH and water intake were measured, fluids replaced, food dispensers refilled and boxes cleaned when needed.

Animals were given a maintenance period of 10 EtOH/water days to ensure a steady level of EtOH drinking. Baseline, drug and postdrug phases each consisted of 5 EtOH/water presentations and their measurements began immediately after the maintenance phase. During the drug phase, AT (1g/kg) was administered IP once per day for five days one hour before the presentation of EtOH (always during the computer shut down period). Half of the animals received AT, while the other half received saline in equal volumes.

## Results

The present experiment examined the changes in ethanol, food and water bout parameters as the result of a drug manipulation. Each bout parameter was analyzed separately by a 3-way mixed design ANOVA (with drug as the between factor and treatment phase and days as the repeated variables). Post hoc Tukey HSD tests were performed to establish significant differences between means. All tests used a significant alpha level of 0.05.

Analysis of Ethanol Intake

Absolute ethanol intake (g/kg) was significantly attenuated following AT administration [$F(8,104)=2.04$, $p<0.0484$]. Conversion of absolute ethanol intake into a percent change from baseline measure showed that the AT group had significantly decreased their ethanol consumption during the drug and postdrug phases compared to the saline control group. [$F(1,13)= 9.91$, $p<0.0077$] (figure 14).

Analysis of bout parameters showed that the decrease in ethanol intake might be the result of a decrease in the total time spent per day on the consumption of ethanol [$F(1,13)=9.26$, $p<0.0094$]. Post hoc analysis showed that the AT group spent less time per day consuming ethanol than the saline group during the drug ($p<0.05$) and postdrug ($p<0.05$) phases when compared to the baseline phase.

The number of ethanol bouts (bout frequency) showed a nonsignificant Drug x Treatment phase interaction [$F(2,26)=2.01$, $p>0.1546$]. When bout frequency was converted to a percent change from baseline measure, the analysis revealed that the main effect of Drug [$F(1,13)=3.23$, $p<0.09$] that was just beyond significance (figure 14). There were no significant Drug x Treatment phase interactions for either ethanol bout size [$F(2,26)=1.1$, $p>0.3494$], bout duration [$F(2,26)= 2.38$, $p<0.1122$] or total ethanol licks [$F(2,26)=0.98$, $p>0.3904$] suggesting that these bout parameters did not contribute to the decrease in ethanol consumption following AT administration.

Ethanol preference ratio (EtOH in mls/ total fluid intake in mls) showed a significant main effect of Treatment phase [$F(2,26)=3.94$, $p<0.032$]. Tukey post hoc tests showed that ethanol preference was greater during the postdrug phase when compared to the drug phase ($p<0.05$). Total fluid intake was not affected by AT administration as shown by a nonsignificant Drug x Treatment phase interaction effect [$F(2,26)=0.33$, $p<0.7199$].

Analysis of Food Intake

Total food intake for the AT treated group, as measured by the number of pellets dispensed per day, was not significantly different from the saline control group [$F(2,26)= 1.207$, $p<0.3154$] (figure 15). Thus, AT did not produce a decrease in food intake compared to the control group. However, there was a significant main effect of Treatment phase [$F(2,26)=7.44$, $p<0.0028$] in which food intake for both groups were

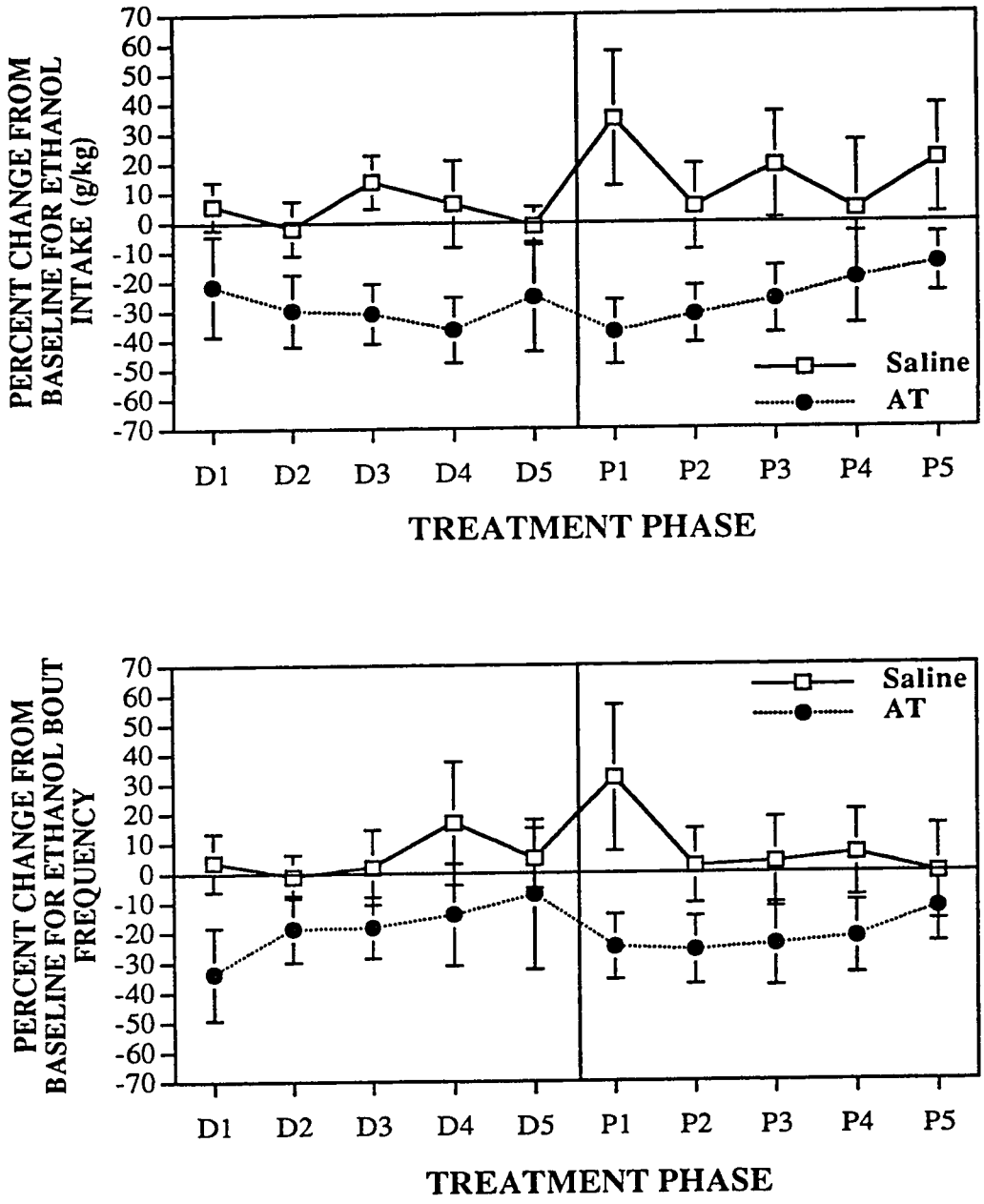


Figure 14. The effect of AT on mean ethanol intake and ethanol bout frequency across baseline ,drug and postdrug phases. Ethanol intake (g/kg) presented as percent cahnge from baseline (top graph). Percent change from baseline for ethanol bout frequency presented in lower graph. Vertical lines represent S.E.M.

