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**The Role of Learning and Environmental Variables on the Acquisition of Ethanol
Self-Administration in Outbred Rodents**

Lana M. Pratt

A Thesis

In

The Department

Of

Psychology

Presented in Partial Fulfillment of the Requirements
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ABSTRACT

The role of learning and environmental variables on the acquisition of ethanol self-administration in outbred rodents.

Lana M. Pratt, Ph.D.
Concordia University, 2004

For several decades, the field of alcohol research has been grappling with a seemingly straightforward question: what factors contribute to or mediate the voluntary intake of a licit yet “addictive” substance such as alcohol? Nevertheless, the search for and description of the mechanisms underlying voluntary alcohol consumption has been plagued with difficulties, in part due to the multiplicity of factors believed to influence alcohol consumption. As alcohol use appears to be a multi-determined behavior, it is suggested that the variability underlying it is apt to be attributed to both pharmacological, as well as, non-pharmacological variables. The present dissertation was designed to assess the role of non-pharmacological variables in the acquisition of voluntary ethanol self-administration in unselected rodents. More specifically, we aimed to determine the role played by the learning ability of individual animals in influencing subsequent ethanol preference. Furthermore, we also wanted to evaluate the role of environmental variables in the acquisition of ethanol intake.

The results of Experiment 1 and Experiment 2 suggested that individual differences in learning ability, as assessed by performance in two different spatial tasks, might be related to the affinity to ingest ethanol. Specifically, it appears that individual differences in spatial ability and its components, such as spatial working memory are likely to be related to ethanol intake. The results of Experiment 3 demonstrated that lesions to the hippocampus, an area largely associated with spatial learning, disrupted the

acquisition of ethanol intake in both a limited and continuous access paradigm.

Experiment 4 demonstrated that superimposing an operant procedure for access to ethanol and providing animals with a distinct environment during acquisition, elevated ethanol consumption as compared to a standard voluntary intake home-cage procedure.

Experiment 5 showed that providing animals with a distinct environment and transport as part of the procedures used during ethanol acquisition increased ethanol intake. Taken together, the studies reported within this dissertation provide support for the role of non-pharmacological factors such as learning ability and environment, in the development of ethanol self-administration within outbred rodents. It is argued that in order to understand the tremendous variability inherent in ethanol self-administration both pharmacological and non-pharmacological factors must be considered.

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Over the years, I have made some incredible friends at Concordia and wish to thank them for their companionship, humor, and support. Although we get busy at times, it is great to know that we are always there for each other.

As a proud Native person, I want to acknowledge the support I have received over the years from my reserve, Gordon's. Part of my quest has always been to shatter the stereotypes of what Native people are capable of. If I have managed to make a small dent in the Native stereotype, then I have truly accomplished my goal.

My mother Anita, my siblings Shaun and Tanya, as well as my extended family have always encouraged me, and in some way made this journey with me.

To my future husband, Dr. Mark Ellenbogen, I wish success in his new endeavors as a Canada Research Chair. Graduate school gave me several things but one of the best is you. Thank you for all that you are, and all that you bring to my life.

In closing, this thesis is dedicated to the one person who has truly shared this experience with me, my son Tristan. You were my inspiration and always will be. Love you.

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Introduction

In today's society, the consumption of beverages containing alcohol is a socially, as well as, legally acceptable behavior notwithstanding individual beliefs and/or personal preferences. A recent population survey indicated that Canadians over the age of 12 consumed 13,475,000 standard drinks a week (Statistics Canada, 1999). Weekly consumption ranged from one to fourteen drinks, with the largest proportion of individuals (44%) consuming one to six drinks and the smallest proportion of individuals (11%) consuming 14 or more drinks weekly. These statistics seemingly reflect both the human proclivity for alcoholic beverages as well as the inherent variability within the spectrum of human alcohol consumption. Unfortunately, alcohol consumption while generally accepted also does result in drastic costs at both the individual and societal level (Altman, Everitt, Glautier, Markou, Nutt et al., 1996) thus, researchers have long attempted to understand the mechanisms underlying its use and abuse. Given the complexity and ethical concerns involved in studying human alcohol consumption, researchers resorted to animal models (Myers & Carey, 1961). These models could provide a practical method to identify and elucidate the factors that may contribute to the differential intake of alcohol with a greater degree of control over experimental variables.

Utilizing animal models of alcohol self-administration researchers have demonstrated that differences in ethanol preference or even ethanol intake in general, may be mediated in part by ethanol's effects on the central nervous system and on neurotransmitter systems which may be involved in the regulation of ethanol's psychopharmacological reinforcing properties (for reviews see Amit & Smith, 1992; Eckardt et al., 1998; Weiss & Porrino, 2002). Given the multiplicity of factors known to

interact and influence ethanol intake in humans (Altman et al., 1996) a parallel area of interest has evolved, aimed at delineating the contribution of behavioral variables in the regulation of ethanol intake. Although less is known about the role of behavioral variables in ethanol consumption, it is extremely likely that they are involved in its regulation, given the complexity and variability inherent in ethanol self-administration. In fact, previous work has suggested that factors other than those directly related to the pharmacological properties of ethanol may also contribute to individual differences in high ethanol intake (Gauvin et al., 1998; Boyle et al. 1994; Goodwin et al. 1996; 1998; Hill, 1978; Wise, 1974). Of particular interest was the finding that rats selectively bred for their presumed learning ability consumed and preferred ethanol in equal or greater quantity than strains selectively bred for high ethanol intake (Amit & Smith, 1992). The finding that rodents bred for factors seemingly unrelated to ethanol, also displayed a distinct pattern of high and low ethanol intake and preference, suggested the possibility that learning ability may be involved in the mediation of the acquisition of ethanol self-administration.

The present dissertation was designed to further assess the role of behavioral variables in the acquisition of voluntary ethanol self-administration in an unselected strain of rats. More specifically, we aimed to determine the role played by the learning ability of individual animals in influencing subsequent ethanol preference. Furthermore, we also wanted to evaluate the role of environmental variables in the acquisition of ethanol intake. Specifically, we wanted to examine the contribution of the type of ethanol exposure procedure(s) used on subsequent ethanol self-administration. As ethanol self-administration is likely to be mediated by multiple mechanisms, the first section of the

thesis presents a synopsis of the data supporting the involvement of several neurotransmitters and biological correlates in mediating the pharmacological or reinforcing effects of ethanol consumption. The next sections of the introduction discuss the preference paradigm and provide a brief overview of its use within the ethanol self-administration literature. The subsequent section will focus on the findings obtained from studies examining the role of behavioral variables and their relation to ethanol consumption. The learning literature as it relates to ethanol self-administration, as well as the brain structures believed to mediate these effects, are briefly reviewed. Reinforcement within the scope of this thesis is defined as a variable or event whose presentation or presence increases the probability of a response occurring in the future.

The search for the mechanisms underlying voluntary ethanol intake.

For several decades, the field of alcohol research has been grappling with a seemingly straightforward question: what factors contribute to or mediate the voluntary intake of a licit yet “addictive” substance such as alcohol? The search for and description of the mechanisms underlying voluntary alcohol consumption has been plagued with difficulties, in part due to the multiplicity of factors believed to influence alcohol consumption. Data from several laboratories have highlighted the fact that individual alcohol intake is a complex behavior seemingly influenced by the interactions of multifaceted psychosocial, environmental, genetic, and pharmacological factors (Altman et al., 1996; Cloninger, Bohman, & Sigvardsson, 1981; Goodwin, Schulsinger, Moller et al., 1974). As alcohol use appears to be a multi-determined behavior, it is suggested that the underlying variability is apt to be attributed to both pharmacological, as well as non-

pharmacological variables. Interestingly, the portion of the variance linked to individual differences in alcohol use and ostensibly related to pharmacological factors is complex and not yet fully understood. As stated by Weiss and Porrino (2002), alcohol's actions at "multiple receptors and neurochemical systems occurring at widespread neuroanatomical sites throughout the brain" pose unique challenges to our understanding of alcohol use and abuse.

Alcohol's pharmacologic profile makes it a uniquely interesting, yet problematic substance to study. First, there exists tremendous variability in the pharmacokinetics of alcohol in humans and animals (Eckardt et al., 1998). As a fat-soluble molecule, ethanol is quickly and evenly dispersed throughout the tissues of the body. Moreover, alcohol's rate of metabolism, distribution, and absorption by the body are affected by many factors such as food intake, percentage body fat, gender, prior drinking experience and even stomach loading or stomach content (Eckardt et al., 1998). Secondly, alcohol is known to interact with every neuronal system within the central nervous system (CNS) including dopamine, GABA, serotonin, glutamate, and the opioid receptors (Eckardt et al., 1998; Kostowski & Bienkowski, 1999; Weiss and Porrino, 2002). Ethanol's effect(s) on the brain's neurotransmitter systems likely mediates some of the behavioral and reinforcing effects associated with its abuse liability (Johnson & Ait-Daoud, 2000). The exact nature of these interactions remains a topic of debate but it is generally recognized that ethanol's effects on these multiple neurotransmitter systems appear to be primarily mediated through ligand-gated ion channels and voltage gated calcium channels (for a review see Eckardt et al., 1998). Thirdly, individual factors such as age, environment, genetic makeup, and gender are also posited to influence/modify ethanol's effect on drinking

behavior (Amit, Weiss, & Smith 2000; Babor et al., 1978; Crabbe et al., 1994; Goodwin et al., 1999).

Neurobiology Of Ethanol Reinforcement: Neurotransmitters and Neuromodulators

As drugs of abuse are commonly considered to be positive reinforcers (e.g. Amit and Smith, 1992; Koob, 1992; Wise and Bozarth, 1987) a large body of research has focused on identifying the neurobiological substrates mediating the reinforcing properties of addictive substances, such as alcohol. Thus, the next section provides an overview focused on our current understanding of the role of several proposed neural substrates underlying both the positive reinforcing properties of alcohol and alcohol seeking behavior.

Dopamine

There has been considerable research directed at further understanding the neural circuits underlying reinforcement and one area of focus has been the mesocorticolimbic dopamine (DA) pathway (for reviews see, Amit & Smith, 1992; Di Chiara, 1995; Robbins et al., 1989; Wise, 1982). The mesocorticolimbic DA system(s) originates in the ventral tegmental area (VTA) and is comprised of DA neurons and their projections to the nucleus accumbens (NAc), amygdala, prefrontal cortex (PFC), hippocampus, and other forebrain regions (Weiss & Porrino, 2002). Given ethanol's ability to act as a reinforcer (Amit & Stern, 1971; Samson, Pfeffer & Tolliver, 1988) dopamine transmission has also been thought to modulate a portion of ethanol's reinforcing effect (Di Chiara, 1997; Koob & Nestler, 1997). Historically, a drug's direct action at dopamine

synapses was taken as evidence for DA's role in mediating its reinforcing properties (Koob, 1992, Wise, 1986). Thus, researchers also sought to demonstrate ethanol's effect on dopamine transmission. Ethanol's ability to increase extracellular dopamine levels in the NAc (Di Chiara and Imperato, 1988) through actions on the VTA DA somata (Yim and Gonzales, 2000; Budygin et al., 2001), and on firing of VTA DA neurons (Gessa et al., 1985; Brodie et al., 1990), supported the notion of ethanol's direct actions on DA neurons in VTA (Brodie et al., 1999). However, the question of whether increased DA activity is a necessary and sufficient condition to produce a reinforcing effect influencing ethanol consumption has not yet been resolved in the literature (e.g. Amit & Smith, 1992; Di Chiara, 1995; Weiss & Porrino, 2002). For example, Engleman and colleagues proposed that ethanol self-administration was in fact not dependent on NAC DA activation, as substantiated by the failure of a selective DA reuptake inhibitor to alter ethanol self-administration (Engleman et al., 2000).

Another problem contributing to this debate is the lack of convergence from various lines of evidence. Researchers studying the effects of manipulating dopamine receptor transmission with pharmacological agents have often reported contradictory results concerning the capacity and specificity of these agents to disrupt ethanol self-administration (Brown et al., 1982; Goodwin et al, 1996; Linesman, 1990; McBride & Li, 1998). Overall, the effect of dopaminergic agents on ethanol self-administration seems to be more consistent within the rodent strain studied, as well as, the experimental procedure used to measure ethanol intake (Boyle et al., 1994; Linesman, 1990). This observation also draws attention to the idea that dopamine's role in ethanol reinforcement

may be indirect and/or linked to external factors such as the type of experimental procedure used to measure ethanol intake.

Further questions concerning DA's critical role in the reinforcement of ethanol self-administration are raised by data suggesting that selective neurotoxin 6-hydroxydopamine (6-OHDA) lesions of the mesolimbic pathway (Corcoran, Lewis & Fibiger, 1983; Rassnick, Stinus & Koob, 1993) or the NAc (Ikemoto, et al., 1997; Koistinen et al., 2001; Shoemaker et al., 2002) failed to disrupt oral ethanol intake, as well as ethanol reinforced lever pressing. In fact, studies have at times found that 6-OHDA lesions produced transient increases in ethanol consumption (Brown & Amit, 1977; Corcoran et al., 1983). The incongruous results of studies attempting to implicate dopamine transmission in the reinforcing effects of ethanol self-administration has contributed to an attempt to reconceptualize dopamine role's as it relates to reinforcement in general, and ethanol self-administration in particular. Several theories concerning DA's involvement in mediating the effects of drugs of abuse have been put forth in the literature. For example, it has been suggested that DA may act as a "teaching signal for associative learning" (Schultz, 1997; 2002) or in contrast that DA may not be involved in the learning process at all but rather in the arousal and preparatory effects of reinforcers prior to the behavioral expression of an already learned response (Robbins & Everitt, 1992; Salamone, Cousins & Snyder, 1997). Unfortunately, a consensus on the exact nature or the specific function of dopamine in reinforcement processes has not been reached (for reviews see Salamone & Correa, 2002; Kelley & Berridge, 2002). However, there appears to be a general agreement that the DA system, in conjunction with other neural systems interacts to collectively shape ethanol's reinforcing properties and

influence ethanol-seeking behavior (Amit & Smith, 1992; Kelley & Berridge, 2002; Kiiianmaa et al., 2003).

Serotonin

Serotonin (5-HT) is a brain neurotransmitter known to influence brain functions such as learning and memory, mood states, and the perception of the environment (Lovinger, 1997). Serotonin also participates in regulating bodily functions such as appetite and sexual behavior. The 5-HT system in general consists of neurons originating in the raphe nuclei with axons projecting to numerous brain regions such as the amygdala and the nucleus accumbens (Cooper, Bloom & Roth, 1991).

Alcohol administration has been shown to have an effect on 5-HT receptor function (LeMarquand, Phil & Benkelfat, 1994; Lovinger 1997). The 5-HT₃-receptor subtype is postulated to contribute to the regulation of ethanol consumption and ethanol reinforcement (Grant, 1995). Support for a role for 5-HT in ethanol intake comes from two main lines of evidence. First, acute administration of ethanol rapidly increases the firing of 5-HT₃ receptors and this stimulation may produce a cascade of similar effects on the neurons in regions receiving 5-HT signals (Lovinger, 1999; Lovinger & Zhou, 1994). Serotonin's interaction with gamma-aminobutyric acid (GABA) receptors, as well as dopamine receptors, may also contribute to its effect on ethanol intake (Campbell & McBride, 1995; Grant, 1995). Second, it has been repeatedly shown that increasing levels of 5-HT at the synapse or blocking 5-HT receptors decreases ethanol intake (for reviews see Gill & Amit, 1989; LeMarquand, Phil & Benkelfat, 1994; Lovinger, 1997). However,

the exact mechanism through which 5-HT agents exert their effect on ethanol consumption is not yet clear.

Gamma-aminobutyric acid (GABA)

γ -Aminobutyric acid (GABA) is a major inhibitory neurotransmitter system within the CNS. Excessive neural inhibition is associated with incoordination, sedation, and anesthesia (Mihic & Harris, 1997). In part due to the similarity between the behavioral consequences of ethanol intoxication and GABA's pharmacologic profile, the role of GABA in mediating the effects of ethanol in the CNS have also been investigated (for a review see Davies, 2003). There are two main types of GABA receptors, GABA_A and GABA_B, distributed throughout the brain (Hill & Bowery, 1981). Acute ethanol exposure is known to have direct effects on the GABA transmitter system (Hunt, 1983) and in particular, to enhance the function of the GABA_A receptor (for reviews see Chester & Cunningham, 2002; Mihic & Harris, 1997). Activation of the GABA_A receptor may mediate some of ethanol's behavioral effects as well as the reinforcement of ethanol self-administration (Chester & Cunningham, 2002; Mihic & Harris 1996). GABA_A antagonists have been repeatedly shown to reduce ethanol intake (Boyle et al., 1993) and ethanol reinforced responding (Rassnick et al., 1993) in rats. The reduction in ethanol intake is suggested to be mediated through blockade of GABA_A receptors located in the anterior VTA (Nowak et al., 1998) and/or in the central nucleus of the amygdala (Hyytia & Koob, 1995). On the other hand, the effects of GABA_A agonists are less clear due to reports of their bidirectional effects on ethanol intake (for a review see Chester & Cunningham, 2002). Administration of a GABA_A agonist has at times increased ethanol

intake and preference (Boyle et al., 1993; Smith, Robidoux & Amit, 1990; Schmitt et al., 2002) or reduced voluntary ethanol intake (Fadda et al., 1983; Petry, 1997). An explanation for the bidirectional effects of GABA_A agonists on ethanol self-administration may make use of the diversity of GABA_A receptors distributed throughout specific brain regions (Davies, 2003). Ethanol's action on GABA_A receptors may be dependent on the specific brain region that is the target of GABAergic manipulation. Alternatively, another explanation may involve the drinking status of the subjects such that decreases in ethanol intake tend to occur with selectively bred high ethanol drinking rodents as opposed to outbred or ethanol naïve rats (Boyle et al., 1993).

Glutamate

Glutamate is a major excitatory neurotransmitter important in regulating brain function (Fonnum, 1984; Cotman & Monaghan, 1988). Activation of these receptors allows the flow of positively charged ions into cells and facilitates activities such as neuron signal transmission (Gonzales & Jaworski, 1997). Glutamate exerts its effect on cells through three types of receptors, AMPA, kainate, and N-methyl-D-aspartate (NMDA) (Gonzales & Jaworski, 1997). It is proposed that the NMDA receptor, important in learning and memory processes, may also act as a “molecular switch” underlying neural plasticity (Gonzales & Jaworski, 1997).

Ethanol specifically induces inhibition or blocks NMDA receptors (Hoffman et al., 1989). The inhibition of NMDA receptors appears, in part, to be involved in the intoxicating and sedative effects of ethanol consumption (Valenzuela & Harris, 1997). Ethanol's ability to inhibit NMDA activity at glutamate receptors may also mediate some

of the reinforcing properties of acute and chronic ethanol intake. In animals, the antagonist acamprosate has been shown to reduce ethanol consumption (Boismare et al., 1984) and ethanol reinforced responding (Rassnick et al., 1992) without disrupting water intake.

Opioids

The opioids are an endogenous peptide system naturally produced in the CNS that function as neuromodulators by modifying the action of other neurotransmitters (Akil et al., 1998). Opioid peptides have the same effects on behavior as opiate compounds such as morphine and heroin (Froehlich, 1997). Through diverse mechanisms, the various endogenous opioid receptors ([e.g.,] mu, delta, and kappa) produce a range of effects including sedation and analgesia (Froehlich, 1997). The presence of opioid receptors and peptide-containing neurons can be detected in many brain regions and organ tissues (Hertz, 1997).

It is thought that the opioid system may play a role in ethanol reinforcement and self-administration (for review see Ulm et al., 1995; Herz, 1997). Overall, drugs that block opioid receptor activity appear to reduce ethanol intake in rodents (Froehlich et al., 1990; Hyytia, 1993). However, the nature of the mechanism mediating this effect is unclear. It has been suggested that the reduction in ethanol intake may likely be, a function of the induction of a conditioned taste aversion (Davidson, 1994). The specificity of blocking opioid receptor activity has also been questioned in the literature, as reductions in ethanol intake have at times been accompanied by general effects on consummatory behavior (Brown and Holtzman, 1979; Weiss et al., 1990; Schwarz-

Stevens et al., 1992). Furthermore, the exact nature of the contribution of the opioid system to ethanol reinforcement is not fully understood. Ethanol may increase extracellular levels of peptides in the NAc and thus may exert its effect on ethanol self-administration through influences on the dopaminergic system (Olive et al., 2001). On the other hand, studies have also shown that lesions of DA terminals in the NAc failed to alter ethanol intake while administration of the antagonist naltrexone reduced ethanol intake in the same animals (Koistinen et al., 2001). Therefore, it appears that the exact role of opioids in ethanol intake remains unclear.

In summary, numerous studies have attempted to identify the neurobiological mechanisms mediating ethanol's reinforcing or psychopharmacological properties. Research findings suggest that ethanol intake may, in part, be mediated by ethanol's effect on the neural systems within the CNS (reviews see Amit & Smith, 1992; Kostowski & Bienkowski, 1999; Weiss & Porrino, 2002). In fact, the literature reviewed demonstrates that several neurotransmitter systems including but not restricted to GABA_A, DA, serotonin, and glutamate may underlie some of ethanol's reinforcing effects. What remains unclear is the extent to which modulating neurotransmitter function on its own, without incorporating the contribution of additional variables such as the environment actually produces a change in the reinforcing efficacy of ethanol intake.

Animal Models

The following section will briefly discuss the original debate surrounding the utility of oral self-administration models of alcohol intake in rodents. The major ethanol self-administration paradigms and procedures employed by alcohol researchers are

presented, followed by a review of the behavioral variables hypothesized to contribute to the development of ethanol intake in rodents. As oral self-administration is a key element to human alcohol intake, the studies contained in this thesis reflect an analogous focus. However, please note that several alternative routes of ethanol self-administration exist and some of these rely on the experimenter to administer ethanol (i.e.: vapor inhalation, place conditioning). In a voluntary self-administration model, rodents are provided with free access to a choice of ethanol and water. Thus, the animal not the experimenter, chooses the amount of ethanol ingested and the pattern of its consumption.

The Preference Paradigm as a Model of Human Alcohol Consumption

The use of animals to model aspects of human alcoholism has been a tool integral to the effort to understand and identify the factors that may underlie human alcohol consumption (Myers & Carey, 1961). A historical yet commonly employed animal model of human alcohol consumption is the preference or voluntary consumption paradigm (Amit, Smith & Sutherland, 1987). The preference paradigm has customarily been used as a tool to identify the critical variables mediating differential ethanol consumption and preference. In the preference paradigm, animals are provided with a free choice between water and ethanol in order to assess ethanol preference, and measure the amount of ethanol consumed voluntarily. Traditionally, the two-bottle preference procedure was employed in the animal's home cage. The amount of absolute ethanol consumed (grams of ethanol/kilogram body weight/day) and the preference (amount of ethanol consumed as a percentage of total daily fluid intake) are frequently calculated and used as indices of ethanol seeking behavior.

Controversy surrounding animal models of human alcohol consumption

Historically, the use of oral self-administration procedures in animals to model human alcohol consumption has been rooted in controversy. Initial attempts to develop animal models of voluntary alcohol self-administration were met with criticism as they supposedly failed to meet the perceived criteria of a comprehensive “animal model of alcoholism” (Cicero, 1979; Li et al., 1979). In other words, early oral drinking paradigms were criticized because they frequently failed to induce ethanol drinking to the point of intoxication and generally did not produce behaviors associated with ethanol dependence following withdrawal (Cicero, 1979). Criticisms of the use of oral self-administration paradigms unfortunately overlooked two important facts. First, humans typically self-administer alcohol orally, thus it makes sense conceptually to employ a similar route of administration in animals. Second, other routes of administration (e.g.: intravenous) have generally failed to produce elevated and consistent levels of ethanol self-administration (Gill, 1989; Numan, 1981). The original debate surrounding oral self-administration paradigms can be traced to a contention over two interrelated points (Amit, Smith & Sutherland, 1988). One is the notion that rodents do not consume alcohol of their own volition (Cicero, 1980; Lester & Freed 1973). Second is the belief that animals must consume alcohol in sufficient quantities to produce intoxication and eventual signs of physical dependence (Cicero, 1980; Lester & Freed 1973).

Of the several animals employed to study alcohol intake, rodents are the most widely utilized. A problem encountered with rodents is their initial reluctance to drink large amounts of alcohol (Amit, Smith & Sutherland, 1987). More precisely, given a

choice between water and ethanol most rodents prefer low ethanol (2%-6%) solutions (Richter & Campbell, 1940). This observation was largely interpreted to suggest that rodents avoided drinking stronger ethanol solutions since they found them aversive. However, it has been demonstrated that animals drink solutions above 6%, although there are considerable individual differences in preference and intake across animals and strains (Boyle et al., 1994; Mendelson & Mello, 1964). The notion that rodents do not consume sufficient amounts of ethanol in an oral self-administration paradigm led to debates over the utility of the paradigm itself (for review see Cicero, 1980; Amit, Smith & Sutherland, 1988), as well as the development of alternative self-administration procedures (i.e.: Falk, Samson & Winger, 1972; Lumeng et al., 1977; Meisch, 1980).

