THE ROLE OF NMDA RECEPTOR BINDING SITES IN ETHANOL'S HEDONIC PROPERTIES

By

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ABBREVIATIONS

ACEA 1021	5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione
ACPC	1-aminocyclopropane carboxylic acid
ADCI	5-aminocarbonyl-10,11-dihydro-5h-dibenzo[a,d] cyclohepten-5,10-imine
AMG	Aminoglutethimide
AMPA	a-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid
ANOVA	Analysis of Variance
AP-5	2-amino-5-phosphonopentanoic acid
AP-7	2-amino-5-phosphonopentanoic acid
BXD RI	C57Bl/6J x DBA/2J Recombinant Inbred
C(8)/E	8 mg/kg CGP-37849/2 g/kg ethanol
C(8)/L	8 mg/kg CGP-37849/6 mEq/kg LiCl
C(8)/S	8 mg/kg CGP-37849/saline
C(15)/E	15 mg/kg CGP-37849/2 g/kg ethanol
C(15)/L	15 mg/kg CGP-37849/6 mEq/kg LiCl
C(15)/S	15 mg/kg CGP-37849/saline
CGP-37849	(*E*)-(±)-2-Amino-4-methyl-5-phosphono-3 pentenoic acid
CGP-39551	(*E*)-(±)-2-Amino-4-methyl-5-phosphono-3-pentenoic acid ethyl ester
CGP-40116	D- isomer of CGP-37849
CGS 19755	cis-4-[phosphomethyl]-piperidine-2-carboxylic acid
CNS	Central Nervous System
CP-101,606	(1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol
CPA	Conditioned Place Aversion
CPP	Conditioned Place Preference
CR	Conditioned response
CRF	Corticotrophin Releasing Factor
CS	Conditioned Stimulus
CTA	Conditioned Taste Aversion
DARPP-32	dopamine and adenosine 3':5'-monophosphate-regulated phosphoprotein
D-CPPene	(R,E)-4-(3-Phosphonoprop-2-enyl)piperazine-2-carboxylic acid
FR	Fixed Ratio
G+	Grid+
G-	Grid-
GABA	Gamma-Aminobutryic Acid
GIRK2	G-protein Coupled Inward Rectifying Potassium Channel 2
(+)-HA-966	(+)- (3-Amino-1-Hydroxypyrrolid-2-One)
HPA	Hypothalamic-Pituitary-Adrenal
ICS-205,930	3-tropanylindole-3-carboxylate hydrochloride
i.p.	intraperitoneal
Kainate	2-carboxy-4-isopropenyl-3-pyrrolidine acetate
KO	knock-out
L-701,324	7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(1H)-quinolinone
LSD	lysergic acid diethylamide
LTP	Long term Potentiation

NMDA	N-methyl-D-aspartate
nNOS	Neuronal Nitric Oxide Synthase
NPC 17742	2R,4R,5S-(2-Amino-4,5-(1,2-cyclohexyl))-7-phosphonoheptanoic acid
MK-801	Dizocilpine
MRZ 2/576	8-chloro-4-hydroxyl-1-oxo-1,2-dihydropyridazino-[4,5b]quinolin-5-oxide choline salt
MRZ 2/579	1-amino-1,3,3,5,5-pentamethyl-cyclohexane hydrochloride
ORL1	Opioid receptor-like 1
PCP	Phencyclidine
p.o.	per oral
Ro-25,6981	R-(R,S)-alpha-(4-Hydroxyphenyl)-beta-methyl-4-(phenyl-methyl)-1-piperidine propanol
Ro 15-4513	Ethyl 8-azido-6-dihydro-5-methyl-6-oxo-4H-imidzo [1,5-a][1,4] benzodiazepine-3-carboxylate
Ro 64-6198	(1S,3aS)-8-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-1,3,8-triaza-spiro[4,5] dexan-4-one hydrochloride
S/E	Saline/2 g/kg ethanol
S/L	Saline/6 mEq/kg LiCl
S/S	Saline/saline
SEM	Standard error of the mean
SSRI	Selective Serotonin Reuptake Inhibitor
U99194A	5,6-dimethoxy-2-(di-u-propylamino) indan
US	Unconditioned Stimulus
WT	Wild-type
5-HT	serotonin

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ABSTRACT

Understanding the neurobiological mechanisms for the behavioral effects of ethanol is important in determining the underlying neurocircuitry involved in ethanol reward. Although the dopamine, serotonin, and gamma aminobutryic acid (GABA) systems have been extensively studied, little is known about the behavioral consequences of the actions of ethanol at N-methyl-D-aspartate (NMDA) receptors. To date, most studies investigating the role of NMDA receptor binding sites in ethanol reward have focused on ethanol self-administration procedures. The experiments that comprise this thesis investigated the role of NMDA receptor binding sites in acquisition of ethanol-induced conditioned place preference (CPP). These studies examined antagonism of NMDA receptor binding sites: ion channel, glycine$_B$, glutamate, and NR2B subunit, in the CPP procedure. Adult male DBA/2J mice received injections of NMDA receptor binding site antagonists/partial agonists and ethanol before exposure to the conditioned stimulus (CS) during CS+ conditioning trials. It was predicted that antagonism of the ion channel, glutamate binding site, and NR2B binding site, but not the glycine$_B$ binding site, would attenuate the acquisition of ethanol-induced CPP based on previous ethanol self-administration, discrimination, and CPP studies.

Pretreatment with dizocilpine (MK-801) and ketamine, NMDA receptor channel blockers; (1S,2S)-1-(4-hydrophenyl)-2-(4-hydorxy-4-phenylpiperidino)-1-propanol (CP-101,606) and ifenprodil, NR2B antagonists; and (+)-(3-Amino-1-Hydropyrrolid-2-One ((+)-HA-966), glycine$_B$ partial agonist; during CS+ trials had no effect on the acquisition of ethanol-induced CPP. On the other hand, (*E*)-(±)-2-Amino-4-methyl-5-phosphono-3 pentenoic acid (CGP-37849) attenuated the acquisition of ethanol-induced CPP. A

second CGP-37849 study replicated these findings and also showed that CGP-37849 does not produce CPP or conditioned place aversion (CPA) alone, thereby showing that CGP-37849 is not rewarding or aversive. However, all NMDA receptor antagonists affected the locomotor stimulation produced by a 2 g/kg dose of ethanol.

Because drugs that interfere with the development of CPP can do so either by reducing ethanol reward or by interfering with learning and memory, we also tested CGP-37849 in an ethanol CPA procedure. Studies from our laboratory have suggested that the rewarding and aversive properties of ethanol are mediated by different neurocircuitry. Consequently, if CGP-37849 affects ethanol reward, it should not affect ethanol-induced CPA. However, if the drug affects general learning, the antagonist could affect both procedures. The results indicate that CGP-37849 blocked the acquisition of ethanol-induced CPA. Therefore, it is difficult to conclude whether the results are due to learning deficits or alterations in ethanol's hedonic properties.

An ethanol-induced conditioned taste aversion (CTA) experiment was conducted to determine if CGP-37849 alters ethanol aversion in another associative conditioning paradigm. CGP-37849, LiCl, and ethanol produced a significant CTA alone and in combination (CGP-37849 + ethanol and CGP-37849 + LiCl) to the 0.2M NaCl paired flavor. To extinguish the association, subjects were given two 24-h access periods to the 0.2M NaCl without drug injections. Subjects previously treated with CGP-37489 during conditioning resisted extinction. Moreover, all CGP-37849 treated groups showed a CTA to a new 0.15% saccharin paired flavor. The robust CTA produced by CGP-37849 injections complicates any conclusion made regarding CGP-37849's effects on LiCl- and ethanol-induced CTA.

In summary, these results indicate that manipulations of the NMDA receptor binding sites alter ethanol-induced locomotor stimulation. Moreover, only the antagonism of the glutamate binding site decreased ethanol-induced CPP. However CGP-37849 also altered ethanol aversion suggesting that CGP-37849 may alter general learning. CGP-37849 produced an aversion for the NaCl and saccharin-paired flavors in the CTA experiment, suggesting that CGP-37849 has some aversive properties. These results suggest that CGP-37849 alters ethanol reward and aversion or the subject's ability to learn these tasks.

INTRODUCTION

Alcoholism is a complex trait affecting nearly 14 million Americans (1 in 13 adults); and alcohol is the most widely used drug in the United States, a problem costing $185 billion per year (NIAAA). However, the behavioral consequences of ethanol's actions within the central nervous system (CNS) are not completely understood. This lack of understanding is due, in part, to ethanol's diverse action within the CNS. The behavioral effects of ethanol can be attributed to its actions at various neurotransmitter systems such as: dopamine, glutamate, GABA, and serotonin. Some of the behavioral effects are due to ethanol's direct inhibition of NMDA receptor function. However, few studies have investigated the role of the NMDA receptor in ethanol reward. Moreover, several binding sites on the NMDA receptor alter the function of the receptor. This thesis will focus on the modulation of the acquisition of ethanol-induced place preference by NMDA receptors to determine the role of the receptor in the neurobiology of ethanol CPP.

Models of ethanol reward and reinforcement

Self-Administration

Self-administration paradigms assess the reinforcing effects of drugs (Meisch, 2001; Koob, 1992; Koob & Weiss, 1990). Home-cage drinking and operant self-administration paradigms are the most commonly used procedures to measure ethanol drinking in rodents. These procedures are widely used as a model of ethanol reward, since in humans, ethanol is ingested rather than injected like cocaine or heroin. Subjects have access to ethanol and another fluid (typically water) for 24 h or for a limited access period during home-cage drinking. Operant self-administration requires subjects to

respond (usually on a lever) for access to ethanol from a dipper or sipper. A change in responding is interpreted as an alteration in the rewarding value of ethanol.

Drug discrimination

The subjective effects of ethanol may mediate a component of ethanol reward and reinforcement. These effects are due to ethanol's interactions with specific receptor systems such as serotonin, NMDA, and GABA (Grant, 1999). Drug discrimination paradigms allow one to measure the subjective and discriminative stimulus effects of the drug as well as to determine the neurocircuitry mediating such effects. The discriminative stimulus properties of drugs, such as ethanol, can reinstate drug-seeking behavior after a period of abstinence (Hodge et al., 2001). There is also evidence that the discriminative stimulus effects of ethanol and ethanol self-administration may be mediated via similar neurocircuitry. For example, self-administered ethanol can serve as a discriminative stimulus and substitute for experimenter-administered ethanol (Hodge et al., 2001).

Food-restricted subjects are first trained to lever press at a set response criteria for a food or sucrose reward. After this behavior stabilizes (2-3 weeks), subjects are trained in a two-lever discrimination task and asked to discriminate ethanol (1, 1.5 or 2 g/kg) from saline. During training, responding on the appropriate lever (ethanol or saline) for a set response requirement (e.g., fixed ratio (FR) 10) results in a food or sucrose reward. Once subjects are responding approximately 80-90% on the appropriate lever, test sessions begin. These test sessions usually occur twice a week during which drugs are tested for their ability to generalize to the ethanol cue. Full substitution requires over 80% of responses on the ethanol-associated lever. Partial substitution occurs when the response on the ethanol-associated lever is between 50-80%.

Place conditioning

Place conditioning has been used extensively to assess the motivational effects of drugs such as cocaine, amphetamine, morphine, and ethanol (Carr et al., 1989; Tzschentke, 1998). Place conditioning is a Pavlovian (or classical) conditioning procedure during which a CS becomes associated with an unconditioned stimulus (US) (Cunningham, 1993). After several CS-US pairings, the CS, when presented in the absence of the US, will elicit a conditioned response (CR; approach drug cues). In ethanol place conditioning, the CS consists of environmental stimuli such as tactile, visual, and/or sensory cues. The US is the drug effect(s). During CS+ conditioning trials, subjects receive distinct environment cue(s) paired with drug treatment. During CS- conditioning trials, subjects receive other distinct environmental cue(s) paired with vehicle treatment. After several CS-US pairings, the subjects undergo a choice test during which no US is presented and subjects have access to both CS+ and CS- cues. The interpretation of the results is if a drug is rewarding, the subject will approach and spend more time in contact with the drug-associated cues. However, if the drug is aversive, the subject will spend less time with the drug-associated cues.

Biased versus unbiased apparatuses and assignment procedures

Place conditioning methodology falls into two general categories: biased and unbiased. A biased apparatus refers to an apparatus in which untrained subjects strongly prefer one of the stimulus alternatives before conditioning. A biased subject assignment procedure refers to assigning the drug-paired side to either the non-preferred or preferred compartment for each subject. An unbiased apparatus refers to an apparatus in which subjects have no basal preference for one cue (or set of cues) over another (Cunningham

et al., 2003). An unbiased subject assignment is done by randomly assigning one cue with the US regardless of individual subject's initial preference.

Several reviews suggest there are interpretational issues when using a biased apparatus and or subject assignment. Biased apparatuses can yield false positives (Bardo & Bevins, 2000; Tzschentke, 1998). For example, it is difficult to distinguish between alterations in the rewarding effects of drugs and alterations in the unconditioned motivational state produced by drug treatment (Cunningham et al., 2003). Therefore, false positives may arise from a drug altering the anxiety or aversion associated with the initially non-preferred cues. Thus, if the drug treatment is anxiolytic, the increased time spent with the non-preferred cue after conditioning may be due to a decrease in anxiety to that compartment independent of alterations in reward. Nevertheless, there may be an increased sensitivity to detect drug effects using a biased apparatus due to a decrease in between-subject variability (Cunningham et al., 2003). Despite this apparent advantage, the use of an unbiased apparatus and subject assignment leads to fewer interpretational issues.

Development of Our Place Conditioning Procedure

Several studies have been conducted to determine the optimal parameters for obtaining ethanol-induced CPP in mice. Those studies have helped establish the conditioning trial length, conditioned stimuli, and doses of ethanol implemented in the current studies. Ethanol place conditioning pairs one distinct environment with ethanol injections and another distinct environment with saline. These are referred to as CS+ and CS- trials during the conditioning phase. Subjects receive drug treatment during CS+ trials and saline during CS- trials. Most ethanol CPP studies have used DBA/2J mice

because an earlier study found that DBA/2J , but not C57BL/6J, mice show a conditioned place preference for ethanol (Cunningham et al., 1992). This initial study utilized two distinct tactile stimuli (mesh and grid floors) as the CSs. Tactile stimuli were used because it is important for the subjects to be in direct contact with the CSs during the conditioning trials in order to experience the conditioned effects of the drug during testing (Cunningham et al., 1992). However, the mesh floor was preferred over the grid floor resulting in a bias. Studies indicated that when another tactile cue (hole floor) was used in addition to the grid floor, there was no basal preference for either floor (Cunningham et al., 1993). Therefore, the grid and hole floors became the distinct tactile stimuli that serve as the CSs. Additionally, a 5 min conditioning trial was implemented based on a previous study indicating that shorter exposure to the CS (5 min instead of 30 min) resulted in a stronger magnitude of ethanol-induced CPP (Cunningham & Prather, 1992).

Two subgroups within each experimental group are used to measure the magnitude of place conditioning within and between experimental groups. Subjects in each drug treatment group are assigned to one of two groups: Grid+ (G+) and Grid – (G-). G+ subjects were given drug treatment (CS+ trials) paired with the grid floor and saline treatment (CS- trials) paired with the hole floor (Figure 1A). G- (or Hole+) subjects were given drug treatment (CS+ trials) paired with the hole floor and saline (CS- trials) paired with the grid floor. After 4 conditioning trials (4 CS+; 4CS-), subjects are placed into the apparatus that contains half grid and half hole floors (Figure 1B). To obtain a within treatment measure of conditioning, the test data are expressed as mean seconds per minute on the grid floor. Therefore, if conditioning occurred and the drug

treatment is rewarding, the subjects in the G+ subgroup will spend more time on the grid floor than subjects in the G- subgroup.

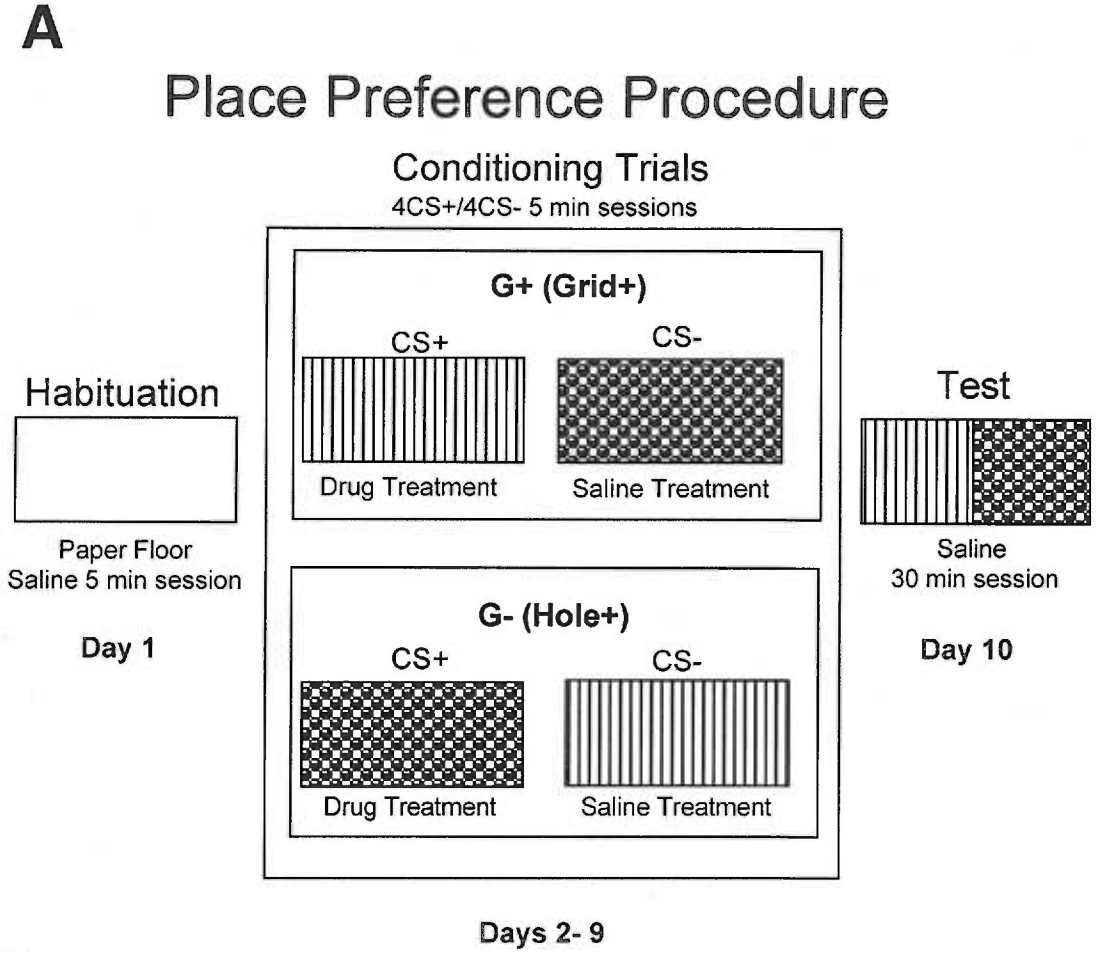
Self-Administration vs. Place Conditioning

Both the self-administration and place conditioning paradigms have been used to measure the reinforcing and rewarding properties of ethanol, respectively. However, results obtained from self-administration and place conditioning studies are not always congruent. For example, phencyclidine (PCP) is self-administered, but it does not produce CPP (see review Bardo & Bevins, 2000). Moreover, lysergic acid (LSD) produces CPP, but it is not self-administered (Meehan & Schechter, 1998). However, ethanol is self-administered (McClearn & Rodgers, 1959) and produces CPP (Cunningham et al., 1993). This suggests that self-administration and place preference may have a dissociable, but overlapping neurocircuitry.

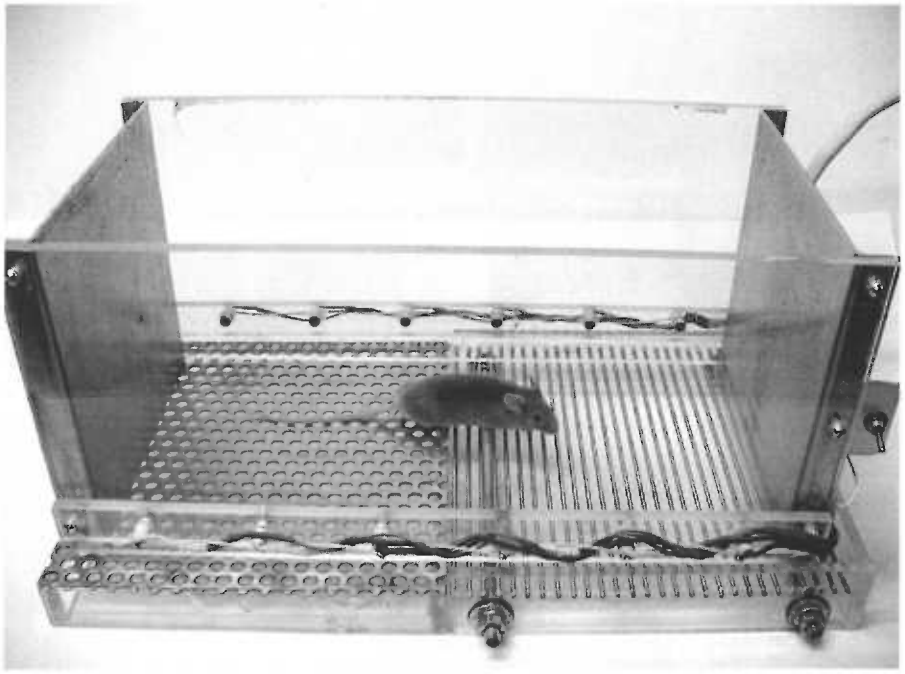
Each paradigm has its own advantages and disadvantages. Place conditioning is less time intensive than self-administration. It requires less training than self-administration. Thus, place preference lends itself to a higher throughput. Furthermore, place conditioning can measure the rewarding and aversive effects of drugs. If the drug administered has rewarding properties, the subjects will spend more time with the drug associated cues, but if the drug is aversive, the subjects will avoid the drug associated cues (Cunningham & Henderson, 2000; Risinger & Oakes, 1995). Moreover, the interpretation of a decrease in self-administration is not always clear. Ethanol self-administration can be influenced by increases or decreases in the rewarding and or aversive value of ethanol. Thus, a drug can decrease ethanol self- administration by decreasing or by increasing the

Figure 1. (A) Diagram of the mouse place conditioning procedure. On day 1 subjects in each drug treatment group are injected with saline and placed on a paper floor within the chamber for 5 minutes. On days 2-9, all subjects undergo the conditioning phase. On alternating days, subjects in the Grid+ (G+) subgroup receive drug treatment paired with the grid floor (CS+ trials) and saline with the hole floor (CS- trials). Animals in the Grid- (G-) subgroup receive saline treatment paired with the grid floor (CS- trials) and drug treatment with the hole floor (CS+ trials). On day 10, the preference test is conducted. All subjects received saline injection before being placed into the apparatus with half grid floor and half hole floor. (B) Picture of a subject in the apparatus during a preference test.

A


Place Preference Procedure
Conditioning Trials
4CS+/4CS- 5 min sessions
Habituation
Paper Floor
Saline 5 min session
Day 1
G+ (Grid+)
CS+
CS-
Drug Treatment
Saline Treatment
G- (Hole+)
CS+
CS-
Drug Treatment
Saline Treatment
Days 2- 9
Test
Saline
30 min session
Day 10

B



rewarding value of ethanol (Cunningham et al., 2000b). If drug treatment makes ethanol more rewarding, subjects can drink less ethanol yet still achieve the same reward state. On the other hand, if a drug treatment reduces ethanol reward, subjects may decrease or stop responding for ethanol. There are fewer interpretational problems with CPP (Bardo & Bevins, 2000). One reason is subjects are drug-free during testing (Carr et al., 1989). Thus, in contrast to self-administration, alterations in locomotor activity during testing usually do not interfere with measuring the motivational effects of ethanol. This is particularly important when testing NMDA receptor compounds; many have been shown to have profound effects on locomotor activity (Broadbent & Weitemier, 1999; Meyer & Phillips, 2003; Shen & Phillips, 1998). However, effects of drug treatment on locomotion during the conditioning phase of CPP could alter the cue interaction. Subjects must continue to respond to obtain ethanol access during most self-administration experiments. Ethanol, at intoxicating doses, produces motor impairments that can interfere with subsequent drinking and responding. This does not occur with CPP. Another advantage of CPP is it is possible to measure both locomotor activity and drug reward. Thus, due to the aforementioned benefits of place conditioning and the problems with interpreting self-administration results, the place preference paradigm will be advantageous to study the effects on NMDA receptor antagonism on ethanol reward.

Drug discrimination vs. Place Conditioning

Drug discrimination studies can provide information regarding changes in the internal state produced by drug administration. The discriminative stimulus properties of a drug may be important for the reinstatement of drug-seeking behavior and may underlie and cause drug abuse (Colpaert, 1987;Hodge et al., 2001; Overton, 1987). This paradigm

has been useful for the identification of neurotransmitters that mediate the effects of drugs. However, little is known about the interactions between the rewarding and the discriminative stimulus effects of drugs (Hodge et al., 2001). Moreover, the subjective effects of drugs may not be related to the abuse potential of the drug. However, there are several advantages to the drug discrimination paradigm. For example, drugs that act in the CNS all produce discriminative stimulus properties (Overton, 1987). Moreover, one can measure the strength of the subjective effects, accuracy of discrimination, speed (sessions to criterion), and the ability of other drugs to substitute for the training drug. On the other hand, there are several disadvantages of this paradigm. The major disadvantage is the time intensive nature of the task. Although these tasks are rapidly learned, it takes on average 2 months to produce a reliable discrimination. However, as previously mentioned, CPP requires minimal training, resulting in a higher throughput. Moreover, drug discrimination can be influenced by alterations in locomotor activity. Drugs that have profound effects on locomotor activity can alter the subject's ability to respond on the drug appropriate lever. On the other hand, CPP tests the subjects with no drug on board (Carr et al., 1989). The CPP paradigm is better suited for the investigation of the effects of NMDA receptor antagonists on ethanol reward due to the disadvantages of the drug discrimination paradigm as well as the possibility that drug discrimination is not related to the rewarding effects of drugs.

NMDA RECEPTOR FUNCTION

Composition

The NMDA receptor is a voltage-dependent ionotrophic glutamatergic receptor that is important for excitatory neurotransmission within the CNS. The NMDA receptor

differs from the other ionotrophic glutamatergic receptors- alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and 2-carboxy-4-isopropenyl-3-pyrrolidine acetate (kainate) receptors in that it is blocked by Mg^{2+} at resting membrane potential and is permeable to Ca^{2+}(Mayer et al., 1984, Nowak et al., 1984). In order for the NMDA receptor to respond to glutamate, the NMDA receptor requires AMPA/kainite-mediated depolarization to remove the Mg^{2+} block (Wu et al., 1996). Then, the NMDA receptor is able to bind glutamate and glycine, which opens the ion channel (Jahr & Stevens, 1987; Johnson & Ascher, 1987). NMDA receptor neurotransmission occurs via slow kinetics (slower rise and decay) that last for a long period of time (Ozawa et al., 1998). The expression of NMDA receptors is localized postsynaptically (Borges & Dingledine, 2003).

There is some debate as to whether the NMDA receptor is a tetramer or a pentamer; however, most functional assays suggest that it is a tetramer composed of two NR1 and two NR2 subunits (Doyle et al., 1998; Laube et al., 1998; Rosenmund et al., 1998; Schorge & Colquhoun, 2003). Moriyoshi and colleagues (1991) were the first to clone and express the NMDA receptor NR1 subunit in *Xenopus* oocytes. Although homomeric NR1 receptors expressed NMDA receptor-related responses, the amplitude of these currents was not consistent with that of currents from cultured brain tissues. Shortly thereafter, the four NR2 receptor subunits (NMDAR2A- NMDAR2D) were cloned by PCR and cross hybridization (Monyer et al., 1992). These NR2 subunits, when expressed with the NR1 subunit, resulted in highly active NMDA receptors (Ikeda et al., 1992; Ishii et al., 1993; Monyer et al., 1992). This suggests that the NR1 and NR2 subunits are both necessary for functional heteromeric NMDA receptors.

The NR2 subunit can be further divided into 4 distinct subtypes (labeled NR2A, NR2B, NR2C, and NR2D). Each of the NR2 subunits has differential distributions and pharmacological properties (Yamakura & Shimoji, 1999). Moreover, functional NMDA receptors can be composed of the same NR2 subunit or of a combination of NR2 subunits (Chazot et al., 1994; Wafford et al., 1993). For example, it was recently shown that the NR2B and NR2D subunits are present in functional NMDA receptors within the cerebellum (Brickley et al., 2003). This was discovered via examination of the kinetics and pharmacological properties of channel conductances. In addition, immunoprecipitation studies have shown that NR2A and NR2B subunits precipitate with NR1 subunits to form functional receptors in the CNS (Blahos & Wenthold, 1996; Chazot & Stephenson, 1997). NR1 subunit proteins are expressed throughout the brain, whereas the NR2 subunits (A-D) have a more limited distribution. The NR2A is expressed mostly in the cerebral cortex, hippocampus, and cerebellum (Ozawa et al., 1998). The NR2B subunit expression overlaps that of the NR2 and also includes the septum, striatum, and olfactory bulb (Laurie et al., 1997; Ozawa et al., 1998). The NR2C subunit distribution is limited to expression in the cerebellum and interneurons of the hippocampus (Monyer et al., 1994). The NR2D is mainly in the thalamus, brain stem, and interneurons of the hippocampus (Ozawa et al., 1998).

Pharmacology of NMDA receptor binding sites

Several binding sites modulate NMDA receptor function. These binding sites are located on either the NR1 or NR2 subunit. The NR1 subunit possesses binding sites for polyamines and glycine, whereas the NR2 subunits bind glutamate. In addition, each NR2 subunit has a unique binding site. However, at present, only specific ligands for the unique

NR2B subunit binding site exist. There is also an ion channel binding site that binds noncompetitive antagonists. This site is often referred to as the PCP binding site since PCP was the original ligand discovered to bind to the ion channel. Modification of any of these binding sites results in alterations in NMDA receptor function.