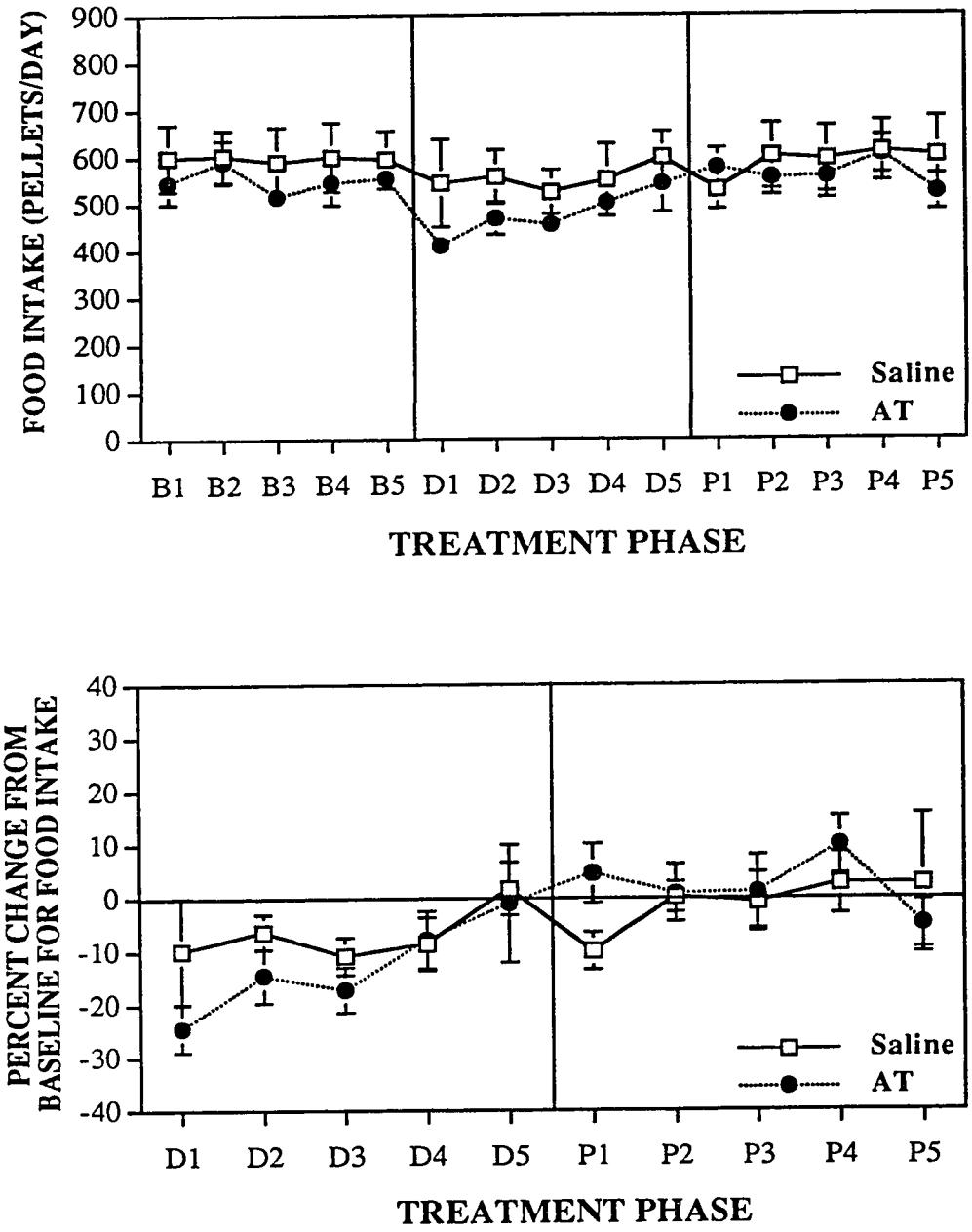


Figure 15. The effect of AT on mean food intake across baseline, drug and postdrug phases. Total food intake (# of pellets) presented in top graph and percent change from baseline for total food intake presented in lower graph. Vertical lines represent S.E.M.

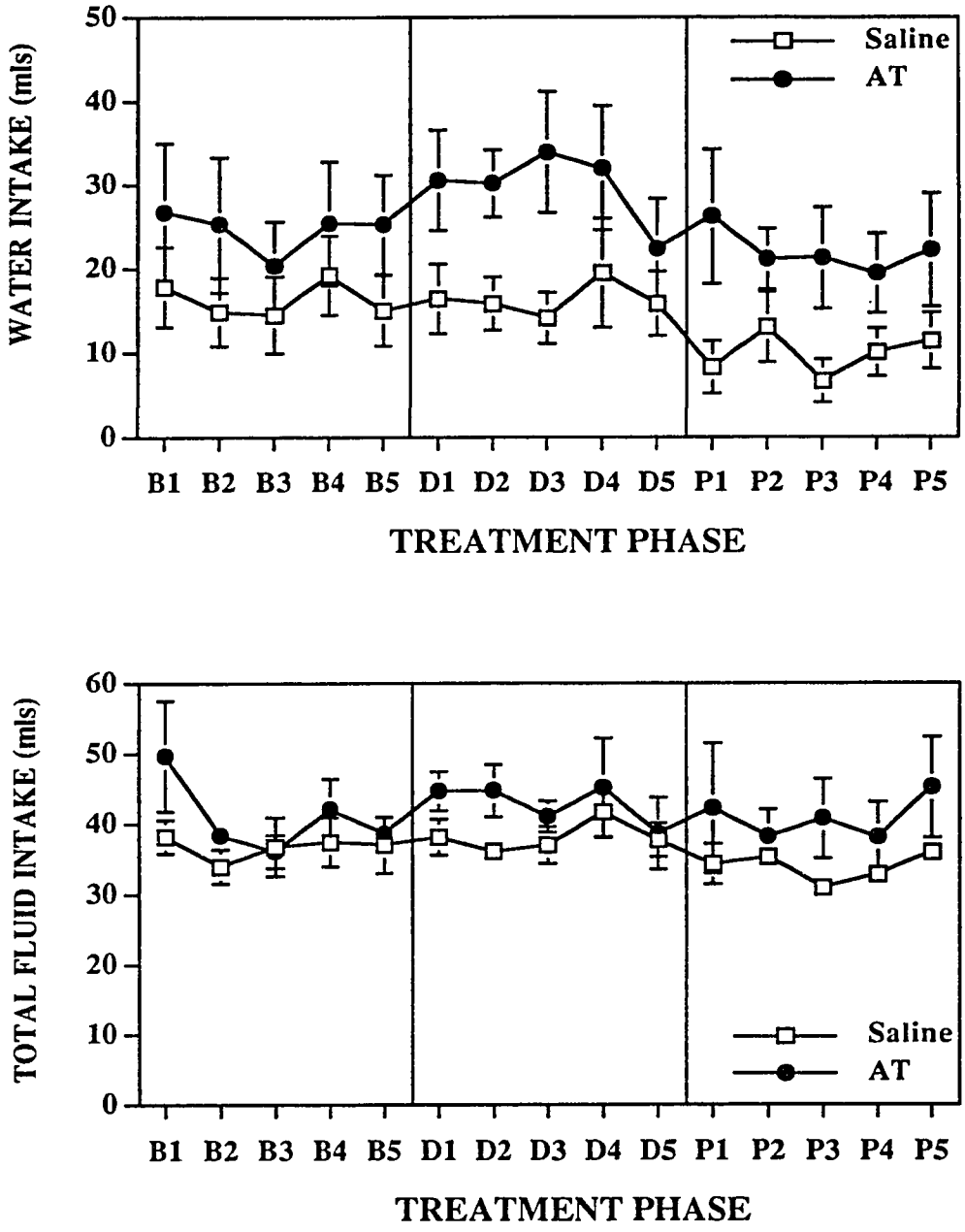


Figure 16. The effect of AT on mean water intake (top graph) and total fluid intake (lower graph) across baseline, drug and postdrug phases. Vertical lines represent S.E.M.

lower during the drug phase compared to the baseline phase. The observed decrease in food intake for both groups during the drug phase was due to a significant decrease in the frequency of food bouts [$F(2,26)=8.99$, $p<0.0011$] and the total time spent feeding per day [$F(2,26)=5.09$, $p<0.0136$].

Analysis of food bout size [$F(2,26)= 2.073$, $p<0.1461$] and time spent per food bout [$F(2,26)= 1.698$, $p<0.2027$] both yielded nonsignificant Drug by Treatment phase interactions suggesting that these variables were not affected following AT administration.

Analysis of Water Intake

The total amount of water consumed for the AT treated group was not significantly different from the saline control group following AT administration [$F(2,26)= 0.546$, $p<0.5855$] (figure 16). However, there was a main effect of Treatment phase for water intake [$F(1,13)=4.6$, $p<0.0313$] (figure 16) showing that animals consumed more water during the drug phase compared to the postdrug phase ($p<0.05$). The lack of a significant decrease in water consumption following AT administrations suggests that AT had a specific effect on ethanol intake rather than a general effect on fluid intake.

## Discussion

The focus of experiment 2 was to monitor the effects of the catalase inhibitor AT on the microstructure of ethanol, food and water consumption. This has become particularly important in view of the discrepancy between the results of experiment 1B and the published reports in the literature (Koechling and Amit, 1994; Aragon and Amit, 1992).

The results of the present experiment showed that the administration of AT significantly attenuated ethanol but not food intake. The significant decrease in ethanol consumption is reflected in the decrease in the total amount of time spent consuming ethanol per day. In addition, it can be argued that the observed decrease in the number of ethanol bouts contributed to the decrease in ethanol intake. The size of ethanol bouts and the time spent consuming ethanol per bout were not affected following AT administration.

AT did not significantly reduce food intake when compared to the saline control group. However, a significant main effect of treatment phase resulted in a decrease in food intake during the drug phase compared to the baseline and postdrug phases and was reflected in a decrease in the frequency of food bouts and the total time consuming food per day. Water intake and total fluid intake were not significantly decreased following AT administration suggesting that the decrease in ethanol intake was not due to a general decrease in fluid intake but to a specific effect on ethanol.

In conclusion, the results of this study revealed that AT produced a decrease in ethanol intake that was not associated with or driven by a concomitant decrease in food intake. According to Tampier et al. (1995), AT produces a decrease in the caloric need of the animal resulting in a decrease in food intake, and subsequently a decrease in ethanol intake. The lack of a decrease in food intake showed that AT did not produce a decrease in the caloric need of the rat. Since AT significantly attenuated ethanol intake, and did not affect either food or water intake, it can be argued that AT's effect was specific to ethanol. These findings of the present experiment are consistent with previous studies reporting an attenuation of ethanol intake following AT administration (Aragon and

Amit, 1992; Koechling and Amit, 1994; Rotzinger, Smith and Amit, 1994). On the other hand, the results of experiment 2 are in contrast to the findings of experiment 1B.

