$GABA_A$ BUT NOT $GABA_B$ RECEPTORS MODULATE ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE AND TASTE AVERSION IN MICE

by

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A DISSERTATION

Presented to the Department of Behavioral Neuroscience
and the Oregon Health Sciences University
School of Medicine
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
August 1998

School of Medicine
Oregon Health Sciences University

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Acknowledgements

The last five years at OHSU have been quite an experience. At first, I thought the rain and Organic Chemistry would never end...but that was just the beginning of the journey! Now that I can finally say that I "love" Oregon (can you believe that coming from a spoiled Californian?), it is time to move on.

Many, many thanks go to my mentor, Chris Cunningham for EVERYTHING! You are truly an excellent advisor and I will always strive to emulate your "properties"; you wise person you. Kari Buck, John Crabbe, Ed McCleskey, and Tamara Phillips were a great advisory committee and made the whole process very enjoyable. You all deserve an award for "best supporting advisor".

It has been a pleasure working with both past and present CLC (CLC-he's dynamite!) lab members. Thanks for all your understanding and help in so many ways. The Mark lab has been very supportive, especially Alan Keys, who of course I would and could never forget! Thank you Deb Finn for all your encouragement and GABA expertise.

Ginger Ashworth (the eye of the storm). Thank you for reminding me not to sweat the small stuff, because it really is small stuff in the grand scheme of things! You are a wonderful friend.

Val Watts, I don't know if I could have done this without you as a role model. Someday I hope to have my own web page too! Gwen Schafer, Erika Simeon, Anita Bechtholt, Heather Hain, Wendy Morrison, Leilani Navarro, Ann Ward, and Sarah Coste- I am very grateful for all the support and friendship you have given me. Finally, I would like to acknowledge the support (there's that word again!) of my family. Thank you for being there on my big day and always.

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Abbreviations

AA - alcohol-accepting

AMG - amygdala

AOAA - aminooxyacetic acid

B-CCE - B-carboline-3-carboxylate

BZ - $GABA_A$/benzodiazepine

cAMP - cyclic adenosine monophosphate

Ca AOTA - calcium-acetyl-homotaurine

CNS - central nervous system

CPA - conditioned place aversion

CPP - conditioned place preference

CS - conditioned stimulus

CTA - conditioned taste aversion

DA - dopamine

DRN - dorsal raphe nucleus

EOS - ethanolamine-O-sulphate

Ex AMG - Extended Amygdala (central amygdaloid nucleus, bed nucleus of the stria terminalis, nucleus accumbens shell)

GABA - gamma-aminobutyric acid

HAD - high alcohol drinking

IPPO - isopropyl-bicyclophosphate

LAD - low alcohol drinking

LORR - loss of righting reflex

PTZ - pentylenetetrazole

P - alcohol-preferring

NACC - nucleus accumbens

NP - alcohol non-preferring

THIP - 4,5,6,7-tetrahydroisoxazolo(5,4-C)pyridine-3-ol

US - unconditioned stimulus

VP - ventral pallidum

VTA - ventral tegmental area

Abstract

Ethanol has been shown to exert many of its biochemical and behavioral effects through an interaction with the gamma-aminobutyric acid (GABA) receptor system. Relatively few studies, however, have examined the role of the GABA receptor system in modulating ethanol's motivational effects. To date, most of the evidence implicating the GABA system in modulating ethanol's motivational properties comes from studies examining oral ethanol self-administration behavior in rats. The purpose of the following studies was to investigate a role for the GABA receptor system in modulating the acquisition of ethanol-induced conditioned place preference (CPP) and conditioned taste aversion (CTA) in DBA/2J mice. These experiments were designed to test the hypothesis that ethanol's rewarding and aversive effects are modulated by activation of both $GABA_A$ and $GABA_B$ receptor subtypes.

The first set of experiments examined the effects of the $GABA_A$ receptor antagonists, bicuculline and picrotoxin, on the acquisition of ethanol-induced CPP and CTA. It was predicted that blockade of $GABA_A$ receptors with picrotoxin and bicuculline would attenuate ethanol-induced CPP and CTA. Opposite to the predicted outcome, both bicuculline (1.0 mg/kg) and picrotoxin (2.0 mg/kg) significantly increased the magnitude of ethanol-induced CPP relative to vehicle-treated controls. A control group showed that picrotoxin alone (2.0 mg/kg) did not produce place conditioning. Ethanol-stimulated locomotor activity was significantly reduced during CPP conditioning trials with picrotoxin (2.0 mg/kg) and higher doses of bicuculline (3.0 and 5.0 mg/kg). Picrotoxin also significantly reduced locomotor activity following saline in the picrotoxin control group. The CTA experiments showed that bicuculline (1.0 and 4.0 mg/kg) did not alter ethanol-induced CTA at either dose; however, picrotoxin (0.75 and 2.5 mg/kg) dose-dependently increased the magnitude of ethanol-induced CTA. The lowest dose of bicuculline and

picrotoxin did not produce a CTA when administered alone. A separate control experiment showed that the effect of picrotoxin is not due to a change in ethanol pharmacokinetics.

Taken together, the results with picrotoxin and bicuculline provide mixed evidence for the hypothesis that ethanol-induced CPP and CTA reflect activation of the same neural substrate (Hunt & Amit, 1987). The picrotoxin studies suggest that the $GABA_A$ receptor modulates ethanol's rewarding and aversive effects, because picrotoxin enhanced both CPP and CTA. Whereas the fact that a low dose of bicuculline enhanced ethanol-induced CPP but not CTA suggests that separate neural mechanisms may mediate ethanol's rewarding and aversive effects in these paradigms. However, this suggestion should be interpreted with caution due to several reasons that may account for the dissociation between bicuculline's effects on ethanol-induced CPP and CTA.

The second set of experiments examined the effects of the $GABA_B$ receptor agonist, baclofen, on the acquisition of ethanol-induced CPP and CTA. It was predicted that baclofen would dose-dependently alter the magnitude of ethanol-induced CPP and CTA. The results of the CPP study showed that baclofen (2.5, 5.0, and 7.5 mg/kg) dose-dependently reduced ethanol-stimulated activity, but did not alter the magnitude of ethanol-induced CPP at any dose. The CTA experiment showed that baclofen (2.0 and 6.0 mg/kg) also did not alter the magnitude of ethanol-induced CTA at either dose. The lowest dose of baclofen did not produce a CTA when administered alone.

In summary, the results of the $GABA_A$ studies indicate that blockade of $GABA_A$ receptors with bicuculline and picrotoxin increases ethanol's rewarding effects in the CPP paradigm; however, only picrotoxin increases ethanol's aversive effects in the CTA paradigm. The $GABA_B$ studies show that activation of $GABA_B$ receptors with baclofen does not alter ethanol's rewarding or aversive effects in the CPP and CTA paradigms. In addition, the results of the CPP studies show a clear dissociation between ethanol's locomotor-stimulant and rewarding effects, because changes in ethanol-stimulated activity

with administration of GABA drugs were unrelated to their effects on ethanol-induced CPP. Overall, the results of these studies suggest that $GABA_A$, but not $GABA_B$ receptors modulate ethanol's motivational effects in the CPP and CTA paradigms in DBA/2J mice.

Introduction

Ethanol is one of the most commonly abused drugs in the United States. However, the physiological mechanisms mediating the acquisition of ethanol-seeking and addictive behavior remain unclear. Attempts to elucidate the neurochemical substrates involved in ethanol's motivational effects have focused on several neurotransmitter systems, including dopamine, serotonin, opioid, glutamate, and gamma-aminobutyric acid (GABA) (for reviews see Harris, Brodie, & Dunwiddie, 1992; Tabakoff & Hoffman, 1996; Koob, 1998). Biochemical, electrophysiological and behavioral studies have shown that ethanol exerts many of its pharmacological and behavioral effects through an interaction with the GABA receptor system (see Ticku, 1990; Korpi, 1994; Mihic & Harris, 1996 for reviews). Despite the many studies implicating the GABA receptor system in mediating ethanol's effects, relatively few studies have examined the role of GABA receptor subtypes in the motivational effects of ethanol. This thesis focused on examining the role of the GABA neurotransmitter receptor system in modulating the rewarding and aversive effects of ethanol in the place and taste conditioning paradigms.

The GABA Receptor System

GABA is the primary inhibitory neurotransmitter in the brain. GABA exerts its inhibitory actions primarily via two distinct receptor subtypes, $GABA_A$ and $GABA_B$, which are responsible for fast and slow synaptic transmission, respectively. GABA receptors are important modulators of diverse physiological and behavioral functions, such as thermoregulation, ingestive behaviors, analgesia, anxiety, and learning and memory processes (see review by Paredes & Ågmo, 1992). Historically, $GABA_A$ and $GABA_B$ receptors were classified based on sensitivity to the pharmacological agents, bicuculline and baclofen, respectively. Bicuculline is the classic $GABA_A$ antagonist that selectively and competitively inhibits the binding of GABA (Curtis, Duggan, Felix, & Johnson, 1970).

$GABA_B$ receptors were defined based on their selective activation by baclofen and insensitivity to bicuculline (Bowery et al., 1980).

$GABA_A$ receptors. The $GABA_A$ receptor is a macromolecular complex consisting of at least five major binding sites for compounds that allosterically modulate ion flux through a chloride channel (for review see Upton & Blackburn, 1997). GABA mediates fast synaptic transmission in the brain by binding to a site on the $GABA_A$ receptor complex, which activates the receptor and causes opening of the chloride channel. The entry of chloride ions produces inhibition of neural activity either through hyperpolarization or reduction in cell membrane resistance (Feltz et al., 1987; Bormann, 1988). In addition to GABA, several other compounds bind to the $GABA_A$ receptor complex, including benzodiazepines, barbiturates, steroids, and picrotoxin. The $GABA_A$ complex is thought to be a pentameric structure comprised of a combination of receptor subunits that form the chloride channel. At least 17 different subunits have been identified and grouped into five distinct classes (6α, 4β, 4γ, 1δ, and 2ρ subunits) based on sequence homology (Möhler et al., 1997). These subunits are assembled in different combinations in the brain, giving rise to a heterogeneous population of $GABA_A$ receptors. In general, a combination of α–, β–, and γ2-subunits are necessary to form fully functional $GABA_A$ receptors. In addition, the affinity and relative efficacy of $GABA_A$ antagonists and agonists depends on the subunit composition of the receptor (Möhler et al., 1997). For example, the presence of a γ subunit allows benzodiazepines to allosterically modulate $GABA_A$ receptors (Pritchett et al., 1989).

$GABA_A$ receptors are located both pre- and postsynaptically throughout the rat central nervous system (Bowery, Hudson, & Price, 1987). The highest concentrations of $GABA_A$ receptors have been found in the frontal cortex, granule cell layer in the cerebellum, olfactory bulb, and thalamic medial geniculate. Although over 10,000 pentameric subunit combinations are possible, it appears that fewer than ten major subtypes of $GABA_A$ receptors exist in the adult mammalian brain (McKernan & Whiting, 1996).

The most abundant $GABA_A$ receptor subtype *in vivo* is the $\alpha1\beta2\gamma2$ subunit combination, which constitutes at least 60% of the $GABA_A$ receptor population (Fritschy & Möhler, 1995). A high concentration of this subtype is present in GABAergic neurons in the cerebellum, basal forebrain, pallidum, substantia nigra, brainstem reticular formation, and interneurons in the hippocampus and cerebral cortex (Fritschy et al., 1992; Gao & Fritschy, 1994).

$GABA_B$ receptors. The $GABA_B$ receptor is a G-protein coupled receptor that is functionally and pharmacologically distinct from the $GABA_A$ receptor. $GABA_B$ receptors are coupled to intracellular effector systems responsible for slow inhibitory synaptic transmission. Activation of $GABA_B$ receptors produces cell hyperpolarization through inactivation of voltage-dependent calcium channels and increases in potassium conductance (see reviews by Mott & Lewis, 1994; Misgeld, Bijak, & Jarolimek, 1995). $GABA_B$ receptor activation also inhibits adenylate cyclase activity, stimulates phospholipase A_2, and modulates inositol phospholipid hydrolysis. $GABA_B$ receptors play a modulatory role in neuronal activity and have been characterized in terms of presynaptic and postsynaptic functions. Presynaptically, $GABA_B$ autoreceptors control the release of GABA, and $GABA_B$ heteroreceptors modulate the release of other neurotransmitters such as dopamine, glutamate, noradrenaline, and serotonin (see review by Waldmeier & Baumann, 1990).

Several lines of evidence indicate heterogeneity of $GABA_B$ receptors. Recently, two highly conserved $GABA_B$ receptor forms from vertebrate nervous systems have been cloned (Kaupmann et al., 1997). Pharmacological studies have indicated the presence of both low and high affinity $GABA_B$ binding sites that have different regional distributions in the brain (e.g., Karbon, Duman, Enna, 1983; Wojcik, Travagli, Costa, & Bertolino, 1990; Bonanno & Raiteri, 1993). Furthermore, there appear to be two main groups of $GABA_B$ receptors, one sensitive and one insensitive to baclofen (Ratieri et al., 1992).

A considerably smaller number of $GABA_B$ receptors than $GABA_A$ receptors exist in the brain. Nevertheless, $GABA_B$ receptors have a widespread distribution and show an expression pattern distinct from $GABA_A$ receptors, although in many brain regions both subtypes appear to co-exist (Bowery et al., 1987). For example, the highest concentrations of $GABA_B$ receptors have been found in the molecular layer of the cerebellum, interpeduncular nucleus, frontal cortex, anterior olfactory nucleus, and thalamic nuclei. Significantly more $GABA_B$ receptors than $GABA_A$ receptors have been found in the globus pallidus, lateral amygdaloid nucleus, temporal cortex, lateral posterior thalamus, superior colliculus, pontine nucleus, raphe magnus, spinal trigeminal tract, and substantia gelatinosa (Bowery et al., 1987).

GABA and the Brain Reward System

One of the areas in the brain thought to play a primary role in the rewarding effects of abused drugs is the mesocorticolimbic dopamine pathway. Cell bodies of this pathway originate in the ventral tegmental area (VTA), or A10 region of the midbrain dopamine system, and project to forebrain regions, including the nucleus accumbens, frontal cortex, amygdala, septal area, and olfactory tubercle. In addition, the nucleus accumbens has a large descending efferent projection to the ventral pallidum (VP). The VP is reciprocally connected to the nucleus accumbens, and also sends efferent projections to the VTA and prefrontal cortex (Lamour, Dutar, & Jobert, 1984). Although dopamine is the primary neurotransmitter in the mesocorticolimbic pathway, GABA also plays an important functional role because it is responsible for mediating dopamine functions (Scheel-Krüger, 1986). It has been estimated that approximately 70% of the afferents impinging on dopamine neurons are GABAergic (Ribak, Vaughn, Saito, Barber, & Roberts, 1976; Smith & Bolam, 1989). Conversely, dopamine has also been shown to modulate the activity of GABAergic neurons (Marco, Mao, Revuelta, Peralta, & Costa, 1978; Kubota,

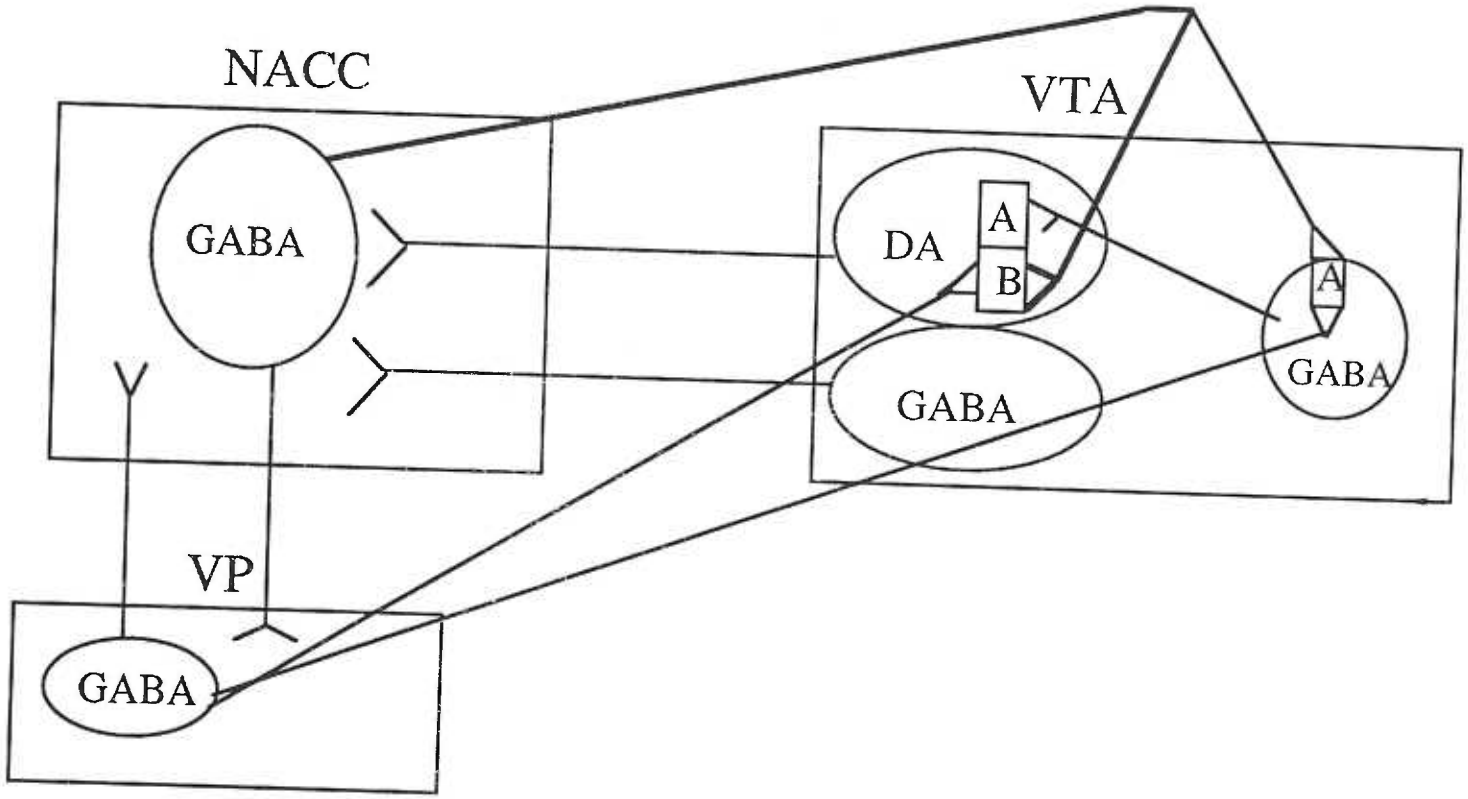
Inagaki, Kito, & Wu, 1987; Reid, O'Connor, Herrera-Marschitz, & Ungerstedt, 1990). Several lines of evidence, including neuroanatomical, electrophysiological, and behavioral, have demonstrated GABA-dopamine interactions in the brain. A comprehensive review of this literature is beyond the scope of this thesis: the topic has been extensively reviewed elsewhere (Scheel-Krüger, 1986). The following section will primarily focus on investigations of GABA-dopamine interactions in the nucleus accumbens, VTA, and VP, areas of the brain that have been implicated as important modulators of the rewarding effects of ethanol and other drugs of abuse (Koob et al., 1991; Amalric & Koob, 1993).

