Effects of Acetaldehyde, GABAergic, and Glutamatergic Manipulation on an

Ethanol Discriminative Taste Aversion

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Abstract

Effects of Acetaldehyde, GABAergic, and Glutamatergic Manipulation on an

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The primary objective of the present thesis was to examine the

contribution of acetaldehyde, as well as GABA receptor agonists and glutamate

receptor antagonists in mediating the discriminative stimulus effects of ethanol

using a discriminative taste aversion (DTA) procedure. The DTA procedure

trained animals to associate the stimulus effects of ethanol or acetaldehyde with

either a saccharin-LiCl or a saccharin-saline pairing. Animals in the LiCl group

learned to decrease their saccharin intake following ethanol or acetaldehyde

injections but not after saline injections. Animals in the Saline groups did not

decrease their saccharin intake when injected with either the training drug or

saline.

Experiment 1a showed that acetaldehyde partially substituted for ethanol

while experiment 1b showed that ethanol partially substituted for acetaldehyde.

Administration of the catalase inhibitor aminotriazole failed to block the

discriminative cue of ethanol. Animals in experiment 2 and 3 were trained to

discriminate ethanol from saline. Generalization tests showed that administration

of the gamma-aminotransaminase inhibitor AOAA, the $GABA_A$ agonists THIP and

pentobarbital, the $GABA_B$ agonist baclofen failed to substitute for ethanol while

the $GABA_A$ antagonist picrotoxin failed to block the ethanol cue. Experiment 3 showed that the NMDA antagonists MK-801 and memantine substituted for ethanol while the AMPA antagonist GYKI 52466 did not, suggesting that inhibition of the NMDA receptor, but not the AMPA receptor contributes to the stimulus effects of ethanol. Overall, the findings from the present thesis showed that the DTA procedure could quickly and reliably train animals to discriminate ethanol, and acetaldehyde, from saline. Generalization tests demonstrated that acetaldehyde and the NMDA receptor, but not the $GABA_A$ receptor, contributes to the stimulus effects of ethanol.

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General Introduction

Alcohol is one of the most abused drugs in Canada with over 1.3 million people, aged 12 years and older, categorized as having a probable case of alcohol dependence (Statistics Canada, 2000-2001). Increased health costs due to alcohol-related injuries, increased unemployment and increased crime rates are some examples of the serious societal and personal costs associated with alcoholism and/or alcohol abuse (Martin, 2001; Rice et al., 1985). Recent work has identified specific effects of alcohol on several neurotransmitter systems including the GABAergic, glutamatergic, serotonergic, dopaminergic and opioidergic systems (for review see Eckardt et al., 1998). The fact that alcohol has effects on multiple systems suggests that multiple pharmacological interventions may be available for treatment of alcohol addiction (Kostowski, & Bienkowski, 1999). Therefore, research examining the neurotransmitter systems that contribute to the behavioural effects of alcohol may be crucial in understanding the biological and psychopharmacological influences mediating alcohol abuse liability (Holtzman, 1990).

The drug discrimination paradigm is a useful and important experimental model for assessing the subjective or stimulus effects of drugs. Alcohol, as well as most drugs that act on the central nervous system produce specific stimulus effects believed to reflect receptor-mediated activity (Colpaert, 1986, 1999). Furthermore, these stimulus effects are thought to contribute to either the initiation of drug taking in intermittent users and/or to the relapse process in drug abusers (Colpaert, 1986, 1999; Stolerman, 1992). The drug discrimination

design provides a method for identifying possible neural mechanisms, including membrane receptor involvement, which may mediate the interoceptive properties of centrally acting drugs. Data from behavioural and psychopharmacological studies have provided evidence suggesting that the interoceptive properties of ethanol, the active ingredient in alcohol, are mediated in part by several compounds and neurotransmitter systems including acetaldehyde, GABA, and glutamate (for review see Kostowski & Bienkowski, 1999).

The present thesis is a drug discrimination study designed to assess the roles of acetaldehyde, as well as the GABAergic and glutamatergic neurotransmitter systems in mediating the discriminative stimulus properties of ethanol using a novel drug discrimination procedure – the discriminative taste aversion procedure. The first three sections of the introduction discuss the drug discrimination procedure, the discriminative stimulus properties of a drug and the discriminative taste aversion procedure respectively. The remainder of the introduction will focus on discussing the roles of acetaldehyde, the $GABA_A$ and the NMDA receptors as possible contributors to the stimulus effects of ethanol. There are two sections that review the metabolism of ethanol into acetaldehyde and the role that acetaldehyde may have on the mediation of ethanol consumption and ethanol-related behaviours. The effects of ethanol on the $GABA_A$ receptor activity, as well as the role of the $GABA_A$ receptor in mediating ethanol-related behaviours will then be discussed followed by a similar review on the effect that ethanol has NMDA receptor function and how inhibition of the NMDA receptor may mediate some of ethanol's behavioural effects. The final

section describes work using immunocytochemical techniques to identify brain structures activated following experimenter- and self-administered ethanol.

Drug discrimination procedure

Several drug discrimination procedures have been developed but by far the most commonly used is based on operant lever responding (Colpaert, 1999). Animals are trained to press a particular lever when the training drug is present and another lever when the training drug is absent. The stimulus effects of the training drug serve as a cue that the animal uses to decide which of the two responses to emit. Under these conditions the behaviour of the animal is controlled by the interoceptive drug stimuli in much the same way as behaviour is controlled by traditional exteroceptive environmental stimuli, such as auditory or visual cues (Colpaert, 1999).

Operant drug discrimination studies are usually conducted over an extended period of time because animals have to be trained to bar press for a reinforcer, usually a food pellet or sweet tasting fluid. Initially, animals are trained to bar press using a fixed ratio (FR) 1 schedule of reinforcement and once stable bar pressing is attained the FR schedule is gradually increased to FR schedules between 10 and 20 (e.g. Grant & Colombo, 1993). Animals are then trained to discriminate the training drug from vehicle or saline and make drug appropriate lever responses. When the training drug is administered animals are trained to press a lever designated as the drug lever. When the stimulus effects of the training drug are absent (saline injections) animals are trained to press a lever

designated as the saline lever. Animals are trained until they press the drug-associated lever above some predetermined set of criteria after administration of the training drug. Discrimination criteria usually require that animals correctly make appropriate drug-lever responses at or above 80% for 5-10 consecutive sessions following the administration of the training drug. The number of sessions typically needed to acquire stable stimulus control in operant paradigms for an ethanol dose of 1.0 g/kg has been reported to take between 30 and 60 training sessions (e.g.; Jarbe et al., 1982; Jeffreys et al., 1990; Stefanski et al., 1996). Lower doses of ethanol, around 0.5 g/kg, have required as many as 100 sessions or more for animals to demonstrate stimulus control, if they do at all (Stefanski et al., 1996).

Once discriminative control has been achieved tests of stimulus generalization (substitution tests) or stimulus blockade (antagonism tests) can be performed. Generalization tests occur when a test drug is administered to an animal trained to discriminate a training drug from saline. If the test drug produces similar stimulus effects as the training drug then the animal should respond by pressing the drug-lever. If the test drug does not share similar stimulus effects with the training drug the animal should press the saline-lever.

The degree of generalization that a test drug displays is a function of how similar the test drug mimics the stimulus effects of the training drug. Substitution of a test drug for a training drug suggests that common receptor systems may be involved in mediating the action of both drugs (Colpaert, 1999). Stimulus generalization tests can produce a range of drug-lever pressing behaviour.

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When a test drug produces drug-lever pressing that is significantly greater than that produced by saline, but less than that produced by the training drug itself, a partial substitution is said to have occurred. Partial substitution has typically been defined as ratio of 40-80% correct responding on the drug-lever over total lever responding following the administration of a test drug (Bienkowski et al., 1998; Hodge & Cox, 1998; Hodge et al., 2001). Complete substitution has usually defined as 80% or more of total lever pressing occurring on the drug-lever after administration of a test drug (Bienkowski et al., 1998; Hodge & Cox, 1998; Hodge et al., 2001).

Antagonism tests are also performed to establish which neurotransmitter systems and, in particular, which specific receptor population may be involved in mediating a drug's discriminative cue. During antagonism tests a test drug is administered prior to the training drug and behavioural testing. If the test drug prevents the training drug from producing its stimulus effects, then the animal would behave as if it were injected with saline and blockade of the discriminative effects would have occurred. Thus, if a particular receptor antagonist blocks the stimulus effects of the training drug, that receptor is thought to contribute to the discriminative cue of the training drug.

Properties of the discriminative stimulus cue

The discriminative stimulus effects of drugs, particularly drugs of abuse, are thought to produce subjective experiences that are believed to be mediated by specific receptor-mediated activity (Colpaert, 1986; Holtzman, 1990). Many

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drugs have compound stimulus cues because they interact with more than one receptor system. The discriminative stimulus cue of ethanol is thought to comprise primarily of GABAergic, glutamatergic and serotonergic components (Green & Grant, 1998; Grant, 1999; Hodge & Cox, 1999) although the neurotransmitter systems dopamine and acetylcholine, as well as the proximate metabolite acetaldehyde have also been shown to make partial contributions (see Kostowski & Bienkowski, 1999).

The stimulus effects of a compound discriminative cue could be perceived in two ways. The stimulus effects of a compound cue could be perceived as the combination of separate, but distinct components (a heterogeneous cue) or by a combination of the separate components resulting in the perception of a single compound cue (a homogeneous cue). Stolerman and colleagues (1987) conducted a drug discrimination study to examine whether the separate components of a compound cue could substitute for the drug mixture or whether each component must be present for substitution to occur. In their study rats were trained to discriminate a mixture of the stimulant drug nicotine and the depressant drug midazolam from saline. Generalization tests with either nicotine or midazolam alone produced complete substitution to the nicotine-midazolam drug mixture. Antagonism tests with either the nicotine antagonist mecamylamine or the benzodiazepine antagonist Ro 15-1788 were also conducted. Mecamylamine or Ro 15-1788 administered alone only partially blocked the stimulus effects of the nicotine-midazolam mixture while administration of both antagonists together completely blocked the stimulus

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effects. These results demonstrate that compound drugs are perceived as a heterogeneous stimulus rather than a homogeneous stimulus complex. Separate components of the drug mixture are sufficient to substitute for the drug compound but complete blockade of the drug mixture requires removing all (in this case two) components of the stimulus cue.

Recent studies have demonstrated the contribution of $GABA_A$, NMDA and $5\text{-}HT_{1B/2C}$ receptor subtypes to the stimulus effects of ethanol (Green & Grant, 1998; Hodge & Cox, 1998, Hodge et al, 2001; Hundt et al., 1998). Green and Grant (1999) trained rats to discriminate ethanol from saline. Generalization tests showed that the $GABA_A$ agonist pentobarbital, the noncompetitive NMDA antagonist MK-801, and the $5/HT_{1B/2C}$ agonist TFMPP all completely substituted for ethanol when administered alone. The fact that each component fully substituted for the stimulus effects of ethanol when administered alone provides evidence that ethanol is a heterogeneous stimulus complex comprised of GABAergic, glutamatergic and serotonergic components and that each contributing component in itself is a sufficient condition for substitution. Furthermore, attempts to block the stimulus effects of ethanol by removing either the NMDA (Bienkowski et al., 1998) or GABA (Bienkowski et al., 1998; Hiltunen & Jarbe, 1988) component separately failed. These results suggest that when one component is removed or blocked, animals trained to discriminate ethanol from saline can still use the remaining, unblocked components to detect the presence of ethanol.

Discriminative taste aversion

There are two aspects of operant drug discrimination studies that may be considered disadvantages (Lucki, 1988). The first aspect is that researchers using operant discrimination procedures often require a significant amount of time to train animals to acquire and maintain discriminative stimulus control. The second aspect is that specialized equipment is needed. Numerous operant chambers, each equipped with at least two levers connected to food or fluid dispensers are needed to conduct operant drug discrimination studies. In addition, computers and specialized software are needed to run and gather data from operant drug discrimination studies. Recently, an alternative procedure for studying drug discrimination has been successfully used to quickly train animals to acquire discriminative stimulus control without the need for any specialized equipment. This particular drug discrimination methodology is based on a conditioned taste aversion procedure (Herrera & Velazquez Martinez, 1997; Lucki, 1988; Mastropaolo et al., 1989; Redila et al., 2000, 2002; Revusky et al., 1982; Smurthwaite & Riley, 1994; Stevenson et al., 1992). This procedure has been given several names including 'conditioned taste aversion as a behavioural baseline for drug discrimination learning' (Mastropaolo et al., 1989) or as 'drug discrimination learning' (Smurthwaite et al., 1992). The more succinct and descriptive term 'discriminative taste aversion' (DTA; Lucki, 1988) will be used for the present thesis.

Although the methods used in studies that have employed the DTA paradigm vary somewhat, there is a common basic procedure used to train and

test discriminative control. In one group of animals the administration of the training drug is followed by a saccharin-LiCl pairing (Drug-LiCl) while in the other group the administration of the training drug is followed by a saccharin-saline pairing (Drug-Saline). Briefly, in this procedure Drug-LiCl animals are injected once every fourth day with the training drug prior to a saccharin-LiCl pairing. Animals in the Drug-Saline group are injected with the training drug followed by a saccharin-saline pairing. On the intervening days both Drug-LiCl and Drug-Saline animals are injected with saline prior to access to the same saccharin. It is assumed that subjects in the Drug-LiCl group will learn to associate the stimulus effects of the training drug with the taste-toxin pairing effects of LiCl. Over training sessions Drug-LiCl animals decrease their saccharin intake whenever administered the training drug but readily consume saccharin when saline is administered prior to the saccharin solution (Lucki, 1988). Thus, animals in the Drug-LiCl group learn to use the stimulus effects of a training drug as a discriminative cue for predicting whether saccharin consumption will be followed by an aversive event or not.

One difference between the operant drug discrimination procedure and the DTA procedure is how unconditioned drug effects are controlled. In operant drug discrimination procedures the rate of lever pressing is used to identify whether or not a drug may exert unconditioned effects on the motivation for food or fluids as well as unconditioned motor effects. That is, a decrease in the rate of lever pressing following administration of a test drug may be the result of a decrease in either consummatory or locomotor behaviours. In the DTA

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procedure unconditioned drug effects can be detected by examining the drinking behaviour of the Drug-Saline group. Since the training drug does not possess any predictive value in the Drug-Saline group saccharin intake should not differ after administration of saline, the training drug, or test drug (Lucki, 1988).

Decreases in saccharin intake observed for the Drug-LiCl group following administration of a test drug can either be the result of common stimulus effects between that test drug and the training drug or to unconditioned motivational or motor effects. Examining the drinking pattern of the Drug-Saline group after test drug injections can facilitate decisions about which of these two explanations is valid. If a test drug were to have unconditioned motivational or motor effects then there would be a decrease in saccharin intake in the Drug-Saline group. Thus, the use of a Drug-Saline group within the DTA procedure enables the researcher to identify those drugs that may produce some type of unconditioned motivational or locomotor effects. Moreover, the Drug-Saline group would identify at which particular dose(s) a test drug would produce unconditioned effects.

The DTA procedure has been successfully used to examine the discriminative stimulus properties of an opiate agonist (Jaeger & Mucha, 1990; Martin et al., 1990), opiate antagonists (Smurthwaite & Riley, 1992; Smurthwaite et al., 1992), the GABA agonist pentobarbital (Jaeger & Mucha, 1990; Riley et al., 1989), serotonin agonists (Lucki, 1988) and the dopamine agonist amphetamine (Herrera & Martinez, 1997). All of these DTA studies have reported a rapid acquisition of discriminative stimulus control. A decrease in

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saccharin intake following administration of the training drug, compared to a saline injection, has been reported to occur within as little as two to three pairing sessions (Herrera & Mucha, 1990; Lucki, 1988). This is a much quicker acquisition of discriminative stimulus control compared to operant drug discrimination procedures that typically require 40-60 training sessions to achieve discriminative control (Jarbe et al., 1982; Jeffreys et al., 1990; Stefanski et al., 1996).

In addition to acquiring discriminative control rapidly to a variety of training drugs, the DTA paradigm has also been shown be as sensitive as the operant drug discrimination procedure at the molecular level. For example, Lucki (1988) used the DTA procedure to train animals to discriminate the stimulus properties of either the selective $5\text{-}HT_{1A}$ receptor agonist DPAT or the selective $5\text{-}HT_{1B/1C}$ receptor agonist TFMPP. Animals in the DPAT-LiCl and TFMPP-LiCl groups quickly acquired discriminative control. DPAT-LiCl and TFMPP-LiCl groups demonstrated a dose-dependent decrease in saccharin intake when injected with various doses of DPAT and TFMPP respectively. Animals in the DPAT-Saline and TFMPP-Saline groups failed to decrease saccharin intake following DPAT and TFMPP generalization tests demonstrating that neither DPAT nor TFMPP produced decreases in the motivation to drink or the ability to move. The DPAT-LiCl group displayed DPAT-like stimulus effects after administration of the $5\text{-}HT_{1A}$ receptor agonists ipsapirone and buspirone while the $5\text{-}HT_{1B/1C}$ receptor agonists TFMPP and m-CPP failed to generalize to the DPAT stimulus cue. Similarly, generalization tests in the TFMPP-LiCl group showed that the $5\text{-}HT_{1B/1C}$ receptor

agonist m-CPP dose-dependently generalized to the TFMPP stimulus cue while DPAT did not. The results of this study demonstrated that only drugs acting at the same receptor site, but not at other receptor sites, substituted for the stimulus effects of the training drug.

The DTA procedure has also been shown to be sufficiently sensitive to detect blockade of the training stimulus cue with antagonism tests. For example, rats trained to discriminate the presence of morphine failed to do so when pretreated with the opioid antagonist naloxone (Martin et al., 1990). The fact that the DTA procedure can quickly train animals to discriminate the presence or absence of a drug, show receptor specificity of the stimulus cue in generalization tests and blockade of the stimulus cue with antagonism tests adds to the attractiveness of this procedure as an alternative to operant procedures.

Ethanol and production of acetaldehyde

Ethanol is a small, fat-soluble molecule that is readily distributed throughout the body following its consumption. Ethanol is metabolized to acetaldehyde by the enzymes alcohol dehydrogenase, catalase and cytochrome P450 2E1 (for review see Topel, 1985). Acetaldehyde is then metabolized into acetate by aldehyde dehydrogenases (Brien & Loomis, 1983). Alcohol dehydrogenase is the major route for ethanol metabolism in the liver while both catalase and cytochrome P450 2E1 seem to play a more minor role (Matsumoto et al., 1994). Trace amounts of alcohol dehydrogenase (ADH) have been detected in brain essentially eliminating the possibility that alcohol

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dehydrogenase can metabolize ethanol into acetaldehyde in brain (Kerr at al., 1989; Rout, 1992). The enzyme catalase has been shown to be capable of metabolizing ethanol into acetaldehyde in brain (Cohen et al., 1980). Catalase reacts with hydrogen peroxide (H_2O_2) to form a complex called Compound I after which it can metabolize ethanol into acetaldehyde (Chance, 1947; Chance & Schonbaum, 1962; Oshino et al., 1973).

Several researchers have reported that catalase is the major route for ethanol metabolism in brain with little or no contribution of either alcohol dehydrogenase or cytochrome P450 2E1 (Aragon et al., 1992; Gill et al., 1992). Aragon and coworkers (1992) demonstrated an increase in acetaldehyde levels in brain tissue incubated with ethanol. Acetaldehyde levels were than measured after the administration of either the catalase inhibitor aminotriazole, the ADH inhibitor pyrazole or the cytochrome P-450 2E1 inhibitor metyraprone. Acetaldehyde levels were reduced following aminotriazole but not pyrazole or metyraprone suggesting that the production of acetaldehyde may depend on catalase rather than ADH or cytochrome P-450 2E1.

Hepatically metabolized ethanol can provide a source of acetaldehyde that may be transported to the brain in some fashion. Baraona and colleagues (1987a, 1987b) have proposed that hepatically produced acetaldehyde may bind to the hemoglobin in red blood cells, pass through the blood brain barrier and act centrally. 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

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acetaldehyde to the brain. Alternatively, small amounts of acetaldehyde may escape hepatic metabolism by aldehyde dehydrogenases. It is unlikely that this free acetaldehyde would be able to enter the brain through the circulatory system since aldehyde dehydrogenases located in the blood brain barrier would be able to metabolize acetaldehyde before it can enter the brain (Zimatkin, 1991).

There is evidence however, that systemically administered acetaldehyde (20 and 100 mg/kg) results in measurable increases in brain acetaldehyde levels (Quertemont & De Witte, 2001; Ward et al., 1997). The increase in brain acetaldehyde levels after intraperitoneally administered acetaldehyde suggests that the capacity of aldehyde dehydrogenases in the blood brain barrier can be overcome allowing acetaldehyde to enter the brain. Thus, when appreciable amounts of acetaldehyde circumvent hepatic aldehyde dehydrogenase metabolism, (i.e. intraperitoneal or intravenous administration) increased levels of brain acetaldehyde are possible.

Ethanol, catalase and acetaldehyde; behavioural effects

It has been proposed that the proximate product of ethanol metabolism, acetaldehyde, plays a role in mediating several ethanol related behaviours as well as in voluntary ethanol consumption (for reviews see McBride et al., 2002; Smith et al., 1997) and in many of the adverse effects of ethanol (Brien & Loomis, 1983; Streissguth et al., 1980). The adverse effect of increasing levels of peripheral acetaldehyde has been the basis for a commonly used treatment for alcoholism. Disulfiram, a drug that inhibits the breakdown of acetaldehyde into

acetate, has been regularly used as a deterrent for alcohol consumption in alcoholics (MacLeod, 1950).

Despite its well-known aversive effects, acetaldehyde has been demonstrated to have reinforcing properties. Wistar rats were shown to acquire and maintain self-administration of acetaldehyde directly into the posterior ventral tegmental area (VTA; Rodd-Henricks et al., 2000). Evidence for the reinforcing properties of acetaldehyde comes from work which demonstrated that rats will self-administer acetaldehyde intravenously (Myers et al., 1982, 1984), intracerebroventricularly (Brown et al., 1979, 1980; Rodd-Henricks, 2002).

A second line of evidence supporting a mediating role for acetaldehyde in ethanol-induced behaviours come from studies demonstrating a relationship with catalase activity. It has been hypothesized that central acetaldehyde production, via the action of the enzyme catalase, may play an important role in mediating ethanol consumption (Smith et al., 1997). Catalase activity has been shown to be significantly and positively correlated with voluntary ethanol consumption in humans (Amit et al., 1999; Koechling & Amit, 1992; Koechling et al., 1995), rats (Amit et al., 1988; Aragon, Spivak et al., 1985; Gill et al., 1996) and mice (Koechling & Amit, 1994). Amit and Aragon (1988) showed a positive correlation between both blood catalase and brain catalase activity with voluntary ethanol consumption in rats. Similarly, ethanol-preferring rats were shown to have higher brain catalase activity than an ethanol-nonpreferring rat strain (Gill et al., 1996).

The role of acetaldehyde in the mediation of several ethanol-induced behaviours was demonstrated using the catalase inhibitor aminotriazole (AT)

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(Aragon & Amit, 1992; Aragon, Spivak et al., 1985, 1991; Koechling & Amit, 1994; Rotzinger et al., 1994). Aminotriazole has been shown to dose dependently decrease catalase activity in the liver and brain (Aragon, Rogan & Amit, 1991). These researchers have shown that a single dose of AT (1 g/kg) produced a greater than 80% decrease in brain catalase activity between three and six hours compared to saline injected animals. Behaviourally, this dose of AT been shown to attenuate several ethanol-induced behaviours. Work from this laboratory has demonstrated that AT administration decreased ethanol consumption and ethanol preference in mice (Koechling & Amit, 1994) and the acquisition of ethanol consumption in rats (Rotzinger et al., 1994). The effect of AT was argued to be specific to ethanol because no concomitant decrease in total fluid intake was observed (Aragon & Amit, 1992; Koechling & Amit, 1994; Rotzinger et al., 1994). In addition to attenuating ethanol consumption AT administration had attenuated an ethanol-induced, but not a morphine- or LiCl-induced conditioned taste aversion (Aragon et al., 1985). AT has also been shown to attenuate ethanol-induced narcosis as well as ethanol lethality (Aragon et al., 1991; Tampier et al., 1988).

The mechanism of action for acetaldehyde has yet to be determined but several researchers have demonstrated an interaction between acetaldehyde and monoamine levels in brain (Heap et al., 1995; Ward et al., 1997). Ward and colleagues (1997) measured the effect of peripherally administered acetaldehyde on monoamine levels in the nucleus accumbens. These researchers showed a decrease in dopamine and serotonin levels following acetaldehyde administration

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whereas the levels of the inhibitory amino acid GABA and the excitatory amino acid glutamate did not change. Taken together these studies support the hypothesis that centrally formed acetaldehyde may be important in mediating ethanol consumption and several ethanol-induced behaviours.

Ethanol's effect on the GABAergic neurotransmitter system

The major inhibitory neurotransmitter γ-aminobutyric acid, GABA, has two major receptor subtypes – the $GABA_A$ and $GABA_B$ receptor. The $GABA_A$ receptor is a multi-subunit ionophore complex containing ligand recognition sites for GABA, benzodiazepines, barbiturates, neurosteroids, alcohols and picrotoxin (DeLorey & Olsen, 1992; Schofield et al., 1987). Activation of the $GABA_A$ receptor site results in a brief opening of the associated chloride ion channel resulting in an influx of Cl^- and hyperpolarization of the postsynaptic cell (Suzdak et al., 1986). Binding of the $GABA_A$ antagonist picrotoxin, at the picrotoxin site, results in a decrease in Cl^- influx in the presence of a GABA agonist. The $GABA_B$ receptor is a G protein-coupled receptor located presynaptically and when activated results in increased GABA release (Misgeld et al., 1995). The effects of ethanol on the function of GABA receptors have provided evidence that ethanol specifically potentiates $GABA_A$ receptor activity rather than $GABA_B$ activity (Allan & Harris, 1985; Suzdak et al., 1986). As a result, increased $GABA_A$ activity has been postulated to underlie some of the behavioural effects of ethanol (for review see Mihic, 1999, Mihic & Harris, 1996).

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In vitro studies have shown that ethanol potentiated GABA-mediated chloride influx (Allan & Harris, 1985; Suzdak et al., 1986). For example, Suzdak and colleagues (1986) showed that when physiologically relevant amounts of ethanol (20-60 mM) were added in the presence of the $GABA_A$ agonist muscimol, Cl^- uptake in rat cerebral cortical synaptoneurosomes was increased when compared to muscimol alone. In vivo electrophysiological studies have also shown that ethanol interacts with the $GABA_A$ receptor (Criswell et al., 1993; Givens & Breese, 1990; Nestoros, 1980). Given and Breese (1990) examined the effects of ethanol on GABA-mediated inhibition of neural activity in the medial septal area and lateral septum. Ethanol was found to enhance the inhibition of neuronal firing rate mediated by GABA demonstrating that ethanol potentiates GABA transmission in the brain. However, ethanol's enhancement of GABA function was not observed in all brain areas. In fact, Givens and Breese (1990) showed that ethanol enhanced neural inhibition in the medial septal area, but not the lateral septum. Similarly, ethanol failed to potentiate GABA inhibition in CA1 neurons in the hippocampus (Mancillas et al., 1986). These findings suggest that the effects of ethanol on $GABA_A$ receptors may depend on the site-specific subunit composition of the $GABA_A$ receptor (Givens & Breese, 1990).

GABA and ethanol related effects

The $GABA_A$ receptor has been shown to be a major target for ethanol (Criswell et al., 1993) and thus it has been hypothesized that $GABA_A$ agonists may mediate some ethanol-induced behaviours (Grobin et al., 1998; Liljequist &

Engel, 1982). Given and Breese (1990) examined the effects of the GABA$_A$ agonist muscimol and the GABA$_A$ antagonist bicuculline on the sedative effects of ethanol. In this study animals injected with ethanol displayed impairment of the aerial righting reflex, a measure of the sedative effects of ethanol. In the aerial righting reflex animals were held by the back of the neck and tail in an inverted position and dropped from varying heights onto a padded flooring. A successful righting reflex required that the animal land with all four feet on the floor. Microinjections of muscimol into the medial septal area, a site reported to be involved in the mediating the sedative properties of ethanol (McCown et al., 1986), enhanced the sedative effects of ethanol. That is, muscimol increased the height at which ethanol-injected animals could successfully right themselves. On the other hand, bicuculline antagonized ethanol's sedative effects, decreasing the height at which ethanol-injection animals successfully righted themselves. These findings suggest that the sedative effects of ethanol may be due to increased function at the GABA$_A$ receptor.