# GENERAL DISCUSSION

The primary metabolite of ethanol, acetaldehyde, has been thought to play a role in mediating both the behavioural effects and the reinforcing properties of ethanol (Smith, Aragon and Amit, 1997). It has been argued that the peroxidatic activity of catalase is the primary route for metabolizing ethanol into acetaldehyde in brain (Aragon, Rogan and Amit, 1991). Inhibition of catalase through the administration of 3-amino-1,2,4-triazole (AT) has been shown to be capable of attenuating many ethanol induced behaviours (Aragon, Spivak and Amit, 1991; Tampier and Quintanilla, 1991;Tampier, Quintanilla, Letelier and Mardones, 1988) as well as voluntary ethanol consumption (Aragon and Amit, 1992). It has been proposed that the decrease in ethanol consumption seen following the administration of AT is due primarily to the attenuation of centrally produced acetaldehyde (Smith, Aragon and Amit, 1997; Gill, Amit and Smith, 1996a; Aragon and Amit, 1985).

However, a group of researchers has argued that AT produces a decrease in the need for calories resulting in an attenuation of food and consequently ethanol consumption (Tampier et al., 1995). Tampier et al. (1995) hypothesized that AT did not decrease ethanol consumption via attenuating the reinforcing properties of ethanol but rather by reducing the caloric requirement of the animal. Thus, the primary purpose of the present thesis was to test the effect of AT on ethanol, food and water intakes and in particular whether AT produced a decrease in caloric requirement.

Experiments 1B and 2 were designed to assess the effects of AT on ethanol, food and water consumption. The findings of experiment 1B demonstrated that AT did not significantly decrease ethanol, water or total fluid intakes but did significantly attenuate

food intake. The finding that AT did not attenuate ethanol consumption in experiment 1B is contrary to several previous reports (Tampier et al., 1995; Koechling and Amit, 1994; Aragon and Amit, 1992) and questions the role of catalase-produced acetaldehyde in the mediation of ethanol consumption. Although AT failed to decrease ethanol consumption it did produce a significant decrease in food intake and as a consequence a decrease in total caloric intake suggesting that AT produces some effect on food consumption. The fact that AT did not produce a decrease in water or total fluid intake suggests that AT does not produce a general effect in fluid consumption. Therefore, it can be argued on the basis of experiment 1B alone that AT produces a specific effect on food, but not ethanol.

Contrary to experiment 1B, experiment 2 showed that AT had significantly decreased ethanol consumption while food and water intakes remained unaffected. A microstructural analysis of ethanol consumption revealed that the decrease in ethanol intake was due to decreases in both the time spent consuming ethanol per day and the number of ethanol bouts. Food intake was not differentially affected by AT. That is, the AT treated group did not differ from the saline treated group in terms of their food intake. As seen in experiment 1B, AT did not significantly affect either water or total fluid intakes suggesting that the decrease in ethanol consumption observed in experiment 2 was not due to a general decrease in the consumption of fluids but rather to a specific effect on ethanol. Therefore, on the basis of experiment 2 alone, it could be concluded that AT produces a specific decrease in ethanol intake while food and water intakes remained unaffected. The results of experiment 2 are consistent with the literature

concerning the ability of AT to attenuate ethanol consumption but are in opposition to the results of experiment 1B.

Comparison of the findings from both experiment 1B and experiment 2 concerning the effect of AT on ethanol consumption are conflicting and present a problem for the notion that catalase produced acetaldehyde is important in mediating ethanol consumption. Administration of AT in experiment 1B failed to attenuate ethanol consumption while in experiment 2 it produced a significant decrease. This observed lack of a decrease in ethanol intake in response to AT administration in experiment 1B is at odds with previous reports that showed an attenuation in ethanol consumption following AT administration (Tampier et al., 1995; Koechling and Amit, 1994; Aragon and Amit, 1992). However, previous unreported work in this laboratory has observed a failure of AT to produce a significant attenuate of ethanol consumption (Amit, personal communication). Given the large number of studies demonstrating an attenuation of ethanol consumption following AT administration the reason for the failure of AT to decrease ethanol intake observed in experiment 1B are still presently inexplicable.

Although the findings of experiment 1B yielded results contrary to the hypothesis that catalase produced acetaldehyde may be mediating ethanol consumption, experiment 2 provided results that support this notion. Analysis of the decrease in ethanol consumption in experiment 2 showed that the pattern of ethanol intake during the drug phase is consistent with the pattern of catalase inhibition following AT administration. Ethanol intake was attenuated throughout the AT injection phase and until the third ethanol presentation day of the postdrug phase. This observation is consistent with the finding that catalase activity returns to baseline levels about 48 hours after an injection of

AT at a dose of 1 g/kg (Aragon, Rogan and Amit, 1991). It can thus be inferred that the decrease in ethanol consumption observered in experiment 2 may be associated with catalase inhibition since the pattern of ethanol intake is consistent with the pattern of catalase inhibition following AT administration.

Analysis of the pattern of food intake in experiment 2 suggests that AT does not produce a general effect on caloric requirement, which would then have resulted in a decrease in food and ethanol intakes. If AT's actions on ethanol intake are presumed to be secondary to the effects on the need for calories, as proposed by Tampier et al (1995), then the decrease in the pattern of ethanol intake should be the result of a general decrease in caloric intake. With this in mind, a decrease in caloric requirement following AT administration would have also produced an attenuation of food intake and that the pattern of food intake should be similar to the pattern of ethanol intake. Food intake had initially decreased during the drug phase but had returned to baseline levels by injection day five. The fact that food intake was not attenuated throughout the drug phase for the AT treated group, as was the case for ethanol intake, suggests that AT did not produce a general decrease in the need for caloric intake.

Furthermore, the results of both experiment 1B and experiment 2, question the hypothesis set forth by Tampier et al. (1995) that AT produces a general decrease in the need for caloric intake. If AT does produce a decrease in the need for calories, then both ethanol and food intake should have decreased following AT administration. Such results did not occur in either experiment 1B or experiment 2. Administration of AT in experiment 1B produced a decrease in food intake without a decrease in ethanol intake while in experiment 2 AT produced a decrease in ethanol intake without a decrease in

food intake. Thus, a general decrease in all calorie-containing substances was not observed following AT administration suggesting that AT may not possess properties that affect the regulation of calories.

A second objective of the present thesis was to assess whether AT exerted its effects by altering the taste reactivity of rats. Previous work in this laboratory has shown that AT administration had decreased the consumption of not only ethanol, but also a saccharin-quinine solution (Rotzinger et al., 1994). It was proposed by Rotzinger et al. (1994) that AT may have altered taste reactivity since the consumption of a flavoured fluid, one that does not possess reinforcing properties by itself, had also decreased following AT administration. These researchers argued that AT might have produced a change in the taste perception of rats making the saccharin-quinine solution less palatable. Thus, experiment 1B was also designed to test the effects of AT on several different tasting foods in order to assess whether AT also produces a change in taste reactivity. It was hypothesized that if AT had an effect on taste reactivity, then presentation of different flavoured foods may result in different degree of attenuation of the foods.

Experiment 1B showed that animals in the saccharin, neutral and quinine-fed groups decreased their consumption of food intake after AT administration compared to their respective saline control groups. That is, there was no significant difference in the degree of attenuation produced by AT between the saccharin, neutral and quinine-fed groups suggesting that AT did not affect taste reactivity for food. This result is contrary to that observed in a previous study that reported that AT had affected the taste reactivity of rats (Rotzinger et al., 1994). The inconsistent results however may be due to

differences in methodologies between the two studies. Rotzinger et al. (1994) assessed the effects of AT on the acquisition of novel-tasting fluids, ethanol and a saccharin-quinine solution. These researchers had argued that AT may have produced an aversion to novel tasting solutions. Thus, if this is the case, AT may not have affected taste reactivity since the flavoured foods in experiment 1B were familiar by the time AT was administered. In addition, Rotzinger et al. (1995) used a saccharin-quinine-flavoured fluid while the present study used a saccharin and a quinine-flavoured powdered mash. The differences in the methodology, the flavours used, as well as the mode of presentation may explain the discrepant results between experiment 1B and the Rotzinger et al. (1994) study.

Finally, analysis of the data from experiment 1B yielded some interesting findings with regard to the type of flavoured food that was presented to the animals. Animals fed the quinine-flavoured food significantly increased their ethanol intake and significantly decreased their food and water intakes compared to the saccharin and neutral fed groups. Experiment 1C was designed to investigate the possible mechanism that may be involved in increasing ethanol intake due to quinine-feeding.

Wang and Grahame-Smith (1992) have shown that quinine, a potassium channel blocker, produced increases in a set of behaviours that are thought to be 5-HT-mediated. In addition, potassium channel blockers have been shown to increase neurotransmitter release (Kumamoto and Kuba, 1985). Thus, it is conceivable that the quinine-fed animals in experiment 1B had increased levels of 5-HT that had affected their consummatory behaviours. Experiment 1C used the 5-$HT_2$ receptor antagonist ritanserin to investigate whether the increase in ethanol consumption was mediated through the 5-$HT_2$ receptor.