GABA and Dopamine

Neurocircuitry. An extensive reciprocal relationship exists between dopamine and GABA throughout the mesocorticolimbic dopamine pathway. The nucleus accumbens has a dense GABAergic innervation (Fonnum, Walaas, & Iverson, 1977). Furthermore, neurons projecting from the nucleus accumbens principally contain GABA (Kalivas, Churchill, & Klitenick, 1993; Walaas & Fonnum, 1980), and these fibers provide inhibitory synaptic inputs to dopamine neurons in the VTA (Hattori, McGeer, Fibiger, & McGeer, 1973; Waddington & Cross, 1978; Waalas & Fonnnum, 1980; Ottersen & Storm-Mathisen, 1984). The presence of GABA containing neurons in the VTA suggests that the VTA may in turn provide inhibitory GABA inputs to the nucleus accumbens (e.g. O'Brien & White, 1987; Churchill & Kalivas, 1994; Van Bockstaele & Pickel, 1995). In addition, many of these GABA containing neurons within the VTA are thought to be inhibitory interneurons (Johnson & North, 1992). The VP contains GABA neurons that project to the nucleus accumbens and VTA , indicating a reciprocal inhibitory circuitry between these regions (Churchill & Kalivas, 1994). Figure 1 shows a schematic representation of this pathway.

GABA receptor subtypes. Both $GABA_A$ and $GABA_B$ receptor subtypes are present throughout the mesolimbic pathway (Bowery et al., 1987; Churchill, Dilts, &

Figure 1. Schematic representation highlighting GABA pathways that innervate the VTA, nucleus accumbens (NACC), and VP within the mesocorticolimbic dopamine (DA) system. These structures are thought to play a primary role in the locomotor-activating and rewarding effects of abused drugs. GABA neurons project from the NACC and VP and primarily innervate $GABA_B$ receptors on dopamine neurons, and $GABA_A$ receptors on GABA interneurons in the VTA. Reciprocal GABA fibers connect the NACC and VP. Within the VTA, it is thought that GABA interneurons primarily stimulate $GABA_A$ receptors on dopamine neurons. The VTA in turn provides inhibitory GABA inputs to the NACC (adapted from Churchill, Dilts et al., 1992; Johnson & North, 1992; Klitenick, DeWitte, & Kalivas, 1992).


NACC
GABA
VTA
DA
A
B
A
GABA
GABA
VP
GABA

Kalivas, 1992). Electrophysiological studies have shown that both receptor subtypes are located on dopamine neurons (Lacey, Mercuri, & North, 1988; Johnson & North, 1992). In addition, it has been suggested that descending GABA neurons from the nucleus accumbens and/or VP stimulate postsynaptic $GABA_B$ receptors on VTA dopamine neurons, whereas intrinsic GABA interneurons stimulate postsynaptic $GABA_A$ receptors on dopamine neurons in the VTA (Johnson & North, 1992; Sugita, Johnson & North, 1992). There are also $GABA_A$ receptors on GABA interneurons that are innervated by descending GABAergic projections from the nucleus accumbens or VP (Grace & Bunney, 1985; Johnson & North, 1992; Churchill, Dilts, et al., 1992). However, it has been suggested that few GABAergic terminals actually contact GABA neurons in the VTA (Pickel, Towle, Joh, & Chan, 1988).

Neurochemical interactions. Both central and peripheral administration of GABA receptor ligands have been shown to alter dopamine neurotransmission. For example, dopamine turnover in the mesolimbic and nigrostriatal system is reduced with systemic administration of muscimol (Lloyd, Worms, Zivkovic, Scatton & Bartholini, 1980) and baclofen (Waldmeier & Maitre, 1978). This is consistent with the finding that GABA agonists reduce both the synthesis and release of striatal and accumbens dopamine (Scatton et al., 1982). However, an increase in the firing rate of dopamine neurons in the A9 region (substantia nigra) has been found with peripheral administration of a $GABA_A$ agonist. This is possibly due to $GABA_A$ receptor activation on GABA interneurons, which results in disinhibition of dopamine neurons (Grace & Bunney, 1979).

Some of the evidence for an interaction between GABA and dopamine systems comes from studies showing that direct microinjection of GABA agonists and antagonists alters dopamine neurotransmission (Santiago & Westerink, 1992; Santiago, Machado, & Cano, 1993). For example, administration of muscimol into the VTA has been shown to produce an increase in extracellular dopamine levels (Klitenick, DeWitte, & Kalivas,

1992). Conversely, dopamine receptor antagonists have been shown to increase the firing rate and GABA turnover in a population of GABA neurons in the VP, indicating the inhibitory influence of dopamine on GABA efferents from the nucleus accumbens (Marco et al., 1978). Furthermore, some evidence suggests that $GABA_A$ activation can indirectly excite GABA neurons. $GABA_A$ agonists were found to decrease, whereas antagonists increased GABA release from substantia nigra, pars compacta tissue slices (Kondo & Iwatsubo, 1978), suggesting that $GABA_A$ agonists may increase dopamine release by presynaptically inhibiting GABA release.

$GABA_B$ receptor activation with baclofen has been shown to decrease impulse generation in dopamine cells in the substantia nigra and VTA (Olpe, Koella, Wolf, & Haas, 1977; Lacey et al., 1988). Direct administration of baclofen into the VTA also decreases extracellular dopamine levels in the VTA (Klitenick et al., 1992). These findings are consistent with another study that showed intravenous administration of baclofen decreased burst firing and increased the regularity of the firing pattern of dopamine neurons in the substantia nigra, and conversely, muscimol, increased burst firing and decreased firing regularity (Engberg, Kling-Peterson, & Nissbrandt 1993). These data suggest that baclofen's inhibitory effect on dopamine cell firing and dopamine release are through activation of $GABA_B$ receptors located on presynaptic dopamine neurons, whereas muscimol may disinhibit dopamine neurons through activation of presynaptic $GABA_A$ receptors on GABA interneurons.

Locomotor activity. The neural pathways thought to be the most important mediators of locomotor activity, including drug-stimulated activity, are those between the VTA, nucleus accumbens, and VP (see Amalric & Koob, 1993; Koob et al., 1991; Mogenson, Jones, & Yim, 1980). Consistent with many of the neurochemical findings, several studies have shown that manipulation of GABA and dopamine systems alters locomotor behavior (see Phillips & Shen, 1996 for review). For example, microinjection

of picrotoxin into the VTA stimulates locomotion (Mogenson, Wu, & Manchanda, 1979), possibly through increased firing of dopamine neurons (Yim & Mogenson, 1980). However, Tanner (1979) has also shown that microinjection of GABA agonists into the VTA increases locomotor activity and $GABA_B$ agonists inhibit locomotor activity. In another study, Kalivas, Duffy, and Eberhardt (1990) examined lower doses of $GABA_A$ and $GABA_B$ agonists and antagonists administered into the VTA, and found that muscimol produced an increase in locomotor activity that was blocked by peripheral administration of a dopamine receptor antagonist, indicating the increase produced by muscimol is dopamine-mediated. In addition, the activation produced by muscimol was blocked with administration of baclofen into the VTA, suggesting that the $GABA_B$ mediated inhibition was due to $GABA_B$ receptors on dopamine cells. Baclofen has also been shown to reduce the motor stimulant effect of several drugs known to act through dopamine increase, such as peripheral injection of cocaine or amphetamine (Kuzcenski, 1983).

Picrotoxin administration in the nucleus accumbens also produces a dose-dependent increase in locomotor activity (Jones & Mogenson, 1980; Morgenstern, Mende, Gold, Lemme, & Oelssner, 1984; Plaznik, Stefanski, & Kostowski, 1990). Conversely, direct administration of GABA receptor agonists, muscimol and baclofen (Plaznik et al., 1990), and GABA itself (e.g., Mogenson & Nielson, 1983; Plaznik et al., 1990), into the nucleus accumbens produces a decrease in locomotor activity. Direct administration of muscimol into the VTA, however, stimulates locomotor activity, whereas baclofen reduces locomotor activity (Klitenick et al., 1992). These effects on locomotor activity are consistent with the fact that muscimol and baclofen increase and decrease dopamine release in the VTA, respectively (Klitenick et al., 1992). Based on these findings, it appears that drugs that modulate GABA receptors may be acting at both pre- and/or postsynaptic $GABA_A$ and $GABA_B$ receptors on dopamine cells or GABA interneurons. For example, $GABA_A$ agonists may stimulate dopamine neurons by presynaptically inhibiting GABA release from

interneurons, resulting in disinhibition of dopamine neurons. $GABA_A$ antagonists may stimulate dopamine neurons by blocking postsynaptic GABA receptors on dopamine neurons, thereby removing the inhibitory influence of a GABA afferent or interneuron (see Figure 1).

There is also evidence to suggest that dopamine-dependent locomotion produced by dopamine receptor stimulation in the nucleus accumbens is mediated by tonic GABAergic inhibition from the VP (Austin & Kalivas, 1988; Jones & Mogenson, 1980; Swerdlow & Koob, 1984). For example, injection of picrotoxin into the VP produces locomotor activation (Austin & Kalivas, 1990; Mogenson & Nielson, 1983), which has been shown to be reduced with intra-accumbens administration of a dopamine antagonist (Austin & Kalivas, 1991). Conversely, injection of muscimol or GABA into the VP inhibits the locomotor response produced by intra-accumbens dopamine injection (Jones & Mogenson, 1980; Austin & Kalivas, 1988).

Behavioral reward studies. Several studies have shown a close interaction between GABA and dopamine systems in modulating the motivational properties of drugs. For example, 6-hydroxydopamine lesions in the nucleus accumbens block conditioned place preference (CPP) induced by diazepam, a $GABA_A$/benzodiazepine (BZ) agonist (Spyraki and Fibiger; 1988). Interestingly, another study showed that the D_1 dopamine receptor antagonist, SCH 23390, blocks conditioned place aversion (CPA) produced by picrotoxin (Acquas, Carboni, Leone, & Di Chiara, 1989). In addition, CPA produced by the BZ inverse agonist, FG 7142, is attenuated by the dopamine receptor antagonist, haloperidol (Di Scala & Sandner, 1989). These studies indicate that the rewarding and aversive effects of GABA receptor ligands may be due to activation of dopamine containing neurons in the nucleus accumbens.

Some studies have demonstrated that animals will self-infuse $GABA_A$ antagonists directly into the VTA. For example, rats will self-infuse picrotoxin and bicuculline into the

anterior VTA, but not the posterior VTA, substantia nigra, or lateral hypothalamus (Ikemoto, Murphy & McBride, 1997). It has been suggested that the rewarding effect of $GABA_A$ antagonist administration into the VTA is due to the fact that intra-VTA administration of these antagonists increases dopamine levels in the nucleus accumbens (Ikemoto, Kohl & McBride, 1997). Another study showed that mice will robustly self-administer bicuculline into the VTA, which is blocked with administration of the D_2 antagonist, sulpiride (David, Durkin, and Cazala, 1997). Thus, these studies suggest that $GABA_A$ receptor blockade in the VTA is rewarding due to an increase in dopamine release in the nucleus accumbens.

As previously discussed, the VP area has been implicated as an important modulator of locomotor activation produced by drugs of abuse (Austin & Kalivas, 1991). In addition, some studies suggest that the VP pathway may be involved in the processing of the rewarding actions of drugs such as cocaine and heroin. For example, lesions of the VP have been shown to attenuate cocaine self-administration (Hubner & Koob, 1990) and acquisition of cocaine (Gong, Neill, & Justice, 1997) and amphetamine-induced CPP (Hiroi & White, 1993). These findings have led to the hypothesis that the VP may be important for modulating the locomotor-activating as well as rewarding properties of drugs.

Several lines of evidence suggest that the rewarding and locomotor-activating effects of drugs may be mediated through reduced GABAergic activity in the VP (Koob & Swerdlow, 1988; Pulvirenti, Swerdlow, Hubner, & Koob, 1991). First, the projection from the nucleus accumbens to the VP is primarily GABAergic (Zahm, Zaborszky, Alones, & Heimer, 1985; Alheid & Heimer, 1988), and picrotoxin administration into the VP has been shown to stimulate locomotion (Mogenson & Nielson, 1983), whereas muscimol inhibits it (Mogenson & Nielson, 1983; Austin & Kalivas, 1988). In addition, a decrease in extracellular GABA in the VP is found with systemic administration of amphetamine and the dopamine agonist, apomorphine (Bourdelais & Kalivas, 1990; 1992).

Thus, drugs of abuse that are known to stimulate dopamine transmission may produce their rewarding and locomotor-activating effects through an inhibition of GABAergic neurons in the VP. This idea was recently tested using the place conditioning paradigm in a study that examined the effect of direct administration of picrotoxin into the VP (Gong, Justice, & Neill, 1997). In this study, picrotoxin stimulated locomotor activity, but did not produce a CPP for the environment paired with picrotoxin administration. Thus, these results indicate a dissociation between locomotor stimulation and reward in this part of the "reward pathway", and suggest that GABA/dopamine interactions in the VP may not be involved in modulating the motivational effects of drugs.

In summary, there is an extensive amount of evidence showing a reciprocal relationship between GABA and dopamine systems within brain reward pathways. Anatomical, neurochemical, and behavioral studies have demonstrated that GABA manipulations within this pathway produce changes in dopamine transmission, which is supported by behavioral studies measuring locomotor activity. Furthermore, evidence suggests that motivational effects of GABA receptor ligands may be due to changes in dopamine activity. Thus, these findings may have direct implications with regard to GABA and dopamine interactions and the motivational effects of abused drugs.

GABA Receptors and Food Reward

There is extensive literature demonstrating that the GABA receptor system plays an important role in modulating feeding and drinking behavior. Administration of BZ receptor compounds has been most frequently reported to produce changes in food consumption (for reviews see Cooper, 1985; 1986; Cooper & Estall, 1985). Although relatively fewer in number, several studies have shown that activation of the $GABA_B$ receptor subtype alters food and water consumption. The literature demonstrating a role for the GABA receptor system in feeding and drinking behavior is extensive and beyond the scope of this thesis; however, some of these studies will be briefly described below.

$GABA_A$ receptors. Stimulation of $GABA_A$ receptors with BZ agonists has been consistently shown to increase food and water consumption in rodents (e.g., Randal & Kappell, 1961; Niki, 1965; Riley & Lovely, 1978; Cole, 1983). It has been suggested that the increase in food consumption with BZ agonists is due to alterations in taste-related mechanisms. Evidence for this comes from many studies showing that BZ agonists increase the consumption of palatable foods (e.g., Cooper, 1980; Yerbury & Cooper, 1989). For example, using a taste reactivity test, chlordiazepoxide has been shown to increase ingestive responses produced by intra-oral infusions of sweet, sour, and bitter tasting solutions (Berridge & Treit, 1986), and this effect is antagonized with the BZ receptor antagonists, Ro 15-1788 and CGS 8216 (Treit, Berridge, & Schultz, 1987). However, aversive responses (e.g., chin rubbing or mouth gapes) to these tastes were unaltered by chlordiazepoxide, suggesting that this BZ agonist increases positive hedonic reactions to taste stimuli. Based on these data, it has been suggested that the increase in food consumption with BZ agonists is at least partially due to an enhancement of the positive palatability of tastes. This is consistent with a study that showed chlordiazepoxide selectively enhanced consumption of preferred foods over non-preferred foods in a food-preference test (Cooper & McClelland, 1980). Conversely, BZ inverse agonists have been shown to reduce the consumption of palatable foods (e.g., Cooper, 1986; Kirkham & Cooper, 1987). Furthermore, changes in consumption of palatable foods with BZ compounds can be blocked with administration of the selective BZ antagonist, Ro 15-1788 (Cooper, Barber, Gilbert, & Moores, 1985; Treit et al., 1987). However, another study examined the ability of a BZ agonist and antagonist, CGS 9896 and CGS 8216, respectively, to modulate the consumption of three diets differing in palatability in combination with three different periods of food deprivation. This study showed that increasing the palatability of food or deprivation state of the animal did not alter the increase and decrease in food consumption with CGS 9896 and CGS 8216, respectively (Chen,

Davies, Loew, 1995), suggesting that neither hunger or taste factors are important in modulating the effect of these BZ compounds on food intake.

Other studies have shown that BZ and other GABA receptor compounds alter aversive, as well as positive, taste properties of food. For example, some studies have shown an increase in consumption of bitter-tasting substances with BZ agonists (Hunt, Poulos, & Cappell, 1988; Cooper & Green, 1993); however, others have shown no effect (Berridge & Treit, 1986). Petry and Heyman (1997) recently showed that chlordiazepoxide increased responding for a bitter solution with a parallel decrease in responding for a sweet solution. The authors suggest this discrepancy may be due to a different type of paradigm used to measure taste palatability. This study also showed that the $GABA_A$ antagonist, picrotoxin, decreased responding for the sweet solution; however, the specificity of this effect is questionable. Interestingly, Söderpalm and Hansen (1998) demonstrated that BZ agonists (midazolam, diazepam, and chlordiazepoxide) increase the consumption of ethanol. Taste reactivity tests showed that these BZs also increase positive orofacial responses to ethanol, suggesting that the increase in ethanol consumption may be due to an increase in the palatability of ethanol.

Some studies have provided contradictory evidence that the $GABA_A$ receptor is involved in modulating taste palatability and food consumption. For example, Sanger (1984) showed evidence that indicates hyperphagia induced by chlordiazepoxide is not mediated by activation of the $GABA_A$ receptor. In this study, the selective $GABA_A$ agonist, muscimol, and antagonists, picrotoxin and bicuculline, did not alter the effect of chlordiazepoxide on food intake. In addition, food consumption was not altered by either picrotoxin or bicuculline when administered alone. However, other studies have reported that picrotoxin and bicuculline antagonize the BZ-induced consumption of food, supporting a role for $GABA_A$ receptors in this effect (Fletcher, Green, & Hodges, 1980; Birk & Noble, 1982; Naruse, Asami, & Koizumi, 1988). Intracerebroventricular injections of

GABA itself or the GABA-transaminase inhibitor, ethanolamine-O-sulphate (EOS), has been shown to reduce food consumption in both food-deprived and -sated rats (Olgiati, Netti, Guidobono, & Pecile, 1980). Interestingly, bicuculline did not block this effect; however, bicuculline alone stimulated eating in both food-deprived and -sated rats. This study also showed that muscimol stimulated feeding in sated rats, which was blocked by bicuculline. This is consistent with another study that showed muscimol injected into the hypothalamus induces feeding in sated rats (Grandison & Guidotti, 1977).