Several research laboratories have shown that the administration of GABA$_A$ receptor agonists increase voluntary ethanol consumption (Boyle et al., 1993; Schmitt et al., 2002; Tomkins & Fletcher, 1996). Work conducted in this laboratory demonstrated that the GABA$_A$ agonist THIP increased ethanol consumption both during acquisition (Boyle et al., 1992; Smith et al., 1992) and maintenance (Boyle et al., 1993) of ethanol self-administration. Supporting evidence from another group of researchers has shown that the GABA$_A$ agonists muscimol (Tomkins et al., 1994) and THIP (Tomkins & Fletcher, 1996) selectively

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increased ethanol, but not water intake, when injected directly into the dorsal raphe nucleus. The role of the $GABA_A$ receptor in modulating ethanol self-administration is further supported by evidence that decreasing or attenuating GABAergic activity decreased ethanol consumption. Administration of the $GABA_A$ antagonist picrotoxin (Boyle et al., 1993), bicuculline (Tomkins & Fletcher, 1996), the benzodiazepine antagonist flumazenil (Ro15-1788; June et al., 1994; Schmitt et al., 2002) or the benzodiazepine inverse agonist Ro15-4513 (June et al., 1992) all decreased voluntary ethanol consumption. It has been argued that the $GABA_A$ receptor, rather than the $GABA_B$ receptor modulates ethanol self-administration since enhancing the activity of the $GABA_B$ receptor with baclofen does not affect ethanol consumption (Smith et al., 1992; Tomkins & Fletcher, 1996).

The GABAergic system has been a major focus in drug discrimination studies based on behavioural and neurochemical evidence implicating the involvement of the $GABA_A$ receptor in mediating several ethanol-related behaviours. The stimulus effects of ethanol have been shown to be partly mediated through the $GABA_A$ receptor complex (Green & Grant, 1998; Hodge & Cox, 1999). The $GABA_A$ receptor agonist pentobarbital has consistently been shown to fully substitute for ethanol when administered peripherally (Hodge et al., 2001). Pentobarbital and muscimol have also been shown to fully substitute for systemically administered ethanol when injected directly into the nucleus accumbens (Hodge & Aiken, 1996; Hodge & Cox, 1998; Hodge et al., 2001). In addition, Hodge and Aiken (1996) showed that the $GABA_A$ antagonist bicuculline

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attenuated the discriminative stimulus effects of ethanol. Other GABA$_A$ agonists such as benzodiazepines (Hiltunen & Jarbe, 1986), barbiturates (York, 1978), and neurosteroids (Bienkowski & Kostowski, 1997; Bowen et al, 1999, Grant et al, 1996, 1997) also substitute for ethanol confirming the role of the GABA$_A$ ionophore as a site important for ethanol's discriminative stimulus effects. Shelton and Balster (1994) demonstrated that the stimulus effects of ethanol may be specifically mediated by the GABA$_A$ receptor since generalization tests with the GABA$_B$ receptor agonist baclofen failed to substitute for ethanol.

Ethanol's effect on the glutamatergic neurotransmitter system

Glutamate receptors are divided into three major subtypes: N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methoxy-4-isoxazole proprionic acid (AMPA) and kainate receptors. The NMDA receptor subtype is associated with a cation channel that allows Ca^{2+} and Na^+ to enter, and K^+ to leave, the cell when activated by glutamate. The NMDA receptor contains several binding sites that can modulate the activity of the ion channel (see Collingridge & Lester, 1989). These sites include the glutamate and NMDA recognition site, a phencyclidine (PCP) binding site within the ion channel, a strychnine-insensitive glycine binding site, a voltage-dependent Mg^{2+} binding site as well as modulatory sites for zinc and polyamines. Recent studies have shown that NMDA receptor activity is sensitive to ethanol (see Tabakoff & Hoffman, 1996).

In vitro electrophysiological studies have demonstrated that ethanol inhibits glutamate-induced bursting activity in neurons (Franklin & Gruol, 1987)

as well as NMDA-evoked ion currents (Hoffman et al., 1989; Lovinger et al., 1989, 1990). Lovinger and colleagues (1989) measured changes in inward ion current in mouse hippocampal neurons. These researchers showed that NMDA-activated ion currents significantly decreased after ethanol (10 to 50 mM) was added to the tissue culture. More importantly, ethanol specifically reduced the amplitude of NMDA-activated ion current. Kainate- or quisqualate-activated ion currents did not significantly change after the addition of ethanol to the culture suggesting that ethanol specifically inhibits NMDA receptors but not the non-NMDA receptors, kainate and AMPA. Several researchers have also supported that notion that NMDA receptor activity is affected to a greater degree than non-NMDA receptor activity (Fink et al., 1992; Nie et al., 1994). There are however contradictory findings demonstrating that ethanol can inhibit non-NMDA stimulated responses (Martin et al., 1995; Wang et al., 1999). Non-NMDA receptors however are thought to be less sensitive to ethanol since the concentrations of ethanol used to produce inhibition had to be significantly increased (66-100 mM) compared to the concentrations (5-50 mM) that inhibit NMDA activity (Martin et al., 1995).

NMDA and ethanol related effects

Data from behavioural studies have revealed that some of ethanol's behavioural effects may be the result of ethanol's ability to inhibit the activity of the NMDA receptor (Dansyz et al., 1992; Grant et al., 1990; Lovinger et al., 1989; Sanna et al., 1993). For example, the occurrence of, and symptoms associated

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with, audiogenic seizures induced by ethanol withdrawal were inhibited by pretreatment with the NMDA antagonist MK-801 suggesting that ethanol may produce seizures through interaction with the NMDA receptor (Dansyz et al., 1992, Grant et al., 1990; Morrisett et al., 1990). Lovinger et al. (1989) have shown that ethanol inhibits the function of NMDA receptors at levels associated with ethanol intoxication (5-50 mM) and have thus argued that the NMDA receptor may mediate that intoxicating effects of ethanol.

A second body of literature that implicates the involvement of NMDA receptors in ethanol-induced behaviours comes from drug discrimination studies. It has generally been demonstrated that NMDA antagonists substitute for ethanol in animals trained to discriminate ethanol from saline (Bowen & Grant, 1999; Grant et al., 1991, 1992; Grant & Colombo, 1993; Shelton & Balster, 1994). The competitive NMDA antagonists CGS 19755 and CPPene partially or completely substituted for ethanol in animals trained to discriminate ethanol from saline (Grant & Colombo, 1993; Sanger, 1993). Similarly, the noncompetitive NMDA antagonists MK-801 (dizocilpine), phencyclidine, memantine and ketamine produced stimulus effects that fully substituted for the stimulus effects of ethanol in rats whether administered intraperitoneally (Bienkowski et al., 1998; Grant & Colombo, 1993; Hundt et al., 1998; Sanger, 1993; Shelton & Grant, 2002) or directly into the brain (Hodge & Cox, 1998).

Drug discrimination studies have also tested drugs that act at NMDA modulatory binding sites to investigate whether ethanol may interact with these sites. The strychnine-insensitive receptor antagonists L-701,324 and MRZ 2/576

both partially substituted for ethanol (Bienkowski et al., 1998). However, several researchers have shown that other strychnine-insensitive receptor antagonists failed to substitute for ethanol (Balster et al., 1995; Hundt et al., 1998). Bienkowski et al. (1998) argued that the differences between these findings may be due to differences in selectivity to the strychnine-insensitive receptor. Polyamine antagonists have also failed to substitute for ethanol in rats trained to discriminate ethanol from saline (Hundt et al., 1998; Sanger, 1993). Together, these results suggest that inhibiting the NMDA receptor at sites other than the NMDA or PCP binding sites may not be sufficient to produce ethanol-like stimulus effects and therefore may not mediate ethanol's effect on the NMDA receptor.

Objectives of the present experiments

The present thesis consists of a series of experiments designed to examine the contribution of acetaldehyde, and the GABAergic and glutamatergic neurotransmitter systems in mediating the discriminative stimulus effects of ethanol. In experiment 1a animals were trained to discriminate ethanol from saline. Antagonism tests with the catalase inhibitor aminotriazole and generalization tests with acetaldehyde were conducted. Experiment 1b was designed to assess whether ethanol would substitute for acetaldehyde in animals trained to discriminate acetaldehyde from saline. Data from experiments 1a and 1b have been published (Redila et al, 2000, 2002). Experiments 2 and 3 were conducted to examine the role of the GABAergic and glutamatergic

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neurotransmitter systems respectively in animals trained to discriminate ethanol from saline.

Experiment 1a

Effect of Acetaldehyde and Aminotriazole on an Ethanol Discriminative Taste Aversion

The proximate product of ethanol metabolism, acetaldehyde, has been proposed to play a role in mediating several ethanol related behaviours including voluntary ethanol consumption (for review see Smith et al., 1997). It has been hypothesized that after ethanol has entered the brain, it is metabolized into acetaldehyde via the action of catalase and that acetaldehyde may mediate both ethanol intake and ethanol-related behaviours (Amit & Aragon, 1988).

Work carried out in our laboratory has provided evidence that catalase plays an important role in mediating ethanol-related behaviours. A positive correlation between catalase activity and voluntary ethanol consumption in both humans (Amit et al., 1999; Koechling & Amit, 1992) and rats (Amit & Aragon, 1988; Gill et al., 1996) has been reported. Pretreatment with the catalase inhibitor 3-amino-1, 2, 4-triazole (AT) has been shown to attenuate the acquisition and maintenance of voluntary ethanol consumption (Aragon & Amit, 1992; Koechling & Amit, 1994; Rotzinger et al., 1994), conditioned taste aversion (Aragon, Abitbol et al., 1991), locomotor depression (Aragon, Abitbol et al., 1991), and narcosis and lethality (Aragon, Spivak et al., 1991).

The purpose of experiment 1a was to examine whether acetaldehyde and ethanol share similar stimulus properties using a discriminative taste aversion (DTA) procedure. In addition, experiment 1a tested whether pre-treatment with

the catalase inhibitor AT would block the stimulus cue of ethanol. If the

hypothesis that centrally produced acetaldehyde mediated some of the effects of

ethanol is correct then acetaldehyde should share stimulus properties with

ethanol and that inhibiting metabolism of ethanol by catalase should decrease

the discriminative properties of ethanol.

Materials and Method

Subjects

Fourteen male Long-Evans rats, each weighing between 225 and 250

gms at the start of the experiment, were used and housed individually in stainless

steel hanging cages. Animals were kept in a colony room maintained at a

constant temperature and on a 12:12 hour light/dark cycle (lights on at 08:00).

The care and use of animals conformed to the standards set by the Canadian

Council of Animal Care (Canadian Council of Animal Care [CCAC], 1993).

Drugs

A 20% v/v ethanol solution was made by diluting a 95% stock ethanol

solution with 0.9% saline. A 0.15 M lithium chloride solution (Fisher Scientific)

was prepared in saline. A 0.1% w/v saccharin solution was prepared by diluting

saccharin sodium (BDH Fine Chemicals) with tap water. A 5% v/v acetaldehyde

solution (Aldrich Chemical Co.) was prepared in cold 0.9% saline. 3-amino-1, 2,

4-triazole (Sigma) was dissolved in 0.9% saline (1g/2ml) and stirred under low

heat until dissolved.

Procedure

Conditioning

Rats were allowed seven days of habituation to the housing conditions. During this time animals were handled and given ad lib access to food and water. Animals were handled daily and weighed every fourth day throughout the experiment. After the habituation period rats were placed on water deprivation for seven days. During water deprivation animals were allowed access to water for only 20 minutes per day. Water intake was measured and recorded following the 20-minute drinking session.

After seven days water was replaced by a 0.1% w/v saccharin solution as the sole fluid presented for the remainder of the experiment. On the third day of saccharin presentation, all animals were injected with saline (5 ml/kg) in order to habituate them to receiving injections. Animals were then assigned to either the Ethanol-LiCl (n=6) or Ethanol-Saline (n=8) group ensuring that the groups had similar saccharin intake during the three previous days.

The discriminative taste aversion procedure consisted of 11 consecutive cycles (four days per cycle) used to train animals to discriminate the presence and absence of ethanol. On day one of each cycle (the pairing day, PD) all animals were injected with ethanol (0.8 g/kg). Thirty minutes later animals were given a 20-min access period to the saccharin solution. The saccharin solution was presented in plastic tubes fitted with ball-bearing steel spouts. Immediately

after the 20-min drinking session animals in the Ethanol-LiCl group were injected with a 0.15 M LiCl (1.8 mEq or 12 mls/kg) solution while animals in the Ethanol-Saline group were injected with an equal volume of saline. On days two, three and four of each cycle (non-pairing days, NPD) all animals were injected with saline (5 mls/kg) 30 minutes prior to the 20-min presentation of the saccharin solution. No injections followed saccharin intake on the NPDs of each cycle.

Pretreatment with 3-amino-1, 2, 4–triazole (AT)

To investigate whether catalase-produced acetaldehyde plays a role in mediating the stimulus properties of ethanol. On the pairing day of the 12th cycle, all rats were injected ip with 3-amino-1, 2, 4-triazole (1g/kg), a catalase inhibitor, five hours before injections of ethanol. Thirty minutes after the ethanol injection animals were given 20 minutes of access to the saccharin solution. No injections followed the saccharin drinking on this day.

Acetaldehyde generalization tests

Cycles 13 through 16 were used to test whether injections of acetaldehyde would generalize to ethanol. Pairing days consisted of ethanol injections followed 30 minutes later by saccharin presentation and then by the administration of LiCl or saline. On the second and fourth days of each cycle all animals were injected with saline 30 minutes prior to saccharin presentation. The third day of each cycle was a test day in which animals received an injection of one of four doses of acetaldehyde 30 minutes prior to saccharin presentation.

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No injections followed saccharin presentation on these days. Each animal received all four doses of acetaldehyde (0.05, 0.1, 0.2 and 0.3 g/kg), one injection per cycle, in a counterbalanced design. All injections during acquisition of the ethanol DTA and during generalization tests were administered intraperitoneally.

The total amount of decrease in saccharin intake during pairing days compared to nonpairing days of the generalization tests (for the Ethanol-LiCl group) was used to make decisions about partial and complete substitution. That is, the amount of decrease observed for animals in the Ethanol-LiCl group on pairing day relative to nonpairing days was considered to be 100%. A partial substitution was defined as a decrease between 40 and 80% of this total decrease following administration of the test drug while complete substitution was defined as an 80% or greater decrease in saccharin intake. For example, if the total decrease in saccharin intake between PD (2 mls) and NPDs (22 mls) for the Ethanol-LiCl group was 20 mls, a partial substitution would occur if a test drug produced saccharin intake between 14 and 6 mls (a 40 and 80% decrease respectively). Complete substitution would occur if an animal consumed 6 mls or less of saccharin following administration of a test drug.

Results

A three-way mixed ANOVA was used to analyze mean saccharin intake during the conditioning phase. The between factor was Conditioning Group while the within factors were training Cycle and Days, where Days consisted of

saccharin intake during the pairing days (PDs) and an average of saccharin intake during the three following non-pairing days (NPDs). A significant three-way interaction was followed by an analysis of simple interactions holding the Group variable constant. Significant simple interactions were further analyzed with a test of simple effects between the Days variable (PD vs. NPDs) and the Cycle variable to determine significant differences between PD and NPDs across training cycles. All tests used an alpha level of .05 to determine significance.

Figure 1 represents mean saccharin intake for the Ethanol-LiCl and Ethanol-Saline groups on pairing and non-pairing days across the 11 training cycles. Results of the analysis yielded a significant three-way interaction, $F(10,120) = 11.49$, $p<.001$. Analysis of simple interaction for the Ethanol-LiCl group was significant, $F(10, 120) = 29.51$, $p<.001$. Analysis of simple effects was performed on Days at each Cycle and showed that PDs and NPDs were significantly different ($p<.05$) for the Ethanol-LiCl group during Cycles 7 through 11. That is, the Ethanol-LiCl group significantly decreased its saccharin intake on pairing days compared to their non-pairing days during Cycles 7 through 11 suggesting that this group acquired and maintained an ethanol discriminative taste aversion.

Simple interaction results for the Ethanol-Saline group demonstrated that saccharin intake during PD significantly differed from saccharin intake during NPDs across cycles, $F(10, 120) = 2.75$, $p<.004$. Simple effects test revealed that saccharin intake on PD was significantly ($p<.05$) different from NPDs on Cycles 1 and 11.

A two-way mixed ANOVA was performed on saccharin intake following pretreatment of AT and on the subsequent three non-pairing days. Analysis of saccharin intake following pretreatment with aminotriazole produced a significant two-way interaction, $F(3,36) = 52.27$, $p<.001$. Tests of simple effects showed that the Ethanol-LiCl group significantly decreased its saccharin intake following pretreatment with AT compared to the subsequent non-pairing days, $F(3,36) = 68.32$, $p<0.001$, suggesting that AT failed to block the stimulus cue of ethanol in the Ethanol-LiCl group (see Figure 2).

Figure 3 represents mean saccharin intake following injections of several doses of acetaldehyde. Analysis of the two-way mixed ANOVA, with conditioning group as the between factor and acetaldehyde dose as the within factor, of saccharin intake during generalization tests revealed that acetaldehyde partially generalized to ethanol as demonstrated by a significant group by dose interaction, $F(4, 48) = 3.17$, $p<.022$. A test of simple effects performed on the Ethanol-LiCl group across acetaldehyde generalization doses was significant, $F(4, 48) = 16.42$, $p<.001$. Posthoc Tukey tests showed that the Ethanol-LiCl group had significantly ($p<.05$) lower saccharin intake following injections of 0.2 (41.71% decrease) and 0.3 g/kg (92.43% decrease) of acetaldehyde compared to the saline NPDs. Test of simple effects for the Ethanol-Saline group was also significant, $F(4, 48) = 8.13$, $p<.001$. Tukey tests showed that the Ethanol-Saline group significantly decreased its saccharin consumption following administration of 0.2 and 0.3 g/kg acetaldehyde ($p<.05$). Finally, the difference in saccharin

intake following 0.3 g/kg acetaldehyde for the Ethanol-LiCl and Ethanol-Saline groups was significant ($p<.05$).

Results of the overall ANOVA also yielded a significant main effect of dose, $F(4, 48) = 22.56$, $p<.001$. Post hoc tests revealed that the 0.2 and 0.3 g/kg doses of acetaldehyde produced greater decreases in saccharin intake compared to the 0.05 and 0.1 g/kg doses suggesting that the two higher doses produce a decrease in saccharin intake regardless of conditioning group.

Figure 1. Mean saccharin intake during ethanol discriminative taste aversion training cycles for the Ethanol-LiCl and Ethanol-Saline groups. Filled and open bars represent mean saccharin intake for the Ethanol-LiCl and Ethanol-Saline respectively on pairing days (PDs). Filled and open squares represent mean saccharin intake across the three non-pairing days (NPDs) of each cycle for the Ethanol-LiCl and Ethanol-Saline groups respectively. Vertical lines represent S.E.M. Where no error bars appear, S.E.M. is smaller than the symbol. The symbol * denotes that saccharin intake was significantly decreased compared to the NPDs of the same cycle ($p<.05$).
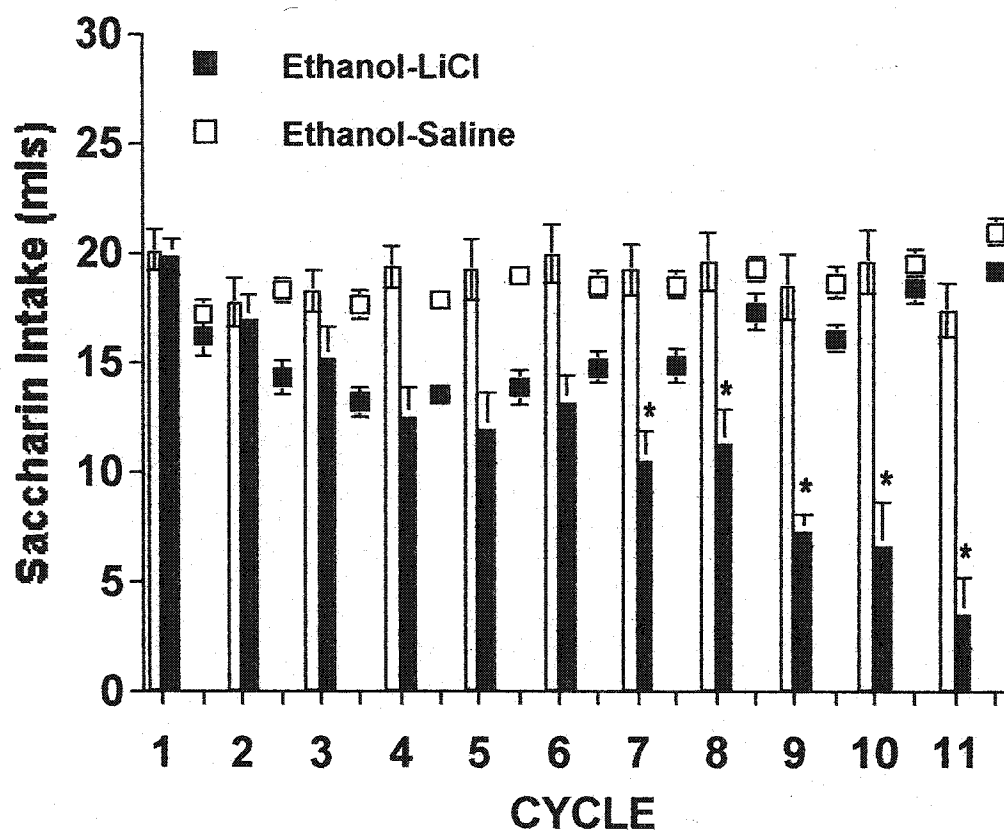
Figure 2. Mean saccharin intake after injection of 3-amino-1, 2, 4-triazole (AT) for the Ethanol-LiCl (filled squares, $n = 7$) and Ethanol-Saline (open squares, $n = 7$) groups. Administration of AT was given 5 hours prior to ethanol injections on pairing day (PD). Mean saccharin intake for the three non-pairing days (NPD) for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open squares) during generalization cycles are also presented. Vertical lines represent S.E.M. The symbol * denotes a significant difference ($p<.05$) between the PD and NPDs within each conditioning group.
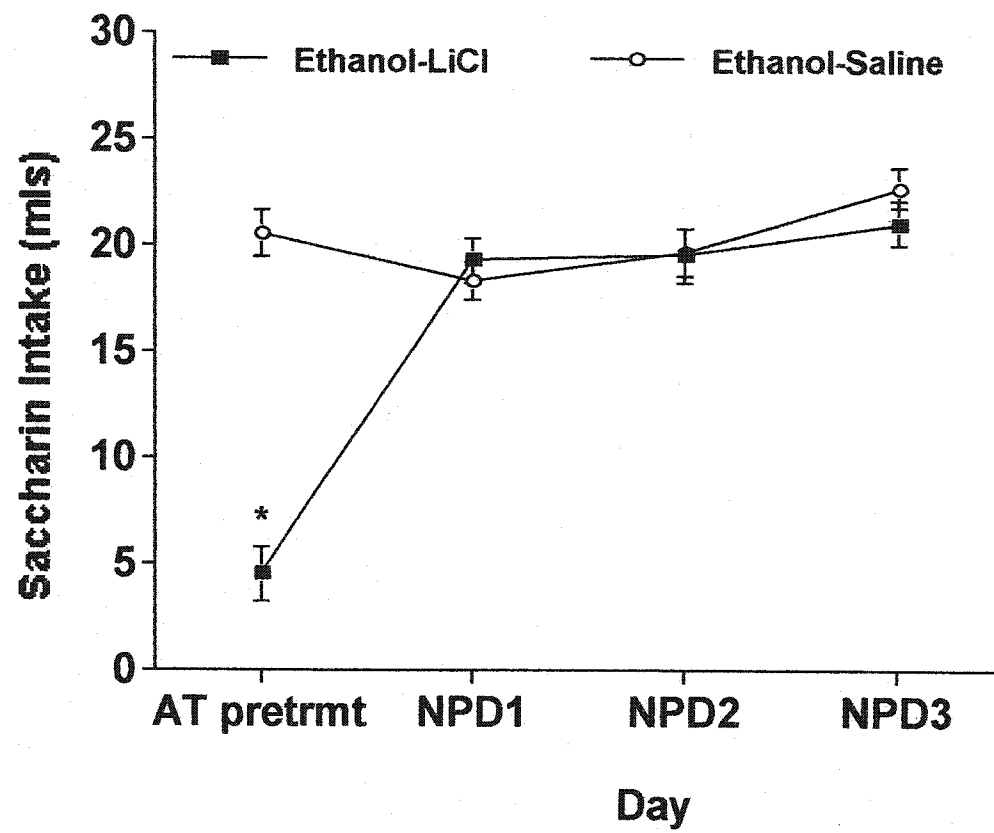
Figure 3. Mean saccharin intake after injections of acetaldehyde for the Ethanol-LiCl (filled squares, $n = 7$) and Ethanol-Saline (open squares, $n = 7$) groups. Acetaldehyde injections were given during the second recovery day (non-pairing day 2) 30-min before saccharin presentation. One dose of acetaldehyde was administered per cycle. Mean saccharin intake for the four ethanol pairing days (PD) and eight non-pairing days (NPD) for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open squares) during generalization cycles are presented for comparison. Vertical lines represent S.E.M. The symbol * denotes a significant difference ($p<.05$) between the acetaldehyde generalization dose and the saline control dose (NPD). The symbol # denotes a significant difference ($p<.05$) between the Ethanol-LiCl and Ethanol-Saline groups.

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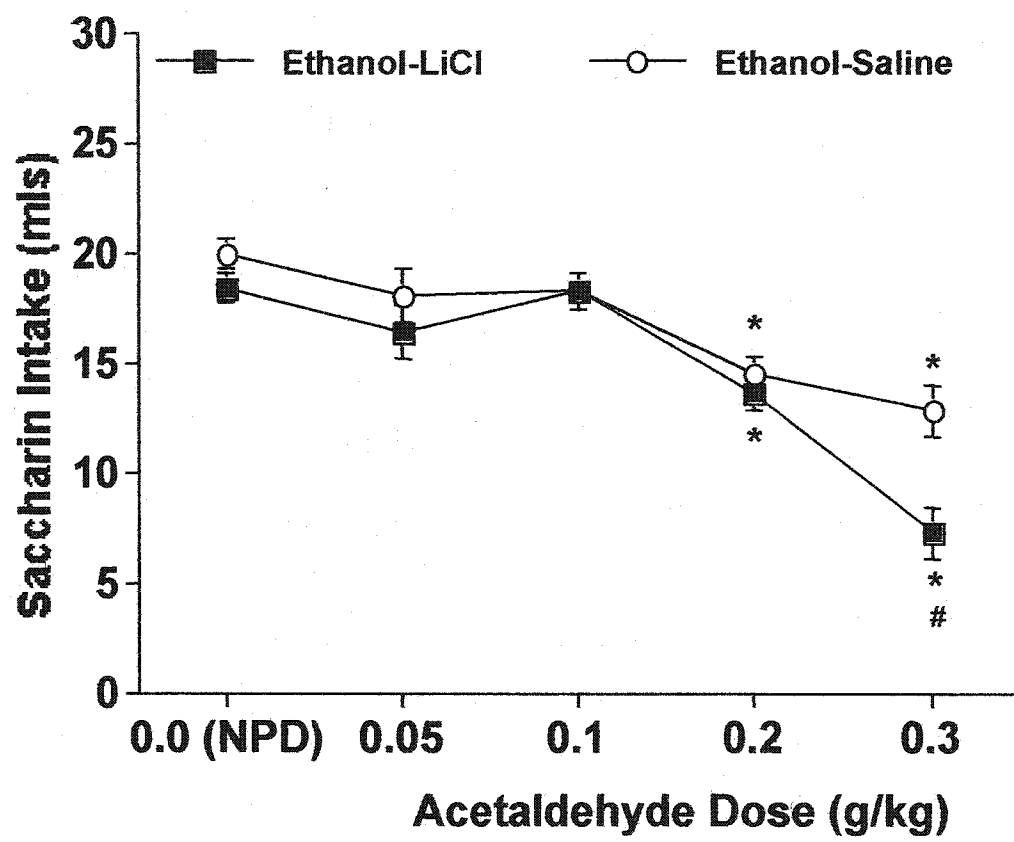
Discussion

The present study showed that the discriminative taste aversion procedure could be successfully used to train animals to discriminate ethanol from saline. Generally, animals in the Ethanol-LiCl group learned to discriminate the presence of ethanol within approximately seven training cycles. During Cycles 4 through 6 animals in the Ethanol-LiCl group had begun to associate the ethanol cue with the aversive state produced by the LiCl injections. Ethanol-LiCl animals began to drink significantly less saccharin during pairing days compared to non-pairing days suggesting that ethanol had begun to provide a cue that signals an upcoming aversive state. That is, ethanol had begun to provide discriminative control on drinking behaviour in the LiCl conditioning situation. In contrast, animals in the Ethanol-Saline group showed no differences in saccharin drinking between pairing days and non-pairing days. Ethanol did not provide cue of an upcoming aversive event since these animals did not receive LiCl injections.