Results of experiment 1C showed that ritanserin did not attenuate the quinine-induced increase in ethanol consumption. In fact, ritanserin also failed to attenuate the decrease in food or water intake seen in the quinine-fed rats. Due to ritanserin's failure to attenuate the quinine-induced increase in ethanol consumption and the decreases in food and water intake, it can be concluded that these changes are not mediated through an interaction with the 5-$HT_2$ receptor. Furthermore, the increase in ethanol consumption seen in the quinine-fed groups could not be explained by compensation for lost calories since a restricted food group had shown no compensation for lost calories by increasing the amount of ethanol consumed.

In summary, the major focus of the present thesis was to assess the effects of the catalase inhibitor AT and whether it produces a decrease in ethanol consumption through an attenuation of centrally produced acetaldehyde or via some mechanism that regulates caloric requirement. On the basis of the present thesis it can be concluded that AT does not produce a general decrease in caloric intake because food intake and ethanol intake were not attenuated simultaneously, or to an equal amount, in either experiment 1B or experiment 2. On the other hand, AT did not produce a decrease in ethanol consumption in experiment 1B raising some questions about the hypothesis that centrally formed acetaldehyde may mediate the reinforcing properties of ethanol. However, although the results of the present thesis showed that AT did not consistently produce a decrease in ethanol intake, it does not preclude a role of central acetaldehyde in mediating the consumption of ethanol.

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# APPENDIX; ANOVA Summary Tables

ANOVA Summary Table for Experiment 1A
Saccharin Concentration Test 1

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Concentration | 795.98 | 5 | 159.197 | 0.8 | 0.5634 |
| Error | 3985.34 | 20 | 199.26 | | |
| Food (F) | 1067.85 | 1 | 1067.85 | 26.24 | 0.0069 |
| Error | 162.81 | 4 | 40.7 | | |
| Days (D) | 134.98 | 4 | 33.74 | 1.62 | 0.2184 |
| Error | 333.88 | 16 | 20.86 | | |
| Conc x F | 690.06 | 5 | 138.01 | 3.86 | 0.013 |
| Error | 714.66 | 20 | 35.73 | | |
| Conc x D | 845.01 | 20 | 42.25 | 2.09 | 0.011 |
| Error | 1616.32 | 80 | 20.2 | | |
| D x F | 1709.01 | 4 | 427.25 | 15.02 | 0.0001 |
| Error | 454.98 | 16 | 28.43 | | |
| Conc x D x F | 1303.46 | 20 | 65.17 | 3.73 | 0.0001 |
| Error | 1399.13 | 80 | 17.48 | | |

ANOVA Summary Table for Experiment 1A
Saccharin Concentration Test 2

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 4.225 | 1 | 4.225 | 0.001 | 0.9224 |
| Error | 3340.375 | 8 | 417.546 | | |
| Concentration | 719.4 | 3 | 239.8 | 1.93 | 0.151 |
| Conc x F | 1570.875 | 3 | 523.625 | 4.22 | 0.0156 |
| Error | 2975.225 | 24 | 123.967 | | |
| Days (D) | 196.15 | 3 | 65.38 | 0.26 | 0.8518 |
| D x F | 313.025 | 3 | 104.341 | 0.42 | 0.7412 |
| Error | 5979.825 | 24 | 249.159 | | |
| Conc x D | 211.45 | 9 | 23.494 | 0.13 | 0.9988 |
| Conc x D x F | 5557.27 | 9 | 617.475 | 3.33 | 0.0018 |
| Error | 13267.75 | 72 | 184.275 | | |

ANOVA Summary Tables for Experiment 1A

Quinine Concentration (0.01% w/w) Test

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 249.6 | 1 | 249.6 | 2.59 | 0.142 |
| Error | 867.53 | 9 | 96.39 | | |
| Days (D) | 118.31 | 3 | 39.43 | 2.01 | 0.1163 |
| D x F | 65.21 | 3 | 21.73 | 1.11 | 0.3628 |
| Error | 529.46 | 27 | 19.61 | | |

Quinine Concentration (0.05% w/w) Test

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 61.96 | 1 | 61.96 | 2.07 | 0.1838 |
| Error | 269.03 | 9 | 29.89 | | |
| Days (D) | 329.78 | 3 | 109.92 | 3.34 | 0.034 |
| D x F | 150.87 | 3 | 50.29 | 1.53 | 0.23 |
| Error | 889.03 | 27 | 32.92 | | |

Quinine Concentration (0.1% w/w) Test

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 249.6 | 1 | 249.6 | 2.59 | 0.142 |
| Error | 867.53 | 9 | 96.39 | | |
| Days (D) | 118.31 | 3 | 39.43 | 2.01 | 0.1361 |
| D x F | 65.21 | 3 | 21.73 | 1.11 | 0.3628 |
| Error | 529.46 | 27 | 19.61 | | |

Quinine Concentration (0.15% w/w) Test

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 2592.8 | 1 | 2592.8 | 16.58 | 0.0028 |
| Error | 1407.83 | 9 | 156.42 | | |
| Days (D) | 75.53 | 3 | 25.17 | 0.3 | 0.8271 |
| D x F | 309.71 | 3 | 103.23 | 1.22 | 0.3221 |
| Error | 2287.83 | 27 | 84.73 | | |

ANOVA Summary Table
Experiment 1B
Ethanol intake (g/kg)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 2009.52 | 2 | 1004.76 | 19.85 | 0.0001 |
| Drug (D) | 5.01 | 1 | 5.01 | 0.1 | 0.7546 |
| F x D | 4.12 | 2 | 2.06 | 0.04 | 0.9601 |
| Error | 2075.61 | 41 | 50.62 | | |
| Treatment (t) | 96.54 | 2 | 48.27 | 12.41 | 0.0001 |
| t x F | 18.37 | 4 | 4.59 | 1.18 | 0.3255 |
| t x D | 1.93 | 2 | 0.97 | 0.25 | 0.7805 |
| t x FD | 1.96 | 4 | 0.49 | 0.13 | 0.9728 |
| Error | 318.93 | 82 | 3.89 | | |
| Days (A) | 8.06 | 4 | 2.01 | 1.78 | 0.1344 |
| A x F | 17.79 | 8 | 2.22 | 1.97 | 0.0533 |
| A x D | 2.98 | 4 | 0.74 | 0.66 | 0.6213 |
| A x FD | 12.38 | 8 | 1.55 | 1.37 | 0.2132 |
| Error | 185.29 | 164 | 1.13 | | |
| tA | 61.35 | 8 | 7.67 | 5.98 | 0.0001 |
| tA x F | 52.58 | 16 | 3.29 | 2.56 | 0.0009 |
| tA x D | 8.72 | 8 | 1.09 | 0.85 | 0.5585 |
| tA x FD | 36.52 | 16 | 2.28 | 1.78 | 0.0325 |
| Error | 420.35 | 328 | 1.28 | | |

ANOVA Summary Table
Experiment 1B
Ethanol preference ratio

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 150394.97 | 2 | 75197.48 | 15.06 | 0.0001 |
| Drug (D) | 223.17 | 1 | 223.17 | 0.04 | 0.8336 |
| F x D | 1811.85 | 2 | 905.93 | 0.18 | 0.8347 |
| Error | 204675.75 | 41 | 4992.09 | | |
| Treatment (t) | 1620.42 | 2 | 810.21 | 2.14 | 0.1242 |
| t x F | 5335.79 | 4 | 1333.95 | 3.52 | 0.0105 |
| t x D | 992.56 | 2 | 496.28 | 1.31 | 0.2752 |
| t x FD | 282.94 | 4 | 70.74 | 0.19 | 0.9446 |
| Error | 31046.48 | 82 | 378.61 | | |
| Days (A) | 1903.1 | 4 | 475.78 | 5.13 | 0.0006 |
| A x F | 3005.77 | 8 | 375.72 | 4.05 | 0.0002 |
| A x D | 540.24 | 4 | 135.06 | 1.46 | 0.2182 |
| A x FD | 756.92 | 8 | 94.61 | 1.02 | 0.4233 |
| Error | 15218.27 | 164 | 92.79 | | |
| tA | 2998.03 | 8 | 374.75 | 2.9 | 0.0039 |
| tA x F | 1335.65 | 16 | 83.47 | 0.65 | 0.8456 |
| tA x D | 843.29 | 8 | 105.41 | 0.82 | 0.5894 |
| tA x FD | 2002.34 | 16 | 125.15 | 0.97 | 0.4916 |
| Error | 42401.99 | 328 | 129.27 | | |

ANOVA Summary Table
Experiment 1B
Total water intake

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 23658.63 | 2 | 11829.31 | 15.18 | 0.0001 |
| Drug (D) | 35.71 | 1 | 35.71 | 0.05 | 0.8316 |
| F x D | 287.49 | 2 | 143.75 | 0.18 | 0.8323 |
| Error | 31956.85 | 41 | 779.44 | | |
| Treatment (t) | 290.86 | 2 | 145.43 | 1.67 | 0.1945 |
| t x F | 1551.9 | 4 | 387.98 | 4.46 | 0.0026 |
| t x D | 75.25 | 2 | 37.62 | 0.43 | 0.6505 |
| t x FD | 24.93 | 4 | 6.23 | 0.07 | 0.9905 |
| Error | 7138.02 | 82 | 87.05 | | |
| Days (A) | 467.55 | 4 | 116.89 | 4.42 | 0.002 |
| A x F | 486.19 | 8 | 60.77 | 2.3 | 0.0232 |
| A x D | 157.48 | 4 | 39.37 | 1.49 | 0.2078 |
| A x FD | 268.73 | 8 | 33.59 | 1.27 | 0.2622 |
| Error | 4336.02 | 164 | 26.44 | | |
| tA | 707.07 | 8 | 88.38 | 2.45 | 0.0136 |
| tA x F | 442.63 | 16 | 27.66 | 0.77 | 0.7218 |
| tA x D | 452.94 | 8 | 56.62 | 1.57 | 0.1322 |
| tA x FD | 505.04 | 16 | 31.57 | 0.88 | 0.5973 |
| Error | 11815.49 | 328 | 36.02 | | |