Ion channel binding site

NMDA receptor function can be affected by noncompetitive NMDA receptor antagonists such as MK-801, PCP, and ketamine and low-affinity channel blockers such as memantine and 5-aminocarbonyl-10,11-dihydro-5h-dibenzo[a,d]cyclohepten-5, 10-imine (ADCI) (Witkin et al., 2003). Physical blockade of the ion channel prevents the influx of Na^+ and Ca^{2+}, and therefore, prevents the channel from opening. These drugs elicit profound locomotor and psychotomimetic effects such as depression, hallucinations, and sedation (Danysz et al., 1994; Hargreaves & Cain, 1992; Krystal et al., 1994; Witkin et al., 2003). These extreme side effects have limited the use of NMDA receptor channel blockers clinically. Although considered a NMDA receptor channel blocker, ketamine has a high affinity for NMDA and D_2 receptors, as well as 5-HT_2 receptors and sigma receptors (Kapur & Seeman, 2002). Due to the low affinity of memantine and ADCI for NMDA receptors, binding at other receptors by these drugs is likely. Therefore, due to its selectively for NMDA receptors, MK-801 is the most advantageous channel blocker to examine the behavioral pharmacology of the NMDA receptor ion channel.

$Glycine_B$ binding site

Glycine is a co-agonist for the NMDA receptor. Moreover, glycine is present in the central nervous system of humans and animals in concentrations above that needed

for activation of NMDA receptors (Leeson & Iversen, 1994). Site-directed mutagenesis of the NR1 subunit substantially decreased glycine binding without altering the binding of glutamate, suggesting that the glycine binding site is located on the NR1 subunit (Hirari et al., 1996; Kuryatov et al., 1994). The glycine$_B$ binding site is strychnine-insensitive (Leeson & Iversen, 1994). This is quite different from the glycine receptor that can be blocked by low concentrations of strychnine. The affinity of the receptor for glycine depends on the subunit composition of the receptor. For example, the NR2A subunit containing receptors have a lower affinity (10 fold lower) for glycine than the other NR2 subunits (Kutsuwada et al., 1992; Buller et al., 1994; Priestley et al., 1996). This suggests there are glycine-dependent and independent desensitization of NMDA receptors.

The glycine binding site is important for locomotor coordination as well as for startle and learning. Mice with a targeted point mutation in the glycine binding site have a reduction in glycine binding affinity and show impairments in motor tasks such as rotarod and grip strength when compared to their WT littermates. In addition, these mice have increased locomotor activity that does not habituate compared to their WT littermates (Ballard et al., 2002). Moreover, the mutated mice have deficits in learning tasks (Morris water maze) and increased startle (Ballard et al., 2002).

Little is known about the behavioral consequences of pharmacological blockade of the glycine$_B$ binding site due to the blood brain barrier impermeability of most glycine$_B$ ligands. However, (+)-HA-966, an amino acid derivative partial agonist for the glycine$_B$ binding site, has high bioavailability and is selective for the glycine$_B$ binding site. (+)-HA-966 has weak efficacy for the glycine$_B$ binding site (<10%) and antagonizes

the seizure activity produced by administration of glycine agonists. Thus, (+)-HA-966 can reduce the effect of a full agonist and thereby diminish the level of facilitation. There are currently only two other glycine$_B$ antagonists that are able to cross the blood brain barrier: 5-nitro-6,7-dicholor-1,4-dihydro-2,3-quinoxalinedione (ACEA 1021) and L-701,324. However, ACEA 1021 has actions at both AMPA and NMDA receptors and would not be ideal for use in determining specific effects of the glycine$_B$ NMDA receptor binding site (Lingenhohl & Pozza, 1998). Moreover, L-701,324 is a glycine$_B$ antagonist with high bioavailability that can be given orally (Leeson & Iverson, 1994). However, little research has been conducted on its effects on behavior.

Glutamate binding site

Glutamate binding to the NMDA receptor is required for NMDA receptor function. Based on X-ray structure modeling of the glutamate binding site, it appears that the glutamate binding site consists of two lobes (beta sheets and alpha helices) that are connected via a hinge (Tikhonova et al., 2002). There are at least two conformations suggested for the glutamate binding site: one for agonist binding and one for competitive antagonists or ligand-free. Binding of the agonist causes the binding site to close, opening the ion channel. Antagonist binding causes the binding site to remain in an open form, thus not activating the ion channel (Tikhonova et al., 2002). Glutamate fits into this pocket, but antagonists are too large and need to turn and bind to different residues (Tikhonova et al., 2002). Most competitive antagonists show no preference for any of the NR2 subunits.

The advantage of competitive NMDA receptor antagonists over the channel blockers is a decrease in the side effects associated with acute or chronic treatment. Two

potent, selective antagonists that can be administered intraperitoneally (i.p.) or per oral (p.o.) route are CGP-37849 and (*E*)-(±)-2-amino-4-methyl-5-phosphono-3-pentenoic acid ethyl ester (CGP-39551). Other antagonists such as 2-amino-5-phosphonopentanoic acid (AP-5), 2-amino-5-phosphonoheptanoic acid (AP-7), and 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid cannot cross the blood-brain barrier, therefore, are only suitable for intracranial administration. CGP-37849 and CGP 3551 are analogs of AP-5 that lack activity at other neurotransmitter binding sites. CGP-37849 competitively inhibits L-[^{3}H]-glutamate binding at levels similar to L-glutamate (Fagg et al., 1990). Furthermore, CGP 37849 does not alter glutamate uptake or release (Fagg et al., 1990).

Competitive antagonists have been typically used as anticonvulsants. For example, CGP-37849 suppresses electroshock-induced seizures (Fagg et al., 2000). In addition, competitive NMDA receptor antagonists have anxiolytic properties. For example, rats pretreated with CGP-37849 showed an increase in shocks accepted during a Vogel conflict drinking test as well as an increase in percent time in open arms and percent open arm entries in the elevated plus maze (Przegalinski et al., 2000). However, like noncompetitive antagonists, competitive NMDA receptor antagonists such as (R,E)-4-(3-phosphonoprop-2-enyl) piperazine-2-carboxylic acid (D-CPPene) and cis-4-[phosphomethyl]-piperidine-2-carboxylic acid (CGS 19755) can cause locomotor stimulation in mice, and high doses of CGP-37849 produce locomotor stimulation and ataxia in rats (Danysz et al., 1994; Waters et al., 1996).

NR2B binding site

NR2B-specific antagonists act by increasing proton inhibition of NMDA receptors, thus reducing the frequency of channel opening (Mott et al., 1998). The NR2B

binding site is inhibited at a high pH. Proton inhibition increases the sensitivity of NMDA receptors by shifting the pKa to the left, thus inhibiting channel function at a physiological pH. Ifenprodil and CP-101,606 donate electrons to the proton sensor which causes the channel to close. The exact location of this binding site on the NR2B subunit is unknown; however, data suggests that the site may reside in the amino terminal domain (Brimecombe et al., 1996). Ifenprodil, CP-101,606, and R-(R,S)-alpha-(4-hydroxypenyl)-beta-methyl-4-(phenyl-methyl)-1-piperidine propanol (Ro-25,6981) are antagonists for the NR2B NMDA receptor binding site that have high bioavailability (Lynch et al., 1995). They are voltage-independent antagonists that lack locomotor impairments and do not produce the neurological impairments that competitive and noncompetitive NMDA receptor antagonists produce (Carter et al., 1989; Witkin et al., 2003). Thus, these compounds have high clinical promise in the treatment of various CNS disorders.

Ifenprodil inhibits 90% of the inward current through the NMDA receptor and also reduces the frequency of the channel opening at high concentrations in whole cell and single-channel patch recording of rat hippocampal neurons (Legendre & Westbrook, 1991). Spermine and glycine do not alter ifenprodil's ability to increase NMDA receptor affinity (Zhang et al., 2000). Although widely used as an NR2B antagonist, ifenprodil also binds to alpha adrenergic receptors as well as to 5-HT3 receptors (McCool & Lovinger, 1995; Lovinger, 1996). CP-101,606 has greater specificity for NR2B subunit binding sites and acts in a similar manner to ifenprodil at NR2B binding sites (Chenard et al., 1995). However, there is recent evidence that CP-101,606 may act at a different population of NR1/NR2B receptors (Chazot et al., 2002).

Neurobiology of ethanol-induced CPP

Understanding the neurobiological mechanisms of ethanol-induced CPP will help to determine the underlying neural circuitry important for the rewarding effects of ethanol. Within the past 10 years, significant advancement has been made in the identification of neurotransmitters involved in the acquisition of ethanol CPP (Tables 1 and 2). Dopamine, GABA, and serotonin receptor systems play an integral role in the acquisition of ethanol-induced CPP, whereas the roles of opiate and stress systems appear to be less of a factor. However, little is known about the role of glutamate in ethanol-induced CPP.

Specific dopamine receptor subtype antagonisms and genetic deletions (KO) lead to alterations in ethanol reward. For example, antagonism of D_3 receptors enhances ethanol-induced CPP, but antagonism of D_2 and D_4 receptors do not alter this behavior (Boyce & Risinger, 2000, 2002; Risinger et al., 1992, Thrasher et al., 1999). However, D_3 receptor KO mice do not show enhanced ethanol-induced CPP when compared to C57BL/6J control mice (Boyce-Rustay & Risinger, 2003). On the other hand, D2 KO mice on a C57BL/6 x DBA/2J background show reduced ethanol-induced CPP in comparison to wild-type (WT) littermates (Cunningham et al., 2000a). This is in contrast to the pharmacological studies that showed effects of D_3 receptor antagonism, but not D_2 receptor antagonism (Boyce & Risinger, 2000, 2002; Risinger et al., 1992a). The disparity may be due to developmental compensation in the KO mice, to background effects that may occlude the development of ethanol-induced CPP, or to the antagonist acting at other receptor subtypes. Dopamine- and adenosine 3':5'-monophosphate-regulated protein (DARPP –32), a protein that phosphorylates dopamine receptors

(Greengard et al., 1999), is also important for ethanol CPP. DARPP-32 KOs show attenuated ethanol-induced CPP in comparison to WT litter mates (Risinger et al., 2001). Moreover, GIRK2 KO mice show a decrease in ethanol-induced CPP in comparison to WT (Hill et al., 2003). Dopamine receptors activate G protein coupled Inward Rectifying Potassium-2 (GIRK2) channels. Taken together, these data suggest that the dopamine receptor system plays a critical role in the acquisition of ethanol-induced CPP.

Components of the GABA system are important for ethanol place conditioning. Bicuculline and picrotoxin, both $GABA_A$ receptor antagonists, enhance ethanol CPP, whereas baclofen, a $GABA_B$ receptor agonist, does not (Chester & Cunningham, 1999a, 1999b). In addition, ethyl 8-azido-6-dihydro-5-methyl-6-oxo-4H-imidzo [1,5-a][1,4] benzodiazepine-3-carboxylate (Ro 15-4513), a partial inverse agonist for the benzodiazepine binding site on the $GABA_A$ receptor, does not either. This suggests that the $GABA_A$ receptor subtype, but not $GABA_B$ or benzodiazepine binding site, is important for the rewarding effects of ethanol as indexed by ethanol-induced CPP.

Several alterations in serotonergic neurotransmission have been shown to alter ethanol CPP. For example, antagonism of 5-HT_{1A} and 5-HT_2, but not 5-HT_3, receptors enhance ethanol CPP, suggesting that specific serotonin receptors are important for the rewarding properties of ethanol (Boyce-Rustay, unpublished observations; Risinger & Boyce, 2002a; Risinger & Oakes, 1996b). Moreover, 5-HT_{1B} receptor KOs show decreased ethanol CPP compared to WT littermates (Risinger et al., 1996). However, blockade of the reuptake of serotonin does not alter ethanol CPP (Risinger, 1997), suggesting that the serotonin transporter may not be involved in ethanol reward. Taken

together, these data suggest the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2 receptors are important for ethanol CPP.

The role of the opiate system in the acquisition of ethanol-induced CPP is less clear. The nonspecific opioid receptor antagonist naloxone and the deletion of the nociceptin gene have no effect on ethanol CPP, but the opioid receptor-like (ORL1) agonists nociceptin and (1S,3aS)-8-(2,3,3a,4,5,6-hexahydro-1H-phenalen-1-yl)-1,3,8-triaza-spiro [4,5] decan-4-one hydrochloride (Ro 64-6198) decrease ethanol CPP (Kuzmin et al., 2003). However, the deletion of the mu-opioid gene results in decreased ethanol CPP (Becker et al., 2002; Hall et al., 2001). However, preproenkephalin KO do not differ from WT in ethanol CPP (Koenig & Olive, 2002). Overall, these data suggest the mu-opioid receptor, but not other opiate receptors, is important for the acquisition of ethanol-induced CPP.

Modulation of the Hypothalamic-Pituitary –Adrenal (HPA) Stress axis provides conflicting results for the involvement of stress responses in ethanol CPP. For example, Corticotrophin Releasing Factor (CRF)-deficient mice show a decrease in ethanol-induced CPP in comparison to controls, suggesting that CRF is important for ethanol reward (Olive et al., 2003). However, pretreatment with allopregnalone and the steroid synthesis inhibitor aminoglutethimide (AMG) do not alter the acquisition (AMG and allopregnalone) or expression (allopregnalone) of ethanol-induced CPP (Chester & Cunningham, 1998; Gabriel et al., submitted). One would have expected that if stress hormones are important for ethanol CPP, AMG would have decreased ethanol CPP. However, since AMG did not alter ethanol CPP, the extent of stress hormone involvement in ethanol reward is unclear.

Table 1. Studies investigating the neurobiology of the acquisition of ethanol-induced CPP.

Receptor	Subtype	Drug	Effect	References
Dopamine				
	D2	Haloperidol	No effect	Risinger et al. (1992a)
	D3	U99194A	Enhancement	Boyce & Risinger (2000, 2002)
	D4	Clozapine	No effect	Thrasher et al. (1999)
GABA				
	A	Bicuculline	Enhancement	Chester & Cunningham (1999a)
		Picrotoxin	Enhancement	Chester & Cunningham (1999a)
	B	Baclofen[a]	No effect	Chester & Cunningham (1999b)
	Benzo	Ro 15-4513[†]	No effect	Risinger et al. (1992b)
Serotonin				
	1A	Pindobind	Enhancement	Risinger & Boyce (2002a)
	2	Mianserin	Enhancement	Risinger & Oakes (1996a)
	3	Ondansetron	No effect	Boyce-Rustay (unpublished)
		ICS-205,930	No effect	Boyce-Rustay (unpublished)
	SSRI	Fluoxetine	No effect	Risinger, 1997
Opioid				
	Nonselective	Naloxone[a]	No effect	Kuzmin et al. (2003)
	ORL1	nociceptin[a]	Decrease	Kuzmin et al. (2003)
		Ro 64-6198[a]	Decrease	Kuzmin et al. (2003)
Glutamate				
	NMDA	MK-801	Decrease*	Biala & Kotlinska (1999)
		L-701,324	Decrease*	Biala & Kotlinska (1999)
	NMDA	Acamprosate	Decrease	McGeehan & Olive (2003)

Table 1. Continued

Receptor	Subtype	Drug	Effect	References
Steroid				
	SSI	AMG	No effect	Chester & Cunningham (1998)
		Allopregnanolone	No effect	Gabriel et al., submitted

Note: All drugs listed are antagonists excepted where noted

† = partial inverse agonist
[a] = agonist
SSRI = selective serotonin reuptake inhibitor
SSI = steroid synthesis inhibitor

* = rats using a biased apparatus
Benzo = benzodiazepine binding site
AMG = aminoglutethimide
ORL= Opioid Receptor Like-1

Table 2. Effects of genetic deletion of a receptor/protein on ethanol-induced CPP

Receptor	Subtype	Effect	References
Dopamine			
	D_2	Decrease	Cunningham et al. (2000)
	D_3	No effect	Boyce-Rustay & Risinger (2003)
	DARPP-32	Decrease	Risinger et al. (1999)
	GIRK-2	Decrease	Hill et al. (2003)
Serotonin			
	5HT-1B	Decrease	Risinger et al. (1996)
Opiate			
	Mu	Decrease	Becker et al. (2002) Hall et al. (2001)
	Preproenkephalin	No effect	Koenig & Olive (2002)

Although there are many data suggesting the involvement of several neurotransmitter systems, such as dopamine, GABA, and serotonin in the neurobiology of ethanol-induced CPP, few data exist on the role of glutamate in ethanol-induced CPP. Acamprosate, a NMDA receptor channel blocker, decreases ethanol-induced CPP in mice (McGeehan & Olive, 2003). In addition, MK-801 and 7-chloro-4-hydroxy-3-(-3-phenalen-1-yl)-1,3,8-triaza-spiro[4,5]dexan-4-one hydrochloride (L-701,324) both attenuated ethanol-induced CPP in rats that were pre-exposed to 0.5 g/kg ethanol for 15 days before the beginning of conditioning and then conditioned in a biased apparatus (Biala & Kotlinska, 1999). Although these data suggest the involvement of NMDA receptors in ethanol CPP, procedural and species differences may make it difficult to compare these rat studies with the mouse literature. Moreover, there are interpretational issues with biased apparatuses and subject assignments (Cunningham et al., 2000b; Tzschentke, 1998). Overall, there are limited data suggesting NMDA receptors are important for the acquisition of ethanol-induced CPP.

NMDA RECEPTORS AND ETHANOL REWARD RELATED BEHAVIORS

Self-Administration

Ion channel binding site

Several studies have shown that low affinity channel blockers decrease ethanol self-administration (Table 3). Memantine, a low-affinity NMDA receptor channel blocker, decreases the conductance of the NMDA receptor ion channel (Kornhuber & Quack, 1995), decreased ethanol drinking during the first hour of a 4 hour limited access

Table 3. Summary of the effects of NMDA receptor antagonists on ethanol reward-related behaviors

Procedure	Compound	Effect	Reference
Self-Administration	memantine[a]	No effect	Bienkowski et al. (2001) Piasecki et al. (1998)
	memantine[a]	Decrease	Bienkowski et al. (2001) Bienkowski et al. (1999)
	PCP[a]	Decrease	Shelton & Balster (1997)
	MRZ 2/579[a]	Decrease	Bienkowski et al. (1999)
		Decrease	Bienkowski et al. (2001)
		No effect	Bienkowski et al. (2001)
	AP-5[b]	Decrease (NAC)	Rassnick et al. (1992)
	CPPene[b]	Decrease	Shelton & Balster (1997)
	MRZ 2/576[c]	No effect	Bienkowski et al. (1999)
Drinking	memantine[a]	Decrease	Piasecki et al.(1998)
Reinstatement	MK-801[a]	Increase[†]	Vosler et al. (2001)
Discrimination-Substitution	MK-801[a]	Full Substitution	Butelman et al. (1993) Grant & Colombo (1993) Harrison et al. (1998) Hundt et al. (1998) Kotlinska & Liljequist (1997) Schechter et al. (1993) Shelton & Grant (2002) Vivian et al. (2002)

Table 3. Continued

Procedure	Compound	Effect	Reference
		Full (NAC/CA1) Substitution †	Hodge & Cox (1998)
	MK-801	No effect (PrLC/ AMY)	Hodge & Cox (1998)
	Ketamine [a]	Full Substitution	Grant et al. (1991) Harrison et al. (1998) Vivian et al. (2002)
	PCP [a]	Full Substitution†	Butelman et al. (1993) Grant & Colombo (1993) Grant et al. (1991)
		Full Substitution	Hundt et al. (1998) Shelton & Balster (1994) Vivian et al. (2002)
	memantine[a]	Full Substitution	Hundt et al. (1998)
	CPP [b$]	No effect (NAC)	Hodge & Cox (1998)
	CPPene [b]	Partial Substitution	Grant & Colombo (1993)
	CGS 19755 [b]	No effect	Grant & Colombo (1993)
		Full Substitution	Sanger (1993)
	CGP 40116 [b]	Full Substitution	Bienkowski & Kostowski (1998) Bienkowski et al. (1996)
	CGP 37849 [b]	Partial Substitution†	Bienkowski et al. (1996)
	NPC 17742 [b]	Partial Substitution	Shelton & Balster (1994)
	ACEA-1021 [b]	No effect	Balster et al. (1995)
	L-701,324[c]	No effect	Hundt et al. (1998)
		Full Substitution	Kotlinska & Liljequist (1997)
	MRZ 2/502 [c]	No effect	Hundt et al. (1998)
	eliprodil[d]	Partial Substitution	Kotlinska & Liljequist (1997)
		No effect	Sanger (1993)

Table 3. Continued

Procedure	Compound	Effect	Reference
Place Conditioning	MK-801[a]	Decrease*	Biala & Kotlinska (1999)
	L-701,324[c]	Decrease*	Biala & Kotlinska (1999)
	Acamprosate	Decrease	McGeehan & Olive (2003)

Note: All studies involve systemic administration of drug treatment except where noted.

Site specific intracranial injection location abbreviations:
NAC = Nucleus accumbens
CA1 = CA1 region of hippocampus
PrLC = Prelimbic cortex
AMY = amygdala

a = NMDA Receptor channel blocker
b = NMDA Receptor competitive antagonist
c = NMDA Receptor $glycine_B$ antagonist
d = NMDA Receptor NR2B subunit antagonist

† = altered response rate in ethanol discrimination studies
$ = 3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid
* = rats using a biased apparatus

paradigm in rats that were trained to drink 8% ethanol (Piasecki et al., 1998). Moreover, this dose of memantine did not alter food intake. However, memantine did not alter operant responding for ethanol in a separate group of animals trained to lever press (FR1) for 8% volume/volume (v/v) ethanol (Piasecki et al., 1998).

Studies utilizing other low-affinity channel blockers have found contradictory results. 1-Amino-1,3,3,5,5-pentamethyl-cyclohexane hydrochloride (MRZ 2/579) dose-dependently decreased responding for 8% v/v ethanol in an FR1 operant paradigm (Bienkowski et al., 1999). However, MRZ 2/579 increased nonreinforced (extinction) responding for ethanol at 2.5 mg/kg, but decreased extinction responding at 5 mg/kg. The decrease in both reinforced and extinction responding for ethanol may be due to alterations in reward (reinforced responding) and craving (extinction responding). However, the results may also be due to learning or locomotor impairments. Locomotor activation caused by the channel blockers could increase the responding at 2.5 mg/kg and may produce impairments at 5 mg/kg.

In another study, MRZ 2/579 administered via minipumps did not alter operant responding for ethanol (8% v/v) or 24 h ethanol consumption in separate groups of rats (Bienkowski et al., 2001). However, repeated i.p. injections of MRZ 2/579 decreased operant responding for ethanol (Bienkowski et al., 2001). Unfortunately, the authors did not investigate repeated injection of MRZ 2/579 on ethanol drinking in this study. The authors suggest one possible interpretation of the data is that the chronic 24-h administration of MRZ 2/579 may have lead to the development of tolerance to the effects of MRZ 2/579.

MK-801 and PCP, both NMDA receptor channel blockers, have also been shown to alter ethanol self-administration. Pretreatment with PCP attenuated the number of ethanol deliveries compared to ethanol deliveries with saline pretreatment, but also decreased the mean saccharin (0.1 % w/v) deliveries during an alternating 5-min FR4 schedule of reinforcement for 60 min (Shelton & Balster, 1997. The effects of PCP on locomotor activity could affect the subject's ability to respond for both saccharin and ethanol. On the other hand, the effect of PCP on ethanol self-administration may be nonspecific, reflecting a more general effect on reward pathways.

MK-801 affects the reinstatement of ethanol seeking behavior in rats. In this procedure, one group was trained to respond for 10% ethanol/ 2% sucrose and another group was trained to respond for 3% sucrose. After extinction, the group trained to respond for ethanol showed an increase in responding on the active lever versus inactive lever when given a 0.5 g/kg ethanol injection, indicating ethanol reinstated ethanol-seeking behavior (Vosler et al., 2001). The group trained with sucrose responded at low levels on both levers. However, both the sucrose and ethanol trained groups increased responding on both the active and inactive levers when injected with 0.175 mg/kg MK-801. Moreover, inactive lever responding was higher than active lever responding in the ethanol trained group, suggesting that pretreatment with MK-801 induced a loss of discriminative control. This study provided a useful control for learning and locomotor alterations. The addition of an inactive lever can be used to measure motor and learning impairments, something that is of great concern when dealing with NMDA receptor antagonists as well as with ethanol.

Overall, these studies suggest that NMDA receptor channel blockers reduce ethanol self-administration. However, procedural differences produce contradictory results. It appears from these studies that although NMDA receptor ion channel blockers decrease the primary reinforcing properties of ethanol, the results may be due to motor incoordination (caused by an additive pharmacological effect of the antagonist and ethanol) or alterations in learning the operant tasks. One cannot determine which of these interpretations is correct using the ethanol self-administration paradigm. It also appears that the secondary reinforcing properties of ethanol (craving) may be altered by antagonism of the NMDA receptor ion channel binding site.

Glycine$_B$ binding site

There are relatively few data regarding NMDA receptor glycine$_B$ binding site regulation of operant ethanol self-administration or ethanol drinking. However, a glycine$_B$ antagonist, 8-chloro-4-hydroxyl-1-oxo-1,2-dihydropyridazino [4,5b] quinolin-5-oxide choline salt (MRZ 2/576), does not affect operant ethanol self-administration in rats (Bienkowski et al., 1999). Although it did not alter ethanol reinforced responding, pretreatment with the antagonist increased nonreinforced responding (measurement of craving) at low doses. In addition, transgenic mice that have 80% decreased affinity for the glycine for the NMDA receptor [Grin 1(D481N)] do not differ from wild-type (WT) mice in ethanol-related behaviors. Both transgenics and WT subjects responded similarly for ethanol in a two-bottle choice drinking experiment (2-16% v/v ethanol versus water) and in forced ethanol (16% v/v) consumption. These results suggest that the glycine$_B$ binding site does not modulate ethanol self-administration or ethanol drinking, but

perhaps is important for the craving or secondary reinforcing properties associated with ethanol self-administration.

Glutamate binding site

There are limited data on the role of NMDA receptor glutamate binding in ethanol self-administration. One probable reason for the lack of data is the effects NMDA receptor competitive antagonists have on locomotor activity. These effects are outlined in a subsequent section (page 32). Although the competitive NMDA receptor antagonists have fewer locomotor impairments than noncompetitive NMDA receptor channel blockers, they still increase locomotor activity at high doses. D-CPPene and (cis-4-[phosphomethyl]-piperidine-2-carboxylic acid (CGS 19755) dose-dependently increase basal locomotor activity in rats and mice (Waters et al., 1996). Thus, in combination with ethanol, significant locomotor impairments can occur. These impairments can alter ethanol self-administration or drinking nonspecifically. Controlling for locomotor impairments through the introduction of inactive levers or responding for water or saccharin can help address these alternative interpretations of ethanol self-administration results.

Two studies addressed the role of the competitive NMDA receptor antagonists AP-5 (Rassnick et al., 1992) and CPPene (Shelton & Balster, 1997) in ethanol self-administration. Bilateral microinjections of AP-5 into the nucleus accumbens decreased responding (FR1) for ethanol (10% v/v) at doses that did not alter responding for water. This suggests that AP-5 decreased the rewarding properties of ethanol in the nucleus accumbens. Moreover, the competitive antagonist CPPene decreased the mean ethanol deliveries in rats responding for 10% v/v ethanol, but also decreased the mean saccharin

(0.1%) deliveries (Shelton & Balster, 1997). However, in this study subjects responded in alternating 5-min blocks for ethanol and saccharin during the 60-min session. This would lead to cumulative ethanol doses and increasing motor deficits that could affect the responding for saccharin. On the other hand, the decrease in responding for ethanol and saccharin suggests that the glutamate binding site may alter general reward or suggests impairments in learning.

Overall, these studies suggest that the glutamate binding site plays a role in the rewarding properties of ethanol. Although both studies showed a decrease in ethanol self-administration, alternative interpretations cannot be excluded. It is possible that the effects of AP-5 are nonspecific. However, the responding for water is difficult to assess in comparison to ethanol self-administration due to such low levels of baseline responding for water. This decrease in responding with CPPene pretreatment may be due to the motor incoordinating effects or due to alterations in learning. Taken together, these results could be due to nonspecific alterations in behavior caused by drug pretreatment, learning, or alterations in ethanol reward. Other studies need to address the specificity of these results.

NR2B binding site

There are no pharmacological data regarding the role of the NR2B subunit binding site in ethanol self-administration. However, there are some data to suggest NR2B subunit protein expression is increased with voluntary ethanol drinking (Henniger et al., 2003). Rats who had access to 5%, 10%, and 20% (v/v) ethanol and water for a 19 month period had an increase in the NR2B subunit protein in the prefrontal cortex, as seen by immunobloting, in comparison to control rats who only had access to water.

More studies are needed to draw any conclusions about the involvement of the NR2B binding site in ethanol self-administration.

Drug Discrimination

Ion channel binding site

There is substantial evidence that the NMDA receptor ion channel mediates, in part, the discriminative stimulus effects of ethanol. MK-801, memantine, ketamine, and PCP consistently substitute for ethanol in various species such as rat, mouse, pigeon, and monkey (Butelman et al., 1993; Grant et al., 1991; Grant & Colombo, 1993; Harrison et al., 1998; Hundt et al., 1998; Kotlinska & Liljequist, 1997; Schechter et al., 1993; Shelton & Balster, 1994; Shelton & Grant, 2002; Vivian et al., 2002). MK-801 fully substituted for 2.0 g/kg ethanol without affecting response rates (Grant & Colombo, 1993). However, MK-801 affected response rates at doses that fully substituted for 1 and 1.5 g/kg ethanol. A similar finding occurred in the Shelton & Balster (1994) and Shelton & Grant (2002) studies. Nonetheless, other studies have shown that MK-801 fully substitutes for the ethanol, but does not alter response rates (Hundt et al., 1998). Findings with PCP are similar, however, PCP decreased the response rates in all studies (Grant & Colombo, 1993; Hundt et al., 1998; Shelton & Balster, 1994).

The neuroanatomical basis for MK-801's ability to substitute for ethanol has been investigated. Hodge & Cox (1998) trained rats on a two-lever discrimination task to discriminate between 1 g/kg ethanol and saline. MK-801 microinjected into the nucleus accumbens core (NAC) and CA1 region of the hippocampus fully substituted for the ethanol cue. However, MK-801 decreased response rate when full substitution occurred

(10 μg/ul) in both the CA1 and the NAC. MK-801 injected into prelimbic cortex produced partial substitution.

Overall, these results suggest that the NMDA receptor ion channel binding site mediates a component of the subjective effects of ethanol. Likewise, the NMDA receptor ion channel binding site may be modulating these effects via the nucleus accumbens core and hippocampus. Nevertheless, it should be noted that the noncompetitive NMDA receptor ion channel blockers altered the response rate in many studies. Response rates are used to control for alterations in motor coordination that can confound interpretations of drug discrimination data.