In the present study it was hypothesized that if acetaldehyde contributed to the discriminative effects of ethanol, then decreasing its central production should result in the blockade of the acetaldehyde-mediated stimulus effects of the ethanol cue. Furthermore, if the contribution of acetaldehyde is an important part of the ethanol cue, then removing it should result in a blockade of the discriminative cue of ethanol. Thus, Ethanol-LiCl animals pretreated with AT should behave as if they were injected with saline and thus consume saccharin accordingly. The results showed that pretreatment with AT failed to alter the discriminative stimulus effects of ethanol. Animals in the Ethanol-LiCl group still

decreased their saccharin intake following ethanol injections when pretreated with AT. This finding suggests that the production of central acetaldehyde is not necessary for animals to perceive the presence of ethanol within the discriminative taste aversion paradigm. It is likely that in the absence of acetaldehyde, other neurotransmitter systems are still available to detect the discriminative stimulus effects of ethanol.

The results of the acetaldehyde generalization tests revealed that animals in the Ethanol-LiCl group significantly decreased their saccharin intake after injections of 0.2 and 0.3 g/kg acetaldehyde. Similarly, animals in the Ethanol-Saline also showed a significant decrease in saccharin intake following injections of 0.2 and 0.3 g/kg acetaldehyde. The fact that both groups of animals decreased their saccharin intake suggests that the higher doses of acetaldehyde may have produced motoric or aversive effects that resulted in a general decrease in saccharin consumption.

The Ethanol-LiCl and Ethanol-Saline groups did however differ in the degree that saccharin intake was decreased following the 0.3 g/kg acetaldehyde dose. The Ethanol-LiCl group showed a greater decrease in saccharin intake following the 0.3 g/kg acetaldehyde injection than the Ethanol-Saline group. In fact, the decrease in saccharin intake for the Ethanol-LiCl group was almost as great as that seen following the ethanol training dose injection. It appears that 0.3 g/kg of acetaldehyde produces similar stimulus properties to the training dose of ethanol for the Ethanol-LiCl group. Thus, the shared stimulus properties

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between acetaldehyde and ethanol may account for the greater decrease in saccharin intake for the Ethanol-LiCl animals.

In conclusion, the results of experiment 1a showed that the DTA procedure could be successfully used to train animals to discriminate the presence or absence of ethanol. Results demonstrated that AT pre-treatment did not affect the discriminative effects of ethanol suggesting that catalase -produced acetaldehyde may not be a major component to the stimulus properties of ethanol. Generalization tests showed that acetaldehyde may partially substitute for ethanol, but only at the highest dose tested (0.3 g/kg) suggesting that acetaldehyde shares similar stimulus properties to ethanol. Taken together, the lack of blockade of the ethanol cue by AT and the partial generalization of acetaldehyde demonstrates that acetaldehyde may contribute partially to the ethanol cue. Although acetaldehyde may share some properties with ethanol, animals may still use stimulus properties from other sources (GABAergic and glutamatergic) to detect the presence of the ethanol cue.

In order to further examine the similarities of the cue provided by ethanol and acetaldehyde experiment 1b tested whether acetaldehyde could be successfully used as the stimulus drug using the discriminative taste aversion procedure and whether ethanol will generalize to acetaldehyde.

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Experiment 1b

Effects of Ethanol on an Acetaldehyde Discriminative Taste Aversion

Analyses of drug discrimination studies have often demonstrated asymmetry of generalization tests. Asymmetrical generalization occurs when animals that are trained to discriminate drug A from saline generalize to drug B, but when drug B is used as the training drug, drug A does not substitute for drug B. This finding usually occurs when drug A has compound stimulus effects and drug B gives rise to a component cue of drug A. Asymmetrical generalization results have been found for ethanol in animals trained to discriminate NMDA antagonists (Balster et al., 1992) and GABA$_A$ agonists (De Vry et al., 1986; York, 1978) from saline. For example, non-competitive NMDA antagonists such as MK-801 or phencyclidine (PCP) have consistently been shown to completely substitute for ethanol in animals that were trained to discriminate ethanol from saline (Bienkowski et al., 1998; Grant & Colombo, 1993). However, ethanol does not substitute for NMDA antagonists in animals trained to discriminate the non-competitive NMDA antagonists NPC 12626 or PCP from saline (Balster et al., 1992). The present study was conducted to examine whether ethanol will substitute for the stimulus effects of acetaldehyde. That is, is there a symmetrical or asymmetrical pattern of generalization tests between animals trained to discriminate acetaldehyde from saline compared to animals trained to discriminate ethanol from saline?

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Materials and methods

Subjects

Thirty-two male Long-Evans rats, each weighing between 225 and 250 gms at the start of the experiment, were used and housed individually in stainless steel hanging cages. Animals were kept in a colony room maintained at a constant temperature and on a 12-:12-h light:dark cycle (lights on at 0800). The care and use of animals conformed to the standards set, by the Canadian Council of Animal Care (CCAC, 1993).

Drugs

A 5% v/v acetaldehyde solution (Aldrich Chemical Co., Milwaukee WI) was prepared in cold saline. A 20% v/v ethanol solution was prepared by diluting a 95% stock ethanol solution with prepared saline. A 0.15 M lithium chloride (Fisher Scientific, Fair Lawn, NJ) solution was prepared with saline. A 0.1% w/v saccharin solution was prepared by diluting sodium saccharin (BDH Fine Chemicals, Toronto, Ont) with tap water.

Procedure

Conditioning

Rats were allowed seven days of habituation to the housing conditions during which time they were handled and given ad lib access to food and water. Following the habituation period rats were placed on water deprivation for seven

days. During the water deprivation period animals were allowed access to water for only 20 min per day and intake was measured. A saccharin solution (0.1% w/v) replaced water at the end of the water deprivation period. On the third day of saccharin presentation, animals were injected with cold saline (5 or 7 ml/kg; equal to the volumes of 0.2 and 0.3 g/kg acetaldehyde injections) in order to habituate them to receiving injections under those conditions. Animals were then assigned to either the 0.2 g/kg (n = 16) or 0.3 g/kg (n = 16) acetaldehyde training dose groups. Animals in each training dose group were then assigned to either the Acetaldehyde-LiCl (Acet-LiCl) or Acetaldehyde-Saline (Acet-Saline) group. Animals were assigned to groups in a manner that ensured that all groups had similar saccharin intake during the three previous days.

The experiment consisted of eight consecutive four-day cycles that were used to train animals to discriminate the presence and absence of acetaldehyde. On day one of each cycle (the pairing day, PD) all animals were injected with acetaldehyde (0.2 or 0.3 g/kg, ip). Thirty minutes later animals were presented with the saccharin solution, allowed 20 min access to drink and then immediately injected with either LiCl (0.15 M, 1.8 mEq, ip) or an equal volume of saline. On days two, three and four of each cycle (non-pairing days, NPD) animals were injected with saline in a volume corresponding to the volume of the acetaldehyde training dose, 30-min before the presentation of the saccharin solution. Animals were then allowed 20-min of access to the saccharin solution. No injections were given after saccharin intake during the non-pairing days of each cycle.

<u>Generalization of ethanol</u>

Cycles 9 through 12 were used to test whether injections of ethanol would substitute for acetaldehyde. Pairing days consisted of acetaldehyde injections followed 30-min later by saccharin presentation and then immediately by LiCl or saline injections. On the second and fourth days of each cycle all animals were injected with saline 30-min before saccharin presentation as usual. The third day of each cycle was designated as a test day in which animals received an injection of one of four doses of ethanol 30-min before saccharin presentation. No injections followed saccharin presentation on these days. Each animal received all four doses of ethanol (0.8, 1.2, 1.6 and 2.0 g/kg), one injection per cycle, in a counterbalanced design. In the same manner as experiment 1a, partial substitution was defined as a decrease between 40 and 80% of the total decrease while complete substitution was defined as greater than 80% of the decrease in saccharin intake.

<div align="center">Results</div>

During acquisition of the acetaldehyde discriminative taste aversion several animals became ill and were subsequently dropped from the study. One animal from the 0.2 g/kg Acet-Saline group and five animals from the 0.3 g/kg group, two from the Acet-LiCl group and three from the Acet-Saline group, failed to complete the study. In addition, one animal from each of the Acet-LiCl groups failed to make criterion during acquisition and their data were eliminated from statistical analyses.

During the training phase of the study a significant decrease in saccharin consumption on PD compared to an average of NPDs were used as an indication of a discriminative taste aversion. In order to simplify analysis the 0.2 g/kg and 0.3 g/kg acetaldehyde groups were analyzed separately with three-way mixed ANOVAs. The between factor was conditioning group (LiCl versus Saline) while the within factors were Cycle (Cycles 1 through 8) and Days (PD versus NPDs). The days within factor consisted of saccharin intake during the pairing days and an average of saccharin intake during the three following non-pairing days. A significant three-way interaction was followed up with an analysis of simple interactions holding the Group variable constant. Significant simple interactions were further analyzed with a test of simple effects between the Days variable (PD vs. NPDs) at each training cycle to determine significant differences between PD and NPDs. All tests used an alpha level of .05 to determine significance.

Figure 4 illustrates mean saccharin intake for the 0.2 g/kg and 0.3 g/kg Acet-LiCl and Acet-Saline groups on pairing and non-pairing days. ANOVA results for the 0.2 g/kg acetaldehyde training groups yielded a significant three-way interaction, $F(7, 84) = 11.62$, $p<.001$. The Cycle by Day simple interaction test for the Acet-LiCl group yielded a significant result, $F(7, 84) = 24.66$, $p<.001$. Tests of simple effects for Acet-LiCl group showed that saccharin intake was significantly decreased on PD and NPD for Cycles 3 through 8 ($p<.05$). The decreased saccharin intake on PDs for the Acet-LiCl group was considered as evidence of discriminative control for drinking behaviour in the presence of acetaldehyde. Analysis of simple interaction for the Acet-Saline group yielded a

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significant interaction, $F(7, 84) = 2.79$, $p<.011$. Saccharin intake between pairing days and non-pairing days was significantly decreased on PD compared to NPD on Cycles 3, 5, and 7.

Analysis of the ANOVA for training cycles showed that saccharin intake for the 0.3 g/kg acetaldehyde training groups differed across days and cycles, $F(7, 56) = 2.61$, $p<.021$. Results of the simple interaction test for the Acet-LiCl group was significant, $F(7, 56) = 32.71$, $p<.001$. Tests of simple effects for the Day by Cycle interaction showed that saccharin intake was significantly decreased on PD compared to NPD on Cycles 2 through 8 ($p<.05$). Saccharin intake for the Acet-LiCl group on PDs during Cycles 4 through 8 was decreased by more than 50% compared to the NPDs of each cycle suggesting that these animals had acquired an acetaldehyde drug discrimination. Results for the simple interaction for the Acet-Saline group was not significant, $F(7, 56) = 1.01$, $p>.434$, demonstrating that saccharin intake during PD and NPDs did not differ. This data suggests that acetaldehyde did not provide a discriminative stimulus cue predicting an aversive event in the Ethanol-Saline group and thus no discriminative taste aversion was learned.

Statistical analyses of the ethanol generalization tests were performed separately for the two acetaldehyde-training doses using two-way mixed ANOVAs. For each ANOVA the between group factor was conditioning group (Group) while the within group factor was the ethanol generalization dose (Dose). Several animals failed to meet the discrimination criteria during generalization tests cycles and thus data for these animals were eliminated from the analysis.

The final number of animals completing all ethanol generalization tests were as follows; 0.2 g/kg Acet-LiCl (n = 7), 0.2 g/kg Acet-Saline (n = 7), 0.3 g/kg Acet-LiCl (n = 5) and 0.3 g/kg Acet-Saline (n = 5).

Results from the two-way ANOVA for the 0.2 g/kg acetaldehyde training group yielded a significant interaction, $F(4, 48) = 2.68$, $p>.042$. Analysis of simple effects for the Acet-LiCl group also yielded a significant result, $F(4, 48) = 5.57$, $p<.001$ and posthoc Dunnett tests revealed that saccharin intake was significantly decreased following the 0.8, 1.2, 1.6 and 2.0 g/kg ethanol doses ($p<.05$) when compared to the saline NPD data. Analysis of the simple effects for the Acet-Saline was significant, $F(4, 48) = 3.48$, $p<.014$. Posthoc Dunnett tests revealed that saccharin intake decreased significantly following only the 2.0 g/kg ethanol generalization dose ($p<.05$).

Results for the 0.3 g/kg acetaldehyde training dose group revealed a significant Group by Dose interaction, $F(4, 48) = 2.68$, $p>.042$. Further analysis showed that the test of simple effects tests, performed on the Acet-LiCl group, was significant $F(4, 48) = 8.01$, $p<.001$. Simple comparisons showed that saccharin intake decreased significantly following administration of the 0.8, 1.2, 1.6 and 2.0 g/kg ethanol ($p<.05$) compared to the saline NPDs. The analysis of simple effects test for the Acet-Saline group produced a significant result, $F(4, 48) = 3.48$, $p<.014$ and simple comparisons showed that saccharin intake significantly decreased after injection with 2.0 g/kg ethanol compared to the saccharin intake following saline injections ($p<.05$).

Figure 4. Mean saccharin intake during acetaldehyde (Acet) discriminative taste aversion training cycles for the Acet-LiCl and Acet-Saline. Filled and open bars represent mean saccharin intake for the Acet-LiCl and Acet-Saline groups respectively on pairing days (PD). Filled and open squares represent mean saccharin intake across the three non-pairing days (NPDs) of each cycle for the Acet-LiCl and Acet-Saline groups respectively. Vertical lines represent S.E.M. Where no error bars appear, S.E.M. is smaller than the symbol. The symbol * denotes that saccharin intake on PD was significantly decreased compared to the NPD of the same cycle ($p<.05$).
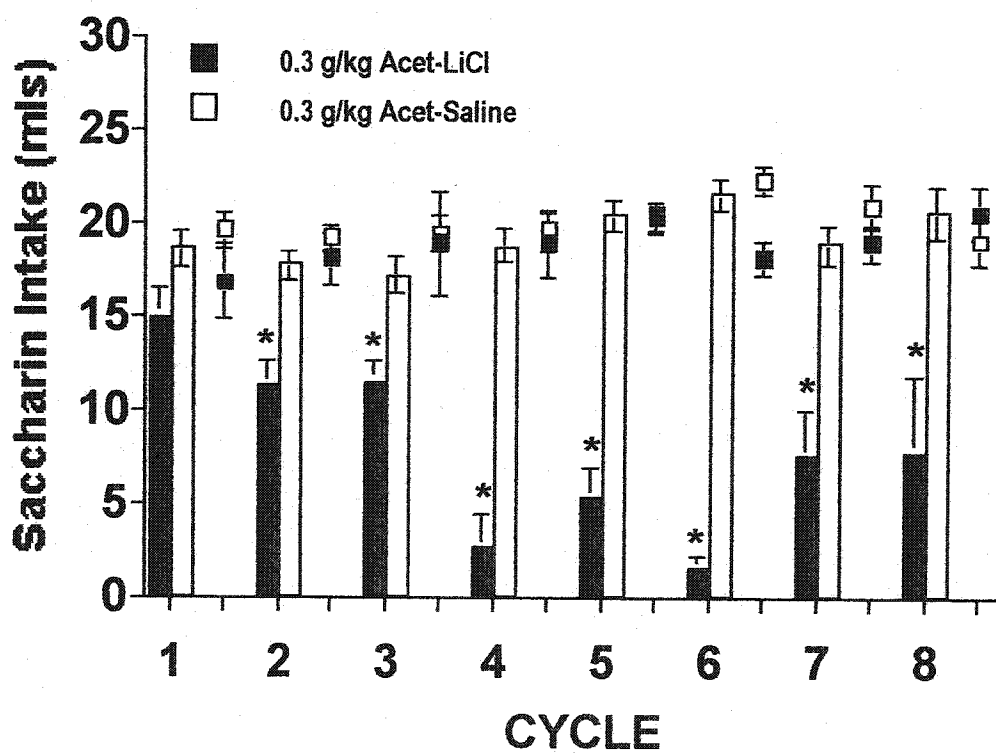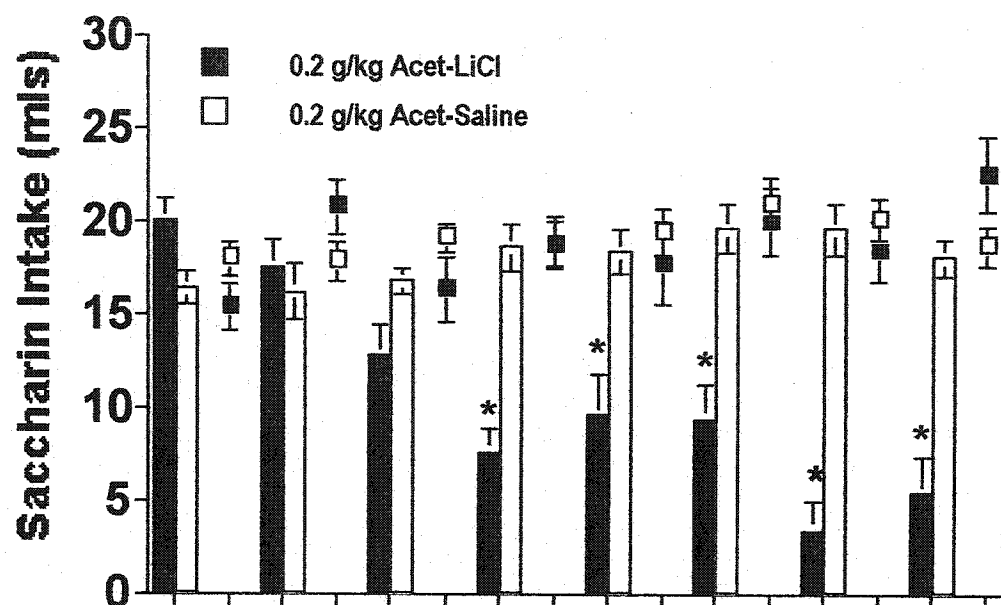
Figure 5. Mean saccharin intake during ethanol generalization tests for the 0.2 g/kg (top panel) and 0.3 g/kg (bottom panel) acetaldehyde-training doses. Filled squares and open circles represent mean saccharin intake for the Acet-LiCl and Acet-Saline groups respectively. Mean saccharin intake for the four acetaldehyde pairing days (PD) and eight non-pairing days (NPD) for the Acet-LiCl (filled squares) and Acet-Saline (open squares) groups during generalization cycles are presented for comparison. Vertical lines represent S.E.M. Where no error bars appear S.E.M. is smaller than the symbol. The symbol * denotes a significant difference ($p<.05$) between the ethanol generalization dose and the saline control dose (NPD).

0.2 g/kg acet training dose

■—— Ethanol-LiCl
○—— Ethanol-Saline

0.3 g/kg acet training dose

PD   0.0 (NPD)   0.8   1.2   1.6   2.0

Ethanol Dose (g/kg)

Saccharin Intake (mls)
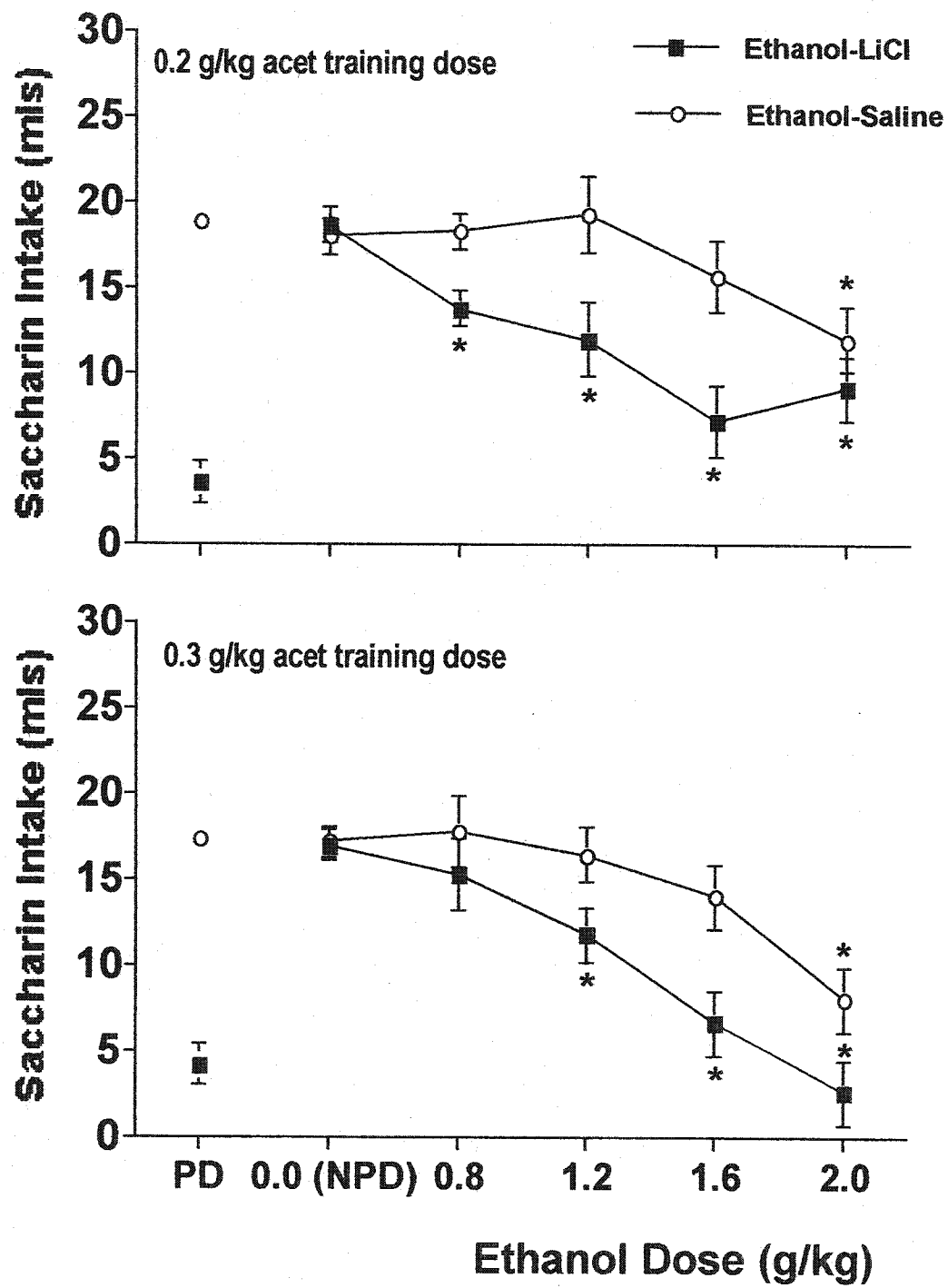
<u>Discussion</u>

The present study used a discriminative taste aversion procedure to examine whether ethanol would generalize to an acetaldehyde drug discrimination. That is, do acetaldehyde and ethanol share similar stimulus properties? The findings from the conditioning phase of the study showed that animals were able to consistently discriminate acetaldehyde from saline after eight training cycles.

The results from the ethanol generalization tests revealed a similar pattern for both training doses of acetaldehyde. For both the 0.2 g/kg and 0.3 g/kg acetaldehyde groups there was a significant decrease in saccharin intake for the Acet-LiCl group after ethanol injections of 1.2 and 1.6 g/kg compared to the Acet-Saline group. Administration of 1.2 and 1.6 g/kg ethanol produced a decrease in saccharin intake of 33% and 60% in the 0.2 g/kg Acet-LiCl group and 34% and 54% for the 0.3 g/kg Acet-LiCl respectively suggesting that these doses of ethanol had produced a partial generalization to acetaldehyde. That is, ethanol produces acetaldehyde-like behaviours at doses of 1.2 and 1.6 g/kg. These partial generalization findings demonstrate that ethanol and acetaldehyde may share similar properties and are consistent with the findings of ethanol conditioned taste aversion studies (Aragon, Abitbol et al., 1986, 1991; Brown et al., 1978).

Operant drug discrimination paradigms employ a particular set of criteria for determining when an animal has acquired discriminative control for the training drug. Typically, an animal will need to meet the discrimination criteria,

80% responding on the correct (drug) lever, for at least 10 consecutive sessions before generalization tests are conducted. By comparison, the criteria for acquiring a drug discrimination are less stringent in this study. Thus, it could be argued that animals might not have acquired a stable background for stimulus control or that animals may have different histories with regard to how long they have met the discrimination criteria. The conditioning phase of this study used eight training cycles to establish discriminative control for acetaldehyde. Overall, both the 0.2 and 0.3 g/kg Acet-LiCl groups had decreases in mean saccharin intake greater than 50% on pairing days, compared to non-pairing days, for the final 5 training cycles. Moreover, saccharin intake for the Acet-LiCl groups was stable on pairing days during the generalization test cycles (see figure 5). Although there seems to be some variation in saccharin intake on pairing days over the acquisition and generalization cycles it can be argued that the Acet-LiCl animals had acquired a stable discrimination.

The main purpose of the present study was to examine whether acetaldehyde and ethanol share similar stimulus properties. The findings demonstrated that animals learned to discriminate acetaldehyde from saline quickly using a discriminative taste aversion procedure. Generalization tests with several doses of ethanol showed partial generalization to 1.2 and 1.6 g/kg of ethanol. These results are similar to experiment 1a, which showed that acetaldehyde will partially generalize to an ethanol discriminative taste aversion suggesting that these two compounds share some stimulus properties.

Experiment 2

The Effects of GABAergic System Manipulation on an Ethanol

Discriminative Taste Aversion

Ethanol has been reported to enhance the effects of the inhibitory

neurotransmitter gamma-butyric acid (GABA; Criswell et al., 1993; Mihic, 1999).

More specifically, ethanol has been shown to potentiate GABA-mediated influx of

chloride ions (Allan & Harris, 1985; Suzdak et al., 1986). It has been

hypothesized that some ethanol-related behaviours may be due to ethanol's

ability to interact with the $GABA_A$ receptor (Liljequist & Engel, 1982). Numerous

studies have shown that altering the function of the $GABA_A$ receptor, by

administering agonists or antagonists, can influence ethanol self-administration

as well as other ethanol-induced behaviours (for review see Chester &

Cunningham, 2002). For example, work conducted in this laboratory has

demonstrated that administration of the $GABA_A$ agonist THIP increased voluntary

ethanol intake (Boyle et al., 1992; Smith et al., 1992) while administration of the

$GABA_A$ antagonist picrotoxin decreased ethanol intake (Boyle et al., 1993).

Drug discrimination studies have supported a role for the $GABA_A$ receptor

in mediating the discriminative stimulus effects of ethanol. Operant drug

discrimination studies have demonstrated that $GABA_A$ agonists such as

pentobarbital and muscimol substituted for the stimulus effects of ethanol in

animals trained to discriminate ethanol from saline (Bowen & Grant, 1998; Grant

et al, 1996, 1997;Hodge, Cox et al., 2001). The data from these drug

discrimination studies suggest that the GABAergic system, particularly through enhancement of $GABA_A$ activity, contributes to the stimulus effects of ethanol. The present study was designed to extend this literature and investigate the contribution of the GABAergic system in contributing to the stimulus effects of ethanol using a discriminative taste aversion procedure. More specifically, the present study was designed to examine whether activation of the $GABA_A$ and/or $GABA_B$ receptors mediate the discriminative cue of ethanol. In addition, pretreatment with a $GABA_A$ antagonist was used to determine whether inhibiting the $GABA_A$ receptor would block the stimulus cues of ethanol.

The drugs used in the present study were chosen for generalization tests because they have either previously been reported to either affect ethanol consumption or were used in previous drug discrimination studies. The GABA aminotransaminase inhibitor aminooxyacetic acid (AOAA; Fuchs et al., 1984) and the $GABA_A$ agonist 4,5,6,7-tetrahydroisoxazolol [5,4-c] pyridin-3-ol (THIP; Smith et al. 1992; Tomkins & Fletcher, 1996) have been shown to increase voluntary ethanol consumption while administration of the $GABA_A$ antagonist picrotoxin decreased ethanol intake (Boyle et al., 1993). Administration of the $GABA_B$ agonist baclofen however, failed to produce any alteration in ethanol consumption suggesting that the $GABA_B$ receptor may not play a role in mediating ethanol consumption (Tomkins & Fletcher, 1996). The above findings are similar to those reported in ethanol drug discrimination studies. Drug discrimination studies have consistently demonstrated that $GABA_A$ agonists, such as pentobarbital substituted for ethanol in rats trained to discriminate

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ethanol from saline (Bowen & Grant, 1999; Hodge, Cox et al., 2001; Hodge, Nannini et al., 2001) while $GABA_B$ agonists such as baclofen failed to substitute for ethanol (Shelton & Balster, 1994). Taken together, data from self-administration and drug discrimination studies suggest a role for the $GABA_A$ receptor, but not the $GABA_B$ receptor, in mediating ethanol consumption and the discriminative stimulus effects of ethanol.