ANOVA Summary Table
Experiment 1B
Total fluid intake

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 2469.63 | 2 | 1234.81 | 1.09 | 0.3463 |
| Drug (D) | 815.75 | 1 | 815.75 | 0.72 | 0.4014 |
| F x D | 449.12 | 2 | 224.56 | 0.2 | 0.8211 |
| Error | 45351.66 | 41 | 1133.79 | | |
| Treatment (t) | 1466.64 | 2 | 733.32 | 7.54 | 0.001 |
| t x F | 443.19 | 4 | 110.79 | 1.14 | 0.3444 |
| t x D | 80.95 | 2 | 40.47 | 0.42 | 0.6611 |
| t x FD | 121.01 | 4 | 30.25 | 0.31 | 0.8699 |
| Error | 7784.21 | 82 | 97.3 | | |
| Days (A) | 94.46 | 4 | 23.62 | 0.66 | 0.6222 |
| A x F | 167.23 | 8 | 20.9 | 0.58 | 0.7914 |
| A x D | 131.48 | 4 | 32.87 | 0.92 | 0.4563 |
| A x FD | 167.67 | 8 | 20.96 | 0.58 | 0.7902 |
| Error | 5743.54 | 164 | 35.89 | | |
| tA | 2686.09 | 8 | 335.76 | 8.43 | 0.0001 |
| tA x F | 850.86 | 16 | 53.18 | 1.34 | 0.1732 |
| tA x D | 836.23 | 8 | 104.53 | 2.63 | 0.0085 |
| tA x FD | 974.71 | 16 | 60.92 | 1.53 | 0.0872 |
| Error | 12738.4 | 328 | 39.81 | | |

ANOVA Summary Table
Experiment 1B
Total food intake (g)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 8191.24 | 2 | 4095.62 | 11.1 | 0.0001 |
| Drug (D) | 1884.67 | 1 | 1884.67 | 5.11 | 0.0292 |
| F x D | 434.72 | 2 | 217.36 | 0.59 | 0.5593 |
| Error | 15121.97 | 41 | 368.83 | | |
| Treatment (t) | 3002.88 | 2 | 1501.44 | 19 | 0.0001 |
| t x F | 781.87 | 4 | 195.47 | 2.47 | 0.0507 |
| t x D | 850.42 | 2 | 425.21 | 5.38 | 0.0064 |
| t x FD | 144.61 | 4 | 36.15 | 0.46 | 0.7667 |
| Error | 64810.24 | 82 | 79.03 | | |
| Days (A) | 2207.48 | 4 | 551.87 | 17.05 | 0.0001 |
| A x F | 452.39 | 8 | 56.55 | 1.75 | 0.0911 |
| A x D | 114.78 | 4 | 28.69 | 0.89 | 0.4733 |
| A x FD | 178.67 | 8 | 22.33 | 0.69 | 0.6998 |
| Error | 5307.22 | 164 | 32.36 | | |
| tA | 2131.37 | 8 | 266.42 | 7.98 | 0.0001 |
| tA x F | 300.26 | 16 | 18.77 | 0.56 | 0.9111 |
| tA x D | 224.48 | 8 | 28.06 | 0.84 | 0.5679 |
| tA x FD | 461.14 | 16 | 28.82 | 0.86 | 0.6129 |
| Error | 10956.18 | 328 | 33.4 | | |

ANOVA Summary Table
Experiment 1B
Total food intake (g/kg)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 2225.91 | 2 | 1112.95 | 0.48 | 0.6193 |
| Drug (D) | 4644.42 | 1 | 4644.42 | 2.02 | 0.1625 |
| F x D | 577.86 | 2 | 288.93 | 0.13 | 0.8821 |
| Error | 94132.36 | 41 | 2295.91 | | |
| Treatment (t) | 29278.03 | 2 | 14639.01 | 39.28 | 0.0001 |
| t x F | 2380.5 | 4 | 595.13 | 1.6 | 0.183 |
| t x D | 4103.99 | 2 | 2051.99 | 5.51 | 0.0057 |
| t x FD | 1229.68 | 4 | 307.42 | 0.82 | 0.5131 |
| Error | 30561.02 | 82 | 372.69 | | |
| Days (A) | 2993.52 | 4 | 748.38 | 3.67 | 0.0068 |
| A x F | 2049.91 | 8 | 256.24 | 1.26 | 0.2696 |
| A x D | 132.99 | 4 | 33.25 | 0.16 | 0.9568 |
| A x FD | 1041.57 | 8 | 130.19 | 0.64 | 0.7444 |
| Error | 33436.14 | 164 | 203.88 | | |
| tA | 11477.72 | 8 | 1434.71 | 7.4 | 0.0001 |
| tA x F | 1649.32 | 16 | 103.08 | 0.53 | 0.9298 |
| tA x D | 957.27 | 8 | 119.66 | 0.62 | 0.7633 |
| tA x FD | 2794.82 | 16 | 174.68 | 0.9 | 0.5684 |
| Error | 63584.86 | 328 | 193.86 | | |

ANOVA Summary Table
Experiment 1B
Ethanol intake (g/kg) for M1 to B5

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 485.41 | 2 | 242.709 | 5.217 | 0.0093 |
| Error | 2046.91 | 44 | 46.521 | | |
| Treatment (t) | 1449.57 | 3 | 483.192 | 85.63 | 0.0001 |
| t x F | 491.28 | 6 | 81.881 | 14.511 | 0.0001 |
| Error | 744.84 | 132 | 5.643 | | |
| Days (A) | 78.2 | 4 | 19.551 | 15.498 | 0.0001 |
| A x F | 4.9 | 8 | 0.613 | 0.486 | 0.8652 |
| Error | 222.02 | 176 | 1.262 | | |
| t x A | 128.82 | 12 | 10.735 | 7.889 | 0.0001 |
| t x F | 25.71 | 24 | 1.072 | 0.787 | 0.7545 |
| Error | 718.48 | 528 | 1.361 | | |

ANOVA Summary Table
Experiment 1B
Food intake (g) for M1 to B5

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 2413.09 | 2 | 1206.545 | 4.177 | 0.0218 |
| Error | 12710.828 | 44 | 288.882 | | |
| Treatment (t) | 481.83 | 3 | 160.61 | 1.787 | 0.1527 |
| t x F | 1208.549 | 6 | 201.425 | 2.242 | 0.043 |
| Error | 11860.559 | 132 | 89.853 | | |
| Days (A) | 622.5 | 4 | 155.625 | 5.499 | 0.0003 |
| A x F | 141.026 | 8 | 17.628 | 0.623 | 0.7579 |
| Error | 4980.99 | 176 | 28.301 | | |
| t x A | 4278.181 | 12 | 356.515 | 8.212 | 0.0001 |
| t x F | 120.721 | 24 | 50.447 | 1.162 | 0.2712 |
| Error | 22921.429 | 528 | 43.412 | | |

ANOVA Summary Table
Experiment 1B
Water intake for M1 to B5

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 9396.127 | 2 | 4698.063 | 4.857 | 0.0125 |
| Error | 41588.877 | 44 | 967.183 | | |
| Treatment (t) | 26776.863 | 3 | 8925.621 | 107.353 | 0.0001 |
| t x F | 5373.307 | 6 | 895.551 | 10.771 | 0.0001 |
| Error | 10725.386 | 132 | 83.143 | | |
| Days (A) | 1263.091 | 4 | 315.773 | 10.542 | 0.0001 |
| A x F | 119.937 | 8 | 14.992 | 0.501 | 0.8547 |
| Error | 5152.035 | 176 | 29.954 | | |
| t x A | 2923.875 | 12 | 243.656 | 8.722 | 0.0001 |
| t x F | 946.575 | 24 | 39.441 | 1.412 | 0.0937 |
| Error | 14414.798 | 528 | 27.936 | | |

ANOVA Summary Table
Experiment 1B
Total caloric intake (Kcal/kg) for M1 to B5

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 17895.981 | 2 | 8947.991 | 3.791 | 0.0303 |
| Error | 103862.379 | 44 | 2360.509 | | |
| Treatment (t) | 455176.832 | 3 | 151725.611 | 82.626 | 0.0001 |
| t x F | 52519.596 | 6 | 8753.266 | 4.767 | 0.0002 |
| Error | 242390.329 | 132 | 1836.29 | | |
| Days (A) | 43825.052 | 4 | 10956.263 | 17.923 | 0.0001 |
| A x F | 1942.6444 | 8 | 242.83 | 0.397 | 0.921 |
| Error | 107587.741 | 176 | 611.294 | | |
| t x A | 136421.552 | 12 | 11368.463 | 13.296 | 0.0001 |
| t x F | 18434.351 | 24 | 468.098 | 0.898 | 0.605 |
| Error | 451445.523 | 528 | 855.01 | | |