Glycine$_B$ binding site

There is a small body of literature that suggests the glycine binding site on the NR1 subunit of the NMDA receptor is not important for the subjective, or discriminative, stimulus properties of ethanol. This conclusion is based primarily on two studies. The first study used Wistar rats that were trained to discriminate (FR10) 1 g/kg ethanol and saline for food reinforcement. L-701,324 and MRZ 2/502 were unable to substitute for the ethanol cue (Hundt et al., 1998). However, L-701,324 fully substituted for ethanol at doses that did not alter locomotor activity in another study (Kotlinska & Liljequist, 1997). Although these compounds did not substitute for ethanol, they did alter response rate, suggesting that behaviorally active doses were being tested. ACEA-1021 was also unable to substitute for ethanol (Balster et al., 1995). These results indicate that behaviorally active doses of glycine$_B$ antagonists do not modulate the discriminative stimulus properties of ethanol.

Glutamate binding site

The literature is mixed regarding the NMDA receptor glutamate binding site's ability to regulate the subjective effects of ethanol. CPPene and 2R,4R,5S-(2-amino-4,5-1,2-cyclohexyl)-7-phosphonoheptanoic acid (NPC 17742) substituted for a 1 g/kg ethanol dose (Grant & Colombo, 1993; Shelton & Balster, 1994). A dose of 5.6 mg/kg CPPene decreased response rate, and 15.6 mg/kg NPC 17742 did not alter response rate, but both partially substituted for 1.0 g/kg ethanol (Shelton & Balster, 1994). Interestingly, CPPene showed greater substitution for 1 g/kg than 1.5 and 2.0 g/kg ethanol (Grant & Colombo, 1993). Other studies have shown that CGS 19755 substituted for ethanol in some studies, but it did not in other studies (Butelman et al., 1993; Grant & Colombo, 1993; Sanger, 1993). The D-isomer of CGP-37849 (CGP-40116) was able to fully substitute for 1 g/kg ethanol in Wistar rats, but CGP-37849 only partially substituted for 1 g/kg ethanol (Bienkowski et al., 1996). Nonetheless, CGP-37849 significantly decreased the response rate at the doses that partial substitution occurred. Furthermore, CGP 40116 enhanced ethanol discrimination by shifting the ethanol dose-response curve to the left (Bienkowski & Kostowski, 1998). These apparent differences may be due to the training dose used. Generalization to lower doses of ethanol is less specific than to higher doses of ethanol (Grant & Colombo, 1993).

Taken together, these results suggest that the glutamate binding site may be important for a component of the discriminative stimulus properties of ethanol at low ethanol training doses. As mentioned previously, it has been suggested that substitution at low, but not high, ethanol training doses may be less specific (Grant & Colombo, 1993). Therefore, more drugs may be able to substitute at lower doses. However, not all

competitive NMDA receptor antagonists substitute for ethanol. The doses at which these antagonists fully or partially substitute for ethanol usually do not alter response rate. Higher doses of competitive antagonists, such as CGP-37849, might fully substitute for ethanol, but the motor impairments caused by these higher doses may interfere with responding and thus confound the interpretation.

NR2B binding site

Little work has been conducted on the ability of the NMDA receptor NR2B binding site to modulate ethanol's subjective effects. There appears to be only one study where eliprodil partially substituted for a 1 g/kg ethanol cue (Kotlinska & Liljequist, 1997). However, another study suggests that eliprodil does not modulate the subjective effects of ethanol (Sanger, 1993). Taken together, the role of NMDA receptor NR2B binding sites in the discriminative stimulus properties of ethanol remain unclear. Additional antagonists (e.g. ifenprodil, CP-101,606) need to be tested in order to make a more definitive conclusion.

Place Conditioning

Ion channel binding site

Ethanol-induced place preference is altered when rats are given pretreatment of NMDA receptor antagonists. MK-801 blocked the acquisition of ethanol-induced CPP in rats that were trained in a biased apparatus (Biala & Kotlinska, 1999). During this procedure, rats were given 15 days of 0.5 g/kg ethanol injections (1 injection per day) before the conditioning phase began. The next phase consisted of a pre-conditioning measurement of time spent in the white compartment and time spent in the black compartment. During conditioning, rats were confined to their non-preferred

compartment and given injections of MK-801 (0.1 mg/kg) and ethanol (0.5 g/kg). On alternate days, they were exposed to the preferred compartment and given injections of water. Rats had access to both compartments during a preference test and were given no injections. Significant place conditioning occurred when rats spent more time on the drug-paired floor (non-preferred) post-conditioning in comparison to pre-conditioning time. It should be mentioned that initial biases, like those in this study, often confound interpretations. A decrease in preference may be actually due to a decrease in aversion to the non-preferred context and not due to an actual decrease in the rewarding effects (Bardo & Bevins, 2000). Moreover, the drug could have profound effects on locomotor activity that can affect the subject's interaction with the cue(s). Furthermore, MK-801 could be reducing the anxiolytic effect of ethanol independent of ethanol reward. This would reduce a place preference for ethanol in a biased design. Therefore it is unclear if MK-801 really decreased the rewarding properties of ethanol, if it reduced the aversion to the non-preferred compartment, or if it reduced the anxiolytic effect of ethanol.

Moreover, it is unclear whether MK-801 has rewarding properties on its own (Table 4). Some studies have shown that MK-801 and ketamine are able to produce CPP in mice and rats (Panos et al., 1999; Papp et al., 1996; Steinpreis et al., 1995; Sukhotina et al., 1998; Suzuki et al., 1999a; Suzuki et al., 2000). Others have shown that MK-801 failed to produce a significant CPP on its own (Kim et al., 1996; Kim & Jang, 1997; Tzschentke & Schmidt, 1995). Different conditioned stimuli (visual versus tactile), species (rats versus mice), and or biased versus unbiased apparatuses and subject assignments make it difficult to compare these studies and to determine why some studies found MK-801 to be rewarding and others not.

Glycine$_B$ binding site

L-701,324, a glycine$_B$ antagonist, decreased the acquisition of ethanol-induced CPP in rats (Biala & Kotlinska, 1999). The procedure used in this study was the same as in the MK-801 study. Briefly, rats were given preinjections of ethanol for 15 days and then were given an initial preference test. All subjects were subsequently conditioned against their initial biases. During the preference test, subjects were given access to both chambers. The results from the post-test minus pre-test indicated that control subjects showed ethanol-induced CPP, whereas the L-701, 324 pretreated subjects did not show ethanol-induced CPP. Furthermore, L-701, 324 and 1-aminocyclopropane carboxylic acid (ACPC) did not produce place preference alone (Kotlinska & Biala, 1999; Papp et al., 1996). These results suggest that antagonism of this site might also mediate ethanol place preference. However, L-701,324 may decrease the anxiolytic properties of ethanol that could result in a decrease the time spent in the non-preferred compartment independent of ethanol reward. Moreover, the majority of the literature indicates that rats exhibit a place aversion for the ethanol-paired cue in place preference studies. More studies need to be conducted to determine if NMDA receptor glycine$_B$ binding site antagonists alter ethanol CPP in an unbiased apparatus.

Glutamate binding site

Competitive NMDA receptor antagonists have not been investigated in ethanol-induced CPP experiments. Competitive antagonists CGP-37849 and CGP-40116 have been shown to produce CPP alone (Papp et al., 1996; Tzschentke & Schmidt, 1995). However, these studies utilized a biased apparatus and the results may be due to a decrease in the aversion for the non-preferred compartment. In support of these

Table 4. Summary of the place conditioning induced by NMDA receptor antagonist alone

Procedure	Compound	Effect	Reference
Place preference			
	MK-801 [a]	CPP	Panos et al. (1999) Papp et al. (1996) Steinpreis et al. (1995) Sukhotina et al. (1998) Suzuki et al. (2000)
	MK-801 [a]	No effect	Kim et al. (1996) Tzschentke et al. (1995)
	Ketamine [a]	CPP	Suzuki et al. (2000)
	CGP 37849 [b]	CPP	Papp et al. (1996)
	CGP 40116 [b]	CPP	Papp et al. (1996)
	Ifenprodil [d]	No effect	Suzuki et al. (1999)

a = NMDA Receptor channel blocker
b = NMDA Receptor competitive antagonist
d = NMDA Receptor NR2B subunit antagonist

alternative hypotheses, CGP-37849 has anxiolytic properties (Jessa et al., 1996; Przegalinski et al., 2000). These results suggest that the glutamate binding site may be a target for ethanol reward and antagonists for this site may be rewarding on their own. However, this conclusion needs to be considered with caution due to interpretational issues of biased designs. Moreover, studies still need to be conducted in order to determine the role of the NMDA receptor glutamate binding site in ethanol CPP.

NR2B binding site

There has been no NMDA receptor NR2B subunit binding site antagonist tested for its ability to alter the rewarding properties of ethanol in place conditioning. The NR2B antagonist ifenprodil has not been tested in ethanol-induced CPP, but does not produce significant CPP on its own in mice (Suzuki et al., 1999b). The doses in that experiment were behaviorally relevant since ifenprodil dose-dependently attenuated the acquisition of morphine-induced CPP at the same doses (Suzuki et al., 1999b). However, the role of the NR2B subunit in ethanol-induced CPP remains unclear.

Overall, MK-801 and L-701,324 are the only NMDA receptor antagonists that have been investigated in ethanol-induced CPP. Both MK-801 and L-701,324 attenuated ethanol-induced CPP in rats pre-exposed to ethanol and trained in a biased apparatus. Those results suggest that the NMDA receptor ion channel and glycine$_B$ binding sites are important for the rewarding properties of ethanol. However, the results from the biased apparatus studies may be due to alterations in unconditioned motivational properties independent of ethanol reward. Moreover, it is unclear whether the glutamate binding site and or the NR2B subunit binding sites are important for ethanol reward.

Ethanol-NMDA Receptor Interactions

In vitro

Ethanol has direct effects on the NMDA receptor, such that it inhibits NMDA receptor current (Lovinger et al., 1989; Lovinger et al., 1990). However, chronic ethanol administration leads to an upregulation of NMDA receptors (Kumari & Ticku, 2000; Samson & Harris, 1992). Although ethanol inhibits the receptor, the exact mechanism for this inhibition is unknown. It is thought that ethanol acts as a noncompetitive NMDA receptor antagonist and may affect one or more binding sites. Results from whole-cell patch clamping of hippocampal neurons have indicated that ethanol's actions at NMDA receptors are voltage-independent and ethanol does not alter the reversal potential of NMDA receptor currents (Peoples et al., 1997). This suggests that the inhibitory effect was not altered with changes in membrane potential. There are also data to suggest that ethanol may not act directly at any of the binding sites (Peoples et al., 1997). Moreover, ethanol sensitivity of NMDA receptors is dependent on NMDA receptor subunit composition. NR1/NR2A- and NR1/NR2B-containing receptors are more sensitive to ethanol than NR1/NR2C- and NR1/NR2D-containing receptors (Masood et al., 1994; Mirshahi & Woodward, 1995). Ethanol may have its actions at NMDA receptors via a postsynaptic mechanism or presynaptically by altering the release of glutamate or inhibiting glutamate clearance from the synapse (Peoples, 2003). Most data suggest that behaviorally relevant effects of ethanol on NMDA receptors are mediated by postsynaptic mechanisms.

In vivo

The behavioral effects of ethanol (i.e., tolerance, locomotor activation, locomotor sensitization, dependence) can be attributed, at least in part, to the direct inhibition of NMDA receptors by ethanol. For example, mice given a chronic liquid diet consisting of 5 % ethanol in skim milk show severe withdrawal signs upon removal of this diet. Subjects that received treatment with ifenprodil (NR2B subunit antagonist) showed a decrease in withdrawal signs in comparison to the subjects given treatment of saline (Narita et al., 2000). In addition, ethanol-dependent subjects had an increase in NR2B protein expression. Moreover, ifenprodil decreased seizures in ethanol-dependent Swiss-Webster mice (Malinowska et al., 1999). There is also evidence that suggests competitive NMDA receptor antagonists alter ethanol withdrawal. CGP 39551, an NMDA receptor competitive antagonist, decreased ethanol withdrawal (Ripley et al., 2002). AP-7 and CGP-37849 decreased the anxiogenic effects of ethanol withdrawal by increasing the percentage of open arm entries and the percentage of open arm time in the elevated plus maze (Gatch et al., 1999). These data suggest that the NR2B and glutamate binding sites are important for ethanol withdrawal.

Only a few NMDA receptor binding sites have been studied for their involvement in the hypnotic effects (sleep time) of ethanol. Ifenprodil, but not MK-801, decreased ethanol-induced sleep time at low doses and increased sleep time at high doses in male Swiss-Webster mice (Malinowska et al., 1999). However, both young and adult rats show a dose-dependent increase in sleep time with pretreatment of MK-801 (Silveri & Spear, 2002). The low-affinity channel blocker memantine also potentiated ethanol-induced

sleep time in rats (Beleslin et al., 1997). These data suggest that the NR2B and channel binding sites are important for the mediation of the hypnotic effects of ethanol.

NMDA receptors also play an important role in the locomotor activating effects of ethanol. MK-801 has been shown to decrease ethanol-stimulated activity in DBA/2J mice (Broadbent & Weitemier, 1999; Camarini et al., 2000; Shen & Phillips, 1998). Along with altering ethanol-stimulated activity, NMDA receptor drugs also affect sensitization to ethanol. The expression of locomotor sensitization (increased locomotor activity following repeated drug injections) can be decreased by administration of MK-801, but not ifenprodil, prior to ethanol injections during the test (Broadbent et al., 2003). Meyer and Phillips (2003) have shown that pretreatment with MK-801 increase ethanol-induced sensitization at low doses and attenuated this behavior at high doses. The opposite effects of low and high doses of MK-801 appear to be due to MK-801's own behavioral effects when coadministered with ethanol. Thus, MK-801 potentiated the ataxic-sedative effects of ethanol. These data suggest that the ion channel binding site plays a role in the acquisition and expression of sensitization, whereas the NR2B binding site does not. However, other NMDA receptor binding sites have not been tested.

Rationale

The foregoing review suggests that NMDA receptors play a role in the intoxicating, rewarding, and discriminative stimulus effects of ethanol. Ethanol directly inhibits NMDA receptor function at doses that are intoxicating to humans. However, data presented here also indicate that there are inconsistencies in results regarding the role of NMDA receptor binding sites in ethanol reward. These differences may be due to species differences in response to ethanol, lack of overlap in the reward measured by self-

administration and place preference, or procedural differences. Furthermore, all NMDA receptor binding sites have not been investigated within a single study. There have been only two studies that investigated the role of the NMDA receptor in ethanol-induced place preference. Furthermore, these studies used rats, a species that does not typically develop a place preference for ethanol (Cunningham et al., 1993; Sherman et al., 1988; Tzschentke, 1998). Since mice, particularly DBA/2J, show robust place preference for ethanol, they are better suited to examine the role of NMDA receptor binding sites in ethanol-induced CPP. Moreover, interpretations of ethanol self-administration and discrimination studies are often confounded by antagonist-induced locomotor impairments at the time of testing. The studies within this dissertation will help characterize the role of NMDA receptor binding sites in ethanol-induced locomotor activity. Furthermore, these studies identify drugs that could be effective medications for the treatment of alcohol abuse and alcoholism.

Data collected for this dissertation are presented in the format of two papers that will be submitted for publication. The first paper (Chapter 1) describes a series of eight experiments that examined the role of the different NMDA receptor binding sites in ethanol place conditioning. The second paper (Chapter 2) describes a taste aversion experiment that examined CGP-37849's (a competitive NMDA receptor antagonist) ability to alter the aversive properties of ethanol and LiCl. This is followed by an overall discussion of the findings from this project. Appendix A contains data that were excluded from Chapter 1 because of a lack of ethanol-induced CPP in the control group (Experiment 1) and lack of alteration of ethanol-induced CPP with MK-801 pretreatment (Experiment 2). Appendix B contains additional locomotor activity were analyzed for the development of locomotor sensitization that develops from repeated injections of 2 g/kg ethanol.

Chapter 1: The role of NMDA receptor bindings sites in ethanol place conditioning

Little is known about the specific role of glutamate, in particular its actions at NMDA receptors, in ethanol reward. Pretreatment with the channel blockers (MK-801 and ketamine), NR2B antagonists (ifenprodil and CP-101,606), and a glycine$_B$ partial agonist ((+)-HA-966) did not alter the acquisition of ethanol-induced conditioned place preference (CPP) in mice. However, pretreatment with the competitive antagonist CGP-37849 attenuated the acquisition of ethanol-induced CPP. Follow-up experiments indicated that CGP-37849 also blocked the acquisition of ethanol-induced CPA but did not produce rewarding or aversive effects on its own. These results suggest that CGP-37849 modulates ethanol place conditioning by modifying ethanol's motivational effects or by impairing the ability to learn these tasks.

Introduction

Binding sites on the NMDA receptor complex have been implicated in the rewarding properties of ethanol (Koob et al., 1998). To date, most studies investigating the role of these binding sites in ethanol reward have used self-administration procedures. However, the literature is sparse and differences across the various NMDA receptor binding sites have not been systematically examined. Based on recent studies, it appears that antagonism of the glutamate binding site and blockade of the NMDA receptor channel alter ethanol self-administration. For example, MRZ 2/579, an NMDA receptor channel blocker, selectively and dose-dependently decreased the operant self-administration of 10% v/v ethanol in rats, but it did not alter two-bottle choice drinking when delivered via osmotic minipumps (Bienkowski et al., 1999). Another study showed that the NMDA receptor channel blocker PCP decreased operant ethanol self-administration (Shelton & Balster, 1997). AP-5, a competitive NMDA receptor antagonist, decreased operant responding for 10% ethanol when injected into the nucleus accumbens of rats (Rassnick et al., 1992). In addition, the competitive antagonist CPPene decreased the mean ethanol deliveries in rats responding in an operant procedure for 10% v/v ethanol (Shelton & Balster, 1997). However, the glycine$_B$ antagonist MRZ 2/576 was found to have no effects on operant ethanol self-administration in rats (Bienkowski et al., 1999). Overall, these studies suggest that antagonism of the NMDA receptor ion channel and glutamate binding site, but not glycine$_B$ binding site, decreases ethanol self-administration. One interpretation of these findings is that the reduction in self-administration is due to an NMDA receptor mediated reduction in ethanol's rewarding effects.

Conclusions about the role of NMDA receptors in ethanol reward based on self-administration studies must be tempered by several considerations. One potential problem is that NMDA receptor antagonists have profound effects on locomotor activity and coordination (Carter, 1994; Waters et al., 1996). For example, PCP pretreatment decreases the number of ethanol (10% w/v) deliveries in comparison to saline pretreated rats, but PCP also decreases mean saccharin deliveries (Shelton & Balster, 1997). Therefore, this decrease in ethanol and saccharin responding could be due either to motor deficits or to general decrements in reward. In addition, self-administration of ethanol can be influenced either by increases or by decreases in the rewarding value of ethanol. Thus, a drug that produces a decrease in ethanol self-administration might do so either by decreasing or by increasing the rewarding value of ethanol (Cunningham et al., 2000b). If drug treatment makes ethanol more rewarding, subjects can drink less ethanol yet still achieve the same reward state. On the other hand, if a drug treatment reduces ethanol reward, subjects may increase responding to achieve a desired effect or they may stop responding completely. In light of these interpretational issues, studies of NMDA receptor antagonists on ethanol self-administration yield ambiguous conclusions about their role in ethanol reward.

Place conditioning offers an alternative method for studying the neurobiology of drug reward (Carr et al., 1989; Tzschentke, 1998). The conditioned place preference (CPP) paradigm potentially avoids some of the interpretational issues of self-administration paradigms because one can separate the acquisition phase from the test phase. Because testing during place conditioning studies is typically conducted in the absence of drug, the likelihood of locomotor differences during testing is substantially

decreased. In addition, it is possible to separately measure ethanol reward and aversion (Cunningham et al., 1997) as well as the rewarding or aversive value of the antagonist treatment itself. Thus, the place conditioning procedure allows more definitive conclusions regarding the direction of effect (increased or decreased) of a drug treatment on ethanol reward.

Relatively few data are available regarding the effects of NMDA receptor antagonists on ethanol-induced CPP. Moreover, all of these data come from studies with rats, a species that typically develops conditioned place aversion with ethanol (Cunningham et al., 1993; Sherman et al.,1988; Tzschentke, 1998). MK-801 and L-701,324 blocked the acquisition of ethanol-induced CPP in rats (Biala & Kotlinska, 1999). However, interpretation of these outcomes as evidence of NMDA receptor modulation of ethanol reward is complicated by the use of a biased apparatus and biased stimulus assignment procedure. That is, rats strongly preferred one of the stimulus alternatives before conditioning and ethanol was paired with the less preferred stimulus. Moreover, biased designs may yield false positives (Bardo & Bevins, 2000; Tzschentke, 1998). Such false positives may be due to the antagonist's effect on the unconditioned motivational state produced by the initial preference, rather than to an alteration in ethanol reward (Cunningham et al., 2003). For example, drug treatment may alter ethanol's anxiolytic effects or have anxiolytic effects on its own. Thus, these alternative interpretations could explain decreases in ethanol-induced CPP with MK-801 and L-701,324.

The present studies systematically examined several of the NMDA receptor binding sites using multiple antagonists. Specifically, the aim of the current studies was

to characterize the role of four NMDA receptor binding sites, specifically, glutamate, NR2B, glycine, and NMDA receptor channel, on ethanol CPP in DBA/2J mice. Although other studies have investigated some of these NMDA receptor binding sites in ethanol CPP, we used an unbiased apparatus and subject assignment procedure in order to avoid interpretational issues raised by use of a biased procedure (Cunningham et al., 2003). Furthermore, mice were used instead of rats. DBA/2J mice develop robust ethanol-induced CPP. In addition, antagonist treatment was given only during the acquisition phase of the studies. Because subjects were drug-free during testing, these studies avoided the antagonist-induced locomotor impairments that sometimes confound the interpretation of self-administration studies. The current studies also assessed the effects of NMDA receptor antagonists on ethanol-induced locomotor activation utilizing several doses of the antagonists.

Method

Subjects

Naive male DBA/2J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. Animals were allowed to acclimate to the colony room for 12 days before training. A 12-h/12-h light/dark cycle was in effect (lights on at 0700), and the colony room was maintained at an ambient temperature of 21°C ± 1°C. Experiments were conducted during the light cycle. Mice were housed four per cage in polycarbonate cages (27.9 x 9.5 x 12.7 cm) with cob bedding. Laboratory chow was continuously available in the homecage. All studies were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*

(1996) and with approval from Oregon Health & Science University Institutional Animal Care and Use Committee.

Apparatus

The place conditioning apparatus consisted of twelve identical acrylic and aluminum chambers (30 X 15 X 15 cm), each within a ventilated, light and sound attenuating box (Coulbourn Model E10-20). Infrared light sources and detectors were positioned opposite each other at 5-cm intervals on the long walls of each chamber, 2.2 cm above the floor surface. Occlusion of the infrared light beams was used both as a measure of locomotor activity and to determine the animal's position in the chamber. Data were recorded each minute by the computer with a 10 ms resolution. The floor of each box consisted of interchangeable halves with one of two distinct textures: "Hole" floors were made from perforated stainless steel with 6.4 mm round holes on 9.5 mm staggered centers; "Grid" floors were composed of 2.3 mm stainless-steel rods mounted 6.4 mm apart in acrylic rails. Naive DBA/2J mice have no basal preference for either floor type (Cunningham, 1995).

Drugs

Ethanol was administered i.p. at a 2 g/kg ethanol dose [20% v/v, 12.5 ml/kg] in all studies. A 2 g/kg ethanol dose was chosen because DBA/2J mice show robust place preference to this ethanol dose (Chester & Cunningham, 1999a; Chester & Cunningham, 1998; Cunningham et al., 2002). NMDA receptor antagonists and partial agonists used are listed in Table 5 along with the time delay before the ethanol injection, doses, and route of administration for all experiments. The time delays were chosen based on other

behavioral studies using these drugs in rodents. All drugs were administered in a volume of 10 ml/kg dissolved in 0.9% (w/v) saline.

Conditioning Procedures

Seven of the eight experiments reported here used place conditioning procedures selected to induce ethanol CPP in vehicle-pretreated control mice (Table 5). Based on initial findings with CGP-37849, however, one of our follow-up experiments used a procedure known to produce conditioned place aversion (CPA) in mice (Experiment 1C). The CPP and CPA procedures are described separately in the following subsections

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Insert Table 5 about here

Conditioned place preference. The experimental sequence consisted of a 5-min habituation session, four conditioning trials of each type (i.e., four CS+ and four CS-), and preference testing. Sessions were conducted 5 days a week, one session per day.

The habituation session (Day 1) consisted of two injections of saline (10 ml/kg and 12.5 ml/kg) separated by the time indicated in Table 5 for each experiment. After the first injection, subjects were returned to the home cage. After the second injection, subjects were immediately placed into the apparatus with paper floor for the 5-min session. The purpose of the habituation session was to minimize the effect of novelty and the stress of the injections.

Subjects were randomly assigned to one of three or four drug treatment groups depending on the experiment (Table 5). Animals were returned to the home cages after the first injection. After the second injection, subjects were placed into the apparatus for a

Table 5. Drug pretreatment information for place conditioning experiments

Exp.	Drug	NMDA receptor binding site	Source		Doses	Group (n)		Drug pretreatment interval
1A	CGP-37849	Glutamate	Tocris Cookson (Ellisville, MO)	CPP	0 mg/kg 5 mg/kg 10 mg/kg 15 mg/kg	0 (24) 5 (24) 10 (23) 15 (23)	i.p.	1 h
1B *				CPP	0 mg/kg 15 mg/kg 15 mg/kg	SE (31) 15CE (30) 15CS (31)	i.p.	1 h
1C				CPA	0 mg/kg 15 mg/kg 20 mg/kg	0 (27) 15 (30) 20 (31)	i.p.	1 h
2	MK-801	Channel	Sigma-RBI (St. Louis, MO)	CPP	0 mg/kg 0.05 mg/kg 0.2 mg/kg	0 (31) 0.05 (30) 0.2 (29)	i.p.	30 min
3	Ketamine	Channel	Fort Dodge Animal Health (Ft. Dodge, IA)	CPP	0 mg/kg 5 mg/kg 10 mg/kg 20 mg/kg	0 (23) 5 (19) 10 (21) 20 (19)	i.p.	15 min
4	Ifenprodil	NR2B	Sigma-RBI (St. Louis, MO)	CPP	0 mg/kg 5 mg/kg 10 mg/kg 20 mg/kg	0 (24) 5 (21) 10 (23) 20 (24)	i.p.	30 min
5	CP-101,606	NR2B	Pfizer (Groton, CT)	CPP	0 mg/kg 5 mg/kg 10 mg/kg 25 mg/kg	0 (24) 5 (23) 10 (20) 25 (24)	s.c.	30 min
6	(+)-HA966	glycineB	Tocris Cookson (Ellisville, MO)	CPP	0 mg/kg 5 mg/kg 15 mg/kg 30 mg/kg	0 (23) 5 (23) 15 (23) 30 (24)	i.p.	30 min

Note: *1B: SE = 0 mg/kg CGP-37849 + 2 g/kg ethanol, 15CE = 15 mg/kg CGP-37849 + 2 g/kg ethanol, 15CS = 15 mg/kg CGP-37849 + 0 mg/kg ethanol

5-min conditioning session. The conditioning sessions were conducted using a between–group discrimination design with full counterbalancing (Cunningham, 1993). Subjects in each drug treatment group were randomly assigned to one of two subgroups (G+: Grid+ or G-: Grid-) and exposed to an unbiased differential conditioning procedure (Days 2-9). On alternate days, subjects received drug treatment (CS+ days) before placement on the grid floor (G+ subgroup) or hole floor (G- subgroup). On CS- days, subjects received saline treatment before placement on the opposite floor (hole for G+; grid for G-). One complete conditioning trial consisted of a CS+ and CS- trial. CS+ and CS- conditioning trials were counterbalanced for order of presentation. The preference test consisted of a 30-min choice session in which half of the apparatus was a 'hole' floor and the other was a 'grid' floor (positions were counterbalanced within groups). All subjects were injected with saline (i.p.) and returned to the homecage. After a delay (designated by pretreatment time during conditioning), subjects were injected with saline (i.p.) and placed into the conditioning chamber. CPP is indicated by more time spent on the grid floor in the G+ subgroup in comparison to the G- subgroup.

Conditioned place aversion. Because drugs that interfere with development of CPP can do so either by reducing ethanol reward or by interfering with learning or memory, we also tested the one drug that blocked ethanol CPP (CGP-37849) in an ethanol CPA procedure. Ethanol CPA can be induced in mice at the same doses that yield CPP simply by injecting ethanol immediately after exposure to the CS rather than immediately before CS exposure (Cunningham et al., 2002; Cunningham & Henderson, 2000; Cunningham et al., 1997). Although the mechanisms underlying ethanol CPA have not been completely elucidated, it does not appear to be due to stress of injection alone in

that post-CS saline injections will not induce CPA (Cunningham et al., 1997). Rather, CPA is based on learning the relationship between the CS and an initial short duration aversive effect that appears to be related to the novelty of the rapid transition from the sober to intoxicated state (Cunningham et al., 2002). Studies from our laboratory have suggested that the rewarding and the aversive properties of ethanol are mediated by different neurocircuitry (Cunningham & Henderson, 2000; Cunningham et al., 2002). Consequently, if the target NMDA receptor binding site only affects ethanol reward, the antagonist should only affect ethanol-induced CPP but should not affect ethanol-induced CPA. If the target binding site has a more general role in learning, the antagonist should alter the subject's ability to learn both tasks. However, the NMDA receptor may be a common receptor within the neurocircuitry of both behaviors even if the behaviors have differing circuitries. Because the competitive NMDA receptor antagonist CGP-37849 altered ethanol-induced CPP (Experiments 1A and 1B), the CPA procedure was used as a control to test for CGP-37849's effects on a different type of ethanol-induced learning.

The experimental sequence for the CPA experiment was generally the same as the CPP experiments. However, a longer test session (60 min) was used in the CPA studies because previous studies have suggested that more time is needed to allow expression of this behavior (Cunningham et al., 1998). In addition, CGP-37849 or saline pretreatment (see Table 1) on CS+ and saline on CS- days was administered 55 min before being placed into the apparatus for a 5 min conditioning session. Subjects received an injection of 2 g/kg ethanol (CS+ trials) or saline (CS- trials) immediately after removal from the apparatus.