$GABA_B$ receptors. A few studies have shown a role for the $GABA_B$ receptor in modulating feeding behavior. For example, the selective $GABA_B$ agonist, baclofen, has been shown to increase feeding in non-fasted rats, and increase operant responding for food (Ebenezer & Pringle, 1992). Recently, it has been shown that the nucleus accumbens appears to play a major role in the hyperphagic effects of baclofen (Stratford & Kelley, 1997). Thus, direct administration of baclofen into the nucleus accumbens increased food intake in rats, and this effect was blocked with the $GABA_B$ antagonist, saclofen. In contrast to Ebenezer and Pringle (1992), another study has reported that systemic administration of baclofen decreases food consumption in non-fasted rats (Zarrindast, Hosseini-Nia, & Allah-Maddadi, 1989). However, the difference between these studies may be due to that fact that higher doses of baclofen (4-14 mg/kg; i.p.) were administered by Zarrindast et al., whereas Ebenezer and Pringle administered baclofen subcutaneously in the range of 1-4 mg/kg. It is possible that the higher doses of baclofen administered by Zarrindast et al. altered motor function that interfered with food consumption. Ebenezer, Houston, and Crook (1992) also examined the effect of baclofen on water intake in rats. Systemic administration of baclofen did not alter water consumption in non-deprived rats. However, baclofen inhibited water intake in 16 hr water-deprived rats. In addition, water intake elicited by i.p. injection of hypertonic saline was also inhibited with baclofen.

In summary, BZ agonists have been consistently shown to increase food

consumption, and this effect may be due to an alteration in hedonic taste mechanisms. However, some inconsistencies exist with regard to the effects of other $GABA_A$ ligands on food consumption and taste palatability. These discrepancies may be due to differences in experimental procedures, route of drug administration or drug doses. Fewer studies have examined the role of the $GABA_B$ receptor in food and water consumption. Administration of baclofen both systemically and directly into the nucleus accumbens has been shown to stimulate feeding in rats, whereas at high doses baclofen suppresses food intake. In addition, baclofen has a suppressive effect on water consumption in thirsty rats. Overall, these studies demonstrate a role for $GABA_A$ and $GABA_B$ receptor subtypes in modulating both food and water consumption.

GABA Receptors in Place and Taste Conditioning

Place and taste conditioning paradigms are Pavlovian conditioning procedures that are often used to assess the motivational effects of many drugs (Bozarth, 1987; Hunt & Amit, 1987; Cunningham, 1993; 1998). The conditioning procedures involve pairing a distinctive stimulus, termed the conditioned stimulus (CS), with administration of a drug, called the unconditioned stimulus (US). With repeated pairings, a learned association is made between the stimulus properties of the CS and physiological and/or behavioral properties of the US. Presentation of the CS in the absence of the drug elicits a conditioned response (CR). The CR of interest is approach or avoidance behavior to the drug-paired CS. The ability of the drug-paired CS to elicit approach or withdrawal behavior provides information about the drug's affective properties. In the place conditioning paradigm, a drug may produce a CPP, in which case the drug is determined to have rewarding motivational effects if an animal spends more time in the environment previously paired with it. Alternatively, if an animal spends more time in the vehicle-paired environment a CPA has developed and the drug is said to have aversive effects.

In the taste conditioning paradigm, a paradoxical situation exists, in that most drugs that normally produce a CPP also produce avoidance of a distinctive flavor previously paired with the drug, known as a conditioned taste aversion (CTA). The mechanism for this avoidance is unknown; however, a number of explanations have been proposed. For example, the CTA paradox may be accounted for by the suggestion that the same neural substrates mediate both rewarding and aversive motivational properties of abused drugs (Hunt & Amit, 1987). Alternatively, CPP and CTA may reflect activation of different neural mechanisms that mediate a drug's rewarding and aversive motivational properties, respectively. Using the taste reactivity test, Parker and colleagues have shown that the CTA produced by rewarding drugs is qualitatively different from that produced by emetic agents, such as lithium chloride. These studies have led to the hypothesis that although an avoidance of a paired flavor CS is observed, CTAs induced by drugs of abuse do not reflect the aversive properties of these drugs (Parker, 1988). This idea has also been supported by Grigson (1997), who suggests that CTA may be explained in terms of a reward comparison hypothesis. Specifically, animals decrease their consumption of a flavor previously paired with a rewarding drug because the motivational value of the taste CS (i.e., a sweet-tasting saccharin solution) is outweighed by a highly rewarding drug. However, there is evidence to suggest that this hypothesis may not account for CTAs produced by ethanol (Risinger & Cunningham, 1995).

The temporal relationship between administration of the CS and US in the place and taste conditioning procedures may be an important factor that determines the direction of the CR (i.e., preference or aversion). In the typical place conditioning procedure, the US immediately precedes the CS, whereas in the taste conditioning procedure, the order of presentation is reversed. Several studies have recently demonstrated that reversal of the CS-US temporal relationship (i.e., US followed by CS) in the place conditioning procedure results in a CPA with several drugs known to produce a CPP, including nicotine

(Fudala & Iwamoto, 1987) and amphetamine (Fudala & Iwamoto, 1990) in rats, and ethanol in mice (Cunningham, Okorn, & Howard, 1997). These findings suggest a paradoxical situation similar to that found with the CTA paradigm, which may be related to the similar order of presentation of the CS and US. It remains to be determined whether a common underlying mechanism is responsible for the formation of CTA and CPA, and whether the same or different mechanism mediates CPP with drugs of abuse.

Place conditioning. The place conditioning paradigm has been used to demonstrate the rewarding and aversive properties of $GABA_A$ receptor ligands in rats and mice. For example, antagonism of $GABA_A$ receptors with negative modulators such as picrotoxin (Spyraki, Kazandjian & Varonos 1985; Acquas et al., 1989), the full BZ inverse agonist, B-carboline-3-carboxylate (B-CCE) (Tsuda, Ida, Nishimura, & Tanaka, 1989), and partial BZ inverse agonists CGS 8216 and FG 7142 (Wagner & Katz, 1984; File, 1986; Di Scala & Sandner, 1989) produce a CPA. In contrast, both preference and aversion has been observed with positive modulators of the $GABA_A$ receptor. For instance, the BZ agonists, diazepam and lorazepam, have been shown to produce a CPP (Spyraki et al., 1985; File, 1986; Nomikos & Spyraki, 1988; Spyraki & Fibiger, 1988), although at least one study failed to find this effect with diazepam (Di Scala, Oberling, Roccha, & Sandner, 1992). In addition, Spyraki et al. (1985) showed that diazepam-induced CPP could be blocked with administration of picrotoxin and the partial inverse agonist, CGS 8816. Di Scala et al. (1992) showed a CPP with the BZ receptor partial agonist, Ro 16-6028, which was blocked with the BZ receptor antagonist, Ro 15-1788. In mice, the neurosteroid 3α-hydroxy-5α-pregnan-20-one (3α-5α-P), which binds to the neurosteroid site on the $GABA_A$ receptor and potentiates GABA-stimulated chloride conductance, has been shown to produce a dose-dependent CPP (Finn, Phillips, Okorn, Chester, & Cunningham, 1997). However, the BZ agonist, chlordiazepoxide, has been shown to produce weak preference (File, 1986) or a place aversion (Parker, Limebeer, &

Simpson, 1998). In addition, phenobarbital, which is an agonist at the barbiturate site on the $GABA_A$ receptor, has also been shown to have aversive effects in the place conditioning paradigm (Wilks & File, 1988).

A few studies have also suggested that GABA receptors modulate the rewarding effects of certain abused drugs in the place conditioning paradigm. For example, microinjection of the $GABA_B$ agonist, baclofen, into the VTA in rats has been shown to dose-dependently suppress morphine-induced CPP (Tsuji, Nakagawa, Ishibashi, Yoshii, Takashima, Shimada, & Suzuki, 1996). The authors suggest the reduction in CPP is possibly due to a suppression of morphine-induced dopamine release in the VTA with baclofen (Klitenick et al., 1992) or a decrease in dopamine release in the nucleus accumbens (e.g., Yoshida, Yokoo, Tanaka, Emoto, & Tanaka, 1994). Another study showed that administration of diazepam enhanced (Leri & Franklin, 1997), and conversely, B-CCE blocked (Franklin & Leri, 1997), the expression of morphine-induced CPP. Diazepam was also shown to interfere with the acquisition and expression of amphetamine-induced CPP (Leri & Franklin, 1997). Consistent with this finding, another BZ agonist, triazolam, has been shown to reduce amphetamine-induced CPP (Pettit, Batsell, & Mueller, 1989). However, another study showed that the GABA agonist, progamide, did not alter amphetamine-induced CPP (Di Scala, Martin-Iversen, Phillips, & Fibiger, 1985).

Overall, these studies show that ligands that bind to the $GABA_A$ receptor possess rewarding and aversive properties in the place conditioning paradigm. In general, $GABA_A$ antagonists have been shown to be aversive, whereas agonists of the receptor have rewarding motivational properties. However, some $GABA_A$ receptor agonists have also been shown to produce a place aversion, indicating that activation of the $GABA_A$ receptor may also produce aversive motivational effects. In addition, some studies suggest the GABA receptor system may play an important role in modulating the motivational effects of some drugs of abuse in the place conditioning paradigm.

Taste Conditioning. Contrary to the place conditioning studies, relatively few studies have demonstrated motivational effects of GABA receptor ligands in the taste conditioning paradigm. For example, intracerebral administration of the $GABA_A$ antagonists, picrotoxin and bicuculline did not produce a CTA in rats, even at a high dose (5 mg/kg) that elicited convulsions (Bures & Buresova, 1989). This is consistent with another study that failed to find a CTA with systemic administration of picrotoxin (Smith, Segal, & Amit, 1989). Ebenezer et al. (1992) showed that systemic administration of baclofen does not produce a CTA. Systemic administration of GABA itself has been shown to produce a CTA; however, this effect appears to be due to a peripheral effect, rather than activation of GABA receptors in the brain (Tews, Repa, & Harper, 1988). Thus, these studies generally suggest that specific ligands for $GABA_A$ and $GABA_B$ receptors do not possess motivational properties in the CTA paradigm.

Ethanol and the $GABA_A$ Receptor System

Ethanol has been shown to exert many of its biochemical and behavioral effects through an interaction with the GABA receptor system . It is well-established that ethanol influences $GABA_A$ receptor functioning; consequently, most of the data reviews to date focus on ethanol's interaction with this receptor subtype (for reviews see Ticku, 1990; Korpi, 1994; Mihic & Harris, 1996). However, a number of studies suggest that the $GABA_B$ receptor subtype also modulates some of ethanol's effects. Furthermore, a few studies have shown that ethanol's actions may be mediated through activation of both receptor subtypes. The following sections will review *in vitro* and *in vivo* evidence for both $GABA_A$ and $GABA_B$ receptor modulation of ethanol's biochemical and behavioral effects.

In vitro studies. Several lines of evidence have demonstrated that ethanol influences $GABA_A$ receptor functioning. Ethanol has a pharmacological profile similar to

BZs and barbiturates, most likely due to its effects on chloride flux through the $GABA_A$ receptor (for review see Yu & Ho, 1990). Biochemical and electrophysiological studies have shown that ethanol interacts with the $GABA_A$ receptor to potentiate GABA-stimulated chloride uptake (for review see Leidenheimer & Harris, 1992). This effect of ethanol is blocked by the $GABA_A$ receptor antagonist bicuculline and the chloride channel blocker picrotoxin, suggesting that ethanol is directly affecting $GABA_A$ chloride channel functioning (Allan & Harris, 1986; Suzdak, Schwartz, Skolnick, & Paul, 1986; Mehta & Ticku, 1988). To date, a specific binding site for ethanol has not been found, although there is some evidence that ethanol may interact with specific $GABA_A$ receptor subunits. For example, several studies have provided evidence that ethanol has a regionally specific action in the brain (Givens & Breese, 1990) by affecting a population of $GABA_A$ receptors with a specific structural composition (Criswell et al., 1993). Furthermore, the γ2L subunit, which shows a specific pattern of distribution in the mouse brain (Wang & Burt, 1991), appears to confer $GABA_A$ receptor sensitivity to ethanol (Wafford et al., 1991; Harris, Mihic, Brozowski, Hadingham, & Whiting, 1997). However, another study showed that coexpression of the rat α1, β1 and γ2L in *Xenopus* oocytes did not produce ethanol-sensitive $GABA_A$ receptors (Sigel, Baur, & Malherbe, 1993; Mihic, Whiting, & Harris, 1994; but see Harris et al., 1997). In behavioral studies, knockout mice lacking the γ2L subunit showed no differences in various ethanol-induced behaviors relative to wild-type counterparts (see review by Homanics, Quinlan, Mihalek, & Firestone 1998), suggesting that this $GABA_A$ receptor subunit may not be important for modulating the effects of ethanol at a behavioral level. Several studies have suggested that the α6 subunit located in cerebellar granular cells may confer sensitivity to ethanol's motor-impairing effects in rats (Lüddens et al., 1990; Korpi, Kleingoor, Kettenmann, & Seeburg, 1993; but see Korpi et al., 1992).

Interestingly, neuroanatomical studies have shown differences in the GABA system between alcohol-preferring and non-preferring rodents. For example, the alcohol-preferring Fawn-Hooded rats have a higher density of $GABA_A$ receptors in cortical regions, substantia nigra pars reticulata, and the ventral pallidum relative to the alcohol non-preferring Wistar-Kyoto strain (Chen, Rezvani, Jarrott, & Lawrence, 1997). In addition, the selectively bred alcohol-preferring (P) and high alcohol drinking (HAD) rat lines have been found to have a 50% greater density of GABAergic terminals in the nucleus accumbens relative to non-preferring (NP) and low alcohol drinking (LAD) rat lines (Hwang, Lumeng, Wu, & Li, 1988). It has been suggested that these observed differences in the GABA system may be responsible for mediating differential sensitivity to ethanol's reinforcing effects in these selected lines.

In vivo studies. Several types of behavioral studies have provided evidence that ethanol interacts with the $GABA_A$ receptor. Ethanol-induced narcosis, or LORR, is reduced with bicuculline (Liljequist & Engel, 1982), picrotoxin (Martz, Deitrich, & Harris, 1983), and isopropylbicyclo-phosphate (IPPO), a picrotoxin-type ligand (Mendelson, Martin, Wagner, Roseberry, Skolnick, Weissman, & Squires, 1985). Microinjection of the $GABA_A$ agonist, muscimol, into the medial septal area has been shown to increase ethanol-induced LORR, whereas bicuculline microinjections decreased LORR. In addition, since microinjections into the lateral septum had no effect, these data suggest that specific brain regions may be involved in ethanol's sedative effects (Givens & Breese, 1990). However, other studies have shown the effects of bicuculline on ethanol-induced LORR depend on the mouse genotype studied and possibly their initial ethanol sensitivity (Dudek & Phillips, 1989; Phillips & Dudek, 1989). Bicuculline has also been shown to reduce ethanol-induced motor impairment in a tilting plane task in rats (Hakkinen & Kulonen, 1976) and inhibition of bar holding in mice (Martz et al., 1983). In addition, direct infusion of the BZ partial inverse agonist, Ro 15-4513, into the cerebellum has been shown

to reduce ethanol-induced motor impairment dose-dependently (Dar, 1995). A potentiation of ethanol's effects has also been observed with $GABA_A$ antagonists. For example, co-administration of bicuculline (Liljequist & Engel, 1982; Phillips & Dudek, 1989) or picrotoxin (Liljequist & Engel, 1982) increases ethanol-induced hypothermia.

$GABA_A$ receptor drugs have also been shown to alter ethanol-stimulated locomotor activity (see review by Phillips & Shen, 1996). For example, the $GABA_A$ agonist THIP (Ägmo & Giordano, 1985) and muscimol (Liljequist & Engel, 1982) are reported to decrease ethanol-stimulated activity. However, Ägmo and Giordano showed that the effects of THIP could not be reversed with bicuculline. Thus, these authors suggest that the effect of THIP on ethanol-stimulated activity may not involve the $GABA_A$ receptor. Conversely, picrotoxin has been shown to increase ethanol-stimulated activity (Liljequist & Engel, 1982). However, another study showed this effect of picrotoxin to be dependent on ethanol dose. In this study, picrotoxin decreased ethanol-stimulated activity with lower ethanol doses and increased stimulated activity when higher ethanol doses were administered (Koechling, Smith, & Amit, 1991). Some studies have demonstrated no effect of Ro 15-4513 on ethanol-stimulated activity in mice (Becker, 1988; Syapin, Jones, Kobayashi, Finn, & Alkana, 1990), whereas another study showed that Ro 15-4513 reduced ethanol's stimulant effects in mice (Risinger, Malott, Riley, & Cunningham, 1992). Ro 15-4513 has also been shown to reduce ethanol's locomotor depressant effects in mice and rats (Becker, 1988; Wood, Healey, Menendez, Verne, & Atrens, 1989). Thus, the effects of GABA antagonists and agonists may depend on ethanol's effect on locomotor activity (stimulation or depression), ethanol dose, and genotype.

In summary, several lines of evidence have shown that ethanol produces many of its effects through an interaction with the $GABA_A$ receptor. Ethanol appears to directly potentiate GABA-stimulated chloride flux through the receptor because this effect can be blocked with the specific $GABA_A$ antagonists, picrotoxin and bicuculline. In addition,

certain $GABA_A$ receptor subunits that are expressed in specific brain regions may be responsible for mediating different biochemical and behavioral effects of ethanol. A number of studies have also shown that $GABA_A$ antagonists reduce, whereas agonists enhance ethanol's behavioral effects. However, $GABA_A$ antagonists have also been shown to enhance some effects of ethanol. Therefore, it is important to consider that the effect of GABAergic drugs may depend on the brain region studied or dose of ethanol administered, as well as genetic variation in the $GABA_A$ receptor system.

Ethanol and the $GABA_B$ Receptor System

In vitro studies. Most studies investigating a role for the GABA system in ethanol's effects have focused on the $GABA_A$ receptor. Recently, however, the development of selective $GABA_B$ receptor ligands has facilitated the investigation of $GABA_B$ receptor pharmacology (see reviews by Mott & Lewis, 1994; Kuriyama & Hirouchi, 1997; Froestl & Mickel, 1997), and may soon advance the study of ethanol's interaction with the $GABA_B$ receptor. *In vitro* studies have provided some evidence that ethanol may alter $GABA_B$ receptor functioning. For example, a recent study by Ichida and Kuriyama (1997) investigated the effects of ethanol on $GABA_B$ receptor-mediated G protein and adenylate cyclase functions. High concentrations of ethanol inhibited [^{3}H] GABA binding to the $GABA_B$ receptor in membrane vesicles obtained from rat brain, and decreased cAMP formation by suppressing the function of G_s stimulatory protein. However, no changes were found in G_i or G_o activity, the inhibitory G proteins through which $GABA_B$ receptors are thought to be coupled (see review by Cunningham & Enna, 1997). Another study showed that [^{3}H] GABA binding to $GABA_B$ receptors was increased in crude synaptic membranes obtained from ethanol-treated mice and mice withdrawn from ethanol (Mizutani, Hashimoto, Nakayasu, & Kuriyama, 1993).