For the purpose of the current experiment several changes were made to the discriminative taste aversion procedure used in Experiments 1a and 1b. The two previous experiments clearly demonstrated discriminative control of drinking behaviour when ethanol was used as the training drug. In these experiments a decrease in saccharin intake of 50% or more for the Ethanol-LiCl group on pairing days was taken to indicate discriminative control for ethanol. The previous methodology however, did not ensure that each animal achieved the same degree of stimulus control before generalization tests were conducted. That is, some animals may have displayed discriminative control over more training cycles than other animals. In order to assure that each animal had an equal amount of experience displaying discriminative control for saccharin intake the following criterion was introduced for experiments 2, 3 and 4. Individual animals had to decrease their saccharin intake by 50% or more on pairing days compared to the three following non-pairing days for 6 cycles before generalization tests were conducted.

The second change to the discriminative taste aversion procedure was to include a dose response curve of generalization to different doses of ethanol.

This dose response curve was included in order to make conclusions about partial generalization to other drugs used during generalization tests possible. The final change to the procedure was to add additional training doses of ethanol. These changes were made to improve on the design of the DTA procedure as well as to make comparisons between the DTA procedure and operant paradigms possible.

Materials and Methods

Subjects

Sixty male Long-Evans rats, each weighing between 225 and 250 at the start of the experiment, were used and housed individually in stainless steel hanging cages. Animals were kept in a colony room maintained at a constant temperature and on a 12:12 hour light/dark cycle (lights on at 08:00). The care and use of animals conformed to the standards set by the Canadian Council of Animal Care (CCAC, 1993).

Drugs

A 20% v/v ethanol solution was made by diluting a 95% stock ethanol solution with 0.9% saline. A 0.15 M lithium chloride (Fisher Scientific) solution was dissolved in saline. A 0.1% w/v saccharin solution was prepared by diluting saccharin sodium (BDH Fine Chemicals) with tap water. Aminooxyacetic acid (AOAA, Sigma, St Louis, MO), 4,5,6,7-tetrahydoisooxazolol [5,4-c] pyridin-3-ol

(THIP HCl, RBI, Natick, MA), baclofen (RBI, Natick, MA), sodium pentobarbital and picrotoxin (Sigma, St Louis, MO) were all prepared in 0.9% saline.

Procedure

Conditioning

Rats were allowed 7 days of habituation to the housing conditions during which time they were handled and given ad lib access to food and water. Animals were handled every day and weighed every other day throughout the experiment. After the habituation period rats were placed on water deprivation for 7 days. During water deprivation animals were allowed access to water for only 20 minutes per day. Water intake was measured following the 20-minute drinking session.

After the water deprivation period a 0.1% (w/v) saccharin solution replaced water as the sole fluid presented. On the third day of saccharin presentation, all animals were injected intraperitoneally (ip) with saline (5 ml/kg) in order to habituate them to receiving injections. Animals were then randomly assigned to one of three training doses of ethanol (0.5, 1.0 and 1.5 g/kg). Within each training dose group half the animals were assigned to the Ethanol-LiCl and the other half to the Ethanol-Saline group.

The discriminative taste aversion procedure consisted of consecutive cycles (4 days per cycle) used to train drug discrimination to ethanol. On day 1 of each cycle (the pairing day, PD) all animals were injected ip with the

appropriate training dose of ethanol. Thirty minutes later animals were allowed 20 minutes access to the saccharin solution. Immediately after the 20-min drinking session animals in the Ethanol-LiCl group were injected ip with LiCl (1.8 mEq) while the animals in the Ethanol-Saline group were injected with an equal volume of saline. On days 2, 3 and 4 of each cycle (non-pairing days, NPDs) all animals were injected with saline 30 minutes prior to the presentation of the saccharin solution. Animals were then allowed 20 minutes of access to the saccharin solution. No injections followed saccharin intake during the non-pairing days of each cycle. Saccharin intake was measured each day to the nearest 0.5 ml.

Ethanol-LiCl animals continued to be trained until they exhibited discriminative control criteria, which were defined as six cycles in which saccharin intake during the pairing days was less than 50% of the saccharin intake during the three non-pairing days. Once an animal in the Ethanol-LiCl group had reached criterion, generalization tests to different doses of ethanol, AOAA, THIP, baclofen, pentobarbital and antagonism tests with the $GABA_A$ receptor antagonist picrotoxin were conducted. At the same time an Ethanol-LiCl animal met discriminative control criteria an animal from the corresponding Ethanol-Saline group (from the same ethanol-training dose group) also started generalization tests. This was done to ensure that the Ethanol-Saline groups had received an equal number of ethanol injections as the Ethanol-LiCl groups before generalization tests were conducted.

Generalization tests

Once criterion had been met animals were given generalization tests to several drugs. The order of the drugs tested was ethanol (0.25, 0.5, 1.0 and 1.5 g/kg), AOAA (5, 10 and 20 mg/kg), THIP (4, 8 and 16 mg/kg), baclofen (2.5, 5 and 10 mg/kg) and pentobarbital (5, 10 and 20 mg/kg). Within each drug tested the order of the doses administered was randomly assigned. All injections were given ip. AOAA was administered four hours prior; THIP and baclofen were injected one hour prior while ethanol and pentobarbital were injected 30 minutes prior to saccharin presentation. All generalization tests were conducted on the second non-pairing day of the cycle. If an animal in the Ethanol-LiCl group did not display a decrease in saccharin intake greater than 50% compared to the previous and following non-pairing days no generalization tests were conducted. Animals continued generalization tests only when they met the 50% decrease criteria on the pairing day of the cycle in question.

During generalization test cycles pairing days consisted of ethanol injections followed 30 minutes later by saccharin presentation and then by LiCl or saline. On the second and fourth days of each cycle all animals were injected with saline 30 minutes prior to saccharin presentation. The third day of each cycle was a generalization test day in which animals received an injection of a test drug prior to saccharin presentation. No injections followed saccharin presentation on these days. The total amount of decrease in saccharin intake during pairing and nonpairing days of the generalization tests (for the Ethanol-LiCl group) was used to make decisions about partial and complete substitution.

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Partial substitution was defined as a decrease between 40 and 80% of the total decrease while complete substitution was defined as greater than 80% of the decrease in saccharin intake.

Pretreatment with picrotoxin

The GABA$_A$ receptor antagonist picrotoxin was used to investigate whether blocking the GABA$_A$ receptor would attenuate the discriminative stimulus cues of ethanol. Picrotoxin (1 and 2 mg/kg) was injected ip 10 minutes prior to the appropriate training dose of ethanol. Rats were then allowed 20 minutes of access to the saccharin solution 30 minutes following the ethanol injection.

Results

Data from a total of ten animals were eliminated from statistical analyses because they either did not reach discriminatory criterion for ethanol after 25 cycles, or became ill sometime during the experiment. Data from a total of five animals from the 0.5 g/kg ethanol-training dose, two animals from the LiCl and three animals from the Saline group, were omitted from analyses. In addition, data from two animals in the 1.0 and 1.5 g/kg ethanol-training dose groups, one each from the Ethanol-LiCl and Ethanol-Saline groups were excluded from statistical analyses

Three separate three-way ANOVAs, one for each ethanol-training dose, were used to examine the acquisition of discriminative control of ethanol across training cycles. The between groups variable was the conditioning group with

two levels (LiCl and Saline). The first within variable was referred to as Days and had two levels: the pairing day (PD) and the average of the three non-pairing days (NPD) of the same cycle. The second within variable was training cycle with 15 levels (the first 15 cycles of the DTA procedure). A significant three-way interaction was followed by an analysis of simple interactions holding the Group variable constant. Significant simple interactions were further analyzed with a test of simple effects between the Days variable (PD vs. NPDs) and the Cycle variable to determine significant differences between PD and NPDs across training cycles. Since simple effects tests were performed on only two groups simple comparisons were not needed. All tests used an alpha level of .05 to determine significance.

Figure 6 depicts saccharin intake across discrimination training Cycles 1 through 15 for animals trained with 0.5, 1.0 and 1.5 g/kg ethanol. Results of the three-way ANOVA for the 0.5 g/kg ethanol-training group yielded a significant Group by Cycle by Days interaction $F(14, 168) = 12.3$, $p<.001$. Simple interactions for the Ethanol-LiCl group yielded a significant result, $F(14, 168) = 28.00$, $p<.001$. Simple effects tests determined that saccharin intake was significantly lower for the Ethanol-LiCl on PD compared to NPD on Cycles 5 through 15 ($p<.05$). The simple interaction test for the Ethanol-Saline group yielded a significant Day by Cycle interaction, $F(14, 168) = 2.05$, $p<.014$. The test of simple effects revealed that saccharin intake on PD was significantly different than saccharin intake on NPDs on Cycles 5 and 13.

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A significant Group by Cycle by Days interaction $F(14, 224) = 18.42$, $p<.001$ was obtained for overall ANOVA performed on the 1.0 g/kg ethanol-training group. The test of simple interaction for the Ethanol-LiCl group was significant, $F(14, 224) = 38.88$, $p<.001$. Further analysis with tests of simple effects showed that animals in the Ethanol-LiCl group drank significantly less on PD than on NPD during Cycles 4 through 15 ($p<.05$). Analysis of the simple interaction for the Ethanol-Saline group resulted in a significant finding, $F(14, 224) = 4.79$, $p<.001$. There was a significant difference between saccharin intake on PD and NPD for the Ethanol-Saline group during Cycles 6 and 12 ($p<.05$).

Examination of the ANOVA results for animals trained with 1.5 g/kg ethanol also yielded a significant three-way interaction $F(14, 224) = 15.75$, $p < .001$. The simple interaction test performed for animals in the Ethanol-LiCl group was significant, $F(14, 224) = 28.97$, $p<.001$ and simple effects tests showed that saccharin intake between PDs and NPDs was significantly different for Cycle 3, and Cycles 6 through 15 ($p<.05$). The test of simple interaction for the Ethanol-Saline group was also significant, $F(14, 224) = 2.68$, $p<.001$. Simple effects tests showed a significant difference between saccharin intake on PD and NPDs on Cycles 2, 7 and 8, $p<.05$.

Figure 6. Mean saccharin intake during ethanol discriminative taste aversion training cycles for the 0.5 g/kg (top panel), 1.0 g/kg (middle panel), and the 1.5 g/kg (bottom panel) ethanol-training dose groups. Filled and open bars represent mean saccharin intake for the Ethanol-LiCl and Ethanol-Saline groups respectively on pairing days (PD). Filled and open squares represent mean saccharin intake across the three non-pairing days (NPD) of each cycle for the Ethanol-LiCl and Ethanol-Saline groups respectively. Vertical lines represent S.E.M. Where no error bars appear, S.E.M. is smaller than the symbol. The symbol * denotes that saccharin intake on PD was significantly decreased compared to the NPDs of the same cycle for the Ethanol-LiCl group ($p<.05$).
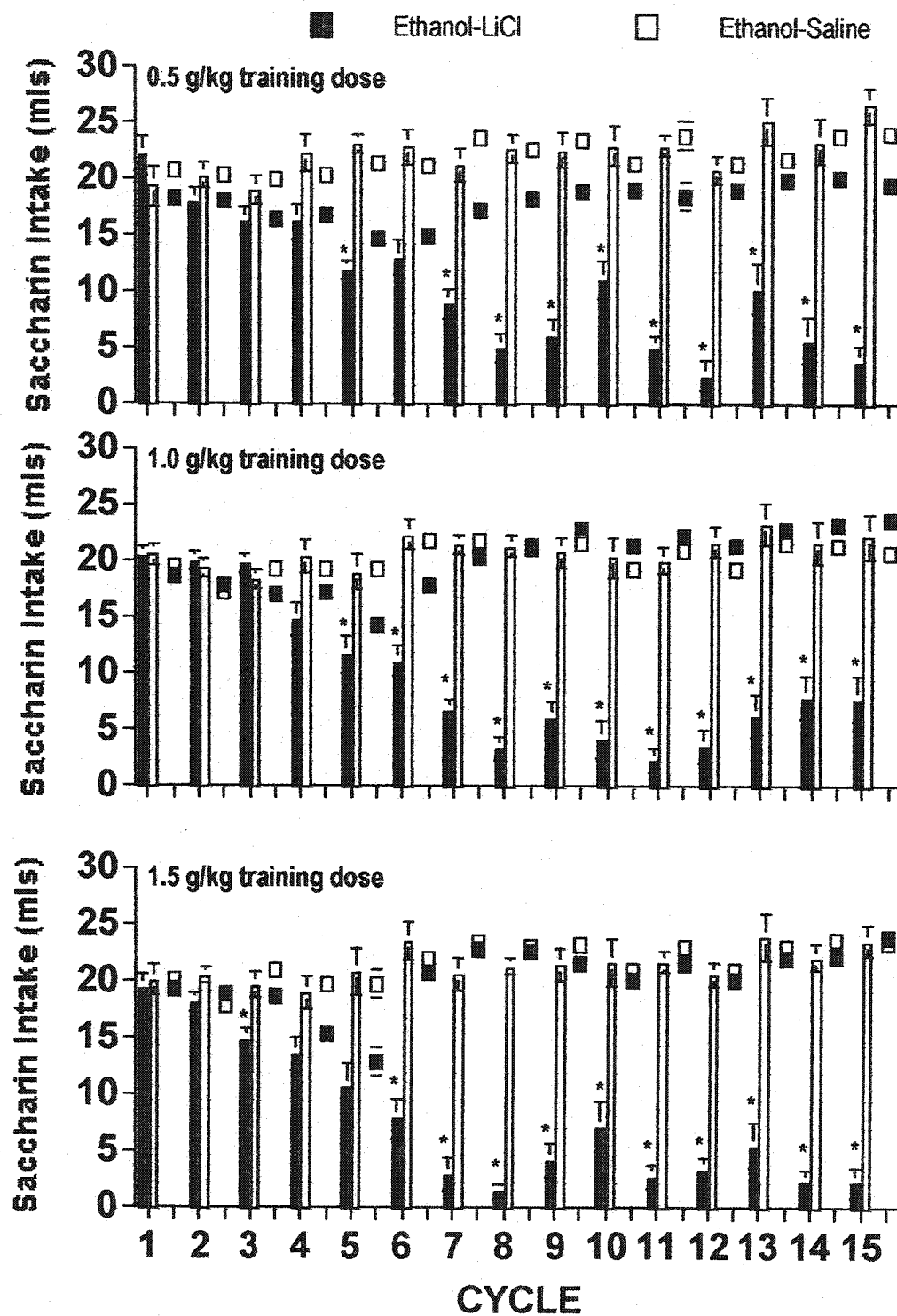
Legend: ■ Ethanol-LiCl   □ Ethanol-Saline

Three panels with y-axis "Saccharin Intake (mls)" and x-axis "CYCLE" (1 through 15). Panels labeled "0.5 g/kg training dose", "1.0 g/kg training dose", and "1.5 g/kg training dose".

Separate two-way ANOVAs, one for each ethanol-training dose, were used to examine the results of generalization tests. The between factor was conditioning Group with two levels (LiCl and Saline) and the within factor was the generalization drug (Dose) with the numbers of levels dependent on the number of doses used. Included in the Dose within factor was data reflecting the average saccharin intake during the saline NPDs of the generalization test cycles. That is, data from NPD 1 and NPD 3 of each generalization test cycle were averaged and included in the analysis. The NPD data was used to represent a 0.0-mg/kg dose of the generalization drug. Significant two-way interactions were further analyzed with tests of simple effects. If the simple effects test of either the Ethanol-LiCl or Ethanol-Saline groups were significant Dunnett posthoc tests were then performed to test significant mean differences in saccharin intake between the NPD saline dose and each generalization dose. All tests used an alpha level of .05 to determine statistical significance. If there were any missing data points for an animal during generalization tests, they were replaced with the mean for that particular group.

Ethanol generalization tests were performed with several doses of ethanol, 0.0 (saline NPDs), 0.25, 0.5, 1.0 and 1.5 g/kg and are illustrated in Figure 7. Analysis of the data from animals trained with 0.5 g/kg ethanol yielded a significant Group by Dose interaction $F(4, 52) = 8.75, p < .001$. Simple effects of Dose holding Group constant showed a significant result for the Ethanol-LiCl group $F(4, 52) = 15.17, p < .001$. Dunnett posthoc tests showed that the Ethanol-LiCl group drank significantly less saccharin after 0.5, 1.0 and 1.5 g/kg ethanol

injections compared to the saline NPDs ($p > .05$). Results of the simple effect of generalization dose holding the group constant revealed a nonsignificant F value for the Ethanol-Saline group $F(4, 52) = 0.46$, $p < .764$.

Analysis of ethanol generalization tests yielded a significant Group by Dose interaction for the LiCl and Saline groups trained with 1.0 g/kg ethanol $F(4, 68) = 14.08$, $p < .001$. Simple effects of ethanol generalization dose for the Ethanol-LiCl group yielded a significant result $F(4, 68) = 29.36$, $p < .001$. Posthoc Dunnett tests revealed that saccharin intake was significantly decreased following administration of 0.5, 1.0 and 1.5 g/kg of ethanol compared to the saline NPDs ($p < .05$). Simple effects test for the Ethanol-Saline group was nonsignificant $F(4, 68) = 2.74$, $p < .553$.

Analysis of the data for the groups trained with 1.5 g/kg ethanol resulted in a significant Group by Dose interaction $F(4, 60) = 14.77$, $p < .001$. Simple effects of ethanol generalization dose for the Ethanol-LiCl group was significant, $F(4, 60) = 31.19$, $p < .001$. Posthoc tests showed that saccharin intake was significantly decreased for the 0.5, 1.0 and 1.5 g/kg ethanol generalization tests when compared to the saline control tests. The results for the simple effects tests performed on the Ethanol-Saline group was nonsignificant, $F(4, 60) = 0.14$, $p < .996$.

Figure 8 displays saccharin intake after aminooxyacetic acid (AOAA) generalization tests. Results of the analyses showed nonsignificant Group by Dose interaction for the 0.5 g/kg, $F(3, 39) = .374$, $p < .772$, 1.0 g/kg , $F(3, 54) =$

.244, $p<.865$ and 1.5 g/kg, $F(3, 48) = .724$, $p<.543$ training doses suggesting that AOAA failed to generalize to ethanol at any of the doses tested.

Administration of either GABA$_A$ receptor agonist THIP or pentobarbital failed to generalize to ethanol across the ethanol-training doses (see figures 9 and 10 respectively). ANOVA results for THIP generalization tests yielded nonsignificant two-way interactions for the 0.5 g/kg [$F(3, 33) = .028$, $p <.993$], the 1.0 g/kg [$F(3, 48) = .349$, $p<.79$], and the 1.5 g/kg [$F(3, 48)= 1.38$, $p<.259$] ethanol-training doses. Similarly, the GABA$_A$ receptor agonist pentobarbital failed to generalize to ethanol at any of the training doses. Nonsignificant Group by Dose interactions were found for the 0.5 g/kg [$F(3, 36) = 1.08.$, $p<.370$], 1.0 g/kg [$F(3, 48) = 1.89$, $p<.143$], and the 1.5 g/kg [$F(3, 48) = 0.522$, $p<.649$].

Analysis of the results for the GABA$_B$ receptor agonist baclofen yielded nonsignificant Group by Dose interactions for the 0.5 g/kg [$F(3, 33) = 0.053$, $p<.984$], the 1.0 g/kg [$F(3, 51) = 2.91$, $p<.073$], and the 1.5 g/kg [$F(3, 48) = 0.744$ $p<.531$] ethanol-training doses (see figure 11). Further analysis of baclofen generalization tests showed significant main effects of Dose for the 0.5 g/kg [$F(3, 33) = 6.79$, $p<.001$], 1.0 g/kg [$F(3, 51) = 36.4$, $p<.001$], and the 1.5 g/kg [$F(3, 48) = 21.1$, $p<.001$] ethanol-training doses. Main comparisons revealed that the 20 mg/kg dose produced a significant decrease in saccharin intake compared to the 0 (NPD), 5, and 10 mg/kg baclofen doses ($p<.05$).

Finally, the GABA$_A$ receptor antagonist picrotoxin was used to examine whether blocking the GABA$_A$ receptor would effectively block the stimulus cue of ethanol. A two-way ANOVA with conditioning Group as the between factor and

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generalization Dose (NPD, 1.0 and 2.0 mg/kg) as the within factor was conducted. A significant Group by Dose interaction was found for animals trained at 0.5 g/kg ethanol, $F(2, 24) = 7.57$ $p<.003$. Simple effects tests showed a significant difference between generalization doses for the Ethanol-LiCl group, $F(2, 24) = 24.25$ $p<.001$, but not for the Ethanol-Saline group, $F(2, 24) = 1.16$ $p<.330$. Dunnett posthoc tests showed that saccharin intake was significantly decreased after 1.0 and 2.0 mg/kg picrotoxin compared to saccharin intake after saline injections for animals in the Ethanol-LiCl group. The omnibus ANOVA results for the 1.0 g/kg ethanol group yielded a significant interaction, $F(2, 30) = 19.24$, $p<.001$. Tests of simple effects produced a significant result for the Ethanol-LiCl group, $F(2, 30) = 37.95$ $p<.001$, but not for the Ethanol-Saline group, $F(2, 30) = 0.277$ $p<.828$. Posthoc tests showed that saccharin intake was significantly reduced after injections of 1.0 and 2.0 mg/kg picrotoxin ($p<.05$) for animals in the Ethanol-LiCl group. Finally, the two-way interaction was significant for the 1.5 g/kg ethanol trained animals, $F(2, 32) = 12.09$ $p<.001$. The results of simple effects tests showed that there was a decrease in saccharin intake following picrotoxin administration for the Ethanol-LiCl group, $F(2, 32) = 32.55$ $p<.001$, but not for the Ethanol-Saline group, $F(2, 32) = 0.75$ $p<.483$. Dunnett posthoc tests performed on saccharin intake for the Ethanol-LiCl animals showed a significant decrease following injection of 1.0 and 2.0 mg/kg picrotoxin compared to saline injections ($p<.05$).

Figure 7. Mean saccharin intake after injections of several doses of ethanol for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open circles) groups across the 0.5 g/kg (top panel), 1.0 g/kg (middle panel) and 1.5 g/kg (bottom panel) ethanol-training groups. Saline was injected on non-pairing days (NPD). Vertical lines represent S.E.M. The symbol * denotes a significant difference ($p<.05$) between the ethanol generalization dose and the saline control dose (NPD).
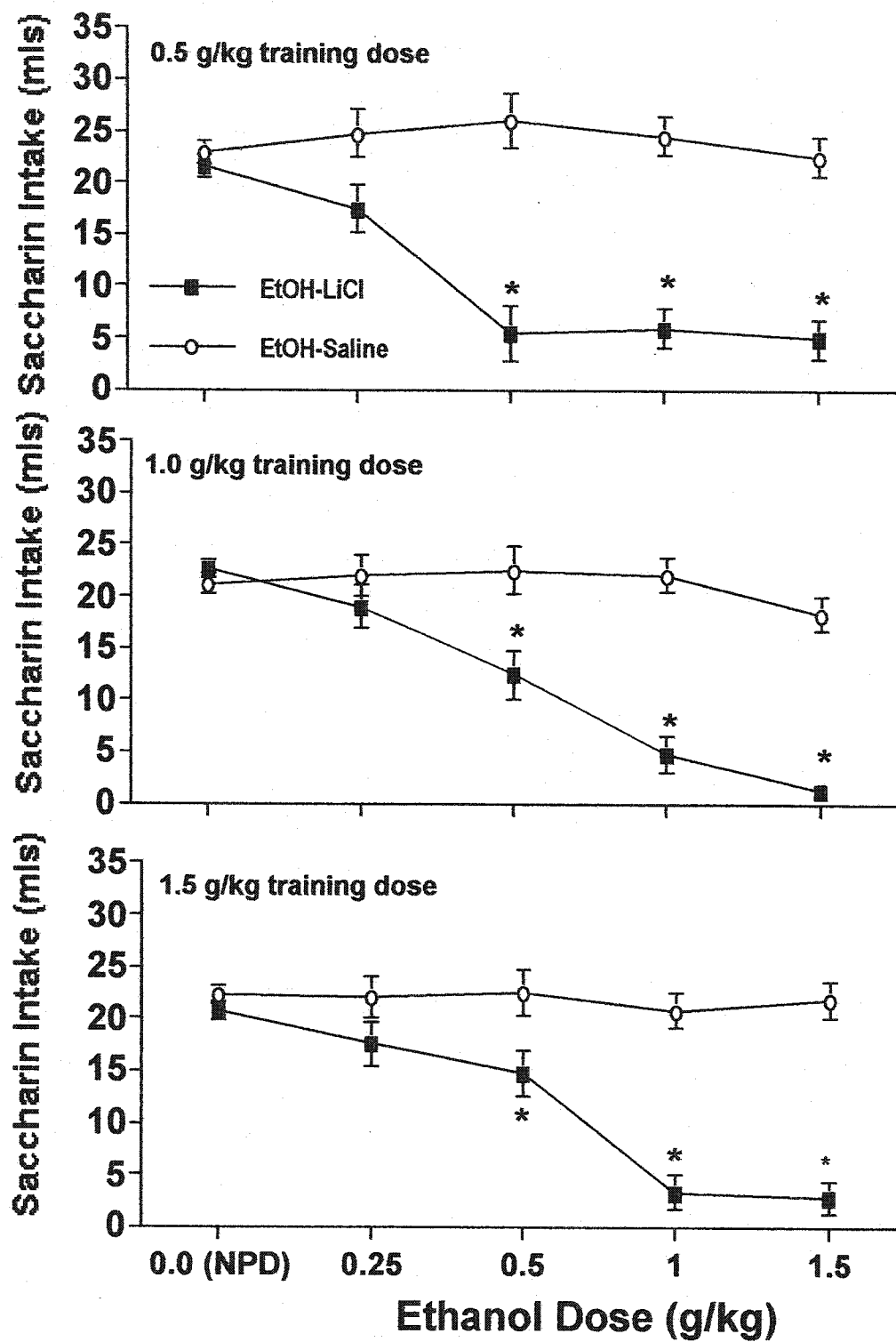
Figure 8. Mean saccharin intake after injections of several doses of aminooxyacetic acid (AOAA) for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open circles) groups across the 0.5 g/kg (top panel), 1.0 g/kg (middle panel) and 1.5 g/kg (bottom panel) ethanol-training groups. Saline was injected on non-pairing days (NPD). Vertical lines represent S.E.M. Where no error bars appear, S.E.M is smaller than the symbol.