In an attempt to overcome these criticisms, several methods were developed to induce animals to drink larger amounts of ethanol. Some early experimental manipulations used to increase ethanol consumption included schedule-induced polydipsia in weight-reduced animals (Meisch & Thomson, 1972), and restricting all fluid to ethanol containing solutions (Campbell, Taylor & Haslett, 1967; Carey, 1972). The merits of these techniques were disputed because animals tended reduce their level of ethanol intake after the discontinuation of the induction technique. An additional concern was the issue of whether the manipulation employed (i.e., fluid or food restriction) reflected actual voluntary intake (for a review see Amit, Smith & Sutherland, 1988). Today common experimental manipulations used to increase ethanol consumption include an alternate day schedule of ethanol presentation (Amit, Stern, & Wise, 1970), a sucrose-fading procedure (Samson, 1986), gradually increasing ethanol concentrations (Myers and Veale, 1972), restricting access to ethanol (Gill, France & Amit, 1986), as

well as selective breeding of high drinking animals (e.g.: Li, Lumeng, McBride, Waller & Hawkins, 1979).

The controversy surrounding the use of oral self-administration procedures has subsided somewhat given the success of these manipulations in producing sustainable levels of oral intake. Thus, rodents do learn to voluntarily drink ethanol in an oral paradigm (e.g.: Gill et al., 1986) and rodents will work or learn to perform an operant response to receive ethanol through the oral route (e.g.: Samson, Pfeffer & Tolliver, 1988). Furthermore, the inclusion of physical dependence as a necessary condition of a valid animal model of human alcoholism was seriously questioned (Amit, Sutherland & White, 1976). In fact, the feasibility of developing a singular animal model incorporating all features of human alcoholism is in all likelihood unrealistic, given its complex and varied nature. For the most part, researchers now concede that it is more viable for animal model(s) of alcohol intake to represent different facets of the alcohol consumption spectrum (Cunningham, Fidler & Hill, 2000). It follows that the model employed should arise from the research question at hand, and not from the quest to satisfy the requirements of a global theoretical model (Amit, Smith & Sutherland, 1988).

Self-administration Procedures

As previously mentioned, critiques of voluntary access or preference paradigms led to the proliferation of procedures aimed to increase ethanol intake, as well as to the development of alternative self-administration paradigms. Consequentially, the methodology used in oral self-administration paradigms varies tremendously across studies.

Voluntary ethanol self-administration paradigms typically differ according to the type of screening methods used to initiate drinking, the length of ethanol access, the ethanol concentration used, and the measures obtained (Boyle et al., 1997). Of these, two common procedural distinctions in oral self-administration paradigms include the length of ethanol access and the behavioral requirement demanded of the animal in order to gain access to ethanol (i.e.: Gill, Mundl, Cabilio, & Amit, 1989; Murphy et al., 1989; Samson, Pfeffer & Tolliver, 1988). The amount of time an animal has access to an ethanol solution differentiates between continuous and limited access procedures. Continuous access typically provides 22-hr to 23-hr of unrestricted access to ethanol (e.g.: Brown et al., 1982) while limited access provides short access periods varying from 10 minutes up to 4hrs, depending on the study (e.g., Linesman, 1990). Restricting access to ethanol was originally developed as a method to increase the amount of ethanol consumed by rodents (e.g., Samson, Roehrs & Tolliver, 1982) but was later embraced as a method that also produced pharmacologically relevant levels of ethanol intake (Gill, France & Amit, 1986). Gill et al., 1986 demonstrated that rodents consumed ethanol in short discrete drinking bouts and that restricting access led to the rapid intake of pharmacologically meaningful levels of ethanol. It was further suggested that a similar process might occur during unrestricted access to ethanol except that there are several drinking bouts occurring throughout the period of ethanol presentation (Gill et al., 1986).

In a standard oral self-administration paradigm, the only behavioral requirement is that the animals lick, and drink the solution(s) presented. On the other hand, operant paradigms require the animal to learn to perform an operant, typically a lever press, in order to gain access to a small quantity of the offered solution(s). The requirement of an

operant can be used in conjunction with either limited or continuous access procedures. However, the location of testing is different according to the type of drinking paradigm employed. In a standard oral self-administration paradigm, animals are traditionally tested in the home cage, however the apparatus necessary to measure operant behavior requires that animals be tested in a separate and suitably equipped room.

Although these paradigms all measure voluntary oral self-administration per se there has been some debate about whether they reflect the same underlying processes (Boyle et al., 1994; Samson & Czachowski, 2003). Deitrich (1992) commented that the multitude of paradigms and a lack of standardization might eventually lead to data that are circumscribed to the paradigm employed. In spite of these concerns, there is not yet a consensus on the paradigm to be used in order to study the factors controlling ethanol self-administration or the appropriate measures to assess the level of voluntary ethanol intake in rodents. The ability to compare and generalize the findings obtained across studies would be enhanced if such were the case.

The role of behavioral variables in ethanol self-administration

In an effort to identify the variables that contribute to the development of alcoholism in human beings, researchers have studied a multitude of factors believed to influence ethanol intake in animals. Initially, the focus of this search was largely to delineate the differential pharmacological responses to ethanol, as well as the neurobiological basis of ethanol intake in various rodent strains [e.g., Eriksson (1972); Korpi et al. (1988); Li et al. (1987); Murphy et al. (1982); Richter & Campbell (1940); Samson et al. (1988); Waller et al. (1986)]. Although informative and of theoretical

importance, these studies did not give much consideration to the likelihood that in addition to the neurobiological and genetic basis of high ethanol consumption, there may be nonpharmacological factors that are nevertheless influencing ethanol intake in rodents. In response to this partial void, there has been more recently a renewed interest [(e.g., Kampov-Polevoy et al. (1990); Overstreet et al. (1997); Salimov & Sinclair (1994)] in studying the role of behavioral and environmental variables and their relation to ethanol consumption.

Results of studies using pairs of selectively bred high-ethanol-preferring and low-ethanol-preferring strains have, at times, revealed behavioral variables such as a preference for saccharin solutions (Colombo et al., 1997; Overstreet et al., 1993; Stewart et al., 1994), as well as some measures of exploratory and avoidance behaviors (Salimov et al., 1996; Stewart et al., 1993; Tuominen et al., 1990; Viglinskaya et al., 1995), to be putative predictors of ethanol consumption. More precisely, the increased ethanol preference displayed by selectively bred strains such as the Indiana P rats (Li et al., 1987) has at times been attributed to behavioral, environmental and taste factors. For example, it has been shown that P rats are more anxious when tested in an elevated plus maze (Stewart et al., 1993), and display an increased preference for sweetened solutions (Sinclair et al., 1992) as compared to their non-preferring (NP) counterparts. Another study by Bice & Kiefer (1990) investigated the role of taste factors in a sample of P and NP rats. Orofacial reactions to a drop of alcohol solution placed on the tongue of P and NP rats did not differ initially and were both essentially neutral. After prolonged experience with alcohol, the lines differed, with P rats showing positive and the NP showing negative reactions to a drop of alcohol solution. One interpretation of these

results is that the reactions to the taste of alcohol were not innate but rather learned, possibly because of associations made with ethanol's post-ingestional effects (Forsander, 1994). It was further suggested that the divergent ethanol intake of the P and NP, using a sucrose fading procedure, might be mediated by their differential "preparedness" to associate the taste of sucrose with the reinforcing properties of ethanol (Gauvin et al., 1998). Overall, these results support the view that "individual gustatory differences" influence initial ethanol acceptance in rats or alternatively that taste plays a role when rats learn to drink ethanol (Kampov-Polevoy et al., 1990).

In another study, Files et al., (1993) demonstrated that the ethanol intake of P rats was modifiable by environmental variables such as the availability of food. In a continuous access condition, increasing the number of lever presses required to obtain food produced a similar increase in lever pressing for ethanol. The significant rise in ethanol intake (g/kg) was largely attributed to an increase in the number of ethanol drinking bouts per day as the requirements to acquire food increased. Thus, as food became more difficult to obtain, P rats increased their responding on the ethanol lever. In summary, manipulating environmental variables such as food availability appears to influence the ethanol intake in P rats, replicating an earlier finding obtained with outbred Long-Evans rats (Tolliver & Samson, 1989). At the time, this observation was interpreted to suggest that P rats, like the outbred Long-Evans rat, were influenced by environmental manipulations, yet the P rats supposedly represented a valid animal model of alcoholism because their overall ethanol intake was consistently higher (Files et al., 1993). However, in the Files et al., (1993) study it was also observed that the ethanol intake of P rats was not consistently elevated and actually varied on a daily basis. In other words, over a 60-

day period, P rats exhibited an “oscillating pattern” of ethanol intake indicating that P rats do not drink excessively at all times.

A more recent study (Gauvin et al., 1998) indicated that environmental factors such as length of the testing situation and induction method may play a significant role in facilitating the differential drinking between pairs of selectively bred rat lines. In this study, P and NP (non preferring) were conditioned to consume ethanol in a limited-access paradigm through the commonly employed sucrose-fading procedure. The animal's daily intake patterns of ethanol were monitored over a 4-month period. Results indicated that although P rats initially consumed larger amounts of ethanol (g/kg), the daily intake patterns of the P and NP were similar across conditioning phases, as was their ethanol intake (g/kg) at the end of the fourth month. Furthermore, at this period there were no significant differences observed in the level of ethanol intake (g/kg) of the P, NP and the two other outbred strains tested. The striking similarity of daily intake patterns and total ethanol intake (g/kg) suggested that the ethanol preference selected in the breeding of the P and NP could be countered by conditioning processes. Furthermore, these results demonstrated the influence exerted by the testing situation employed to assess the level of ethanol intake.

Several of the strains bred to prefer ethanol over water were developed via a specific protocol that involved providing rats with 10% ethanol as their sole fluid for four days, prior to a period of free choice between 10% ethanol and water (e.g.: Li et al., 1979). Presently, the forced choice protocol is regularly employed to verify the high level of ethanol intake associated with selected strains. The technique of forced choice is known to increase ethanol intake in rodents (Boyle et al., 1997) but it is interesting to

note that several researchers have shown (Bice & Kiefer, 1990; Gill 1989; Lankford et al., 1991) that unless a forced exposure to ethanol is employed prior to a standard two-bottle choice test, P rats do not exhibit their phenotypic drinking behavior. Furthermore, Gill (1989) demonstrated that when a forced exposure to ethanol is given to outbred Long-Evans rats and P rats, the high drinking animals in both strains displayed a similar pattern of ethanol consumption.

Despite the renewed interest in studying the role of behavioral and environmental factors in ethanol intake, the data obtained from behavioral studies have at times been difficult to replicate outside of the originating lab and are frequently strain specific (George, 1987; Overstreet et al., 1999; Wilson, Neill, & Costall, 1997). This observation has contributed to the need to reconsider the importance of several variables identified as possible factors mediating ethanol intake in rodents.

A study by Salimov (1999) using multivariate statistics to reevaluate previous behavioral findings across high-ethanol-drinking strains, confirmed that certain variables, such as saccharin intake, indices of cross-maze exploration, and behavior in inescapable situations, are factors that are correlated with increased ethanol intake. Specifically, a two-factor solution, obtained from the principal component analysis of behavioral data for eight pairs of selectively bred high-alcohol-drinking rodents, revealed that the factors labeled initial timidity/meekness and novelty seeking/persistence shared common variance with high ethanol intake. A note of caution however remains that the relationship of these individual variables to ethanol intake was not consistent across high drinking strains. For example, the high drinking strains such as the HAD1 and HAD2 were more timid and passive in the tests than their low drinking counterparts. However,

the high drinking P and AA rats displayed more escape/avoidance behavior in the slip funnel test and were relatively more effective in exploring the crossmaze, as compared to their low drinking counterparts. The results of these studies lend further support to the body of research suggesting that factors other than those directly related to the pharmacological properties of ethanol may also contribute to individual differences in high ethanol intake (Gauvin et al., 1998; Hill, 1978; Moolten & Kornetsky, 1990; Wise, 1974).

As reviewed above, several variables or factors not directly linked to ethanol's action on the CNS have been found to play a mediation role in the development of ethanol self-administration in rodents. For the most part, these studies have used pairs of selectively bred high-ethanol and low-ethanol-preferring strains, but it has been proposed that behavioral and environmental factors may also contribute to increased ethanol intake in non-selected strains (Amit & Smith, 1992; Goodwin et al., 1999; Schenk, Gorman & Amit, 1990). Several rat strains bred for variables seemingly unrelated to ethanol intake do nevertheless exhibit a distinct pattern of high and low ethanol intake and preference. For example, high levels of ethanol intake have been observed in Fawn hooded (Rezvani et al., 1990), Maudsley Reactive (Adams, Hannah & Henry, 2000; Koechling & Amit, 1990), and Tryon-maze-bright (Amit & Smith, 1992) strains of rats, all of which were bred for factors unrelated to ethanol intake. Specifically, the Maudsley Reactive (MR) strain was originally selected for emotional reactivity in a novel environment (Broadhurst, 1960). The ethanol intake of the MR, as well as the Harrington derivation of the strain (MR/Har) has been shown to be influenced by environmental factors, such as the method of food delivery and cage type (Adams et al., 2000). Furthermore, the ethanol

intake of the male MR/Har rat is known to be quite variable (Adams & Oldham, 1996; Goodwin et al., 1996) and the expression of high ethanol intake seems to require the use of a forced exposure to ethanol (Adams et al., 2001). While not widely accessible, the MR and the Fawn hooded strains can be procured from their originating laboratories and as such have been the subjects of multiple studies (e.g.: Adams et al., 2000; Goodwin et al., 1996; Hall et al., 1998). Unfortunately, the Tryon colony no longer exists but the last published study involving the strain as subjects evaluated the Tryon's level of ethanol self-administration (Amit & Smith, 1992). Of particular interest was the finding that Tryon-maze-bright animals preferred, as well as consumed, greater amounts of ethanol as compared with the average intake of ethanol-preferring strain of rats (Amit & Smith, 1992).

The Present Investigation

The research contained in this thesis was aimed at further investigating the influence of behavioral and environmental factors on ethanol self-administration in outbred strains. The first study was intended as a further exploration of the Amit & Smith (1992) study that assessed the level of ethanol intake in Tryon rats. Thus, Experiment 1 examined the relationship of a behavioral trait, maze competency, to ethanol consumption using a traditional preference paradigm. The range of consumption in the sample was determined in order to classify animals according to their drinking level and facilitate the examination of within group differences in the behaviors under investigation.

The second study was designed to replicate and extend the findings obtained in Experiment 1 with the addition of another learning task, the radial maze. The radial maze is a commonly used measure of short-term spatial memory in rodents (Olton, 1979). The radial maze task was specifically chosen to assess the role of individual differences in short-term spatial memory (Olton, 1979) in mediating the observed relationship between maze competency and ethanol intake (Pratt et al., 2002). Given that the findings obtained in the first two experiments can be considered spatial tasks, the third study explored the role of the hippocampus, a brain structure implicated in the mediation of spatial memory and orientation.

The variability observed in the ethanol consumption of outbred as well as selected strains, in fact, across phylogenies, may be viewed as a methodological nuisance or as a reflection of the underlying nature of ethanol self-administration behavior. Furthermore, as the individual differences observed within and across strains cannot be solely attributed to ethanol's pharmacological properties, it is likely that non-pharmacological factors contribute, perhaps even control the individual differences in alcohol consummatory patterns. Thus, the fourth and fifth experiments were designed to assess the influence of environmental variables on individual differences in ethanol intake. Specifically, we hypothesized that the type of procedure used to initiate intake would have an effect on subsequent ethanol self-administration. Thus, in Experiment 4 we were interested in examining the interrelationship amongst different drinking paradigms, as well as any influence that the drinking paradigm itself might exert on ethanol intake. Although the various drinking paradigms all measure oral self-administration, the location of testing is different according to the type of drinking paradigm employed. As a

follow-up to Experiment 4, the influence of the drinking context, in particular the effect of transporting animals to the testing environment employed to initiate ethanol intake, was explored in Experiment 5.

Alcoholism is often characterized as a chronically relapsing disorder that evolves from occasional, controlled drinking to loss of control over drinking behavior (Cloninger, 1987). It follows logically that the behavioral processes that lead to excessive drinking are likely represented to a degree in most individual regulated drinking. Thus, if one's goal is to understand the extremity of excessive human alcohol intake then it is imperative to explore all variables that may contribute to its development in the first place.

Experiment 1: Is there a relationship between maze learning ability and ethanol intake in non-selected rodents?

The main purpose of this experiment was to examine the relationship between learning ability and ethanol intake, using a complex T-maze to assess learning ability. The T-maze was a replica of the maze employed by Tryon (1929) to assess the inheritance of maze learning ability in rats. The finding that Tryon-maze-bright animals, bred for high-maze-learning scores, exhibited elevated ethanol intake levels (Amit & Smith, 1992) suggested the possibility that a common mechanism may mediate the actions of the seemingly unrelated behaviors of ethanol drinking and maze learning ability. It was thus hypothesized that individual differences in learning ability, as measured by the ability to perform in a T-maze, may be related to the affinity to ingest ethanol. The goal of the present study was therefore to assess the role of a behavioral factor such as learning ability in mediating the acquisition and maintenance of ethanol intake in an unselected strain of rats. An outbred strain of rats was specifically chosen because we hypothesized that if learning ability is a basic trait that contributes to the development of high ethanol intake, a relation between these variables must be observable without any prior selective breeding.

Method

Subjects

Subjects were 60, male, Long-Evans rats (Charles River, St. Constant, Quebec, Canada), each weighing between 150 and 175 g when received. Rats were given a 2-week acclimatization period during which they were handled daily. Animals were housed

individually in hanging stainless steel cages in a colony room maintained and controlled for temperature, humidity, and a 12 h light/12 h dark cycle (lights on at 0800). All testing took place during the light portion of the cycle. Because a pilot study conducted in our lab demonstrated that animals could complete the maze without food deprivation, food (Purina Rat Chow) and water were provided ad libitum throughout the experiment. For the duration of the ethanol consumption phase, fluids were presented in Richter tubes attached to the front of the cage. All animals were treated in accordance with the guidelines of the Canadian Council for Animal Care, and the Animal Care Committee of Concordia University approved all procedures.

Drugs

Ethanol solutions were prepared through a dilution of a 95% ethyl alcohol stock solution with tap water.

Apparatus

A 16-unit modular T-maze was constructed to assess the learning ability of each rat. In essence, the maze was a replica of the one used by Tryon (1929) except that one less T-unit was used because of room size restrictions. A unit was composed of a runway and two opposing arms. One arm of each unit was closed and the other was open and connected to the runway of the next T-unit. Each section of a unit was equipped with photocells positioned 4 cm above the floor. The photocells relayed information about the location of the animals in the maze to a computer (486 IBM-compatible PC). The T-maze was constructed with the use of dark gray polyvinyl chloride plastic for the walls, clear

polyvinyl chloride plastic for the ceiling, and wire mesh for the floor. Each section of a T-unit measured 28 cm in height, 30.5 cm in length, and 12 cm in width. Thus, the distance from the start box to the goal box, without committing an error, would be approximately 488 cm in length.

Procedure

After acclimatization to the colony room, animals were given a daily trial in the T-maze for 19 consecutive days. The first day was provided as an exploratory trial and was not included in the calculation of an animal's competency score. Testing order was randomized for each trial. A trial began at the start box position and ended when the animal either reached the goal box or a predetermined time limit (15 min) had elapsed before completion. Each trial was rewarded with sugarcoated cereal (Kellogg's Honey Rice Krispies) bits placed in the goal box. Animals were given 2 min to consume the five pieces of cereal before being returned to their holding cage. An error was signaled when an animal disrupted the photocell beam that was installed 6 cm inside the entrance of a closed arm. The number of errors and time to complete each trial were monitored and recorded by the computer. The mean error score and mean time (seconds) was obtained for each rat over the 18-day period of recorded trials.

Subsequent to the completion of maze testing, animals were given 4 days to acclimatize to the presentation of fluids in Richter-type drinking tubes. Water was the only fluid presented during this period. Animals were subsequently introduced to ethanol through a graduated ethanol acquisition paradigm (Brown et al., 1982). This consisted of the presentation of ethanol solutions and water in a free-choice situation on alternate

days. Water was the sole fluid presented on intervening days. Beginning with a 2% (vol./vol.) ethanol solution, the concentration was raised in increments of 2% until a final concentration of 10% (vol./vol.) was reached. Rats received two exposures to each ethanol concentration before the concentration was increased to the next level. Animals were then maintained on daily presentations of a 10% (v/v) ethanol solution in free choice with water for 10 additional days. Ethanol and water intakes were measured on a daily basis to the nearest milliliter. The position of the fluids was switched on each ethanol presentation day to control for the influence of side preferences. Ethanol intake during the final 5 days of the maintenance period was used to calculate the mean ethanol intake score in grams per kilogram of body weight per day (g/kg/day).

Data analysis

Data collected from the experiment included individual mean maze errors and individual mean times (seconds) to complete the maze, as well as ethanol intake (grams per kilogram of body weight per day). To assess overall maze performance, each dependent variable measured in the maze was first transformed to similar scales that could then be used to reflect performance relative to the group. Dividing an animal's score on either maze errors or time by the corresponding highest score in the group provided a comparable measure for each maze variable. The transformed data points for errors and time were then added together to allot each animal with an index of overall maze performance. The combination of the individual variables was undertaken as it was believed that this index would better capture and represent the overall performance of the animal and that this would be a more accurate reflection of the animal's rate of learning

for this task. Thus, a higher score on the maze index indicates that an animal took more time and made more errors in completing the maze and thus reflects a poorer performance relative to the group.

As our interest was to examine any relation between maze performance and differential ethanol intake, animals were subdivided for statistical purposes according to their level of ethanol intake. Animals were classified as high drinkers or low drinkers if their mean ethanol consumption (grams per kilogram of body weight per day) was greater or less than 2.5 g/kg/day, respectively. Mean group differences on individual maze variables were assessed by *t* tests, for which equal variances were not assumed (Keppel, 1991). Maze competency data were analyzed by using a Pearson product moment correlation (Keppel, et al., 1992). Data were analyzed with the use of the Systat software application for an IBM computer. An alpha level of .05 was used for all analyses.

Results

Mean scores for ethanol intake, mean maze error scores, and mean time scores according to groups are presented in Table 1. Inspection of the specific maze variables in Table 1 seems to indicate that the high drinkers group committed a larger number of errors than the low drinkers group, but they took slightly less time to complete the maze. However, neither of these differences was significant [$t(21.49) = 1.351, p > .05$ and $t(34.64) = -0.496, p > .05$, respectively]. Because our primary goal was to assess whether individual differences in learning processes were a plausible variable contributing to increased ethanol intake, the index of maze competency for each animal was viewed as the critical measure for further analysis. The maze competency index was calculated by

transforming each variable to a similar scale and then combining the animal's score on both error and time variables. Thus, a lower score on the maze index indicates that an animal took less time and made fewer errors in completing the maze and thus reflects a better performance relative to the group. The combination of the individual variables was undertaken as it was believed that this index would better capture and represent the overall performance of the animal and that this would be a more accurate reflection of the animals' rate of learning for this task. As shown in Fig. 1, a significant negative correlation between intake of a 10% ethanol solution (grams per kilogram of body weight per day) during the final 5 days of the maintenance period and the index of maze competency was obtained for the high drinkers group, [$r(14) = -0.54, p < .05$]. In other words, animals with a lower maze index score, indicative of overall better performance on the combined maze variables, were also the highest ethanol drinkers. As can be seen in Fig. 2, a significant correlation between ethanol intake (grams per kilogram of body weight per day) and index of maze competency was not obtained for the low drinkers group, [$r(42) = -0.044, p > .05$]. Similarly, no correlation between maze ratio and ethanol intake was obtained for the group containing all animals [$r(58) = -0.038, p > .05$ (data not shown)]. From these results, it can be suggested that although the singular variables of errors and time failed to differentiate the high drinkers from low drinkers, the index or composite of these variables, believed to more accurately reflect maze performance, was correlated with the level of daily ethanol intake for the high drinkers group.

Table 1Mean ethanol intake, mean time, and mean maze error scores by Group (\pm S.E.M.)