Wan, Berton, Madamba, Francesconi, and Siggins (1996) demonstrated that $GABA_B$ receptor activation may inhibit the ability of a low ethanol concentration to enhance $GABA_A$ receptor function in rat hippocampus. This is consistent with a study showing that baclofen can inhibit muscimol-stimulated chloride uptake in an *in vitro* membrane preparation from mouse cerebellum via G-protein mediated phosphorylation of the $GABA_A$ receptor (Hahner, McQuilkin, & Harris, 1991). However, in mouse cortex, $GABA_B$ receptor activation appears to be required for a low concentration of ethanol to enhance $GABA_A$ receptor chloride uptake (Allan et al., 1991; but see Mehta & Ticku, 1990). Overall, these studies provide evidence on a cellular level that ethanol interacts with the $GABA_B$ receptor. Moreover, $GABA_B$ receptors may modulate $GABA_A$ receptor functioning in both a facilitatory and inhibitory fashion. However, the direction of the modulatory effect on $GABA_A$ functioning appears to depend on the brain region and species that is studied.

In vivo studies. Most of the evidence that ethanol interacts with $GABA_B$ receptors comes from studies showing that $GABA_B$ receptor activation modulates various behavioral effects of ethanol. For example, administration of the $GABA_B$ antagonist, phaclofen, blocks acute ethanol-induced hypothermia, motor incoordination, and sleep time (Allan & Harris, 1989). More recently, Dar (1996) showed that direct microinfusion of phaclofen into the cerebellum significantly reduced the potentiation of ethanol-induced motor impairment produced by the $GABA_B$ agonist, baclofen. In addition, infusion of pertussis toxin into the cerebellum completely blocked this effect of baclofen and also significantly reduced ethanol-induced motor impairment. These findings suggest that the pertussis toxin sensitive G proteins (such as G_i and G_o) may be directly involved in modulating ethanol's interaction with $GABA_B$ receptors in the cerebellum.

Stimulation of $GABA_B$ receptors with the selective agonist, baclofen, potentiates ethanol-induced sleep time (Martz et al., 1983). Baclofen administration also reduces

ethanol-stimulated locomotor activity in mice (Cott, Carlsson, Engel, & Lindquist, 1976; Humeniuk, White, & Ong, 1993; Shen, Dorow, Harland, Burkhart-Kasch, & Phillips, in press), which is blocked by the selective $GABA_B$ antagonist, CGP 35348 (Humeniuk et al., 1993; Shen et al., in press). Since ethanol has been shown to affect locomotor behavior in a biphasic manner, with stimulation at low doses and depression at higher doses (Crabbe, Johnson, Gray, Kosobud, & Young, 1982), this effect of baclofen could be due to a facilitation of ethanol's depressant effects on locomotor activity via $GABA_B$ receptor activation. In addition, phaclofen has been shown to block low-dose ethanol-stimulated locomotor activity (Allan & Harris, 1989; Humeniuk et al., 1993), suggesting that the $GABA_B$ receptor may also modulate behavioral responses to low doses of ethanol.

In support of some of the *in vitro* findings, there is also behavioral evidence that the $GABA_B$ receptor modulates $GABA_A$ receptor functions. For instance, phaclofen has been shown to attenuate ethanol's protective effect against picrotoxin-induced seizures (Rastogi & Ticku, 1986; Mehta & Ticku, 1990). In another study, Malcangio, Malmberg-Aiello, Giotti, Ghelardini, and Bartolini (1992) showed that CGP 35348 antagonized the antinociceptive effect of intracerebroventricular injections of picrotoxin and bicuculline. Thus, these findings suggest that some $GABA_A$-mediated effects may depend on activation of the $GABA_B$ receptor. Furthermore, both receptor subtypes may be involved in mediating certain behavioral effects of ethanol.

In summary, the findings of several different types of studies suggest that ethanol interacts with the $GABA_B$ receptor. The mechanism for this interaction is unclear; however, there is some evidence that ethanol may alter the function of G proteins coupled to the $GABA_B$ receptor. In addition, changes in G protein pathways may in turn modulate $GABA_A$ receptor activity. Behavioral studies, however, have provided most of the evidence that $GABA_B$ receptors modulate ethanol's effects. The $GABA_B$ receptor antagonist, phaclofen, has been shown to reduce ethanol-induced behaviors such as sleep

time and motor impairment, while the $GABA_B$ agonist, baclofen, potentiates these ethanol-induced behaviors. Furthermore, the effects of baclofen can be blocked with administration of the $GABA_B$ antagonists, phaclofen and CGP 35348, showing that baclofen's effects are specifically mediated through the $GABA_B$ receptor.

GABA Receptors and the Motivational Effects of Ethanol

Evidence for an involvement of the GABA receptor system in modulating the rewarding and aversive effects of ethanol comes from several types of behavioral drug reward models. Most of the studies to date have investigated the effects of $GABA_A$ receptor compounds on ethanol self-administration behavior. In general, these studies have provided evidence for an involvement of the $GABA_A$ receptor in modulating ethanol consumption in rats using several types of self-administration paradigms, including limited-access, free-access, and response-contingent procedures. Although relatively fewer in number, a role for the GABA system in ethanol's motivational properties has also been examined in other drug reward models, including taste and place conditioning paradigms. Table 1 provides a summary of the effects of GABAergic manipulations on several reward-related behaviors. The studies listed here will be briefly described in subsequent sections.

Ethanol Self-Administration

Convulsants. Several studies have shown that $GABA_A$ antagonists that bind to the convulsant site within the chloride channel decrease ethanol self-administration in rats. For example, Boyle, Segal, Smith and Amit (1993) demonstrated that picrotoxin selectively decreased the maintenance of ethanol self-administration without altering water intake. IPPO, a picrotoxin-type ligand, has also been shown to decrease motivated responding for ethanol in a limited-access, free-choice operant paradigm (Rassnick, D'Amico, Riley & Koob, 1993). In another recent study, the effect of pentylenetetrazole (PTZ) was examined on the acquisition and maintenance of ethanol self-administration in a limited-access

Table 1. Summary of the effects of GABAergic manipulations on ethanol reward-related behaviors.

Table 1

Procedure	Compound	Effect	Reference
Self-Administration	PTZ[a]	Increase (chronic)	Buczek et al. (1994)
	Picrotoxin[a]	Decrease	Boyle et al. (1993)
	IPPO[a]	Decrease	Rassnick et al. (1993)
	PTZ	Decrease (chronic)	Buczek et al. (1998)
	SR95531[a]	Decrease (Ex AMG)	Hyytiä & Koob (1995)
	PTZ	No effect (acute)	Buczek et al. (1998)
	Bicuculline[a]	No effect	Boismare et al. (1984)
	THIP[a*]	Increase	Smith et al. (1992)
			Boyle et al. (1992; 1993)
	Muscimol[a*]	Increase (DRN)	Tomkins et al. (1994)
		Decrease (NACC)	Hodge et al. (1995)
		Decrease (AMG)†	Roberts et al. (1996)
	Ro 15-4513[ai]	Increase (chronic)	Buczek et al. (1997)
			June et al. (1992)#
		Decrease (acute)	Buczek et al. (1997)
		Decrease	Samson et al. (1987; 1989)
			McBride et al. (1988)
			June et al. (1991)
			Rassnick et al.(1993)
			Petry (1995)
	Ro 19-4603[ai]	Decrease	Balakleevsky et al. (1990)
			Wegelius et al. (1994)
			June et al. (1994; 1996; 1998)
		Decrease (NACC)	June et al. (1998)
	chlordiazepoxide[af]	Increase (low dose)	Petry (1995)
	Midazolam[af]	Increase	Wegelius et al. (1994)
	chlordiazepoxide	Decrease (high dose)	Petry (1995)
		Decrease	Samson & Grant (1985)
			Chan,Schanley, et al. (1983)
		No effect	Chan, Leong, et al. (1983)
			Beaman et al. (1984)
			Rassnick et al. (1993)
	Bretazenil[ap]	Increase	Wegelius et al. (1994)
	CGS 9895[ap]	Decrease	Wegelius et al. (1994)
	Abecarnil[ap]	No effect	Wegelius et al. (1994)
	ZK 91296[ap]	No effect	Wegelius et al. (1994)

Table 1: continued

Procedure	Compound	Effect	Reference
Self-Administration	gamma-butyrolactone[n]	Decrease	Fadda et al. (1983)
	Ca AOTA[n]	Decrease	Boismare et al. (1984)
	AOAA[n]	Decrease	Daoust et al. (1987)
	Baclofen[b*]	Increase	Smith et al. (1992)
		Decrease	Daoust et al. (1987)
		No effect (DRN)	Tomkins & Fletcher (1996)
CPP	Picrotoxin[a]		
	(Diazepam[d])	Decrease	Spyraki et al. (1985)
	Ro 15-4513[ai]	No effect	Risinger et al. (1992)
CTA	Picrotoxin[a]	Decrease	Smith et al. (1989)
	Ro 15-4513[ai]	No effect	Jeffreys et al. (1990)
			June et al. (1992)

a = $GABA_A$ antagonist
a* = $GABA_A$ agonist
ai = $GABA_A$/BZ partial inverse agonist
af = $GABA_A$/BZ full agonist
ap = $GABA_A$/BZ partial agonist
n = non-selective GABA agonist
b* = $GABA_B$ agonist

d = Diazepam-induced CPP
† = in ethanol-dependent rats
= Ro 15-4513 prevented a normal decline in ethanol consumption during a chronic self-administration period

AMG - Amygdala
DRN - Dorsal Raphe Nucleus
Ex AMG - Extended Amygdala (central amygdaloid nucleus, bed nucleus of the stria terminalis, nucleus accumbens shell)
NACC - Nucleus Accumbens

procedure, with free-choice between ethanol and water (Buczek, Lê, Sellers, & Tomkins, 1998). Acute administration of PTZ did not alter ethanol intake during the maintenance phase of the procedure; however, PTZ administered chronically (12 days) during the maintenance phase produced a gradual dose-dependent reduction of ethanol intake beginning on day 7. In addition, chronic treatment with PTZ during the acquisition phase also dose-dependently reduced ethanol intake. The authors suggest one mechanism for this effect may be a change in the density and/or function of $GABA_A$ receptors with repeated PTZ administration. Thus, a progressive increase in sensitivity to the effects of PTZ may account for the delayed reduction in ethanol consumption. However, only one rat in this study showed evidence of kindled-induced convusions, which sometimes occurs when administration of an initially subconvulsant dose of PTZ eventually produces convulsant activity after repeated administration. The finding that PTZ decreased ethanol consumption during the maintenance phase is inconsistent with a previous study that showed PTZ administration increased ethanol intake in rats maintained on ethanol during the limited-access procedure (Buczek, Tomkins, Higgins, & Sellers, 1994). The authors suggest that the discrepancy could be due to methodological differences that resulted in more or less stressful conditions that influenced ethanol intake. Thus, PTZ increased ethanol intake when consumption was relatively low (Buczek et al., 1994) and decreased ethanol intake when consumption was relatively high (Buczek et al., 1998). Overall, these studies suggest that ligands that bind to the convulsant site on the $GABA_A$ receptor reduce ethanol self-administration, possibly due to a reduction in ethanol-induced chloride influx. However, this effect may depend on environmental conditions or initial preference for ethanol in experimental subjects.

Partial inverse agonists. Many investigators have shown that $GABA_A$/BZ partial inverse agonists reduce ethanol consumption in several different rodent populations. Samson, Tolliver, Pfeffer, Sadeghi, and Mills (1987) were the first to report that the partial

inverse agonist, Ro 15-4513, reduces ethanol self-administration in non-deprived rats using a sucrose-fading procedure (Samson, 1986). Ro 15-4513 has also been shown to reduce ethanol consumption during a limited-access procedure in rats given a choice between sweetened ethanol and water (June, Lummis, Colker, Moore, & Lewis, 1991). Rassnick et al. (1993) showed that Ro 15-4513 selectively reduced motivated responding for ethanol over water in a limited-access, free-choice operant paradigm. In addition, Ro 15-4513 did not alter responding for a saccharin reinforcer, which is consistent with previous studies (e.g., Samson, Haraguchi, Tolliver, & Sadeghi, 1989; June et al., 1991; Petry, 1995) that suggest the effect of Ro 15-4513 is selective for ethanol reinforcement.

Other studies have shown that inverse agonists produce a reduction in ethanol self-administration in rats selectively bred to prefer alcohol. In the P rat line, Ro 15-4513 has been shown to selectively reduce ethanol consumption in a 2-hr limited-access paradigm. This effect was completely blocked with the specific BZ receptor antagonist, Ro 15-1788 (McBride, Murphy, Lumeng, & Li, 1988). Another partial inverse agonist, Ro 19-4603 has been shown to reduce ethanol consumption in the Sardinian alcohol-preferring rat line during a 24-hr free-access period (Balakleevsky, Colombo, Fadda, & Gessa, 1990). In this study, Ro 19-4603 administered three times per day significantly reduced ethanol intake and increased water intake by the second day of treatment. However, in a separate group of animals, Ro 19-4603 failed to alter water consumption under similar conditions. Thus, the authors suggest that Ro 19-4603 selectively reduced ethanol consumption that was accompanied by a compensatory increase in water consumption, since total fluid intake in Ro 19-4603-treated animals was not different from controls. Ro 15-4513 and Ro 19-4603 has been shown to decrease ethanol intake in the alcohol-accepting (AA) rat line allowed limited-access to ethanol for 1 or 4 hrs per day (Wegelius, Honkanen, & Korpi, 1994). The authors of this study also reported that Ro 19-4603 produced convulsant effects in some animals that could have interfered with ethanol self-administration behavior.

However, another study examined lower doses of Ro 19-4603 and found a selective decrease in ethanol intake in P rats within the first 15 min of a 4-hr limited-access period, and this effect was reversed with the BZ receptor antagonist CGS 8216 (June et al., 1996). This rapid decrease in ethanol consumption by Ro 19-4603 is inconsistent with Balakleevsky et al. (1990); however, the discrepancy may be due to differences in the selected lines, dose of Ro 19-4603 administered, or ethanol access period (free- vs limited-access). Other studies have demonstrated Ro 19-4603 produces a selective and prolonged decrease (up to 32 hrs post injection) in ethanol self-administration in P rats maintained on 24-hr free-access (June, Murphy, Mellor-Burke, Lumeng, & Li, 1994) and in an operant self-administration paradigm (June et al., 1998).

Ro 15-4513 has been shown to produce different effects in studies examining chronic ethanol consumption. For example, Ro 15-4513 has been shown to reduce ethanol consumption during the first half of a chronic ethanol self-administration period (60 days), but not during the last half when ethanol consumption declined in vehicle treated controls (June, Colker et al., 1992). Interestingly, the Ro 15-4513 treated group maintained a constant level of ethanol self-administration across the 60-day period. Furthermore, the specific BZ receptor antagonist, Ro 15-1788 blocked the effect of Ro 15-4513 on ethanol consumption, suggesting a role for the BZ receptor in modulating chronic ethanol drinking patterns. A recent study by Buczek, Tomkins, Lê, and Sellers (1997) found opposite effects of Ro 15-4513 on the acquisition and maintenance of ethanol drinking behavior in Wistar rats. Acute and chronic effects of Ro 15-4513 were examined during the maintenance phase of a limited-access procedure, with free-choice between ethanol and water. In addition, chronic effects of Ro 15-4513 were tested during the acquisition phase of ethanol self-administration. Consistent with previous studies, acute administration of Ro 15-4513 reduced ethanol self-administration during the maintenance phase without altering water intake. However, when Ro 15-4513 was administered chronically (8 days),

an initial decrease in consumption was observed followed by an increase after the third day of treatment to the level of vehicle-treated controls. In addition, chronic treatment (30 days) with Ro 15-4513 during the acquisition phase significantly increased ethanol self-administration. The authors suggest this effect was not due to the development of tolerance to Ro 15-4513, although this was not directly tested.

Overall, these studies show that administration of several different $GABA_A$ antagonists and inverse agonists reduce ethanol consumption using various types of self-administration procedures. However, Ro 15-4513 has also been shown to produce an increase, as well as prevent a normal decline, in ethanol consumption during periods of chronic self-administration. In these studies, interpretation is complicated by the possibility of tolerance to the effects of Ro 15-4513. Nevertheless, these findings suggest a role for the $GABA_A$ receptor in modulating both acute and chronic ethanol self-administration behavior. Moreover, these studies indicate that the type of self-administration procedure employed (acute vs chronic) may be important in determining the effect of a $GABA_A$ antagonist or inverse agonist on ethanol consumption.

$GABA_A$ Agonists. Changes in ethanol self-administration behavior have also been demonstrated with administration of GABA agonists. For example, the selective $GABA_A$ agonist THIP has been shown to increase both the acquisition (Smith, Robidoux, & Amit, 1992) and maintenance (Boyle, Segal, Smith, & Amit, 1993) of ethanol self-administration in rats, and this effect appears to be selective for ethanol consummatory behavior (Boyle, Smith, & Amit, 1992). In contrast, the non-selective GABA agonists, gamma-butyrolactone (Fadda, Argiolas, Melis, De Montis, & Gessa, 1983), AOAA (a GABA decarboxylase inhibitor) (Daoust et al., 1987), and calcium-acetyl-homotaurine (Ca AOTA) (Boismare et al., 1984) have been shown to decrease voluntary ethanol consumption in a free-access paradigm. Although Ca AOTA is a relatively non-selective GABA agonist, the specific $GABA_A$ antagonist, bicuculline, attenuated the decrease in

ethanol intake observed with Ca AOTA. This finding suggests that the $GABA_A$ receptor is involved in mediating the effect of Ca AOTA on ethanol intake.

The BZ agonist, chlordiazepoxide, has also been shown to decrease ethanol self-administration in rats (Samson & Grant, 1985) and mice (Chan, Schanley, & Leong, 1983) under a variety of experimental conditions. However, other studies have shown no effect of chlordiazepoxide on ethanol drinking behavior (Beaman, Hunter, Dunn, & Reid, 1984; Chan, Leong, & Schanley, 1983; Rassnick et al., 1993). It is possible the effect of chlordiazepoxide is dependent on the dose, since Petry (1995) showed increases in ethanol self-administration at low doses and decreases at high doses in rats in a concurrent or free-choice procedure. Wegelius et al. (1994) tested several other BZ compounds, such as the full agonist, midazolam, and partial agonists abecarnil, ZK 91296, bretazenil, and CGS 9895 on ethanol consumption in AA rats. Midazolam increased ethanol consumption, but the partial agonists generally decreased ethanol consumption or produced no effect (see Table 1). In addition, these compounds also produced significant changes in food intake, suggesting that the changes observed in ethanol consumption are not due to a selective alteration in ethanol's reinforcing effects. Indeed, many studies have shown that BZ receptor compounds modulate feeding and drinking behavior (see earlier section on GABA receptors and food reward).