ANOVA Summary Table
Experiment 1B
Weights (g)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 550961.348 | 2 | 275480.674 | 12.76 | 0.0001 |
| Drug (D) | 7191.784 | 1 | 7191.784 | 0.333 | 0.567 |
| F x D | 20235.007 | 2 | 10117.503 | 0.469 | 0.6291 |
| Error | 885096.839 | 41 | 21587.728 | | |
| Treatment (t) | 308120.389 | 2 | 154060.194 | 575.57 | 0.0001 |
| t x F | 3492.378 | 4 | 873.094 | 3.262 | 0.0156 |
| t x D | 3341.629 | 2 | 1670.815 | 6.242 | 0.003 |
| t x FD | 783.631 | 4 | 195.908 | 0.73 | 0.5728 |
| Error | 21948.558 | 82 | 267.665 | | |
| Days (A) | 35695.81 | 4 | 8923.953 | 223.265 | 0.0001 |
| A x F | 555.824 | 8 | 69.478 | 1.738 | 0.0931 |
| A x D | 259.915 | 4 | 64.979 | 1.626 | 0.1702 |
| A x FD | 622.953 | 8 | 77.869 | 1.948 | 0.0562 |
| Error | 6555.112 | 164 | 39.97 | | |
| tA | 2090.676 | 8 | 261.335 | 6.094 | 0.0001 |
| tA x F | 1042.858 | 16 | 65.179 | 1.52 | 0.0905 |
| tA x D | 287.363 | 8 | 35.92 | 0.838 | 0.57 |
| tA x FD | 1010.541 | 16 | 63.159 | 1.473 | 0.1076 |
| Error | 14066.257 | 328 | 42.885 | | |

ANOVA Summary Table
Experiment 1B
Caloric intake from ethanol (Kcal/kg) for M1 to B5

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 24134.352 | 2 | 12067.176 | 5.15 | 0.0098 |
| Error | 103099.699 | 44 | 2343.175 | | |
| Treatment (t) | 73075.947 | 3 | 24358.649 | 87.523 | 0.0001 |
| t x F | 25996.016 | 6 | 4332.669 | 15.568 | 0.0001 |
| Error | 36737.302 | 132 | 278.313 | | |
| Days (A) | 3843.438 | 4 | 960.859 | 17.227 | 0.0001 |
| A x F | 288.691 | 8 | 36.086 | 0.647 | 0.7374 |
| Error | 9816.8 | 176 | 55.777 | | |
| t x A | 6291.885 | 12 | 524.324 | 7.958 | 0.0001 |
| t x F | 1271.056 | 24 | 52.961 | 0.804 | 0.7337 |
| Error | 34788.688 | 528 | 65.888 | | |

ANOVA Summary Table
Experiment 1B
Caloric intake from food (Kcal/kg) for M1 to B5

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 5444.55 | 2 | 2722.27 | 0.77 | 0.4687 |
| Error | 155373.68 | 44 | 3531.22 | | |
| Treatment (t) | 893603.14 | 3 | 297867.71 | 137.69 | 0.0001 |
| t x F | 9165.94 | 6 | 1527.65 | 0.71 | 0.6452 |
| Error | 285556.81 | 132 | 2163.31 | | |
| Days (A) | 71850.15 | 4 | 17962.53 | 29.22 | 0.0001 |
| A x F | 1997.59 | 8 | 249.69 | 0.41 | 0.916 |
| Error | 108210.24 | 176 | 614.83 | | |
| t x A | 133151.27 | 12 | 11095.94 | 12.65 | 0.0001 |
| t x F | 19001.93 | 24 | 791.74 | 0.9 | 0.5987 |
| Error | 763020.17 | 528 | 876.93 | | |

ANOVA Summary Tables for Experiment 1C
Ethanol intake (g/kg)

**Quinine-Vehicle vs. Quinine-Ritanserin**

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 34.672 | 1 | 34.672 | 0.636 | 0.4406 |
| Error | 654.062 | 12 | 54.505 | | |
| Treatment (t) | 836.852 | 4 | 209.213 | 35.737 | 0.0001 |
| t x F | 38.236 | 4 | 9.559 | 1.633 | 0.1813 |
| Error | 281 | 48 | 5.854 | | |
| Days (A) | 15.694 | 4 | 3.924 | 3.153 | 0.0222 |
| F x A | 9.32 | 4 | 2.33 | 1.873 | 0.1306 |
| Error | 59.724 | 48 | 1.244 | | |
| At | 86.797 | 16 | 5.425 | 3.694 | 0.0001 |
| At x F | 34.578 | 16 | 2.161 | 1.472 | 0.1135 |
| Error | 281.946 | 192 | 1.468 | | |

**Quinine-Vehicle vs. Neutral-Vehicle**

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 142.059 | 1 | 142.059 | 2.353 | 0.1533 |
| Error | 664.081 | 12 | 60.371 | | |
| Treatment (t) | 286.016 | 4 | 71.504 | 12.148 | 0.0001 |
| t x F | 79.051 | 4 | 19.763 | 3.358 | 0.0175 |
| Error | 258.99 | 48 | 5.886 | | |
| Days (A) | 10.322 | 4 | 2.581 | 1.744 | 0.1574 |
| F x A | 8.606 | 4 | 2.152 | 1.454 | 0.2324 |
| Error | 65.095 | 48 | 1.479 | | |
| At | 54.228 | 16 | 3.389 | 3.135 | 0.0001 |
| At x F | 27.469 | 16 | 1.717 | 1.588 | 0.0761 |
| Error | 190.291 | 192 | 1.081 | | |

**Quinine-Vehicle vs. Restricted Food**

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 87.35 | 1 | 87.35 | 1.899 | 0.1956 |
| Error | 505.944 | 12 | 45.995 | | |
| Treatment (t) | 351.67 | 4 | 87.917 | 19.936 | 0.0001 |
| t x F | 43.391 | 4 | 10.848 | 2.46 | 0.0592 |
| Error | 194.041 | 48 | 4.41 | | |
| Days (A) | 16.835 | 4 | 4.209 | 3.769 | 0.0101 |
| F x A | 5.159 | 4 | 1.29 | 1.155 | 0.3437 |
| Error | 49.135 | 48 | 1.117 | | |
| At | 55.977 | 16 | 3.499 | 3.208 | 0.0001 |
| At x F | 30.542 | 16 | 1.909 | 1.75 | 0.0415 |
| Error | 191.949 | 192 | 1.091 | | |

ANOVA Summary Tables for Experiment 1C
Food intake (g)

**Quinine-Vehicle vs. Quinine-Ritanserin**

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 5.283 | 1 | 5.283 | 0.016 | 0.9019 |
| Error | 3996.77 | 12 | 333.065 | | |
| Treatment (t) | 10429.24 | 4 | 2607.31 | 35.787 | 0.0001 |
| t x F | 74.131 | 4 | 18.533 | 0.254 | 0.9056 |
| Error | 3497.1098 | 48 | 72.856 | | |
| Days (A) | 470.183 | 4 | 117.546 | 4.221 | 0.0052 |
| F x A | 223.474 | 4 | 55.869 | 2.006 | 0.1086 |
| Error | 1336.823 | 48 | 27.85 | | |
| At | 1192.703 | 16 | 74.544 | 2.922 | 0.0002 |
| At x F | 399.469 | 16 | 24.967 | 0.979 | 0.4816 |
| Error | 4898.149 | 192 | 25.511 | | |

**Quinine-Vehicle vs. Neutral-Vehicle**

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 98.409 | 1 | 98.409 | 0.142 | 0.7134 |
| Error | 7619.942 | 12 | 692.722 | | |
| Treatment (t) | 3781.69 | 4 | 945.422 | 9.092 | 0.0001 |
| t x F | 1847.302 | 4 | 461.826 | 4.441 | 0.0042 |
| Error | 4575.301 | 48 | 103.984 | | |
| Days (A) | 161.727 | 4 | 40.432 | 1.721 | 0.1625 |
| F x A | 281.493 | 4 | 70.3736 | 2.995 | 0.0285 |
| Error | 1033.787 | 48 | 23.495 | | |
| At | 991.503 | 16 | 61.969 | 1.587 | 0.0763 |
| At x F | 615.552 | 16 | 38.472 | 0.985 | 0.4747 |
| Error | 6871.137 | 192 | 39.041 | | |

**Quinine-Vehicle vs. Restricted Food**

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 1413.162 | 1 | 1413.162 | 2.146 | 0.1709 |
| Error | 7244.229 | 12 | 658.566 | | |
| Treatment (t) | 5677.392 | 4 | 1419.348 | 22.454 | 0.0001 |
| t x F | 1097.762 | 4 | 274.44 | 4.342 | 0.0048 |
| Error | 2781.248 | 48 | 63.21 | | |
| Days (A) | 201.563 | 4 | 50.391 | 2.623 | 0.0474 |
| F x A | 194.732 | 4 | 478.683 | 2.534 | 0.0535 |
| Error | 845.2 | 48 | 19.209 | | |
| At | 735.349 | 16 | 45.959 | 2.542 | 0.0015 |
| At x F | 433.011 | 16 | 27.063 | 1.497 | 0.1053 |
| Error | 3182.657 | 192 | 18.083 | | |