Group	n	Ethanol (g/kg/day)	Maze errors	Time (s)
All animals	60	2.09 + 0.19	5.07 + 0.22	265.84 + 15.24
High drinkers	16	4.16 + 0.26	5.62 + 0.51	254.68 + 24.13
Low drinkers	44	1.34 + 0.11	4.87 + 0.23	269.90 + 18.96

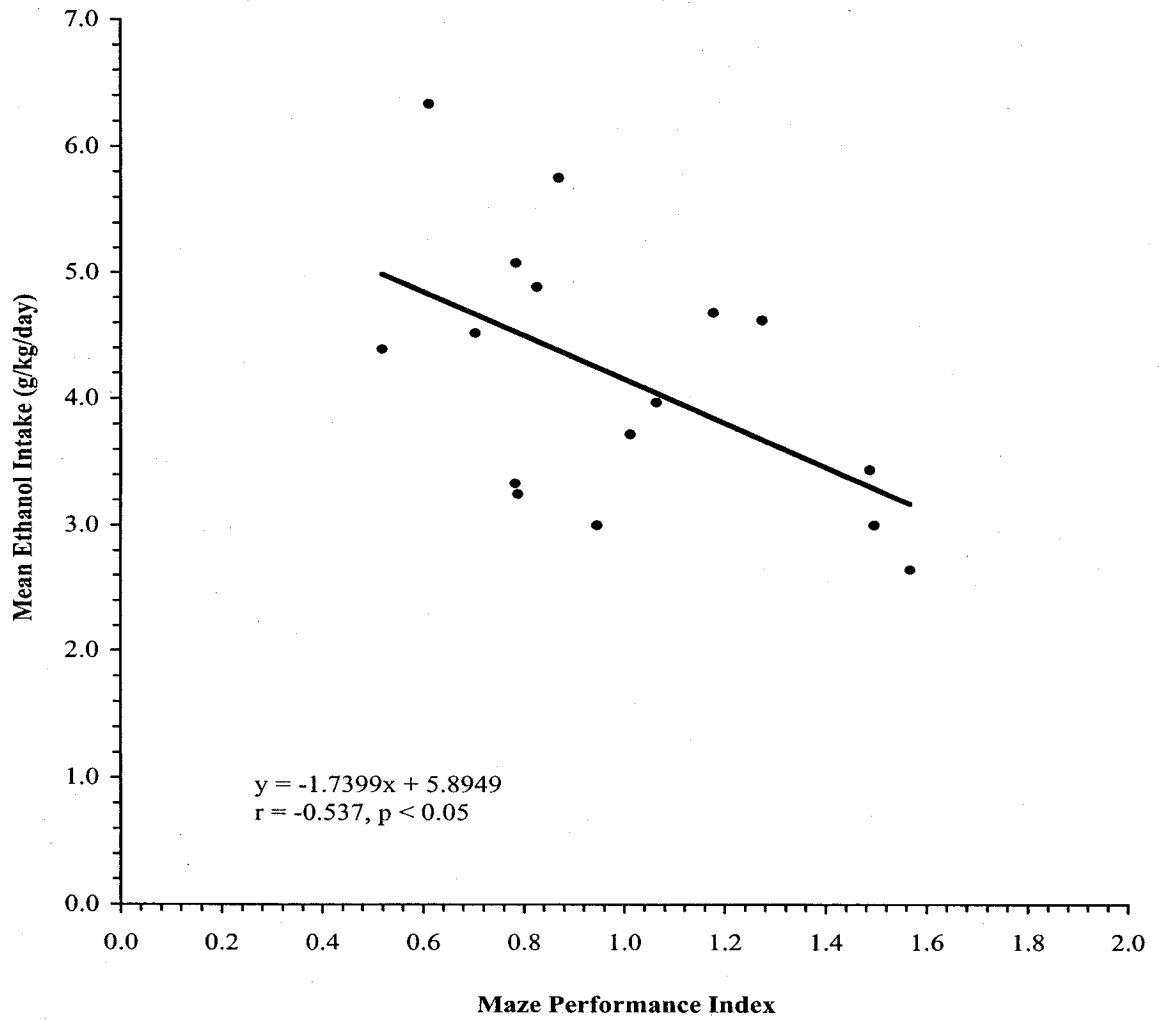


Figure 1. Scatterplot of mean ethanol intake (grams per kilogram of body weight and the index of maze performance in the high drinkers group (n = 16).

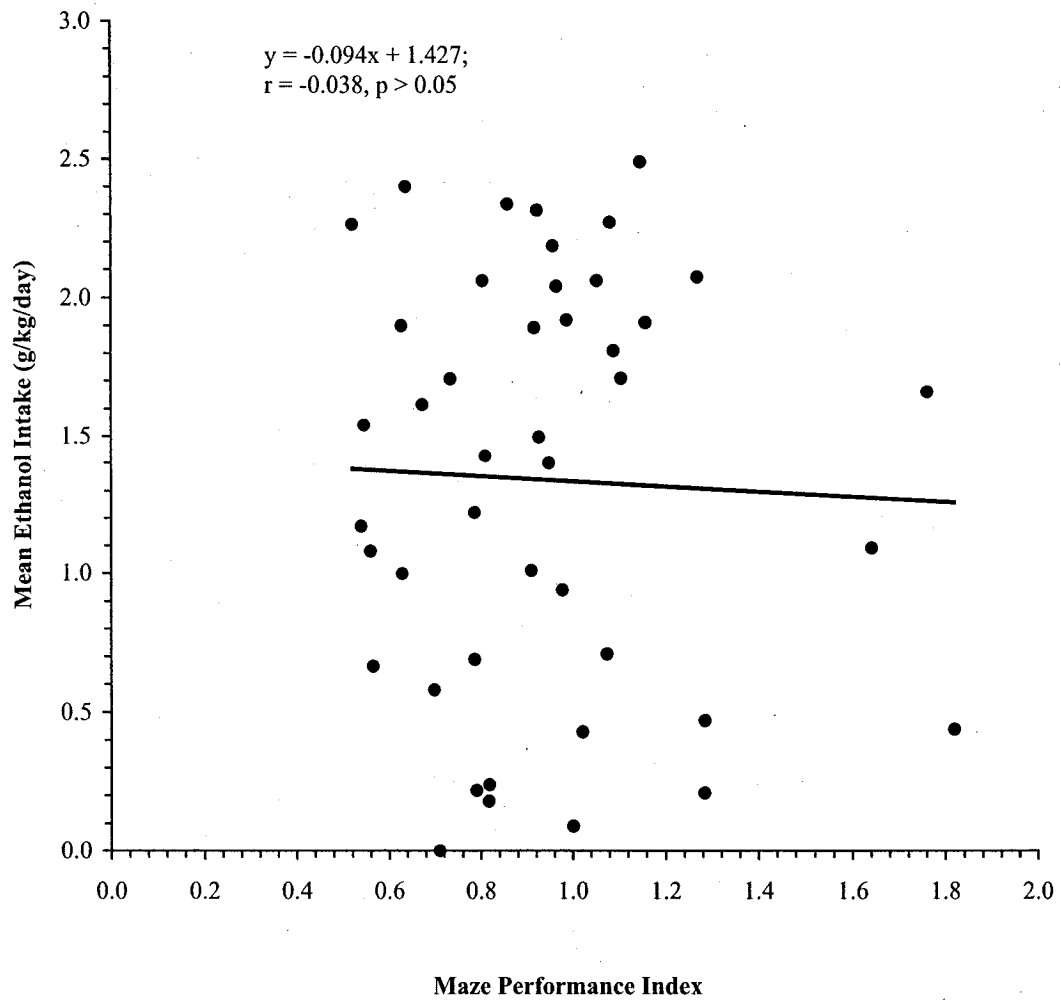


Figure 2. Scatterplot of mean ethanol intake (grams per kilogram of body weight) and the index of maze performance in the low drinkers group (n = 44).

Discussion

The purpose of this experiment was to assess the role of a specific behavioral variable such as learning ability in mediating at least in part the acquisition and maintenance of ethanol intake in an unselected strain of rats. The major finding of this study is that among high ethanol drinkers, an animal's competency to complete a 16-arm T-maze seems to be an underlying factor related to subsequent increased ethanol intake. In other words, animals that had a smaller index of time and errors in the maze were also the highest drinkers. No significant correlation was found between maze competency index and subsequent ethanol intake in the low drinkers group. The results of this study thus seem to indicate that individual differences in learning ability, defined operationally as the competency to complete a complex T-maze (Tryon, 1929), were related to increased ethanol intake. The finding that individual differences in maze competency can explain 29% of the variability of ethanol intake in the high drinkers group highlights the importance of investigating the contribution of behavioral factors such as learning ability to the acquisition and maintenance of high ethanol intake in outbred rodent strains.

A role for learning ability in ethanol self-administration seems in line with the notion that all animals must first learn to drink ethanol to develop an affinity and preference for its later chronic consumption. It is important to note in this context that the initial reaction to moderate concentrations of ethanol in most naive animals is normally to reject the offered solution (Eriksson, 1968; Myers, 1968). We hypothesize that efficient use of distal and proximal cues may facilitate an animal's ability to pair the pharmacological effects of ethanol with the gustatory and olfactory cues associated with consumption of ethanol, and thus may promote increased levels of ethanol intake. In

other words, rodents that display a superior ability to proficiently navigate a complex maze over a period of 19 trials may also use this innate ability to effectively pair the external cues associated with the presentation of ethanol to the initially unfamiliar and internal sensations provided by the pharmacological effects of ethanol. It is proposed that animals possessing this increased learning competence, as demonstrated by a smaller maze competency ratio, should thus approach and consume more ethanol in the long term because they have formed a stronger association between presentation and consumption of ethanol. Moreover, our results seem to indicate that although learning ability may be related and contribute to ethanol self-administration in high-drinking outbred animals, it does not seem to account for the behavior of the animals in the low-drinking group. Despite similar group performance on the singular maze variables of errors and time, individual low-drinking animals do not seem to use this ability to facilitate their acquisition of ethanol self-administration. Thus, performance in the T-maze only correlated with level of ethanol intake when individual animals displayed good results in both the numbers of errors and time taken to complete the maze. Group performance between high drinkers and low drinkers may have been similar but individual low-drinking animals tended to have good performance on only one of two variables. If we hypothesize that myriad factors contribute to the acquisition of ethanol intake in rodents, it is plausible that the specific factors contributing to intake vary across individuals and strains. Thus, it may be that other factors, such as aversion to the taste of ethanol or higher rejection levels of ethanol, are more salient in the low-drinking animals and thus may impede initial ethanol self-administration.

The explanation that our low-drinking group may find the gustatory properties of

ethanol more aversive actually fits with previous findings demonstrating that both the NP and the alcohol-avoiding ANA low-drinking strains find ethanol to be less palatable, as compared with findings for their high-drinking counterparts (Badia-Elder & Kiefer, 1999; Bice & Kiefer, 1990). Of interest in the study by Badia-Elder and Kiefer (1999) was the finding that differences in the reaction of the P and NP animals to a drop of ethanol developed only after prolonged experience with ethanol. These results may be interpreted to support the suggestion that the reactions to the taste of ethanol were not innate but, rather, learned, possibly as a result of postingestional effects (Forsander, 1994).

Experiment 2: An examination of learning and memory variables and their relationship to ethanol self-administration.

In the previous experiment, (Pratt et al., 2002), we found that the competency to navigate a complex maze was related to increased ethanol intake in Long-Evans rats. In other words, Experiment 1 demonstrated that individual differences in learning ability, defined operationally as the competency to complete a complex T-maze (Tryon, 1929), were positively correlated with increased ethanol intake among high drinkers. Tryon rats were originally bred on the basis of their maze scores and it is known that rats use their spatial ability to solve mazes (Olton, 1979). Thus, it is highly plausible that the high drinkers in Experiment 1 also possess a superior spatial ability to solve mazes. A goal of the present study was to further assess the role of learning, as indicated by performance in an additional spatial task, the radial maze, in mediating increased ethanol intake. Olton (1979) has suggested that in the radial maze, an animal learns the maze by building a cognitive map of the maze environment and then uses working memory to keep track of where it has been. In a naturalistic setting, the spatial ability of rodents would be imperative for their survival as they must be able to integrate information gleaned from the environment in order to orient them in the physical space.

In Experiment 1, it was proposed that animals endowed with enhanced learning ability, should thus approach and consume more ethanol in the long term because they have formed a stronger association between the presentation and consumption of ethanol. In other words, rodents that display a superior ability to proficiently navigate a complex maze may also use this innate ability to effectively pair the external cues associated with the presentation of ethanol to the initially unfamiliar and internal sensations provided by

the pharmacological effects of ethanol. We thus reasoned that high drinking animals that overcome the initially aversive properties of ethanol, (e.g., smell, taste) may be doing so by effectively pairing the external cues associated with the presentation of ethanol to the internal sensations provided by the ingestion of ethanol.

The notion that rats learn to drink ethanol is in line with the fact that over time rodents can be induced to drink large amounts of ethanol through a variety of exposure techniques. While undergoing the experience of acquisition, it is likely that the animal learns something about the properties of the drug presented, in addition to information about the environmental context of its presentation. Logically, it follows that any information or learned associations surrounding the drug's consumption are then stored in memory. It has been hypothesized that any process that can enhance or aid in the formation of this association may result in the facilitation of the acquisition of ethanol self-administration (Boyle et al., 1997). In fact, White (1996) previously suggested that learning and memory processes might contribute to ethanol reinforcement. He stated that the information required for the self-administration of a drug may be stored in multiple, independent memory systems and thus a singular concept such as reinforcement isn't likely to explain addictive behavior in general or self-administration in particular.

Since self-administration of ethanol is carried out almost exclusively orally, a delay likely occurs between the ethanol drinking behavior and the onset of any central pharmacological effects (Nurmi, Kiianmaa, & Sinclair, 1994). Furthermore, the smell and taste of the ethanol solution likely represent salient cues to an animal about to engage in ethanol consumption. Given the time delay between ingestion of ethanol and the onset of any pharmacological effect, an animal with a superior short-term memory may be more

likely to form an association between the internal sensations produced by ethanol ingestion and the external features linked to its presentation. Thus, in addition to the hypothesis that learning ability is related to the consumption of ethanol, it was also hypothesized that individual difference in short-term spatial memory may contribute to or mediate the observed relationship between maze competency and ethanol intake (Pratt et al., 2002). The radial maze task was specifically chosen to assess the role of individual differences in short-term spatial memory (Olton, 1979) in mediating the observed relationship between maze competency and ethanol intake (Pratt et al., 2002).

Finally, it has been previously suggested that the findings obtained from behavioral studies are difficult to replicate and frequently depend on the testing protocol employed by the researchers (e.g.: Overstreet et al., 1999; Ritz, Wilson, Neill, & Costall, 1996). Thus another goal of Experiment 2 was to partially replicate our initial study while determining if the order of testing conditions in Experiment 1 was vital to the finding that maze competency correlated with increased ethanol intake.

Method

Subjects

Twenty-one male Long-Evans rats weighing 150-175 g at the start of the experiment were individually housed in stainless steel cages in a room regulated for constant temperature and humidity. The colony had a 12: 12 hr light/dark illumination cycle. Water and food were available ad libitum throughout the experiment except during the radial maze portion of the study. Prior to and during testing in the radial maze, animals were food deprived and maintained at 85% of their free-feeding body weight. All

animals were treated in accordance with the guidelines of the Canadian Council for Animal Care, and the Animal Care Committee of Concordia University approved all procedures.

Apparatus

Radial Maze

An eight-arm radial maze was used to assess the rat's spatial learning and memory capacity. The maze rose 74 cm above the floor in a room that contained several extra-maze cues (e.g.: laboratory counter running length of room and posters affixed on remaining three walls). Each arm of the maze measured 51cm in length and 11.5 cm in width and extended at 45-degree angles from an octagonal central platform. A recessed food cup was installed at the end of each arm. A guillotine door restricted access to each of the radial arms. The radial maze was constructed using clear PVC for the walls and doors and wood painted black for the floor.

T-Maze

A 16-unit T-maze was employed to assess the rat's learning ability. A unit was comprised of a runway and two opposing arms (open/closed). Each section of a unit was equipped with photocells positioned 4 cm above the floor. The photocells relayed information about the location of the animals in the maze to a computer (486 IMB compatible PC). The T-maze was constructed using dark gray PVC for the walls, clear PVC plastic for the ceiling and wire mesh for the floor.

Procedure

Phase 1: Ethanol Acquisition

Following a two-week acclimatization period, animals were introduced to ethanol through a gradual acquisition procedure. Ethanol and water solutions were presented daily in a free-choice paradigm. Beginning with a 2% (v/v) ethanol solution, the concentration was increased in increments of 2% until reaching a final concentration of 10% (v/v). Rats received two exposures of each ethanol concentration before it was increased to the next level. An additional 10 days of 10% ethanol were presented and constituted the maintenance period. Ethanol and water intakes were measured on a daily basis to the nearest milliliter. The position of the fluids was switched on each ethanol presentation day to control for the influence of side preferences. Ethanol intake during the final 5 days of the maintenance period was used to calculate the mean ethanol intake score in grams per kilogram of body weight per day (g/kg/day).

Phase 2: Radial Maze Task

After the completion of phase 1, animals were given a five-day period without any experimental manipulations except daily handling. Animals were then food deprived and maintained at 85% of their free-feeding weight. Prior to maze testing, each animal was given a daily 10-min period to adapt to the apparatus for 3 days. During this period, food pellets (45-mg Bio-Serv) were scattered throughout the maze. Following this period, animals were each given one daily test trial in the maze. Two pellets were placed in the receptacle of each arm. A session was determined to be complete when animals had visited each of the eight baited arms or 10 minutes had elapsed. Entries into baited arms

were recorded as correct responses, and re-entries were recorded as errors. The number of correct responses before committing the first error (initial correct), the number of errors, and the running time were used as the indices of radial arm performance (RAM) (Li et al., 1997). When a rat made no errors or only one error after the seventh choice for 5 consecutive days, a delay-interposed task was given. The delay-interposed task involved returning the rat to his home cage for a 5-min delay between the third and fourth choices. The number of correct choices before committing an error (re-entry) after the delay and the running time were recorded as measures of choice accuracy and RAM performance.

Phase 3: T-maze Task

Animals were given a minimum of one week to eat freely and return to their free-feeding weight. After regaining their weight, animals were given a daily trial in the T-maze for 19 consecutive days. The first day was provided as an exploratory trial and was not included in the calculation of an animal's competency score. Testing order was randomized for each trial. A trial began at the start box position and ended when the animal either reached the goal box or a predetermined time limit (10 min) had elapsed before completion. Each trial was rewarded with Reese's Peanut Butter Pieces placed in the goal box. Animals were given 2 min to consume the two Reese's pieces before being returned to their holding cage. An error was signaled when an animal disrupted the photocell beam that was installed 6 cm inside the entrance of a closed arm. The number of errors and time to complete each trial were monitored and recorded by the computer. The mean error score and mean time (seconds) was obtained for each rat over the 18-day period of recorded trials.

Data analysis

As a goal of this experiment was to replicate our findings in the first study, data from Phase 1(ethanol acquisition) and Phase 3 (T-maze) were collected and synthesized with the same protocol as in Experiment 1. However, given that there were fewer subjects in Experiment 2, a median split of the individual mean ethanol consumption (grams per kilogram of body weight per day) over the five final days of acquisition was used to classify animals as high drinkers or low drinkers. In Phase 3 individual maze variables such as the number of initial correct, number of errors, time (sec), number of choices per trial, and the number of trials to criterion were collected. As a measure of timed choice accuracy (competency) in the radial maze task, the numbers of choices made was divided by the time taken and provided an indication of the time taken per choice. In the delay-interposed task the number of correct choices made prior to committing an error after the delay, the number of errors, number of choices, and the running time were recorded as measures of choice accuracy and RAM performance. Mean group differences on individual maze variables were assessed by *t* tests (Keppel, 1991). A directional *t*-test in the delay-interposed task was chosen because we hypothesized that the high drinkers group would have a better performance on the behavioral variables as compared to the low drinkers group. The relationship between maze performance and ethanol intake were analyzed by using a Pearson product moment correlation (Keppel, et al., 1992). Data were analyzed with the use of the Systat software application for an IBM computer. An alpha level of .05 was used for all analyses.

Results

Six of the twenty-one subjects failed to acquire ethanol self-administration by the

end of the ethanol acquisition phase and were excluded from further participation in the study. Another subject's data were excluded from all analyses due to illness during Phase 3 of the study.

Table 2 represents the mean and standard errors of the behavioral measures according to group. T-tests conducted on the variables presented in Table 2 reveal that the high drinkers group and low drinkers group do not differ in their acquisition of either maze. In the T-Maze, the number of errors committed and the time taken to complete the maze did not differ between the high drinkers and the low drinkers, [$t(12) = 0.686, p > .05$ and $t(12) = -0.067, p > .05$, respectively]. Similarly, in the radial maze, there were no differences in the number of errors or in the amount of time taken by rats of the high drinkers group as compared to rats of the low drinking group [$t(12) = -1.310, p > .05$ and $t(12) = 0.694, p > .05$, respectively]. The high drinkers group and the low drinkers group did not differ in the number of trials required to reach criterion prior to the delay-interposed task, $t(12) = -0.077, p > .05$.

Although a standard radial maze task did not highlight any group differences, the more demanding delay interposed task revealed significant differences in maze performance and choice accuracy. In the delay-interposed task the high drinkers tended to make fewer errors than the low drinkers but this was not a significant difference, $t(12) = 1.044, p > .05$. In contrast, the high drinkers group made a greater number of initial correct choices and took less time to complete the delay task than the low drinkers group [$t(12) = -1.721, p = .055$ and $t(12) = 2.143, p < .05$, respectively]. Furthermore, the high drinkers group and the low drinkers group differed significantly in the amount of time they took to make each choice, such that the high drinkers took less time per choice made

$[t(12) = 2.30, p < .05]$.

Since the goal was to replicate our findings in Experiment 1, as well as assess whether individual differences in learning processes may be variables contributing to differential ethanol intake, the individual index of maze competency in the T-maze was subjected to further analysis. As previously observed in Experiment 1 and shown in Fig. 3, a significant negative correlation between intake of a 10% ethanol solution (grams per kilogram of body weight per day) during the final 5 days of the maintenance period and the index of maze competency was obtained for the high drinkers group, $r(6) = -0.67, p < .05$. In other words, animals with a lower maze index score, indicative of overall better performance on the combined T-maze variables, were also the highest ethanol drinkers. A significant correlation between ethanol intake (g/kg/day) and index of maze competency was not obtained for the low drinkers group, $r(6) = -0.52, p > .05$.

As our main goal was to assess whether individual differences in learning and memory processes, as measured in an additional spatial task, were related to increased ethanol intake, the behavioral variables in the radial maze task were viewed as important measures for further analysis. As can be seen in Fig. 4, a significant correlation between ethanol intake and the time taken in the delay task was obtained for all animals, $r(13) = -0.63, p < .05$, such that animals that took less time to complete the task after a delay were also the highest drinkers.

In spite of significant group differences, no other radial maze variable was correlated with level of ethanol intake. As shown in Fig 5, individual performance across spatial learning tasks was stable, $r(13) = 0.77, p < .05$. For example, animals that performed poorly in the T-maze also took more time per choice made in the radial maze.

Table 2Mean ethanol intake, mean time, and mean maze error scores by Group (\pm S.E.M.)

Measure	All animals (N = 14)	High drinkers (n = 7)	Low drinkers (n = 7)
Ethanol (g/kg/day)	2.01 \pm 0.22	2.65 \pm 0.17	1.36 \pm 0.18
T-Maze			
T-maze errors	3.96 \pm 0.37	3.67 \pm 0.36	4.18 \pm 0.67
Time (s) in T-maze	150.89 \pm 15.26	151.96 \pm 26.33	149.82 \pm 17.77
Radial Maze			
Radial maze errors	2.30 \pm 0.15	2.49 \pm 0.20	2.11 \pm 0.21
Time (s) in R-maze	164.23 \pm 7.95	158.61 \pm 10.09	169.87 \pm 12.70
Delay Interposed Task			
Errors	1.29 \pm 0.41	0.86 \pm 0.46	1.71 \pm 0.68
Initial correct	3.64 \pm 0.40	4.29 \pm 0.36	3.00 \pm 0.66 *
Time (s)	91.64 \pm 4.78	82.57 \pm 4.86	100.71 \pm 6.93 *
Time per choice(s)	9.80 \pm 0.30	9.34 \pm 0.32	10.40 \pm 0.34 *

* Significant mean difference between high drinkers and low drinkers

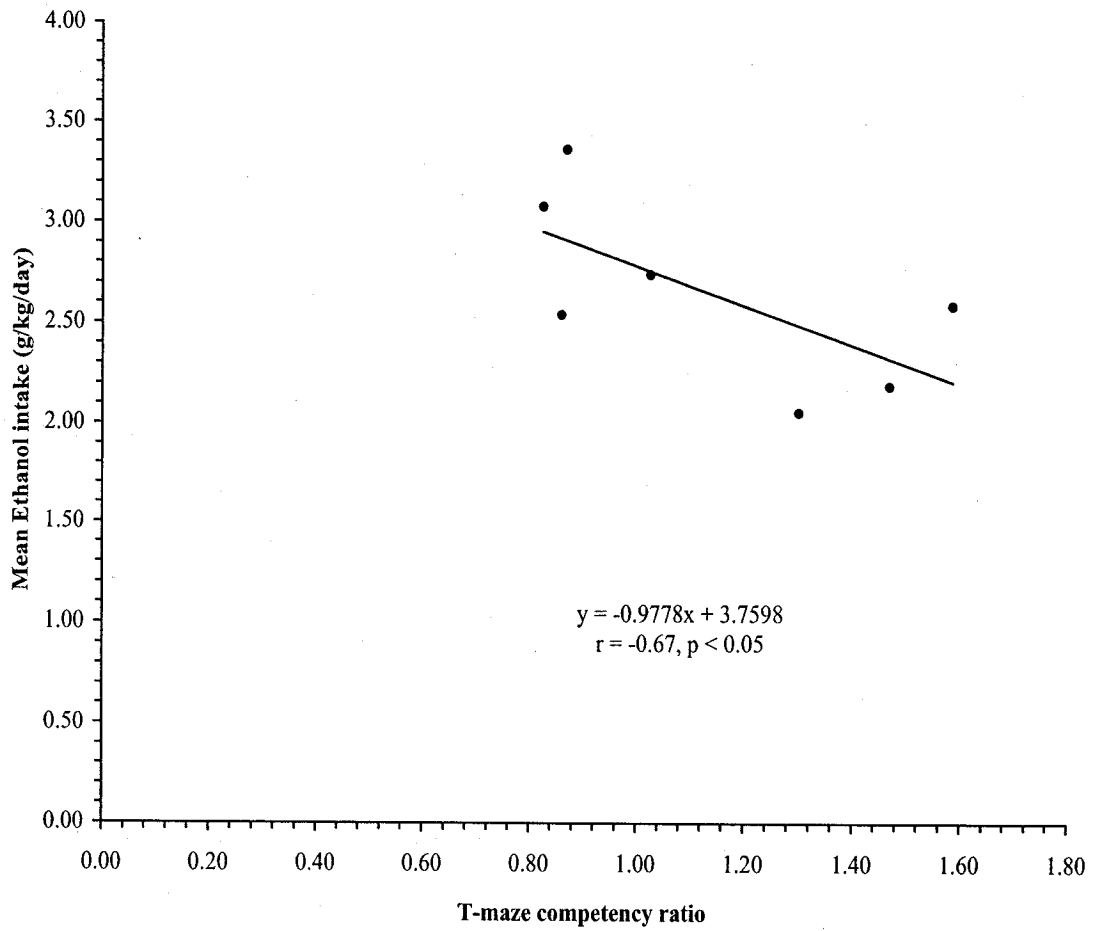


Figure 3. Scatterplot of mean ethanol intake of the “high” drinking animals and the index of maze competency in completing a 16-arm T-Maze. Ethanol intake values were obtained by averaging the final five days of 10% ethanol intake during the maintenance period.