Overall, these studies indicate that agonists of the $GABA_A$ receptor produce both increases and decreases in ethanol self-administration. Although these findings suggest the $GABA_A$ receptor modulates ethanol's motivational properties, it is unknown why $GABA_A$ agonists produce bidirectional effects on ethanol self-administration. It is possible these inconsistencies are due to differences in ethanol dose, efficacy of the $GABA_A$ agonist, experimental conditions, or non-specific effects of the agonists on consummatory behavior.

Site-specific injections. Other studies have reported changes in ethanol self-administration behavior following administration of $GABA_A$ receptor ligands to specific

areas of the brain thought to be involved in ethanol's motivational effects. For example, Hodge, Chappelle, and Samson (1995) demonstrated in rats that administration of the $GABA_A$ agonist, muscimol, into the nucleus accumbens decreased ethanol self-administration by terminating ethanol-reinforced responses after a period of approximately 10 min, when responding normally lasted about 20 min. In addition, this effect was blocked by co-administration of bicuculline. This study indicates that $GABA_A$ receptor activation in the nucleus accumbens is involved in the termination of ethanol self-administration. However, microinjection of the partial inverse agonist, Ro 19-4603, into the nucleus accumbens has also been shown to produce a significant reduction in ethanol-responding in P rats, with no change in saccharin-responding (June et al., 1998). It is difficult to interpret these findings, since administration of both an agonist and antagonist of the $GABA_A$ receptor into the nucleus accumbens reduces ethanol self-administration. It is possible that the discrepancy is due to differences in self-administration procedures or in the rat strains utilized [selectively bred P rats (June et al.) vs outbred Long Evans rats (Hodge et al.)]. Nevertheless, these studies suggest that $GABA_A$ receptors in the nucleus accumbens may modulate ethanol's motivational effects in the self-administration paradigm.

In a free-choice, limited-access paradigm, Hyytiä and Koob (1995) examined the effects of microinjections of the potent $GABA_A$ receptor antagonist, SR95531, into the extended amygdala on operant ethanol self-administration in rats. SR95531 reduced motivated responding for ethanol when injected into the central amygdaloid nucleus, the bed nucleus of the stria terminalis, and the shell of the nucleus accumbens, suggesting a role for $GABA_A$ receptors in the extended amygdala in modulating voluntary ethanol consumption. In another study, Roberts, Cole, and Koob (1996) used an extended ethanol access paradigm to examine the effects of intra-amygdala infusions of muscimol on operant responding for ethanol in dependent and non-dependent rats. Ethanol-dependent rats

responded more for ethanol than non-dependent rats, and intra-amygdala muscimol significantly reduced responding in dependent rats, but did not affect responding in non-dependent rats. These results suggest that $GABA_A$ receptor activation in the amygdala is involved in modulating ethanol's motivational properties in dependent rats. The authors suggest the muscimol-induced decrease in ethanol-responding in dependent rats is possibly due to a decrease in the aversive motivational effects of ethanol withdrawal, resulting in a decrease in the amount of ethanol required to alleviate aversive withdrawal symptoms. Another study examined the effects of muscimol injections into the dorsal and median raphe nuclei in non-dependent rats trained to consume ethanol in a limited-access procedure (Tomkins, Sellers, & Fletcher, 1994). Muscimol administered into the dorsal raphe selectively increased ethanol intake, whereas median raphe injections increased both ethanol and water consumption. In addition, peripheral administration of bicuculline has been shown to block the effect of muscimol at a dose that did not alter ethanol intake when administered alone (Tomkins & Fletcher, 1996). These results suggest that $GABA_A$ receptor activation in the dorsal, but not the median raphe nucleus is selectively involved in modulating ethanol consumption. Furthermore, the authors suggest the effect of muscimol in the dorsal raphe nucleus could be due to $GABA_A$ receptor interactions with other neurotransmitter systems. For example, muscimol injections have been shown to decrease serotonin release in several brain areas (Nishikawa & Scatton, 1985), and increase dopamine turnover in the nucleus accumbens (Bendotti, Berettera, Invernizzi & Samanin, 1986). In summary, these studies suggest that
$GABA_A$ receptor activation in specific brain regions differentially modulates voluntary ethanol consumption in dependent and non-dependent rats, presumably by altering ethanol's hedonic value.

$GABA_B$ agonists. As previously discussed, most studies investigating a role for the GABA receptor system in ethanol's motivational effects have focused on the

$GABA_A$ receptor. Very few studies have examined manipulations of the $GABA_B$ receptor, most likely due to the lack of selective antagonists for this receptor subtype. Thus, studies that have examined a role for the $GABA_B$ receptor in ethanol self-administration have utilized the selective $GABA_B$ agonist, baclofen. These investigations have provided contradictory evidence that $GABA_B$ receptors modulate ethanol self-administration. Smith et al. (1992) examined the effects of baclofen on the acquisition of voluntary ethanol intake in rats. These investigators found that baclofen (10 mg/kg) increased ethanol intake, but also increased total fluid intake, suggesting the effect of baclofen was not selective for ethanol's motivational effects. In another study, baclofen (3 mg/kg) was shown to decrease voluntary ethanol intake without altering total fluid intake (Daoust et al., 1987). Although the subjects in both studies were male Long Evans rats, the discrepancy between these two studies is possibly due to different doses of baclofen used or different procedures used to measure ethanol self-administration. For example, Smith et al. administered baclofen daily during an acquisition phase of self-administration, while Daoust et al. selected rats that were ethanol preferring (≥60% of their total fluid intake was ethanol) before examining the effect of daily baclofen administration on the maintenance of ethanol self-administration behavior. More recently, Tomkins and Fletcher (1996) showed that direct injections of baclofen into the dorsal raphe nucleus had no effect on ethanol or water consumption. Thus, the role of $GABA_B$ receptors in modulating ethanol self-administration remains unclear.

In summary, these findings suggest that activation of the $GABA_A$ receptor plays an important role in modulating ethanol's reinforcing effects in the self-administration paradigm. In general, administration of $GABA_A$ receptor antagonists either systemically or into specific brain regions reduces ethanol intake in several different types of self-administration procedures. However, a few studies have also shown that certain $GABA_A$ antagonists increase ethanol intake under chronic self-administration conditions. The effect

of $GABA_A$ agonists on ethanol self-administration is less clear, since agonists have been shown to both increase and decrease ethanol self-administration. It is possible that these discrepancies are due to the ubiquitous distribution and diversity of $GABA_A$ receptors in the brain. Several studies suggest that the effect of $GABA_A$ receptor activation on ethanol self-administration may be regionally specific, since site-specific injections of muscimol have been shown to produce both increases and decreases in ethanol intake depending on the anatomical location of injection. In addition, the few studies that have examined a role for the $GABA_B$ receptor in ethanol self-administration have provided contradictory results. Thus, the role of the $GABA_B$ receptor in ethanol self-administration remains to be elucidated. It is important to keep in mind that many of the inconsistencies with regard to the role of the GABA receptor system in ethanol self-administration may also be due to genetic and environmental factors that influence ethanol drinking behavior. Nevertheless, these studies suggest that the GABA receptor system modulates ethanol's motivational effects in the self-administration paradigm.

GABA Receptors in Ethanol-Induced CPP and CTA

Place conditioning. Many abused drugs such as amphetamine, heroin, morphine, and cocaine produce a CPP in rodents (Carr, Fibiger, & Phillips, 1989). Interestingly, one of the most commonly abused drugs, ethanol, generally produces a place aversion in rats (Cunningham, 1981; van der Kooy, O'Shaughnessy, Mucha, & Kalant, 1983; Stewart & Grupp, 1986) although there are a few reports of place preference (e.g., Bozarth, 1990). In contrast, several inbred and selectively bred lines of mice have shown a reliable and robust place preference for the environment paired with ethanol (e.g., Chester & Cunningham, 1998; Chester, Risinger, & Cunningham, 1998; Cunningham et al., 1991; Cunningham, Niehus, Malott, & Prather, 1992).

Only one study to date has investigated the role of the GABA receptor system in mediating ethanol-induced CPP in mice (Risinger, Malott, Riley, & Cunningham, 1992). This study showed that administration of Ro 15-4513 during ethanol conditioning trials did not alter the acquisition of place preference. These results, however, are consistent with other studies showing that Ro 15-4513 is not effective in antagonizing other ethanol reward-related behaviors (Schaefer & Michael, 1989; Hiltunen & Jarbe, 1988; Jeffreys, Pournaghash, Glowa, & Riley, 1990; June, June et al., 1992). In addition, several other studies have shown that Ro 15-4513 is ineffective in antagonizing the pharmacological and behavioral effects of ethanol (e.g., Hellevuo & Korpi, 1988). It may be that the intrinsic actions of Ro 15-4513 are only effective in reversing specific effects of ethanol at the $GABA_A$ receptor (see review by Lister & Nutt, 1987)

Taste conditioning. Few studies have investigated the possible involvement of the GABA system in mediating ethanol-induced CTA. Smith, Segal, and Amit (1989) tested the effect of picrotoxin and Ro 15-4513 administration on the acquisition of amphetamine- and ethanol-induced CTA in rats. Picrotoxin selectively attenuated the CTA produced by ethanol, whereas it had no effect on the magnitude of amphetamine-induced CTA. However, Ro 15-4513 attenuated both ethanol- and amphetamine-induced CTA, suggesting that Ro 15-4513 may not be selective for ethanol's motivational effects in the CTA paradigm. The results of this study suggest that activation of the $GABA_A$ receptor may modulate the motivational properties of ethanol responsible for the acquisition of CTA. However, other studies have failed to find an effect of Ro 15-4513 on the magnitude of ethanol-induced CTA (Jeffreys et al., 1990; June, June et al., 1992). Clearly, more studies are needed to resolve this issue.

Measurement of Ethanol's Motivational Effects in Self-Administration vs Conditioning Paradigms

A significant amount of evidence has implicated the GABA receptor system in modulating the motivational properties of ethanol. As previously reviewed, most of the studies that have investigated a role for the GABA receptor system in ethanol's motivational effects have focused on the oral self-administration paradigm. However, relatively few studies have examined manipulations of the GABA receptor system on other ethanol reward-related behaviors. In addition, a potential problem in interpreting self-administration studies is that GABAergic manipulations may be affecting mechanisms involved in consummatory behavior rather than affecting a mechanism mediating ethanol's motivational effects. Indeed, the GABA receptor system has been shown to play a primary role in modulating the consumption of both food and water (see earlier section on GABA receptors and food reward). There is also evidence to suggest that changes in ethanol consumption following GABAergic manipulations may be due to alterations in the palatability of ethanol (Söderpalm & Hansen, 1998). Furthermore, interpretation of the self-administration studies is complicated by the fact that both agonists and antagonists of the GABA receptor system have been shown to reduce the consumption of ethanol. This could be due to the fact that ethanol self-administration is influenced by both rewarding and aversive effects of ethanol, which may be mediated by independent neural mechanisms. Accordingly, changes in self-administration behavior following pharmacological manipulations could be due to either an increase or decrease in ethanol's rewarding or aversive properties. In addition, administration of GABA receptor compounds produce alterations in motor activity (see earlier GABA/locomotor activity section), which can interfere with measurement of the self-administration response.

The experiments in this thesis utilized the place and taste conditioning paradigms to examine the rewarding and aversive properties of ethanol. There are several advantages to

conditioning paradigms relative to other animal models designed to assess a drug's motivational properties. For example, the dose of a drug is controlled by the experimenter rather than the subject, which allows for a more precise assessment of a drug's motivational effects and avoids between- and within-group dose variability. In addition, the expression of place or taste conditioning does not require the presence of the drug. This is important if a drug's pharmacological or behavioral effects may interfere with the measurement of its motivational properties (e.g., motor effects that impair responding). Furthermore, these paradigms avoid possible non-specific effects of an agonist or antagonist on consummatory behavior, which would interfere with interpretation of selective effects on a drug's motivational properties. Another advantage of these paradigms is that they can be used to separately measure both rewarding and aversive drug effects. In this regard, they are also useful for assessing the effects of drugs that may increase or decrease the magnitude of place or taste conditioning, and these drugs can be assessed independently for their own affective properties as a measure of control.

Despite the many advantages of place and taste conditioning paradigms, there are several disadvantages associated with the use of these procedures relative to other models of drug reward. For example, it is often difficult to observe clear dose-response relationships in the place conditioning procedure. Thus, there may be a small range between a drug's minimally effective dose and a dose that produces a maximal effect. There has also been some concern regarding what is being measured in place or taste conditioning paradigms because these procedures measure responses elicited by a secondary reinforcer (taste or tactile CS) in the absence of the drug. For example, in the place conditioning procedure, it has been suggested that a drug's effect on locomotor activity may increase or decrease the level of familiarization with the CS relative to vehicle-treated animals, which could affect the propensity of an animal to approach/avoid the CS during a place conditioning test. Another complicating factor with conditioning paradigms

is that a drug could alter an animal's ability to learn or remember the stimulus properties of the CS, which may produce place or taste conditioning results that do not reflect motivational effects of the drug.

As previously mentioned, conditioning paradigms avoid potential drug effects on motor performance that could interfere with the direct measurement of a drug's motivational effect. However, it is important to keep in mind that conditioned locomotor responses may occur with repeated CS-US pairings, which could interfere with expression of the CR. In the following studies, locomotor activity is simultaneously assessed during expression of place conditioning but not taste conditioning. Thus, it is difficult to know whether reduction in fluid consumption in the taste conditioning studies is specifically due to a conditioned motivational response or to other behavioral CRs that interfere with consumption of the CS.

Rationale

The purpose of the following experiments is to investigate the role of the GABA receptor system in modulating the acquisition of ethanol-induced CPP and CTA in DBA/2J mice. The overall hypothesis for these studies is that ethanol's rewarding and aversive effects are modulated by activation of both $GABA_A$ and $GABA_B$ receptor subtypes. This may be due to a direct action of ethanol on the GABA receptor system or via indirect GABA-mediated changes in other neurotransmitter systems, such as dopamine (see GABA/dopamine section; Harris et al., 1992).

The following studies examined the effects of the selective $GABA_A$ antagonists, bicuculline and picrotoxin, and the selective $GABA_B$ agonist, baclofen, on the acquisition of CPP and CTA in the DBA/2J inbred mouse strain. These mice were chosen because they consistently display a robust CPP with ethanol (e.g., Chester & Cunningham, 1998; Cunningham & Prather, 1992; Cunningham, Niehus, & Noble, 1993; Risinger,

Dickinson, & Cunningham, 1992; Risinger, Malott, Riley, & Cunningham, 1992). In addition, DBA/2J mice are sensitive to ethanol's aversive effects in the CTA paradigm relative to other mouse strains (Horowitz & Whitney, 1975; Risinger & Cunningham, 1992; 1995). The use of inbred mice is advantageous because they are all genetically identical. Thus, in controlled environmental conditions, behavioral differences between groups within an experiment and between experiments can be attributed mostly to the independent variable (e.g., administration of a drug) and not to genotype. However, a disadvantage in using an inbred strain is that generalization of the obtained results to the mouse species as a whole may be limited.

The first series of experiments examined the effects of picrotoxin and bicuculline on the acquisition of ethanol-induced CPP and CTA. These classical $GABA_A$ antagonists were chosen because they are

among the most selective and have high affinity for the $GABA_A$ receptor. In addition, they are among the most effective antagonists *in vivo* because they readily pass through the blood brain barrier (Curtis et al., 1970; Andrews & Johnston, 1979). In addition, picrotoxin and bicuculline have been shown to block many of the effects of ethanol both *in vitro* (Allan & Harris, 1986; Suzdak et al., 1986; Mehta & Ticku, 1988) and *in vivo* (Liljequist & Engel, 1982; Martz et al., 1983). Of particular relevance to the present studies are the findings that picrotoxin reduces ethanol self-administration (Boyle et al., 1993) and ethanol-induced CTA in rats (Smith et al., 1989). Finally, $GABA_A$ receptor antagonists (rather than agonists) were chosen for the first series of experiments because a greater number of studies report effects of $GABA_A$ antagonists on ethanol-reward-related behaviors (primarily self-administration), and the data appear to be more consistent relative to the few studies that have reported effects of selective $GABA_A$ agonists (see Table 1). There also are no reports in the literature showing effects of $GABA_A$ agonists on ethanol-induced place or taste conditioning. Lastly, one drawback of the place conditioning

paradigm is the potentially small range between minimal and maximal drug effects. Thus, it is possible that effects of a $GABA_A$ agonist on ethanol-induced CPP would not be detected due to a ceiling effect, since the predicted outcome with a $GABA_A$ agonist would be enhancement of ethanol-induced CPP.

The second series of experiments examined the effects of the $GABA_B$ agonist, baclofen, on the acquisition of CPP and CTA. Baclofen was chosen because it is the most well-characterized and selective ligand for the $GABA_B$ receptor that is commercially available (see Froestl & Mickel, 1997). This agonist has been shown to alter a number of ethanol-induced behaviors (Martz et al., 1983; Cott et al., 1976; Humeniuk et al., 1993), suggesting that ethanol interacts with the $GABA_B$ receptor to produce many of its behavioral effects. In addition, there is some evidence to suggest that baclofen may alter ethanol's motivational effects in the self-administration paradigm, although the direction of this effect is unclear (Daoust et al., 1987; Smith et al., 1992). Although the $GABA_B$ receptor antagonist, phaclofen, has been reported to reduce many of ethanol's behavioral effects (Allan & Harris, 1989; Humeniuk et al., 1993), this antagonist has been shown to have relatively weak affinity for the $GABA_B$ receptor (Kerr, Ong, Prager, Gynther, & Curtis, 1987) and may have partial agonist properties *in vivo* (Humeniuk et al., 1993). At the time these studies were conducted, a selective $GABA_B$ antagonist was not commercially available. The following experiments are the first to report the effects of the GABA receptor ligands, picrotoxin, bicuculline, and baclofen, on ethanol-induced CPP and CTA in mice. Specific predictions for each experiment will be discussed below.

$GABA_A$ Antagonists Facilitate the Acquisition of Ethanol-Induced Conditioned Place Preference and Taste Aversion in Mice

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This research was supported by NIAAA grants AA07468, AA07702 and AA05489 and a Tartar Trust Fellowship.