0.5 g/kg training dose

EtOH-LiCL
EtOH-Saline

1.0 g/kg training dose

1.5 g/kg training dose

0 (NPD) 5 10 20

AOAA Dose (mg/kg)
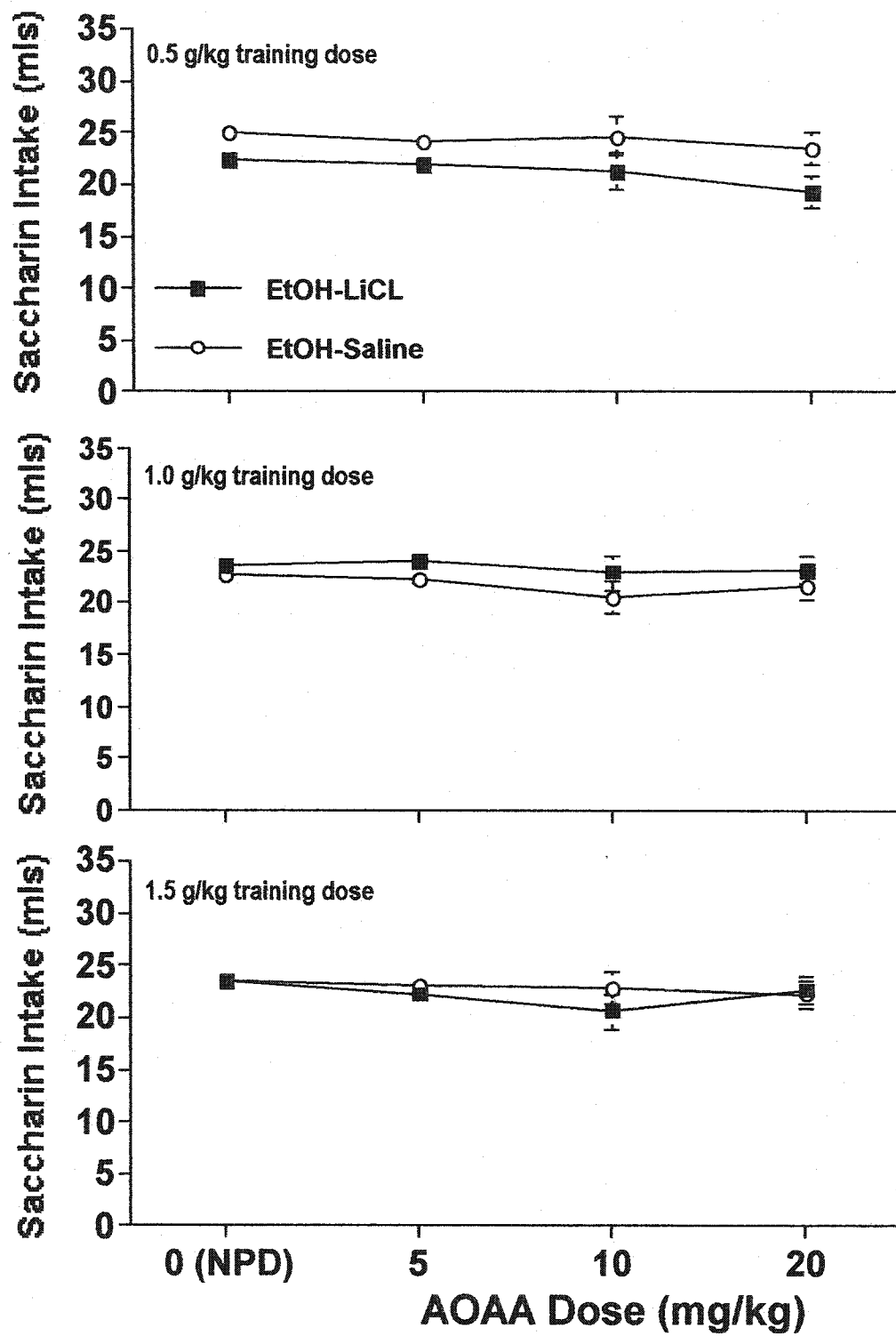
Figure 9. Mean saccharin intake after injections of several doses of the $GABA_A$ receptor agonist THIP for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open circles) groups across the 0.5 g/kg (top panel), 1.0 g/kg (middle panel) and 1.5 g/kg (bottom panel) ethanol-training groups. Saline was injected on non-pairing days (NPD). Vertical lines represent S.E.M. Where no error bars appear, S.E.M. is smaller than the symbol.
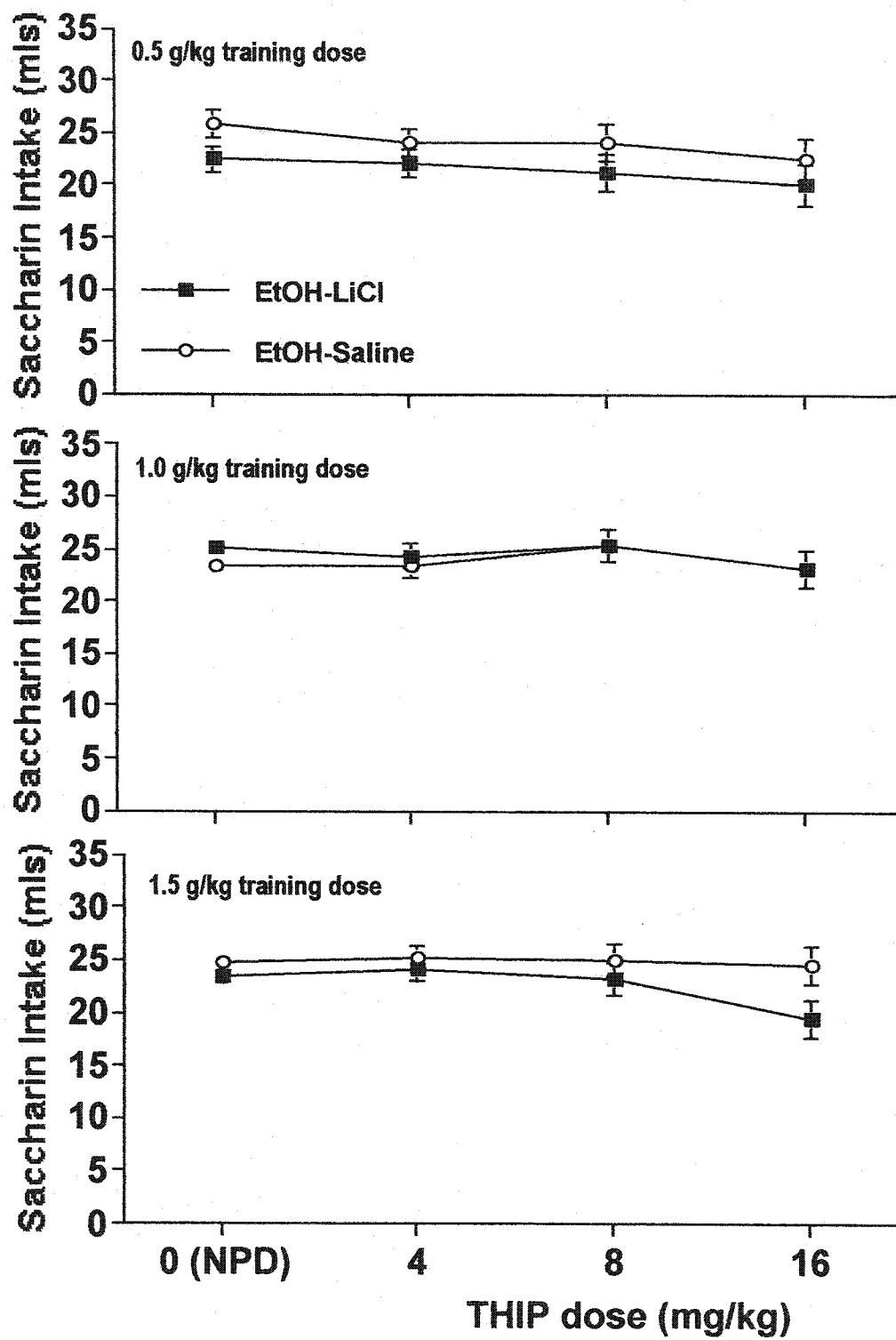
Figure 10. Mean saccharin intake after injections of several doses of sodium

pentobarbital for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open

circles) groups across the 0.5 g/kg (top panel), 1.0 g/kg (middle panel) and 1.5

g/kg (bottom panel) ethanol-training groups. Saline was injected on non-pairing

days (NPD). Vertical lines represent S.E.M. Where no error bars appear, S.E.M.

is smaller than the symbol.
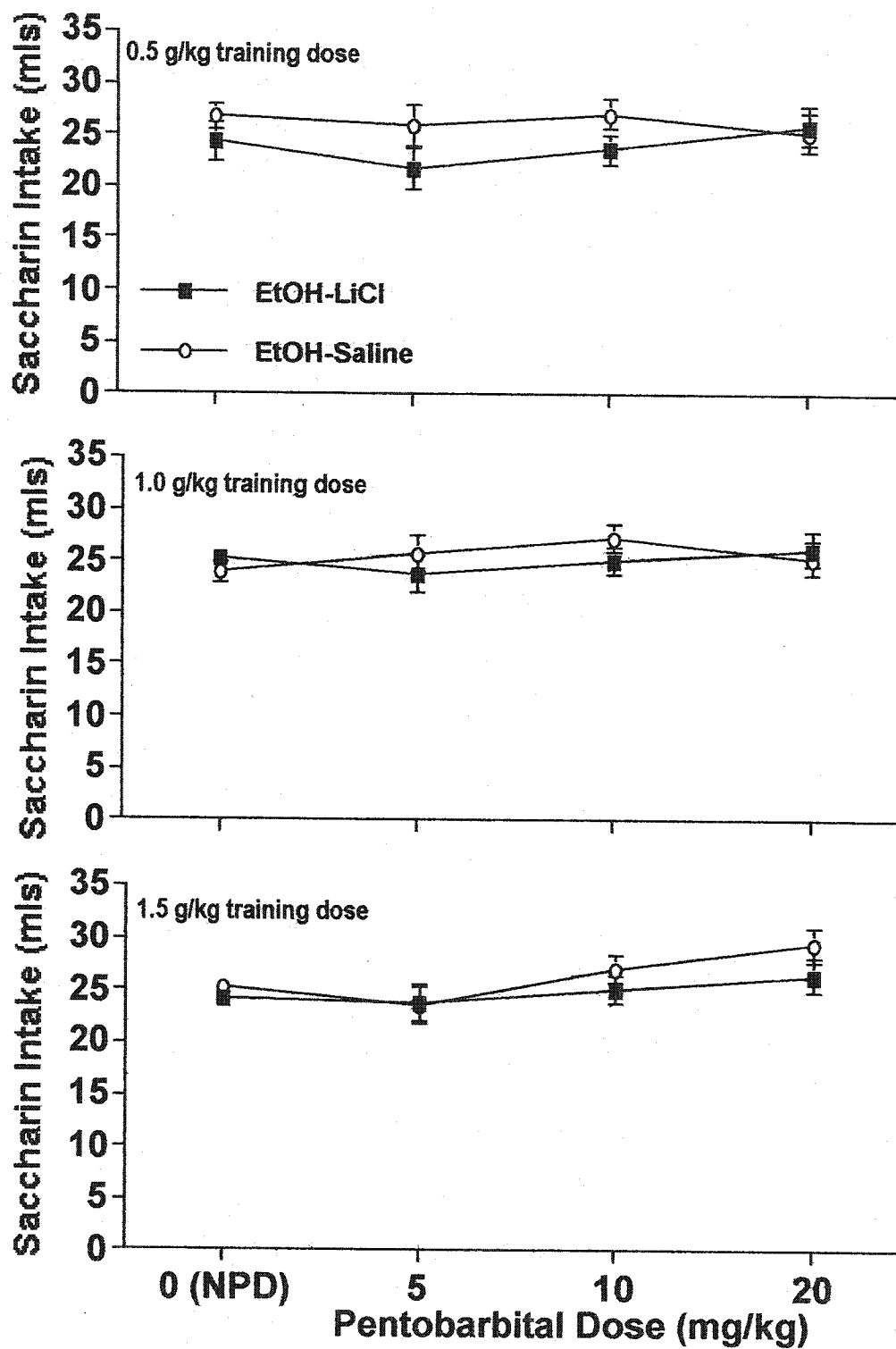
Figure 11. Mean saccharin intake after injections of several doses of baclofen for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open circles) groups across the 0.5 g/kg (top panel), 1.0 g/kg (middle panel) and 1.5 g/kg (bottom panel) ethanol-training groups. Saline was injected on non-pairing days (NPD). Vertical lines represent S.E.M. The symbol * denotes a significant difference ($p<.05$) between the generalization dose and NPD within each group.
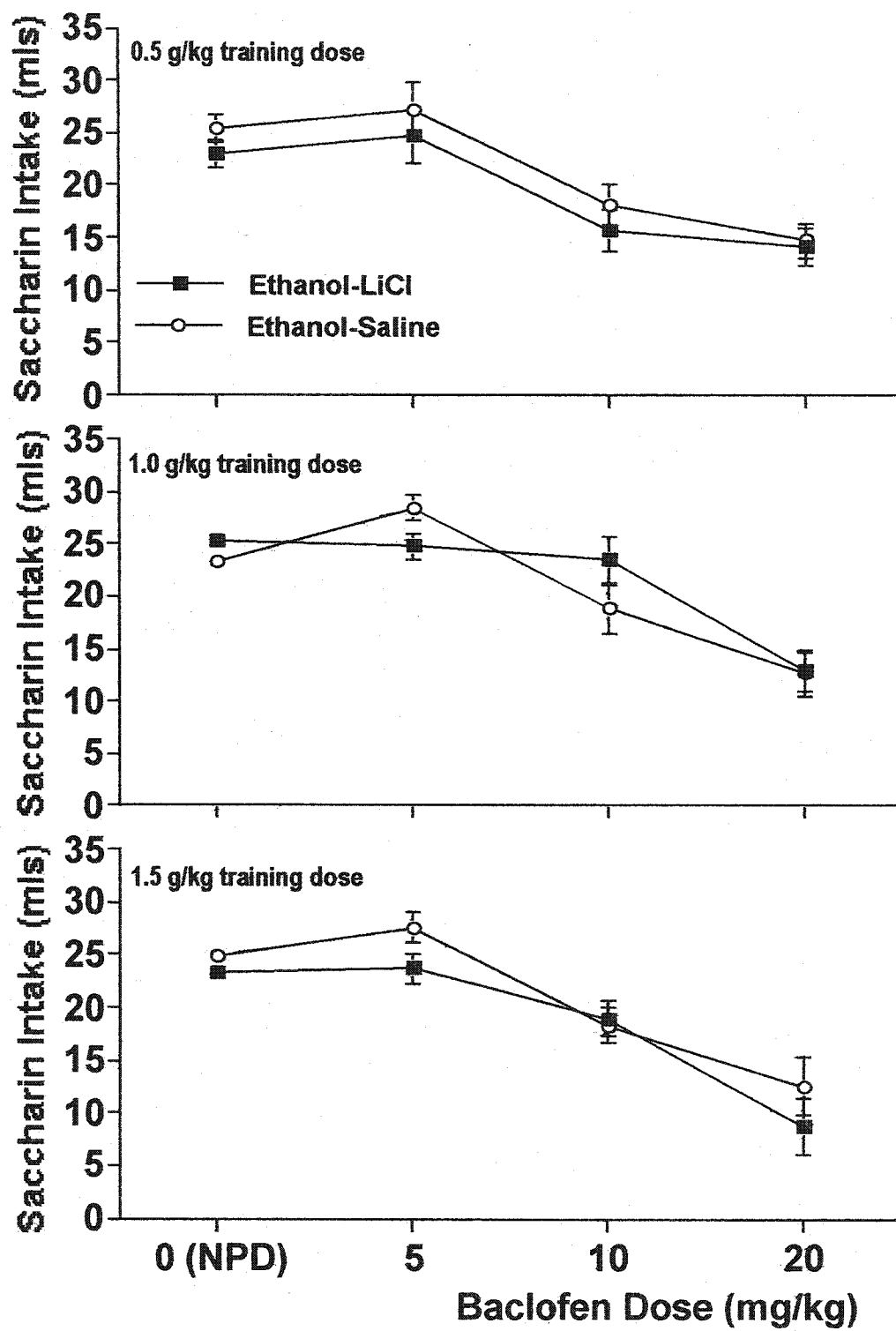
Figure 12. Mean saccharin intake after injections of several doses of the $GABA_A$ receptor antagonist picrotoxin for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open circles) groups across the 0.5 g/kg (top panel), 1.0 g/kg (middle panel) and 1.5 g/kg (bottom panel) ethanol-training groups. Picrotoxin injections were administered 5 minutes before ethanol injections. Vertical lines represent S.E.M. The symbol * denotes a significant difference ($p<.05$) between the picrotoxin dose and NPD.
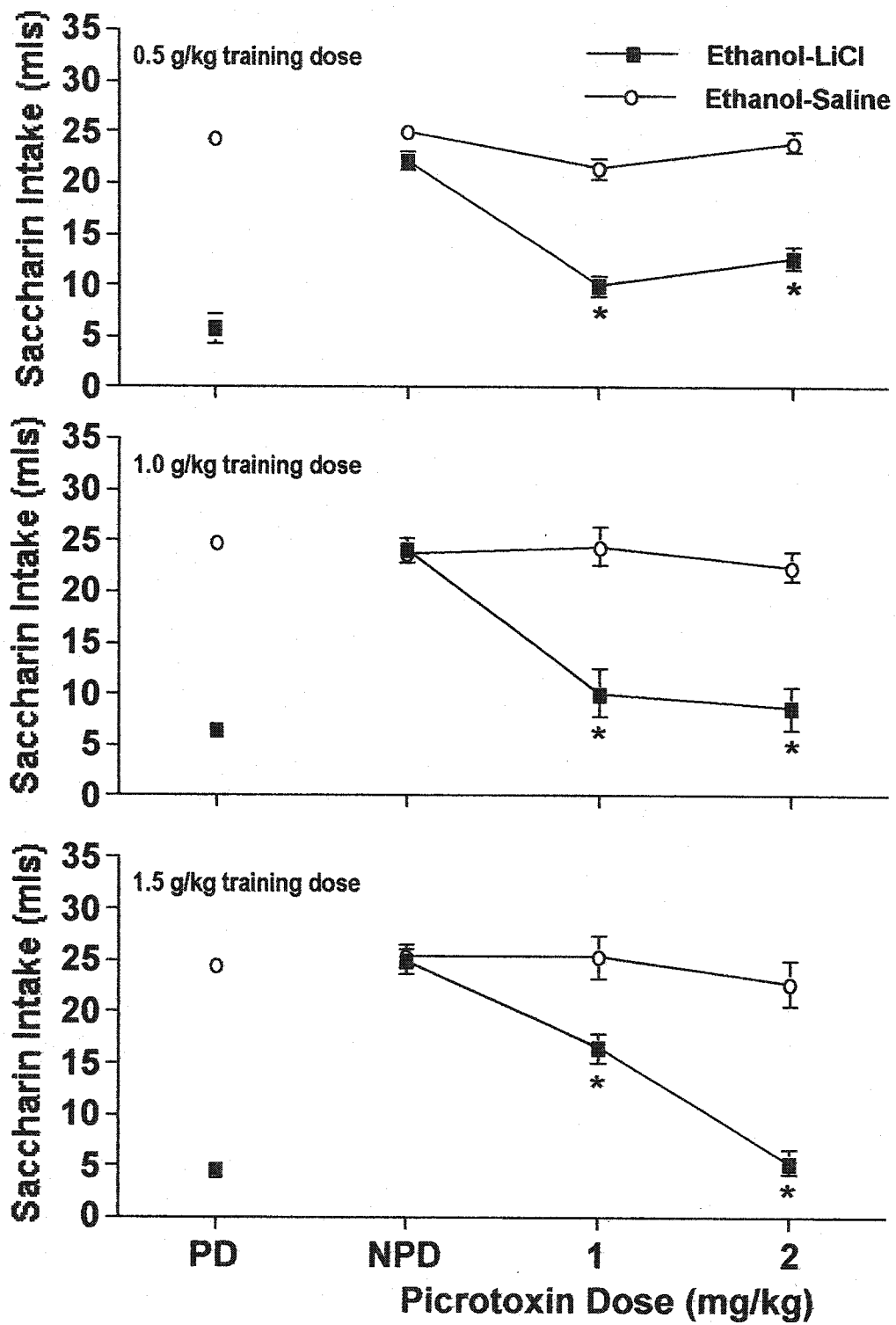
Discussion

The present study used a discriminative taste aversion procedure to characterize the contribution of the GABAergic system in mediating the discriminative stimulus effects of ethanol. The GABA agonist AOAA, the $GABA_A$ receptor agonists THIP and pentobarbital, and the $GABA_B$ receptor agonist baclofen were used to determine whether activation of either, or both, GABA receptor subtypes would contribute to the stimulus effects of ethanol. In addition, the $GABA_A$ antagonist picrotoxin was used to examine whether inhibiting the action of the $GABA_A$ receptor would block the stimulus cue of ethanol.

Three doses of ethanol were used to train animals to discriminate between ethanol and saline. As can be seen in Figure 6, each training dose of ethanol was able to produce stable discriminative control on drinking behaviour using the DTA procedure. Animals in the Ethanol-LiCl group met criteria for discriminative control after an average of 14.3, 12.6, and 10.7 training cycles for the 0.5, 1.0 and 1.5 g/kg ethanol-training dose groups respectively. As expected, animals in the Ethanol-Saline groups did not consistently decrease their saccharin intake on pairing days (PDs) compared to non-pairing days (NPDs) demonstrating that the Ethanol-Saline groups did not acquire a discriminative taste aversion or that ethanol produced any unconditioned effects that would affect fluid consumption.

Ethanol generalization tests showed a dose-dependent substitution to ethanol in the Ethanol-LiCl groups. Generally, ethanol doses close to or greater than the ethanol-training dose fully substituted for ethanol. Ethanol doses of 0.5, 1.0 and 1.5 g/kg completely substituted for ethanol in the 0.5 g/kg Ethanol-LiCl

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group. The ethanol dose of 0.5 g/kg partially substituted while 1.0 and 1.5 g/kg ethanol completely substituted for ethanol in the Ethanol-LiCl animals trained with 1.0 g/kg ethanol. Lastly, 1.0 g/kg and 1.5 g/kg ethanol completely substituted for the 1.5 g/kg ethanol-training dose in the Ethanol-LiCl group. The lowest test dose of ethanol, 0.25 g/kg, did not substitute for ethanol at any of the ethanol-training doses tested suggesting that this dose of ethanol did not produce sufficient stimulus effects to be recognized as being ethanol.

The overall findings of the generalization tests showed that the GABA agonist AOAA, the $GABA_A$ agonists THIP and pentobarbital as well as the $GABA_B$ agonist baclofen all failed to substitute for ethanol. The goal of the present study was to extend the findings concerning the contribution of $GABA_A$ activation in mediating the stimulus effects of ethanol. Pentobarbital has been consistently reported to substitute for the stimulus effects of ethanol (Bowen et al., 1999; Green & Grant, 1998; Hodge, Cox, et al., 2001; Shelton & Grant, 2002;). Increased activation of the $GABA_A$ receptor by positive modulators such as neurosteroids has also supported the hypothesis that the $GABA_A$ receptor contributes to the stimulus effects of ethanol (Bowen & Grant, 1999; Hodge, Cox, et al., 2001). Data from the present study however showed that both pentobarbital and THIP failed to substitute for ethanol at any of the doses used. This finding suggests that activation of the $GABA_A$ receptor did not produce stimulus effects similar to ethanol when assessed with the discriminative taste aversion procedure.

Although previous reports have showed that pentobarbital fully substituted for ethanol, there is a study that showed a failure of THIP to substitute for ethanol (Shelton & Grant, 2002). THIP belongs to a class of drugs referred to as direct $GABA_A$ agonist since it acts at the $GABA_A$ receptor site. This is opposed to indirect $GABA_A$ agonists that act at other binding sites on the $GABA_A$ receptor. Shelton and Balster (1994) demonstrated that the direct $GABA_A$ agonist muscimol failed to substitute for ethanol while the indirect agonist pentobarbital completely substituted for ethanol in animals trained to discriminate ethanol from saline. As a result, these researchers suggested that direct $GABA_A$ agonists such as THIP and muscimol do not produce stimulus effects that resemble the stimulus effects of ethanol. This notion was supported by studies that demonstrated a failure of direct $GABA_A$ agonists to substitute for the indirect $GABA_A$ agonist pentobarbital in animals trained to discriminate pentobarbital from saline (Grech & Balster, 1993; Nielsen et al., 1983). In light of these findings, the results of THIP and pentobarbital generalization tests in the present study support and contradict previous findings respectively. Therefore, testing other direct and/or indirect $GABA_A$ agonists with the DTA procedure would be needed to determine the role of the GABAergic system in mediating the stimulus effects of ethanol.

Antagonism tests with the $GABA_A$ antagonist picrotoxin demonstrated a failure to completely block the stimulus effects of ethanol for the Ethanol-LiCl animals trained with 0.5 and 1.0 g/kg ethanol. Data from the 0.5 g/kg Ethanol-LiCl group showed a partial substitution on test days suggesting that picrotoxin

may have at least partially blocked the stimulus effects of ethanol. A closer look at the data showed that three Ethanol-LiCl animals displayed partial substitution while the other three Ethanol-LiCl animals still demonstrated complete substitution for ethanol when pretreated with picrotoxin. The majority of animals in the 1.0 g/kg Ethanol-LiCl group (six of eight animals) decreased their saccharin intake when picrotoxin was administered prior to ethanol to the same degree as when ethanol was administered alone. Thus, data from the 1.0 g/kg Ethanol-LiCl group suggests that blocking the activity of the $GABA_A$ receptor does not attenuate the discriminative stimulus cue of ethanol. Finally, results from the 1.5 g/kg Ethanol-LiCl group showed that 1.0 mg/kg picrotoxin pretreatment partially blocked the stimulus effects of ethanol while 2.0 mg/kg picrotoxin failed to block the stimulus effects of ethanol. These results suggest that the discriminative stimulus effects of 1.5 g/kg ethanol may be mediated partially through the $GABA_A$ receptor. The results of picrotoxin antagonism provided mixed results and thus the role of picrotoxin cannot be clearly ascertained without running additional sample of animals or other $GABA_A$ receptor antagonists.

Generalization tests with the gamma-amino-transaminase inhibitor AOAA failed to substitute for ethanol. Gamma-amino-transaminase is an enzyme responsible for the metabolism of GABA on the synaptic cleft. When AOAA is present, GABA cannot be metabolized and as a result GABA remains available to activate GABA receptors rather than being metabolized. AOAA thus acts as an GABA agonist. The hypothesis was that the increase in available GABA due

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to AOAA would result in activation of both types of GABA receptors. In light of the failure of the GABA$_A$ agonists to substitute for ethanol, the failure of AOAA to substitute for ethanol may also stem from the fact that GABA$_A$ receptors may not mediate the stimulus effects of ethanol.

The GABA$_B$ agonist baclofen was used to differentiate the contribution of the two GABA receptor subtypes in mediating the ethanol discriminative cue. Generalization tests with the GABA$_B$ receptor agonist baclofen failed to substitute for ethanol suggesting that GABA$_B$ activation does not contribute to the stimulus effects of ethanol. Baclofen was not expected to substitute for ethanol since previous studies have reported a failure of the GABA$_B$ receptor to contribute to ethanol discriminative cue (Shelton & Balster, 1994).

Experiment 2 demonstrated the ease with which animals were able to acquire discriminative control of drinking behaviour using the DTA procedure. The fact that animals readily learned to discriminate a low dose of ethanol, 0.5 g/kg, using the DTA procedure makes it possible to examine the stimulus effects of low doses of ethanol with the DTA procedure. The failure of both pentobarbital and THIP to substitute for ethanol in the present study suggests that activation of the GABA$_A$ receptor does not contribute to the stimulus effects of ethanol within the DTA paradigm. Since previous studies have proposed that the stimulus effects of ethanol is partially mediated by the glutamatergic neurotransmitter systems (Hodge & Cox, 1998, Hodge et al, 2001; Hundt et al., 1998) experiment 3 was designed to assess the contribution of the glutamatergic system in contributing to the stimulus effect of ethanol.

Experiment 3

The Effects of Glutaminergic System Manipulation on an Ethanol

Discriminative Taste Aversion

A growing literature has implicated glutamatergic neurotransmission in mediating the effects of ethanol (see Dansyz et al., 1992). Electrophysiological studies have shown that ethanol inhibited the activity of the N-methyl-D-aspartate (NMDA) receptor. Specifically, ethanol inhibited neuronal firing (Franklin & Gruol, 1987) and decreased glutamate-stimulated cyclic GMP production (Hoffman et al., 1989) in cultured brain cells. The ability of ethanol to antagonize NMDA receptor function has led to the hypothesis that several ethanol-related behaviours may result from ethanol's interaction with the NMDA receptor (Lovinger et al., 1989, 1990; Tabakoff & Hoffman, 1996; Woodward, 1999). For example, ethanol withdrawal symptoms (hyperexcitability, tremors, convulsions) are typically observed in animals chronically exposed to ethanol and then are suddenly withdrawn from ethanol exposure. These ethanol withdrawal symptoms are thought to reflect upregulation of NMDA receptors and removal of ethanol results in hyperexcitability (Grant et al., 1990). Treatment with the NMDA antagonist MK-801 attenuated ethanol withdrawal symptoms and eliminated ethanol withdrawal seizures in rats chronically exposed to high concentrations of ethanol (Grant et al., 1990). This result suggests that ethanol withdrawal symptoms may be mediated through NMDA receptor subtypes.

Several drug discrimination studies have shown the involvement of the glutamatergic system in contributing to the stimulus effects of ethanol (Grant et al., 1991; Sanger, 1993; Schechter et al., 1993). Researchers have consistently shown that noncompetitive NMDA antagonists such as MK-801 (Hodge & Cox, 1998; Hodge, Cox, et al., 2001; Schechter et al., 1993) and memantine (Bienkowski et al., 1998) completely substituted for ethanol. The glutamatergic component of the discriminative cue of ethanol has been shown to be mediated specifically through inhibition of the NMDA receptor since administration of the AMPA antagonist GYKI-52466, the glycine antagonist L-701,324, and the polyamine site antagonist arcaine all failed to substitute for ethanol (Hundt et al., 1998).