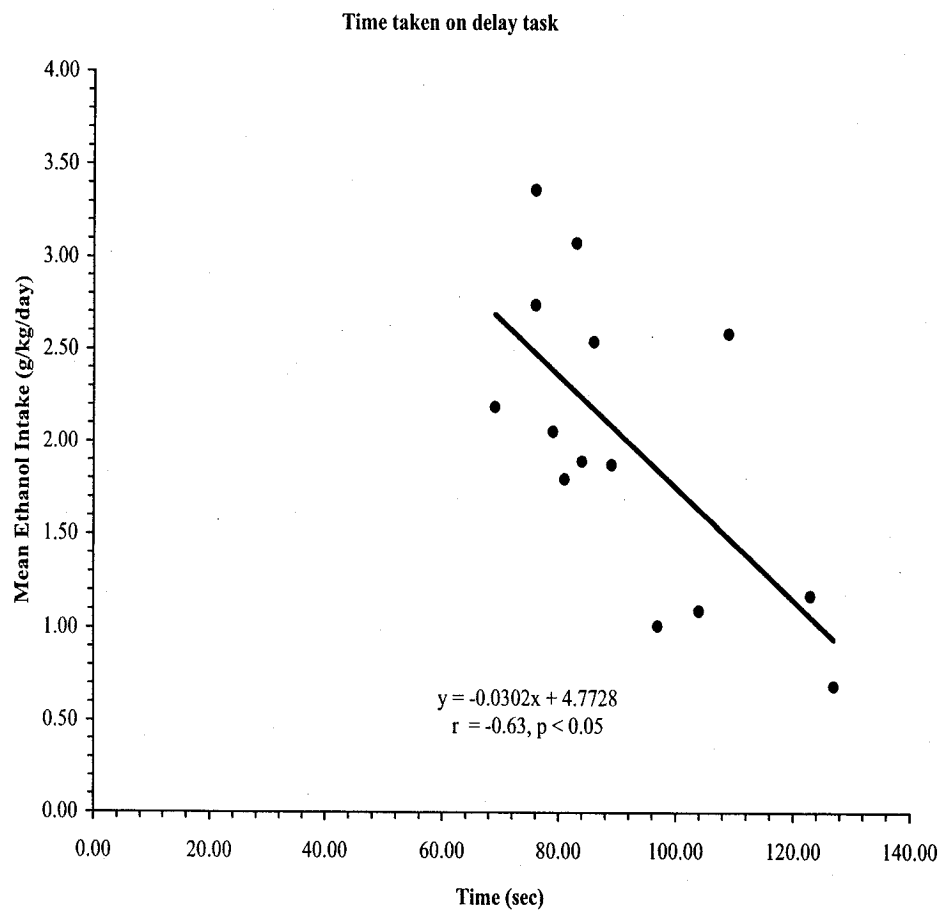


Figure 4. Scatterplot of mean ethanol intake and the time (sec) taken to complete the delay-interposed task in the radial maze. Ethanol intake values were obtained by averaging the final five days of 10% ethanol intake during the maintenance period.

Performance across learning tasks

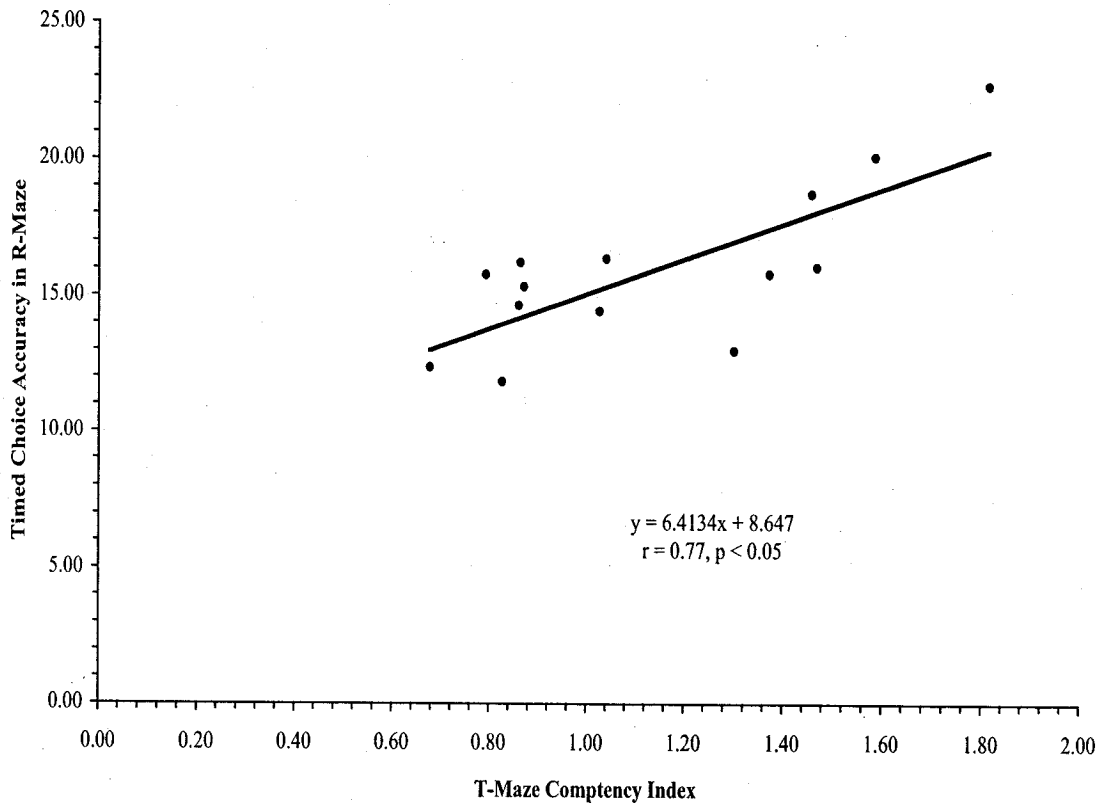


Figure 5. Scatterplot of individual's average time taken per choice in the 8-arm radial maze and the T-maze performance index derived from the ratio of a rats' errors and time in the T-maze.

Discussion

The main goal of Experiment 2 was to assess whether individual differences in learning and memory processes, as measured in a complex T-maze and a radial maze, were related to increased ethanol intake. Consistent with the findings obtained in Experiment 1, the results of Experiment 2 revealed that individual differences in learning ability, defined operationally as the competency to solve a complex T-maze, were correlated with increased ethanol intake. Thus, animals that had a lower index score of time and errors in the T-maze were also the highest drinkers. No comparable significant relationship between mean T-maze performance and ethanol intake was found in the "low drinking" group.

Since rats use their spatial orienting ability to solve mazes (Olton, 1979) we hypothesized that the findings observed in Experiment 1 may be, in part, mediated by individual differences in spatial ability. The results of Experiment 2 suggest that performance on variables measured during the acquisition of the radial maze did not differentiate the high drinkers from the low drinkers. The failure to find group differences in the number of trials to criterion, errors, time, and number of correct choices does not appear to support the notion that individual differences in spatial ability may mediate ethanol intake. However, it is also plausible to suppose that the radial maze task employed to assess spatial ability may have been too simple and thus readily and indiscriminately acquired by most animals.

Thus, the findings obtained in the delay-interposed task were of particular interest. Our results indicate that individual variations in choice accuracy, as revealed by the amount of time spent in the delay task, may be associated with the development of

increased ethanol intake. Regardless of group membership, rats that required less time to successfully complete the delay-interposed task also drank more ethanol.

Furthermore, in the delay-interposed task the low drinkers group made fewer correct choices and took more time to complete the task as compared to the high drinkers group. Since there were no significant group differences prior to the delay task, these results seem to suggest that the delay produced a greater disruption in the radial maze performance of the low drinkers as compared to the high drinkers. It is further speculated that the decrease in performance may be linked to individual differences in short-term spatial memory and that these differences may underlie the observed relationship between maze competency and ethanol intake in Experiment 1 (Pratt et al., 2002). In other words, animals endowed with an enhanced spatial memory may exhibit higher levels of ethanol intake, as they are able to effectively integrate and memorize information about the external and internal cues associated with ethanol consumption.

In the present experiment, individual performance across learning tasks was consistent. Overall, animals that were less competent in the T-maze were also the animals that took more time per choice while learning the initial radial maze task. For example, in the low drinkers group, competency in the T-maze was related to choice accuracy in the radial maze. These results seem to suggest that individual maze learning competence is comparable across maze types and that poor maze competence is related to decreased ethanol intake. Alternatively, our results may be interpreted to suggest that among low drinkers, timidity in maze exploration, as gleaned from the increased time taken on the delay task, may have impaired maze performance. A role for a behavioral factor such as timidity/meekness, in mediating the affinity to ingest ethanol has previously been

suggested by Salimov (1999). In reviewing the findings obtained from several behavioral studies, he found that rodents that displayed timidity/meekness across behavioral tasks were also likely to be classified as low ethanol drinkers. However, it is important to note that in the Salimov study (1999) the timidity/meekness factor was defined by behavior on multiple maze tasks, which did not include the radial, or T-mazes.

In summary, the results of Experiment 1 and Experiment 2 suggest that individual differences in learning ability, as assessed by performance in two different spatial tasks, seems to be related to the affinity to ingest ethanol. Specifically, it appears that individual differences in spatial ability and its components, such as spatial working memory are likely to be related to ethanol intake. If spatial learning per se, is involved in mediating the ethanol intake of outbred rodents then manipulating the brain structure most closely implicated in spatial learning should affect the acquisition of ethanol intake. Therefore, the next experiment was designed to address this hypothesis by examining the effect of hippocampal lesions on ethanol intake within a 23-hour continuous access paradigm. Furthermore, it was deemed important to determine if the effect of the lesion on ethanol intake in a continuous access paradigm was comparable to its effect in a different drinking paradigm such as the 1-hr limited access. The addition of an operant variable in the limited access paradigm would also allow us to detect any gross motor deficit that might result from the lesions.

Experiment 3: The effect of hippocampal lesions on the acquisition of ethanol intake in an operant paradigm and during subsequent 23-hr home cage free-choice ethanol drinking.

Given the notion that all animals must first learn to drink ethanol in order to develop an affinity and preference for its later continued consumption, we argued that learning and memory processes may be involved in the mediation of ethanol drinking behavior. The results observed in Experiment 1 and Experiment 2 support the hypothesis that individual differences in learning and memory processes may be an important factors in mediating, at least in part, the acquisition of ethanol intake in laboratory rodents. In particular, it appears that individual differences in spatial learning ability and spatial working memory may in fact be in some way related to the development of ethanol intake. We thus hypothesized that if spatial learning and spatial working memory are involved in mediating ethanol intake, then lesioning the brain structure most closely implicated in spatial learning should affect the acquisition of ethanol intake.

The role of the hippocampus in learning, specifically spatial learning (for a review see Good, 2002) continues to be at the center of an ongoing debate. The details of this debate are beyond the scope of this thesis but it is generally accepted that the brain hippocampal formation is an important central structure implicated in the mediation of learning and memory processes (Squire, 1992). Previous studies have also indicated that the hippocampus may play an essential role during learning of spatial tasks such as the radial maze (Nadel & Moscovitch, 1997). Through the accumulation of data obtained from testing in the radial maze, it has also been proposed that the hippocampus is a primary brain region involved in spatial working memory (Olton & Papas, 1979). Given

the findings of Experiment 1 and Experiment 2 suggesting the involvement of spatial learning and memory in ethanol intake, the purpose of the present study was thus to examine the effect of hippocampal lesions on the acquisition of ethanol intake in both limited-operand and 24-hr oral access paradigms.

Method

Subjects

Twenty-four male Wistar rats weighing 200g at the start of the experiment were individually housed in stainless steel cages in a room regulated for constant temperature and humidity. The colony had a 12: 12 hr light/dark illumination cycle. Water and food were available ad libitum throughout the experiment except during each two-hour limited access session. All animals were treated in accordance with the guidelines of the Canadian Council for Animal Care, and the Animal Care Committee of Concordia University approved all procedures.

Drugs

Ethanol solutions were prepared through a dilution of a 95% ethyl alcohol stock solution with tap water.

General Procedure

Following a two-week acclimatization period, animals were randomly assigned to one of four groups (n = 6 per group): hippocampal-lesion-ethanol (HLE), hippocampal-lesion-water (HLW), control-ethanol (CE), or control-water (CW). Following recovery,

animals were initially exposed to a 2hr limited-access operant paradigm for ascending concentrations of ethanol (2-10%). Animals were subsequently presented with 24hr access to ascending concentrations of ethanol (2-10%) in their home cage.

Surgical procedures

Bilateral lesions of the hippocampal formation were obtained by microinjecting the neurotoxin N-methyl-D-aspartate (Sigma Chemical Company) while the rats were anesthetized with sodium pentobarbital. Control animals received sham operations with the same procedures but without NMDA administration. See Appendix A for the stereotaxic coordinates used. The lesions were made with injections of a 5.1M solution of NMDA dissolved in 0.1M phosphate buffered saline. The NMDA solution was infused at a flow rate of 0.15 microlitres per minute at each site for 2.5 minutes and then allowed to diffuse for an additional 2.5 minutes following each injection.

Histology

When data analysis was complete, animals were given an overdose of sodium pentobarbital and then perfused transcardially with 0.9% saline followed by 10% buffered formalin. Each brain was removed immediately and then fixed in a 10% sucrose and formaldehyde solution. Brains were cut on a cryostat in the coronal plane at 30 um with every fifth slice kept for staining. Sections were stained with cresyl violet, according to standard procedures, for localization of the lesion site and size.

Operant Responding

A standard operant chamber (Coulbourn Instruments) equipped with a response lever connected to a liquid dipper apparatus was used. The activation of the dipper via depression of the lever resulted in presentation of 0.1 ml of liquid. Each animal was placed in an operant chamber for three consecutive 24-hr periods where the availability of water was contingent on the activation of the dipper. A fixed ratio (FR1) schedule of reinforcement was imposed during the first two days followed by an FR4 on the final training day. At the end of this 3-day training period, animals returned to their home cage where they were housed for the remainder of the study. After a one-day break, each animal received a daily two-hour test session in the operant chamber where they could lever press (FR4) for access to increasing concentrations of ethanol. An initial concentration of 2% (v/v) was presented for three-days. Each ethanol concentration was presented for a three-day period. Subsequently, ethanol solutions were raised in ascending concentrations of 2% until a final 10% concentration was reached. The 10% ethanol solution was then presented for an additional 4 days in order to establish stable ethanol intake. The number of responses, as well as the volume of ethanol consumed during each test sessions were recorded.

24-Hour Re-acquisition

Following the operant acquisition procedure described above, all animals were presented with a 24-hour ethanol acquisition paradigm that involved a free choice of ethanol along with tap water. Ethanol solutions, starting at 2%, were presented on a daily basis. The concentration of ethanol was increased by an increment of 2% every 2 days

until a final concentration of 10% ethanol was reached. Four additional days of 10% were given and constituted the maintenance period. The position of the fluids was switched on each ethanol presentation day to control for the influence of side preferences.

Data Analysis

Data collected from the experiment included mean ethanol intake (g/kg/day) in both the limited and continuous access paradigms. In the operant task, the number of responses as well as the volume of ethanol consumed (milliliters) during each test session were recorded and the latter was converted into grams of absolute ethanol consumed per kilogram of body weight (g/kg). Potential group differences during the training period were assessed by a one-way analysis of variance with group as the main factor. Potential group differences within the limited access and continuous access paradigms were assessed by separate 4 (group) x 6 (concentration), analysis of variance (ANOVAs). Simple main effects and simple comparisons were employed to determine the source of interactions. The data were analyzed using either the CLR ANOVA software application for a Macintosh computer or the SYSTAT software application for an IBM computer. An alpha level of .05 was assumed for all analyses.

Results

Histology

Figure 6 illustrates the extent of the smallest and largest of the hippocampal lesions, respectively. The NMDA injections produced extensive cell loss in dentate gyrus, subiculum and all principle subfields of the HPC for each lesion rat. The damage to the

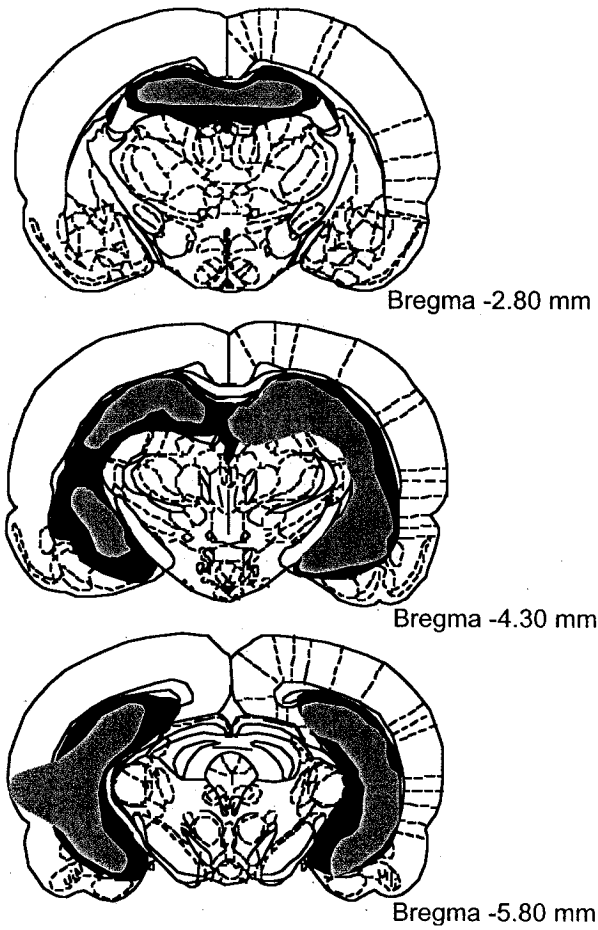


Figure 6. The location and extent of the hippocampal lesions is illustrated in coronal sections. The top section is located 2.8 millimeters (mm) posterior to bregma, the middle section is 4.3 mm posterior to bregma, and the bottom section is 5.8 mm posterior to bregma. In all three sections, the lightly shaded area indicates the largest lesion and the darkly shaded area indicates the smallest lesion.

dorsal HPC was pronounced in all rats, but in 4 rats, there was some minor sparing of dentate granule cells. There was minor sparing of cells in the dorsal lateral CA fields bilaterally in 3 rats, and unilaterally in one rat. The extent of damage to the ventral HPC was also extensive, where there was bilateral loss of cells in this area in each rat. However, in 2 rats there was some minor sparing of the CA fields unilaterally and 2 rats with minor bilateral sparing. In all HPC rats, there was also some damage to the posterior parietal cortex where the injection cannulae were inserted. Some rats also sustained minor damage to the fornix/fimbria. No damage was found in the thalamus or the rhinal cortex except for one rat that had unilateral damage to the rhinal cortex.

Training Phase

Data for mean lever presses for access to obtain water by each group during the training phase are presented in Figure 7. A one way analysis of variance conducted on the lever press data found no significant group [hippocampal-lesion-ethanol (HLE), hippocampal-lesion-water (HLW), control-ethanol (CE) and control-water (CW)] differences in lever pressing for water during the training phase [$F(3, 17) = 0.916, p > 0.05$]. Thus suggesting that the hippocampal lesions did not disrupt an animal's ability to learn to lever-press for water in an operant paradigm.

Phase 1: Acquisition of lever pressing for limited access ethanol.

As shown in Figure 8, the mean number of lever presses differed significantly across groups [$F(3, 17) = 4.778, p = 0.014$]. A post hoc Tukey analysis revealed that the CE group pressed significantly more for access to ethanol as compared to the HLE group

($p < 0.05$). Furthermore, there were no significant differences in the number of lever presses carried out by the control group (CW), and the hippocampal lesion group (HLW) when presented with water ($p > 0.05$). This supported the finding initially observed during the training phase. Interestingly, there were no significant differences in the number of lever presses observed between the CW, HLW, HLE groups ($p > 0.05$), despite the fact that the HLE was presented with ethanol and the other groups (CW & HLW) received water. These results seem to suggest that the lesions of the hippocampus had a specific effect on the HLE group's lever pressing for access to ethanol in a 2-hr limited access paradigm.

The main effect of concentration [$F(5, 15) = 1.119, p = 0.357$] was not significant, thus indicating that the rats did not differ in their level of lever pressing across concentrations. A significant interaction between group and ethanol concentration [$F(15, 85) = 1.886, p = 0.035$], was investigated with a test of simple effects. The analysis of simple effects reflected the finding that experimental groups differed significantly in lever pressing at individual concentrations of 6% [$F(3, 17) = 5.820, p = 0.006$], 8% [$F(3, 17) = 3.374, p = 0.043$], 10% [$F(3, 17) = 6.863, p = 0.003$], and 10% maintenance [$F(3, 17) = 6.936, p = 0.003$]. Analysis of simple comparisons revealed that the control-ethanol group (CE) emitted significantly more lever presses than the other groups across the concentrations with the exception being at the 8% ethanol solution. The only significant group difference at 8% occurred between the control ethanol group and the hippocampal lesion group.

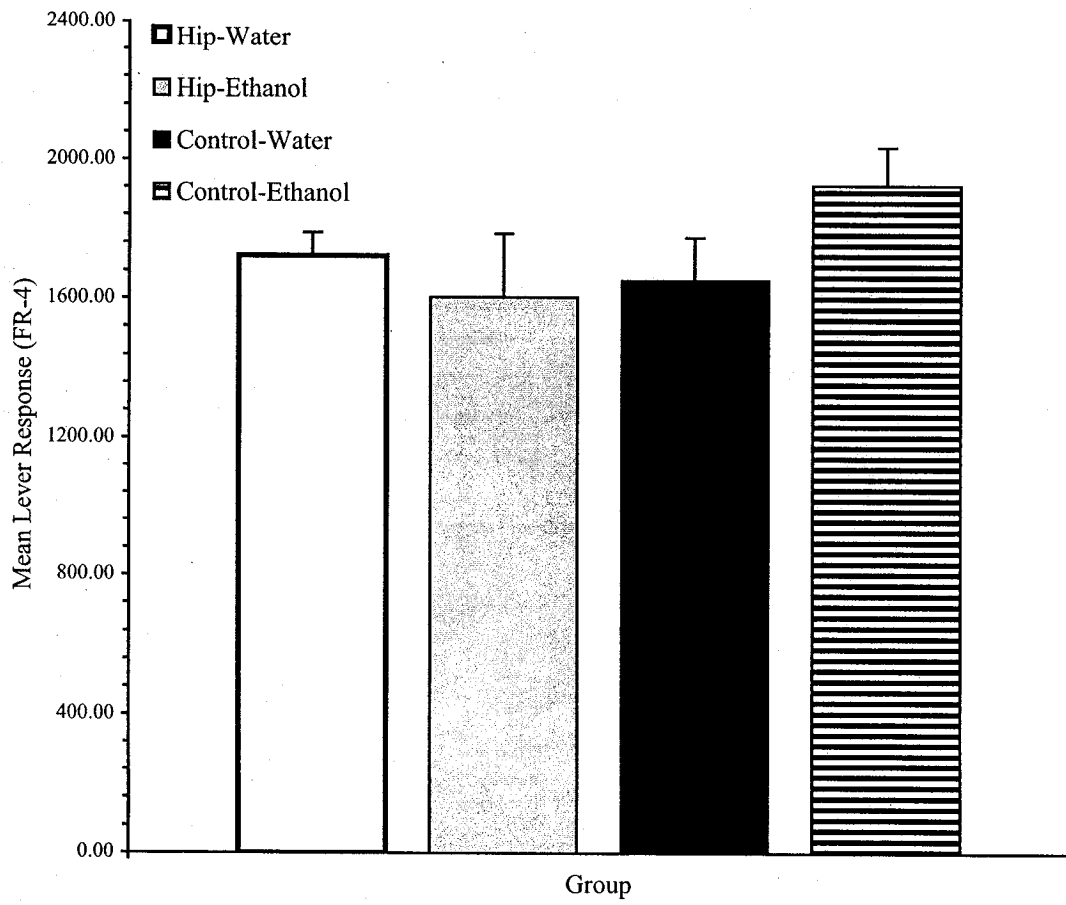


Figure 7. Group mean lever responses (FR-4) for water during the 24-hr training session prior to the start of experimental sessions.