Running Head: $GABA_A$ Antagonists and Ethanol Reward and Aversion

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Abstract

The present experiments examined the effects of the $GABA_A$ receptor antagonists, bicuculline and picrotoxin, on the acquisition of ethanol-induced conditioned place preference (CPP) and conditioned taste aversion (CTA) in male DBA/2J mice. Mice in the CPP experiments received four pairings of ethanol (2 g/kg) with a distinctive floor stimulus for a 5-min conditioning session (CS+ sessions). During CS+ sessions, mice also received bicuculline (0.0, 1.0, 3.0, or 5.0 mg/kg) or picrotoxin (2.0 mg/kg) before an injection of ethanol. On intervening days (CS- sessions), the pretreatment injection was always vehicle followed by saline injections that were paired with a different floor type. For the preference test, all mice received saline injections and were placed on a half grid and half hole floor for a 60-min session. Both picrotoxin and the lowest dose of bicuculline (1.0 mg/kg) significantly increased the magnitude of CPP relative to vehicle-treated controls. Picrotoxin alone did not produce place conditioning. Ethanol-stimulated locomotor activity was significantly reduced during conditioning trials with picrotoxin and the higher doses of bicuculline (3.0 and 5.0 mg/kg). For the CTA experiments, mice were adapted to a 2-hr per day water restriction regimen followed by five conditioning trials every 48 hrs. During conditioning trials, subjects received an injection of vehicle, bicuculline (1.0 and 4.0 mg/kg), or picrotoxin (0.75 and 2.5 mg/kg) before injection of 2 g/kg (picrotoxin study) or 3 g/kg (bicuculline study) ethanol or saline following 1-hr access to a saccharin solution. Bicuculline did not alter ethanol-induced CTA; however, picrotoxin dose-dependently increased the magnitude of ethanol-induced CTA. Bicuculline and picrotoxin did not produce CTA when administered alone. Overall, these results suggest that blockade of $GABA_A$ receptors with bicuculline and picrotoxin enhances

ethanol's rewarding effects in the CPP paradigm; however, only picrotoxin enhances ethanol's aversive effects in the CTA paradigm.

Key Words: Alcohol, DBA/2J, Reward, Aversion, GABA, Locomotor Activity, Place Conditioning, Taste Conditioning

Introduction

Biochemical, electrophysiological and behavioral studies have shown that ethanol exerts many of its pharmacological and behavioral effects through an interaction with the $GABA_A$ receptor (see Ticku, 1990; Korpi, 1994; Mihic & Harris, 1996 for reviews). Despite the many studies implicating the $GABA_A$ receptor in modulating ethanol's effects, relatively few studies have examined the role of the $GABA_A$ receptor in the motivational effects of ethanol.

To date, much of the evidence implicating the $GABA_A$ receptor in ethanol's motivational effects comes from studies examining the effects of $GABA_A$ antagonists on ethanol self-administration in rats. For example, ethanol self-administration is reduced with administration of the chloride channel blockers picrotoxin (Boyle et al., 1993) and isopropyl-bicyclophosphate (IPPO), a picrotoxin-type ligand (Rassnick et al., 1993). In addition, many studies using several different types of self-administration procedures have demonstrated that acute administration of $GABA_A$/benzodiazepine receptor partial inverse agonists reduce ethanol consumption in outbred rats (Samson et al., 1987; June et al., 1991; Rassnick et al., 1993; Buczek et al., 1997) and rats selectively bred to prefer alcohol (McBride et al., 1988; Balakleevsky et al., 1990; Wegelius et al., 1994; June et al., 1994; 1996). Other studies have reported reductions in ethanol self-administration following microinjections of a potent $GABA_A$ receptor antagonist, SR95531, into the extended amygdala (Hyytiä & Koob, 1995) and a partial inverse agonist, Ro 19-4603, into the nucleus accumbens in ethanol-preferring P rats (June et al., 1998).

In contrast to the self-administration studies, evidence is sparse for $GABA_A$ receptor modulation of ethanol's motivational effects in other drug reward paradigms, such as taste aversion and place preference conditioning. For example, one study has reported that picrotoxin selectively reduces ethanol-induced conditioned taste aversion (CTA) in rats (Smith et al., 1989). However, other studies have shown that ethanol-induced CTA is not

altered with the partial inverse agonist, Ro 15-4513 (Jeffreys et al., 1990; June et al., 1992). Only one study has investigated the role of the $GABA_A$ receptor in mediating ethanol-induced conditioned place preference (CPP) in mice (Risinger et al., 1992). In this study, Ro 15-4513 administered during conditioning trials did not alter the acquisition of CPP in DBA/2J mice. These results, however, are consistent with other studies showing that Ro 15-4513 is not effective in antagonizing other ethanol reward-related behaviors (Schaefer & Michael, 1989; Hiltunen & Jarbe, 1988). Clearly, more studies are needed to resolve this issue.

As previously discussed, most of the studies that have investigated a role for the GABA receptor system in ethanol's motivational effects have utilized the oral self-administration paradigm. However, a potential problem in interpreting the self-administration studies is that GABAergic manipulations may be affecting mechanisms involved in consummatory behavior rather that affecting a mechanism modulating ethanol's motivational properties. In fact, $GABA_A$/ benzodiazepine partial inverse agonists have been shown to decrease the consumption of a palatable diet in non-deprived rats (Cooper, 1986). In addition, ethanol self-administration may be influenced by both rewarding and aversive effects of ethanol, which may be mediated by independent neural mechanisms. Accordingly, changes in self-administration behavior following pharmacological manipulations could be due to either an increase or decrease in ethanol's rewarding or aversive properties.

The present experiments used the place and taste conditioning paradigms to examine the effects of $GABA_A$ receptor antagonists on the rewarding and aversive properties of ethanol. There are several advantages to the place and taste conditioning procedures relative to the oral self-administration paradigm. For example, the dose of a drug is controlled by the experimenter rather than the subject, which allows for a more precise assessment of a drug's motivational effects and avoids between and within-group dose

variability. Furthermore, these paradigms avoid interpretive problems regarding possible non-specific effects of an agonist or antagonist on consummatory behavior, because pharmacological agents are not administered during expression of place or taste conditioning. Another advantage of these paradigms is that they can be used to separately measure both rewarding and aversive effects of ethanol. In this regard, they are also useful for assessing the effects of drugs that may increase or decrease the magnitude of place or taste conditioning, and these drugs can be assessed independently for their own motivational properties as a measure of control.

The purpose of the following four experiments was to examine the effects of two $GABA_A$ receptor antagonists, picrotoxin and bicuculline, on the acquisition of ethanol-induced CPP and CTA. It was hypothesized that $GABA_A$ receptor activation is an important factor modulating ethanol's rewarding and aversive effects during conditioning. Based on previous findings (Boyle et al., 1993; Smith et al., 1989), administration of picrotoxin or bicuculline before conditioning trials with ethanol was expected to reduce the magnitude of both ethanol-induced CPP and CTA.

Method

Subjects

Subjects in all experiments were adult male inbred mice (DBA/2J) obtained from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. For the place conditioning studies, mice were housed in polycarbonate cages (27.9 x 9.5 x 12.7 cm) in groups of four. For the taste conditioning studies, mice were housed individually in hanging stainless-steel cages (12 x 18 x 18) with wire mesh fronts and bottoms. Animals were allowed to acclimate to the colony room for 12-14 days before training. During place conditioning, animals were allowed free access to food and water. During taste conditioning, lab chow was continuously available; however, daily access to fluids was

restricted according to the procedure described below. Ambient temperature was maintained at 21± 1° C. Experimental procedures were conducted during the light phase of a 12:12 light/dark cycle (lights on at 0700).

Apparatus

The place conditioning apparatus consisted of twelve identical acrylic and aluminum boxes (30 x 15 x 15 cm) that were separately enclosed in ventilated, light and sound-attenuating chambers (Coulbourn Model E10-20). Six sets of infrared light sources and photodetectors were mounted opposite each other at 5-cm intervals along the length of each box, 2.2 cm above the floor. Occlusion of the infrared light beams was used to measure general activity and location of the animal (left or right) within the box. Total activity counts were recorded every minute by computer (10 msec resolution). The floor of each box consisted of interchangeable halves of one of two distinct textures. "Grid" floors consisted of 2.3 mm stainless steel rods mounted 6.4 mm apart in acrylic rails. "Hole" floors consisted of perforated 16 gauge stainless steel with 6.4 mm round holes on 9.5 mm staggered centers. This combination of floor textures was selected on the basis of previous studies showing that drug-naive DBA/2J mice spend approximately equal time on each floor type during drug-free preference tests (Cunningham, Niehus, et al., 1992; Cunningham & Noble, 1992; Cunningham, 1995). The floors and the inside of the boxes were wiped with a damp sponge and the litter paper beneath the floors was changed between animals.

The taste conditioning experiments were conducted in the home cages. Water and saccharin solutions were presented at room temperature in 25 ml graduated glass cylinders fitted with stainless-steel drinking spouts inserted through the front of the cage. Consumption was measured to the nearest 0.1 ml and was corrected for evaporation and spillage by subtracting the mean fluid loss measured in two drinking tubes placed on empty cages for an equal amount of time.

Drugs

Ethanol (20% v/v) was prepared from a 95% stock solution using saline as the vehicle. The dose of ethanol was varied by manipulating the injection volume. Picrotoxin (Research Biomedicals International, Natick, MA) was dissolved in saline and (+) Bicuculline (Sigma Chemical Company, St. Louis, MO) was dissolved in two drops of glacial acetic acid (Sigma) and saline. Picrotoxin and bicuculline were administered intraperitoneally in an injection volume of 10 ml/kg. Vehicle-treated groups in the bicuculline experiments received saline adjusted with 2 drops of glacial acetic acid. Bicuculline was made fresh daily and kept on ice during experimental procedures to minimize its conversion to less active compounds (Olsen, Ban, Miller, & Johnston, 1975).

Procedure

Place Conditioning Studies. The place conditioning studies involved one habituation session, eight conditioning sessions, and one test session. A 2-day weekend break occurred between the first four and last four conditioning sessions. For the habituation session, mice received an injection of saline immediately before being placed in the conditioning box for 5 min on a smooth paper floor.

For conditioning, mice were randomly assigned to one of two conditioning subgroups (G+ or G-) within each drug treatment group, and exposed to a Pavlovian differential conditioning procedure. On alternating days, mice in the G+ group received an intraperitoneal (IP) injection of ethanol (2 g/kg; 12.5 ml/kg) immediately before a 5 min session on the grid floor (CS+ sessions). On intervening days, these mice received saline immediately before exposure to the hole floor (CS- sessions). Conversely, mice in the G- group received ethanol paired with the hole floor and saline paired with the grid floor. During conditioning trials, all mice had access to both sides of the apparatus on a homogeneous floor type. Conditioning subgroups were matched for overall exposure to CS type (grid or hole) and drug treatment, and the order of drug exposure was

counterbalanced within groups. Thus, this procedure provides control over exposure to both the CS (floor type) and the US (ethanol) in both G+ and G- subgroups, with subgroups differing only in the specific floor-ethanol pairing (Cunningham, 1993). The dose of ethanol (2 g/kg) was chosen based on previous studies showing that it produced a strong preference for the paired tactile stimuli (e.g., Cunningham et al., 1991; Cunningham & Prather, 1992). The 5 min session duration was chosen based on previous studies showing that it produced a stronger conditioned place preference with ethanol in DBA/2J mice than did longer session durations (Cunningham & Prather, 1992).

For the bicuculline study, mice were randomly assigned to one of four bicuculline dose groups: 0.0 (vehicle), 1.0, 3.0, and 5.0 mg/kg (n=20-24 per group). All mice received two IP injections before each conditioning session. On CS+ sessions, G+ subjects received an injection of 0.0 (vehicle), 1.0, 3.0, or 5.0 mg/kg bicuculline immediately before an injection of 2 g/kg ethanol and were placed on the grid floor for a 5 min session. Bicuculline was administered immediately before ethanol to avoid bicuculline-induced convulsions, particularly in the higher bicuculline dose groups, which have been shown to occur within 1-2 min following injection (Schecter & Tranier, 1977; Freund, Marley, & Wehner, 1987; Phillips et al., 1989). Previous studies have shown these doses of bicuculline are within the range shown to attenuate ethanol's behavioral effects (Liljequist & Engel, 1982; Dudek & Phillips, 1989). On CS- sessions, these mice received two back-to-back vehicle/saline injections before a 5 min session on the hole floor. Conversely, G- subjects received vehicle/ethanol or bicuculline/ethanol paired with the hole floor and saline injections paired with the grid floor.

For the picrotoxin study, mice were assigned to three drug treatment groups: PICRO (n=28), ETOH (n=30), and PICRO/ETOH (n=30). The design of this study differed from the bicuculline experiment in order to include a control group (PICRO) to assess the possible rewarding or aversive effects of picrotoxin alone. The dose of

picrotoxin (2 mg/kg) and pretreatment interval was chosen because they are within the range shown to alter ethanol's behavioral effects (Liljequist & Engel, 1982). All mice received two IP injections before each conditioning session. On CS+ sessions, G+ subjects in the PICRO and PICRO/ETOH groups received picrotoxin (2 mg/kg) 15 min before the conditioning session, and the ETOH group received an injection of saline. A saline (PICRO group) or ethanol injection was given immediately before a 5 min session on the grid floor. On CS- sessions, these mice received two saline injections 15 min apart before a 5 min session on the hole floor. Conversely, G- subjects received picrotoxin/saline, saline/ethanol, or picrotoxin/ethanol paired with the hole floor and saline/saline paired with the grid floor.

Prior to the 60-min test sessions, all mice received two injections of vehicle/saline back-to-back (bicuculline study) or saline/saline 15 min apart (picrotoxin study) to match handling and injection cues during conditioning days. The floor of each box was half grid and half hole with left/right position counterbalanced within groups.

Taste Conditioning Studies. Subjects were adapted to a water restriction schedule (2 h water per day) over a 7-day period. At 48-h intervals over the next 10 days, all mice received 1-h access to a solution of saccharin (0.15% w/v sodium saccharin in tap water). Subjects were weighed daily before experimental procedures began. No subjects were excluded from studies based on body weight criteria (≥25% loss of body weight prior to first water restriction day).

For the bicuculline study, mice were randomly assigned to one of five drug treatment groups: vehicle/saline (V/S; n=12), bicuculline (1.0 mg/kg)/saline [B(1.0)/S; n=12], vehicle/ethanol (V/E; n=10), bicuculline (1.0 mg/kg)/ethanol [B(1.0)/E; n=10], and bicuculline (4.0 mg/kg)/ethanol [B(4.0)/E; n=4]. Immediately after 1-h access to saccharin, mice received injections of vehicle or bicuculline immediately before injections of saline or ethanol (3.0 g/kg). The dose of ethanol was chosen in order to test a wider

dose range of bicuculline and to avoid bicuculline-induced convulsions (Phillips et al., 1989). The drug treatment groups were similar for the picrotoxin study. Mice were randomly assigned to one of five drug treatment groups (n=12/group): saline/saline (S/S), picrotoxin (0.75 mg/kg)/saline [P(0.75)/S], saline/ethanol (S/E), picrotoxin (0.75 mg/kg)/ethanol [P(0.75)/E], and picrotoxin (2.5 mg/kg)/ethanol [P(2.5)/E]. Immediately after 1-h access to saccharin, mice received injections of saline or picrotoxin 15 min before injections of saline or ethanol (2.0 g/kg). All mice also received 30-min access to tap water 5 h after each saccharin access period, in order to prevent dehydration. Two-h access to tap water was given during intervening days.

Blood Ethanol Concentration Analyses. Separate groups of naive DBA/2J mice were used to determine the effect of picrotoxin on blood ethanol concentration at three time points after injection of 2 g/kg ethanol. Mice received an injection of saline (n=9), 0.75 mg/kg picrotoxin (n=8), or 2.5 mg/kg picrotoxin (n=9) 15 min before an injection of ethanol. At 15, 60, and 120 min after the ethanol injection, mice were removed from the home cage and a 20 µl sample of blood was taken from a small cut at the tip of the tail. Samples were prepared and analyzed by gas chromatography as previously described elsewhere (Crabbe et al., 1982).

Statistical Analyses

Data were analyzed by analysis of variance (ANOVA) with the alpha level set at 0.05. All probability values reported for followup comparisons (post-hoc F tests) are Bonferroni corrected.

Results

Effects of Bicuculline on Ethanol-Induced CPP

Five subjects died (4 from 5.0 mg/kg and 1 from 0.0 mg/kg group) and one subject received incorrect drug treatment (0.0 mg/kg group). Data from these subjects were

excluded from all analyses. Due to equipment failure, data from one additional subject (1.0 mg/kg group) were excluded from conditioning trial analyses. Chi-square analysis indicated a significant loss of subjects due to drug treatment in the 5.0 mg/kg dose group ($\chi^2 = 11.5$, df=3).

Conditioning. Figure 1 shows mean activity counts per min during conditioning trials 1-4 averaged across each bicuculline dose group. Ethanol produced significant locomotor activation during CS+ sessions in the 0.0 mg/kg group relative to CS- sessions with saline. The lowest dose of bicuculline (1.0 mg/kg) did not alter ethanol-stimulated activity relative to the 0.0 mg/kg group. However, the higher doses of bicuculline (3.0 and 5.0 mg/kg) produced dose-dependent reductions in ethanol-stimulated activity.

Insert Figure 1 about here

Overall analysis of CS+ session data (Dose x Trials ANOVA) showed a significant effect of Dose [$F(3,86)=20.7$, $p<0.001$]. No effect of Trials or Dose x Trials interaction was found ($ps \geq 0.2$). Followup comparisons of drug treatment groups showed significant differences between all bicuculline dose groups ($ps \leq 0.05$), except between 0.0 and 1.0 mg/kg groups ($p=1.0$). Two-way ANOVA of CS- session data yielded a significant effect of Trials [$F(3,255)=5.5$, $p=0.001$], indicating habituation to experimental procedures across trials. No effect of Dose [$F(3,85)=2.3$, NS] or interaction [$F(9,255)=1.2$, NS] was found.

Preference Testing. Figure 2 shows the mean (±sem) sec per min spent on the grid floor collapsed across the 60-min session by both G+ and G- conditioning subgroups in each bicuculline dose group. G+ subgroups spent significantly more time on the grid floor relative to G- subgroups, indicating the development of ethanol-induced preference for the grid floor. The 1.0 mg/kg bicuculline dose group showed a larger magnitude of preference

relative to the 0.0 mg/kg group, suggesting that the lowest dose of bicuculline enhanced ethanol-induced preference. This enhancement was not observed at the higher bicuculline doses (3.0 and 5.0 mg/kg).