The primary object of the present study was to characterize the contribution of the glutamatergic neurotransmission to the stimulus effects of ethanol using the DTA procedure. The NMDA antagonists MK-801 and memantine and the AMPA antagonist GYKI-52466 were used to examine the effects of differentially inhibiting glutamate neurotransmission on mediating the discriminative stimulus cue of ethanol. A second objective was to assess whether the failure of pentobarbital to substitute for ethanol in experiment 2 was due to an altered perception of ethanol's discriminative cue. The failure of pentobarbital to substitute for ethanol is contradictory to operant drug discrimination studies that have reported that pentobarbital substituted for ethanol in rats trained to discriminate ethanol from saline (Bowen & Grant, 1999; Hodge, Cox, et al., 2001). It may be possible that once trained to discriminate

ethanol from saline with the discriminative taste aversion procedure, separate components of the ethanol cue cannot be distinguished alone. Generalization tests with pentobarbital were thus conducted earlier in the DTA procedure in order to test this hypothesis.

Materials and Methods

Subjects

Two groups of male Long-Evans rats were used to complete the present study. The first group consisted of 49 animals and the second group consisted of 30 animals. Animals weighed between 225 and 250 g at the start of the experiment and were housed individually in stainless steel hanging cages in a colony room maintained at a constant temperature. The first group of animals was maintained in a colony room with a 12:12 hour light/dark cycle that had lights on at 19:00 while the second group had lights on at 08:00. The care and use of animals conformed to the standards set by the Canadian Council of Animal Care (CCAC, 1993).

Drugs

A 20% v/v ethanol solution was made by diluting a 95% stock ethanol solution with 0.9% physiological saline. A 0.15M lithium chloride (Fisher Scientific) was dissolved in saline. A 0.1% w/v saccharin solution was prepared by diluting saccharin sodium (BDH Fine Chemicals) with tap water. Dizocilpine maleate [(+)-MK-801; Sigma, St Louis, MO], sodium pentobarbital, and

memantine hydrochloride (RBI, St Louis, MO) were dissolved in saline. GYKI 5266 hydrochloride (RBI, St Louis, MO) was dissolved in a 10% cremaphor solution (Fluka/Sigma-Aldrich, Oakville, Ont).

Procedure

Conditioning

After a habituation and water deprivation period rats were allowed access to water for only 20 minutes per day. After 7 days saccharin (0.1% w/v) replaced water as the sole fluid presented. On the third day of saccharin presentation, all animals were injected (ip) with saline (5 ml/kg) in order to habituate them to receiving injections. On day 1 of each cycle (pairing day, PD) all animals were injected ip with the appropriate training dose of ethanol. Thirty minutes later animals were allowed 20 minutes access to the saccharin solution and then injected with LiCl (0.15 M, 1.8 mEq) or an equal volume of saline. On days 2, 3, and 4 of each cycle (non-pairing days; NPDs) animals were injected with saline 30 minutes prior to the presentation of the saccharin solution. Animals were then allowed 20 minutes of access to the saccharin solution and no injections ensued. Saccharin intake was measured each day to the nearest 0.5 ml.

Discriminative control of saccharin intake was defined as six cycles in which saccharin intake during the pairing days was less than 50% of the saccharin intake during the following three non-pairing days of the same cycle. These criteria were used to ensure that all Ethanol-LiCl animals displayed the

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same degree of discriminative stimulus control before generalization tests were conducted. This criterion did not apply to the Ethanol-Saline group. The number of cycles to reach discriminative criteria differed across animals. Once an animal in the Ethanol-LiCl group had reached discriminatory criterion, it and an animal in the Ethanol-Saline group began generalization tests. This procedure was used to ensure that animals in the Ethanol-LiCl and Ethanol-Saline groups had similar exposure to ethanol prior to generalization tests. Generalization tests to different doses of ethanol, the noncompetitive NMDA antagonists MK-801 (dizocilpine maleate) and memantine, the $GABA_A$ receptor agonist pentobarbital, and the AMPA receptor antagonist GYKI 52466 hydrochloride were conducted.

Animals in the first group were randomly assigned either to the 0.5, 1.0 or 1.5 g/kg ethanol-training dose. Animals in the first group were given generalization tests to ethanol and MK-801 but failed to maintain discriminative control and could not complete generalization tests with the other drugs. In addition, Ethanol-LiCl animals trained with 0.5 g/kg ethanol failed to meet discriminative stimulus control. Therefore, a second group of animals were needed to complete the generalization tests. Animals in the second group were randomly assigned to either the 1.0 or 1.5 g/kg ethanol-training dose. A group trained with 0.5 g/kg ethanol was not included in the second group since this dose did not produce stable discrimination in group 1 animals. Within each ethanol-training dose group half the animals were assigned to the Ethanol-LiCl group while the other half were assigned to the Ethanol-Saline group.

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Generalization tests

Once stimulus control criterion had been reached animals were given generalization tests to several drugs. Several doses of ethanol and MK-801 were used as generalization drugs for the first group of animals. The second group was tested for generalization to ethanol with several doses of ethanol, MK-801, pentobarbital, GYKI 52466, and memantine. Drugs were tested in the following order for the first group; ethanol (0.25, 0.5, 1.0 and 1.5 g/kg) and MK-801 (0.05, 0.1, 0.25 and 0.5 mg/kg). The second group received drugs in the following order: ethanol (0.25, 0.5, 1.0 and 1.5 g/kg), MK-801 (0.025, 0.05, 0.1, and 0.25 mg/kg), pentobarbital (5, 10 and 20 mg/kg), memantine (2, 5, 10, and 15 mg/kg), GYKI 52466 (1, 2 and 5 mg/kg). The order in which drug doses were administered was randomly assigned to each animal within each drug treatment. All injections were given intraperitoneally 30-min prior to saccharin presentation.

Generalization tests were conducted in the same manner as in the previous study. If an animal failed to discriminate ethanol from saline (<50% saccharin intake on PD compared to the average of the previous and following NPDs) then generalization tests were not performed until the animal had met criterion, a decrease of more than 50% on a subsequent pairing day. Partial substitution was defined as a decrease between 40 and 80% of the total decrease while complete substitution was defined as greater than 80% of the decrease in saccharin intake observed during pairing day and nonpairing days.

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<u>Results</u>

Separate three-way ANOVAs were used to examine the acquisition of discriminative control of ethanol through the first fifteen cycles of the experiment. Separate three-way ANOVAs were conducted for each ethanol-training dose and for each group of animals tested (Group 1 and Group 2 animals). The between groups variable was called Group and consisted of two levels (LiCl and Saline). The first within variable was referred to as Days and had two levels, the pairing day (PD) and the average of the three non-pairing days (NPDs) of the same cycle. The second variable was labeled Cycle that included 15 levels.

Significant Group by Cycle by Day interactions were further analyzed with a test of simple interaction holding the Group variable constant. That is, the test of simple interaction tested whether there is a significant interaction between Cycle and Days for each level of the conditioning group (Ethanol-LiCl and Ethanol-Saline). If the simple interaction was significant then simple effects tests were conducted to test whether there was a difference in saccharin intake between the pairing day and non-pairing day at each training cycle for each training group. All analyses used a significance level of .05.

The three-way ANOVA for the first group of animals trained at the 0.5 g/kg ethanol training dose revealed a significant Group by Cycle by Day interaction, $F(14, 168) = 5.08$, $p<.05$. The test of simple interaction for the Ethanol-LiCl group produced a significant result, $F(14, 168) = 7.3$, $p<.001$. Simple effects tests showed that the LiCl group had significantly decreased their saccharin intake on PD compared to NPDs on Cycles 7 through 15 ($p<.05$). Analysis of the

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Ethanol-Saline group also yielded a significant simple interaction $F(14, 168) = 3.58$, $p<.001$. Saccharin intake was significantly different PD compared to NPDs on Cycles 2, 6, and 9 ($p<.05$).

The three-way ANOVA results for the first group of animals trained with 1.0 g/kg ethanol yielded a significant Group by Cycle by Day interaction $F(14, 182) = 17.91$, $p<.001$. Simple interaction test for the Ethanol-LiCl group yielded a significant Cycle by Days interaction, $F(14, 182) = 30.29$, $p<.001$. Animals in the Ethanol-LiCl group had significantly lower saccharin intake on pairing days compared to non-pairing days on Cycles 4 through 15 ($p>.05$). Simple effects test for the Ethanol-Saline group produced a nonsignificant result, $F(14, 182) = 1.09$, $p>.369$.

A significant three-way interaction was obtained for the group 1 animals trained with 1.5 g/kg, $F(14, 196) = 13.28$, $p<.001$. The test of simple interaction resulted on a significant finding for the Ethanol-LiCl group, $F(14, 196) = 19.11$, $p<.001$. Saccharin intake for the Ethanol-LiCl group was significantly decreased on PD compared to NPDs for Cycles 2 through 15 as demonstrated by significant simple effects tests ($p<.05$). Simple interaction test for the Ethanol-Saline group was significant, $F(14, 196) = 3.96$, $p<.001$. Tests of simple effects showed that saccharin intake on PD was different from NPDs on Cycles 2, 9, 12,and 15 ($p<.05$).

Similar results were found for the acquisition of an ethanol discriminative taste aversion for the second group of animals tested. A significant Group by Cycle by Days interaction was obtained for the second group of animals trained

with 1.0 g/kg, $F(14, 168) = 12.42$, $p<.001$. The simple interaction test for the Ethanol-LiCl group yielded a significant result, $F(14, 168) = 29.39$, $p<.001$. Further analysis with tests of simple effects showed that animals in the Ethanol-LiCl group significantly decreased their saccharin intake on following ethanol injections compared to the days in which they were injected with saline on Cycles 4 through 15 ($p<.05$). The test for simple interaction performed on the Ethanol-Saline group was nonsignificant, $F(14, 168) = 1.27$, $p>.231$.

The overall ANOVA for group 2 animals trained with 1.5 g/kg yielded a significant three-way interaction, $F(14, 168)= 9.46$, $p<.001$ Analysis of the simple interaction test showed a significant Day by Cycle interaction for the Ethanol-LiCl group, $F(14, 168) = 23.75$, $p<.05$. The simple effects tests showed that saccharin intake was significantly decreased on PD compared to NPD for Cycles 4 through 15 ($p<.05$). There was no significant simple interaction for the Ethanol-Saline group, $F(14, 168) = 0.72$, $p>.05$.

Figure 13. Mean saccharin intake during ethanol discriminative taste aversion

training cycles for the 0.5 g/kg (top panel), 1.0 g/kg (middle panel), and 1.5 g/kg

(bottom panel) ethanol-training dose for group 1 animals. Filled and open bars

represent mean saccharin intake for the Ethanol-LiCl and Ethanol-Saline groups

respectively on pairing days (PD). Filled and open squares represent mean

saccharin intake across the three non-pairing days of each cycle for the Ethanol-

LiCl and Ethanol-Saline groups respectively. Vertical lines represent S.E.M.

Where no error bars appear, S.E.M. is smaller than the symbol. The symbol *

denotes a significant difference ($p<.05$) between pairing day and non-pairing

days for the Ethanol-LiCl group.
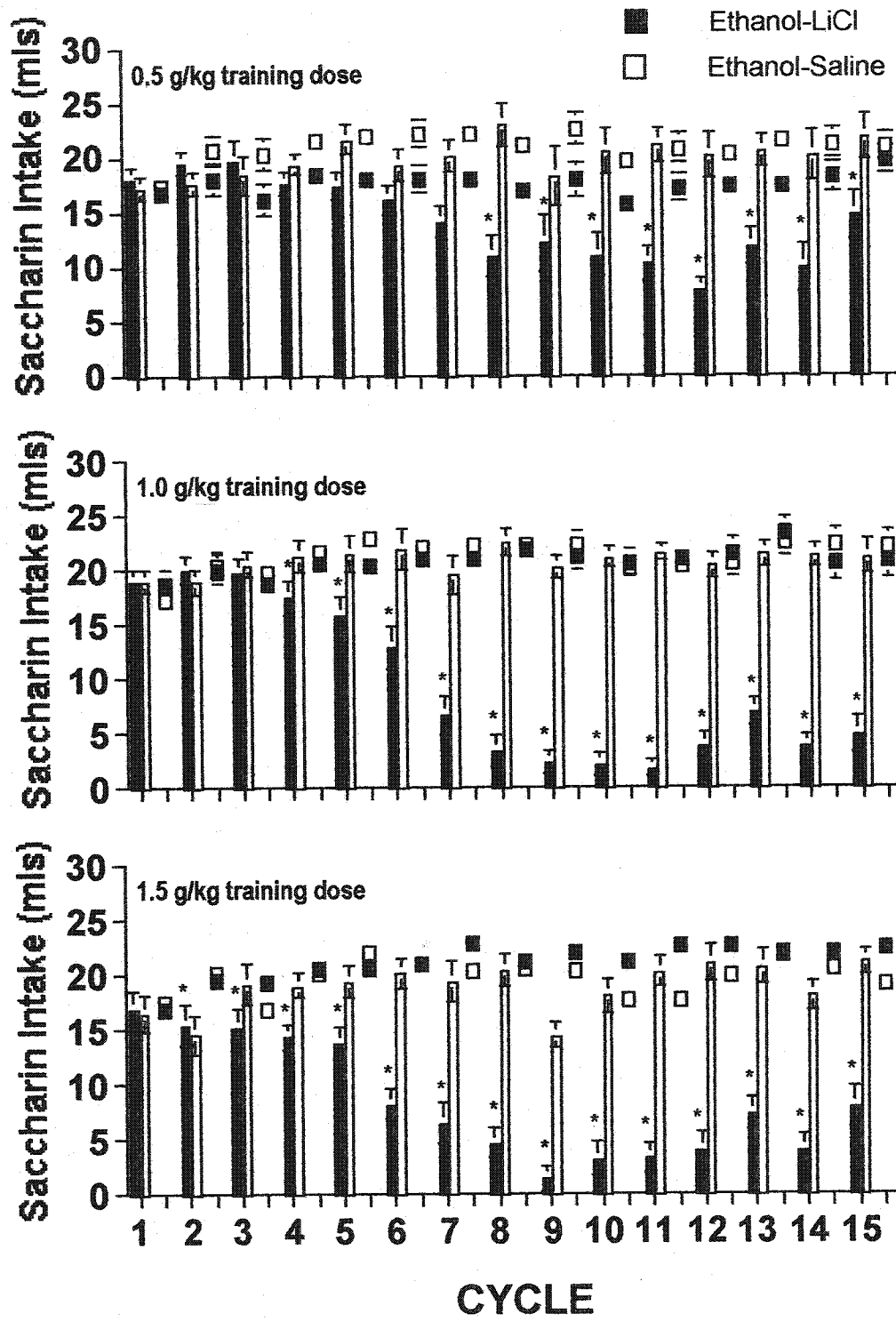
Figure 14. Mean saccharin intake during ethanol discriminative taste aversion training cycles for the 1.0 g/kg and 1.5 g/kg ethanol-training dose groups (group 2). Filled and open bars represent mean saccharin intake for the Ethanol-LiCl and Ethanol-Saline groups respectively on pairing days (PD). Filled and open squares represent mean saccharin intake across the three non-pairing days of each cycle for the Ethanol-LiCl and Ethanol-Saline groups respectively. Vertical lines represent S.E.M. Where no error bars appear, S.E.M. is smaller than the symbol. The symbol * denotes a significant difference ($p<.05$) between pairing day and non-pairing days for the Ethanol-LiCl group.
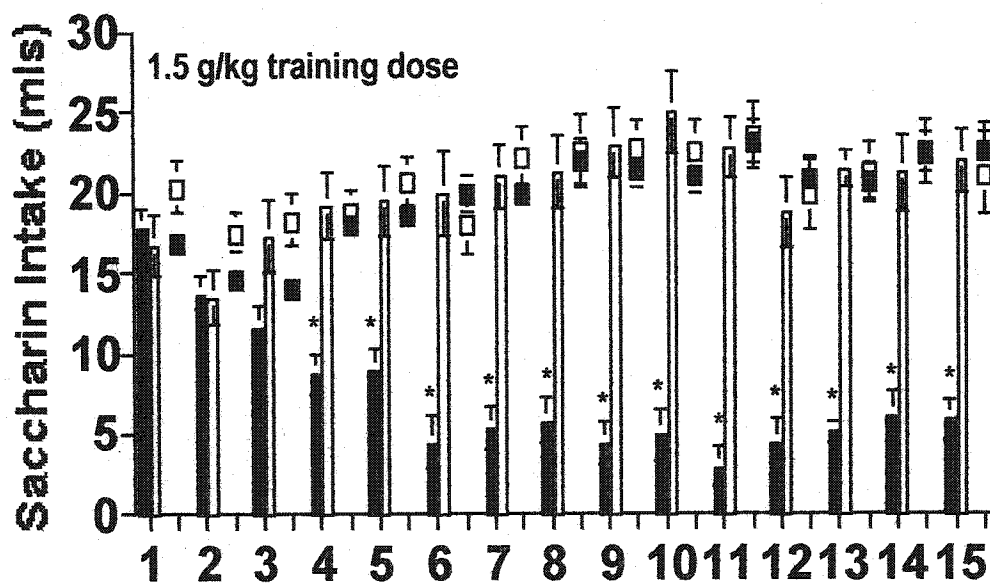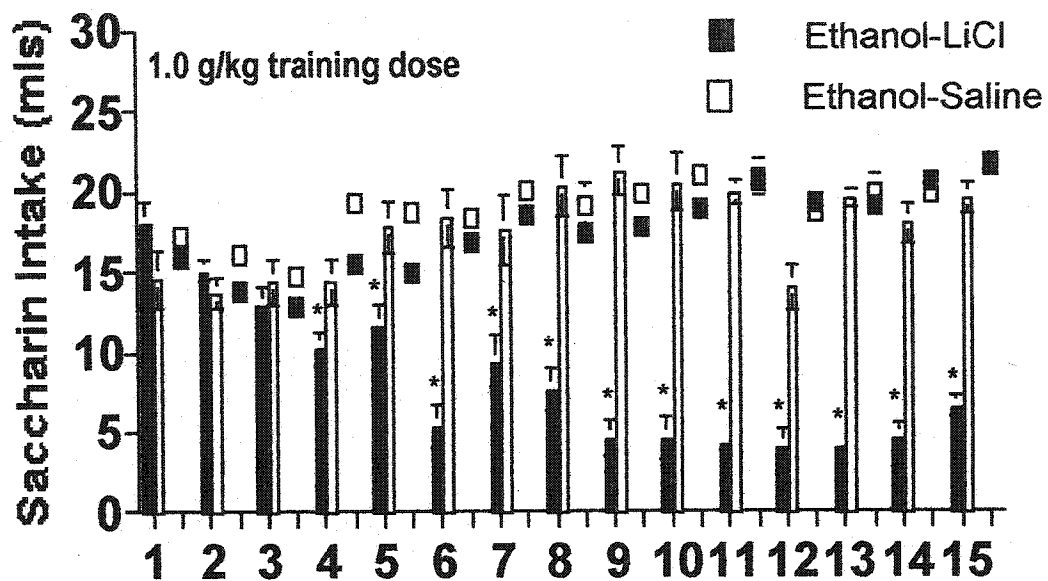
Analysis of Generalization Tests

Separate two-way ANOVAs, one for each ethanol-training dose, were used to examine the results of generalization tests. The between factor was conditioning Group with two levels, LiCl and Saline and the within factor was the generalization drug dose (Dose) with the number of levels dependent on the number of doses used. Included in the within factor is data representing the average saccharin intake during saline NPDs for the duration of the generalization tests cycles of a particular generalization drug. The NPD data was used as a saline control dose for the generalization drug. Significant interactions were followed with tests of simple effects. If simple effects of generalization tests doses at the group factor were significant then Dunnett posthoc tests between mean saccharin intake on saline days and each generalization tests day were conducted. All tests used an alpha level of .05 to establish significance. Missing data that occurred during generalization tests were replaced with the mean for that particular group.

Generalization tests for animals in the first group consisted of ethanol and MK-801. Towards the end of the MK-801 generalization tests (around Cycle 20) most Ethanol-LiCl animals had failed to maintain stimulus control for ethanol. That is, animals failed to meet criterion for ethanol discrimination consistently. In addition, most animals in the 0.5 g/kg ethanol-LiCl group did not reach criterion at the time it was decided to end the study. Therefore, generalization data were not collected for the 0.5 g/kg Ethanol-LiCl group. A second group of rats were trained to discriminate between ethanol and saline and were again tested with

generalization tests using ethanol, MK-801. In addition, several doses of pentobarbital, GYKI 52466, and memantine were tested for generalization to ethanol.

Analysis for the ethanol generalization tests performed on the first group trained with 1.0 g/kg ethanol yielded a significant Group by Dose interaction, $F(4, 56) = 16.72$, $p<.001$. Simple effects for the Ethanol-LiCl group was significant, $F(4, 56) = 27.00$, $p<.001$ and Dunnett tests showed that saccharin intake significantly decreased after administration of the .05, 1.0 and 1.5 g/kg ethanol doses compared to the saline NPDs ($p<.05$). Simple effects test for the Ethanol-Saline group resulted in a nonsignificant interaction, $F(4, 56) = 0.54$, $p>.746$.

The Group by Dose interaction was significant for animals trained with 1.5 g/kg ethanol across ethanol generalization tests, $F(4, 56) = 12.57$, $p<.001$. Analysis of simple effects for the Ethanol-LiCl group was significant, $F(4, 56) = 33.79$, $p<.05$. Further analysis showed that Ethanol-LiCl animals significantly decreased their saccharin intake following administration of the 1.0 and 1.5 g/kg ethanol generalization doses. Simple effects test for the Ethanol-Saline group revealed a nonsignificant result, $F(4, 56) = 0.01$, $p>.465$.

Figure 15. Mean saccharin intake after injections of several doses of ethanol for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open circles) groups across the 1.0 g/kg (top panel) and 1.5 g/kg (bottom panel) ethanol-training groups (group 1 animals). Saline was injected on non-pairing days (NPD). Vertical lines represent S.E.M. The symbol * denotes a significant difference ($p < .05$) between the Ethanol-LiCl and Ethanol-Saline groups.

Figure 16. Mean saccharin intake after injections of several doses of ethanol for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open circles) groups across the 1.0 g/kg (top panel) and 1.5 g/kg (bottom panel) ethanol-training groups (group 2 animals). Saline was injected on non-pairing days (NPD). Vertical lines represent S.E.M. The symbol * denotes a significant difference ($p<.05$) between the Ethanol-LiCl and Ethanol-Saline groups.
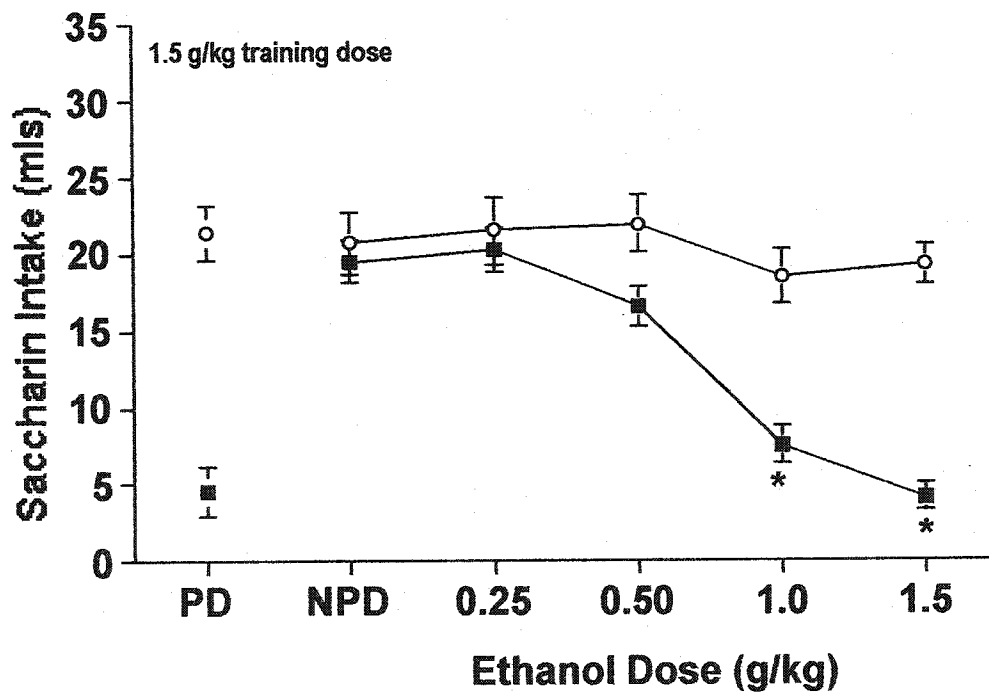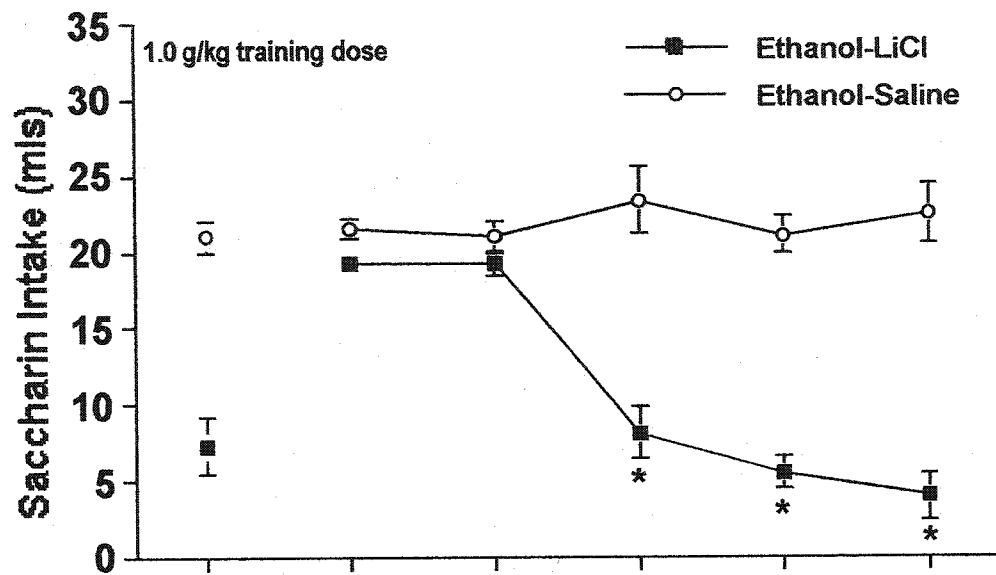
Animals in the first group were tested with MK-801 doses of 0.05, 0.1, 0.25 and 0.5 mg/kg. Analysis of MK-801 generalization tests for group 1 animals trained with 1.0 g/kg resulted in a significant Group by Dose interaction, $F_{(4, 56)} = 8.49$, $p<.001$. Simple effects test for the Ethanol-LiCl group was significant, $F_{(4, 56)} = 20.47$, $p<.001$, and simple comparisons showed that saccharin intake was significantly decreased after injections of 0.1, 0.25 and 0.5 mg/kg MK-801 doses. Simple effects test for the Ethanol-Saline group was nonsignificant, $F_{(4, 56)} = 2.27$, $p<.073$.

MK-801 generalization tests produced a significant overall two-way ANOVA for group 1 animals trained with 1.5 g/kg ethanol, $F_{(4, 56)} = 3.49$, $p<.014$. The simple effects test performed on the Ethanol-LiCl group 1 animals was significant, $F_{(4, 56)} = 11.62$, $p<.001$, and Dunnett tests showed that saccharin intake was significantly decreased following administration of 0.1, 0.25 and 0.5 mg/kg MK-801 compared to saline NPDs ($p<.05$). Simple effects test performed on data from the Ethanol-Saline group yielded significant Dose by Group interaction, $F_{(4, 56)} = 4.41$, $p<.004$. Posthoc tests showed that saccharin intake significantly decreased following administration of the 0.5 mg/kg MK-801 dose compared to saline NPDs.

Animals in the second group were tested with MK-801 doses of 0.025, 0.05, 0.1 and 0.25 mg/kg. Saccharin intake following MK-801 generalization tests for animals in group 2 is illustrated in figure 18. Analysis of the two-way ANOVA revealed a significant Dose by Group interaction, $F_{(4, 52)} = 3.67$, $p<.01$, for animals trained with 1.0 g/kg ethanol. Simple effects test for the Ethanol-LiCl

group yielded a significant result, $F(4, 52) = 19.70$, $p<.001$. Dunnett posthoc comparisons showed that animals decreased their saccharin intake after 0.05, 0.1, and 0.25 mg/kg MK-801 compared to the saline NPDs. The simple effects tests for the Ethanol-Saline group also produced a significant result, $F(4, 52) = 3.80$, $p<.009$. Dunnett tests demonstrated that animals injected with 0.5 mg/kg of MK-801 significantly decreased their saccharin intake compared to saline NPDs. Results of the two-way ANOVA produced a significant Dose by Group interaction for animals trained with 1.5 g/kg of ethanol, $F(4, 48) = 4.73$, $p<.003$. Simple effects tests for the Ethanol-LiCl group showed that saccharin intake was significantly different after injections of several doses of MK-801, $F(4, 48) = 35.57$, $p<.001$. Tests of simple comparison demonstrated that saccharin intake was significantly decreased following administration of 0.05, 0.1 and 0.25 mg/kg MK-801 compared to saline injections on NPDs ($p<.05$). The simple effects test for the Ethanol-Saline group was nonsignificant, $F(4, 48) = 1.65$, $p>.177$, and thus further analysis was not necessary.