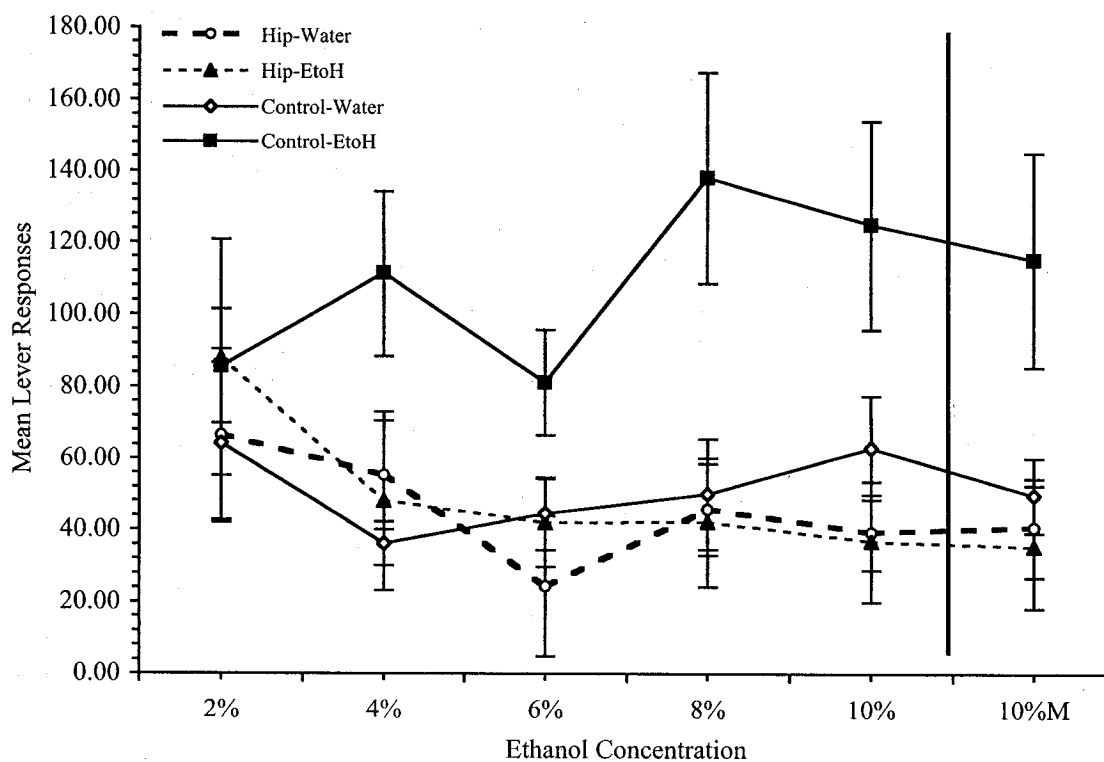


Figure 8. Mean lever responses for each 2-hr test session across days. Animals received either sham or hippocampal lesions and the fluid presented were either water or ethanol. Reference to ethanol concentration in the x-axis applies only to animals receiving ethanol.

Phase 2: Home cage acquisition of ethanol drinking in a continuous access paradigm.

Data for mean ethanol intake (g/kg) across ethanol concentrations within a 23-hr continuous access paradigm are presented in Figure 9. Analysis of ethanol intake (g/kg) across days revealed significant group differences [$F(3, 17) = 12.959, p = 0.0001$]. Post-hoc Tukey tests indicated that overall the CE group consumed more ethanol (g/kg) than the other three groups ($p < 0.05$). Ethanol intake (g/kg) for the CW, HLE, and HLW groups did not differ ($p < 0.05$). A significant main effect of concentration [$F(5, 15) = 16.608, p = 0.0001$], and an interaction between group and ethanol concentration [$F(15, 85) = 3.321, p = 0.002$] were obtained. The analysis of simple effects reflected the finding that the control-ethanol group drank significantly more ethanol than the other groups at the concentrations of 2% [$F(3, 17) = 6.103, p = 0.005$], 4% [$F(3, 17) = 7.705, p = 0.002$], 6% [$F(3, 17) = 10.783, p = 0.000$], 8% [$F(3, 17) = 10.699, p = 0.000$], 10% [$F(3, 17) = 3.863, p = 0.028$], and 10% maintenance [$F(3, 17) = 7.10, p = 0.003$]. However, simple comparisons revealed that the 10% ethanol intake of the control-ethanol group was elevated only as compared to the hippocampal-lesion group. During the maintenance period the control-ethanol group consumed more ethanol as compared to the hippocampal ethanol lesion and control water groups.

Data for mean water intake (mls) during 23-hr continuous access are presented in Figure 10. The main effect of group [$F(3, 17) = 1.409, p = 0.274$] and the interaction between group and concentration [$F(15, 85) = 1.254, p = 0.278$] were not significant, thus indicating that the groups did not differ in their level of water intake. A significant main effect of concentration [$F(5, 15) = 26.122, p = 0.000$] indicated that water intake varied

across the ethanol concentration presented. In other words, as the ethanol concentration increased, all groups increased their water intake.

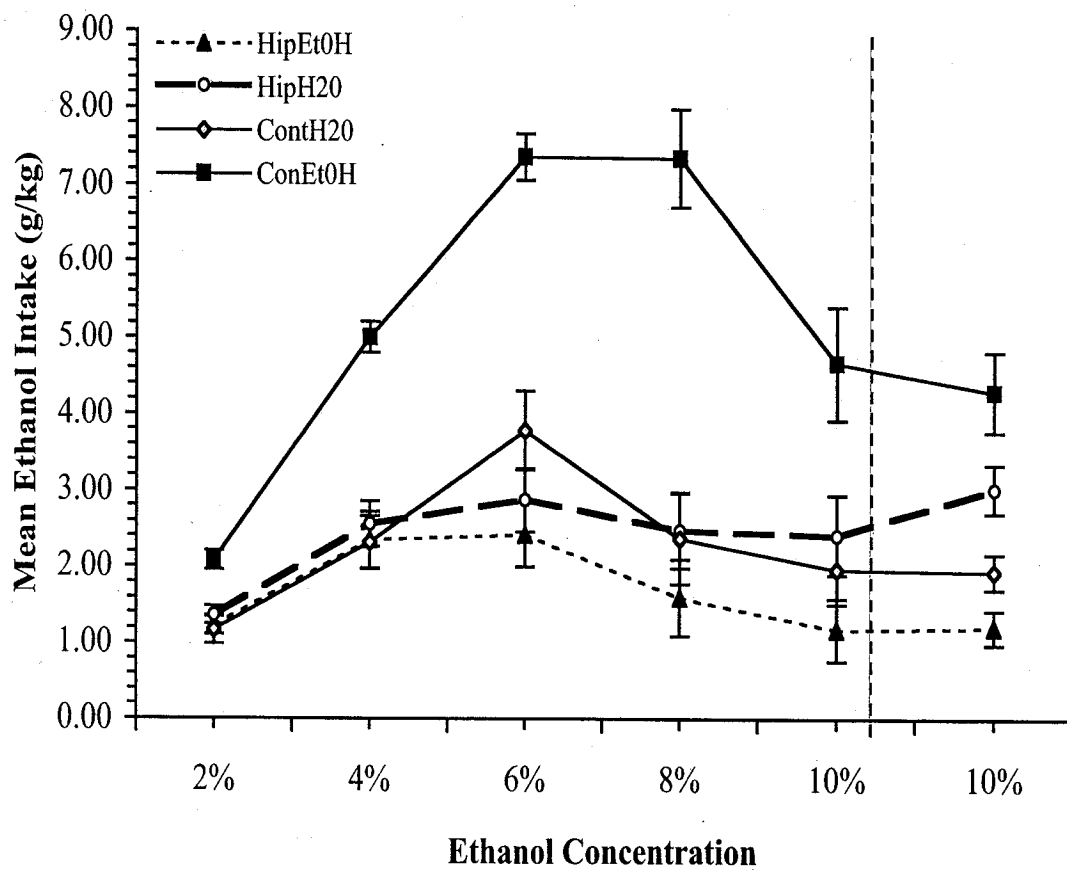


Figure 9. Mean ethanol intake (g/kg) for each 24-hr test session across ethanol concentrations. Animals previously exposed to 2-hr limited operant access paradigm were then exposed to a 24-hr home cage re-acquisition to ethanol.

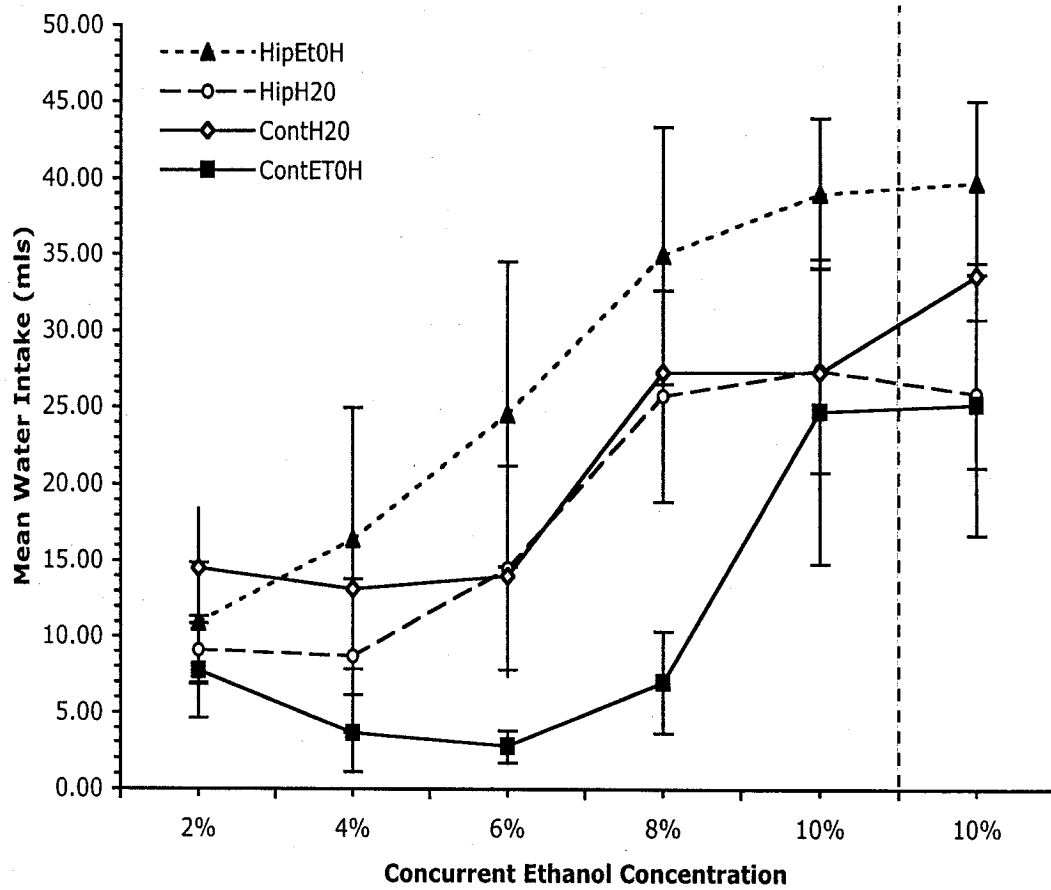


Figure 10. Group mean water intake (mls) for each 24-hr test session across ethanol concentrations.

Discussion

Given that the findings of Experiment 1 and Experiment 2 suggested the involvement of spatial learning and memory processes in ethanol intake, the goal of Experiment 3 was to assess the effects of hippocampal lesions on ethanol and water consumption within a limited operant paradigm as well as within a 23-hr continuous access paradigm. The analysis of the lever press data suggested that lesions to the hippocampus appear to disrupt an animal's ability to acquire ethanol-drinking behavior when examined within a limited access operant paradigm. Specifically, the hippocampal-lesion-ethanol group (HLE), as compared to its control group (CE), displayed a significantly reduced amount of lever pressing for access to ethanol. Interestingly, the lesions did not have an effect on water consumption during limited access as the amount of lever presses for access to water by the hippocampal lesion water (HLW) and its control group (CW) did not differ. These results appear to provide initial support for the notion concerning the involvement of the hippocampus in mediating the acquisition of ethanol intake in a limited access operant paradigm. It is hypothesized that the hippocampal lesions may have affected ethanol intake indirectly by disrupting the processes necessary for the animals to associate the behavioral response of lever pressing with the ingestion of ethanol and thus any post-ingestional effects associated with its pharmacological properties. Motor deficits cannot account for this finding, since lever pressing during training was similar for all groups.

Our results do not support previous findings suggesting that lesions to the hippocampus produced higher levels of operant responding (Shull & Holloway, 1985). The disparity in results obtained by Shull & Holloway (1985) and in Experiment 3 may

be in part explained by strain differences, as Sprague-Dawley rats were employed in the Shull & Holloway study. Further testing differences such as the use of a variable interval schedule for access to food pellets (Shull & Holloway, 1985) may also in part account for the discrepancy between the results of these two studies.

The results of the ANOVA on the continuous access data revealed that lesions to the hippocampus had a significant effect on ethanol intake (g/kg) as compared to the ethanol controls. In fact, the ethanol intake of the hippocampal lesion group (HLE) was decreased across all concentrations when compared to the control-ethanol group. Any differences in prior exposure to ethanol cannot explain our findings in the continuous access paradigm, as both groups were equally exposed to ethanol in the limited access paradigm. The results obtained in the continuous access paradigm corroborate the findings previously obtained in the limited access paradigm. Thus, it appears that the hippocampus may also be involved in mediating the acquisition of ethanol intake in a continuous access paradigm.

Interestingly, the ethanol intake of the hippocampal lesion group (HLW) and the control water group, both previously presented only with water in the limited access paradigm, did not differ. It is difficult to explain these findings as one might expect the control water group to display ethanol intake approaching that of the control ethanol group. However, it is important to remember that the groups were not exposed to ethanol to the same extent and thus were not likely to have formed adequate comparison groups. Our results cannot be explained by a general fluid effect, as water intake was not different across groups. As is the case in the limited access paradigm, we speculate that the lesions affected ethanol intake during continuous access by disrupting processes necessary for

animals to make the association between ethanol intake and its post-ingestional properties.

In summary, the results of Experiment 3 suggest that the hippocampus may in part mediate the acquisition of ethanol intake in both a limited and continuous access paradigm. The finding that lesions to the hippocampus affect ethanol intake also lends support to the notion that individual differences in learning and spatial ability may be related to ethanol intake. Given the exploratory nature of the present study, in the future studies should consider examining the effects of lesions to specific regions of the hippocampus on ethanol intake. However, the main purpose of Experiment 3 was to determine if lesions to the hippocampus, an area largely associated with spatial learning, would affect the acquisition of ethanol intake.

Given the possible role for learning and memory processes in mediating ethanol self-administration, a more interesting and pertinent issue for this thesis was whether individual differences in ethanol self-administration are in fact, independent of any environmental contributions (e.g.; type of paradigm, location, type of housing). Thus, the purpose of Experiment 4 and Experiment 5 was to assess the influence of environmental variables on individual differences in ethanol intake. Experiment 4 was designed specifically to assess any influence that a drinking paradigm itself might exert on ethanol intake, as well as the interrelation amongst commonly employed drinking paradigms.

Experiment 4: The effect of drinking paradigm on the acquisition of ethanol intake.

Within the context of animal models of self-administration, several drinking paradigms have been used to study the processes regulating the acquisition and maintenance of ethanol intake (Gill, et al., 1986; Linesman, 1990; Marcucella et al., 1984; Samson, 1986). Length of access to ethanol, and the mode of ethanol presentation (oral intake presentation vs. operant training) are only two of several variables commonly manipulated as a means to measure the level of voluntary ethanol intake. However, it has been suggested that the abundance of methods to promote and measure voluntary ethanol intake has created a wealth of data but also a lack of uniformity that makes comparisons across paradigms difficult (Deitrich, 1992; Myers, 1996).

Embedded in the differential use of these drinking paradigms is the assumption of the presence of core processes that underlie ethanol intake, and are invariant to the procedure of inducing ethanol intake employed. If the assumption that similar core processes underlie voluntary ethanol intake regardless of the induction procedures used were true, then one would expect a high degree of consistency in the results obtained across different paradigms. It has however, been suggested that the body of data generated by various drinking paradigms may in fact be intrinsically tied to the type of paradigm employed to initiate ethanol consumption (Boyle et al., 1994; Deitrich, 1992; Myers, 1996). The possibility that a drinking paradigm may influence subsequent ethanol intake suggests that individual animals may consume different amounts of ethanol according to the drinking paradigm employed. An earlier study conducted in our laboratory (Boyle et al., 1994) found that the type of ethanol exposure paradigm used to initiate drinking could in fact affect subsequent ethanol intake. Furthermore, a positive

correlation was obtained between the amount of ethanol intake in a 24 hr access paradigm and the amount consumed in a subsequent limited access paradigm. These findings were taken to suggest that the core processes regulating voluntary oral intake in a 24 hr access paradigm and oral intake in a limited access paradigm were likely to have a common basis.

While of interest, the results obtained by Boyle et al., 1994 would be enhanced by a further elaboration that will include the commonly utilized method of operant responding for access to ethanol. Additionally, for comparison purposes, a distinct cage for oral access to ethanol should be included, since the procedure of operant responding for ethanol typically involves the removal of animals from their home cage and subsequently provides them with a distinct environment (operant chamber) for the duration of the ethanol access period. The purpose of the present study was to further explore the interrelation of drinking levels across paradigms by examining the relationship between operant responding for limited access to ethanol, home cage limited access, distinct-cage limited access and 24-hr continuous access ethanol procedures.

Method

Subjects

Sixty male Wistar rats (Charles River, Quebec) weighing 200 g at the start of the experiment were individually housed in stainless steel cages in a room regulated for constant temperature and humidity. The colony had a 12:12 hr light/dark illumination cycle. All testing took place during the light portion of the cycle. Rats were given a two week acclimatization period during which they were handled daily. Water and food

(Purina Rat Chow) were available ad libitum throughout the experiment except during each two-hour test session. All animals were treated in accordance with the guidelines of the Canadian Council for Animal Care, and the Animal Care Committee of Concordia University approved all procedures.

Drugs

Ethanol solutions were prepared through a dilution of a 95% ethyl alcohol stock solution with tap water.

Apparatus

A standard operant chamber (Coulbourn Instruments, Allentown, Penn.) equipped with a response lever connected to a liquid dipper apparatus was used for the operant portion of this study. The retractable lever was situated on the end wall and adjacent to it was a receptacle containing an electronically activated dipper. The activation of the dipper via depression of the lever resulted in presentation of 0.1 ml of liquid, which was available for 4 seconds.

Procedure

Phase 1.

Following a one-week acclimatization period, animals were randomly assigned to one of three conditions. Due to a limited number of operant boxes and distinct cages, 30 animals were exposed to the limited access home cage procedure, 18 were exposed to an operant responding for access to ethanol paradigm and the final 12 were exposed to a

limited access oral procedure in a distinct-cage setting. Ethanol was the sole fluid presented during the limited access period.

Group 1 or the operant group, was exposed to an ethanol acquisition paradigm that required the performance of a lever press to gain access to the ethanol solution. To facilitate the acquisition of lever pressing, each animal was placed in an operant chamber for three consecutive 24-hr periods where the availability of water was contingent on the activation of the dipper via a depression of the response lever. A fixed ratio (FR1) schedule of reinforcement was used for the first two days followed by an FR4 on the final training day. At the end of this 3-day training period, animals were returned to their home cage where they were housed for the remainder of the study. After a one-day pause, each animal received a daily two-hour test session in the operant chamber where they could lever press (FR4) for access to increasing concentrations of ethanol. An initial concentration of 2% (v/v) was presented for three-days. Each ethanol concentration was presented for a three-day period. Subsequently, ethanol solutions were raised in ascending concentrations of 2% until a final 10% concentration was reached. An additional 4-days of 10% ethanol were presented and constituted the maintenance period. The number of responses, as well as the volume of ethanol consumed during each test sessions were recorded.

The second group of animals was presented with a 2-hr limited access of increasing ethanol concentrations in their home cage (Home Cage Group). The presentation schedule and concentrations of ethanol presented were as described in the operant procedure. The presentation schedule and concentrations of ethanol provided to the third group were as described in the home cage procedure with the exception that

animals in this group were presented with a 2-hr limited access of increasing concentrations in an operant-like chamber (Distinct Cage Group). Access was non-operant and only required the animal to drink the fluid presented.

Phase 2.

Following the acquisition procedures described above, all animals were presented with a home cage 24-hour ethanol acquisition paradigm that involved a free choice of ethanol along with tap water. The position of the ethanol and water tubes was alternated on a daily basis to prevent the development of a position bias. Ethanol solutions, starting at 2%, were presented daily. The concentration of ethanol was increased by an increment of 2% every 2 days until a final concentration of 10% ethanol was achieved. Four additional days of 10% ethanol was given and constituted the maintenance period.

Data Analysis

Data collected from the experiment included mean ethanol intake (g/kg/day) in both the limited and continuous access paradigms. In the operant task, the number of responses as well as the volume of ethanol consumed (milliliters) during each test session was recorded and converted into grams of absolute ethanol consumed per kilogram of body weight (g/kg). Since we were interested in examining any relationship between the types of drinking paradigm employed and differential ethanol intake, the level of ethanol consumed was first examined by comparing group means and then individual intake in limited access was correlated with subsequent 23-hours continuous access intake. Potential group differences within the limited access and continuous access paradigms

were assessed by separate 3 (training group) x 19 (day) and 3 (training group) x 14 (day) respectively, analysis of variance (ANOVAs) with the last factor as a repeated measure. Simple main effects and simple comparisons were employed to determine the source of interactions. The relationship between individual drinking levels across drinking paradigms was analyzed using a Pearson product-moment correlation (Keppel, Saufley, Tokunaga, 1992). The data were analyzed using either the CLR ANOVA software application for a Macintosh computer or the SYSTAT software application for an IBM computer. An alpha level of .05 was used for all analyses.

Results

Within the limited access paradigm, mean ethanol intake (g/kg) across days is presented in Figure 11. The analysis of variance revealed a significant main effect of group [$F(2, 1026) = 16.18, p < 0.0001$], day [$F(18, 1026) = 30.56, p < 0.0001$] and an interaction between group and day [$F(36, 1026) = 4.240, p < 0.0001$] suggesting that, with the exception of day 3 (2%) and day 4 (4%), rats trained with the operant acquisition procedure consumed more ethanol across days when compared to rats that were exposed to ethanol in their home cage ($p < 0.05$). When compared to rats exposed to ethanol in a distinct cage setting, the operant group consumed larger amounts of ethanol from day 9 (6%) to day 19 (10%), excluding day 15 (10%) ($p < 0.05$). Animals that received their ethanol in a distinct cage setting as compared to their home cage counterparts, consumed larger amounts of ethanol on days 6-10 (6% and 8%) and days 17 and 19 (10%).

Data for the mean ethanol intake (g/kg) within a 23-hr continuous access paradigm, across days are presented in Figure 12. Similar to the results obtained in the

limited access paradigm, ANOVA revealed a significant main effect of group [$F(2, 741) = 11.68, p < 0.0001$], day [$F(13, 741) = 24.27, p < 0.0001$], and an interaction between group and day [$F(26, 741) = 10.26, p < 0.0001$]. These results suggested that on average across days, the operant group consumed larger amounts of ethanol than either the home cage group or the distinct cage group. Analysis of simple effects and simple comparisons revealed that the operant group, as compared to the home cage and distinct cage groups, maintained their higher level of ethanol consumption across concentrations ranging from 4% to 10% ($p < 0.05$). The distinct cage group consumed larger amounts of ethanol than the home cage group at day 2 (2%) and day 6 (6%). However, on day 11 (10%) and day 13 (10%) the home cage group consumed more ethanol than the distinct cage group ($p < 0.05$).