ANOVA Summary Tables for Experiment 1C
Water intake (mls)

**Quinine-Vehicle vs. Quinine-Ritanserin**

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 66.883 | 1 | 66.883 | 0.066 | 0.8016 |
| Error | 12163.211 | 12 | 1013.604 | | |
| Treatment (t) | 41230.943 | 4 | 10307.736 | 85.228 | 0.0001 |
| t x F | 229.331 | 4 | 57.333 | 0.474 | 0.7545 |
| Error | 5805.246 | 48 | 120.943 | | |
| Days (A) | 370.771 | 4 | 92.693 | 3.128 | 0.023 |
| F x A | 171.16 | 4 | 42.79 | 1.444 | 0.234 |
| Error | 1422.389 | 48 | 29.633 | | |
| At | 2102.571 | 16 | 131.411 | 3.127 | 0.0001 |
| At x F | 513.554 | 16 | 32.098 | 0.764 | 0.725 |
| Error | 8069.154 | 192 | 42.027 | | |

**Quinine-Vehicle vs. Neutral-Vehicle**

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 143.795 | 1 | 143.795 | 0.224 | 0.6456 |
| Error | 7075.854 | 12 | 643.259 | | |
| Treatment (t) | 18762.722 | 4 | 4690.68 | 48.029 | 0.0001 |
| t x F | 2721.737 | 4 | 680.434 | 6.967 | 0.0002 |
| Error | 4297.198 | 48 | 97.664 | | |
| Days (A) | 358.992 | 4 | 89.748 | 3.47 | 0.0151 |
| F x A | 56.407 | 4 | 14.102 | 0.545 | 0.7034 |
| Error | 1138.036 | 48 | 25.864 | | |
| At | 1786.137 | 16 | 111.637 | 3.645 | 0.0001 |
| At x F | 614.506 | 16 | 38.407 | 1.254 | 0.2319 |
| Error | 5389.697 | 192 | 30.623 | | |

**Quinine-Vehicle vs. Restricted Food**

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Food (F) | 2063.315 | 1 | 2063.315 | 2.652 | 0.1317 |
| Error | 8558.894 | 12 | 778.081 | | |
| Treatment (t) | 21088.254 | 4 | 5272.064 | 60.495 | 0.0001 |
| t x F | 1913.202 | 4 | 478.3 | 5.488 | 0.0011 |
| Error | 3834.558 | 48 | 87.149 | | |
| Days (A) | 298.734 | 4 | 74.684 | 3.068 | 0.0259 |
| F x A | 131.497 | 4 | 32.874 | 1.35 | 0.2666 |
| Error | 1071.063 | 48 | 24.342 | | |
| At | 1771.953 | 16 | 110.747 | 3.986 | 0.0001 |
| At x F | 471.559 | 16 | 29.472 | 1.061 | 0.3961 |
| Error | 4889.604 | 192 | 27.782 | | |

ANOVA Summary Table
Experiment 2
Ethanol intake (g/kg)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 21.52 | 1 | 21.52 | 0.51 | 0.4893 |
| Error | 552.46 | 13 | 42.49 | | |
| Treatment (t) | 14.32 | 2 | 7.16 | 2.07 | 0.1462 |
| t x D | 19.51 | 2 | 9.75 | 2.82 | 0.0778 |
| Error | 89.85 | 26 | 3.46 | | |
| Days (A) | 6.58 | 4 | 1.65 | 2.05 | 0.1012 |
| A x D | 1.07 | 4 | 0.26 | 0.33 | 0.8538 |
| Error | 41.79 | 52 | 0.8 | | |
| tA | 8.42 | 8 | 1.05 | 1.39 | 0.2098 |
| tA x D | 12.38 | 8 | 1.54 | 2.04 | 0.0484 |
| Error | 78.82 | 104 | 0.76 | | |

ANOVA Summary Table
Experiment 2
Percent change from baseline
Ethanol intake (g/kg)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 53375.57 | 1 | 53375.57 | 9.91 | 0.0077 |
| Error | 69993.24 | 13 | 5384.09 | | |
| Treatment (t) | 2188.95 | 1 | 2188.95 | 0.61 | 0.4486 |
| t x D | 777.64 | 1 | 77.64 | 0.22 | 0.6492 |
| Error | 46618.52 | 13 | 3586.04 | | |
| Days (A) | 2322.05 | 4 | 580.51 | 0.65 | 0.6273 |
| A x D | 2355.25 | 4 | 588.81 | 0.66 | 0.6209 |
| Error | 46222.32 | 52 | 888.89 | | |
| tA | 847.37 | 4 | 211.84 | 0.34 | 0.8513 |
| tA x D | 4004.53 | 4 | 1001.13 | 1.6 | 0.1892 |
| Error | 32613.76 | 52 | 627.19 | | |

ANOVA Summary Table
Experiment 2
Total Ethanol Bout Duration
(seconds per day)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 668639.16 | 1 | 668639.16 | 0.37 | 0.5531 |
| Error | 23450480.37 | 13 | 1803883.11 | | |
| Treatment (t) | 930153.73 | 2 | 465076.86 | 1.91 | 0.1678 |
| t x D | 1576230.6 | 2 | 788115.29 | 3.24 | 0.0553 |
| Error | 6320048.07 | 26 | 243078.58 | | |
| Days (A) | 163759.98 | 4 | 40939.99 | 0.33 | 0.8543 |
| A x D | 395003.35 | 4 | 98750.84 | 0.80 | 0.5283 |
| Error | 6387764.22 | 52 | 122841.62 | | |
| tA | 1079140.1 | 8 | 134892.51 | 1.16 | 0.3292 |
| tA x D | 707438.68 | 8 | 88429.83 | 0.76 | 0.637 |
| Error | 12072936.2 | 104 | 116085.93 | | |

ANOVA Summary Table
Experiment 2
Ethanol bout frequency

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 8.69 | 1 | 8.69 | 0.20 | 0.6622 |
| Error | 565.69 | 13 | 43.51 | | |
| Treatment (t) | 131.09 | 2 | 65.54 | 1.55 | 0.2306 |
| t x D | 169.43 | 2 | 84.72 | 2.01 | 0.1546 |
| Error | 1097.12 | 26 | 42.19 | | |
| Days (A) | 49.86 | 4 | 12.46 | 1.31 | 0.2777 |
| A x D | 6.25 | 4 | 1.56 | 0.16 | 0.9554 |
| Error | 793.9 | 52 | 9.49 | | |
| tA | 101.13 | 8 | 12.64 | 1.12 | 0.353 |
| tA x D | 49.27 | 8 | 6.16 | 0.55 | 0.8177 |
| Error | 1168.85 | 104 | 11.24 | | |

ANOVA Summary Table
Experiment 2
Percent change from baseline
Ethanol bout frequency

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 27436.471 | 1 | 27436.4 | 3.32 | 0.0917 |
| Error | 107586.25 | 13 | 8275.87 | | |
| Treatment (t) | 0.17 | 1 | 0.17 | 0.00 | 0.9912 |
| t x D | 446.42 | 1 | 446.42 | 0.32 | 0.5787 |
| Error | 17886.94 | 13 | 1375.92 | | |
| Days (A) | 1380.18 | 4 | 345.04 | 0.39 | 0.815 |
| A x D | 4949.09 | 4 | 1237.27 | 1.40 | 0.2478 |
| Error | 46038.18 | 52 | 885.35 | | |
| tA | 3445.26 | 4 | 861.32 | 1.05 | 0.3909 |
| tA x D | 595.37 | 4 | 148.84 | 0.18 | 0.9471 |
| Error | 42681.51 | 52 | 820.79 | | |

ANOVA Summary Table
Experiment 2
Ethanol bout size

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 9.16 | 1 | 9.16 | 0.67 | 0.4273 |
| Error | 177.39 | 13 | 13.65 | | |
| Treatment (t) | 5.06 | 2 | 2.53 | 2.71 | 0.0855 |
| t x D | 2.05 | 2 | 1.02 | 1.10 | 0.3494 |
| Error | 24.29 | 26 | 0.93 | | |
| Days (A) | 1.59 | 4 | 0.39 | 1.31 | 0.2802 |
| A x D | 2.03 | 4 | 0.51 | 1.66 | 0.1743 |
| Error | 15.91 | 52 | 0.31 | | |
| tA | 3.45 | 8 | 0.43 | 1.05 | 0.4055 |
| tA x D | 2.33 | 8 | 0.29 | 0.71 | 0.6852 |
| Error | 42.82 | 104 | 0.41 | | |