Insert Figure 2 about here

Two-way ANOVA (Dose x Conditioning Group) yielded a significant effect of Conditioning Group [$F(1,82)=64.5$, $p<0.001$] and Dose x Conditioning Group interaction [$F(3,82)=2.9$, $p<0.05$]. To investigate the interaction, followup ANOVAs were conducted comparing each bicuculline dose group (1.0, 3.0 and 5.0 mg/kg) with the 0.0 mg/kg group. Only the comparison of 0.0 and 1.0 mg/kg groups showed a significant Dose x Conditioning Group interaction [$F(1,42)=7.1$, $p=0.01$]. Followup comparisons between G+ and G- subgroups were significant within both the 0.0 mg/kg ($p=0.003$) and 1.0 mg/kg ($p<0.001$) groups. Comparisons of 3.0 and 5.0 mg/kg groups with 0.0 mg/kg group were not significant ($Fs<1$). These analyses indicate that administration of the lowest dose of bicuculline (1.0 mg/kg) during conditioning trials increased magnitude of preference relative to the saline (0.0 mg/kg) group. The higher doses of bicuculline (3.0 and 5.0 mg/kg) did not alter the magnitude of ethanol-induced preference.

Mean (±sem) activity counts per min during the 60-min test were 28.6±1.8, 26.8±1.1, 25.2±1.6, and 28.3±2.7 for the 0.0, 1.0, 3.0, and 5.0 mg/kg bicuculline groups, respectively. One-way ANOVA showed no significant effect of Bicuculline Dose on test activity levels [$F(3,86)= 0.7$, NS].

Effects of Picrotoxin on Ethanol-Induced CPP

Conditioning. Figure 3 shows mean activity counts per min during conditioning trials 1-4 averaged across each drug treatment group. Ethanol produced significant

locomotor activation during CS+ sessions in the ETOH group relative to CS- sessions with saline. Picrotoxin significantly reduced ethanol-stimulated activity during CS+ sessions in the PICRO/ETOH group relative to the ETOH group. Picrotoxin alone also reduced locomotor activity during CS+ sessions in the PICRO group relative to saline during CS- sessions.

Insert Figure 3 about here

Because the performance of the ETOH group compared to the PICRO/ETOH group was of primary interest, these data were included in one set of analyses. Separate analyses were conducted for the PICRO control group to determine whether picrotoxin alone altered locomotor activity or produced place conditioning. Analysis of CS+ session data for ETOH and PICRO/ETOH groups (two-way ANOVA: Drug Treatment x Trials) yielded significant main effects of Drug Treatment [$F(1,58) = 132.7$, $p < 0.001$] and Trials [$F(3,174) = 5.7$, $p=0.001$]. The interaction was not significant. The effect of Trials indicates that locomotor sensitization to ethanol's stimulant effects occurred across the four conditioning trials. The lack of significant interaction indicates that picrotoxin did not alter ethanol-induced locomotor sensitization. The CS- session ANOVA for ETOH and PICRO/ETOH groups showed a significant effect of Drug Treatment [$F(1,56) = 4.2$, $p=0.05$], Trials [$F(3,168) = 20.1$, $p<0.001$] and Drug Treatment X Trials interaction [$F(3,168)=4.7$, $p<0.01$]. The interaction appeared to be due to a greater decrease in saline activity levels in the PICRO/ETOH group relative to the ETOH group across CS- trials. Followup analyses of Drug Treatment at each CS- conditioning trial revealed a significant effect of Drug Treatment on trials 3 [$F(1,58)=12.4$, $p=0.001$] and 4 [$F(1,58)=4.2$, $p<0.05$], but not on trials 1 and 2.

Separate two-way ANOVA (Drug Type x Trials) of CS+ and CS- conditioning trial activity data for the PICRO group also showed significant effects of Drug Type [$F(1,27)= 4.6$, $p<0.05$], Trials [$F(3,81)=57.0$, $p=0.001$], and interaction [$F(3,81)=2.7$, $p=0.05$]. This analysis indicates that picrotoxin on CS+ sessions reduced locomotor activity relative to saline on CS- sessions. Followup analyses of Trials within each drug type showed a significant effect of Trials for CS- session data [$F(3,81)=9.2$, $p<0.001$], but not for CS+ session data.

Preference Testing. The amount of time spent on the grid floor in both the G+ and G- subgroups was nearly constant throughout the test session. Therefore, the data were collapsed across the 60-min session. Figure 4 shows the mean (±sem) sec per min spent on the grid floor by both G+ and G- conditioning subgroups in each drug treatment group. G+ subgroups in the ethanol-treated groups spent significantly more time on the grid floor relative to G- subgroups, indicating the development of ethanol-induced preference for the grid floor. Moreover, the PICRO/ETOH group showed a larger magnitude of preference relative to the ETOH group, suggesting that picrotoxin enhanced ethanol-induced preference. Picrotoxin alone did not produce place conditioning in the PICRO group.

Insert Figure 4 about here

Two-way ANOVA (Drug Treatment x Conditioning Group) of ethanol-treated groups yielded a significant effect of Conditioning Group [$F(1,56)=34.0$, $p < 0.001$] and a Drug Treatment x Conditioning Group interaction [$F(1,56)=5.3$, $p=0.03$]. The effect of Conditioning Group signifies a conditioned place preference for the ethanol-paired grid floor, and the interaction indicates that picrotoxin administered during conditioning trials increased the magnitude of preference relative to the ETOH group. Followup comparisons of G+ and G- subgroups within each ethanol-treated group were significant in both the

ETOH group ($p=0.03$) and PICRO/ETOH group ($p<0.001$). Separate one-way ANOVA of the PICRO group data showed no effect of Conditioning Group ($F<1$), indicating that picrotoxin alone did not produce place preference or aversion for the drug-paired floor.

Mean (±sem) activity counts per min during the 60-min test were 34.3±1.3, 36.8±1.3, and 31.2±1.0 for the PICRO, ETOH, and PICRO/ETOH groups, respectively. One-way ANOVA showed a significant effect of Drug Treatment on activity levels during the 60-min test [$F(2,85)= 5.5$, $p<0.01$]. Followup pairwise comparisons of each Drug Treatment group showed a significant difference between ETOH and PICRO/ETOH groups only ($p<0.01$). No other comparisons were significant.

Effects of Bicuculline on Ethanol-Induced CTA

Mean (±sem) consumption of saccharin on trial 1 (before conditioning) for each drug treatment group was 2.79±0.14, 2.83±0.11, 2.72±0.10, 2.77±0.17, and 2.83±0.05 for V/S, B(1.0)/S, V/E, B(1.0)/E and B(4.0)/E, respectively. One-way ANOVA of trial 1 intakes indicated no significant difference between groups in preconditioning consumption of saccharin ($p>0.9$). Nevertheless, to offset minor initial differences in saccharin intake and facilitate presentation of the data, difference scores were calculated for each subject by subtracting the ml of saccharin consumed on trial 1 from the amount consumed on subsequent conditioning trials. Figure 5 shows mean difference scores for each drug treatment group across conditioning trials 2-6. Twelve subjects died during the course of the experiment [2 from V/E, 2 from B(1.0)/E, and 8 from B(4.0)/E] and were removed from all analyses. Chi-square analysis indicated a significant loss of subjects due to drug treatment in the B(4.0)/E group ($\chi^2 = 9$, $df=2$).

Insert Figure 5 about here

Ethanol-saccharin pairings produced reductions in saccharin intake across trials, indicating the development of CTA in group V/E. Two-way ANOVA of V/S and V/E groups (Drug Treatment x Trials) showed significant effects of Drug Treatment [$F(1,20)=62.8$, $p<0.001$], Trials [$F(4,80)=12.1$,$p<0.001$], and interaction [$F(4,80)=28.2$, $p<0.001$], signifying the development of ethanol-induced CTA across trials in the V/E group. All ethanol-treated groups [V/E, B(1.0)/E, B(4.0)/E] showed a similar development of CTA across conditioning trials. Two-way ANOVA of ethanol-treated groups (Drug Treatment x Trials) showed a significant effect of Trials [$F(4,84)=49.5$, $p<0.001$], but no effect of Drug Treatment or interaction ($ps>0.1$). This analysis indicates that bicuculline (1.0 or 4.0 mg/kg) did not alter the acquisition of ethanol-induced CTA. A separate two-way ANOVA of V/S and B(1.0)/S showed a significant effect of Trials [$F(4,88)=4.8$, $p=0.001$], but no effect of Drug Treatment or interaction, indicating that bicuculline (1.0 mg/kg) did not produce a CTA. In general, the effect of Trials appears to be due to an initial decrease in consumption after conditioning trial 1 followed by an increase over trials.

Effects of Picrotoxin on Ethanol-Induced CTA

Mean (±sem) consumption of saccharin on trial 1 for each drug treatment group was 3.00±0.13, 2.66±0.10, 2.99±0.09, 2.66±0.19, and 2.86±0.12 for S/S, P(0.75)/S, S/E, P(0.75)/E and P(2.5)/E, respectively. One-way ANOVA of trial 1 intakes indicated no significant difference between groups in initial consumption of saccharin ($p>0.1$). Consistent with the bicuculline study, data are presented as difference scores to offset minor initial differences in saccharin consumption. Figure 6 shows mean difference scores for each drug treatment group across conditioning trials 2-6.

Insert Figure 6 about here

Ethanol-saccharin pairings produced reductions in saccharin intake across trials, indicating the development of CTA in group S/E. Two-way ANOVA of S/S and S/E groups (Drug Treatment x Trials) showed significant effects of Drug Treatment [$F(1,22)=18.1$, $p<0.001$] and a Drug Treatment x Trials interaction [$F(4,88)=3.2$, $p=0.02$], signifying the development of ethanol-induced CTA across trials in the S/E group. Picrotoxin produced a dose-dependent enhancement of ethanol-induced CTA [P(0.75)/E and P(2.5)/E groups], but did not produce a CTA when administered alone [P(0.75)/S group]. Analysis of ethanol-treated groups (Drug Treatment x Trials ANOVA) showed a significant effect of Drug Treatment [$F(2,33)=34.7$, $p<0.001$], Trials [$F(4,132)=66.9$, $p<0.001$], and a Drug Treatment x Trials interaction [$F(8,132)=11.5$, $p<0.001$]. Followup one-way ANOVAs showed significant effects of Drug Treatment on each conditioning trial [all $Fs(2,33)\geq 8.0$, $ps\leq 0.001$]. Followup pairwise comparisons of drug treatment groups showed significant differences between all ethanol-treated groups on each conditioning trial (all $ps<0.05$), except between the S/E and P(0.75)/E group on conditioning trials 2, 3, and 5. A separate two-way ANOVA of S/S and P(0.75)/S groups showed no significant main effects or interaction ($ps>0.2$), indicating that administration of picrotoxin alone (0.75 mg/kg) did not produce a CTA.

Effects of Picrotoxin on Blood Ethanol Concentration

Table 1 shows mean (±sem) blood ethanol concentrations (mg/ml) at 15, 60, and 120 min following 2 g/kg ethanol in mice pretreated with saline or picrotoxin (0. 75 and 2.5 mg/kg). Blood ethanol concentration continued to rise after the 15 min time point followed by a decrease at the 120 min time point. Two-way ANOVA (Drug Pretreatment

X Time) showed a main effect of Time [$F(2,46)=29.6$, $p<0.00$], but no effect of Drug Pretreatment or interaction, indicating that picrotoxin did not alter ethanol pharmacokinetics.

Discussion

The present experiments examined a role for the $GABA_A$ receptor in modulating ethanol's rewarding and aversive effects in the CPP and CTA paradigms. The results of the CPP studies showed that the $GABA_A$ antagonists, bicuculline and picrotoxin, facilitated the acquisition of ethanol-induced CPP. The CTA studies showed that ethanol-induced CTA was dose-dependently increased by picrotoxin, but not by bicuculline.

The enhancement of ethanol-induced CPP with picrotoxin and bicuculline is opposite to the predicted outcome that blockade of $GABA_A$ receptors would reduce the magnitude of ethanol-induced CPP. This prediction was based on previous studies showing that picrotoxin reduces other ethanol-reward related behaviors (Boyle et al., 1993; Smith et al., 1989). Taken together, these results suggest that $GABA_A$ receptor blockade increases ethanol's rewarding properties in the place conditioning paradigm. The bicuculline CPP experiment showed that only the lowest dose of bicuculline (1.0 mg/kg) increased the magnitude of CPP. Consistent with the effects of bicuculline, the picrotoxin CPP experiment showed that picrotoxin (2.0 mg/kg) enhanced ethanol-induced CPP and did not produce place conditioning when administered alone. The lack of place conditioning in the picrotoxin control group suggests that this dose of picrotoxin does not possess motivational properties in DBA/2J mice in the place conditioning paradigm. Thus, picrotoxin's enhancement of ethanol-induced CPP cannot be explained in terms of a summation of rewarding effects produced separately by each drug. Rather, this outcome appears to reflect a selective effect of picrotoxin on ethanol's rewarding properties. Future studies will need to assess the possible rewarding or aversive effects of 1.0 mg/kg

bicuculline alone. Although picrotoxin did not produce place conditioning in the present study, previous studies have shown that picrotoxin produces a place aversion in rats (Spyraki et al., 1985; Acquas et al., 1989). However, differences in the place conditioning procedure or a species difference in sensitivity to an aversive motivational effect of picrotoxin may account for these findings. It should also be mentioned that the increase in magnitude of CPP in the PICRO/ETOH group may related to the finding that activity levels during the preference test were significantly lower in the PICRO/ETOH group relative to the ETOH group. However, this explanation cannot account for the increased magnitude of CPP with 1.0 mg/kg bicuculline, since no differences in preference test activity were seen in the bicuculline CPP study.

It is interesting that higher doses of bicuculline (3.0 and 5.0 mg/kg) did not alter the magnitude of ethanol-induced CPP. One possibility is that higher doses of bicuculline produce a place aversion that interferes with the expression of ethanol conditioned preference. Unfortunately, it is not possible to test this idea because repeated administration of these doses of bicuculline alone produces severe convulsions and death in mice (Freund et al., 1987; Engstrom & Woodbury, 1988; Phillips et al., 1989). Another possible reason for this dose effect is that bicuculline increases ethanol's rewarding properties only in a low dose range. The mechanism for such an effect is unknown, but may be due to differential sensitivity to bicuculline in certain neuronal populations located in reward-related pathways. For example, several studies have reported differential sensitivity of dopamine versus non-dopamine (e.g., GABA) cells in the ventral mesencephalon to GABA agonists. Specifically, GABA and muscimol have been shown to preferentially inhibit non-dopamine versus dopamine neurons in both *in vivo* and *in vitro* preparations (Grace & Bunney, 1979; Waszczak, Eng, & Walters, 1980; Klitenick et al., 1992). Furthermore, Klitenick et al. observed a biphasic dose-response curve for extracellular dopamine with increasing doses of muscimol, possibly due to a greater

number of $GABA_A$ receptors on non-dopamine relative to dopamine neurons (Churchill, Dilts, et al., 1992). A similar mechanism may underlie the observed dose-response relationship for bicuculline's effect on ethanol-induced CPP. This could also be the mechanism by which picrotoxin enhances ethanol-induced CPP; however, a wider range of picrotoxin doses should be tested to determine if a similar dose-response relationship exists for both $GABA_A$ antagonists. Furthermore, lower doses of bicuculline (below 1.0 mg/kg) should be examined in order to determine the full dose-response pattern for bicuculline's effect on ethanol-induced CPP.

Unlike the CPP studies, picrotoxin and bicuculline did not have the same effect on ethanol-induced CTA. Bicuculline did not alter ethanol-induced CTA at any dose, whereas picrotoxin dose-dependently increased the magnitude of CTA. The picrotoxin effect is opposite to the prediction that $GABA_A$ receptor blockade would attenuate ethanol CTA. Measurement of blood ethanol concentrations indicate that the enhancement of ethanol-induced CTA is not due to an effect of picrotoxin on ethanol pharmacokinetics. This is also supported by other studies showing that picrotoxin does not alter ethanol pharmacokinetics in mice (Koechling et al., 1991). The outcome of the picrotoxin CTA experiment is inconsistent with a previous study that showed picrotoxin selectively reduces ethanol-induced CTA in rats (Smith et al., 1989). This discrepancy is possibly due to a species difference in sensitivity to ethanol and/or picrotoxin. Alternatively, the discrepancy could also be due to procedural differences in the CTA paradigm. For example, Smith et al. administered picrotoxin 30 min before animals received 20 min access to a saccharin solution that was immediately followed by ethanol. In the present study, picrotoxin was administered immediately after 1 hr access to a saccharin solution and 15 min before ethanol. Thus, in the Smith et al. study, it is possible that picrotoxin altered the stimulus properties of saccharin which could have disrupted conditioning, resulting in the observed attenuation of CTA.

The lowest doses of bicuculline and picrotoxin did not produce a CTA when administered alone. This is consistent with several studies that failed to find a CTA in rats with picrotoxin (Bures & Buresova, 1989; Smith et al., 1989) or bicuculline (Bures & Buresova, 1989). The lack of taste conditioning in the picrotoxin control group (0.75 mg/kg) suggests that the effect of 0.75 mg/kg picrotoxin on ethanol-induced CTA is not due to a summation of an aversive motivational effect of picrotoxin alone with ethanol's aversive effects. Rather, these data suggest that picrotoxin selectively enhances ethanol's aversive properties in the taste conditioning paradigm. The mechanism for this effect is unclear, but it may be the same mechanism that was suggested to underlie the enhancement of CPP (e.g., a GABA/dopamine interaction). Indeed, there is evidence to suggest that dopamine modulates ethanol-induced CTA, because dopamine antagonists have been shown to reduce the acquisition of CTA (Risinger, 1994).

A role for the $GABA_A$ receptor in modulating ethanol's motivational effects has been most often reported in studies with rats using the oral self-administration paradigm. The results of the present experiments suggest that reductions in ethanol consumption produced by $GABA_A$ antagonists in previous studies may be due to an increase in either the rewarding or the aversive effects of ethanol in the self-administration paradigm. Thus, rats may consume less ethanol because $GABA_A$ antagonists increase sensitivity to ethanol's rewarding effects, thereby reducing the amount of ethanol needed to obtain the same motivational effect. Alternatively, blockade of $GABA_A$ receptors may increase ethanol's aversive effects, resulting in a decrease in ethanol consumption. Another interpretation of the self-administration data is that the effect of $GABA_A$ antagonists are unrelated to ethanol's motivational effects. Several studies have reported that GABA/benzodiazepine agents alter taste mechanisms (Berridge & Treit, 1986), food consumption (Cooper, 1980; 1986), and the palatability of ethanol (Söderpalm & Hansen, 1998). Thus, the reduction in ethanol self-administration may be accounted for by an alteration in ethanol's taste or

orosensory properties. Finally, it is important to consider that the effects of $GABA_A$ antagonists on ethanol self-administration may not be related to their effects on ethanol-induced CPP and CTA, since separate neural mechanisms may modulate ethanol's motivational effects in these paradigms.