As shown in Fig. 13 and Fig. 14 a significant positive correlation between limited access intake of a 10% ethanol solution (g/kg) during the final 4 days of the phase 1 acquisition period and the continuous access intake of the 10% ethanol solution (g/kg) during the final 4 days of the phase 2 acquisition period was obtained for the distinct cage group [$r(10) = +0.67, p < .05$] and the home cage group [$r(28) = +0.66, p < .05$].

In other words, individual animals maintained their relative drinking status across both drinking paradigms. Animals that were high drinkers in limited access were also high drinkers in the subsequent continuous access paradigm. As can be seen in Fig. 15, no significant correlation was obtained between limited access and continuous access for the operant group. Thus, animals in the operant group did not maintain their relative drinking status across drinking paradigms.

The results of Experiment 4 seem to suggest that the use of an operant to train

animals to consume ethanol produced a higher level of ethanol intake than home cage or distinct cage settings. However, it also appears that while the use of an operant increased the group's mean ethanol intake during both limited and continuous access, the same animals did not contribute to this finding as no correlation was obtained between limited and continuous access.

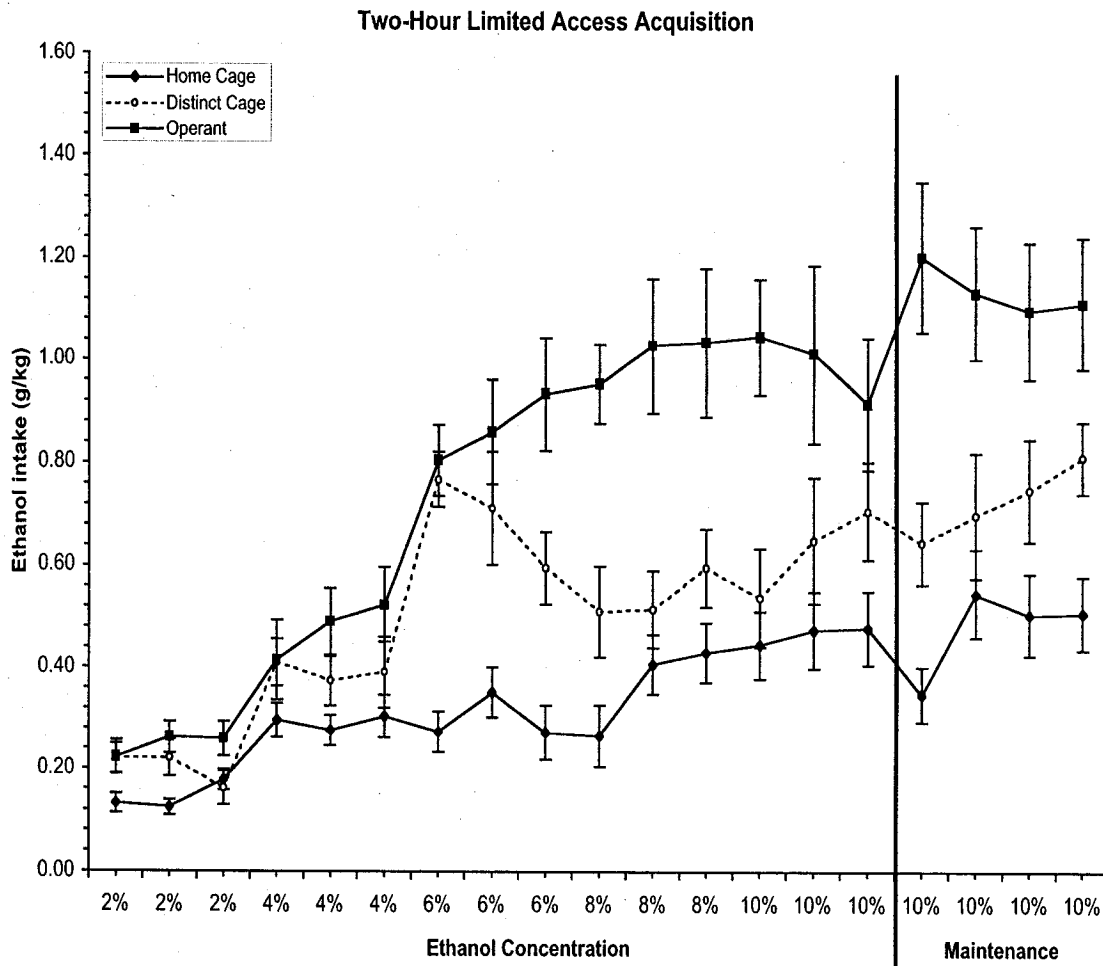


Figure 11. Mean ethanol intake (g/kg) for each 2-hour test session across ethanol concentrations. Animals were exposed to an ethanol acquisition procedure in one of three paradigms: home cage-oral, a distinct cage-oral, or operant chamber-lever press. Vertical lines represent S.E.M.

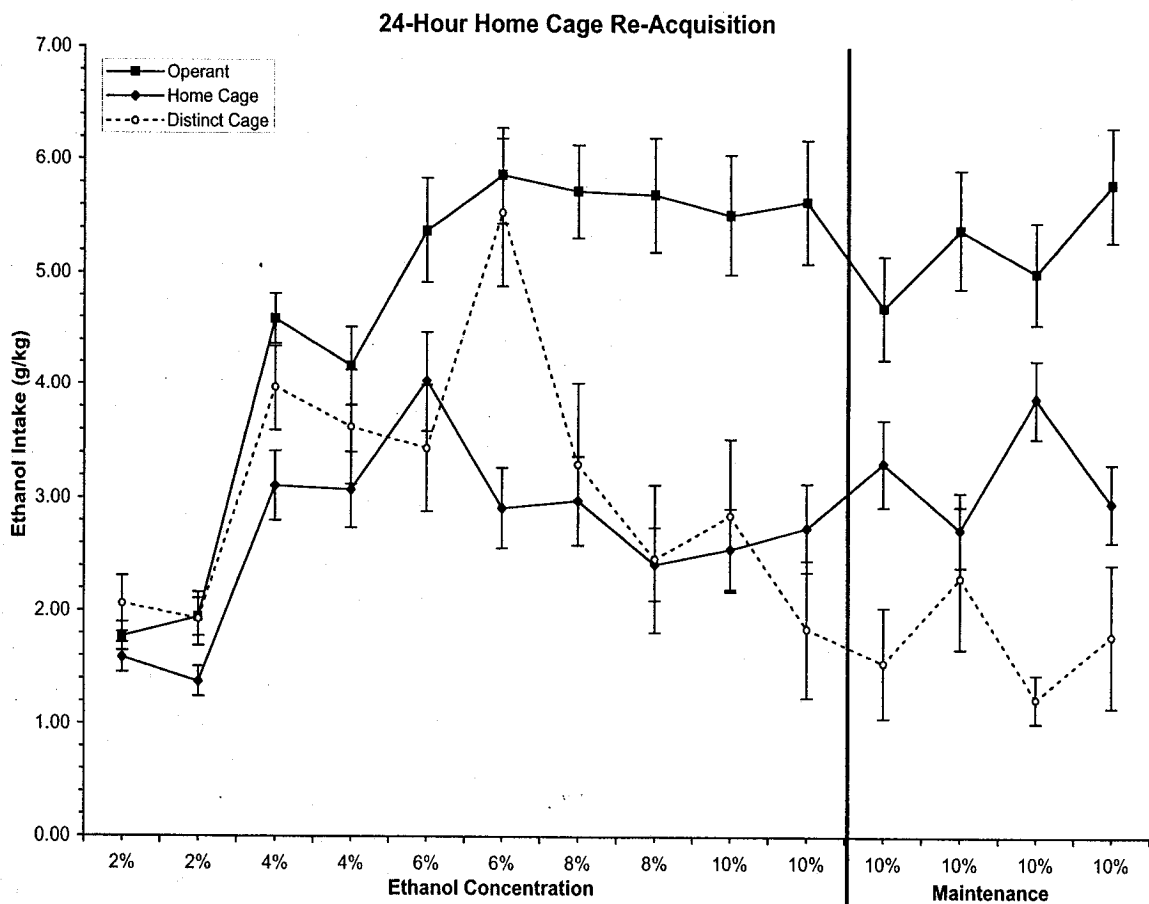


Figure 12. Mean ethanol intake (g/kg) for each 24-hour test session across ethanol concentrations. Animals previously exposed to one of three limited access procedures were then exposed to a 24-hour home cage re-acquisition to ethanol.

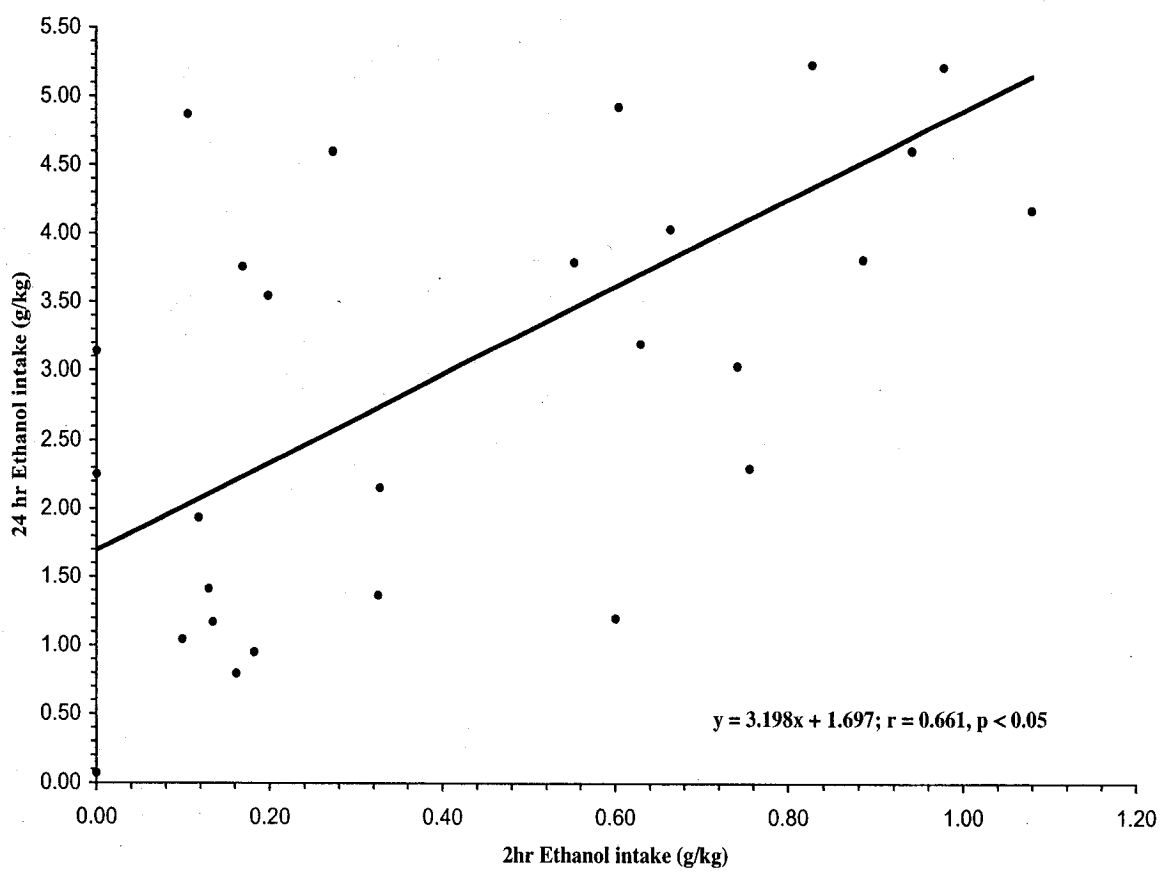


Figure 13. Scatterplot of mean ethanol intake (g/kg) for animals exposed to the initial 2-hr home cage limited access procedure and subsequent ethanol intake during the 24-hr home cage re-acquisition procedure.

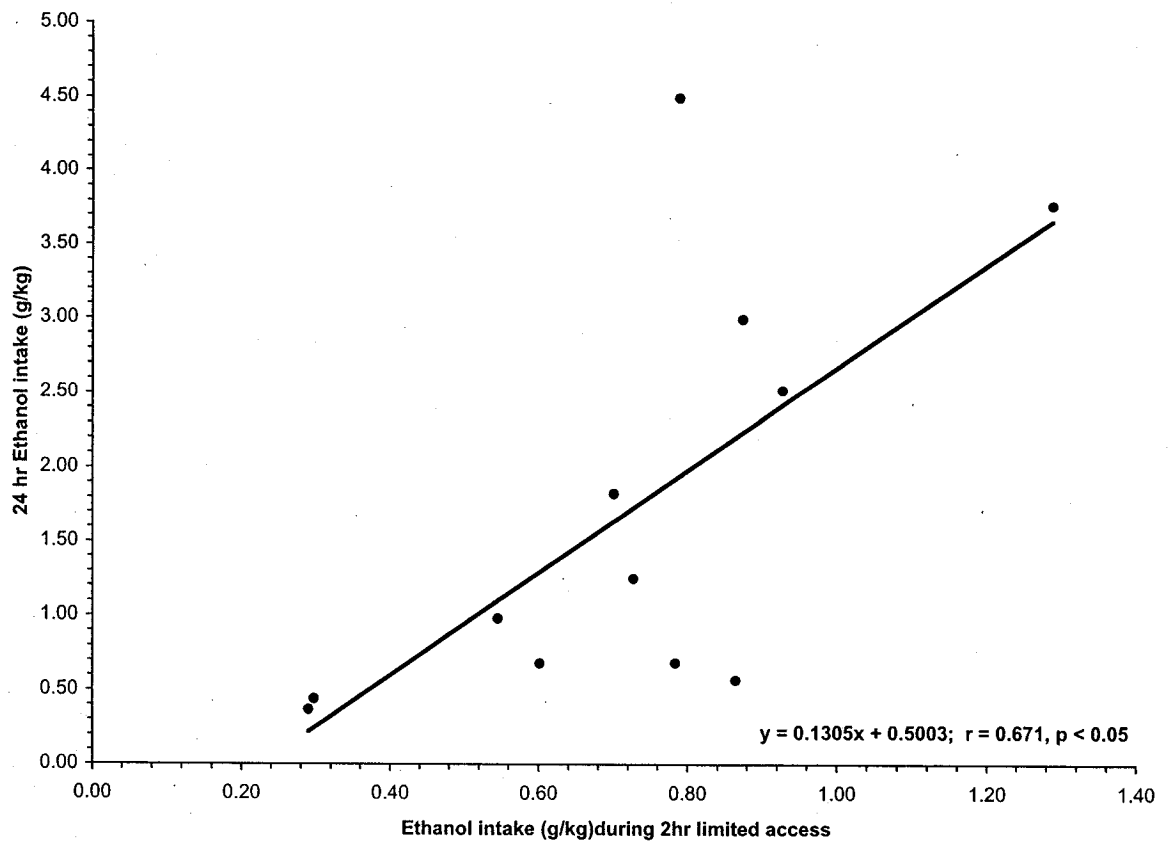


Figure 14. Scatterplot of mean ethanol intake (g/kg) for animals exposed to the initial 2-hr distinct cage limited access procedure and subsequent ethanol intake during the 24-hr home cage re-acquisition procedure.

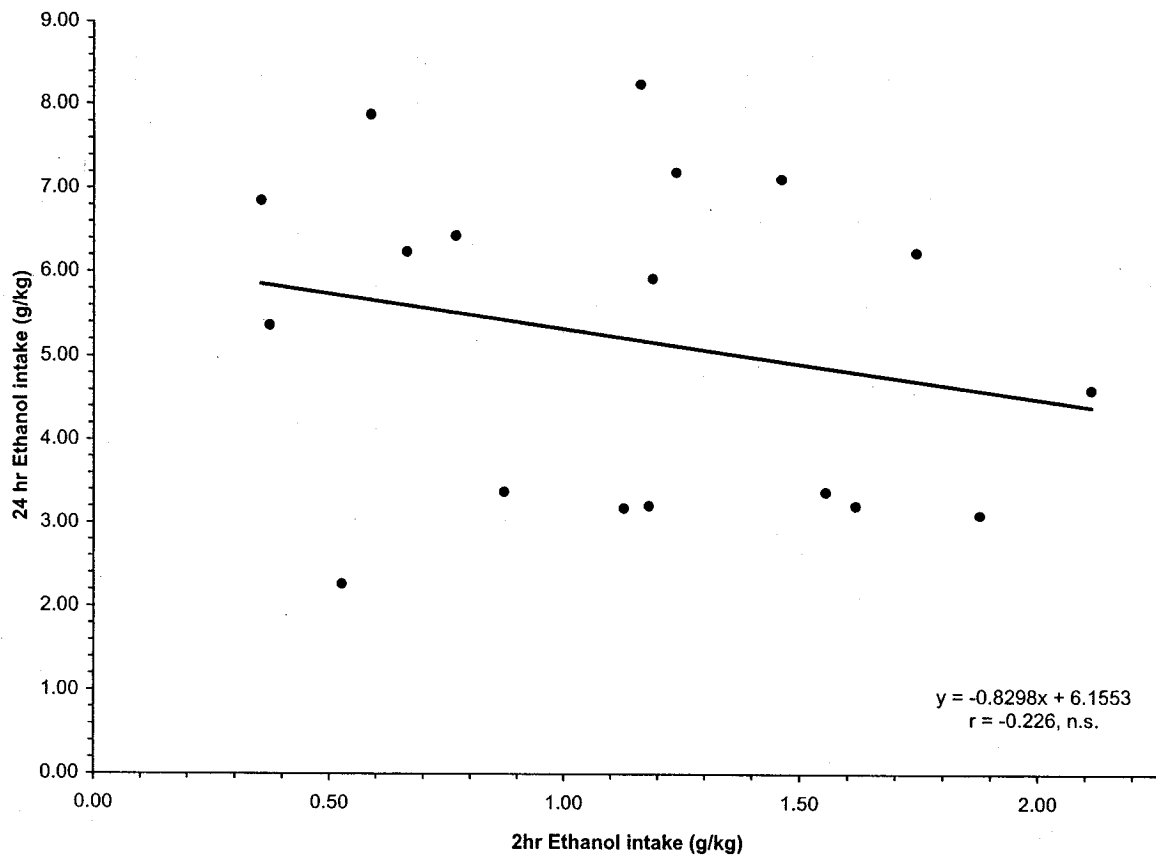


Figure 15. Scatterplot of mean ethanol intake (g/kg) for animals exposed to the initial 2-hr operant limited access procedure and subsequent ethanol intake during the 24-hr home cage re-acquisition procedure.

Discussion

The main purpose of this experiment was to assess the stability of drinking patterns across paradigms by examining the relationship between limited access and 24-hr continuous access procedures for ethanol. The primary finding of this study was that animals exposed to a limited-access operant procedure consumed more ethanol than animals exposed to either a limited access in the home cage or limited access in a distinct cage procedure.

During limited access, the groups were undistinguishable in their ethanol drinking until the more pharmacologically relevant concentration of (6%) was reached. From this point on the operant group consumed more ethanol than either the distinct cage or home cage groups. Interestingly, merely providing animals with a distinct drinking environment also appeared to increase ethanol intake as animals exposed to a limited access procedure in a distinct-cage consumed more ethanol than their home cage counterparts. Thus, it seems that providing animals with a distinct location for their ethanol consumption as well as superimposing an operant on the availability of ethanol results in increased ethanol intake. It is interesting that in the present study operant self-administration was associated with higher levels of ethanol intake than unconditioned free access. Previous studies have demonstrated that adding an operant can decrease ethanol intake (Samson et al., 1988). For example, Samson et al. (1988) have shown that the introduction of an operant schedule decreased 24-hour intake as compared to 24-hour free access. The discrepancy between the findings of the present study and the Samson study may be linked to procedural differences as we examined the effect of adding an operant within a 2-hr limited access paradigm while they employed a 24-hr operant paradigm.

In the second phase of our study, the group previously exposed to an operant procedure during limited access consumed more ethanol during the 24-hr re-acquisition paradigm than the other groups. Thus, overall the group that previously learned to drink ethanol in the operant paradigm, continued to drink larger amounts of ethanol even when tested in a different ethanol acquisition paradigm.

Since we were primarily interested in the relationship of drinking status across ethanol acquisition paradigms, we conducted within group correlations between the level of limited access ethanol intake and subsequent continuous access ethanol intake. We hypothesized that if the variability in drinking behavior observed between rodents is primarily mediated through ethanol's pharmacological properties then where animals consume ethanol after they have learned to drink should not be an important factor in determining any subsequent ethanol intake. However, examinations of the correlations between mean ethanol intake during the initial 2hr access paradigm and ethanol intake during subsequent 24hr access revealed an unsymmetrical relationship between individual ethanol intakes across paradigms type. A positive correlation was found between ethanol intake in both the 2hr home cage and distinct cage paradigms and subsequent ethanol intake during the 24 hr home cage re-acquisition. Thus, animals that were high drinkers or low drinkers (home cage or distinct cage) in the first part of the study remained high drinkers in the 2nd part of the study. However, unlike the findings obtained by Boyle et al., (1994) no significant correlation was found between ethanol intake within a 2hr operant paradigm and subsequent 24hr access. In other words, animals that were high drinkers in the operant paradigm were not necessarily the animals that were high drinkers in the continuous access paradigm. These results may be

interpreted to suggest that the regulatory processes controlling individual variability in ethanol self-administration within an operant paradigm may be different and unrelated to those involved in ethanol self-administration in a non-operant paradigm. However, it is important to point out the existence of procedural difference between the present study and Boyle et al. (1994). In the present study, animals were first introduced to ethanol within a 2-hr limited access paradigm followed by 23-hr continuous access to ethanol while Boyle et al. (1994) first exposed animals to ethanol within a 24-hr continuous access followed by a 10-minute limited access paradigm.

It is argued that animals maintaining their relative level of ethanol intake across drinking situations would be more suggestive of animals consuming ethanol because of its pharmacological properties. In other words, stable drinking across drinking situations would suggest the minimal involvement of non-pharmacological variables such as drinking environment and acquisition paradigm. Taken together these results seem to suggest that there may be an additional variable modulating the acquisition of ethanol intake in the operant paradigm other than merely the pharmacological response to ethanol. It may be possible that factors specifically related to the testing situation surrounding the use of an operant paradigm add a unique influence over and above that which is provided by the pharmacological response to ethanol itself.

Experiment 5: The effect of testing situation and transport on ethanol acquisition.

In the previous experiment, we found that the type of drinking paradigm employed during ethanol acquisition had an effect on the subsequent level of ethanol consumed. In other words, Experiment 4 demonstrated that beginning with an operant procedure for access to ethanol and providing animals with a distinct environment during acquisition elevated ethanol consumption as compared to a standard voluntary intake-home cage procedure. Thus, the results obtained in Experiment 4 supported the notion that environmental variables might influence overall ethanol intake. We speculated that the learning of an operant might facilitate the acquisition of ethanol consumption by facilitating the association between the behavioral responses related to ethanol intake and the reinforcing properties of ethanol. However, it is possible that ethanol self-administration in an operant paradigm may also involve the influence of variables that are unrelated to the individual pharmacological response to ingested ethanol.

Voluntary oral self-administration in an operant paradigm differs from a standard free choice self-administration paradigm on two points. First, operant paradigms require the animal to learn to perform a lever press in order to gain access to the ethanol solution. Second, the apparatus necessary to measure operant behavior typically requires that animals be tested in a separate and suitably equipped room. Testing animals in an operant paradigm thus requires an animal to be removed from his home cage and transported to a different environment for the duration of the exposure to ethanol. The change in environment and transport is a common experience for laboratory rats but it is plausible that this experience may also have an effect on their subsequent ethanol related behavior. In fact, it has recently been demonstrated that environmental disturbances such as those

described previously increased ethanol intake in mice (O'Callaghan et al., 2002). The main purpose of this experiment was to examine the effect of the testing situation and of environmental disturbances on ethanol acquisition within a limited access paradigm. Specifically, we wanted to further explore the findings obtained in Experiment 4 by examining the effect of providing animals with a distinct drinking cage, as well as the effect of transporting animals to a distinct testing situation on their subsequent ethanol intake. In order for the results of this experiment to be comparable to the results obtained in Experiment 4, a limited access paradigm was chosen.

Method

Subjects

Subjects were 27, male, Long-Evans rats (Charles River, St. Constant, Quebec, Canada), each weighing between 150 and 175 g when received. Rats were given a 2-week acclimatization period during which they were handled daily. Animals were housed individually in hanging stainless steel cages in a colony room maintained and controlled for temperature, humidity, and a 12 h light/12 h dark cycle (lights on at 0800). All testing took place during the light portion of the cycle. All animals were treated in accordance with the guidelines of the Canadian Council for Animal Care, and the Animal Care Committee of Concordia University approved all procedures.