Data Analyses

Data were analyzed by analysis of variance (ANOVA) with the alpha level set at 0.05. Drug treatment and Conditioning group (G+/G-) were treated as between-group factors, and trial type (CS+/CS-) was treated as a within-group factor. Significant interactions were analyzed by follow-up ANOVAs (Keppel, 1991). Pairwise comparisons were Bonferroni corrected.

Results

Preference Test

Table 6 lists the initial ANOVAs (Drug treatment x Conditioning group) for the preference test grid times in each of the eight experiments. Follow-up analyses are described below for those experiments in which the Drug treatment x Conditioning group interactions were significant.

Insert Table 6 about here

CGP-37849 (Experiments 1A, B, C)

Figure 2 shows the mean (+ standard error of the mean: *SEM*) s/min spent on the grid floor during the preference test for the G+ and G- subgroups within each Drug treatment group in the CGP-37849 studies. As can be seen by examining the first pair of bars in each figure, the saline pretreated G+ subgroups (black bars) spent more time on the grid floor in comparison to the saline pretreated G- subgroups (hatched bars), indicating a significant ethanol-induced place preference in experiments 1A and 1B.

Table 6. Analysis of Variance of grid times for all 30 minutes of the place preference test

Exp.	Drug Treatment	CPP/CPA	Source	*F*(df)
1A	CGP-37849	CPP	Drug treatment	$F(3, 86) = .6$
			Conditioning group	$F(1, 86) = 39.2^{**}$
			Drug treatment x Conditioning group	$F(3, 86) = 2.7^{*}$
1B	CGP-37849	CPP	Drug treatment	$F(2, 86) = 3.2^{*}$
			Conditioning group	$F(1, 86) = 67.3^{**}$
			Drug treatment x Conditioning group	$F(2, 86) = 17.5^{**}$
1C	CGP-37849	CPA	Drug treatment	$F(2, 82) = .2$
			Conditioning group	$F(1, 82) = .2$
			Drug treatment x Conditioning group	$F(2, 82) = 3.2^{*}$
2	MK-801	CPP	Drug treatment	$F(2, 84) = 1.7$
			Conditioning group (G+/G-)	$F(1, 84) = 98.8^{**}$
			Drug treatment x Conditioning Group	$F(2, 84) = 1.8$
3	Ketamine	CPP	Drug treatment	$F(3, 74) = .4$
			Conditioning group	$F(1, 74) = 93.8^{**}$
			Drug treatment x Conditioning Group	$F(3, 74) = .04$
4	Ifenprodil	CPP	Drug treatment	$F(3, 84) = .8$
			Conditioning group	$F(1, 84) = 60.3^{**}$
			Drug treatment x Conditioning group	$F(3, 84) = .7$
5	CP-101,606	CPP	Drug treatment	$F(3, 82) = .3$
			Conditioning group	$F(1, 82) = 70.5^{**}$
			Drug treatment x Conditioning group	$F(3, 82) = .8$
6	(+)-HA-966	CPP	Drug treatment	$F(3, 85) = .4$
			Conditioning group	$F(1, 85) = 79.9^{**}$
			Drug treatment x Conditioning group	$F(3, 85) = .1$

Note. $^{*}p < .05$, $^{**}p < .001$

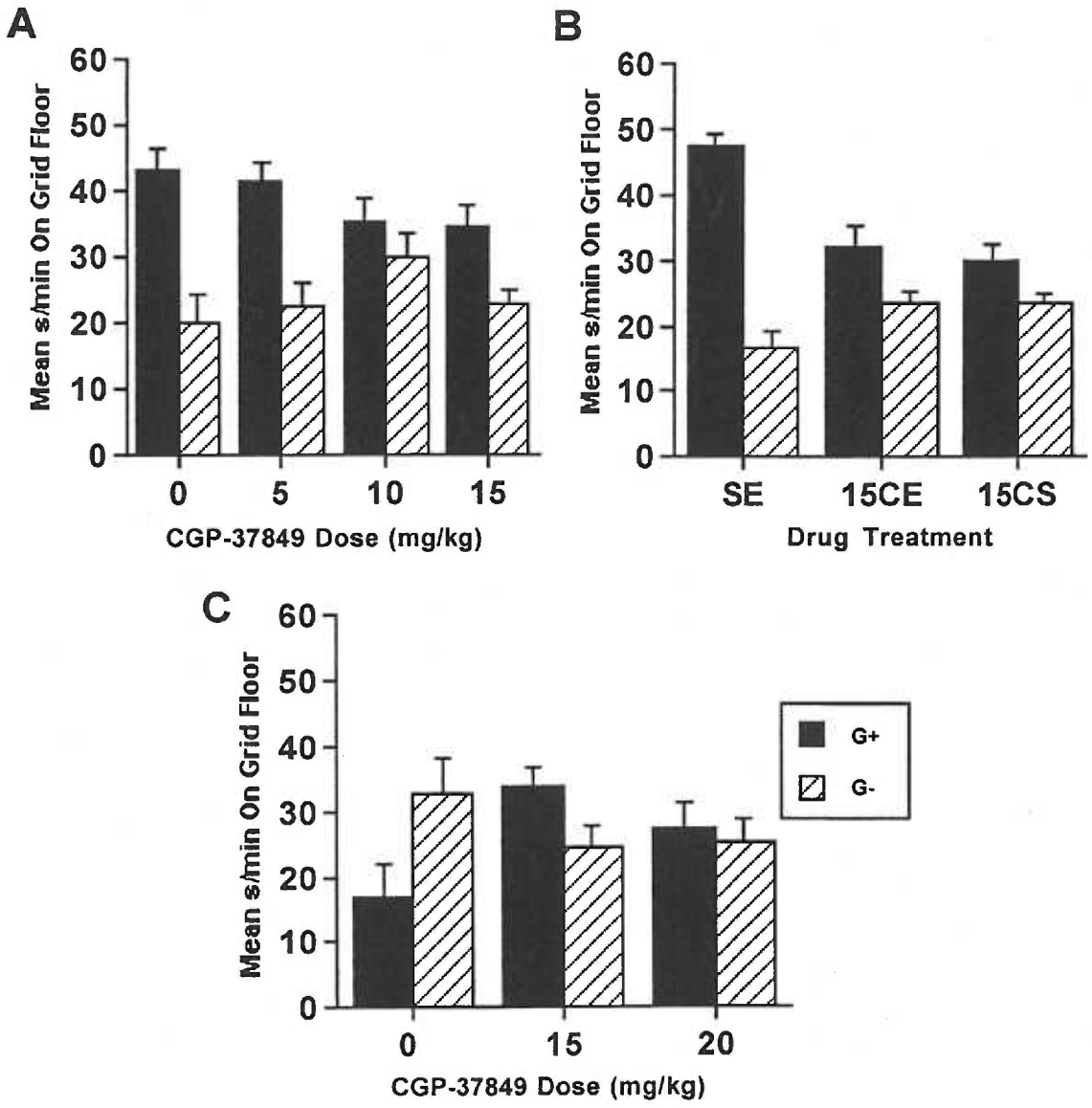
In contrast, the saline-pretreated G+ subgroup in experiment 1C spent less time on the grid floor than the G- comparison subgroup, reflecting development of conditioned place aversion. For all three of these studies, however, pretreatment with high doses of CGP-37849 interfered with the acquisition of place conditioning as indicated by significant Drug treatment x Conditioning group interactions (Table 6).

Insert figure 2 about here

Experiment 1A. To evaluate the interaction, separate two-way follow-up ANOVAs were conducted to compare each CGP-37849 pretreated group (5, 10, 15 mg/kg) with the saline group (0 mg/kg). These analyses indicated that the 10 mg/kg CGP-37849 group differed from the saline group [$F(1, 43) = 5.9, p = .02$]. In addition, the 15 mg/kg CGP-37849 was marginally different from the saline pretreated group [$F(1, 43) = 2.9, p = .09$]. Pairwise comparisons of the conditioning subgroups (G+ vs. G-) within each drug treatment group were also conducted. The 0 mg/kg and 5 mg/kg CGP-37849 groups showed a difference between conditioning subgroups [$ps < .001$], however the 10 and 15 mg/kg CGP-37849 groups did not [$p = .3, p = .07$]. Overall, these results suggest that pretreatment with CGP-37849 reduced ethanol-induced CPP.

Experiment 1B. Given the trend observed at the highest dose in Experiment 1A, this experiment was run to address the reliability of the antagonist's effect on ethanol-induced place preference. Furthermore, we wanted to determine if CGP-37849 given alone has any rewarding or aversive properties. Results from this study confirmed that pretreatment with CGP-37849 reduced ethanol-induced CPP in group 15CE (Figure 2B).

Figure 2. Mean (+*SEM*) s/min spent on the grid floor by conditioning subgroups G+ and G- for each CGP-37849 dose group collapsed across the 30 min (1A and 1B) or 60 min (1C) of the preference test. Conditioned place preference is shown when time spent on the grid floor by the G+ group exceeds the time spent on the grid floor by the G- group (1A and 1B). Conditioned place aversion is shown when time spent on the grid floor by the G- group exceeds the time spent on the grid floor by the G+ group (1C). (A) During conditioning, subjects in the G+ subgroup received pre-CS CGP-37849 (0, 5, 10, or 15 mg/kg) and ethanol (2 g/kg) paired with the grid floor and pre-CS saline paired with the hole floor. Subjects in the G- subgroup received pre-CS CGP-37849 (0, 5, 10, or 15 mg/kg) and ethanol (2 g/kg) paired with the hole floor and pre-CS saline paired with the grid floor. (B) During conditioning, subjects in the G+ subgroup received pre-CS injections of CGP-37849 (15mg/kg), 15CS; ethanol (2g/kg), SE; or both, 15CE, paired with the grid floor and pre-CS saline paired with the hole floor. Subjects in the G- subgroup received CGP-37849, ethanol, or both paired with the hole floor and saline paired with the grid floor. (C) During conditioning, subjects in the G+ subgroup received pre-CS CGP-37849 (0, 15, or 20 mg/kg) and post-CS ethanol (2 g/kg) paired with the grid floor and pre- and post-saline paired with the hole floor. Subjects in the G- subgroup received pre-CS CGP-37849 (0, 15, or 20 mg/kg) and post-CS ethanol (2 g/kg) paired with the hole floor and pre- and post-CS saline paired with the grid floor.


A
Mean s/min On Grid Floor
60
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20
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0
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5
10
15
CGP-37849 Dose (mg/kg)
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Mean s/min On Grid Floor
60
50
40
30
20
10
0
SE
15CE
15CS
Drug Treatment
C
Mean s/min On Grid Floor
60
50
40
30
20
10
0
0
15
20
CGP-37849 Dose (mg/kg)
G+
G-

Moreover, the antagonist did not produce significant place conditioning on its own (group 15CS), suggesting that it is neither rewarding nor aversive at this dose.

The foregoing conclusions were supported by planned between group comparisons (two-way ANOVAs) between the SE and 15CE treatment groups as well as between the 15CE and 15CS treatment groups. These analyses showed that the 15CE differed from the SE group [$F(1, 57) = 21.6, p < .001$], but the 15CE and 15CS groups did not differ [$p = .6$]. Pairwise comparisons of conditioning group (G+ vs. G-) within each drug treatment group were also conducted. The 15CE and SE groups showed a significant G+ versus G- difference suggesting these groups developed place preference [$p = .03, p < .001$, respectively]. However, the group that received only CGP-37849 (15CS) did not show a significant G+/G- difference [$p = .1$], suggesting that the antagonist does not induce place conditioning at this dose.

Experiment 1C. This experiment was run as a learning control for the results seen in experiments 1A and 1B to determine if CGP-37849 also alters ethanol's aversive effects. As noted earlier, the place aversion induced by post-CS injection was attenuated in the 15 and 20 mg/kg CGP-37849 groups. Two-way follow-up ANOVAs were conducted to evaluate the source of the significant interaction in the overall ANOVA (Table 6). These analyses indicated that the 0 mg/kg CGP-37849 group differed from the 15 and 20 mg/kg CGP-37849 groups [$F(1, 53) = 9.2$, $p = .004$ and $F(1, 54) = 4.0$, $p = .05$; respectively]. The 15 and 20 mg/kg CGP-37849 groups did not differ from each other [$p = .33$]. Pairwise comparisons of the conditioning subgroups (G+ vs. G-) within each drug treatment group revealed a significant effect of conditioning only in the 0 mg/kg CGP-37849 group [$p = .03$]. There was no significant effect of conditioning group in the 15

and 20 mg/kg CGP-37849 groups [p = .3, p >.99, respectively]. Taken together, experiments 1A, 1B, and 1C suggest that the competitive NMDA receptor antagonist decreases ethanol-induced CPP and CPA.

Other Drugs (Experiments 2 - 6)

Figure 3 shows the mean (+ *SEM*) s/min spent on the grid floor collapsed across the 30-min preference test for the G+ and G- subgroups within each Drug treatment group. The G+ subgroups (black bars) spent more time on the grid floor in comparison to the G- subgroups (hatched bars) indicating a significant ethanol-induced place preference in all groups, regardless of drug pre-treatment. This observation was supported by a significant main effect of conditioning group in each experiment (Table 6). However, there was no main effect or interaction with drug treatment, suggesting that MK-801, ketamine, ifenprodil, CP-101,606 and (+)-HA-966 did not affect the acquisition of ethanol-induced CPP. Thus, these results suggest the ion channel, NR2B subunit, and $glycine_B$ binding sites do not modulate ethanol reward as indexed by the place conditioning task.

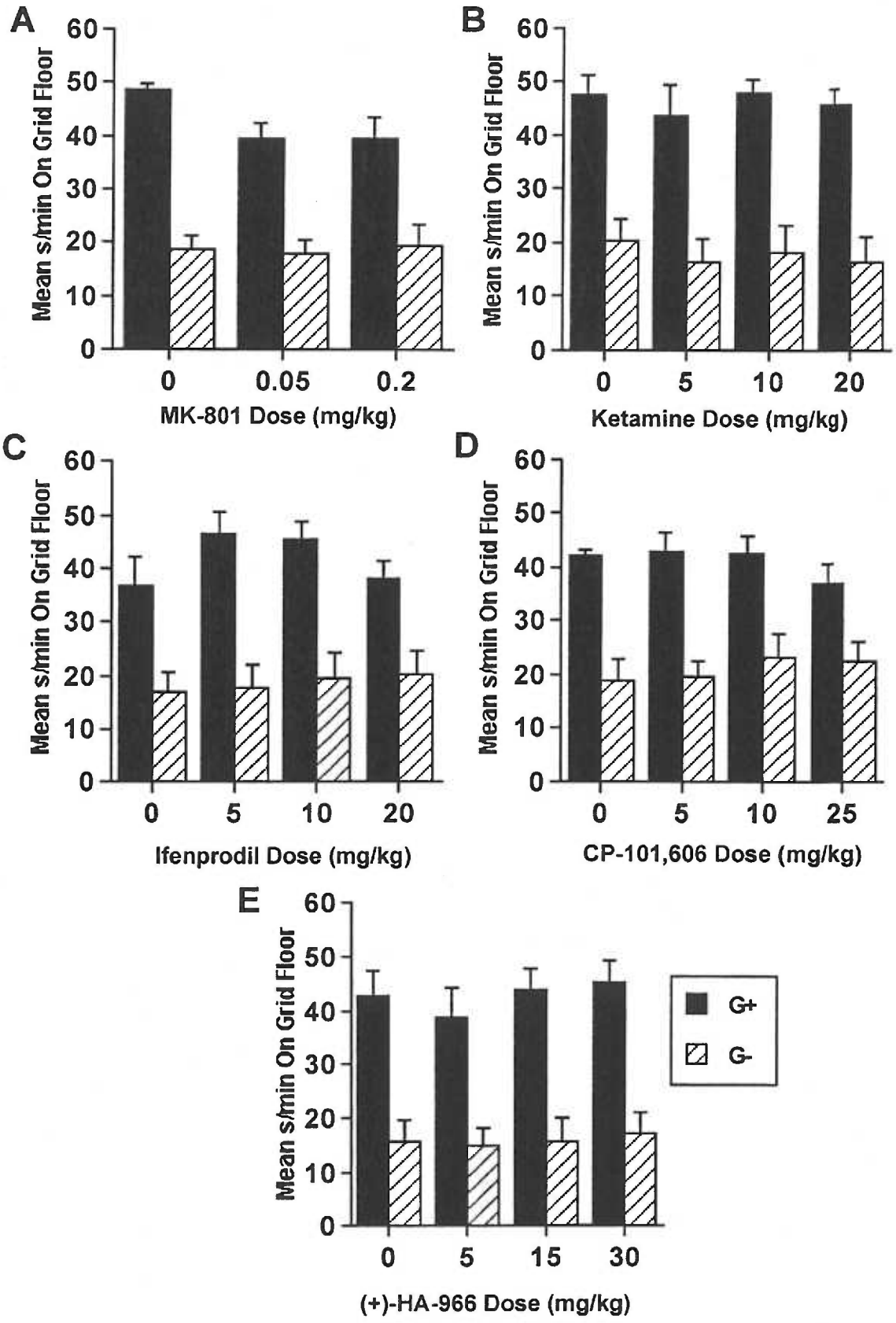
Insert Figure 3 about here

Test activity

The mean (±*SEM*) activity counts/min during the preference test are listed in Table 7. Information on test session activity is important for determining whether group differences in strength of place conditioning might have been influenced by activity levels (Cunningham, 1995).

Figure 3. Mean (+*SEM*) s/min spent on the grid floor by conditioning subgroups G+ and G- in each experiment for each drug treatment dose group collapsed across the 30 min of the preference test. Drug treatment (antagonist and ethanol) was given pre-CS in all experiments. All subjects in each of these experiments received varying doses of antagonists and 2 g/kg ethanol on CS+ days and saline on CS- days (Table 1). In all panels, conditioned place preference is shown when time spent on the grid floor by the G+ group exceeds the time spent on the grid floor by the G- group. (A) During conditioning, subjects in the G+ subgroup received MK-801 (0, 0.05, or 0.2 mg/kg) paired with the grid floor and saline paired with the hole floor. Subjects in the G- subgroup received MK-801 (0, 0.05, or 0.2 mg/kg) paired with the hole floor and saline paired with the grid floor. (B) During conditioning, subjects in the G+ subgroup received ketamine (0, 5, 10, or 20 mg/kg) paired with the grid floor and saline paired with the hole floor. Subjects in the G- subgroup received ketamine (0, 5, 10, 20 mg/kg) paired with the hole floor and saline paired with the grid floor. (C) During conditioning, subjects in the G+ subgroup received ifenprodil (0, 5, 10, or 20 mg/kg) paired with the grid floor and saline paired with the hole floor. Subjects in the G- subgroup received ifenprodil (0, 5, 10, or 20 mg/kg) paired with the hole floor and saline paired with the grid floor (D) During conditioning, subjects in the G+ subgroup received CP-101,606 (0, 5, 10, or 25 mg/kg) paired with the grid floor and saline paired with the hole floor. Subjects in the G- subgroup received CP-101,606 (0, 5, 10, 25 mg/kg) paired with the hole floor and saline paired with the grid floor. (E) During conditioning, subjects in the G+ subgroup received (+)-HA-966 (0, 5, 15, or 30 mg/kg) paired with the grid floor and saline paired with the

hole floor. Subjects in the G- subgroup received (+)-HA-966 (0, 5, 15, or 30 mg/kg) paired with the hole floor and saline paired with the grid floor.


A
Mean s/min On Grid Floor
60
50
40
30
20
10
0
0
0.05
0.2
MK-801 Dose (mg/kg)
B
Mean s/min On Grid Floor
0
5
10
20
Ketamine Dose (mg/kg)
C
Mean s/min On Grid Floor
0
5
10
20
Ifenprodil Dose (mg/kg)
D
Mean s/min On Grid Floor
0
5
10
25
CP-101,606 Dose (mg/kg)
E
Mean s/min On Grid Floor
0
5
15
30
(+)-HA-966 Dose (mg/kg)
G+
G-

CGP-37849 (Experiments 1 A, B, C)

One-way ANOVA of test activity in experiment 1B showed a significant effect of drug treatment, which was given during CS+ conditioning trials only, on test activity levels [$F(2, 89) = 3.6, p = .03$]. The 15CE group had significantly lower activity levels in comparison to the SE group [$p = .05$]. CGP-37849 given during CS+ trials did not affect locomotor activity during the preference test in experiments 1A and 1C. The fact that prior exposure to 15 mg/kg CGP-37849 had a minor effect on test activity in only one of these three experiments suggests that the effect observed in experiment 1B may have been due to sampling error. Moreover, given previous data suggesting a negative correlation between test activity and expression of CPP (Cunningham, 1995), it seems unlikely that the antagonist effect on CPP in experiment 1B is explained by its apparent effect on test activity.

Insert Table 7 about here

MK-801 (Experiment 2)

A one-way ANOVA showed a significant effect of drug treatment, which was given only during CS+ conditioning trials, on test activity levels [$F(2, 87) = 6.4$, $p = .003$]. The 0.2 mg/kg MK-801 group showed less activity during the test than both 0 and 0.05 mg/kg dose groups [ps < .001]. This decrease in activity during the test for the 0.2 mg/kg MK-801 dose group is not an issue since this group did not differ from the 0 and 0.05 mg/kg dose groups in time spent on the grid floor.

Table 7. Mean (± SEM) activity counts/min during the preference test

Experiment	Drug Treatment	Group	Means (±SEM)
1A	CGP-37849	0 mg/kg	31.29 (± 1.42)
		5 mg/kg	28.39 (± 1.36)
		10 mg/kg	29.31 (± 1.26)
		15 mg/kg	29.45 (± 1.04)
1B	CGP-37849	0 mg/kg/ 2 g/kg	34.28 (±1.47)
		15 mg/kg/ 2g/kg	29.67 (±1.17)
		15 mg/kg/ 0 g/kg	33.77 (±1.29)
1C	CGP-37849	0 mg/kg	16.80 (± 1.59)
		15 mg/kg	20.00 (± 1.19)
		20 mg/kg	20.42 (± 1.22)
2	MK-801	0 mg/kg	32.83 (± 1.19)
		0.05 mg/kg	32.31 (± 1.36)
		0.2 mg/kg	26.64 (± 1.49)
3	Ketamine	0 mg/kg	30.88 (± 1.65)
		5 mg/kg	27.97 (± 1.70)
		10 mg/kg	30.02 (± 1.30)
		20 mg/kg	31.39 (± 1.64)
4	Ifenprodil	0 mg/kg	31.98 (± 1.82)
		5 mg/kg	26.82 (± 2.05)
		15 mg/kg	30.50 (± 1.19)
		20 mg/kg	31.18 (± 1.16)
5	CP-101,606	0 mg/kg	34.18 (± 1.38)
		5 mg/kg	31.67 (± 1.18)
		10 mg/kg	31.48 (± 1.50)
		25 mg/kg	32.05 (± 1.17)
6	(+)-HA-966	0 mg/kg	29.25 (± 1.21)
		5 mg/kg	28.29 (± 1.45)
		15 mg/kg	27.60 (± 1.07)
		30 mg/kg	29.85 (± 1.42)

Other Drugs (Experiments 3- 6)

Treatment with ketamine, ifenprodil, CP-101,606, and (+)-HA-966 during CS+ trials did not effect locomotor activity during the preference test when no drug was present [*ps* > .05].

Conditioning activity

Table 8 shows the overall ANOVAs for conditioning activity in each of the eight experiments. Initial analyses of conditioning activity in each experiment considered trial number as a factor (data not shown). However, there were no consistent effects of trial number on overall conclusions about effects of NMDA receptor antagonism on ethanol-stimulated activity when the data were collapsed across trials 1-4. Therefore, for simplicity, we have presented conditioning activity collapsed across conditioning trials 1-4.

Insert Table 8 about here

CGP-37849 (Experiments 1A, B, C)

Figure 4 shows the mean (+*SEM*) activity counts per min collapsed across conditioning trials 1-4 for each drug treatment group. In the CPP experiments (1A and 1B), a 2 g/kg ethanol dose produced locomotor stimulation in the 0 mg/kg CGP-37849 groups on CS+ trials (white bars) in comparison to activity during CS- sessions with saline injections (black bars). This observation was supported by the main effect of trial type (Table 8). Furthermore, there were main effects and interactions with drug treatment in both experiments, suggesting that CGP-37849 altered locomotor activity. The follow-

Table 8. Analysis of Variance for mean conditioning activity counts per minute collapsed across trials 1-4.

Exp.	Drug Treatment	Source	*F*(df)
1A	CGP-37849	Drug treatment	$F(3, 90) = 323.2^{**}$
		Trial type	$F(1, 90) = 142.1^{**}$
		Drug treatment x Trial type	$F(3, 90) = 293.6^{**}$
		CS+ only: Drug treatment	$F(3, 90) = 433.4^{**}$
		CS- only: Drug treatment	$F(3, 90) = 1.8$
1B	CGP-37849	Drug treatment	$F(2, 89) = 270.3^{**}$
		Trial type	$F(1, 89) = 282.8^{**}$
		Drug treatment x Trial type	$F(2, 89) = 335.3^{**}$
		CS+ only: Drug treatment	$F(2, 89) = 340.3^{**}$
		CS- only: Drug treatment	$F(2, 89) = 4.1^{**}$
1C	CGP-37849	Drug treatment	$F(2, 85) = 55.6^{**}$
		Trial type	$F(1, 85) = 102.0^{**}$
		Drug treatment x Trial type	$F(2, 85) = 22.2^{**}$
		CS+ only: Drug treatment	$F(2, 85) = 44.6^{**}$
		CS- only: Drug treatment	$F(2, 85) = 14.9^{**}$
2	MK-801	Drug treatment	$F(2, 87) = 27.7^{**}$
		Trial type	$F(1, 87) = 1266.8^{**}$
		Drug treatment x Trial type	$F(2, 87) = 37.6^{**}$
		CS+ only: Drug treatment	$F(2, 87) = 36.4^{**}$
		CS- only: Drug treatment	$F(2, 87) = 1.0$
3	Ketamine	Drug treatment	$F(3, 78) = 19.6^{**}$
		Trial type	$F(1, 78) = 1848.7^{**}$
		Drug treatment x Trial type	$F(3, 78) = 21.4^{**}$
		CS+ only: Drug treatment	$F(3, 78) = 23.0^{**}$
		CS- only: Drug treatment	$F(3, 78) = .3$
4	Ifenprodil	Drug treatment	$F(3, 88) = 137.2^{**}$
		Trial type	$F(1, 88) = 1399.7^{**}$
		Drug treatment x Trial type	$F(3, 88) = 160.0^{**}$
		CS+ only: Drug treatment	$F(3, 88) = 179.0^{**}$
		CS- only: Drug treatment	$F(3, 88) = 2.4$
5	CP-101,606	Drug treatment	$F(3, 86) = 9.4^{**}$
		Trial type	$F(1, 86) = 2267.6^{**}$
		Drug treatment x Trial type	$F(3, 86) = 15.2^{**}$
		CS+ only: Drug treatment	$F(3, 86) = 12.5^{**}$
		CS- only: Drug treatment	$F(3, 86) = 4.3^{**}$
6	(+)-HA-966	Drug treatment	$F(3, 89) = 12.5^{**}$
		Trial type	$F(1, 89) = 2127.8^{**}$
		Drug treatment x Trial type	$F(3, 89) = 19.7^{**}$
		CS+ only: Drug treatment	$F(3, 89) = 17.1^{**}$
		CS- only: Drug treatment	$F(3, 89) = 2.9^{*}$

Note. $^{*}p < .05$, $^{**}p < .001$

up statistics for these analyses are located in the figure caption of Figure 4. In experiment 1A, CGP-37849 decreased ethanol-stimulated activity at all doses. Experiment 1B replicated this finding at the high dose of CGP-37849 (15CE). Experiment 1B also showed that CGP-37849 given alone (15CS) increased activity but not as high as when ethanol was given alone (SE). On CS- trials, subjects in the SE group had higher locomotor activity than the CGP-37849 pretreated groups (15CS and 15CE).

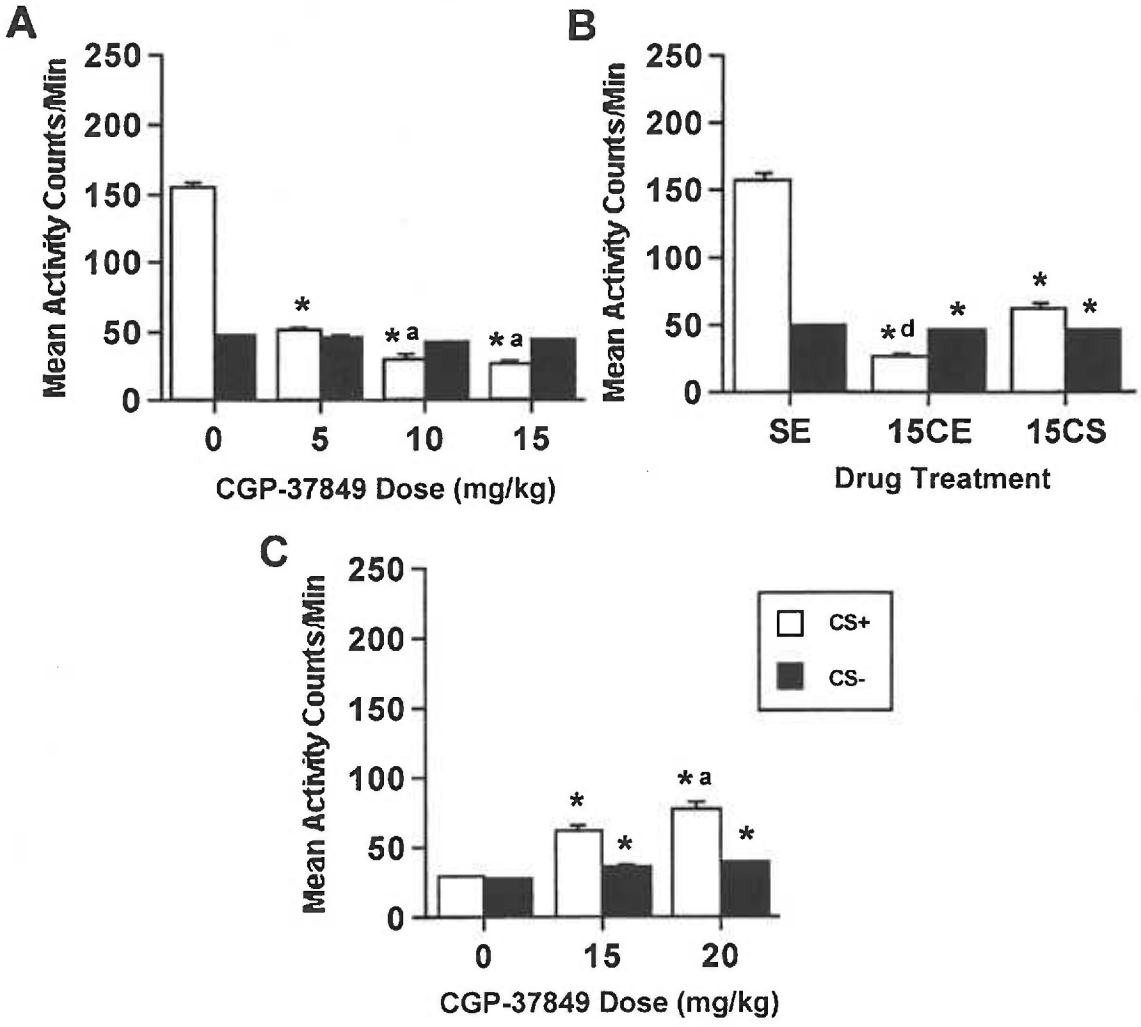
The locomotor stimulating effect of CGP-37849 was also observed in the CPA experiment (1C). This observation was supported by the main effect of trial type (Table 8). There were also main effects and interactions with drug treatment in the CPA experiment suggesting that CGP-37849 produces locomotor stimulation. The significant effect of drug treatment on CS- trials is due to the 15 and 20 mg/kg CGP-37849 groups having higher activity levels than the 0 mg/kg CGP-37849 group.