Figure 17. Mean saccharin intake after injections of several doses of MK-801 for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open circles) groups across the 1.0 g/kg (top panel) and 1.5 g/kg (bottom panel) ethanol-training groups (group 1 animals). Mean saccharin intake during the four ethanol pairing days (PD) for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open squares) during generalization cycles are presented as unconnected symbols for comparison. Saline was injected on non-pairing days (NPD). Vertical lines represent S.E.M. The symbol * denotes a significant difference ($p<.05$) between the Ethanol-LiCl and Ethanol-Saline groups.
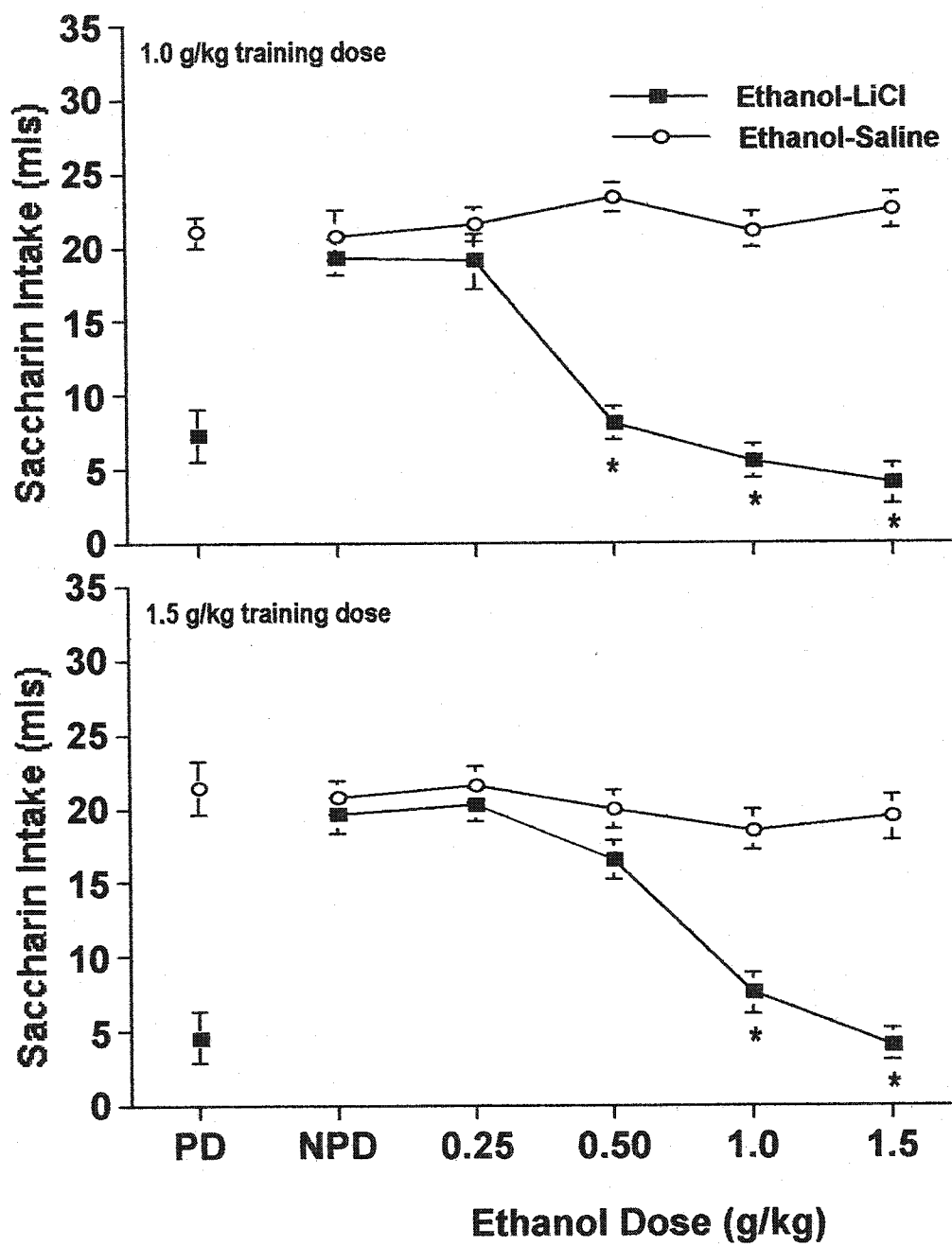
Figure 18. Mean saccharin intake after injections of several doses of MK-801 for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open circles) groups across the 1.0 g/kg (top panel) and 1.5 g/kg (bottom panel) ethanol-training groups (group 2 animals). Mean saccharin intake during the four ethanol pairing days (PD) for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open squares) during generalization cycles are presented as unconnected symbols for comparison. Saline was injected on non-pairing days (NPD). Vertical lines represent S.E.M. The symbol * denotes a significant difference ($p<.05$) between the ethanol generalization dose and the saline control dose (NPD).
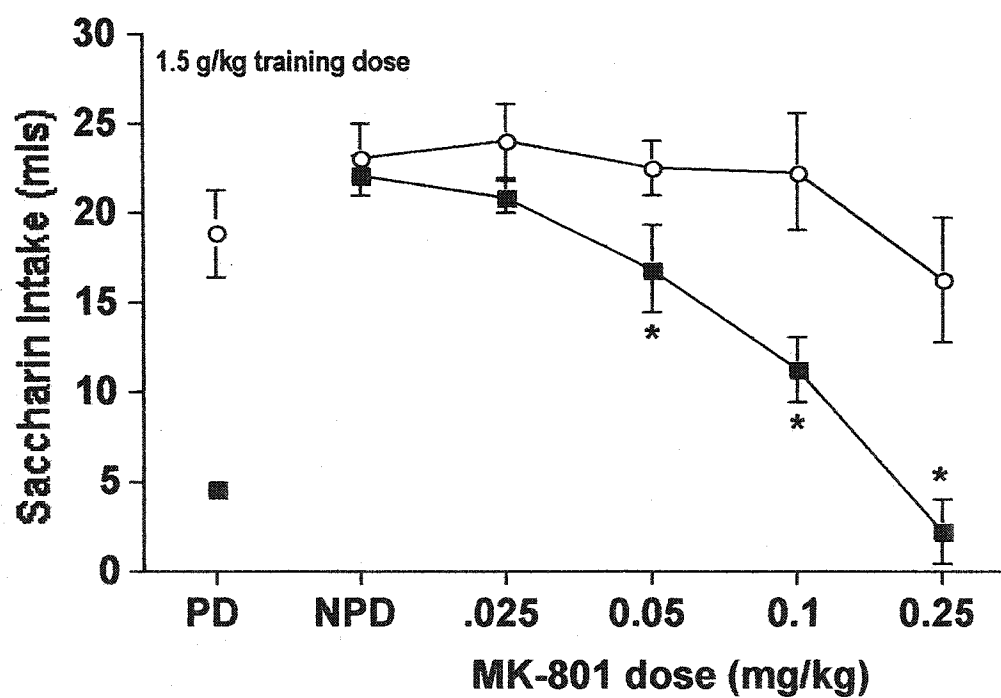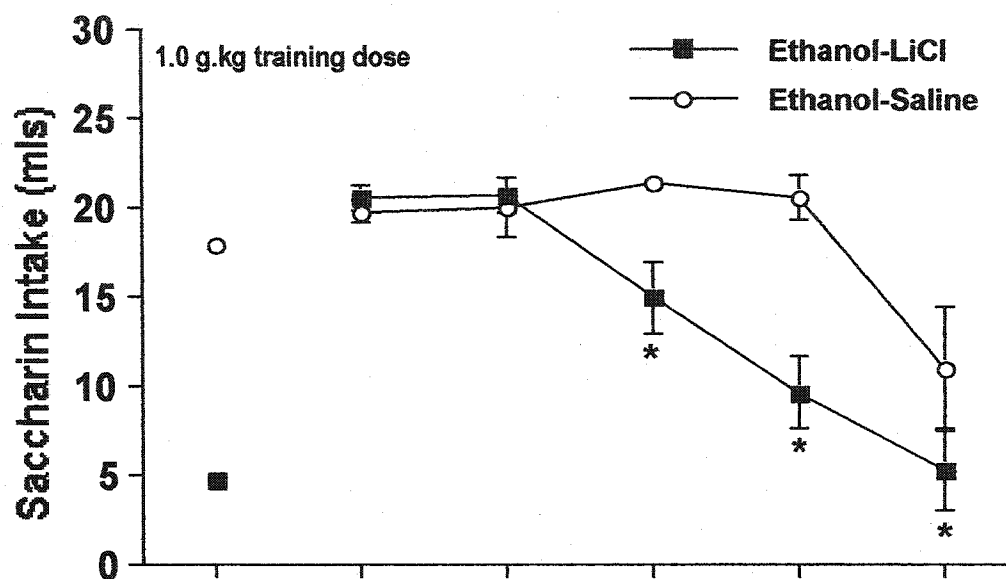
Animals in the second group were given generalization tests with doses of 5, 10 and 20 mg/kg of pentobarbital. The results for the overall ANOVA for animals trained with 1.0 g/kg ethanol, $F(3, 39) = .413$, $p > .745$, and 1.5 g/kg ethanol, $F(3, 36) = 4.95$, $p > .688$, were nonsignificant demonstrating that saccharin intake did not decrease following administration of any dose of pentobarbital when compared to saline injections (see figure 19).

Figure 20 depicts saccharin intake for the 1.0 and 1.5 g/kg ethanol groups following generalization tests with the NMDA antagonist memantine. Analysis of saccharin intake during memantine generalization tests for the animals trained with 1.0 g/kg ethanol yielded a significant Group by Dose interaction, $F(4, 52) = 2.53$, $p < .05$. Simple effects tests for generalization dose for the Ethanol-LiCl group was significant $F(4, 52) = 23.6$, $p < .001$. Simple comparisons showed that saccharin intake following administration of 10 and 15 mg/kg of memantine was significantly less than saccharin intake following saline (NPD), 2, and 5 mg/kg doses of memantine ($p < .05$). Tests of simple effects for the Ethanol-Saline group across memantine generalization tests doses was nonsignificant, $F(4, 52) = 2.47$, $p > .059$.

Results of the omnibus ANOVA for memantine generalization for the 1.5 g/kg ethanol training doses showed a significant Group by Dose interaction, $F(4, 44) = 12.66$, $p < .001$. Simple effects tests for the Ethanol-LiCl group was significant $F(4, 44) = 47.43$, $p < .05$. Simple comparisons showed that the 10 and 15 mg/kg doses of memantine produced a significant decrease in saccharin intake compared to the 0 (NPD) dose ($p < .05$). Simple effects tests for the

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Ethanol-Saline group revealed a nonsignificant results, $F(4, 44) = 0.74$, $p>.05$, suggesting that saccharin intake did not differ across memantine generalization doses.

The AMPA receptor antagonist GYKI-52466 was used to determine whether specific glutamatergic receptor subtypes differ in their generalizability to ethanol (see figure 21). The two-way ANOVA for the animals trained with 1.0 g/kg ethanol was nonsignificant, $F(3, 39) = 1.51$, $p<.226$. Similarly, the Ethanol-LiCl group animals trained with 1.5 g/kg ethanol failed to show ethanol-like behaviours following GYKI-52466 injections, $F(3, 33) = 2.01$, $p<.131$.

Figure 19. Mean saccharin intake after injections of several doses of

pentobarbital for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open

circles) groups across the 1.0 g/kg (top panel) and 1.5 g/kg (bottom panel)

ethanol-training groups. Saline was injected on non-pairing days (NPD). Vertical

lines represent S.E.M.
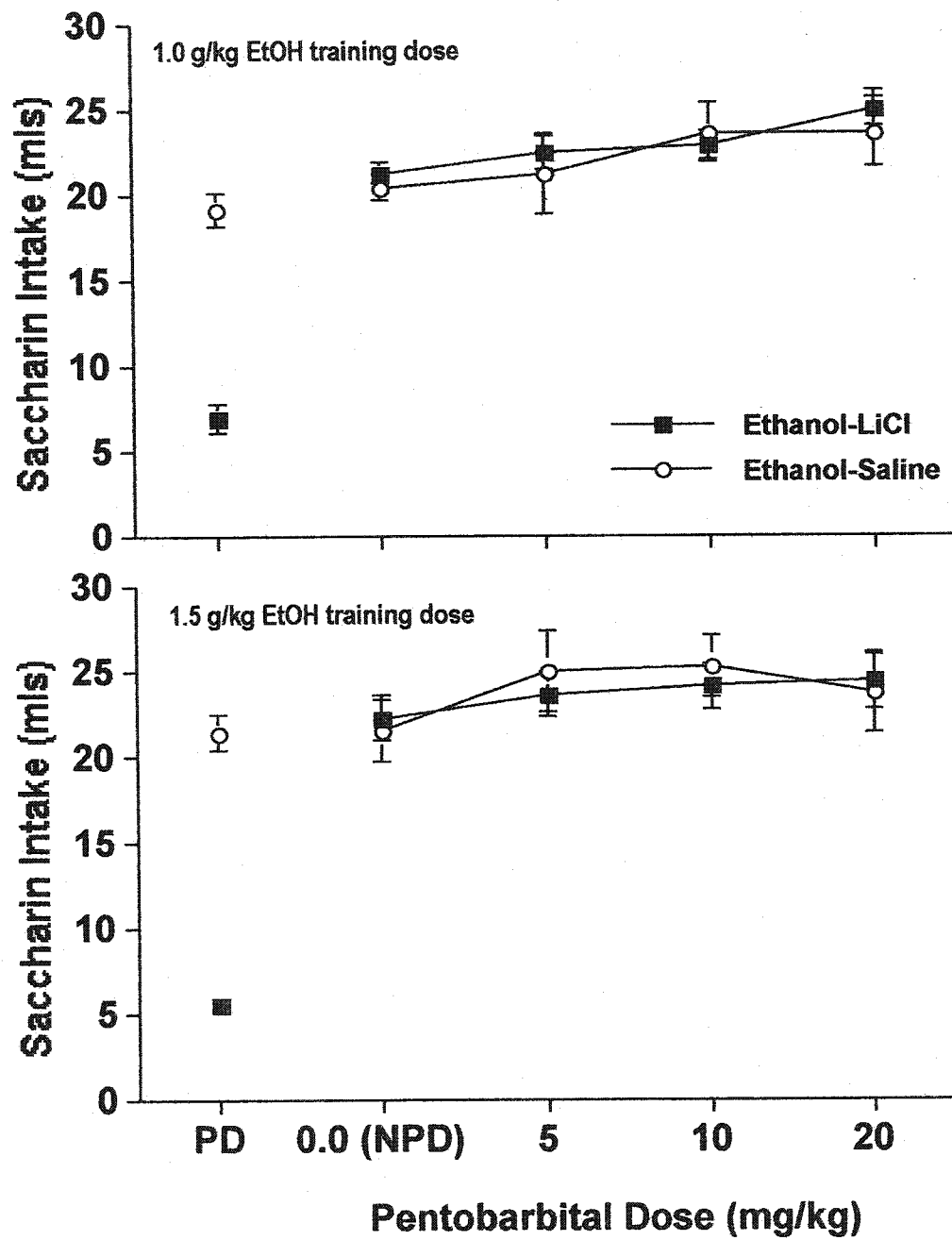
Figure 20. Mean saccharin intake after injections of several doses of memantine

hydrochloride for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open

circles) groups across the 1.0 g/kg (top panel) and 1.5 g/kg (bottom panel)

ethanol-training groups. Mean saccharin intake for the four ethanol pairing days

(PD) and for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open squares)

during generalization cycles are presented as unconnected symbols for

comparison. Saline was injected on non-pairing days (NPD). Vertical lines

represent S.E.M. The symbol * denotes a significant difference ($p < .05$) between

the ethanol generalization dose and the saline control dose (NPD).
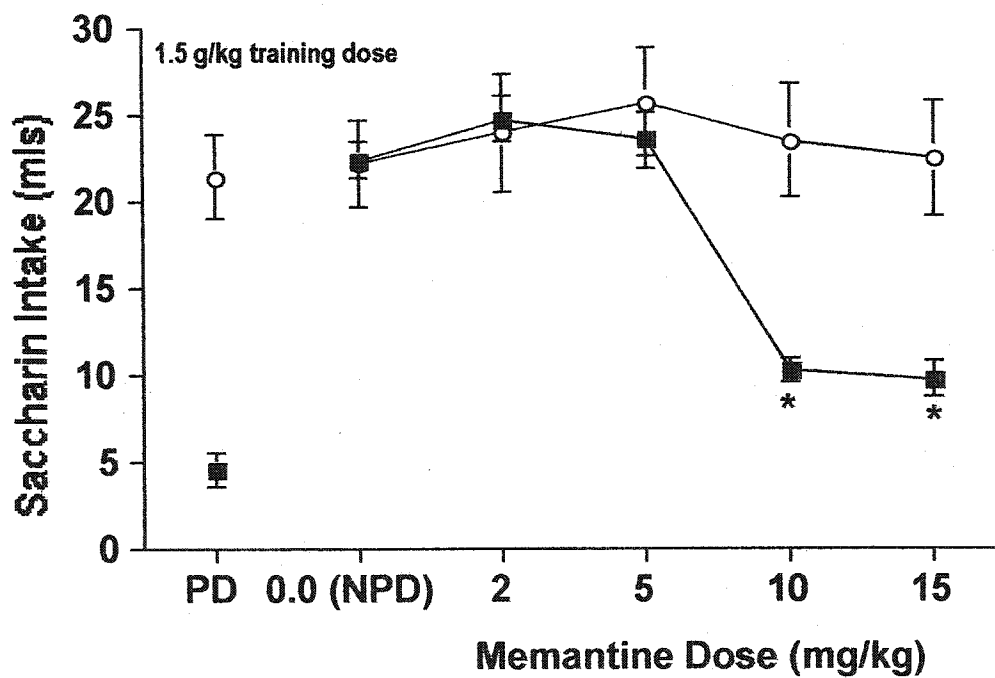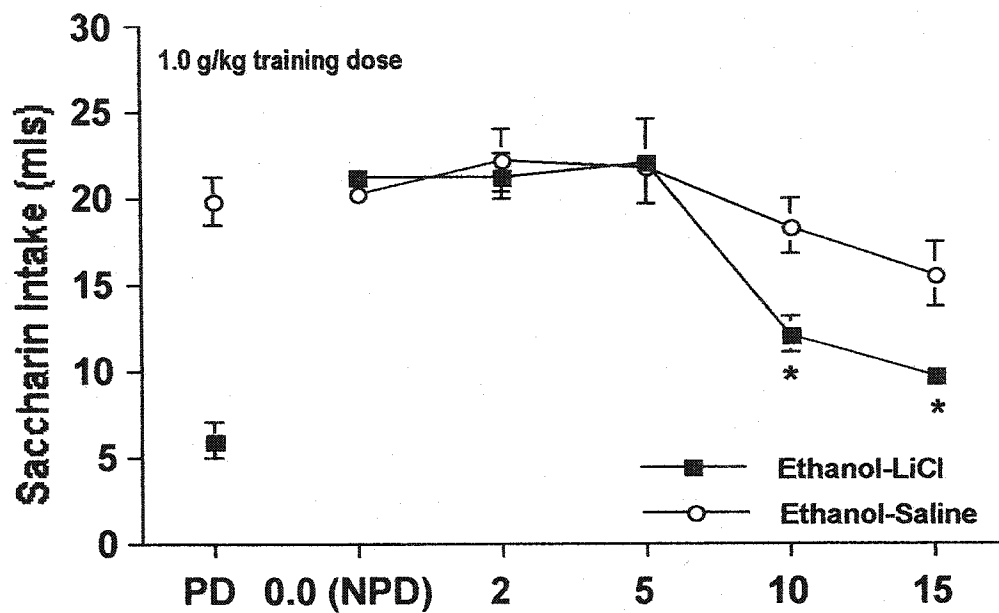
Figure 21. Mean saccharin intake after injections of several doses of GYKI

52466 for the Ethanol-LiCl (filled squares) and Ethanol-Saline (open circles)

groups across the 1.0 g/kg (top panel) and 1.5 g/kg (bottom panel) ethanol-

training groups. Saline was injected on non-pairing days (NPD). Vertical lines

represent S.E.M. The symbol * denotes a significant difference ($p<.05$) between
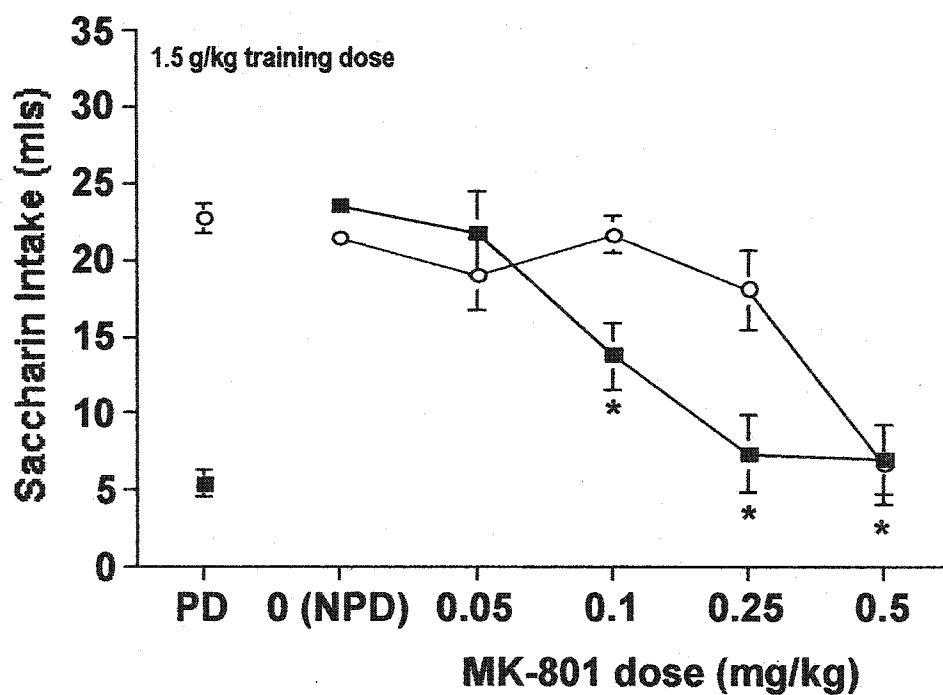
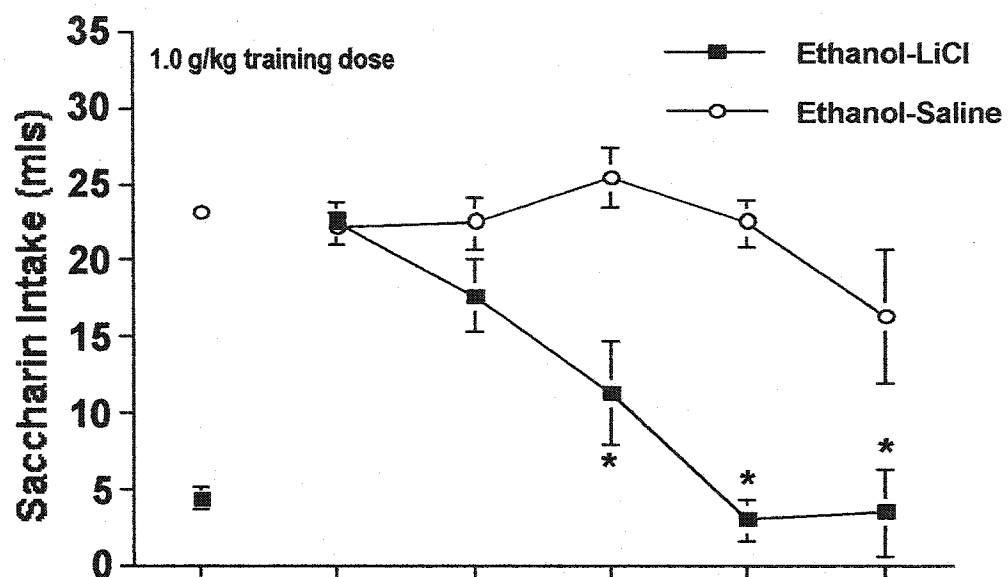the Ethanol-LiCl and Ethanol-Saline groups.
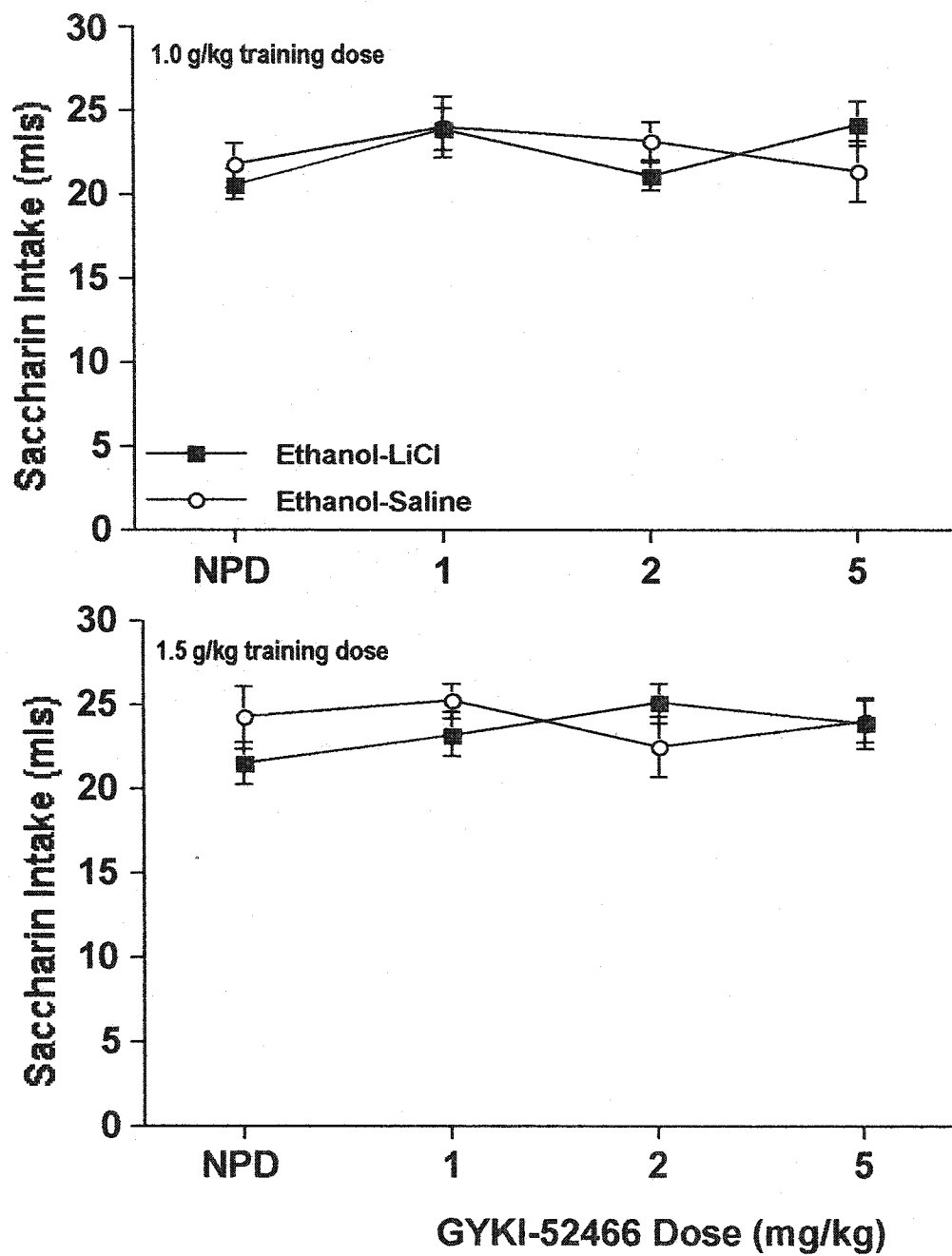
<u>Discussion</u>

The present study was designed to assess whether the excitatory amino acid glutamate plays a role in mediating the ethanol stimulus cue using the discriminative taste aversion procedure. The acquisition of an ethanol DTA was successful for the 1.0 and 1.5 g/kg Ethanol-LiCl groups in both group 1 and group 2 animals. There was however a possible problem among the first sample of animals (group 1) tested. Animals trained with 0.5 g/kg failed to either maintain discriminative control of saccharin intake or to failed to meet discriminative control criteria. Two animals in the 0.5 g/kg Ethanol-LiCl group had reached criterion but failed to consistently maintain discriminative control after 20 cycles. The rest of the animals in this group (n=6) failed to meet criterion. These Ethanol-LiCl animals displayed inconsistent saccharin consumption on PDs. That is, the degree of decrease in saccharin intake on PDs ranged from about 40 to 70% when compared to the three following NPDs. Due to this lack of discriminative control of saccharin intake, animals in the 0.5 g/kg group were not given generalization tests. On the other hand all group 1 animals in the 1.0 and 1.5 g/kg Ethanol-LiCl groups met discriminative control criterion and received generalization tests to ethanol and the noncompetitive NMDA antagonist MK-801. Group 1 animals in both the 1.0 and 1.5 g/kg Ethanol-LiCl groups however, also became inconsistent in maintaining discriminative control sometime during or after completion of the MK-801 generalization tests. That is, these group 1 animals failed to maintain discriminative control even after 20 to 25 cycles and therefore further

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generalization tests could not be conducted. In order to test other glutamatergic

acting drugs a second group of animals were trained to discriminate ethanol from

saline. Group 2 Ethanol-LiCl animals trained with 1.0 and 1.5 g/kg ethanol met

discrimination criteria after 11 and 9 cycles respectively and maintained

discriminative control throughout the entire study.