Drugs

Ethanol solutions were prepared through a dilution of a 95% ethyl alcohol stock solution with tap water.

Apparatus: (Distinct cage)

A distinct cage was created by modifying the standard operant chamber (Coulbourn Instruments, Allentown, Penn.) used in Experiment 4. Thus, the retractable levers and the dipper receptacle were removed and a standard plastic test tube fitted with a ball bearing spout was positioned on the end wall, where the dipper receptacle would typically be located.

Apparatus: (Transport)

Nalgene buckets (13.2 liters) with lids (Fisher Scientific, Nepean, Ontario, Canada) were used to transport animals.

Procedure

Phase 1.

Following a one-week acclimatization period, animals were randomly assigned to one of three conditions (N=6 for all groups). Group 1 (Home Cage/HC) was exposed to an ethanol acquisition paradigm in their home cage. To facilitate the acquisition of ethanol consumption within a limited access paradigm, each animal was given two days of 1-hr access to an initial concentration of 2% (v/v). Subsequently, ethanol solutions were presented for a three-day period and raised in ascending concentrations of 2% until a final 10% concentration was reached. Four additional days of 10% ethanol were provided and constituted the maintenance period. Ethanol intake was measured on a daily basis to the nearest milliliter. Ethanol intake during the maintenance period was used to

calculate the mean ethanol intake score in grams per kilogram of body weight per day (g/kg/day).

The second group of animals (Transport/TR), randomly divided into sub-groups of 3 animals, were first placed in buckets, and then transported by the experimenter to the testing rooms. Transport involved descending in an elevator 3 floors to the testing rooms and then allowing a period of 2 minutes to elapse prior to returning via the elevator back to the animal colony. Animals were then immediately presented with a 1-hr limited access to increasing ethanol concentrations in their home cage. The presentation schedule and concentrations of ethanol presented were as described in the home cage procedure. The presentation schedule and concentrations of ethanol provided to the third group (Distinct Cage/DC) were as described in the home cage procedure with the exception that animals in this group were presented with a 1-hr limited access of increasing concentrations in an operant-like chamber. Access to ethanol only required the animal to drink the fluid presented. Animals in the third group were transported to their Distinct Cage testing room in the same manner as Group 2, with the exception that they remained in the testing room for the duration of the ethanol presentation before being returned to their home cage.

Phase 2.

The goal of Phase 2 was to assess whether the levels of ethanol intake established in Phase 1 were stable or would change as a function of the type of drinking environment employed. Following a washout period of three weeks, the groups were re-introduced to ethanol using a different method of ethanol presentation as compared to Phase 1. Group 1

(HC) that previously was exposed to ethanol in their home cage was subsequently exposed to ethanol in a Distinct Cage (HC/DC). The Transport group (TR/HC), and the Distinct Cage (DC/HC) from Phase 1 both received exposure to ethanol in their Home Cage. The procedure used was identical to that described in Phase 1 with the exception that a 10% ethanol solution was the only concentration utilized during this phase. Since animals were already established drinkers, an eight day limited access of 10% ethanol was employed. The first day of 10% ethanol access was used to evaluate any changes in the level of intake as compared to the maintenance period of Phase 1. The subsequent seven days of 10% ethanol presentation were provided to ensure that an adequate sample of ethanol intake was assessed.

Data Analysis

Data collected in the experiment included mean ethanol intake (g/kg/day) in both phases of the limited access paradigms. In order to provide a score for individual mean ethanol intake at each ethanol concentration presented in Phase 1, a mean of the ethanol intake (g/kg) over three days at each concentration was calculated.

A 10% ethanol solution was the sole fluid tested in Phase 2 thus daily ethanol intake (g/kg/day) was the measure collected. As our interest was to examine any influence of the type of drinking environment employed on differential ethanol intake, the level of ethanol consumed was examined by comparing group means. Potential group differences within the limited access paradigm of Phase 1 and Phase 2 were assessed by separate 3 (group environment) x 6 (concentration) and 3 (group environment) x 8 (day) respectively, analysis of variance (ANOVAs) with the last factor as a repeated measure.

Simple main effects and simple comparisons were employed to determine the source of interactions. Repeated measures *t*-tests were used to assess within group mean differences in ethanol intake (g/kg) at the end of Phase 1 as compared to the first day of Phase 2. The data were analyzed using the CLR ANOVA software application for a MAC computer. An alpha level of .05 was used for all analyses.

Results

Phase 1

Group mean ethanol intake (g/kg) across concentrations is presented in Figure 16. The analysis of variance revealed a significant main effect of group [$F(2, 24) = 4.611, p < 0.05$], concentration [$F(5, 120) = 17.111, p < 0.05$], and interaction [$F(10, 120) = 2.531, p < 0.05$]. The significant interaction between group drinking condition and ethanol concentration was further analyzed with tests of simple effects and simple comparisons. The analysis revealed several significant simple effects of drinking condition on ethanol consumption. Specifically, the analysis indicated that the group ethanol intake differed significantly at concentrations of 2% [$F(2, 24) = 3.592, p < 0.05$], 4% [$F(2, 24) = 6.799, p < 0.05$], 6% [$F(2, 24) = 11.305, p < 0.05$], and 8% [$F(2, 24) = 3.984, p < 0.05$]. Simple comparisons confirmed that animals in the Distinct Cage group consumed more ethanol than the Transport Group across all of the previously mentioned concentrations (2%-8%) and differed from the Home Cage group at the concentrations of 4%, 6%, and 8%. Ethanol intake of the Home Cage group as compared to the Transport Group was not significantly different across ethanol concentrations.

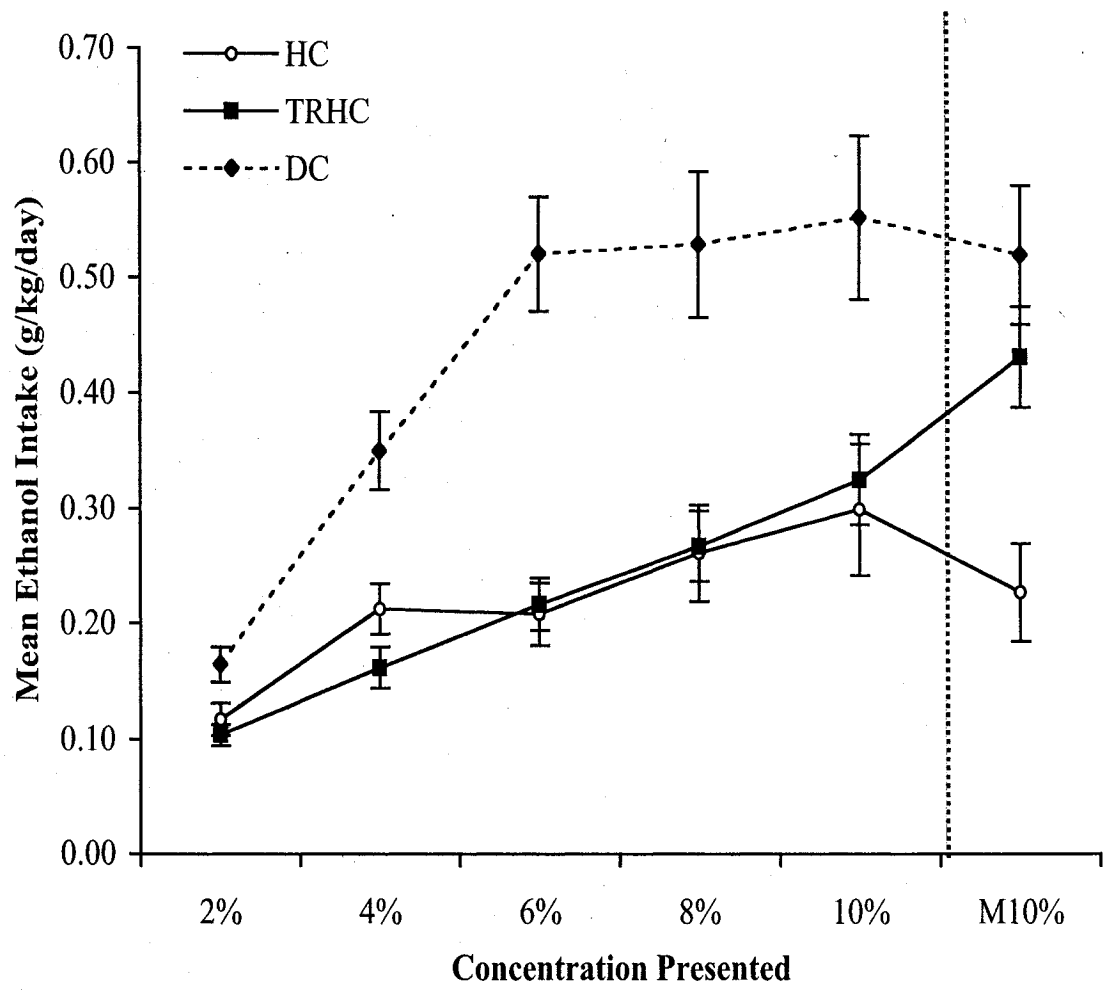


Figure 16. Group mean ethanol intake (g/kg/day) \pm S.E.M. across concentration. Each concentration presented reflects the average intake over a 3-day period.

Phase 2

Mean intake of a 10% ethanol solution at two different times across groups is presented in Table 3. Analysis of group differences indicated a significant increase in ethanol intake in the Home Cage group switched to a Distinct Cage and a significant decrease in ethanol intake for the Transport group subsequently exposed to ethanol in their home cage [$t(8) = -3.808, p < 0.05$ and $t(8) = 2.550, p < 0.05$, respectively]. No significant difference in ethanol intake was observed in the Distinct Cage group later exposed to an ethanol acquisition in the Home Cage ($t(8) = 0.623, p > 0.05$).

Figure 17 shows the level of ethanol intake (g/kg/day) across days for groups as a function of re-introducing ethanol using a different method of ethanol presentation. Analysis of variance revealed no significant main effect of group [$F(2, 24) = 2.251, p > 0.05$] and a significant main effect of day [$F(7, 168) = 4.081, p < 0.05$] suggesting that while ethanol intake varied across days the groups did not differ in the overall level of ethanol consumed. A significant interaction [$F(14, 168) = 5.509, p < 0.05$] was followed by a test of simple effects. The analysis reflected the finding of significant differences in ethanol intake on day 4 [$F(2,24) = 4.973, p < 0.05$], day 6 [$F(2,24) = 8.947, p < 0.05$], and day 8 [$F(2,24) = 6.412, p < 0.05$]. Simple comparisons revealed that on day 4 the Home Cage group previously exposed to a Distinct Cage (DC/HC) consumed more ethanol than either the Home Cage exposed to a Distinct Cage group (HC/DC) or the Transport/Home Cage group (TR/HC). On day 6, the HC/DC consumed less ethanol than the other two groups, which drank similar amounts. Analysis of day 8 of the ethanol presentation indicated that the DC/HC group drank more ethanol as compared to both the HC/DC group and the TR/HC group.

Table 3

Mean ethanol intake (g/kg) at the end of Phase 1 and day one of Phase 2 according to group (\pm S.E.M.)

<u>Group Condition</u>		<u>Ethanol intake (g/kg)</u>	
Phase 1/ Phase 2	n	Phase 1	Phase 2
Home Cage/Distinct Cage	9	0.226 \pm 0.064	0.504 \pm 0.099
Transport/ Home Cage	9	0.430 \pm 0.080	0.301 \pm 0.042
Distinct Cage/Home Cage	9	0.519 \pm 0.113	0.469 \pm 0.080

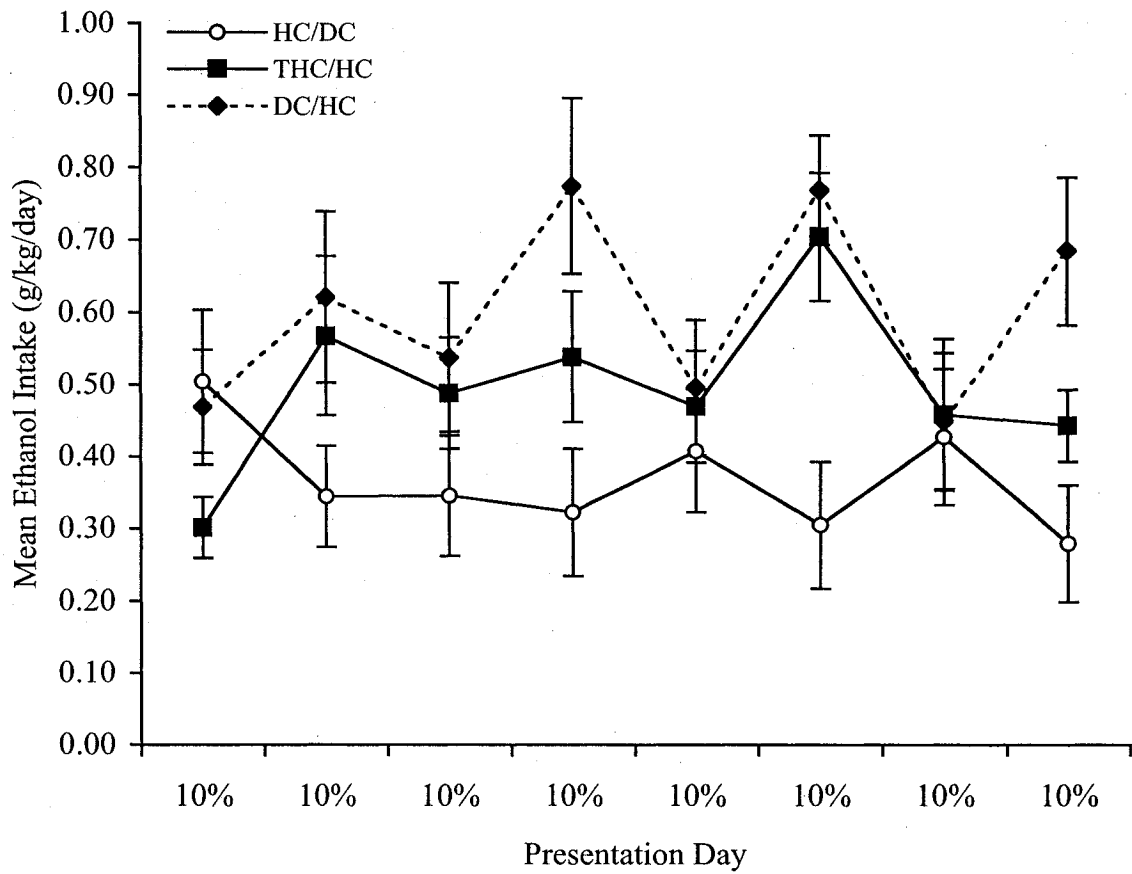


Figure 17. Group mean ethanol intake (g/kg/day) \pm S.E.M of 10% ethanol across days in Phase 2. In Phase 2, groups were reintroduced to ethanol with a different procedure than the procedure used in Phase 1.

Discussion

The present study employed a limited access paradigm in an attempt to assess the effects of providing animals with a distinct drinking environment on subsequent voluntary ethanol intake. Another goal of Experiment 5 was to assess the effect of transporting animals to a distinct setting, different than their home cage on subsequent ethanol acquisition. The findings of Phase 1 suggested that the type of drinking environment employed to facilitate ethanol acquisition might in fact influence the level of ethanol consumed. Animals subjected to a Distinct Cage environment during exposure to a limited access acquisition paradigm consumed significantly greater amounts of ethanol than those animals consuming ethanol in their home cage or animals exposed to transport followed by home cage ethanol acquisition procedure. Ethanol intake was not significantly different between the Home Cage and Transport groups. Thus, in the present study an increase in ethanol intake was only observed when both a distinct environment and transport were part of the procedures used during ethanol acquisition. It is noteworthy that a distinct drinking environment and transport are also variables common to the procedure used in Experiment 4 to initiate ethanol intake within an operant paradigm.

Comparisons of the intake of a 10% ethanol solution at the end of Phase 1 and of the intake on the first day of Phase 2 revealed that manipulating the drinking environment could produce significant changes in ethanol intake on the first day of ethanol re-exposure. Specifically, a significant increase in ethanol intake on the first day of Phase 2 was obtained for the Home Cage group that was switched to a Distinct Cage for the duration of Phase 2. On the other hand, switching drinking environments produced a

significant decrease in ethanol intake for the Transport/Home Cage group. Although there was a slight decrease, no significant differences in ethanol intake were observed for the animals initially exposed to a Distinct Cage who were then subsequently provided with ethanol in their home cage.

Despite the findings of a significant difference in ethanol intake on the first day of ethanol re-exposure, the results of the Phase 2 analysis indicated that changing the drinking environment after the initial ethanol acquisition paradigm, failed to alter the overall level of ethanol intake among the three groups. A further analysis of the interaction between group and day, found significant differences in ethanol intake on days four, six, and eight. However, contrary to the findings obtained in Phase 1, the group now exposed to a Distinct Cage (HC/DC) in Phase 2 did not drink more ethanol than the group now exposed to ethanol in their home cage (DC/HC).

Interestingly, the significant differences in ethanol intake during Phase 2 on days four, six, and eight were the result of the DC/HC group drinking more ethanol as compared to the HC/DC group. In other words, animals that were exposed to a distinct cage in Phase 1 and subsequently exposed to ethanol in their home cage during Phase 2 continued to drink more ethanol than animals initially exposed to ethanol in their home cage followed by exposure to a distinct cage acquisition in Phase 2. These results may suggest that an animal's initial experience while learning to consume ethanol is an important; perhaps even critical experience that is difficult to modify, especially when subsequent drinking conditions are reversed. In other words, an animal's initial experience with ethanol may determine how the animal will behave later on regardless of any changes in their drinking conditions. Furthermore, it seems that the distinct cage

condition exerts its effect only on naive animals. Once the animals were exposed to another condition, for example, home cage first; the distinct cage setting seems to lose its effect.

Taken together the results of Experiment 5 support the notion that at least in part, the level of ethanol intake in naïve rodents seems to be influenced by environmental factors such as the testing situation during acquisition and the type of drinking paradigm employed to initiate ethanol consumption.

GENERAL DISCUSSION

The purpose of this thesis was to assess the role of individual differences in learning and memory processes on the acquisition of voluntary ethanol self-administration using unselected rodent strains. Another aim of this thesis was to evaluate the influence and contribution of environmental variables on individual and group patterns of ethanol self-administration.

What factors contribute to or mediate the voluntary intake of a licit yet “addictive” substance such as alcohol? For more than half a century, the field of alcohol research has been grappling, with this seemingly straightforward question (e.g.: Amit & Stern, 1971; Eriksson, 1968; Lester & Freed, 1973; Richter & Campbell, 1940). Utilizing animal models of alcohol self-administration researchers have demonstrated that differences in ethanol preference or even ethanol intake in general, may be mediated in part by ethanol’s effects on the central nervous system and on neurotransmitter systems which may be involved in the regulation of ethanol’s psychopharmacological reinforcing properties (for reviews see Amit & Smith, 1992; Eckardt et al., 1998; Weiss & Porrino, 2002). Given the multiplicity of factors known to interact with and influence ethanol intake in humans (Altman et al., 1996) a parallel area of investigation has evolved, aimed at delineating the role and contribution of behavioral variables in the regulation of ethanol intake (e.g.: Goodwin et al., 2000; Overstreet et al., 1997; Samson et al., 2003). Although less is known about the role of behavioral variables in ethanol consumption in rodents, it is extremely likely that they are involved in its regulation, given the complexity and variability inherent in the processes underlying the evolution of ethanol self-administration. In fact, previous work has suggested that factors other than those

directly related to the pharmacological properties of ethanol may also contribute to individual differences in high ethanol intake (Gauvin et al., 1998; Boyle et al. 1994; Goodwin et al. 1996; 1998; Hill, 1978; Wise, 1974). Of particular interest was the finding that rats selectively bred for their presumed learning ability consumed and preferred ethanol in equal or greater quantity than strains selectively bred for high ethanol intake (Amit & Smith, 1992; Russell & Stern, 1973). The finding that rodents bred for their maze learning ability, a factor seemingly unrelated to ethanol, also displayed a distinct pattern of high and low ethanol intake and preference, suggested the possibility that learning ability may be involved in the mediation of the acquisition of ethanol self-administration.

The data contained within this thesis reflects a desire to explore the role of individual differences in learning and memory processes, in the acquisition of voluntary ethanol self-administration in unselected rodent strains. As opposed to being viewed as a methodological nuisance, the inherent variability observed in the learning capacity of rodents was viewed as a reflection of the underlying nature of ethanol self-administration behavior. The possible involvement of the hippocampus, a brain region important in mediating spatial learning and memory processes was also examined (for a review see Good, 2002). Specifically, we hypothesized that if individual differences in spatial learning and spatial working memory are involved in mediating ethanol intake, then lesioning the brain structure most closely implicated in these areas should affect the acquisition of ethanol intake.

Another goal was to evaluate the contribution of environmental variables such as the nature of the ethanol-drinking environment and the type of drinking paradigm

employed to initiate ethanol self-administration. It was thought necessary to examine the role of environmental variables on ethanol acquisition, as it is obvious that behavior does not occur independently of the environment in which it is taking place (e.g.: Krank, 1989). Finally, given the proliferation of procedures used to measure voluntary oral self-administration there has been some debate whether different paradigms reflect the same underlying processes (Boyle et al., 1994; Dietrich, 1992; Samson, 2003). Thus, it was important to assess the stability of both individual and group ethanol consumption patterns across the different types of voluntary ethanol intake paradigms employed within this thesis.

If one's goal is to understand the extremity of excessive alcohol intake then it is imperative to explore all variables that may contribute to its development in the first place. In fact, the data presented in this thesis provide support for the notion that learning, behavioral and environmental factors contribute to and actually play a vital role in the acquisition of ethanol self-administration of outbred rodents.

The aim of the first study in this thesis was to further explore a finding obtained by Amit and Smith (1992). These researchers examined the differential ethanol intake of Tryon-maze-bright and Tryon-maze-dull rats and reported that the Tryon-maze-bright animals, bred for high maze-learning scores, exhibited elevated ethanol intake levels. The finding of elevated intake levels in a strain bred for a trait seemingly irrelevant to ethanol consumption suggested the possibility that a common mechanism may mediate the behaviors of ethanol self-administration and maze learning ability. In designing Experiment 1, we specifically chose to employ an outbred rodent strain as we hypothesized that if learning ability was a basic trait that contributed to the development

of high ethanol intake, a relation between these variables must be observable without any prior selective breeding. Thus, Experiment 1 assessed the relationship of a behavioral trait, maze learning ability, to ethanol consumption using a complex 16-arm T-maze to assess learning ability. The T-maze was a replica of the maze employed by Tryon (1929) to assess the inheritance of maze learning ability in rats.

The results of Experiment 1 revealed that the high drinkers group and the low drinkers group did not differ in the number of errors committed or time taken to complete the T-maze. However, as we were interested in exploring the role of individual differences in learning capacity on ethanol acquisition we combined the error and time variables to provide an index of overall maze performance and competency. The index of overall maze performance that resulted from the combination of the individual variables was undertaken as it was believed that this index would better capture and represent the overall performance of the animal and that this would be a more accurate reflection of the animal's rate of learning of this task. Thus, the major finding of Experiment 1 was that an animal's ability to competently solve a complicated maze was significantly correlated with increased ethanol intake. In other words, animals that were classified as "high drinkers" were also more competent in completing the T-maze task. No relationship between the level of ethanol intake and the index of maze competency was observed in the low drinkers group.

The results of Experiment 1 lend support to a previous finding obtained with Tryon rats (Amit & Smith, 1992) and endorse the notion that individual differences in learning ability, defined operationally as the competency to complete a T-Maze (Tryon, 1929), may be related to increased ethanol self-administration. In Experiment 1, we

hypothesized that the efficient use of distal and proximal cues might facilitate an animal's ability to pair the pharmacological effects of ethanol with the gustatory and olfactory cues associated with ethanol consumption and thus might promote increased levels of ethanol intake. Thus, animals that displayed superior maze competency may have also used this innate ability to effectively pair the external cues (e.g.: smell and taste) associated with the presentation of ethanol to its post-ingestive effects and thus approached and consumed more ethanol over time.

The notion that rodents likely learn to drink ethanol prior to developing regular intake patterns intuitively fits with the observation of alcohol consumption patterns in humans. Alcohol consumption in humans can be characterized as typically occurring in smaller, discrete drinking bouts during the individual's initial experience prior to the onset of regular and/or chronic use (Cloninger, 1987). More importantly, the fact that several techniques were developed out of a perceived need to elevate the levels of drinking in rodents (Cicero, 1979; Li et al., 1979) suggests that ethanol intake evolves rather than occurs naturally. In many rodents, even those selectively bred to prefer ethanol, ethanol appears to be an "acquired taste" (e.g.: Gauvin, et al., 1998; Kampov-Polevoy, et al., 1990). For example, initial reactions to a drop of ethanol by the ethanol preferring P rats, the high alcohol drinking (HAD) rats and their non-preferring counterparts were similar (Bice & Kiefer, 1990; Kiefer, Badia-Elder, & Bice, 1995). Moreover, the P and the HAD rats only demonstrated a preference reaction to a drop of ethanol after prolonged experience (Bice & Kiefer, 1990; Kiefer, Badia-Elder, & Bice, 1995) suggesting that the reactions to the taste of ethanol were possibly learned, as a result of associations that have been made with ethanol's post-ingestive effects.