Insert Figure 4 about here

Other Drugs (Experiments 2 - 6)

Figure 5 shows the mean (+*SEM*) activity counts per min collapsed across conditioning trials 1-4 for each drug treatment group. In all studies, a 2 g/kg ethanol dose produced locomotor stimulation in all groups on CS+ trials (white bars) in comparison to activity during CS- sessions with saline injections (black bars). This observation was supported by the main effect of trial type (Table 8). Furthermore, there were main effects and interactions with drug treatment, suggesting MK-801, ketamine, ifenprodil, CP-101,606, and (+)-HA-966 altered activity. Follow-up statistics for these analyses are

Figure 4. Mean (+*SEM*) activity counts/min following ethanol (CS+) and saline (CS-) for drug treatment groups collapsed across conditioning trials 1-4 for each experiment. All subjects in each experiment received 2 injections of saline on CS- days. * $p < .05$ different from 0 mg/kg or SE; [a] $p < .05$ different from low dose; [b] $p < .05$ different from high dose; [d] $p < .001$ different from 15CS. (A) On CS+ days, mice received 0, 5, 10, or 15 mg/kg CGP-37849 1 h before a 2 g/kg ethanol injection. (B) On CS+ days, subjects in the SE group received an injection of saline 1 h before a 2 g/kg ethanol injection; subjects in the 15CE group received a 15 mg/kg CGP-37849 injection 1 h before a 2 g/kg ethanol injection; subjects in the 15CS group received a 15 mg/kg CGP-37849 injection 1 h before a saline injection. (C) On CS+ days, mice received 0, 15, or 20 mg/kg CGP-37849 55 min before a 5 min conditioning trial. Subjects received an ethanol injection (2g/kg) immediately after the CS+ trial.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15
CGP-37849 Dose (mg/kg)
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Mean Activity Counts/Min
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SE
15CE
15CS
Drug Treatment
C
Mean Activity Counts/Min
250
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15
20
CGP-37849 Dose (mg/kg)
CS+
CS-

located in the caption of Figure 5. Separate one-way ANOVAs showed significant effects of Drug treatment during CS+ trials (Table 8), suggesting these NMDA receptor antagonists altered ethanol-stimulated activity. All doses of ifenprodil, all doses of (+)-HA-966, and the high doses of ketamine, CP-101,606, and MK-801 decreased ethanol stimulated activity. However, the lowest dose of MK-801 and CP-101,606 increased ethanol-stimulated activity, suggesting biphasic dose effect curves.

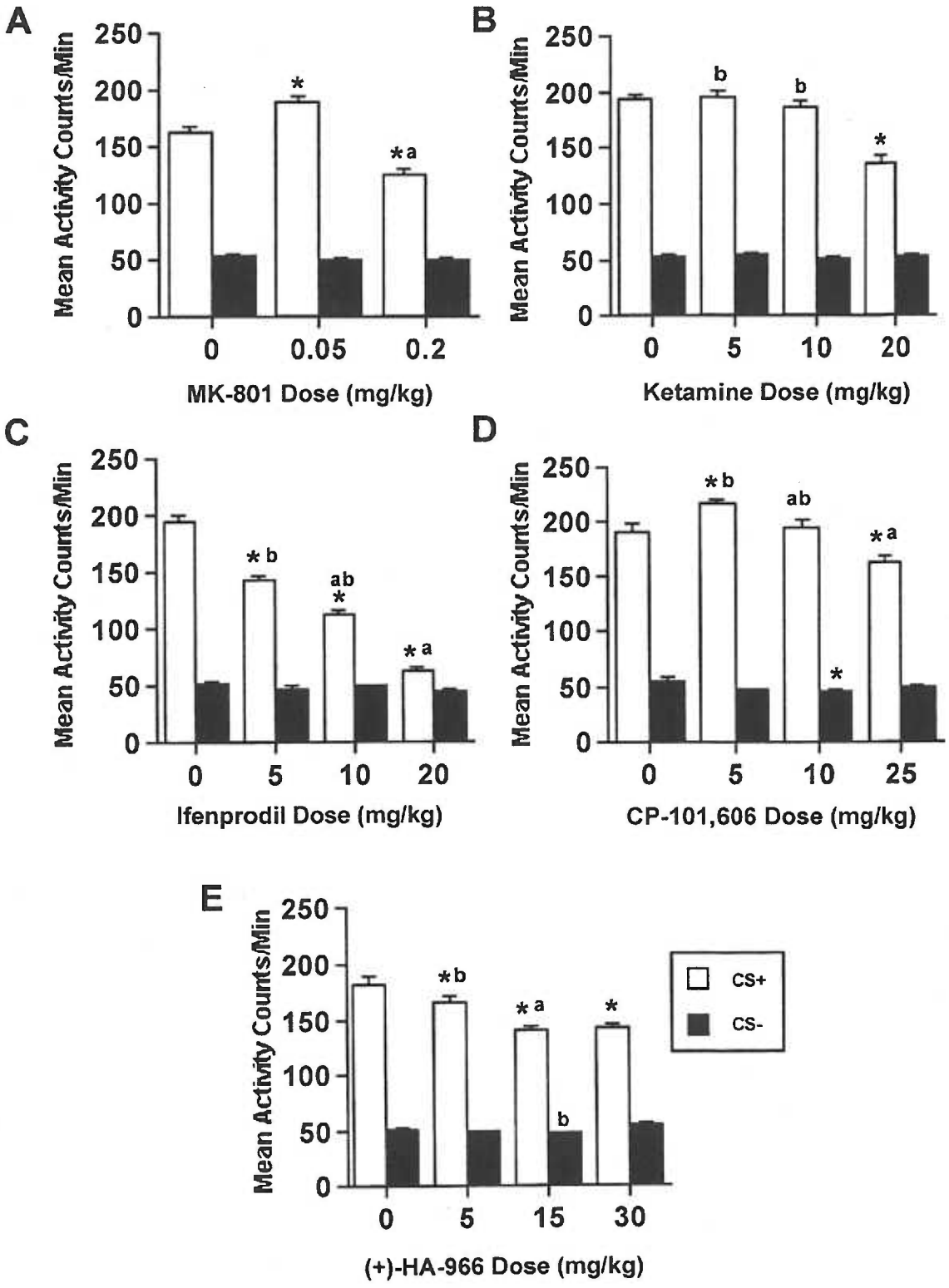
Insert Figure 5 about here

Separate one-way ANOVAs showed a significant effect of Drug treatment on CS- saline activity with CP-101,606 and (+)-HA-966, such that the group pretreated with 15 mg/kg (+)-HA-966 on CS+ trials had lower activity levels on CS- saline treated trials than those treated with 30 mg/kg (+)-HA-966. Moreover, those treated with 10 mg/kg CP-101,606 had fewer activity counts than the 0 mg/kg CP-101,606 group. The nonsystemic nature of these effects on CS- trials suggests they may reflect sampling error rather than a true effect of prior exposure to these drugs.

Discussion

The present studies show that CGP-37849 (a NMDA receptor competitive antagonist for the glutamate binding site) was able to decrease ethanol-induced CPP and CPA. Furthermore, CGP-37849 reduced ethanol-stimulated activity. The fact that CGP-37849 affected both ethanol reward and aversion suggests that competitive antagonism of

Figure 5. Mean (+*SEM*) activity counts/min following ethanol (CS+) and saline (CS-) for drug treatment groups collapsed across conditioning trials 1-4 for each experiment. All subjects in each experiment received 2 saline injections on CS- days. * $p < .05$ different from 0 mg/kg; [a] $p < .05$ different from low dose; [b] $p < .05$ different from high dose. (A) On CS+ days, mice received 0, 0.05, or 0.2 mg/kg MK-801 30 min before a 2 g/kg ethanol injection. (B) On CS+ days, subjects received 0, 5, 10, or 20 mg/kg ketamine 15 min before a 2 g/kg ethanol injection. (C) On CS+ days, mice received 0, 5, 10, or 20 mg/kg ifenprodil 30 min before a 2 g/kg ethanol injection. (D) On CS+ days, subjects received 0, 5, 10, or 25 mg/kg CP-101,606 30 min before a 2 g/kg ethanol injection. (E) On CS+ days, mice received 0, 5, 15, or 30 mg/kg (+)-HA-966 30 min before a 2 g/kg ethanol injection.


A
Mean Activity Counts/Min
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MK-801 Dose (mg/kg)
B
Mean Activity Counts/Min
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Ketamine Dose (mg/kg)
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Mean Activity Counts/Min
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5
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Ifenprodil Dose (mg/kg)
D
Mean Activity Counts/Min
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ab
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0
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25
CP-101,606 Dose (mg/kg)
E
Mean Activity Counts/Min
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b
CS+
CS-
0
5
15
30
(+)-HA-966 Dose (mg/kg)

the NMDA receptor influences both of these motivational effects. Alternatively, this overall pattern of results might be explained in terms of an alteration in the ability to learn these tasks. Moreover, these results suggest blockade of the ion channel, NR2B, and $glycine_B$ binding sites alter ethanol-stimulated activity, but have no effect on ethanol reward or on the ability to learn this task (Table 9).

Insert Table 9 about here

Competitive NMDA receptor antagonists affect spatial and non-spatial learning tasks. Thus, the attenuation of ethanol-induced CPP could be due to alterations in the subject's ability to learn the task. For example, CGP-37849 impairs a delayed match-to-sample task (Gutnikov & Rawlins, 1996). The authors suggest the impairment may be due to a reference memory deficit, working memory deficit, impairment of perception, or impairment of behavioral inhibition. However, spatial and non-spatial learning tasks can be confounded by locomotor impairments (Deacon & Rawlins, 1995). Therefore, the results in those experiments may be due to alterations in motor activity and not in learning. We assessed CGP-37849's ability to alter the aversive properties of ethanol since place conditioning can be altered by changes in learning or reward. Based on recent studies, it appears that CPP and CPA are mediated via different mechanisms. Specifically, US pre-exposure affects ethanol-induced CPA but not CPP (Cunningham et al., 2002). Moreover, there is a lack of genetic correlation between ethanol-induced CPP and CPA in mice that were selectively bred for high and low ethanol place preference (Hill et al., 2002). CGP-37849 decreased ethanol-induced CPA. Therefore, the decrease

Table 9. Summary of findings in the CPP and CPA experiments

Exp. Number	Drug	NMDA Receptor Binding Site		EtOH Place Conditioning	EtOH Locomotor Stimulation
1A	CGP-37849	Glutamate	CPP	↓	↓
1B	CGP-37849	Glutamate	CPP	↓	↓
1C	CGP-37849	Glutamate	CPA	↓	↓
2	MK-801	Ion Channel	CPP	—	↑↓
3	Ketamine	Ion Channel	CPP	—	↓
4	Ifenprodil	NR2B	CPP	—	↓
5	CP-101,606	NR2B	CPP	—	↑↓
6	(+)-HA-966	$Glycine_B$	CPP	—	↓

Note: —, no effect; ↓, attenuation; ↑, enhancement

in ethanol-induced CPP and CPA is most likely due to alterations in learning and not alterations in reward and aversion. However, since ethanol-induced CPA has not been extensively characterized there is still a possibility that there is some overlap in neurocircuitry between ethanol-induced CPP and CPA.

The current results support the notion that competitive antagonism of NMDA receptors leads to a decrease in ethanol reward or alters learning and memory. However, our results are in disagreement with the rat CPP literature showing MK-801 and L-701,324 were able to block ethanol-induced CPP and the competitive NMDA receptor antagonists CGP-37849 and CGP-40116 produced CPP alone in rats (Biala & Kotlinska, 1999; Papp et al., 1996; Papp & Moryl, 1994). We initially expected MK-801, ketamine, and L-701,324 to attenuate ethanol-induced CPP in mice. However, our results showed that MK-801, ketamine, and L-701,324 were unable to alter ethanol-induced CPP at doses that altered ethanol-induced locomotor stimulation using an unbiased design and an unbiased apparatus. Moreover, the current study found that CGP-37849 had no rewarding or aversive properties, but interfered with the acquisitioned of ethanol-induced CPP. Given problems with the interpretation of studies conducted in biased apparatuses (Cunningham et al., 2003; Tzschentke, 1998), it is possible that those previous reports of CPP and alterations of ethanol CPP with those compounds in rats may be due the antagonists' effects on general anxiety or unconditioned motivational states independent of alterations in reward. In fact, CGP-37849 has been found to increase the percent open arm time as well as open arm entries in an elevated plus maze task, suggesting CGP-37849 has anxiolytic properties (Przegalinski et al., 2000). Moreover, MK-801 has been shown to increase percent open time during a plus maze experiment in mice, suggesting

an effect on general anxiety (Fraser et al., 1996). Taken together, the current results suggest that the decrease ethanol consumption during operant ethanol self-administration by NMDA receptor channel blockers may be due disturbances in locomotor activity or in anxiety states caused by MK-801 or ketamine pretreatment and not due to alterations in ethanol reward.

NR2B antagonists such as ifenprodil, eliprodil, and CP-101,606 inhibit the channels of NR1/NR2B NMDA receptors by binding to an unique binding site on the NR2B subunit (Yamakura & Shimoji, 1999). The current studies show that ifenprodil and CP-101,606 do not alter ethanol-induced CPP. Furthermore, ifenprodil dose dependently decreased ethanol-induced locomotor stimulation while CP-101,606 increased ethanol-locomotor stimulation at 5 mg/kg and attenuated ethanol-induced locomotor stimulation at high doses. The difference between these antagonist effects on ethanol-stimulated activity may be due to the lack of effects at 5-HT3 receptors and alpha adrenergic receptors with CP-101,606 (Mott et al., 1998). Thus, it appears that the NMDA receptor NR2B binding site is not important for ethanol CPP.

The current results also suggest there is a dissociation between alterations in ethanol-induced locomotor activation and ethanol reward. Although CGP-37849 decreased ethanol-induced locomotor activation and ethanol-induced CPP and CPA, the other compounds (MK-801, ketamine, ifenprodil, CP-101,606, and (+)-HA-966) affected ethanol-induced locomotor activation but not ethanol-induced CPP. A similar lack of correlation between ethanol-stimulated activity and ethanol reward has been reported previously (Boyce & Risinger, 2000, 2002; Chester & Cunningham, 1999a, 1999b, Cunningham, 1995; Risinger & Boyce, 2002a; Thrasher et al., 1999).

These experiments are the first to investigate the effects of antagonism of the NMDA receptor glutamate binding sites, NR2B subunit binding sites, and glycine$_B$ binding sites on ethanol-induced locomotor activation. Previous studies have shown that MK-801, ketamine, and CGP-37849, but not (+)-HA-966, alter spontaneous locomotor activity in rats (Danysz et al., 1994). MK-801, ketamine, CGP-37849, and CGP-39551 also alter the locomotor coordination of mice during a rotarod task (Carter, 1994). MK-801 has been shown to increase ethanol-induced locomotor activation at low doses and decrease activation at high doses in DBA/2J mice (Shen & Phillips, 1998). The current results replicate the findings from that study. In addition, these results show that ketamine, ifenprodil, CGP-37849, and (+)-HA-966 decrease ethanol-induced locomotor stimulation. Furthermore, CP-101,606 has a similar effect as MK-801, increasing ethanol-induced locomotor activation at low doses and decreasing activation at high doses.

Although the NR2B antagonists ifenprodil and CP-101,606 did not alter ethanol CPP, the NMDA receptor NR2A subunit may nevertheless be important for CGP-37849's alterations in ethanol CPP and CPA. Competitive NMDA receptor antagonists, such as CGP-37849, alter the functioning of NMDA receptors containing NR1/NR2A-D subunits, whereas NR2B antagonists only affect NR2B containing NMDA receptors. Antagonists for the glutamate binding site show no preference for the different NR2 subtypes (Tikhonova et al., 2002). Because ethanol shows preferential actions at NR2A and NR2B containing receptors (Masood et al., 1994; Mirshahi & Woodward, 1995), it is possible that the NR2A subunit is important for CGP-37849's effects on ethanol CPP and CPA. Mice lacking the NR2A subunit have reduced NMDA receptor current,

decreased long-term potentiation (LTP), and altered spatial learning (Sakimura et al., 1995). However, there are no NR2A subunit specific antagonists currently available to investigate this hypothesis. Testing NR2A knockout mice in the current procedure would shed light on the role of the NR2A subunit in ethanol reward.

Although previous results from the self-administration and rat place conditioning studies suggested that MK-801and ketamine might decrease ethanol reward, it is not unexpected that CGP-37849 attenuated ethanol-induced CPP whereas MK-801 and ketamine did not. There are several explanations for the differing effects of these compounds. First, these drugs do not substitute for each other in a drug discrimination paradigm, providing evidence for a difference in their subjective effects (Zajaczkowski et al., 1996). Such data suggests differences in the behavioral consequences of competitive and noncompetitive antagonist binding. Moreover, competitive NMDA receptor antagonists, but not channel blockers, block the discriminative stimulus effects of NMDA (Willetts & Balster, 1989). These data suggest the subjective effects of NMDA are mediated by the glutamate binding site and not the NMDA receptor ion channel. Moreover, some of the pharmacological effects of competitive and noncompetitive NMDA receptor antagonists differ. For example, channel blockers increase dopamine release and competitive antagonists decrease or have no effect on dopamine release (Svensson et al., 1991; Waters et al., 1996). Taken together, these data suggest that although both competitive and noncompetitive NMDA receptor antagonists alter NMDA receptor function, they may do so in different ways that result in distinctive pharmacological and behavioral actions.

Regardless, these results demonstrate that the NMDA receptor is important for the acquisition of ethanol-induced CPP and CPA in DBA/2J mice using an unbiased apparatus and design. These studies have also shown that CGP-37849 is not rewarding alone, suggesting NMDA receptor glutamate antagonists may have a reduced abuse liability in comparison to NMDA receptor channel blockers. In addition, these studies also show that blockade of the NMDA receptor ion channel, glycine$_B$ binding site, as well as the NR2B binding site does not alter ethanol-induced CPP. However, antagonism of all of these binding sites altered ethanol-stimulated activity. Ethanol's actions at NMDA receptors and the NMDA receptor's role in the rewarding properties of ethanol involve complicated interactions. Further studies must be conducted to evaluate the role of the glutamate binding site on other ethanol-related behaviors. In addition, the development of selective NR2 subunit compounds will help determine the importance of specific NMDA receptor NR2 subunits in the rewarding effects of ethanol.

The current results suggest that some of the conclusions drawn initially from ethanol self-administration studies may be confounded by alterations in locomotor activity. Although results from those studies suggested that NMDA receptor channel blockers decrease ethanol reward (Bienkowski et al., 1999; Shelton & Balster, 1997), results from the current studies suggest that NMDA receptor channel blockers may have decreased ethanol self-administration indirectly by altering locomotor activity. On the other hand, the current results are in agreement with the self-administration literature suggesting that competitive NMDA receptor antagonists decrease ethanol reward. Moreover, the lack of an effect of (+)-HA-966 on ethanol-induced CPP is consistent with the ethanol self-administration studies showing that MRZ 2/576 had no effect on operant

ethanol self-administration (Bienkowski et al., 1999). Taken together, these results suggest that alterations in locomotor activity and coordination must be considered when drawing conclusions about effects of antagonist treatments on ethanol self-administration.

Chapter 2: NMDA Receptor Glutamate Binding Site and Ethanol- and LiCl-induced Conditioned Taste Aversion

CGP-37849, a competitive NMDA receptor antagonist, attenuated ethanol-induced place preference and place aversion. Two interpretations of these data are (1) CGP-37849 decreased the rewarding and aversive properties of ethanol or (2) CGP-37849 interfered with the subject's ability to learn the associative learning tasks. To investigate these interpretations, a conditioned taste aversion (CTA) procedure was run during which male DBA/2J mice received five 1-h access periods to 0.2 M NaCl. After the first two access periods, subjects received injections of CGP-37849 (0 or 15 mg/kg) and ethanol (0 or 2 g/kg) or LiCl (6 mEq/kg). As expected, LiCl and ethanol produced an aversion to the NaCl solution. In addition, CGP-37849 alone produced aversion relative to zero, the combination of CGP-37849 + ethanol or CGP-37849 + LiCl did not alter the aversion produced by CGP-37849 alone. To extinguish the association, subjects were given two 24-h access periods to the 0.2 M NaCl solution. During extinction, subjects previously treated with CGP-37849 during conditioning resisted extinction. After extinction to the 0.2 M NaCl flavor, the same animals received three 1-h access periods to a novel .015% saccharin. After the first two access periods, subjects received injections of CGP-37849 (0 or 8 mg/kg) and ethanol (0 or 2 g/kg) or LiCl (6 mEq/kg). All groups treated with 8 mg/kg CGP-37849 showed a significant CTA; there was no difference in the magnitude of CTA between these groups. Taken together, these data suggest NMDA receptor competitive antagonists have aversive properties that may influence the aversive properties of ethanol.

Introduction

Previous research suggests that competitive NMDA receptor antagonists alter ethanol reward and aversion. Competitive NMDA receptor antagonists AP-5 and CPPene decrease ethanol self-administration (Rassnick et al., 1992; Shelton & Balster, 1997). Moreover, the competitive NMDA receptor antagonist CGP-37849 attenuated the acquisition of both ethanol-induced conditioned place preference (CPP) and conditioned place aversion (CPA) (Boyce-Rustay & Cunningham, in preparation). Taken together, these data suggest competitive NMDA receptor antagonists may be important for both the rewarding and aversive properties of ethanol. However, studies from our laboratory also suggest the rewarding and aversive properties of ethanol are mediated via different neurocircuitry (Cunningham & Henderson, 2000; Cunningham et al., 2002). Therefore, if the competitive NMDA receptor antagonist affects ethanol reward, the antagonist should only affect ethanol-induced CPP and not CPA. However, if the competitive NMDA receptor antagonist alters learning processes, it could alter the subject's ability to learn both tasks. This alternative hypothesis is plausible since competitive NMDA receptor antagonists alter learning and memory tasks (Gutnikov & Rawlins, 1996).

Another method used to examine the aversive properties of drugs is the conditioned taste aversion (CTA) paradigm (Hunt & Amit, 1987). It is an associative learning paradigm in which a CS (palatable fluid) becomes associated with the US (drug injection). After several pairings, the presentation of the CS elicits a CR (avoidance of the CS). There are some data to suggest NMDA receptor antagonists can produce CTA on their own. For example, injections of PCP, MK-801, and ifenprodil all produce taste aversion to a saccharin-paired solution in rats (Jackson & Sanger, 1989; Bienkowski et

al., 1998). These data suggest that NMDA receptor antagonists may have aversive properties that could influence or interact with ethanol's hedonic properties.

The current study was conducted in order to determine CGP-37849's effects on ethanol-induced CTA. Conditioned taste aversion produced by ethanol appears to reflect the aversive properties of ethanol (Broadbent et al., 2002; Risinger and Boyce, 2002b). However, there are no data regarding the role of NMDA receptors in ethanol-induced CTA. Based on our previous studies, we hypothesized that CGP-37849 altered the learning of the place conditioning task. Furthermore, if CGP-37849 affected only the learning of ethanol CPP and CPA, it should interfere with CTA to both LiCl and ethanol. However, if CGP-37849's effects are specific to ethanol's hedonic properties, it should not affect LiCl-induced CTA. Literature suggests that NMDA receptor antagonists produced a CTA (Jackson & Sanger, 1989; Bienkowski et al., 1998). Therefore, we hypothesize that CGP-37849 may also possess aversive properties and produce CTA to the NaCl- and saccharin-paired solutions. If CGP-37849 produces a CTA, it is also possible that CGP-37849 will enhance ethanol-induced CTA due to the summation of the aversive effects of CGP-37849 and ethanol.

Method

Subjects

Adult male DBA/2J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 7 weeks of age and were allowed to acclimate to the colony room for 1 week prior to onset of the experiment. On arrival, subjects were housed in groups of four in polycarbonate cages (33 x 16 x 13 cm) with carefresh bedding. Food and water were available ad libitum. The colony room was maintained on a normal 12 h light-dark cycle

(lights on at 0700) at an ambient temperature of 21±1°C. After acclimation to the colony room, subjects were individually housed in stainless-steel hanging cages (14 x 18 x 18 cm) with wire mesh fronts and bottoms. Fluid access was then restricted as described below. Animal housing, care, and procedures were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (1996) and were approved by the Oregon Health & Science University Institutional Animal Care and Use Committee.

Drugs

Ethanol was mixed in sterile saline at a concentration of 20% v/v and given in an injection volume of 12.5 ml/kg. Lithium chloride was mixed in sterile water and given in an injection volume of 20 ml/kg. CGP-37849 was mixed with saline and given in an injection volume of 10 ml/kg.

Procedure

Fluids were presented in a 25-ml graduated glass cylinder with a stainless steel drinking spout. The cylinders were attached to the fronts of the individual wire-mesh cages. Consumption was measured to the nearest 0.1 ml. Evaporation loss was measured by placing an empty cage on each rack and the volumes were recorded each day at the same time. Subjects were randomly assigned to one of 6 drug treatment groups (n = 10/group): saline/saline (S/S), CGP-37849/saline (C(15)/S), saline/ethanol (S/E), saline/LiCl (S/L), CGP-37849/ethanol (C(15)/E), and CGP-37849/LiCl (C(15)/L). Mice were weighed and moved into the individual housing after 1 week adaptation to the colony. Food and water were available ad libitum for an additional week while subjects acclimated to individual housing. Twenty-three h water intakes were measured at 0900

each day. After 1 week of individual housing, water tubes were removed from each subject's cage at 0900. Subjects were weighed and returned to their individual cage with no water access until 0900 the next morning. For the next 6 days, water access was restricted to 2-h per day (0900-1100). Then at 48-h intervals, subjects had 1-h access to 0.2 M NaCl in tap water (0900-1000). Immediately following the removal of the NaCl solution on conditioning trials 1 and 2, subjects were injected with 0 or 15 mg/kg CGP-37849 followed 1-h later by injection of 0 or 2 g/kg ethanol or 6 mEq/kg LiCl. On trials 3-5, no drug injections were given On treatment days, subjects had access to water for 30-min 5 hours after removal of the 0.2 M NaCl solution. On intervening days, subjects received access to water for 2 h (0900-1100).

In order to more rapidly extinguish NaCl as a conditioned stimulus, the 0.2 M NaCl solution remained on the cage for 24 h following conditioning trial 5. Subjects were then given 1-h access to water to prevent dehydration. After the 1-h access to water, the 0.2 M NaCl solution was placed back on the cage for a second 24 hours (1030-1030). For the next three days, subjects had 2-h access to water (0900-1100). Following 3 days of water access, subjects had 1-h access to 0.15% saccharin in tap water (0900-1000) at 48-h intervals. Subjects remained in the same treatment groups as they had been in during the NaCl phase. On conditioning trials 1 and 2, subjects were injected with 0 or 8 mg/kg CGP-37849 immediately upon removal of the saccharin followed 1-h later by injections of 0 or 2 g/kg ethanol or 6 mEq/kg LiCl. During conditioning trial 3, no drug injections were given. On conditioning days, subjects had access to water for 30-min 5 hours after removal of the saccharin solution.

Statistical analyses

ANOVA was used for all initial comparisons, with an alpha level set at .05. Difference scores were obtained by subtracting the intakes on conditioning trials 2-5 from the intake on conditioning trial 1 to correct for individual differences on intake on trial 1. Significant interactions were analyzed by follow-up ANOVAs (Keppel, 1991). Pairwise comparisons were Bonferroni corrected.

Results

0.2 M NaCl.

Mean (± SEM) consumption of the 0.2 M NaCl solution during conditioning trial 1 for each group was as follows: 1.78 ± .18, 2.03 ± .12, 2.33 ± .09, 1.84 ± .14, 1.76 ± .25, 1.95 ± .18 for S/S, C(15)/S, S/E, C(15)/E, S/L, and C(15)/L, respectively. Two-way ANOVA (CGP-37849 x Drug treatment) of conditioning 1 trial intakes showed a marginally significant CGP-37849 x Drug treatment interaction [$F(2, 54) = 2.88, p = .06$]. This interaction appears to be due to higher consumptions in the S/E group. Therefore, differences scores were analyzed.