Generalization tests with several doses of ethanol showed a dose-

dependent substitution for ethanol. Group 1 Ethanol-LiCl animals trained with

1.0 g/kg ethanol fully substituted for ethanol following injections of 0.5, 1.0 and

1.5 g/kg ethanol. More specifically, ethanol generalization tests produced

decreases of 93, 114, and 127% following administration of 0.5, 1.0 and 1.5 g/kg

respectively. Similarly, group 1 Ethanol-LiCl animals trained with 1.5 g/kg fully

substituted for ethanol at the 1.0 and 1.5 g/kg ethanol test doses with decreases

of 80 and 103% respectively. Animals in the second sample had similar pattern

of decreases in saccharin intake following ethanol test doses as seen in figure

16. Neither the 1.0 g/kg nor the 1.5 g/kg Ethanol-LiCl group generalized to a

0.25 g/kg ethanol dose suggesting that this dose was below the threshold for the

detection of ethanol stimulus properties. Animals in the Ethanol-Saline groups,

trained with either dose of ethanol, did not show any change in saccharin intake

following ethanol generalization tests suggesting that these doses of ethanol did

not produce unconditioned effects that would decrease saccharin consumption.

The $GABA_A$ agonist pentobarbital was tested again in order to replicate

the findings of experiment 2. Data from the pentobarbital generalization tests

showed that pentobarbital failed to substitute for ethanol in either the 1.0 or 1.5

g/kg Ethanol-LiCl group. Animals in the Ethanol-LiCl groups consumed the same amount of saccharin following administration of 5, 10 and 20 mg/kg pentobarbital as they did following saline injections suggesting that pentobarbital does not possess similar stimulus effects as ethanol. This finding replicated the results from experiment 2 that pentobarbital failed to substitute for ethanol using the DTA procedure. It was hypothesized that the failure of pentobarbital to substitute for ethanol in experiment 2 may have been due to a shift in the contribution of the GABA component with increased experience with ethanol. In experiment 2 pentobarbital was the second to last drug tested among the generalization drugs used and at least 13 generalization test cycles passed before pentobarbital was examined. To exclude the possibility that the discriminative cue of ethanol changed with experience to the DTA procedure pentobarbital was tested earlier (second drug tested) in experiment 3. Pentobarbital again failed to substitute for ethanol in experiment 3 supporting the findings of experiment 2 that the $GABA_A$ receptor does not contribute significantly to ethanol stimulus effects of ethanol. In addition, the fact that pentobarbital failed to substitute for ethanol whether tested during the early or late stages of the DTA procedure suggests that the discriminative cue of ethanol does not change with increased experience.

Several researchers have previously shown that NMDA antagonists readily generalize to the ethanol stimulus cue (Bowen & Grant, 1999; Bowen et al., 1997; Grant & Colombo, 1993). The finding that NMDA antagonists substitute for ethanol was extended in the present thesis. The NMDA antagonists MK-801 and memantine both substituted for ethanol. MK-801 doses

of 0.1, 0.25 and 0.5 mg/kg were found to significantly decrease saccharin intake for the 1.0 and 1.5 g/kg Ethanol-LiCl groups in the first sample of animals. The dose of 0.1 mg/kg partially substituted for ethanol producing decreases of 61.42 and 53.00% for animals in the 1.0 and 1.5 g/kg Ethanol-LiCl groups respectively. Similar to the findings of group 1 animals, MK-801 doses of 0.1 and 0.25 mg/kg produced partial and full substitution respectively in animals trained with 1.0 and 1.5 g/kg ethanol. Animals in the Ethanol-Saline groups (group 1) displayed a significant decrease in saccharin intake following only the 0.5 mg/kg MK-801 dose suggesting that this dose of MK-801 may have produced some type of unconditioned effect that resulted in decreased fluid consumption in animals.

The NMDA antagonist memantine also produced ethanol-like behaviours during generalization tests. Animals in the Ethanol-LiCl group of the second sample, trained either with 1.0 or 1.5 g/kg ethanol, partially substituted for ethanol following administration of 10 and 15 mg/kg, but not 2 and 5 mg/kg of memantine. Saccharin intake was not affected by memantine injections in either of the Ethanol-Saline groups suggesting that memantine does not possess nonspecific effects. These findings extend previous findings that the noncompetitive NMDA antagonist memantine substitutes for ethanol (Hundt et al., 1998).

Generalization tests using the AMPA receptor antagonist GYKI-52466 failed to substitute for ethanol. Saccharin intake was not altered for either the Ethanol-LiCl or Ethanol-Saline groups after injections of several doses of GYKI-52466. The failure of GYKI-52466 to substitute for ethanol in the present study

supports a previous finding that demonstrated that the stimulus effects of ethanol are not mediated by the AMPA receptor (Hundt et al., 1998). Taken together, the findings of the present experiment provides further evidence that inhibition of the NMDA, but not the AMPA receptor contributes to the stimulus effects of ethanol.

General Discussion

The present thesis was designed to assess whether acetaldehyde, $GABA_A$ agonists and NMDA antagonists play a role in the mediation of the discriminative stimulus effects of ethanol using a discriminative taste aversion (DTA) procedure. In a series of DTA studies animals were trained to discriminate a training drug, ethanol or acetaldehyde, from saline. Through the DTA procedure two groups of animals were trained - a lithium-chloride-group (LiCl group) and a Saline group. Animals in the Ethanol-LiCl and the Acetaldehyde-LiCl (Acet-LiCl) group were trained to associate the stimulus effects of ethanol or acetaldehyde with the "illness" produced by a saccharin-LiCl pairing (e.g. Schafe & Bernstein, 1996). These animals learned that the stimulus effects of ethanol or acetaldehyde predicted an upcoming aversive event, the illness produced by LiCl and associated with the saccharin solution. Animals in the LiCl groups learned to refrain from drinking the saccharin solution when the stimulus effects of ethanol or acetaldehyde were present but to drink readily when they were not. The Ethanol-Saline and Acetaldehyde-Saline (Acet-Saline) groups were trained to associate the stimulus effects of ethanol and acetaldehyde with a saccharin-saline pairing. These groups were used as a control group since saline injections administered after saccharin consumption did not produce aversive effects. Thus, the stimulus effects of ethanol and acetaldehyde did not serve as a predictive cue to guide their drinking behaviour. As such, animals in these groups should readily consume saccharin following ethanol or acetaldehyde administration.

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Data obtained from the present series of DTA studies demonstrated that animals in the Ethanol-LiCl and Acet-LiCl groups decreased saccharin intake following ethanol and acetaldehyde injections respectively, but not after saline injections. This finding suggested that the stimulus effects of the training drug were able to exert discriminative control on saccharin consumption. On the other hand, animals in the Ethanol-Saline and Acet-Saline groups did not vary in their drinking pattern after injections of either ethanol or acetaldehyde. That fact that ethanol and acetaldehyde injections did not produce decreases in saccharin intake suggests that the training doses chosen for these drugs did not produce unconditioned effects that would in themselves decrease drinking behaviour.

Training animals to discriminate ethanol from saline was achieved in a shorter time frame using the DTA procedure compared to operant drug discrimination studies, particularly with the lowest dose. Animals trained with 1.0 g/kg ethanol reached criterion in 12 cycles, or 48 days, a number comparable to operant studies (e.g. Bienkowski et al., 1998). Experiment 2 however showed that animals trained with 0.5 g/kg ethanol acquired discriminative control in 14 cycles or 56 sessions when nonpairing days were included. This time frame is significantly shorter than the 100 plus sessions needed to achieve stimulus control for 0.5 g/kg ethanol using an operant drug discrimination procedure (Stefanski et al., 1996). Overall, the present thesis clearly demonstrated that the DTA procedure could be successfully used to quickly train animals to discriminate ethanol and acetaldehyde from saline.

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Generalization tests were used to identify common mechanisms of action between a test drug and the training drug and were conducted once animals in the Ethanol-LiCl and Acet-LiCl groups acquired stable discriminative stimulus control. Test drugs were administered in order to assess if they produced stimulus effects similar to either ethanol or acetaldehyde. If test drugs produced similar stimulus effects to those of the training drug then the animal should avoid consuming saccharin. That is, the test drug is said to substitute for the training drug. On the other hand, if the stimulus effects of the test drug and the training drug do not overlap, then the animal should consume saccharin. Generalization tests were conducted with acetaldehyde and drugs acting on the GABAergic and glutamatergic neurotransmitter systems. Overall, generalization tests showed that acetaldehyde partially substituted while the NMDA antagonists MK-801 and memantine completely substituted for ethanol suggesting that acetaldehyde as well as NMDA antagonists contribute to the stimulus effects of ethanol. Generalization tests with the $GABA_A$ agonists pentobarbital and THIP, as well as the AMPA antagonist GYKI 52466 failed to substitute for ethanol suggesting that activation of the $GABA_A$ receptor or inhibition of the AMPA receptor do not produce similar stimulus effects as ethanol when using the discriminative taste aversion procedure. Taken together these generalization results suggest that $GABA_A$ and $GABA_B$ agonists, as well as AMPA antagonists do not mediate the discriminative cue of ethanol, at least the cues involved in the DTA procedure.

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Contribution of acetaldehyde to the stimulus effects of ethanol

Experiment 1a trained animals to discriminate ethanol from saline and then tested several doses of acetaldehyde, the primary metabolite of ethanol, for its ability to substitute for ethanol. Data from acetaldehyde generalization tests showed that animals in both the Ethanol-LiCl and Ethanol-Saline groups decreased their saccharin intake following administration of 0.2 and 0.3 g/kg of acetaldehyde compared to saline nonpairing days (NPDs). There was no significant difference between the Ethanol-LiCl and Ethanol-Saline groups in the percent decrease in saccharin intake following 0.2 g/kg acetaldehyde generalization test. There was however, a significant difference in saccharin intake between the two conditioning groups following administration of 0.3 g/kg acetaldehyde. The fact that saccharin intake was decreased in the Ethanol-Saline group suggests that 0.3 g/kg acetaldehyde produced some unconditioned effects that affected fluid consumption. It is thus likely that some of the decrease in saccharin intake observed in the Ethanol-LiCl animals may be due to these same unconditioned effects. Moreover, the remaining decrease in saccharin intake could be attributed to similar stimulus effects between acetaldehyde and ethanol. Therefore, the greater decrease in saccharin intake for the Ethanol-LiCl group may be due to a partial generalization of acetaldehyde to the stimulus effects of ethanol.

The finding that acetaldehyde shares some stimulus properties with ethanol is in line with reports that acetaldehyde does generalize to the stimulus effects of ethanol in animals trained to discriminate ethanol from saline when

tested in a T-maze discrimination procedure (York, 1978, 1981). The partial

substitution of acetaldehyde for ethanol is however contrary to a recent study that

examined whether acetaldehyde contributed to the stimulus effects of ethanol

using an operant drug discrimination procedure (Quertemont & Grant, 2002). In

this study animals were trained to discriminate 1.0 or 2.0 g/kg ethanol,

administered intragastrically, from saline and then tested with a several doses of

acetaldehyde (0-0.3 g/kg, ip). Generalization tests showed that acetaldehyde

failed to substitute for ethanol in both the 1.0 or 2.0 g/kg ethanol training dose

group. These authors argued that acetaldehyde did not produce discriminative

stimulus effects similar to ethanol and thus does not play a role in mediating the

stimulus effects of ethanol.

Quertemont & Grant (2002) argued that one reason for the discrepancy

between the findings of a partial substitution of acetaldehyde for ethanol in the

present thesis and the lack of substitution of acetaldehyde in their study may be

due to the different discrimination procedures used. These researchers reported

that animals significantly decreased bar pressing following 0.3 g/kg acetaldehyde

injections suggesting that this dose of acetaldehyde may have had motoric or

aversive effects that interfered with that specific operant behaviour.

Acetaldehyde (0.3 g/kg) decreased saccharin intake in Ethanol-Saline animals

but did not eliminate it completely suggesting that the operant procedure may be

more sensitive to unconditioned effects than the DTA procedure. Thus, the

greater sensitivity of the operant procedure, relative to the DTA procedure, to the

unconditioned effects may have prevented these authors from detecting substitution effects of acetaldehyde that were seen in the DTA procedure.

Quertemont and Grant (2002) offered two other explanations for the discrepancy in findings between the two studies. These authors argued that animals in the DTA study had about 3 to 4 ethanol training sessions in which they reliably discriminated ethanol from saline while in their operant study animals had 14 to 18 sessions experience with ethanol. Thus, animals in the DTA study may not have tolerated to the aversive effects of ethanol and the stimulus effects were thus based on the aversive properties of ethanol whereas animals in the operant study based the ethanol discrimination on other properties of ethanol. Although Ethanol-LiCl animals had fewer sessions in which they displayed discriminative stimulus control this group did receive 11 ethanol administrations. Over the 11 pairing days it was observed that animals tolerated to the sedative effects of ethanol. Although not measured in any manner, animals showed depressed motor activity following the first ethanol injection, but not after the last ethanol injection. Therefore, it is likely that if the depressant effects of ethanol had tolerated after 11 injections other aversive properties may have also been tolerated.

A second explanation for the discrepancy between these studies may involve the route of administration. Quertemont and Grant (2002) had administered ethanol intragastrically while it was injected intraperitoneally in the present thesis. It is possible that the partial generalization of acetaldehyde to ethanol seen in experiment 1a may be due to similar peripheral effects of both

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drugs rather than central stimulus effects. That is, both ethanol and acetaldehyde injected ip may produce peripheral effects (e.g. abdominal irritation) specifically associated with this route of administration (ip). Quertemont and Grant (2002) argued that since intragastrically administered ethanol may control for these peripheral cues, they may not have been part of the ethanol stimulus effects and as such acetaldehyde would not have substituted to intragastrically administered ethanol. A DTA study that administered ethanol intragastrically would be necessary to clarify this position.

Data obtained from experiment 1a showed that pre-treatment with aminotriazole failed to block the discriminative stimulus effects of ethanol. Rats pre-treated with aminotriazole and subsequently injected with ethanol decreased their saccharin intake compared to days when saline injections were administered. This finding suggests that the production of central acetaldehyde via catalase may not be a major component of the ethanol stimulus cue as measured in the DTA procedure. Although acetaldehyde was able to partially substitute for ethanol, the blockade of central acetaldehyde production was not enough to substantially alter the stimulus effects of ethanol. The compound nature of the ethanol stimulus cue may account for the failure of AT to block the ethanol cue. That is, other components of the ethanol stimulus cue are still available for the animal to make a decision about whether ethanol was administered or not.

The findings of experiment 1b showed that ethanol substituted for acetaldehyde in animals trained to discriminate acetaldehyde from saline

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suggesting that ethanol and acetaldehyde share stimulus properties. The symmetrical finding that acetaldehyde and ethanol substitute for ethanol and acetaldehyde respectively support the notion and acetaldehyde mediates some of the stimulus properties of ethanol. In conclusion, acetaldehyde has been shown to contribute to the stimulus effects of ethanol as examined by the DTA procedure.

Contribution of the GABA system to the stimulus effects of ethanol

The findings of experiment 2 demonstrated that the $GABA_A$ receptor agonists pentobarbital and THIP failed to substitute for the stimulus effects of ethanol. The failure of pentobarbital to substitute for ethanol is contrary to the majority of studies that have reported that administration of pentobarbital produced complete or partial generalization to an ethanol cue (Bowen & Grant, 1998; Grant et al, 1996, 1997; Hodge & Cox, 1998; Hodge et al., 2001a, 2001b; Shelton & Balster, 1994; Shelton & Grant, 2002). The most obvious explanation for the lack of substitution by $GABA_A$ agonists in the present thesis is based on the difference in methodological procedures between the DTA and operant drug discrimination procedures. In the DTA procedure ethanol predicts an aversive event and requires animals to avoid consuming a saccharin solution whenever the stimulus effects of ethanol are present. On the other hand, animals in operant procedures are trained to approach and press one of two levers in order to receive a reinforcer when ethanol is administered. Therefore, the demand characteristics of the DTA and operant discrimination procedures may be

sufficiently different that animals may attend to different aspects of the discriminative cue of ethanol depending on the procedure used. If animals attend to different components of the stimulus effects of ethanol in the DTA procedure, relative to the operant procedure, it is conceivable that the DTA procedure may recruit different neurotransmitter systems and/or brain structures than the operant procedures. Therefore, the lack of substitution for pentobarbital and THIP suggests that the GABAergic system does not mediate the stimulus effects of ethanol when examined with the DTA procedure, but does when examined with an operant procedure.

Antagonism tests with the GABA$_A$ receptor antagonist picrotoxin failed to block the stimulus effects of 0.5 and 1.0 g/kg ethanol. Pre-treatment with picrotoxin failed to alter the stimulus effects of ethanol in Ethanol-LiCl animals trained with 0.5 and 1.0 g/kg ethanol. That is, animals still avoided the saccharin solution following ethanol injections even though the contribution of the GABA$_A$ receptor had been removed. This finding is in line with previous studies that have demonstrated a failure of GABA$_A$ antagonists to block the stimulus cue of ethanol (Bienkowski & Kostowski, 1997; Hiltunen & Jarbe, 1988; Hodge & Aiken, 1996). The failure of a GABA$_A$ antagonist to completely antagonize the stimulus effects of ethanol is not unexpected due to the heterogeneous nature of the ethanol discriminative cue. Without the GABAergic component animals would still have other components (i.e. glutamatergic, serotonergic) on which the ethanol discrimination can be based. Antagonism tests with 1.0 mg/kg picrotoxin, but not 2.0 mg/kg picrotoxin, was shown to partially block the stimulus

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effects of ethanol only for Ethanol-LiCl animals trained with 1.5 g/kg ethanol. It is

unclear why the low dose of picrotoxin partially blocked the stimulus effects of

ethanol for the 1.5 g/kg Ethanol-LiCl animals given the fact that $GABA_A$ agonists

failed to substitute for ethanol. The fact that picrotoxin partially blocked the

stimulus effects of ethanol may suggest that the $GABA_A$ receptor may contribute

somewhat to the discriminative cue of a dose of 1.5 g/kg ethanol. It is just as

likely however, that this result may have been due to some artifact or confound in

these particular animals. Retesting the effects of picrotoxin on antagonizing the

stimulus effects of 1.5 g/kg ethanol would be needed to confirm or refute the

present findings.

In conclusion, the results of generalization tests showed that the $GABA_A$

agonists pentobarbital and THIP failed to substitute for ethanol. Thus, it can be

concluded that the $GABA_A$ receptor does not mediate the stimulus effects of

ethanol when using the DTA procedure. The failure of the $GABA_B$ agonist

baclofen to substitute for ethanol demonstrates that the $GABA_B$ receptor also

does not mediate the stimulus effects of ethanol. Taken together, it can be

concluded that activation of the GABAergic neurotransmitter system does not

mediate ethanol's discriminative cue as determined by the DTA procedure. The

disparate findings between the present thesis and operant procedures in regards

to the involvement of the GABAergic system may stem from different demand

characteristics. The DTA procedure may involve those components of the

ethanol cue that are associated with predicting aversive events while that operant

procedure may involve components associated with positive reinforcers. In this

context it may be important to note that this may be the first study reporting the dissociation of the separate stimulus effects of ethanol associated with two different drug discrimination procedures.

Contribution of the glutamatergic system to the stimulus effects of ethanol

Inhibition of the glutamatergic neurotransmitter system, more specifically the NMDA receptor subtype, has been reported to mediate the discriminative cue of ethanol (Bowen & Grant, 1999; Grant et al., 1991, 1992; Grant & Colombo, 1993; Shelton & Balster, 1994). Experiment 3 examined whether administration of NMDA and/or AMPA receptor antagonists produce stimulus effects similar to that of ethanol. The findings of experiment 3 demonstrated that inhibition of NMDA receptor activity substituted for ethanol. More specifically, NMDA antagonists substituted for ethanol in rats trained to discriminate 1.0 and 1.5 g/kg ethanol. The NMDA antagonist MK-801 completely partially substituted for ethanol after administration of the 0.1 mg/kg dose and fully substituted for ethanol after administration of the 0.25 and 0.5 mg/kg doses for group 1 Ethanol-LiCl animals trained with 1.0 and 1.5 g/kg ethanol. Similar results were observed for group 2 animals: the 0.1 mg/kg MK-801 produced partial substitution while 0.25 mg/kg MK-801 produced full substitution. The present finding that MK-801 fully substituted for ethanol have also been reported in operant drug discrimination studies (Bowen & Grant, 1999; Hundt et al., 1998; Grant & Colombo, 1992; Green & Grant, 1998). Generalization tests with the NMDA

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antagonist memantine produced partial substitution to ethanol in animals trained with 1.0 and 1.5 g/kg ethanol. These findings are in line with a previous study that showed that memantine completely substituted for ethanol (Bienkowski et al., 1998; Hundt et al., 1998). Finally, generalization tests with the AMPA receptor GYKI-52466 failed to substitute for ethanol in animals trained with 1.0 and 1.5 g/kg ethanol. This finding is in line with a previous study that showed GYKI-52466 failed to substitute for 1.0 g/kg ethanol (Hundt et al., 1998).

Overall, the findings of experiment 3 supports the majority of the operant drug discrimination studies which demonstrated that substitution to ethanol occurred with NMDA receptor antagonists, but not AMPA receptor antagonists (Bowen & Grant, 1999; Green & Grant, 1998; Hodge et al., 1998, 2001). The fact that inhibition of NMDA receptors, but not AMPA receptors, was observed using both the DTA and operant discrimination procedures suggests that the NMDA receptor plays an important role in mediating the stimulus effects of ethanol whether tested with the DTA procedure or an operant discrimination procedure.

Overshadowing and the heterogeneous ethanol cue.

Although the demand characteristics of the DTA procedure may account for the failure of $GABA_A$ agonists to substitute for ethanol, an alternative explanation may involve the overshadowing of the GABA component by the NMDA component. One aspect of a mixed compound like ethanol is that the separate components do not necessarily contribute equally to the stimulus

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effects (Grant, 1999). In other words, the stimulus effects of one component usually contribute significantly more, or overshadow the stimulus effects of other components (Green & Grant, 1998; Mariathasan et al., 1999). That is, a relatively weak component may not be detected because a more dominant component overshadows its contribution. Several researchers have reported that the relative contribution of each component of the ethanol cue change depending on the dose of ethanol used to train discriminative control (Grant, 1999; Grant & Colombo, 1993; Green & Grant, 1998). Green and Grant (1998) trained animals to discriminate either 1.0 g/kg or 2.0 g/kg ethanol from saline and then conducted generalization tests with the $GABA_A$ agonist pentobarbital, the serotonin agonist TFMPP and the NMDA antagonist MK-801. These researchers showed that pentobarbital and TFMPP substituted for ethanol at lower doses when animals trained with 1.0 g/kg and 2.0 g/kg were compared. That is, the dose response curve for both pentobarbital and TFMPP was shifted to the right when the training dose of ethanol was increased. There was no change in the dose-response curve for MK-801 substitution demonstrating that the contribution of the glutamatergic system remained the same at increasing doses of ethanol. The authors argued that the $GABA_A$ and 5-HT components were overshadowed by the NMDA component within the context of a higher ethanol-training dose.

The results of the present DTA studies showed that pentobarbital failed to generalize to the stimulus effects of 0.5, 1.0 and 1.5 g/kg ethanol while NMDA antagonists substituted for 1.0 and 1.5 g/kg ethanol. It is possible that the failure of pentobarbital to substitute for ethanol is the result of overshadowing by the

NMDA component. That is, the DTA procedure may have somehow changed the relative contribution of the separate components , compared to operant drug discrimination procedures, such that the $GABA_A$ component became relatively less important in mediating the stimulus effects of ethanol than the NMDA component. AS a result the failure of $GABA_A$ agonists to substitute for ethanol may be due to overshadowing by the NMDA receptor. This explanation however is based on findings that $GABA_A$ agonists substitute for ethanol using operant discrimination procedures. An overshadowing explanation for the lack of substitution of pentobarbital would only be plausible if pentobarbital substituted for ethanol in the DTA procedure. The fact that pentobarbital failed to substitute for ethanol suggests that the GABAergic system does not contribute to the stimulus effects of ethanol in the DTA procedure rather than being overshadowed by the NMDA component.

Conclusions and future directions

The present thesis examined the roles of the primary metabolite of ethanol, acetaldehyde, $GABA_A$ agonists and NMDA antagonists in mediating the stimulus effects of ethanol using the discriminative taste aversion procedure because previous operant drug discrimination studies have implicated these compounds in mediating the discriminative cue of ethanol (Bowen & Grant, 1999; Grant et al., 1991, 1992; Grant & Colombo, 1993; Redila et al., 2000, 2002; Shelton & Balster, 1994; York, 1978, 1981). The results of the present thesis clearly demonstrated that ethanol could be successfully used as a training drug

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when the DTA procedure is used. Generalization tests showed that acetaldehyde partially substituted, NMDA antagonists fully substituted while $GABA_A$ agonists failed to substitute for ethanol. The finding that MK-801 and memantine substituted for ethanol is consistent with operant drug discrimination studies that implicate inhibition of the NMDA receptor in contributing to the stimulus effects of ethanol (Bowen & Grant, 1999; Hundt et al., 1998; Grant & Colombo, 1992). On the other hand, the findings that $GABA_A$ agonist failed to substitute and acetaldehyde partially substituted for ethanol are at odds with previous studies that have shown that $GABA_A$ agonists fully substituted, and acetaldehyde did not substitute, for ethanol (Bowen & Grant, 1998; Grant et al, 1996, 1997; Hodge et al., 2001; Quertemont & Grant, 2002). Thus, the findings of the present thesis showed that the glutamatergic neurotransmitter, as well as the proximate metabolite acetaldehyde, play a role in mediating the stimulus effects of ethanol while the GABAergic neurotransmitter system does not play a role in mediating the ethanol cue as tested with the discriminative taste aversion procedure.

The findings of the present thesis suggest that the methodological differences between the DTA and operant procedures change the demand characteristics in such a manner that the two paradigms are no longer equivalent. Clearly, the manner in which the animals are trained to discriminate ethanol from saline has a large impact on how the separate components of the ethanol cue are perceived. This may be the first study that shows that the type of drug discrimination procedure used may affect how animals attend to or how they

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utilize the separate components of the ethanol cue. Training animals to avoid a saccharin solution when ethanol is administered seems to involve at least an acetaldehyde and a glutamatergic component while training animals to receive a reinforcer following ethanol injections involves at least a GABAergic and glutamatergic component. It is unclear at this time whether animals trained with the DTA procedure based their discrimination on the aversive nature of the paradigm or whether animals trained in the operant procedures based their discrimination on the reinforcing nature of that design. However, given the nature of conditioned taste aversion studies and the speed at which they are learned, it would be reasonable to assume that, as a minimum, conditioned taste cues may play a differential role in the two paradigms. It would seem on the surface that this may be a possibility but future studies would be needed to clarify this question.

In addition to defining what differences between the DTA procedure and operant procedure are responsible for the different outcomes, future DTA studies should follow the lead of operant studies. That is, discriminative taste aversion studies should examine the effect of intracranial microinjections in specific brain sites, the effects of particular brain lesions on substitution as well as the role of other neurotransmitter systems such as the serotonergic, dopaminergic and opioidergic systems. In summary, the DTA procedure has proven to be a good alternative to the operant procedure in studying the stimulus effects of ethanol. The DTA procedure has demonstrated the ability to detect separate components of the compound cue of ethanol and in that way, to underscore the fact that a

variety of cues participate in helping animals to recognize ethanol. Moreover, the DTA procedure was able to differentiate between different receptor subtypes, (NMDA vs. AMPA) in mediating the stimulus effects of ethanol. Taken together, the discriminative taste aversion procedure can be useful in understanding the mechanisms involved in mediating the stimulus effects of ethanol.

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