That selectively bred rodent strains exhibit increased levels of drinking suggests there may be a heritable component to high ethanol intake. However, the exact nature or processes through which an inherited predisposition is translated into behavior is not yet clearly understood. In particular, it is still unclear what processes underlie any organism's propensity to voluntarily ingest ethanol, regardless of any putative genetic contribution. Although there is an apparent increase in the study of behavioral factors in strains of rats selectively bred for consumption of ethanol, there have been relatively few comprehensive studies of the behavioral factors that may influence the acquisition and maintenance of ethanol intake in unselected strains of rats. The findings presented in this study offer an additional piece of evidence that may help us understand the processes that contribute to the tremendous variability in individual ethanol intake levels observed across animals. Given the interest in identifying genetic contributions to human alcoholism, the present findings underlie the importance of considering non-pharmacological along with pharmacological variables with regards to human alcohol intake.

Given the fact that the major findings in Experiment 1 were obtained through testing with a specific learning task such as the T-maze, it was of interested to determine if we would obtain similar results with another learning task. Thus, Experiment 2 assessed whether the relationship between learning ability and increased ethanol intake observed in Experiment 1 was task specific or possibly the result of a more generalized role for learning capacity in ethanol self-administration.

Since rats use their spatial orienting ability to solve mazes (Olton, 1979), we hypothesized that the findings observed in Experiment 1 may in part be mediated by

individual differences in spatial ability. It was argued that if a general role for learning ability exists and in part mediates the acquisition of ethanol, then a relationship between ethanol intake, radial maze performance, and T-maze performance should be observed. The results of Experiment 2 suggested that the number of errors committed and the time taken to complete the T-maze, as well as the radial maze, failed to distinguish the high and low drinkers. On the other hand, the more demanding delay interposed task in Experiment 2 revealed that individual variations in choice accuracy appear to be related to the development of high ethanol intake. For example, the high drinkers made a greater number of correct choices and took less time to complete the delay task than the low drinkers group. Since there were no significant group differences prior to the delay task, these results seem to suggest that the delay produced a greater disruption in the radial maze performance of the low drinkers as compared to the high drinkers. It is further speculated that the decrease in performance may be linked to individual differences in short-term spatial memory and that these differences may underlie the observed relationship between maze competency and ethanol intake in Experiment 1 (Pratt et al., 2002). In other words, animals endowed with an enhanced spatial memory may exhibit higher levels of ethanol intake, as they are able to effectively integrate and memorize information about the external and internal cues associated with ethanol consumption. Our interpretation of the previous finding actually fits with an earlier theory (White, 1996), which suggested that several independent learning and memory processes might contribute to ethanol reinforcement. In part, White (1996) proposed that a neural system that includes the hippocampus might mediate “declarative learning” whereby the information about the cues in the environment would be stored and used to direct future

behavior.

Another major finding in Experiment 2 was the significant relationship between ethanol intake and the index of T-maze competency for the high drinkers group. A relationship between ethanol intake and maze competency provides further support for our initial hypothesis that learning ability, defined operationally as the competency to complete a T-maze, was related to differential ethanol intake. Furthermore, animals that performed poorly in the T-maze also were less accurate in making choices during the delay task, suggesting that individual performance across the learning tasks employed in Experiment 2 was stable. These results seem to suggest that individual maze learning competency is comparable across maze types and that poor maze competence is related to decreased ethanol intake. Alternatively, our results may be interpreted to suggest that among low drinkers, timidity in maze exploration, as gleaned from the increased time taken on the delay task, may have impaired maze performance. A role for a behavioral factor such as timidity/meekness, in mediating the affinity to ingest ethanol has previously been suggested by Salimov (1999). In reviewing the findings obtained from several behavioral studies, he found that rodents that displayed timidity/meekness across behavioral tasks were also likely to be classified as low ethanol drinkers. However, it is important to note that in the Salimov study (1999) the timidity/meekness factor was defined by behavior on multiple maze tasks, which did not include the radial or T-mazes.

Taken together the results of Experiment 1 and Experiment 2 lend support to hypothesis that individual differences in learning and memory processes, as assessed in two differential spatial tasks, seem to be important variables contributing to ethanol acquisition in outbred rodent strains. Furthermore, our results support the notion that in

order to understand the variability in ethanol self-administration across rodent strains it is important to assess individual differences in performance on a variety of learning tasks seemingly unrelated to ethanol intake or ethanol's direct actions of the CNS.

The results observed in Experiment 1 and Experiment 2 supported the hypothesis that individual differences in learning and memory processes appear to be important factors influencing, at least in part, the acquisition of ethanol intake in laboratory rodents. In particular, it appears that individual differences in spatial learning ability and spatial working memory may in fact be in some way related to the development of ethanol intake. As a natural extension of the previous experiments, Experiment 3 used brain lesions as a biological-assay of our behavioral hypothesis. Furthermore, lesions of the hippocampus would allow for the testing of White's (1996) hypothesis about the role of learning and memory processes in addiction. In particular, we wanted to explore the hypothesized involvement of the hippocampus in "learning about the relationship of affective states to external cues" (White, 1996).

Thus, in Experiment 3 we hypothesized that if spatial learning per se is involved in influencing the ethanol intake of outbred rodents then manipulating the brain structure most closely implicated in spatial learning (Olton, 1979) should affect the acquisition of ethanol intake. Experiment 3 examined the effect of hippocampal lesions on the acquisition of ethanol intake in both a limited-operant and 23-hr oral access paradigms. The major finding obtained in Experiment 3 suggested that hippocampal lesions disrupt an animal's ability to acquire ethanol-drinking behavior in both a limited and continuous access paradigms. Animals with hippocampal lesions lever pressed for access to ethanol significantly less than their sham controls. In fact, the effect of the lesion appeared to be

specific to lever pressing for ethanol as there were no differences in lever pressing for access to water. Our findings cannot be explained by the presence of lesion induced motor deficits, since rate of lever pressing during training was similar for all groups. We hypothesized that the hippocampal lesions may have affected ethanol intake indirectly by disrupting the processes necessary for the animals to associate the behavioral response of lever pressing with the ingestion of ethanol but not with water and thus any post-ingestional effects associated with its pharmacological properties. A similar finding was obtained in the continuous access paradigm. Thus, animals that received lesions to the hippocampus consumed significantly less ethanol than their controls yet water intake was not different amongst the groups. Any differences in prior exposure to ethanol cannot explain our findings in the continuous access paradigm, as both groups were exposed to ethanol in the limited access paradigm. Thus, it appears that the hippocampus may also be involved in mediating the acquisition of ethanol intake in a continuous access paradigm. It was hypothesized that the lesions affected ethanol intake during continuous access by disrupting processes necessary for animals to make the association between ethanol intake and its post-ingestional properties. The findings of Experiment 3 also provide support for the assertion that the hippocampus is part of a neural system that mediates and possibly even stores the information about the learned associations between external cues and internal states (White, 1996).

The finding that lesions to the hippocampus affect ethanol intake lends support to the notion that individual differences in learning and spatial orientation ability may be related to ethanol intake. Given this proposed role for learning and memory processes in influencing ethanol self-administration as suggested by the first three experiments, a

pertinent issue for this thesis became whether individual differences in ethanol self-administration were independent of any environmental contributions (e.g.; type of paradigm, location of testing, type of housing). Examining the possible influence exerted by environmental variables was deemed important as a means to help identify and better understand the variables that might affect individual differences in oral ethanol self-administration. Several researchers (e.g.: Boyle et al., 1994; Deitrich, 1992; Myers, 1996; Samson & Czachowski, 2003) have previously underscored the need for this type of investigation particularly in view of the multitude of techniques and paradigms used to measure oral-self administration, which necessarily rely on a variety of environmental parameters.

Thus, the aim of Experiment 4 and Experiment 5 was to assess the influence of environmental variables on individual differences among laboratory rodents in the acquisition of oral ethanol self-administration.

Experiment 4 investigated the influence that a drinking paradigm might exert on ethanol intake, as well as the stability of ethanol intake across commonly employed drinking paradigms. More precisely, one of the goals of Experiment 4 was to expand on a result previously obtained by Boyle et al., 1994. These researchers demonstrated that the type of drinking paradigm employed to initiate ethanol consumption influenced the subsequent level of ethanol intake (Boyle et al., 1994). However, a significant correlation was obtained between the level of ethanol intake within a 24-hr oral access paradigm and the subsequent level of ethanol intake within a 10-minute limited oral access paradigm. This result was taken to suggest the likely existence of common core processes regulating voluntary oral self-administration, despite the differential use of techniques used to

measure ethanol intake (Boyle et al., 1994).

For the purposes of this thesis, it was of interest to further elaborate on the results obtained by Boyle et al., (1994), by including the commonly utilized method of operant responding for access to ethanol. Operant procedure testing sessions typically involve the removal of animals from their home cage and subsequently provide them with a distinct environment (operant chambers) for the duration of the ethanol access period. Thus, a distinct-cage for testing the impact of oral access to ethanol was included to isolate the effect of providing animals with a distinct drinking environment. The main finding of Experiment 4 was that animals exposed to a limited-access operant procedure consumed more ethanol than animals exposed to either a limited access in the home cage or limited access in a distinct cage procedure. Interestingly, providing animals with a distinct drinking environment also appeared to increase ethanol intake as animals exposed to a limited access procedure in a distinct cage consumed more ethanol than their home cage counterparts. The finding that operant self-administration was associated with higher levels of ethanol intake than unconditioned free access does not seem to be in line with previous studies that have demonstrated that adding an operant can decrease ethanol intake (Samson et al., 1988). As previously mentioned, the discrepancy between the present findings and those obtained by Samson (1988) may be linked to procedural differences since we examined the effect of adding an operant within a 2-hr limited access paradigm while they employed a 24-hr operant paradigm. In the second phase of Experiment 4, the group previously exposed to an operant procedure during limited access consumed more ethanol during the 24-hr. re-acquisition paradigm than as compared to the other groups. Thus, overall the group that previously learned to drink

ethanol in the operant paradigm, continued to drink larger amounts of ethanol even when tested in a different, unconditioned, ethanol acquisition paradigm.

Another goal of Experiment 4 was to assess the stability of drinking patterns across paradigms. Within group correlations between the level of limited access ethanol intake and subsequent continuous access to ethanol were conducted in order to examine the relationship between limited access and 24-hr continuous access procedures for ethanol consumption. We hypothesized that if the variability in drinking behavior observed between rodents is primarily mediated through ethanol's pharmacological properties then the place where animals consume ethanol after they have already learned to drink, should not be a significant factor in determining any subsequent ethanol intake. Interestingly, the relationship between individual ethanol intakes across paradigms was unsymmetrical. Specifically, a positive correlation was found between ethanol intake in both the 2hr home cage and distinct cage paradigms and subsequent ethanol intake during the 24 hr home cage re-acquisition. Contrary to the findings obtained by Boyle et al., (1994) yet supporting another finding (Koros et al., 1999), no significant correlation was found between ethanol intake within a 2hr operant paradigm and subsequent 24hr access in the home cage. Thus, in the distinct cage or home cage group, animals that were high drinkers in the first part of the study remained high drinkers also in the second part of the study. On the other hand, animals that were high drinkers in the operant paradigm were not necessarily the animals that were high drinkers in the continuous access paradigm. These results may be interpreted to suggest that the regulatory processes controlling individual variability in ethanol self-administration within an operant paradigm may be different and unrelated to those involved in the regulation of ethanol self-administration

in a non-operant paradigm. However, it remains possible that procedural differences between Experiment 4 and Boyle et al. (1994) may have influenced the results. In Experiment 4, animals were first introduced to ethanol within a 2-hr limited access paradigm followed by 23-hr continuous access to ethanol while Boyle et al. (1994) first exposed animals to ethanol within a 24-hr continuous access followed by a 10-minute limited access paradigm. Finally, the access to ethanol in the operant paradigms described above varied from 10-minutes (Boyle et al., 1994) to 2-hrs in Experiment 4. Perhaps, a 24-hr operant paradigm would have been a better comparison group for animals drinking ethanol in the 24-hr voluntary oral paradigm.

In summary, the notion that environmental variables influence overall ethanol intake was supported by the finding that superimposing an operant procedure on the access to ethanol, as well as providing animals with a distinct drinking environment, increased ethanol intake. The learning of an operant response may facilitate the acquisition of ethanol consumption by strengthening the association between the lever pressing, ingestion of ethanol, and the reinforcing properties of ethanol. If the variability in drinking behavior observed in the Experiment 4 is primarily related to ethanol's pharmacological properties, then it should make no difference where animals consume their ethanol once they have learned the behavior. If an animal is a high drinker in one situation, it should remain a high drinker anywhere. However, our results seem to indicate that where and under what circumstance an animal imbibes ethanol does make a significant difference. It is possible that ethanol self-administration in an operant paradigm may be exposing animals to the influence of variables that are unrelated to the individual pharmacological response to ingested ethanol.

Although commonly employed drinking paradigms all measure oral self-administration, the location of testing is often different according to the type of drinking paradigm employed. In particular, testing animals in an operant paradigm requires an animal to be removed from its home cage and transported to a different environment for the duration of the exposure to ethanol. The change in environment and transport is a common experience for laboratory rats but it is plausible that this experience may also have an effect on their subsequent ethanol related behavior (e.g.: O'Callaghan et al., 2002). As a follow-up to Experiment 4, the influence of a distinct drinking environment, in particular, the effect of transporting animals to the testing environment employed to initiate ethanol intake, was explored in Experiment 5.

As expected, in Phase 1 animals subjected to a Distinct Cage environment during exposure to a limited access acquisition paradigm consumed significantly greater amounts of ethanol than those animals consuming ethanol in their home cage or animals exposed to transport followed by a home cage ethanol acquisition procedure. Conversely, ethanol intake was not significantly different between the Home Cage and Transport groups. The finding that transport in, and of itself, did not have an effect on ethanol intake does not support a previous finding by O'Callaghan et al., 2002. These researchers demonstrated that moving the low drinking C57 mice to a new location increased their ethanol intake. However, the increase in ethanol intake observed in the C57 mice was only observed after a 2-month period (O'Callaghan et al., 2002). It remains possible that the change in environment used to define our Transport group was not long enough in order to observe any strong effects of environmental disturbances on ethanol intake. In fact, it appears that an increase in ethanol intake was only observed when both a distinct

environment and transport were part of the procedures used during ethanol acquisition.

It is noteworthy that a distinct drinking environment and transport are also variables included in the procedure used in Experiment 4 to initiate ethanol intake within an operant paradigm. Furthermore, the results of Experiment 5 may provide a context in which to understand the findings of Experiment 4. We surmised that in naïve rodents, any element that renders an ethanol presentation episode more salient would likely increase the possibility that an association will be formed between the presentation of ethanol and its post-ingestional effects. Furthermore, it is also hypothesized that this aforementioned process would also increase the likelihood that the rodent would approach and consume ethanol in future drinking episodes.

The results of Phase 2 in Experiment 5 suggested that manipulating the drinking environment could produce significant changes in ethanol intake on the first day of ethanol re-exposure. Specifically, switching drinking environments produced a significant increase in ethanol intake for the Home Cage group that was switched to a Distinct Cage for the duration of Phase 2. On the other hand, switching drinking environments produced a significant decrease in ethanol intake for the Transport group changed to Home Cage. There were no significant differences in ethanol intake observed for the animals initially exposed to a Distinct Cage that were then subsequently provided with ethanol in their home cage. Thus, it is suggested that for the HC/DC group and the TR/HC group, any differences observed in ethanol intake may be attributed to changes in the cues that normally would be associated with the onset of a drinking episode. For example, in Phase 1, the HC/DC group normally consumed ethanol in the home cage. Subsequently introducing a transport component to this group on the first day of Phase 2

may have introduced a variable that transiently increased ethanol intake. On the other hand, in the case of the TR/HC group, transport always preceded ethanol presentation and the absence of this signal on the first ethanol presentation day may have reduced ethanol intake. The lack of difference in ethanol consumption in the DC/HC group may at first seem surprising and even contrary to our findings of observed differences in the other groups. However, since the DC/HC group consumed the most ethanol in Phase 1, it is plausible or even likely that their ethanol drinking behavior was sufficiently established, so as not to be disturbed by a change in procedure.

A major finding of Experiment 5 was that despite a significant difference in ethanol intake observed on the first day of Phase 2 (ethanol re-exposure), changing the drinking environment after the initial ethanol acquisition paradigm failed to alter the overall level of ethanol intake among the three groups. Contrary to the findings obtained in Phase 1, the group now exposed to a Distinct Cage (HC/DC) in Phase 2 did not drink more ethanol than the group now exposed to ethanol in their home cage (DC/HC). Thus, it seems that the distinct cage condition exerts its effect only on naive animals. Once the animals were exposed to another condition, for example, home cage first; the distinct cage setting seems to lose its effect. In other words, once ethanol intake is established, it appears that further manipulations do not seem to have additional effects. Furthermore, it looks as if the impact of environmental factors is primarily on the acquisition, or learning to drink ethanol. This interpretation fits with previous data suggesting that environmental stimuli easily become associated with the effects of self-administered drugs (Robbins & Everitt, 2002). Moreover, this associative process is likely to be robust during the initial phases of learning or acquiring a new behavior when neural synapses may be modified

(Kelley, 2004). During the animal's initial experience with ethanol, elements of the environment are likely incorporated and associated with the internal state produced in part by ethanol's post-ingestional effects (White, 1996). If these assertions are true, then it would follow that the initial learning context would exert a strong influence on any future occurrence of the behavior. In relation to ethanol self-administration, this may be taken to suggest that the initial experience with ethanol will strongly determine how the animals will behave later on regardless of any changes in their drinking conditions.

Taken together, the findings obtained in Experiment 4 and Experiment 5 support the hypothesis that environmental variables strongly influence the degree to which ethanol self-administration will be acquired in rodents. Furthermore, as the individual differences observed cannot be solely attributed to ethanol's pharmacological properties non-pharmacological factors must contribute, perhaps even control the individual differences in alcohol consummatory patterns. In fact, it may be stated that to ignore the influence of the environment when trying to explain the variability underlying the acquisition of ethanol self-administration may misconstrue our true understanding of the complex variables affecting ethanol intake.

Conclusions and Future Considerations

The present thesis examined the influence of non-pharmacological variables such as learning capacity, spatial ability, type of drinking paradigm and drinking environment on individual differences in the acquisition of ethanol self-administration within outbred rodent strains. The results of the studies reported in this thesis reveal a number of important findings regarding the contribution of non-pharmacological variables to voluntary ethanol self-administration. First, it was demonstrated that an animal's learning

capacity, as measured in two different maze-learning tasks, is in fact related to its subsequent level of ethanol intake (Pratt et al., 2001). Thus, animals that obtained better scores in the maze-learning tasks also seemed to rely on this capacity in order to learn to drink ethanol. Indeed, although rodents may initially find ethanol to be aversive (Eriksson, 1968; Myers, 1968), a portion of each sample still do approach and consume ethanol on subsequent ethanol presentations suggesting that rodents do learn to drink ethanol. Furthermore, there exists a body of literature documenting that at low concentrations (e.g.: 2-3%), most rodents actually prefer ethanol (e.g.: Boyle et al., 1994; Gill, 1989; Richter & Campbell, 1940; Richter, 1941). In fact, this observation served as the basis for the development of some of the induction procedures, such as exposing rats to increasing concentrations of ethanol (e.g.: Amit & Stern, 1971; Gill et al., 1986). If rats did not voluntarily consume ethanol, at least in lower concentrations, then the use of induction procedures would not have been successful. In this context, it would seem unfortunate that there is such a strongly held belief that rodents do not voluntarily drink ethanol without the use of induction techniques (e.g., Samson, 1986). This belief appears to have shifted the focus of the research from an examination of the evolution and development of voluntary ethanol consumption to an exclusive preoccupation with final levels of intake.

As a means to answer the question of what type of learning may be involved in the process of ethanol acquisition and in which animals this would be reflected most significantly, it was proposed that animals who could effectively pair the external cues in their environment with the internal state produced by ethanol's pharmacological properties would be the ones that would further approach and consume ethanol. In view

of the fact that rats use their spatial orienting ability to navigate a maze (Olton, 1979) we argued that individual differences in spatial ability might contribute to the relationship between learning capacity and ethanol intake. Moreover, if one assumes a relationship between the post-ingestional reinforcing properties of ethanol and learning or memory processes (e.g.: White, 1996; Robbins & Everitt, 2002) then it is conceivable that the hippocampus may mediate the previously observed relationship between maze performance and increased ethanol intake. The finding that lesions to the hippocampus disrupted the acquisition of ethanol intake is consistent with the theoretical viewpoint that implicates the hippocampus in mediating the associations between internal states and environmental stimuli (e.g.: White, 1996). In support of White (1996), it is proposed that the hippocampus is an important neural structure contributing to the development and maintenance of ethanol self-administration. Further research will be required to verify and delineate the role of the hippocampal formation and its sub-regions.

The findings of the present thesis suggested that in order to acquire ethanol self-administration in significant amounts, rodents must first learn to approach and consume ethanol. More precisely, animals must be able to effectively pair the external cues in their environment with the internal state produced by ethanol's pharmacological properties in order to develop increased levels of ethanol self-administration. It is noteworthy that the type of drinking paradigm, as well as the drinking environment employed to initiate ethanol self-administration, had an effect on the level of ethanol intake. These findings clearly suggest that the manner in which rodents are trained to drink ethanol has a large impact on their ability to make the reinforcing associations required in order to consume ethanol. In particular, it was reported in this thesis that the presence and use of variables

that render a drinking episode during ethanol acquisition more memorable (e.g.: use of an operant; a distinct drinking environment, etc.) facilitate an animal's learning to drink. More importantly, once associations between the internal state produced by ethanol ingestion and the external cues associated with a drinking episode are made, the associations established seem quite resilient to further changes in the environment, in terms of their future impact on ethanol intake. On the other hand, it remains possible that environmental disturbances need to be drastic and/or consistent in order for an animal to incorporate this information and form new associations that would allow ethanol self-administration to be modified (O'Callaghan et al., 2002).

It is clear that non-pharmacological factors such as environmental variables impact the development of ethanol self-administration in rodents. What remains unclear and requires further clarification is the nature of the associations being made when ethanol self-administration is being acquired. Future research may consider examining whether the associations being made are the result of instrumental (Dickenson & Balleine, 1994), incentive conditioning (e.g.: Bindra, 1974), Pavlovian (e.g.: Krank & O'Neill, 2002), or a combination of those (e.g.: Robinson & Berridge, 2000). The associations may perhaps even be the result of other types of learning processes.

The findings concerning the environmental contributions to the acquisition and subsequent pattern of ethanol consumption in rodents may also be extrapolated to support the results of human studies. It has been shown that in addition to any putative genetic contribution, the development of human alcohol consumption is strongly influenced by variables such as taste preferences (Rozin & Schulkin, 1990), cognitions, or attributions about alcohol's effect (Prescott et al., 2004). Of particular relevance is the finding that the

environmental stimuli associated with alcohol consumption (e.g.: visual and olfactory) have been shown to influence the subjective and reinforcing effects of alcohol consumption in humans (Perkins, et al., 2003). The findings in this thesis suggest that rodents, like humans learn to drink and develop a preference for ethanol. Furthermore, as was found with human subjects (Perkins, et al., 2003) the environmental variables associated alcohol consumption influence subsequent ethanol self-administration.

It is at times difficult to apply the findings obtained from animal studies to the treatment of human behavior. Given the results reported in this thesis, a plausible target for the treatment of individuals who misuse alcohol may be to address the environmental variables maintaining use, as well as, the cognitions established over time.

In summary, the present thesis has provided strong support for the role of non-pharmacological factors in the development of ethanol self-administration within outbred rodent strains. In addition, the results have suggested learning as a plausible variable that may underlie the documented individual differences in the development of ethanol self-administration. Finally, it is further argued that non-pharmacological factors play a vital and necessary role, over and above any pharmacological influence, in mediating the acquisition of ethanol self-administration.

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Appendix A

The stereotaxic coordinates used to make NMDA lesions of the hippocampal formation.

All values are listed as the number of millimeters relative to bregma.

<u>Site</u>	<u>anterior posterior</u>	<u>mediolateral</u>	<u>dorsoventral</u>
1	-3.1	+/-1.0	-3.6
2	-3.1	+/-2.0	-3.6
3	-4.1	+/-2.0	-4.0
4	-4.1	+/-3.5	-4.0
5	-5.0	+/-3.0	-4.1
6	-5.0	+/-5.2	-5.0
7	-5.0	+/-5.2	-7.3
8	-5.8	+/-4.4	-4.4
9	-5.8	+/-5.1	-6.2
10	-5.8	+/-5.1	-7.5