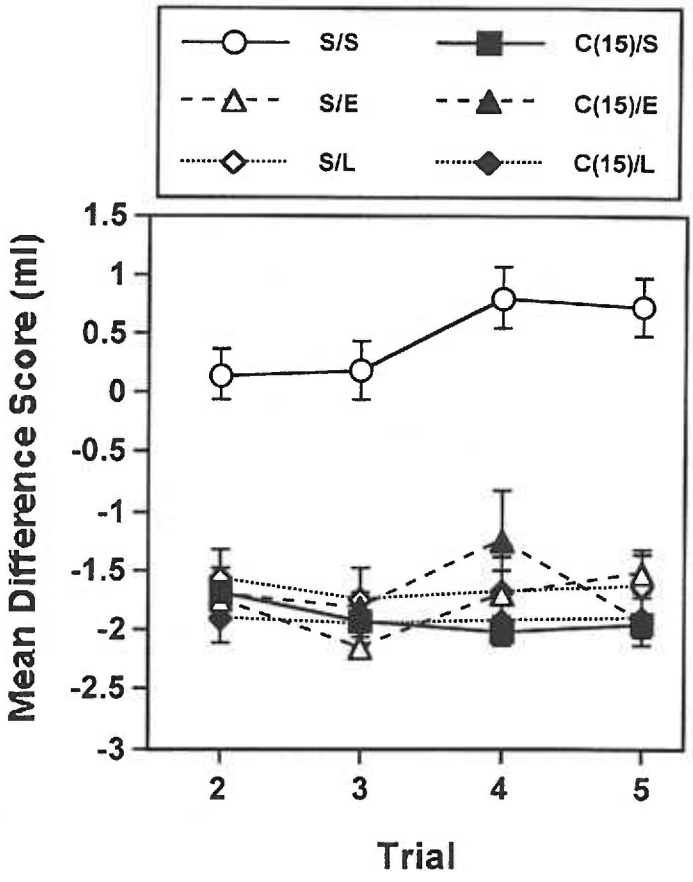
Figure 6 shows the mean (± SEM) differences scores for trials 2-5 when 0.2 M NaCl was the conditioned stimulus. Ethanol-, LiCl-, and CGP-37849-NaCl pairings produced a reduction in NaCl consumption across trials indicating a significant CTA developed in all but group S/S. In addition, all drug treated groups [C(15)/S, S/E, C(15)/E, S/L, C(15)/L] showed a similar development of CTA. This suggests CGP-37849 produced a significant CTA similar to that for ethanol and LiCl alone, and similar to the combination of CGP-37849+ethanol/LiCl.

Insert figure 6 about here

A three-way ANOVA (CGP-37849 x Drug Treatment x Trial) of difference scores yielded significant main effects of CGP-37849 [$F(1, 54) = 30.28, p < .001$], Drug Treatment [$F(2, 54) = 21.29, p < .001$], and Trial [$F(3, 162) = 5.63, p < .001$] as well as CGP-37849 x Drug treatment [$F(2,54) = 27.04, p < .001$], CGP-37849 x Trial [$F(3, 162) = 3.09, p = .03$], and CGP-37849 x Drug Treatment x Trial [$F(6, 162) = 3.43, p = .003$] interactions. Follow-up two-way ANOVA (Drug treatment x Trial) yield a significant effect of Drug treatment [$F(2, 57) = 8.8, p < .001$] but no interaction [$p = .27$]. Two-way ANOVA (CGP-37849 x Trial) yielded a significant effect of CGP-37849 [$F(1, 174) = 11.7, p < .001$] Trial [$F(3, 174) = 5.1, p = .002$] and an interaction [$F(3, 174) = 2.8, p = .04$]. Bonferroni corrected pairwise comparisons indicate that the 15 mg/kg CGP-37849 groups showed significantly greater difference scores on all trials in comparison to those receiving saline (0 mg/kg CGP-37849) ($ps < .001$). These analyses indicate that all experiment groups (except S/S) control showed a significant CTA to the NaCl paired flavor that did not differ from one another.

The mean (± SEM) absolute consumptions in mL for trial 2 for the ethanol and LiCl drug treatment group are as follows: .34 ± .18, .57 ± .13, .14 ± .1, .2 ± .04, and .05 ± .05; C(15)/S, S/E, C(15)/E, S/L, and C(15)/L respectively. That is, all drug-treated groups decreased their NaCl intakes to almost zero after 1 conditioning trial. Thus, the lack of

Figure 6. Mean (± SEM) difference scores (ml) during taste conditioning trials 2-5 for each drug treatment group (n = 10/group). After 60-min access to 0.2 M NaCl on trials 1 and 2, groups received 15 mg/kg CGP-37849 (C(15)/S, C(15)/E, C(15)/L) or saline (S/S, S/E, S/L), followed 1-h later by ethanol at 0 (S/S) or 2 g/kg (S/E, C(15)/E) or 6 mEq/kg LiCl (S/L, C(15)/L). No drugs were injected on Trials 3 to 5. Difference scores were calculated by subtracting the volume of NaCl consumed on trial 1 from consumptions on trials 2-5.


S/S
C(15)/S
S/E
C(15)/E
S/L
C(15)/L
Mean Difference Score (ml)
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
2
3
4
5
Trial

differences between C(15)/S and C(15)/E and between C(15)/S and C(15)/L may be due to a floor effect.

Extinction of NaCl.

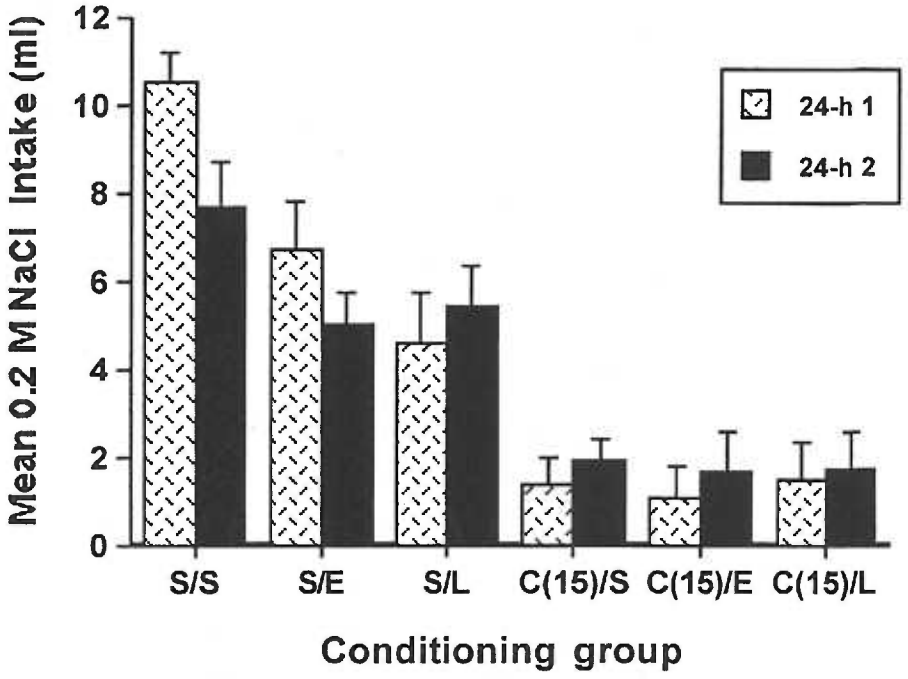
The rapid development of CTA in all groups precludes drawing conclusions about CGP-37849's ability to interfere with or enhance CTA. Therefore, we continued to extinguish NaCl as the conditioned stimulus to see if there was a difference in the rate of extinction between groups after the presentation of NaCl with injections on trials 3-5. Groups that received CGP-37849 [C(15)/S, C(15)/E, and C(15)/L] resisted extinction of the 0.2 M NaCl when no longer paired with drug injection. Figure 7 shows the mean (± SEM) 0.2 M NaCl intakes for both 24-h access periods for each drug treatment group.

Insert figure 7 about here

A three-way ANOVA (CGP-37849 x Drug Treatment x Trial) on consumptions of 0.2 M NaCl for 24-h access period yielded a significant main effects of CGP-37849 [$F(1,5\ 4) = 62.36, p < .001$] and Drug Treatment [$F(2, 54) = 3.96, p = .02$] and interactions of CGP-37849 x Drug treatment [$F(2, 54) = 3.49, p = .04$], CGP-37849 Dose x Trial [$F(1, 54) = 12.09, p = .001$], Drug Treatment x Trial [$F(2, 54) = 3.98, p = .02$], and CGP-37849 x Drug treatment x Trial [$F(2, 54) = 5.76, p = .005$].

The two-way interaction (CGP-37849 x Drug treatment) was significant in the first 24-h access period, but not during the second 24-h access period. Two-way ANOVAs (CGP-37849 Dose x Drug treatment) on the first 24-h 0.2 M NaCl access

Figure 7. Mean (± SEM) 0.2 M NaCl intake during extinction. All subjects had 24-h access to 0.2 M NaCl for two access periods without drug injections. Group designations listed on the X axis are in reference to the drug treatment given with the NaCl paired flavor during conditioning. Group designations are: S/S (saline + saline), S/E (saline + 2 g/kg ethanol), S/L (saline + 6 mEq/kg LiCl), C(15)/S (15 mg/kg CGP-37849 + saline), C(15)/E (15 mg/kg CGP-37849 + 2 g/kg ethanol), C(15)/L (15 mg/kg CGP-37849 + 6 mEq/kg LiCl). During the first 24h consumption period (24-h 1) and second 24h consumption period (24-h 2), no drug injections were given.


Mean 0.2 M NaCl Intake (ml)
12
10
8
6
4
2
0
24-h 1
24-h 2
S/S
S/E
S/L
C(15)/S
C(15)/E
C(15)/L
Conditioning group

period yielded significant main effects of CGP-37849 [$F(1, 54) = 71.38, p < .001$] and Drug Treatment [$F(2, 54) = 5.97, p = .005$] as well as a CGP-37849 x Drug Treatment interaction [$F(2,54) = 6.11, p = .004$]. Separate one-way ANOVAs of Drug treatment for 0 and 15 mg/kg CGP-37849 dose groups were also conducted. There was a significant effect of Drug treatment in the 0 mg/kg CGP-37849 groups [$F(2, 27) = 9.6, p < .001$, but not in the 15 mg/kg CGP-37849 group [$p > .9$]. These data suggest that C(15)/S, C(15)/E, and C(15)/L groups did not differ from each other. Bonferroni corrected pairwise comparisons indicate that the group S/S consumed significantly more NaCl than the S/E and S/L groups [$ps < .03$].

Two-way ANOVAs (CGP-37849 x Drug treatment) on the second 24-h 0.2 M NaCl access period yielded significant main effect of CGP-37849 (0 versus 15 mg/kg) [$F(1, 54) = 39.09, p < .001$] but no interaction suggesting that the groups receiving CGP-37849 did not differ from each other and the saline pretreated groups (S/S, S/E, and S/L) extinguished NaCl as an CS. This also suggests that the groups receiving CGP-37849 during conditioning resisted extinction to the flavor cue, but the groups receiving only saline, ethanol, or LiCl partially extinguished the avoidance of NaCl. This greater resistance to extinction with CGP-37849 pretreatment suggests that there is an enhanced CTA in those groups.

0.15% saccharin.

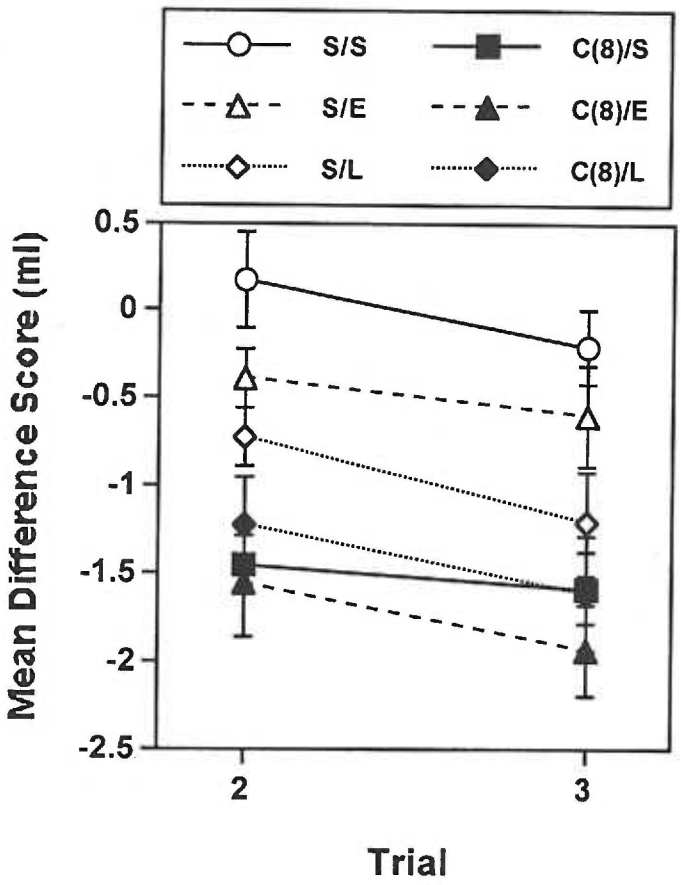
The purpose of the extinction was to determine if CGP-37849 + ethanol/LiCl produced a stronger aversion than CGP-37849 alone. However, all groups treated with CGP-37849 resisted extinction. The possibility exists that CGP-37849 may enhance ethanol- and LiCl-induced CTA. CGP-37849 produced such a robust CTA on its own that

resulted in a floor effect, consequently, we were unable to detect an enhancement of ethanol- and or LiCl-induced CTA. Therefore, the conditioning was repeated in the same subjects with another flavor, 0.15% w/v saccharin, after extinction and with a lower dose of CGP-37849 (8 mg/kg) to optimize the ability to see an enhancement of ethanol-induced CTA. Saccharin was used as the CS since DBA/2J mice show a less robust ethanol-induced CTA to saccharin than to NaCl (Risinger & Cunningham, 1995; Risinger & Boyce, 2002b).

Figure 8 shows the mean (± SEM) differences scores for trials 2 and 3 when 0.15% saccharin was the conditioned stimulus. All subjects remained in the same drug treatment groups (with the exception of a lower CGP-37849 dose) as in NaCl conditioning. Six subjects (One S/E, one S/S, one C(15)/L, one C(15)/E, and two C(15)/S) were removed from the analysis when saccharin served as the CS due to no intake on conditioning trial 1. The mean (± SEM) absolute consumptions in mL for trial 1 for all groups are as follows: 3.02 ± .2; 2.05 ± .23; 2.8 ± .2; 2.06 ± .2; 3.34 ± .3; 2.34 ± .2 for S/S, C(15)/S, S/E, C(15)/E, S/L, and C(15)/L respectively. A Two-way ANOVA (CGP-37849 x Drug Treatment) of trial 1 consumptions yielded a significant main effect of CGP-37849 [$F(1, 48) = 22.498, p < .001$], so difference scores were used for the analyses. As expected, there was less robust ethanol-induced CTA with the saccharin CS. In addition, 8 mg/kg CGP-37849 produced a significant CTA that did not differ between CGP-37849 given alone and CGP-37849 given with 2 g/kg ethanol or 6 mEq/kg LiCl.

Insert figure 8 about here

Figure 8. Mean (± SEM) difference scores (ml) during taste conditioning trials 2 and 3 for each drug treatment group (n = 9-10/group). After 60-min access to .015% saccharin, groups received 8 mg/kg CGP-37849 (C(8)/S, C(8)/E, C(8)/L) or saline (S/S, S/E, S/L), followed 1-h later by ethanol at 0 (S/S) or 2 g/kg (S/E, C(8)/E) or 6 mEq/kg LiCl (S/L, C(8)/L). Difference scores were calculated by subtracting the volume of saccharin consumed on trial 1 from consumptions on trials 2 and 3.


S/S
C(8)/S
S/E
C(8)/E
S/L
C(8)/L
Mean Difference Score (ml)
0.5
0
-0.5
-1
-1.5
-2
-2.5
2
3
Trial

A three-way ANOVA (CGP-37849 Dose x Drug Treatment x Trial) on difference scores yielded significant main effects of CGP-37849 [$F(1,48) = 32.09, p < .001$] and Trial [$F(1,48) = 15.23, p < .001$] but no interactions [$ps > .07$].

Discussion

The current study examined the role of the NMDA receptor glutamate binding site in the aversive properties of ethanol and LiCl. These results indicate that blockade of the NMDA receptor glutamate binding site produces a CTA alone. Moreover, ethanol and LiCl produced taste aversion. It is unclear if CGP-37849 produces an additive effect on ethanol- and LiCl-induced CTA since CGP-37849 at doses of 8 and 15 mg/kg produced almost maximal aversion to the NaCl- and saccharin-paired solutions within one conditioning trial. These data suggest that competitive NMDA receptor antagonists possess aversive properties that may have additive effects on ethanol- and LiCl-induced CTA. Further studies need to be conducted with a lower dose of CGP-37849 to determine if CGP-37849 enhances ethanol- and LiCl-induced CTA.

The CGP-37849-induced CTA was not expected based on previous place conditioning studies. Results from those studies suggested that competitive NMDA receptor antagonist CGP-37849 does not produce a preference or aversion on its own in place conditioning studies (Boyce-Rustay & Cunningham, submitted). It was expected that if CGP-37849 altered ethanol's hedonic properties in ethanol-induced CPP and CPA studies, CGP-37849 would decrease ethanol-induced CTA. However, the current results are consistent with other studies showing various NMDA receptor antagonists produce a taste aversion to the saccharin paired flavor when administered alone (Jackson & Sanger, 1989; Bienkowski et al., 1998).

The experiences during the first phase of conditioning (0.2 M NaCl) may have had residual effects on the CTAs produced during the second phase of conditioning (0.15% saccharin). Subjects remained in the same drug treatment groups during both phases; therefore, the experience with the CS-US pairing could have altered responding in phase 2. The baseline differences in saccharin consumption (CGP-37849 pretreated subjects drank less saccharin) suggest that this did in fact occur. Moreover, previous studies have shown that ethanol pre-exposure decreases subsequent ethanol-induced CTA (Risinger & Cunningham, 1995). This decrease in the magnitude of ethanol-induced CTA from phase 1 to phase 2 could be attributed to tolerance development to the US. Furthermore, the switch from saccharin produces a less robust ethanol-induced CTA than NaCl (Risinger & Boyce, 2002) that could have also contributed to the decreased ethanol-induced CTA in phase 2. Tolerance development to the aversive properties of CGP-37849 has not yet been investigated. However there are no data indicating that tolerance develops to the anticonvulsant effects of CGP-37849 (De Sarro et al., 1996). The lack of tolerance development to the aversive properties of CGP-37849 could explain why subjects given CGP-37849 still showed a robust CTA even when the dose of CGP-37849 was reduced. Another experiment needs to be conducted with a lower dose of CGP-37849 (2.5 mg/kg) on ethanol- and LiCl-induced CTA to the saccharin-paired flavor in order to determine if CGP-37849 pretreatment enhances these behaviors in naive mice.

One possible explanation for the opposing results in ethanol-induced CPA and CTA studies is the timing of the CGP-37849 injections. CGP-37849 was given 55 – 60 min before experience with the CS in the place conditioning experiments, but after exposure to the CS in the taste conditioning experiment. CGP-37849 was given post-CS

in the CTA experiment because CGP-37849 given before access to the CS would cause locomotor impairments that would confound the interpretation. CGP-37849 given after exposure to the CS is aversive in a CTA paradigm. Therefore, the timing is beneficial for producing a CTA by itself and for summating with the later aversive effect of ethanol to most likely enhance CTA. However, CGP-37849 given 1 hour before exposure to the CS (in the CPP experiments) is also aversive, but the longer delay (60 min) prevents it from becoming associated with the CS, reducing CPP through a motivational interaction of conditioned reward and conditioned aversion. Moreover, the delay prevents it from enhancing CPA. On the other hand, because CGP-37849 is still active at 1 h, it can interfere with the learning of CPP and CPA.

However, why does not CGP-37849 interfere with CTA conditioned by ethanol? Several possibilities exist. First, it is possible that CGP-37849 does interfere with the learning of ethanol CTA. But because it produces such a robust CTA on it own, CGP-37849 could potentially mask an effect on ethanol CTA. However, these results may suggest that CGP-37849 did not interfere with the learning of ethanol-induced CPP and CPA. There may have been a different outcome if CGP-37849 was given 1 hour before the CS-ethanol pairings are in the place conditioning experiments. Nonetheless, our place conditioning studies showed that CGP-37849 alone causes locomotor activation, therefore CGP-37849 might have altered the drinking of the flavor leading to interpretational issues. However, previous studies have shown that other drugs such as LiCl produce a CTA when given before exposure to the CS. Moreover, results from several other studies suggest that NMDA receptor blockade does not alter the acquisition

associative learning tasks (Bolhuis & Reid, 1992; Deacon & Rawlins, 1996; Gutnikov & Rawlins, 1996).

Overall, these results in combination with the ethanol CPP and CPA studies make it difficult to conclude whether or not CGP-37849 is altering ethanol's hedonic properties or the learning of associative learning tasks. It is possible that the lack of a preference or aversion with CGP-37849 alone was due to such a long delay before exposure to the CS Moreover, other experiments need to be conducted in order to come to a definitive conclusion regarding how CGP-37849 is able to alter ethanol place conditioning as well as if CGP-37849 can enhance ethanol CTA.

GENERAL DISCUSSION

The central aim of this thesis was to evaluate the role of NMDA receptor binding sites in the rewarding properties of ethanol, specifically in the acquisition of ethanol-induced place preference. MK-801, ketamine, ifenprodil, CP-101,606, and (+)-HA-966 were all tested to determine the ability of the NMDA receptor ion channel, NR2B, and $glycine_B$ binding sites to modulate ethanol reward. These antagonists were able to alter ethanol-stimulated activity, but were unable to alter ethanol-induced CPP. These results suggest that these binding sites do not modulate the rewarding effects of ethanol. On the other hand, the competitive NMDA receptor antagonist CGP-37849 attenuated ethanol-stimulated activity and the acquisition of ethanol-induced CPP. These data suggest the glutamate binding site may modulate the rewarding properties of ethanol. The next study replicated those results showing that CGP-37849 attenuated ethanol-induced CPP, and the study also extended these findings to show that CGP-37849 was not rewarding or aversive on its own. Furthermore, CGP-37849 when given alone slightly, but significantly, increased locomotor activity.

Drugs that interfere with the development of CPP can do so by reducing ethanol reward or by interfering with learning and memory. Thus far, data from our laboratory suggest that the rewarding and aversive properties of ethanol are mediated via different mechanisms (Cunningham et al., 2002; Cunningham & Henderson, 2000). Therefore a drug that affects ethanol reward should not affect ethanol aversion, unless it alters general learning. However the same drug when administered systemically may have effects on both behaviors, but producing these effects via different mechanisms. To investigate the effects of CGP-37849 on ethanol aversion, we tested CGP-37849's ability to alter

ethanol-induced CPA. Results from this study showed that CGP-37849 blocked ethanol-induced CPA. Thus, one possible conclusion from this study is that CGP-37849 has a non-specific detrimental effect on associative learning. However, it is still possible that CGP-37849 is altering ethanol reward and aversion through NMDA receptors that are contained in different brain areas and or different neurocircuitry. Investigation of site-specific microinjections of CGP-37849 during CPP and CPA will help to investigate this alternative explanation.

CGP-37849 effects on ethanol-and LiCl-induced CTA were examined in order to determine if CGP-37849 alters ethanol aversion or learning and memory in another associative learning task. The results from the CTA experiment show that CGP-37849 produced a strong CTA on its own, which was not expected based on our previous results. Moreover, the lack of a decrease in ethanol- or LiCl-induced CTA with CGP-37849 was not consistent with theory that CGP-37849 altered the learning of associative learning tasks. However, because all groups treated with CGP-37849 decreased their consumption to almost zero within one conditioning trial, we were unable to see if CGP-37849 enhanced or interfered with CTA with ethanol and or LiCl. Overall, these results suggest all NMDA receptor binding sites modulate ethanol-induced locomotor activation, but only the glutamate binding site modulates ethanol-induced CPP and CPA. However, the alterations in CPP and CPA may be due to learning and memory impairments. The results from the CTA experiments make it difficult to make any conclusions regarding the ability of CGP-37849 to alter ethanol and LiCl CTA since CGP-37849 produced a robust CTA alone.

Interpretations of results

Competitive versus noncompetitive antagonists. Although it was expected that MK-801and ketamine would also attenuate ethanol-induced CPP, there are several explanations why CGP-37849 attenuated the behavior but MK-801 and ketamine did not. CGP-37849 and MK-801 do not substitute for each other in a drug discrimination paradigm, providing evidence for a difference in the subjective effects of these compounds (Zajaczkowski et al., 1996). In addition, the noncompetitive NMDA receptor antagonist MK-801 (0.3 and 0.8 mg/kg) increases basal locomotor activity in mice, whereas D-CPPene (3 and 8 mg/kg) modestly increases locomotor activity at the high dose (Svensson et al., 1991). These data suggests that some aspects of the behavioral consequences of competitive and noncompetitive antagonist binding differ. Moreover, competitive NMDA receptor antagonists, but not channel blockers, block the discriminative stimulus effects of NMDA (Willetts & Balster, 1989). These data suggest the subjective effects of channel blockers are not mediated by direct blockade of NMDA receptors. Moreover, some of the pharmacological effects of competitive and noncompetitive NMDA receptor antagonists differ. For example, channel blockers increase dopamine release and competitive antagonists decrease or have no effect on dopamine release (Svensson et al., 1991; Waters et al., 1996). The differences regarding dopamine release may be due to activation of different corticostriatal glutamatergic pathways. One pathway is tonically active (inhibiting dopamine neuronal activity) and mediates negative feedback, whereas the other is phasically active and mediates positive feedback (stimulates dopamine neuronal activity). Taken together, these data suggest that although both competitive and noncompetitive NMDA receptor antagonists alter NMDA

receptor function, they may do so in different ways that result in distinctive pharmacological and behavioral actions.

Sensory deficits. Since CGP-37849 attenuated ethanol-induced CPP and CPA, but not ethanol-induce CTA, an alternative explanation of these results is CGP-3849 caused peripheral sensory deficits in the CPP and CPA experiments. Sensory deficits would mean that the subjects were unable to sense the tactile cues and ultimately the hole and grid floors were not salient cues in those studies. Therefore, subjects would be able to still form an association between the NaCl or saccharin flavor and the drug injections since the flavors would be gustatory stimuli. There are little data to argue for or against the ability of NMDA receptor antagonists to produce peripheral sensory deficits.

Learning and memory deficits. NMDA receptors are important in learning and memory. Of importance to the current studies, the acquisition of associative learning depends in part on activation of NMDA receptors. Therefore, an alternative interpretation of the current data is that CGP-37849 altered associative learning and memory and not ethanol reward when given before exposure to the CS. However, blockade of several NMDA receptor binding sites alter learning processes. Numerous studies have shown that manipulation of NMDA receptor function alters various learning processes such as spatial and nonspatial learning, as well as avoidance learning. For example, pre-training injections of MK-801 disrupted contextual fear conditioning in rats, but do not affect cued fear conditioning (Gould et al., 2002). Pretreatment of PCP (Danysz et al., 1988) and MK-801 (Mondadori et al., 1989) produce a decrement on working memory. MK-801 also impaired responding in a delayed nonmatch-to-sample task, whereas competitive NMDA receptor antagonists, NMDA receptor NR2B subunit antagonists,

and glycine$_B$ binding site antagonists do not alter this behavior (Willmore et al., 2001). These data suggest that the ion channel binding site might be more involved in the learning and memory of these tasks than other NMDA receptor binding sites.

However, depending on the study, competitive NMDA receptor antagonists have or do not have an effect on acquisition of learning tasks. CGP-37849 altered working memory (delayed matched-to-sample task) at doses that it did not affect locomotor activity (Gutnikov & Rawlins, 1996). This decrement in working memory was not stimulus-specific and may result from a reference memory deficit, working memory deficit, or impairments of perception. Competitive NMDA receptor antagonists CPP and CGS 19755 impaired alternating behavior in a Y-maze, a measure of working memory, but also attenuated locomotor activity at those doses (Parada-Turska & Turski, 1990). These data suggest that competitive NMDA receptor antagonists alter working memory or the acquisition of new learning tasks. However, it should be noted that spatial and non-spatial learning tasks could be confounded by alterations in locomotor activity caused by competitive NMDA receptor antagonist treatment (Deacon & Rawlins, 1995). Other studies have shown a lack of involvement of the NMDA receptor glutamate binding site in learning tasks. CGP 40116 did not alter the acquisition of a visual object-in-place memory task in male rhesus monkeys (Gutnikov & Gaffan, 1996). In addition, competitive NMDA receptor antagonists D-CPPene and NPC 17742 do not alter the accuracy or discriminability in a delayed-nonmatch-to sample or place task (Ballard & McAllister, 2000; Willmore et al., 2001).

It is difficult to conclude whether the results from the current data are due to alterations in learning and memory or to ethanol's hedonic properties based on the fact

that NMDA receptor channel blockers, glycine$_B$ binding site antagonists, and competitive antagonists do not consistently alter the acquisition of several learning and memory tasks. Nevertheless, if the results are due to a global learning deficit, it remains to be explained why CGP-37849 altered the acquisition of the associative conditioning tasks and why MK-801 and ketamine did not. Moreover, CGP-37849 produced a CTA on its own, suggesting CGP-37849 did not attenuate learning in this associative learning paradigm. Therefore, the alterations in the current studies may not be due to alterations in learning, but CGP-37849 may alter the rewarding properties of ethanol due to the apparent aversive properties CGP-37849.

Alterations in ethanol reward. It is still unclear whether CGP-37849 is altering ethanol's hedonic properties or learning of the place conditioning tasks. The conditioned taste aversion experiment provided insight to the mechanism of attenuation of ethanol-induced CPP and CPA. If CGP-37849 attenuated ethanol-induced CTA, but not LiCl-induced CTA, it could be concluded that CGP-37849 decreased ethanol sensitivity and did not do so by a learning and memory mechanism. However, since CGP-37849 produced CTA on its own, it appears that CGP-37849 has aversive properties that could decrease the rewarding properties of ethanol. However, the CTA experiment needs to be conducted with a lower dose of CGP-37849 to determine if CGP-37849 alters ethanol aversion in that paradigm. Nevertheless, the attenuation of ethanol-induced CPA can be explained by context blocking that is independent of changes in ethanol aversion. Previous studies from our laboratory suggest ethanol-induced CPA, but not CPP, are susceptible to proximal pre-exposure effects. Specifically, injections of ethanol 60-min before each CS+ (drug treatment) trials decreased aversion to ethanol given after

exposure to the tactile CS but had no effect on preference to ethanol given before exposure to the tactile CS (Cunningham et al., 2002). One interpretation by Cunningham and colleagues (2002) suggested proximal pre-exposure could reduce the effectiveness of the US (ethanol). This explanation could be applied to the current studies. It is possible that CGP-37849 given 60-min before exposure to the target CS (tactile stimuli) could decrease the ability of ethanol to serve as the US. It is apparent from the CTA study that CGP-37849 produces a strong interoceptive cue. Moreover, CGP-37849 can be discriminated from saline (Zajaczkowski et al., 1996). Therefore, the pre-CS CGP-37849 could reduce the salience of the post-CS ethanol injection.