ANOVA Summary Table
Experiment 2
Ethanol bout duration
(seconds per bout)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 17840.48 | 1 | 17840.48 | 1.13 | 0.3073 |
| Error | 205406.12 | 13 | 15800.47 | | |
| Treatment (t) | 2327.27 | 2 | 1163.63 | 1.07 | 0.3576 |
| t x D | 5157.35 | 2 | 2578.67 | 2.37 | 0.1132 |
| Error | 28271.6 | 26 | 1087.37 | | |
| Days (A) | 1354.68 | 4 | 338.67 | 0.54 | 0.7104 |
| A x D | 598.47 | 4 | 149.62 | 0.24 | 0.9162 |
| Error | 32899.12 | 52 | 632.68 | | |
| tA | 11632.61 | 8 | 1454.08 | 2.13 | 0.0392 |
| tA x D | 3481.19 | 8 | 435.15 | 0.64 | 0.7445 |
| Error | 70974.08 | 104 | 682.44 | | |

ANOVA Summary Table
Experiment 2
Ethanol licks per day

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 9465440.5 | 1 | 9465440 | 0.13 | 0.7213 |
| Error | 925508602.5 | 13 | 71192969.42 | | |
| Treatment (t) | 6587588.4 | 2 | 3293794.2 | 1.02 | 0.3735 |
| t x D | 6281882.8 | 2 | 3140941.4 | 0.98 | 0.3904 |
| Error | 83714483.9 | 26 | 3219787.84 | | |
| Days (A) | 6289874 | 4 | 1572468.5 | 1.49 | 0.2194 |
| A x D | 1214792.5 | 4 | 303698.13 | 0.29 | 0.8849 |
| Error | 54965770 | 52 | 1057034.04 | | |
| tA | 6394791.8 | 8 | 799348.97 | 0.96 | 0.4699 |
| tA x D | 12661552 | 8 | 1582693.9 | 1.90 | 0.067 |
| Error | 86425723 | 104 | 831016.57 | | |

ANOVA Summary Table
Experiment 2
Ethanol preference ratio

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 14000.915 | 1 | 14000.915 | 1.94 | 0.1867 |
| Error | 93666.054 | 13 | 7205.081 | | |
| Treatment (t) | 5667.16 | 2 | 2833.581 | 3.94 | 0.032 |
| t x D | 3623.78 | 2 | 1811.892 | 2.52 | 0.0999 |
| Error | 18690.55 | 26 | 718.68 | | |
| Days (A) | 996.689 | 4 | 249.17 | 1.31 | 0.277 |
| A x D | 907.54 | 4 | 226.88 | 1.20 | 0.3234 |
| Error | 9860.51 | 52 | 189.62 | | |
| tA | 738.32 | 8 | 92.29 | 0.83 | 0.6266 |
| tA x D | 2025.95 | 8 | 253.24 | 1.47 | 0.179 |
| Error | 17979.18 | 104 | 172.87 | | |

ANOVA Summary Table
Experiment 2
Total fluid intake (mls)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 1603.35 | 1 | 1603.35 | 1.97 | 0.1843 |
| Error | 10599.94 | 13 | 815.38 | | |
| Treatment (t) | 355.44 | 2 | 177.725 | 1.46 | 0.2501 |
| t x D | 80.89 | 2 | 40.44 | 0.33 | 0.7199 |
| Error | 3159.32 | 26 | 121.51 | | |
| Days (A) | 460.51 | 4 | 115.13 | 1.71 | 0.1611 |
| A x D | 162.29 | 4 | 40.57 | 0.60 | 0.6617 |
| Error | 3494.44 | 52 | 67.2 | | |
| tA | 664.44 | 8 | 83.05 | 1.48 | 0.1749 |
| tA x D | 400.86 | 8 | 50.11 | 0.89 | 0.5274 |
| Error | 5852.41 | 104 | 56.27 | | |

ANOVA Summary Table
Experiment 2
Total food intake (# of pellets)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 1422309.44 | 1 | 142309.44 | 0.68 | 0.4241 |
| Error | 2716609.35 | 13 | 208969.95 | | |
| Treatment (t) | 173314.2 | 2 | 86657.1 | 7.44 | 0.0028 |
| t x D | 28191.99 | 2 | 14095.99 | 1.21 | 0.3144 |
| Error | 302822.96 | 26 | 11647.04 | | |
| Days (A) | 50033.03 | 4 | 12508.26 | 2.04 | 0.1022 |
| A x D | 3957.74 | 4 | 989.43 | 0.16 | 0.9569 |
| Error | 318688.49 | 52 | 6128.62 | | |
| tA | 67416.41 | 8 | 8427.05 | 1.05 | 0.4021 |
| tA x D | 54374.26 | 8 | 6796.78 | 0.85 | 0.5619 |
| Error | 832521.89 | 104 | 8005.02 | | |

ANOVA Summary Table
Experiment 2
Food bout frequency

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 102.78 | 1 | 102.78 | 1.53 | 0.2382 |
| Error | 874.21 | 13 | 67.25 | | |
| Treatment (t) | 130.14 | 2 | 65.07 | 8.99 | 0.0011 |
| t x D | 18.67 | 2 | 9.34 | 1.29 | 0.2922 |
| Error | 188.14 | 26 | 7.23 | | |
| Days (A) | 20.50 | 4 | 5.13 | 1.51 | 0.2121 |
| A x D | 8.56 | 4 | 2.14 | 0.63 | 0.6425 |
| Error | 176.27 | 52 | 3.39 | | |
| tA | 72.38 | 8 | 9.05 | 2.54 | 0.0146 |
| tA x D | 31.31 | 8 | 3.91 | 1.10 | 0.3712 |
| Error | 371.02 | 104 | 3.57 | | |

ANOVA Summary Table
Experiment 2
Total food bout duration
(seconds per day)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 2906579.2 | 1 | 2906579.2 | 0.30 | 0.5912 |
| Error | 124605563 | 13 | 9585043.3 | | |
| Treatment (t) | 6151857.6 | 2 | 3075928.8 | 5.09 | 0.0136 |
| t x D | 13122.79 | 2 | 6561.31 | 0.01 | 0.9892 |
| Error | 15701772.4 | 26 | 603914.33 | | |
| Days (A) | 2694092 | 4 | 673523.01 | 1.79 | 0.1445 |
| A x D | 1192479.7 | 4 | 298119.93 | 0.79 | 0.535 |
| Error | 19545944.2 | 52 | 375883.54 | | |
| tA | 4913619.5 | 8 | 614202.44 | 1.83 | 0.0795 |
| tA x D | 2395657.8 | 8 | 299457.22 | 0.89 | 0.5256 |
| Error | 34892756.3 | 104 | 335507.24 | | |

ANOVA Summary Table
Experiment 2
Food bout size

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 9222.84 | 1 | 9222.84 | 2.19 | 0.1627 |
| Error | 54742.94 | 13 | 4210.99 | | |
| Treatment (t) | 4147.32 | 2 | 2073.66 | 6.36 | 0.0056 |
| t x D | 1362.91 | 2 | 681.46 | 2.09 | 0.144 |
| Error | 8476.87 | 26 | 326.03 | | |
| Days (A) | 896.69 | 4 | 224.17 | 1.33 | 0.272 |
| A x D | 114.05 | 4 | 28.51 | 0.17 | 0.9533 |
| Error | 8779.11 | 52 | 168.82 | | |
| tA | 4239.92 | 8 | 529.99 | 3.14 | 0.0032 |
| tA x D | 2101.14 | 8 | 262.64 | 1.55 | 0.1477 |
| Error | 17573.29 | 104 | 168.97 | | |

ANOVA Summary Table
Experiment 2
Food bout duration
(seconds per bout)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 23594179 | 1 | 23594179 | 2.05 | 0.1755 |
| Error | 16092166 | 13 | 123785.89 | | |
| Treatment (t) | 44361.22 | 2 | 22180.61 | 4.47 | 0.0215 |
| t x D | 18491.46 | 2 | 9245.73 | 1.86 | 0.1754 |
| Error | 129053.39 | 26 | 4963.59 | | |
| Days (A) | 41756.45 | 4 | 10439.11 | 3.27 | 0.0183 |
| A x D | 2777.36 | 4 | 694.34 | 0.22 | 0.9276 |
| Error | 166125.33 | 52 | 3194.72 | | |
| tA | 47545.32 | 8 | 5943.17 | 2.13 | 0.0391 |
| tA x D | 33822.28 | 8 | 4227.79 | 1.52 | 0.1605 |
| Error | 290014.21 | 104 | 2788.59 | | |

ANOVA Summary Table
Experiment 2
Total water intake

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Drug (D) | 7158.33 | 1 | 7158.32 | 3.81 | 0.0727 |
| Error | 24407.89 | 13 | 1877.53 | | |
| Treatment (t) | 1926.09 | 2 | 963.05 | 3.97 | 0.0314 |
| t x D | 267.04 | 2 | 133.52 | 0.55 | 0.5835 |
| Error | 6311.9 | 26 | 242.77 | | |
| Days (A) | 261.68 | 4 | 65.42 | 0.64 | 0.6367 |
| A x D | 206.57 | 4 | 51.64 | 0.50 | 0.7323 |
| Error | 5319.21 | 52 | 102.29 | | |
| tA | 476.94 | 8 | 59.62 | 0.64 | 0.7441 |
| tA x D | 447.93 | 8 | 55.99 | 0.60 | 0.7766 |
| Error | 9717.1 | 104 | 93.43 | | |