Dissociation of Locomotor Activation & Ethanol Reward

The results from this dissertation extend previous findings indicating a dissociation of ethanol-induced locomotor activation from ethanol reward. The apparent dissociation is in disagreement with the psychomotor stimulation theory of reward. This theory states that locomotor activation and the positive reinforcing effects of psychomotor stimulants are under the control of the same neurocircuitry (Wise & Bozarth, 1987). However, there is mixed evidence that this holds true for ethanol reward. There are some data that are in agreement with this theory. The inhibition of neuronal nitric oxide synthase (nNOS) and the loss of the DARPP-32 gene block both locomotor activation to ethanol and ethanol-induced conditioned place preference in mice (Itzhak & Martin, 2000; Risinger et al., 2001). Some of the current data are in support of the psychomotor stimulation theory of reward. The competitive NMDA receptor antagonist CGP-37849 was able to attenuate the acquisition of ethanol-induced CPP and was also able to attenuate ethanol-stimulated activity. On the other hand, MK-801, ketamine, (+)-

HA-966, ifenprodil, and CP-101,606 all decreased ethanol-induced locomotor stimulation at high doses, but did not alter ethanol reward. There are many more ethanol place preference studies that argue against the common mechanism. $GABA_A$ and $5\text{-}HT_2$ antagonism enhance ethanol-induced CPP but decreases ethanol-induced locomotor activation (Chester & Cunningham, 1999a; Risinger & Oakes, 1996b). D_3 and $5\text{-}HT1_A$ antagonism enhance ethanol-induced CPP but have no effect on ethanol-induced activation (Boyce & Risinger, 2000, 2002; Risinger & Boyce, 2002). Furthermore, $GABA_B$, D_2, and D_4 antagonism decreases ethanol-induced locomotor activation, but has no effect on ethanol-induced place conditioning (Chester & Cunningham, 1999b; Risinger et al., 1992; Thrasher et al., 1999). Further support of dissociation comes from C56Bl/6J x DBA/2J Recombinant Inbred (BXD RI) mice. Ethanol-induced locomotor stimulation was not correlated with the magnitude of ethanol-induced CPP (Cunningham, 1995). In addition, there is also a lack of correlation between locomotor activation and 10% v/v ethanol drinking in BXD recombinant inbred mice (Phillips et al., 1995). Taken together, these data suggest that the locomotor activating effects of ethanol and the rewarding effects of ethanol are largely mediated via different neurocircuitry.

Relationship to Self-Administration Studies

Some of the results from the present studies differ quite noticeably from the self-administration studies in the literature since channel blockers alter ethanol self-administration. Specifically, NMDA receptor channel blockers such as memantine, MK-801, MRZ 2/579, and PCP decrease reinforced responding for ethanol in rats (Bienkowski et al., 1999; Piasecki et al., 1998; Shelton & Balster, 1997). However, most of these compounds alter other behaviors such as locomotor activity. Therefore, the self-

administration results may not be due to alterations in ethanol reward. For example, MRZ 2/579 decreased responding for ethanol, but also altered nonreinforced (i.e. craving) responding (Piasecki et al., 1998). These results suggest that MRZ 2/579 may be altering both the primary and secondary reinforcing properties of ethanol or alternatively affecting motor coordination. It is most probable that the latter interpretation is correct. Support for the alternative interpretation comes from channel blockers' ability to significantly alter locomotor activity and motor coordination. A low dose of MK-801 potentiates the stimulant effect of ethanol, which could lead to an increase in general responding, whereas high doses of MK-801 attenuate ethanol's stimulant effects and potentiate the sedative effects, which could lead to a decrease in responding (Shen & Phillips, 1998; Meyer & Phillips, 2003). In addition, PCP decreased responding for saccharin as well as for ethanol (Shelton & Balster, 1997). The effects on both behaviors may indicate that PCP may alter general reward pathways or alter motor coordination.

In place conditioning, testing is conducted when the subjects are drug free, so alteration in locomotor activity with these drugs is not a potential confound. As mentioned previously, this is one strong advantage of this paradigm. It is possible that NMDA receptor channel blockers are altering self-administration behavior by changes in locomotor activity and not ethanol reward. This could account for the difference in results. Another possibility is a species difference. Perhaps MK-801 and other channel blockers affect ethanol-reward in rats but not mice. Another possibility is that the ion channel binding site is important for self-administration and not place preference since data from the two paradigms suggest that they do not have a complete overlap in neurocircuitry (Bardo & Bevins, 2000). This alternative hypothesis is probable.

The present results also suggest that the NMDA receptor glutamate binding site is important for ethanol place conditioning and the glycine$_B$ and NR2B binding sites are not. Competitive NMDA receptor antagonists AP-5 and CPPene both decrease reinforced responding for ethanol (Rassnick et al., 1992; Shelton & Balster, 1997). The present studies show that CGP-37849 decreases ethanol-induced CPP. This suggests that the glutamate binding site modulates ethanol reinforcement and reward. However, CPPene altered responding for saccharin as well as ethanol responding (Shelton & Balster, 1997). It should be mentioned that the subjects in that study were required to respond for ethanol and saccharin on an alternating schedule, therefore, previous responding may have affected saccharin responding. Further studies need to be conducted to determine if competitive NMDA receptor antagonists do in fact alter saccharin reward. There are no data regarding the role of the NMDA receptor NR2B subunit in the rewarding and reinforcing properties of ethanol. The present data are the first to suggest a lack of involvement, however, additional studies need to be conducted with CP-101,606 on ethanol-self administration to come to a decisive conclusion. The present studies are in agreement with the data from Bienkowski and colleagues (1999) suggesting that the NMDA receptor glycine$_B$ binding site does not modulate ethanol reinforcement.

Relationship to Ethanol-Induced Place Preference Studies

The current results differ from the previous rat ethanol place conditioning studies. Biala & Kotlinska (1999) have shown that MK-801 (NMDA receptor channel blocker) and L-701,324 (glycine$_B$ antagonist) were both able to attenuate ethanol-induce CPP in rats. The current studies have shown that MK-801 and ketamine, NMDA receptor channel blockers, were unable to alter the acquisition of ethanol-induced CPP at doses

that had a behaviorally relevant effect (i.e., alterations in ethanol-stimulated activity). We have also shown that (+)-HA-966, a glycine$_B$ partial agonist, did not alter the acquisition of ethanol-induced CPP. There are several possible reasons for the discrepancies, which will be discussed below.

The species difference may account for the conflicting results. Several strains of mice have been shown to exhibit a place preference for ethanol (Cunningham et al., 1991; Cunningham et al., 1992; Cunningham et al., 1993; Risinger & Oakes, 1996a), but most studies have shown that rats exhibit a place aversion for ethanol (Cunningham et al., 1993; Sherman et al., 1988). Of the mouse strains that show place preference, DBA/2J mice show the most robust ethanol-induced place preference when given 5 min conditioning trials (Cunningham et al., 1992). DBA/2J mice spend approximately 80% of their time on the drug-paired floor during the preference test (Cunningham & Prather, 1992). Thus, there may be a significant species difference in response to ethanol. There is further evidence for a species difference in response to ethanol. HS-Ibg stock mice show activation to 1.5 g/kg ethanol, whereas Holtzman albino rats do not (Cunningham et al., 1993). These data show that mice and rats respond very differently to experimenter administered ethanol and suggest that the species difference may in part account for the contradictory results.

Procedural differences could also explain the discrepancy. In order to show significant place preference in rats, Biala & Kotlinska (1999) gave 15 days of 0.5 g/kg ethanol injections to pre-expose the subjects to ethanol. This pre-exposure to ethanol makes the experiments quite different since our mice are ethanol-naive and theirs are ethanol-experienced. MK-801 and L-701,324 could have different results in ethanol-

experienced animals, thus possibly accounting for the difference in results. In addition, initial biases often confound interpretations of place conditioning experiments. When a decrease in preference is obtained using a biased design, the decrease could be due to a decrease in reward or could also be due to a decrease an unconditional motivational state associated with the non-preferred context. The ethanol pre-exposure in combination with the biased apparatus could be producing a decrease in aversion and or a decrease in the anxiolytic effects of ethanol and not a decrease in reward.

CONCLUSIONS

These studies provide information into the role of the NMDA receptor binding sites in neurobiology of ethanol-induced CPP and ethanol-stimulated activity. All of the NMDA receptor binding sites tested modulate ethanol-stimulated activity. The place conditioning studies demonstrate that the NMDA receptor glutamate binding site is important for the acquisition of ethanol-induced CPP and CPA. It is hypothesized that CGP-37849 given before exposure to the CS in the ethanol-induced CPA experiment may make the post-CS ethanol cue less salient. The place conditioning studies have also shown that CGP-37849 is not rewarding or aversive alone, however the data from the CTA study suggests that CGP-37849 has aversive properties. The 60-min pre-CS injection timing used in the place conditioning studies may not be optimal for the expression of the aversive properties. Future studies will provide greater insight to how CGP-37849 alters ethanol aversion. Overall, these studies are the first to demonstrate the involvement of the NMDA receptor glutamate binding site in ethanol –induced place preference and lack of involvement of the NMDA receptor ion channel, $glycine_B$ binding site, and NR2B binding site in ethanol-induced CPP.

FUTURE DIRECTIONS

CGP-37849 and aversive properties of ethanol

Three additional studies could provide more support for the interpretation that CGP-37849 attenuated ethanol reward, but the reduction of ethanol-induced CPA was due to a decrease in salience of the post-CS ethanol cue. One study examining post-CS injections of both CGP-37849 and ethanol could provide additional information of CGP-37849's ability to alter ethanol aversion. By giving the CGP-37849 and ethanol injections post-CS 60 min apart, it is possible that CGP-37849 would enhance ethanol CPA and produce CPA on its own. Giving CGP-37849 60-min pre-CS, was not optimal to determine if CGP-37849 had aversive properties on its own.

Another study would be a similar study to the CTA study in this dissertation, however, the CS would be 0.15% saccharin to optimize an enhancement of ethanol-induced CTA and the dose of CGP-37849 lowered to 2-5 mg/kg. In this proposed study, it may be possible to determine if there is an additive effect of CGP-37849 and ethanol on aversion.

In addition, to investigate the possibility that CGP-37849 has different effects given pre-CS (CPP) versus post-CS (CTA), another CTA study should be conducted. During this study, CGP-37849 would be administered before exposure to the 0.15% saccharin CS. However, this study may be difficult to conduct since CGP-37849 causes locomotor impairments that could confound interpretations. One way to get around the locomotor issue would be to get a saccharin intake baseline, inject the saccharin into the subject's mouth on a subsequent day after the CGP-37849 injection, then 48-h later take readings of saccharin consumption.

CGP-37849 and other ethanol-mediated behaviors

Further studies need to be conducted on the role of the glutamate binding in other ethanol-related behaviors such as ethanol-sensitivity and ethanol drinking in order to determine how large of a role the NMDA receptor glutamate binding site plays in ethanol-mediated behaviors. Little has been done regarding the involvement of the NMDA receptor glutamate binding site and ethanol-related behaviors. Although CGP-37849 does cause motor impairments, it would be important to examine this drug's effects on ethanol drinking behavior in mice. This could be accomplished via 24-h two-bottle choice drinking and 30-min limited access drinking in C57BL/6J mice. Moreover, CGP-37849 should be investigated in ethanol withdrawal as well as in ethanol's hypnotic effects.

Mechanism for effects of CGP-37849

Other studies need to be conducted to determine how the competitive antagonists alter ethanol-induced CPP and learning and memory. Some learning and memory studies need to be conducted in DBA/2J mice using the same NMDA receptor drugs, doses and pretreatment times in order to determine the role of competitive NMDA receptor antagonists in the learning of associative conditioning tasks in DBA/2J mice. Moreover, investigation of the NMDA receptor NR2A subunit may provide information about whether or not the NR2A subunit is important in ethanol's hedonic properties. It is possible that the NMDA receptor mediation of ethanol reward is due to alteration in NR2A subunits. This hypothesis is based on the equal preference of CGP-37849 for all NR2 subunits. The NR2A subunit could be important for the ability of CGP-37849 to alter ethanol's hedonic properties. Moreover, ethanol has a greater effect at NR1/NR2A-

and NR1/NR2B-containing receptors. The current studies have shown the NR2B subunit does not modulate ethanol-induced CPP. Unfortunately, there are no NR2A specific compounds currently available to test this hypothesis. However, an alternative would be to use NMDA receptor NR2A subunit KO mice. I would hypothesize that NR2A KO mice would show attenuated ethanol-induced CPP in comparison to WTs. Little has been done to determine the role of the NR2A subunit in the rewarding properties of ethanol in these mice. NMDA receptor NR2A subunit KO mice could provide useful information regarding how CGP-37849 alters ethanol-induced CPP.

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APPENDIX A

The following experiments are included in the appendix because the data did not fit with those of chapters one and two. In the case of the first experiment presented in the appendix, the magnitude of CPP in the control group was not what was seen with the control groups in the other CPP experiments contained in this dissertation suggesting that the lack of an effect was due to sampling error. There was no significant effect of conditioning group (G+ versus G-) within the 0 mg/kg MK-801 dose group, therefore, this experiment was run again (Experiment 2 in Chapter 1).

The second experiment reported here was conducted in order to determine if MK-801 might actually enhance ethanol-induced CPP. In Experiment 2 of Chapter 1, it seemed possible that there was a ceiling effect that prevented our ability to see greater ethanol-induced CPP with MK-801 pretreatment in comparison to saline pretreated controls since all groups showed a very robust CPP. In order to determine if MK-801 could enhance ethanol CPP, the same dose of ethanol was used in this experiment. However, a preference test was conducted earlier during conditioning (after 2 instead of 4 conditioning trials) in order to optimize the ability to determine if MK-801 enhances ethanol CPP since maximal CPP in the control group occurred after 4 conditioning trials. The results from this experiment showed that MK-801 did not enhance (or retard) ethanol-induced CPP. Although the outcome was consistent with our other studies, it was not included with the other CPP experiments in chapter 1 because the test strategy differed.

Materials and Methods

For details on subjects, apparatus, and drugs, please see materials and methods in Chapter 1.

Conditioned place preference

Experiment 1

The methods used for conditioned place preference were the same as used previously (see chapter 1). Briefly, subjects were randomly assigned to one of four MK-801 dose groups (0, 0.05, 0.1, or 0.2 mg/kg) and subjects in each drug treatment group were randomly assigned to one of two subgroups (G+ or G-) and exposed to an unbiased differential conditioning procedure. The preference test consisted of a 60-min choice session.

Experiment 2

The experiment was run identically to those used previously in chapter 1 with the exception of the experimental sequence. In this experiment, subjects received the same habituation session as in experiment 1, but then underwent two conditioning trials (2 CS+; 2 CS-), preference test, two additional conditioning trials, and a second preference test. Subjects were assigned to one of Drug treatment groups: SE (0 mg/kg MK-801 + 2 g/kg ethanol), 0.2ME (0.2 mg/kg MK-801 + 2 g/kg ethanol), or 0.2MS (0.2 mg/kg MK-801 + 0 g/kg ethanol).

Data Analyses

Data were analyzed by analysis of variance (ANOVA) with the alpha level set at 0.05. Drug treatment and Conditioning group (G+/G-) were treated as between-group factors, and trial type (CS+/CS-) was treated as a within-group factor. Significant

interactions were analyzed by follow-up ANOVAs (Keppel, 1991). Pairwise comparisons were Bonferroni corrected. Initial analyses of conditioning activity in each experiment considered trial number as a factor (data not shown). However, there were no consistent effects of trial number on overall conclusions about effects of NMDA receptor antagonism on ethanol-stimulated activity when the data were collapsed across trials 1-4. Therefore, for simplicity, we have presented conditioning activity collapsed across conditioning trials 1-4.

Results

Conditioning Activity

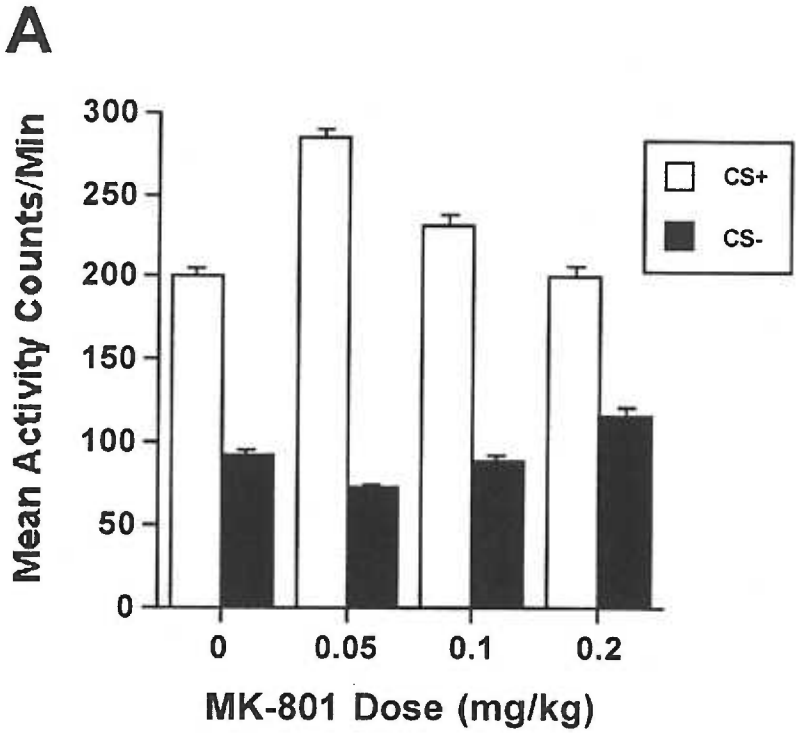
Experiment 1

Figure 9A shows the mean (+*SEM*) activity counts per min collapsed across conditioning trials 1-4 for each MK-801 dose group. A 2 g/kg ethanol dose produced locomotor stimulation on CS+ trials (white bars) in comparison to activity during CS- sessions with saline injections (black bars). This observation was supported by the main effect of trial type. Furthermore, there were main effects and interactions with MK-801 dose, suggesting that MK-801 altered ethanol-stimulated locomotor activity. MK-801 increased ethanol-stimulated activity at low doses and decreased ethanol-stimulated activity at high doses.

Insert Figure 9 about here

A two-way ANOVA (MK-801 dose x Trial type(CS+/CS-)) of conditioning activity yielded significant main effects of MK-801 dose [$F(3, 88) = 10.5, p < .001$] and

Figure 9. (A) Mean (+*SEM*) activity counts/min following ethanol (CS+) and saline (CS-) for each MK-801 dose group collapsed across conditioning trials 1-4. On CS+ days, mice received 0, 0.05, 0.1, or 0.2 mg/kg MK-801 30 min before a 2 g/kg ethanol injection. All subjects received 2 saline injections on CS- days. (B) Mean (+*SEM*) s/min spent on the grid floor by conditioning subgroups G+ and G- for each MK-801 dose group collapsed across the 60 min of the preference test. Drug treatment (antagonist and ethanol) was given pre-CS in all experiments. During conditioning, subjects in the G+ subgroup received MK-801 (0, 0.05, or 0.2 mg/kg) paired with the grid floor and saline paired with the hole floor. Subjects in the G- subgroup received MK-801 (0, 0.05, or 0.2 mg/kg) paired with the hole floor and saline paired with the grid floor. Conditioned place preference is shown when time spent on the grid floor by the G+ group exceeds the time spent on the grid floor by the G- group.


A
Mean Activity Counts/Min
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100
50
0
CS+
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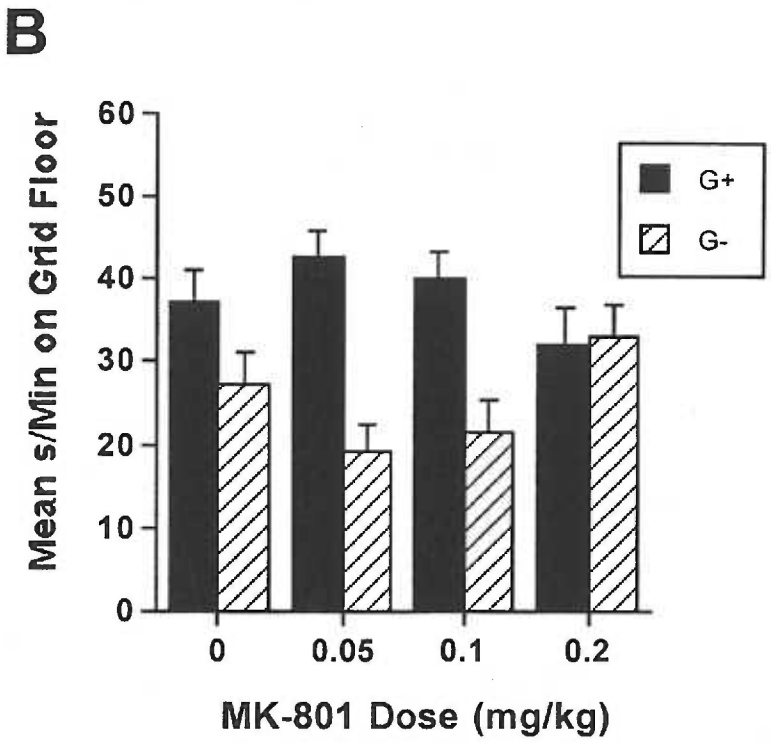
MK-801 Dose (mg/kg)


B
Mean s/Min on Grid Floor
60
50
40
30
20
10
0
G+
G-
0
0.05
0.1
0.2
MK-801 Dose (mg/kg)

Trial type [$F(1, 88) = 1931.1, p < .001$] and a MK-801 dose x Trial type interaction [$F(3, 88) = 25.6, p < .001$]. Separate one-way ANOVA of CS+ trials showed a significant effect of MK-801 dose on CS+ activity [$F(3, 88) = 19.1, p < .001$]. Follow-up analyses indicate that the 0.05 mg/kg MK-801 dose group showed greater ethanol-stimulated activity than the 0, 0.1, and 0.2 mg/kg MK-801 dose groups [$ps < .04$]. Moreover, the 0.2 m/kg MK-801 dose group showed less ethanol-stimulated activity than the 0 and 0.1 mg/kg MK-801 groups [$ps < .02$]. Separate one-way ANOVA of CS- trials yielded no significant differences, suggesting that all MK-801 dose groups showed similar activity after saline injections [$p = .4$]. These results suggest that MK-801 at low doses increases ethanol stimulated activity and at high doses decreases ethanol stimulated activity.

Experiment 2

Figure 10A shows the mean (+*SEM*) activity counts per min collapsed across conditioning trials 1-4 for each Drug Treatment group. In the SE group, a 2 g/kg ethanol dose produced locomotor stimulation on CS+ trials (white bars) in comparison to activity during CS- trials with saline injections (black bars). Moreover in the 0.2MS group, MK-801 produced locomotor stimulation on CS+ trials in comparison to activity during CS- trials indicating that MK-801 produced locomotor stimulation alone. Furthermore, this replicates the previous data indicating that high doses of MK-801 decreased ethanol-stimulated activity.

A two-way ANOVA (Drug treatment x Trial type (CS+/CS-)) of conditioning activity yielded significant main effects of Drug treatment [$F(2,86) = 20.2, p < .001$] and Trial type [$F(1, 86) = 1709.6, p < .001$] as well as a Drug treatment x Trial type interaction [$F(2, 86) = 27.0, p < .001$]. Separate one-way ANOVA of CS+ trial activity

yielded a significant effect of Drug treatment [$F(2, 86) = 25.7, p < .001$]. Follow-up analyses indicate that the SE and 0.2MS groups had higher activity than the 0.2ME group [$ps < .001$]. Separate one-way ANOVA of CS- trial yielded no significant effects, suggesting that all groups had similar activity levels with saline injections [$p = .5$].

Insert Figure 10 about here

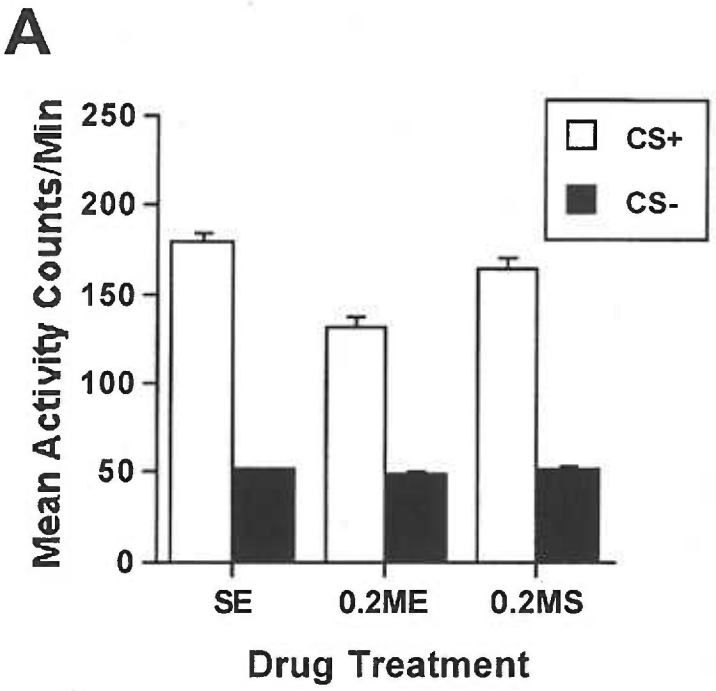
Preference test

Experiment 1

Figure 9B shows the mean (+ *SEM*) s/min spent on the grid floor during the preference test for the G+ and G- subgroups within each MK-801 dose group for the 60 min preference test. Unlike the experiments in chapter one of this thesis, all 60 min preference test data were analyzed because there was a significant interaction at this time point.

A two-way ANOVA (MK-801 dose x Conditioning group) for all 60 min of the preference test yielded a significant effect of Conditioning group [$F(1, 84) = 24.6, p < .001$] and a MK-801 x Conditioning group interaction [$F(3, 84) = 4.2, p = .008$]. Two-way follow-up ANOVAs were conducted to investigate the Drug treatment x Conditioning group interaction. These analyses indicate that the 0.1 mg/kg MK-801 and 0.05 mg/kg MK-801 dose groups showed a greater magnitude of place conditioning than the 0.2 mg/kg MK-801 dose group [$F(1, 42) = 6.3, p = .02$ and $F(1, 42) = 10.9, p = .002$]. The 0 mg/kg MK-801 dose group did not differ from the other groups [$ps > .06$]. Pairwise comparisons of conditioning group (G+ vs. G-) within each MK-801 treatment

Figure 10. (A) Mean (+*SEM*) activity counts/min following ethanol (CS+) and saline (CS-) for each drug treatment group collapsed across conditioning trials 1-4. On CS+ days, mice received 0 (SE) or 0.2 mg/kg MK-801 (0.2ME and 0.2MS) 30 min before a 2 (SE and 0.2ME) or 0 (0.2MS) g/kg ethanol injection. All subjects received 2 saline injections on CS- days. (B) Mean (+*SEM*) s/min spent on the grid floor by conditioning subgroups G+ and G- for each drug treatment group collapsed across the 30 min of the first (left) and second (right) preference test. The first preference test was after 2 conditioning trials; whereas the second was after an additional 2 conditioning trials (4 total). Drug treatment (antagonist and ethanol) was given pre-CS in all experiments. During conditioning, subjects in the G+ subgroup received drug treatment paired with the grid floor and saline paired with the hole floor. Subjects in the G- subgroup received drug treatment paired with the hole floor and saline paired with the grid floor. Conditioned place preference is shown when time spent on the grid floor by the G+ group exceeds the time spent on the grid floor by the G- group.


A
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CS+
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SE
0.2ME
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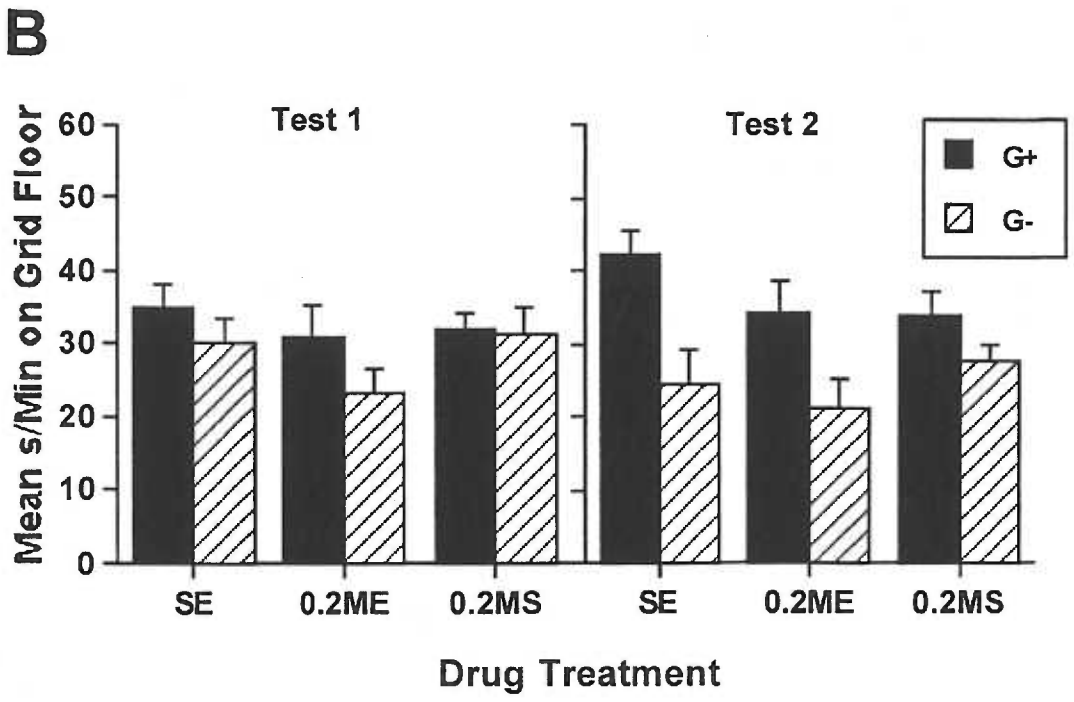
Drug Treatment


B
Mean s/Min on Grid Floor
60
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0
Test 1
Test 2
G+
G-
SE
0.2ME
0.2MS
SE
0.2ME
0.2MS
Drug Treatment

group were also conducted. These analyses showed that the 0.05 and 0.1 MK-801 dose groups showed significant differences of conditioning group [$p < .001$, $p = .002$, respectively], however, the 0 and 0.2 MK-801 MK-801 dose groups did not [$ps > .03$]. This suggests that the 0 mg/kg and 0.2 mg/kg MK-801 dose groups did not show a place preference for the ethanol-paired floor.

Experiment 2

Figure 10B shows the mean (+ *SEM*) s/min spent on the grid floor during the preference test for the G+ and G- subgroups within each MK-801 dose group for the 30 min preference test. Test 1 (after 2 conditioning trials) is shown on the right panel and test 2 (after 2 additional conditioning trials) is shown on the left panel.

A two-way ANOVA (Drug treatment x Conditioning group) of test 1 grid times yielded a significant main effect of Conditioning group [$F(1, 83) = 5.1$, $p = .03$] but not Drug treatment or Drug treatment x Conditioning group interaction [$ps < .5$]. This suggests that there was significant place conditioning, but no difference between Drug treatment groups.

A two-way ANOVA of test 2 grid times again yielded a main effect of Conditioning group [$F(1, 83) = 22.6$, $p < .001$], but no Drug treatment or Drug treatment x Conditioning group interaction [$ps < .6$]. This suggests that there was again place conditioning, but no difference between groups. Moreover, this outcome also suggests that MK-801 pretreatment does not enhance ethanol-induced CPP.

Discussion

Although the magnitude of ethanol-induced place conditioning in the control group (0 mg/kg MK-801) in the experiment 1 was inconsistent with that of the studies in

Chapter 1, the locomotor alterations produced by MK-801 during the acquisition of conditioning was similar to the MK-801 in that chapter. In both experiments low doses of MK-801 enhanced ethanol-induced locomotor stimulation and high doses attenuated ethanol-induced locomotor stimulation. Likewise, 0.2 mg/kg MK-801 enhanced ethanol stimulated activity in experiment 2. Moreover, ethanol produced locomotor stimulation in the control groups. These results suggest that although the preference data was not as robust as usual in the control subjects, there was still the same degree of locomotor stimulation with 2 g/kg ethanol. In addition, MK-801 consistently attenuates ethanol-induced locomotor activation at high doses and enhances ethanol-induced locomotor activation at low doses.

APPENDIX B

The data in appendix B are the conditioning activity from the place conditioning studies in Chapter 1. This appendix will be looking at the development of sensitization to repeated 2 g/kg ethanol injections. Sensitization is the increase in the effect of a drug after repeated exposure.

Materials and Methods

Experiment numbers will be consistent with those of Chapter 1. The methods for the CS+ and CS- conditioning trials were described in Chapter 1. Briefly, Subjects were randomly assigned to one of three or four drug treatment group depending on the experiment (Table 1). Subjects were given a preinjection and returned to the home cages after the first injection for the time delay indicated in Chapter 1. After the second injection, subjects were placed into the apparatus for a 5-min conditioning session. On alternate days, subjects received drug treatment (CS+ days) or saline treatment (CS- days).

Data Analyses

Data were analyzed by ANOVA with the alpha level set at 0.05. Drug treatment was treated as the between-group factor and trial type (CS+/CS-) and trial number were treated as within-group factors. Significant interactions were analyzed by follow-up ANOVAs (Keppel, 1991).

Results

Table 10 shows the overall ANOVAs for CS+ conditioning activity across trials in each of the eight experiments. Follow-up analyses are described below for those

experiments in which the Drug treatment x Trial number interactions were significant. CS- activity was not included in the analysis because no drug was on board during these trials, therefore should not affect locomotor sensitization during CS+ trials. All subjects received 2 injections of saline on CS- trials.

Insert Table 10 about here

Experiment 1A & 1B

Figures11A and 11B show the mean (±*SEM*) CS+ activity counts per min during conditioning trials 1-4 in each CGP-37849 treatment group for both studies. As can be seen by examining the solid squares, the saline pretreated group increased their ethanol-stimulated activity across trials, indicating that the group developed locomotor sensitization. Overall, CGP-37849 decreased ethanol-stimulated activity as indicated by a main effect of CGP-37849 dose in both studies. Separate two-way ANOVAs showed a significant CGP-37849 dose x Trial number interaction (Table 10) during CS+ trials, suggesting that CGP-37849 pretreatment altered the development of ethanol-induced locomotor sensitization. These results may have been due to tolerance to the sedative effects of ethanol, CGP-37849 or the combination. However, most data suggests that tolerance does not develop to competitive NMDA receptor antagonists. Moreover, it should be noted that the magnitude of locomotor sensitization in the control group was not as robust as others have previously reported (Shen & Phillips, 1998). The reduction in activity during trial 1 with CGP-37849 pretreatment complicates the interpretation. As

Table 10. Analysis of Variance for mean conditioning activity counts per minute across trials 1-4.

Exp	Drug treatment	Source	F(df)
1A	CGP-37849	Drug treatment	$F(3, 90) = 433.4$ **
		Trial number	$F(3, 270) = 19.7$ **
		Drug treatment x Trial number	$F(9, 270) = 2.9$ *
1B	CGP-37849	Drug treatment	$F(1, 59) = 687.9$ **
		Trial number	$F(3, 177) = 11.1$ **
		Drug treatment x Trial number	$F(3, 177) = 2.9$ *
2	MK-801	Drug treatment	$F(2, 87) = 36.4$ **
		Trial number	$F(3, 261) = 23.8$ **
		Drug treatment x Trial number	$F(6, 261) = 2.7$ *
3	Ketamine	Drug treatment	$F(3, 78) = 23.0$ **
		Trial number	$F(3, 234) = 66.2$ **
		Drug treatment x Trial number	$F(9, 234) = 2.0$ *
4	Ifenprodil	Drug treatment	$F(3, 88) = 179.0$ **
		Trial number	$F(3, 264) = 78.0$ **
		Drug treatment x Trial number	$F(9, 264) = .9$
5	CP-101,606	Drug treatment	$F(3, 86) = 12.5$ **
		Trial number	$F(3, 258) = 24.2$ **
		Drug treatment x Trial number	$F(3, 258) = 1.0$
6	(+)-HA-966	Drug treatment	$F(3, 89) = 17.1$ **
		Trial number	$F(3, 267) = 11.9$ **
		Drug treatment x Trial number	$F(9, 267) = 9.6$ **

Note. *$p < .05$, **$p < .001$

noted in Meyer and Phillips (2003), the stimulant effects of ethanol may need to be experienced for the development of sensitization to occur.

Insert Figure 11 about here

Experiment 1A

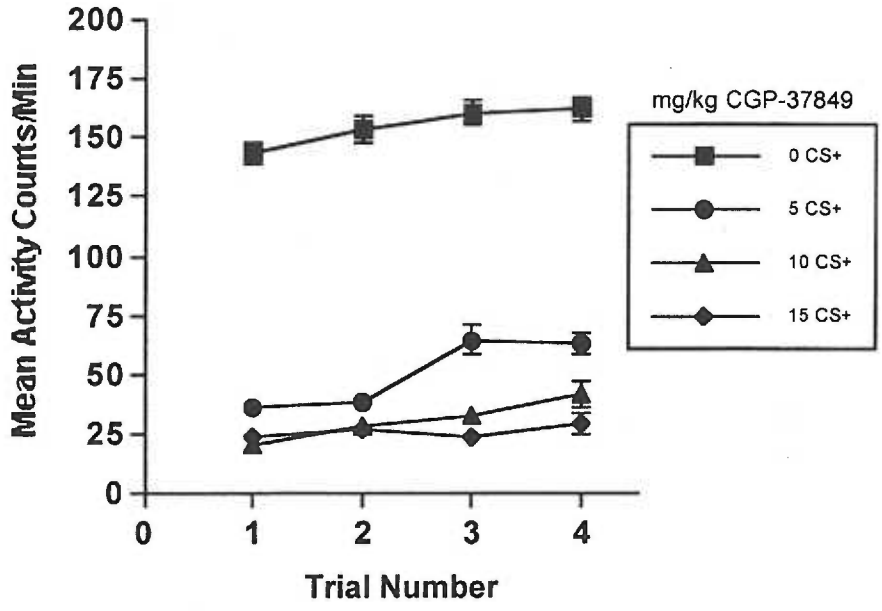
To evaluate the interaction, planned between group comparisons (two-way ANOVAs) were also conducted to compare each CGP-37849 pretreated group (5, 10, 15 mg/kg) with the saline group (0 mg/kg). These analyses indicated that the 0 mg/kg CGP-37849 dose group only differed from the 5 mg/kg CGP-37849 group across trials [$F(3, 138) = 2.6$, $p = .05$]. This difference was due to the 5 mg/kg CGP-37849 showing tolerance to the initial locomotor suppressant effects of CGP-37849 + 2 g/kg ethanol. One-way ANOVAs of activity across CS+ trials indicated that the 0, 5, and 10 mg/kg CGP-37849 pretreated groups, but not the 15 mg/kg CGP-37849 ($p = .7$), developed locomotor sensitization [$Fs > 5.1$, $ps < .008$].

Experiment 1B

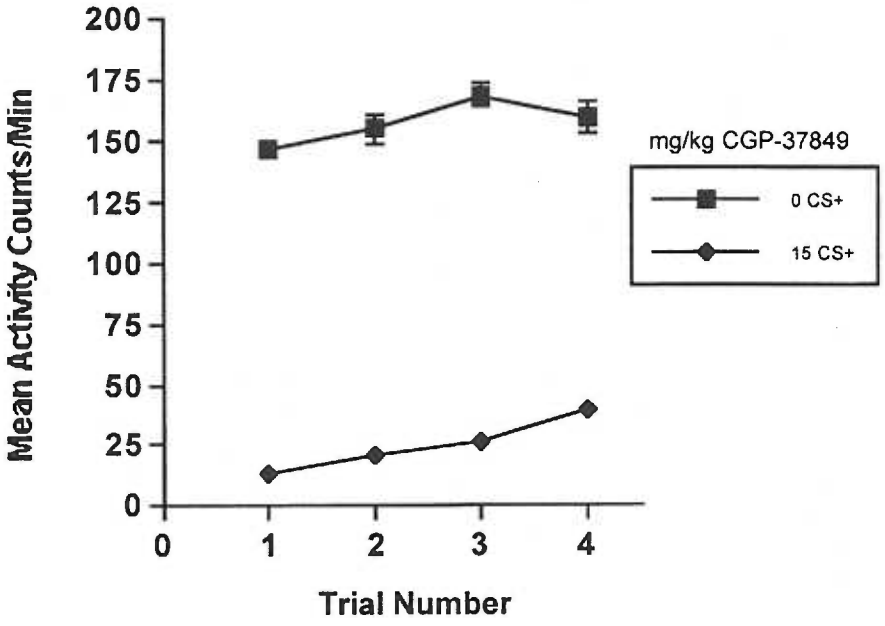
A two-way ANOVA (CGP-37849 Dose x Trial number) of CS+ activity yielded significant main effects of CGP-37849 dose [$F(1, 59) = 687.9$, $p < .001$] and Trial number [$F(3, 177) = 11.1$, $p < .001$] as well as a CGP-37849 dose x Trial number interaction [$F(3, 177) = 2.9$, $p = .03$]. This interaction suggests that the 0 and 15 mg/kg CGP-37849 dose groups differed in their locomotor activity across trials. This difference appears to be due to the 15 mg/kg CGP-37849 showing tolerance to the initial locomotor suppressant effects of CGP-37849 + 2 g/kg ethanol.

Figure 11. Mean (± *SEM*) activity counts/min following ethanol (CS+) for drug treatment groups across the 4 conditioning trials. (A) On CS+ trials, mice received 0, 5, 10, or 15 mg/kg CGP-37849 60 min before a 2 g/kg ethanol injection. (B) On CS+ trials, mice received 0 or 15 mg/kg CGP-37849 60 min before a 2 g/kg ethanol injection.

A


Mean Activity Counts/Min
200
175
150
125
100
75
50
25
0
0
1
2
3
4
Trial Number
mg/kg CGP-37849
0 CS+
5 CS+
10 CS+
15 CS+

B


Mean Activity Counts/Min
200
175
150
125
100
75
50
25
0
0
1
2
3
4
Trial Number
mg/kg CGP-37849
0 CS+
15 CS+

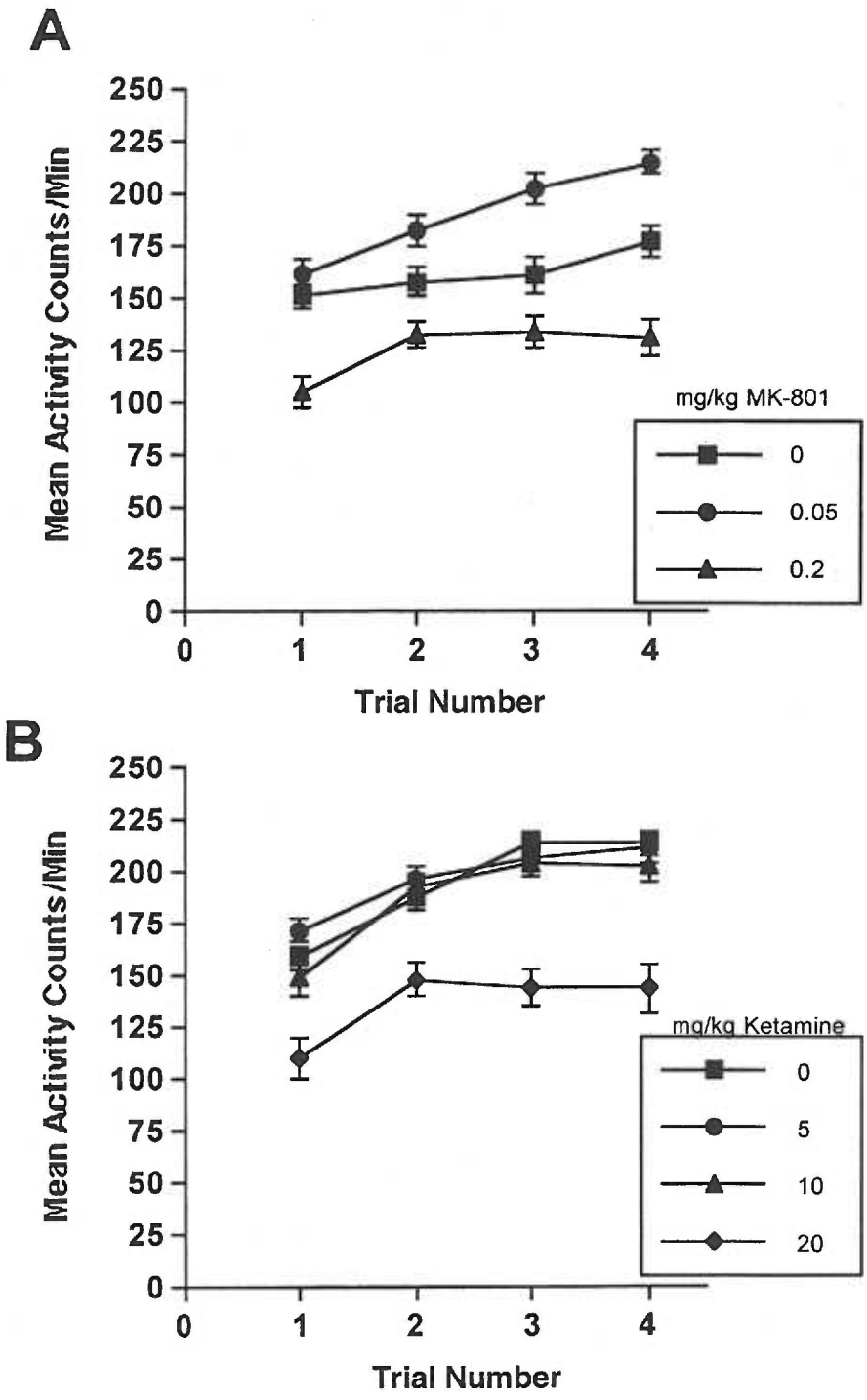
Experiment 2

Figure 12A shows the mean (± SEM) activity counts per minute during for CS+ trials 1-4 in each drug treatment group. A 2 g/kg ethanol dose produced locomotor sensitization across trials in the control group (black squares). There was a MK-801 dose x Trial number interaction suggesting the groups differed in the magnitude of sensitization. This observation was supported by two-way follow-up ANOVAs that showed that the 0.05 mg/kg MK-801 dose group showed greater sensitization than controls (0 mg/kg). This data is consistent with a study showing low doses of MK-801 enhance ethanol-induced sensitization, but high doses attenuated this effect (Meyer & Phillips, 2003).

Insert Figure 12 about here

To evaluate the interaction, separate planned between group comparisons (two-way ANOVAs) were conducted to compare the MK-801 pretreated groups (0.05, 0.2 mg/kg) with the saline group (0 mg/kg). These analyses showed that 0 mg/kg MK-801 dose group differed from the 0.05 mg/kg MK-801 dose group across trials [$F(3, 177) = 3.7$, $p = .01$]. In addition, separate one-way ANOVAs of Trial number within each MK-801 dose group revealed a significant effect of Trial number in each MK-801 dose group [$Fs > 4.7$ $ps < .004$], suggesting all groups developed locomotor sensitization. However, 0.2 mg/kg MK-801 decreased the stimulant effect of ethanol which may have affected the development of sensitization in this group.

Figure 12. Mean (± *SEM*) activity counts/min following ethanol (CS+) for drug treatment groups across the 4 conditioning trials. (A) On CS+ trials, mice received 0, 0.05, or 0.2 mg/kg MK-801 30 min before a 2 g/kg ethanol injection. (B) On CS+ trials, mice received 0, 5, 10, or 20 mg/kg ketamine 15 min before a 2 g/kg ethanol injection.


A
Mean Activity Counts/Min
250
225
200
175
150
125
100
75
50
25
0
0
1
2
3
4
Trial Number
mg/kg MK-801
0
0.05
0.2
B
Mean Activity Counts/Min
250
225
200
175
150
125
100
75
50
25
0
0
1
2
3
4
Trial Number
mg/kg Ketamine
0
5
10
20

Ketamine

Figure 12B shows the mean (± *SEM*) activity counts per minute during CS+ trials 1-4 for each ketamine dose group. A 2 g/kg dose of ethanol produced locomotor sensitization across trials in the 0 mg/kg ketamine group (black squares). Only the highest dose of ketamine was able to alter ethanol locomotor sensitization. However, this dose of ketamine, much like the highest dose of MK-801 and CGP-37849, altered the initial stimulant response to ethanol on trial 1. This reduction of ethanol's stimulant effect may have impeded the development of sensitization in 20 mg/kg ketamine group.

To evaluate the Ketamine dose x Trial number interaction, separate planned between group comparisons were conducted to compare the ketamine pretreated groups (5, 10, 20 mg/kg) with the saline group (0 mg/kg) across trials. The 5 mg/kg and 20 mg/kg ketamine dose groups differed from the 0 mg/kg ketamine dose group across trials [$F(3, 120) = 2.7$, $p = .05$; $F(3, 120) = 3.3$, $p = 0.02$]. The differences between groups was due to the 5 and 20 mg/kg ketamine groups showing less locomotor sensitization than the 0 mg/kg ketamine dose group. However, the CS+ activity during trial 1 was much lower in the 20 mg/kg ketamine group compared to all other ketamine dose groups. Moreover, follow-up one-way ANOVAs of activity by trial number within each group showed that all groups showed locomotor sensitization [Fs > 5.8, ps $< .005$].

(+)-HA-966

Figure 13 shows the mean (± *SEM*) activity counts per minute for CS+ trials 1-4 for each (+)-HA-966 treated group. A 2 g/kg dose of ethanol (black squares) produced locomotor stimulation and locomotor sensitization across trials. There were main effects

of drug treatment and trial number in each of these studies, suggesting (+)-HA-966 altered locomotor activity the (+)-HA-966 dose groups developed ethanol-induced locomotor sensitization. Moreover, 15 mg/kg and 30 mg/kg (+)-HA-966 altered the development of sensitization. However, the administration of (+)-HA-966 altered the initial locomotor response to 2 g/kg ethanol. Therefore, the alterations in sensitization may again be due to the drug decreasing the stimulant effect of ethanol that may be required for the development of sensitization in these groups.

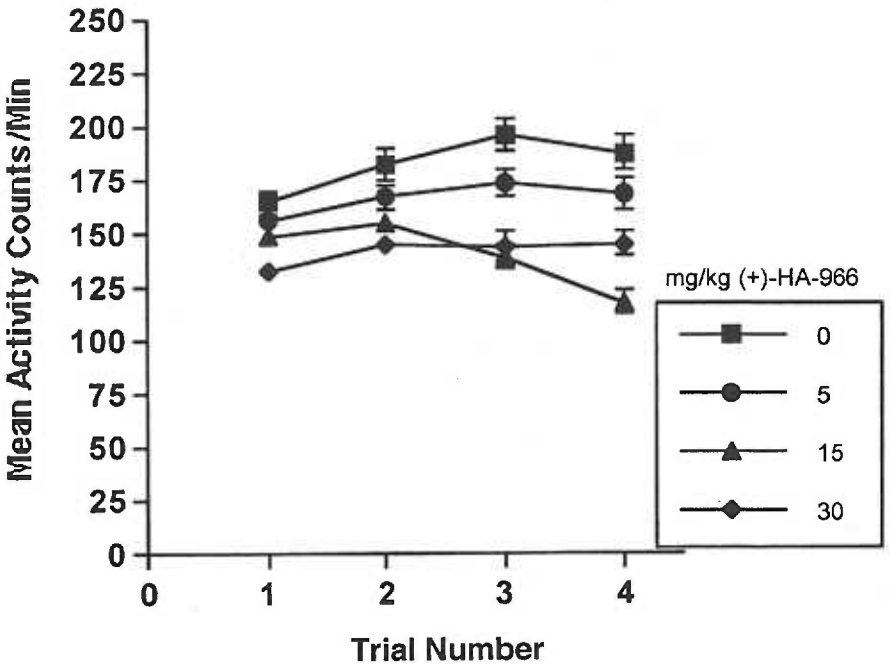
Insert Figure 13 about here

To evaluate the interaction, planned between group comparisons were conducted to compare each (+)-HA-966 group (5, 15, 30 mg/kg) with controls (0 mg/kg) on their development of ethanol-induced locomotor sensitization. These analyses indicated that the 15 and 30 mg/kg (+)-HA-966 dose groups differed from the saline controls (0 mg/kg) across trials 1-4 [$F(3, 132) = 25.8, p < .001$, $F(3, 135) = 2.6, p = .05$]. However, one-way ANOVAs of activity within each (+)-HA-966 dose group indicated that the locomotor activity changed as a function of trials in all dose groups [Fs > 2.8, ps $< .04$], suggesting that all groups developed locomotor sensitization.

NR2B antagonists (Experiments 3 and 4)

Figure 14 shows the mean (± *SEM*) activity counts per minute for CS+ trials 1-4 for each drug treatment group. In all studies, a 2 g/kg ethanol dose (black squares) produced locomotor stimulation and locomotor sensitization across trials. There were main effects of drug treatment and trial number in each of these studies, suggesting that

Figure 13. Mean (± *SEM*) activity counts/min following ethanol (CS+) for drug treatment groups across the 4 conditioning trials. (A) On CS+ trials, mice received 0, 5, 15, or 30 mg/kg (+)-HA-966 30 min before a 2 g/kg ethanol injection.


Mean Activity Counts/Min
250
225
200
175
150
125
100
75
50
25
0
0
1
2
3
4
Trial Number
mg/kg (+)-HA-966
0
5
15
30

ifenprodil and CP-101,606 (both NMDA receptor NR2B subunit antagonists) altered locomotor activity, but did not alter the development of ethanol-induced locomotor sensitization.

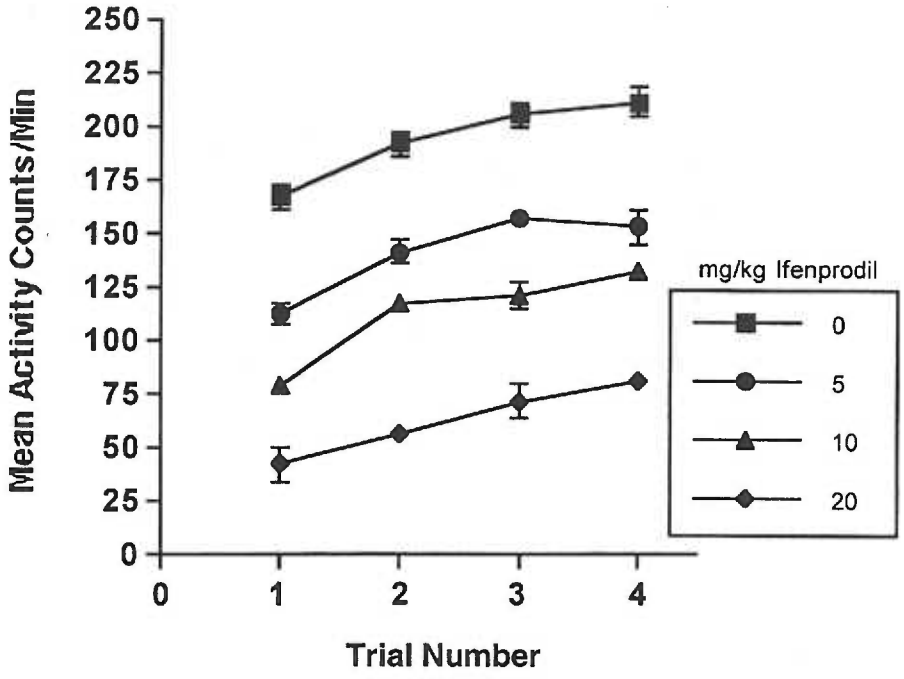
Insert Figure 14 about here

Discussion

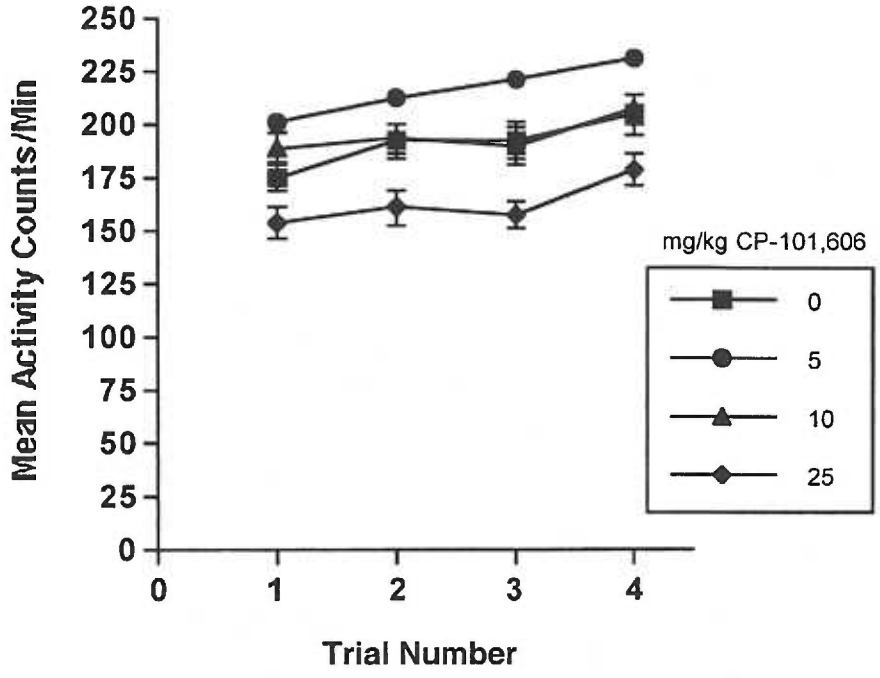
These results suggest that the NMDA receptor glutamate, ion channel, and glycineB binding sites may be important for the development of ethanol-induced locomotor sensitization. Moreover, these results suggest that the NMDA receptor NR2B binding site does not play a role in ethanol sensitization even though NR2B binding site antagonists attenuate the initial stimulant response of ethanol. However, these results need to be interpreted with caution because all drugs decreased the initial stimulant response to ethanol. As mentioned previously, this initial response to ethanol may in fact be important for the development of locomotor sensitization. Furthermore, additional studies need to be conducted to investigate the role of these antagonists in an ethanol sensitization paradigm with an ethanol challenge (no antagonist on board) to get a better measurement of the effects of these drugs on the acquisition of sensitization. Moreover, utilization of doses that do not attenuate the initial stimulant response to ethanol will help to elucidate the involvement of NMDA receptor binding sites in ethanol-induced locomotor sensitization.

Figure 14. Mean (± *SEM*) activity counts/min following ethanol (CS+) for drug treatment groups across the 4 conditioning trials. (A) On CS+ trials, mice received 0, 5, 10, or 20mg/kg ifenprodil 30 min before a 2 g/kg ethanol injection. (B) On CS+ trials, mice received 0, 5, 10, or 25 mg/kg CP-101,606 30 min before a 2 g/kg ethanol injection..

A


Mean Activity Counts/Min
250
225
200
175
150
125
100
75
50
25
0
0
1
2
3
4
Trial Number
mg/kg Ifenprodil
0
5
10
20

B


Mean Activity Counts/Min
250
225
200
175
150
125
100
75
50
25
0
0
1
2
3
4
Trial Number
mg/kg CP-101,606
0
5
10
25

These results also suggest that there is dissociation between ethanol-induced CPP and locomotor sensitization. For example, 0.05 mg/kg MK-801 enhanced locomotor sensitization to 2 g/kg ethanol without affecting the initial stimulant response, but it did not alter ethanol-induced CPP. Moreover, (+)-HA-966, a partial agonist for the NMDA receptor $glycine_B$ binding site appeared to decrease ethanol-induced locomotor sensitization, but did not affect ethanol-induced CPP. However, the NMDA receptor NR2B binding site antagonists altered the acute stimulant response to ethanol, but CP-101,606 and ifenprodil did not affect the magnitude of ethanol-induced locomotor sensitization. The place preference data showed that both drugs also did not affect the acquisition of ethanol-induced CPP. These results would argue that the magnitude of locomotor sensitization is related to the magnitude of ethanol-induced CPP. On the other hand, only the highest dose (15 mg/kg) of CGP-37849 decreased locomotor sensitization in Experiment 1A. In this study, however, 10 mg/kg CGP-37849 decreased ethanol-induced CPP, but not locomotor sensitization. While the current studies show that there is generally dissociation between ethanol-induced locomotor sensitization and ethanol-induced CPP, there is some overlap in the receptor subtypes that mediate these behaviors. These NMDA receptor drugs need to be tested in a locomotor sensitization paradigm with doses that do not alter the acute ethanol locomotor response and with an ethanol only test in order to make conclusions regarding the association or dissociation between ethanol reward and locomotor sensitization.