The finding that bicuculline failed to enhance ethanol-induced CTA may be interpreted in several ways. One possibility is that picrotoxin and bicuculline have different pharmacological actions on ethanol's aversive effects in the CTA paradigm. This may be related to the fact that bicuculline and picrotoxin differ with respect to blockade of GABA-binding to $GABA_A$ receptors in the brain (Zukin, Young, & Snyder, 1974; Simmonds, 1980) and release of $[H^3]$-GABA from brain slices (Johnston & Mitchell, 1971). In addition, there is evidence to suggest that bicuculline also produces changes in calcium currents (Heyer, Nowak, & McDonald, 1981; Johnson & Seutin, 1997) and inhibits the production of acetylcholinesterase (Olsen, Ban, & Miller, 1976). Bicuculline and picrotoxin also differ with regard to their mechanism of seizure production in mice (Schechter & Tranier, 1977; Engstrom & Woodbury, 1988). It is also important to keep in mind that the difference between picrotoxin and bicuculline's effect on ethanol-induced CTA could be due to variations in $GABA_A$ receptor subunit composition throughout the brain. For example, the brain pathway(s) mediating ethanol-induced CTA may contain a higher proportion of $GABA_A$ receptor subunits preferentially sensitive to picrotoxin binding. Indeed, regional differences in $GABA_A$ receptor sensitivities to bicuculline and picrotoxin have been reported (Krnjevic, 1974).

It is also possible that the dose of ethanol tested in combination with bicuculline was too high to observe an enhancement of CTA. A dose of 3 g/kg ethanol was chosen in order to examine a wide dose range of bicuculline, but still avoid bicuculline's pro-convulsant effects (Phillips et al., 1989). However, the magnitude of CTA produced with 3 g/kg ethanol may have masked any enhancement of ethanol-induced CTA with

bicuculline. In addition, a significant number of animals were lost in the high bicuculline dose group (4.0 mg/kg), indicating that this dose combination was detrimental to the health of the subjects during the course of the CTA experiment. Thus, the high subject attrition in the 4.0 mg/kg bicuculline dose group may have precluded detection of bicuculline's effect on ethanol-induced CTA. A study is currently being conducted to examine a lower dose of ethanol (i.e., 2 g/kg) in combination with lower doses of bicuculline to determine if picrotoxin and bicuculline indeed have different effects on ethanol-induced CTA.

It has previously been suggested that a positive relationship exists between ethanol's motor stimulant effects and its rewarding effects (Wise & Bozarth, 1987). However, the results of the CPP studies show no relationship between ethanol-stimulated activity and ethanol-induced CPP. In the bicuculline CPP experiment, ethanol-stimulated activity was unaltered with 1.0 mg/kg bicuculline, but the 1.0 mg/kg dose significantly increased CPP. Moreover, the higher bicuculline doses decreased ethanol-stimulated activity, but had no effect on the magnitude of CPP. In addition, picrotoxin significantly reduced ethanol-stimulated activity, but increased ethanol-induced CPP. Thus, these results are consistent with previous studies (Risinger, Dickinson, et al., 1992; Risinger, Malott, et al., 1992; Risinger et al., 1994; Cunningham, 1995; Chester & Cunningham, 1998) that have demonstrated a clear dissociation between ethanol-stimulated locomotor activity and the rewarding effects of ethanol in the place conditioning paradigm.

In summary, the present studies suggest a role for the $GABA_A$ receptor in modulating ethanol's motivational effects in the CPP and CTA paradigms in DBA/2J mice. In contrast to the expected outcome, picrotoxin increased the magnitude of both ethanol-induced CPP and CTA. A low dose of bicuculline also enhanced ethanol-induced CPP, but did not alter the magnitude of CTA, suggesting that bicuculline and picrotoxin may

differ with regard to their effect on ethanol's aversive properties in the CTA paradigm. Overall, these results suggest that blockade of $GABA_A$ receptors increases ethanol's rewarding and aversive effects in these paradigms.

Figure Captions

Figure 1. Mean (±SEM) activity counts per min following ethanol (CS+ sessions) and saline (CS- sessions) for each bicuculline dose group (n=20-24/group) during conditioning trials 1-4. On CS+ days, mice received vehicle (0.0 mg/kg) or bicuculline (1.0, 3.0, or 5.0 mg/kg) immediately before 2 g/kg ethanol. All mice received vehicle/saline injections on CS- days. Data are shown collapsed across the 5-min conditioning sessions.

Figure 2. Mean (+SEM) sec per min spent on the grid floor by conditioning subgroups (G+ and G-; n=10-12/subgroup) of each bicuculline dose group during the preference test. During conditioning, mice in the G+ subgroups received vehicle (0.0 mg/kg group) or bicuculline (1.0, 3.0, or 5.0 mg/kg) immediately before ethanol (2 g/kg) paired with the grid floor and saline paired with the hole floor. Conversely, mice in the G- subgroups received vehicle or bicuculline paired with the hole floor and saline paired with the grid floor. Data are shown collapsed across the 60-min test session.

Figure 3. Mean (±SEM) activity counts per min following ethanol (CS+ sessions) and saline (CS- sessions) for PICRO (n=28), ETOH (n=30), and PICRO/ETOH (n=30) groups during conditioning trials 1-4. On CS+ days, mice received either saline or picrotoxin (2 mg/kg) 15 min before 2 g/kg ethanol. All mice received saline/saline injections on CS- days. Data are shown collapsed across the 5-min conditioning sessions.

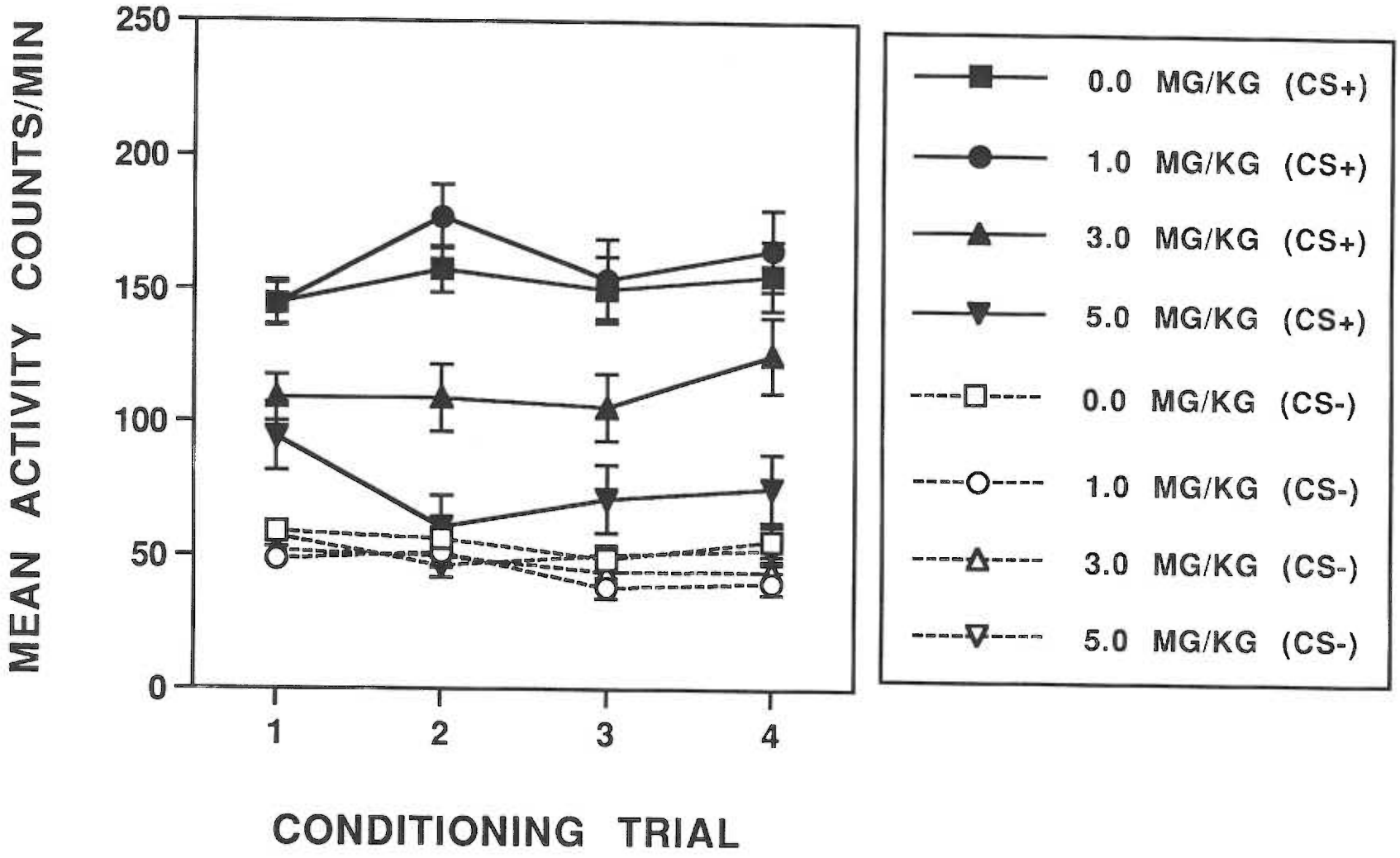
Figure 4. Mean (+SEM) sec per min spent on the grid floor by conditioning subgroups (G+ and G-; n=14-15/subgroup) of the PICRO, ETOH, and PICRO/ETOH groups during the preference test. During conditioning, mice in the G+ subgroups received picrotoxin (2.0 mg/kg), ethanol (2 g/kg), or both paired with the grid floor and saline paired with the hole floor. Conversely, mice in the G- subgroups received picrotoxin, ethanol, or both paired with the hole floor and saline paired with the grid floor. Data are shown collapsed across the 60-min test session.

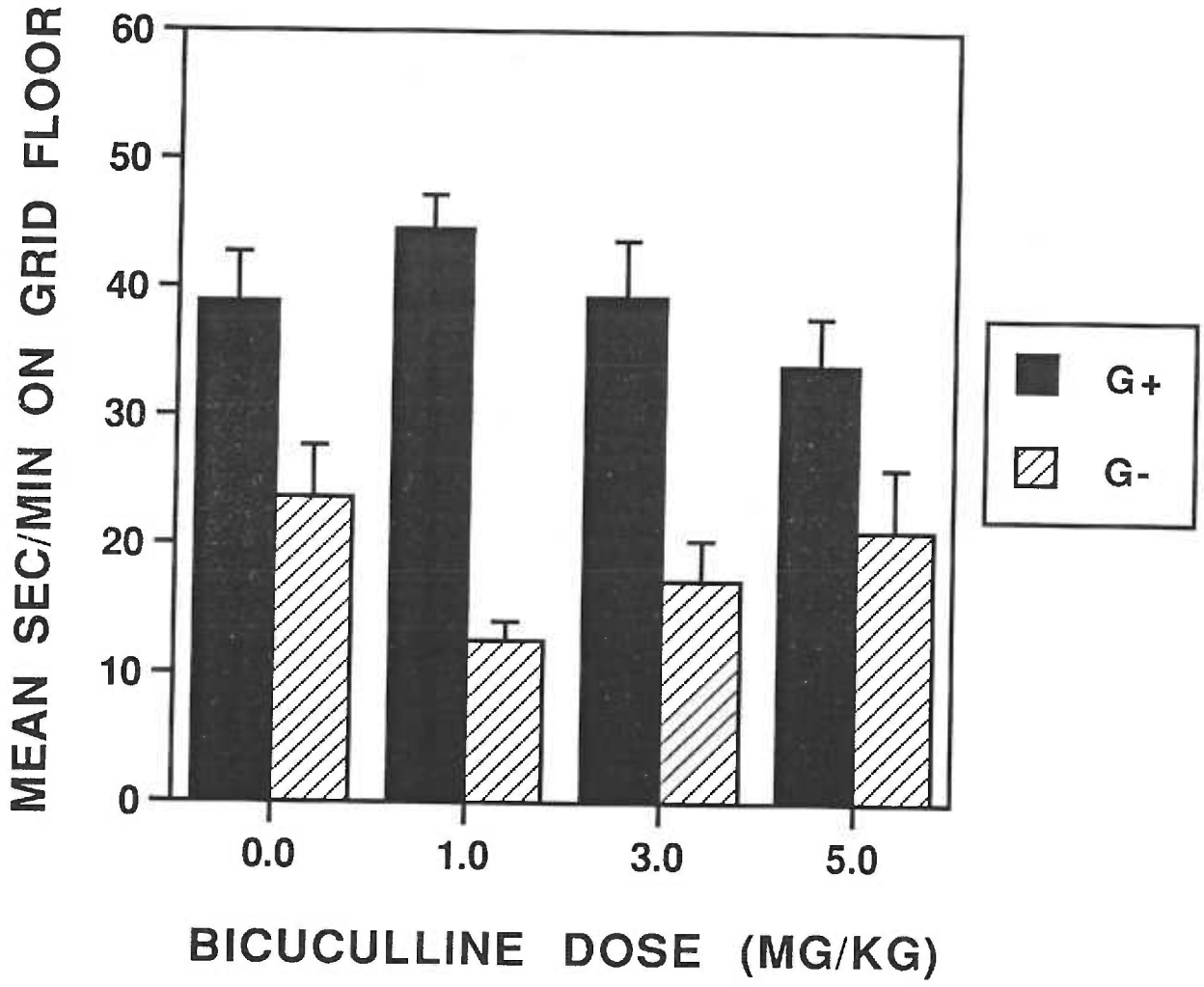
Figure 5. Mean (±SEM) difference scores (ml) during taste conditioning trials 2-6 for each drug treatment group (n=4-12/group). During conditioning, mice received 1-h access to saccharin followed by injections of vehicle or bicuculline (1.0 or 4.0 mg/kg) immediately before injections of vehicle or ethanol (3.0 g/kg). Group abbreviations in legend refer to drug treatment on conditioning trial days: V/S (vehicle/saline), B(1.0)/S [bicuculline (1.0 mg/kg)/saline], V/E (vehicle/ethanol), B(1.0)/E [bicuculline (1.0 mg/kg)/ethanol], and B(4.0)/E [bicuculline (4.0 mg/kg)/ethanol]. Difference scores were calculated by subtracting the ml of saccharin consumed on trial 1 from the amount consumed on subsequent conditioning trials.

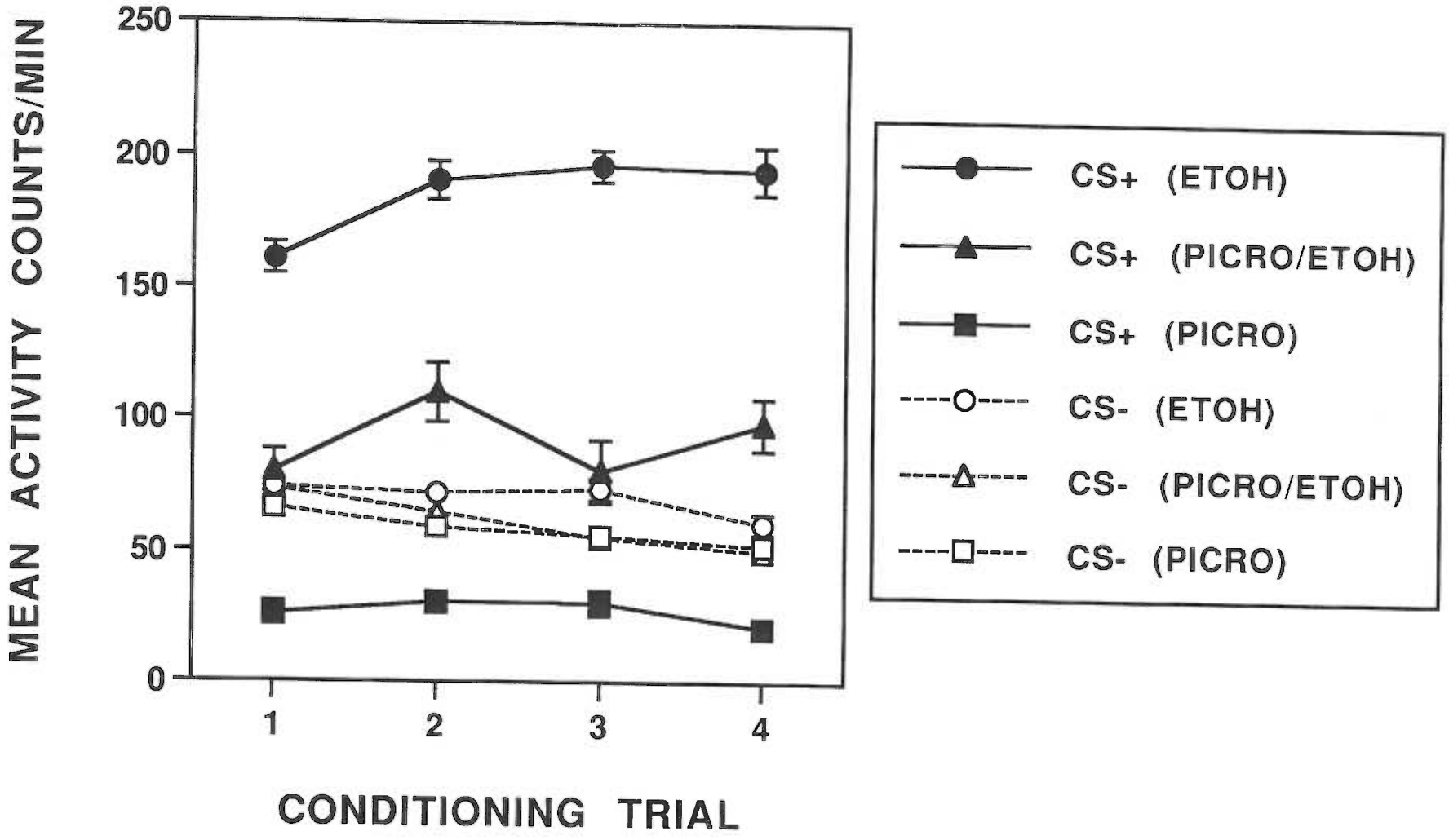
Figure 6. Mean (±SEM) difference scores (ml) during taste conditioning trials 2-6 for each drug treatment group (n=12/group). During conditioning, mice received 1-h access to saccharin followed by injections of saline or picrotoxin (0.75 or 2.5 mg/kg) 15 min before injections of saline or ethanol (2.0 g/kg). Group abbreviations in legend refer to drug treatment on conditioning trial days: S/S (saline/saline), P(0.75)/S [picrotoxin (0.75 mg/kg)/saline], S/E (saline/ethanol), P(0.75)/E [picrotoxin (0.75 mg/kg)/ethanol], and P(2.5)/E [picrotoxin (2.5 mg/kg)/ethanol]. Difference scores were calculated by subtracting the ml of saccharin consumed on trial 1 from the amount consumed on subsequent conditioning trials.

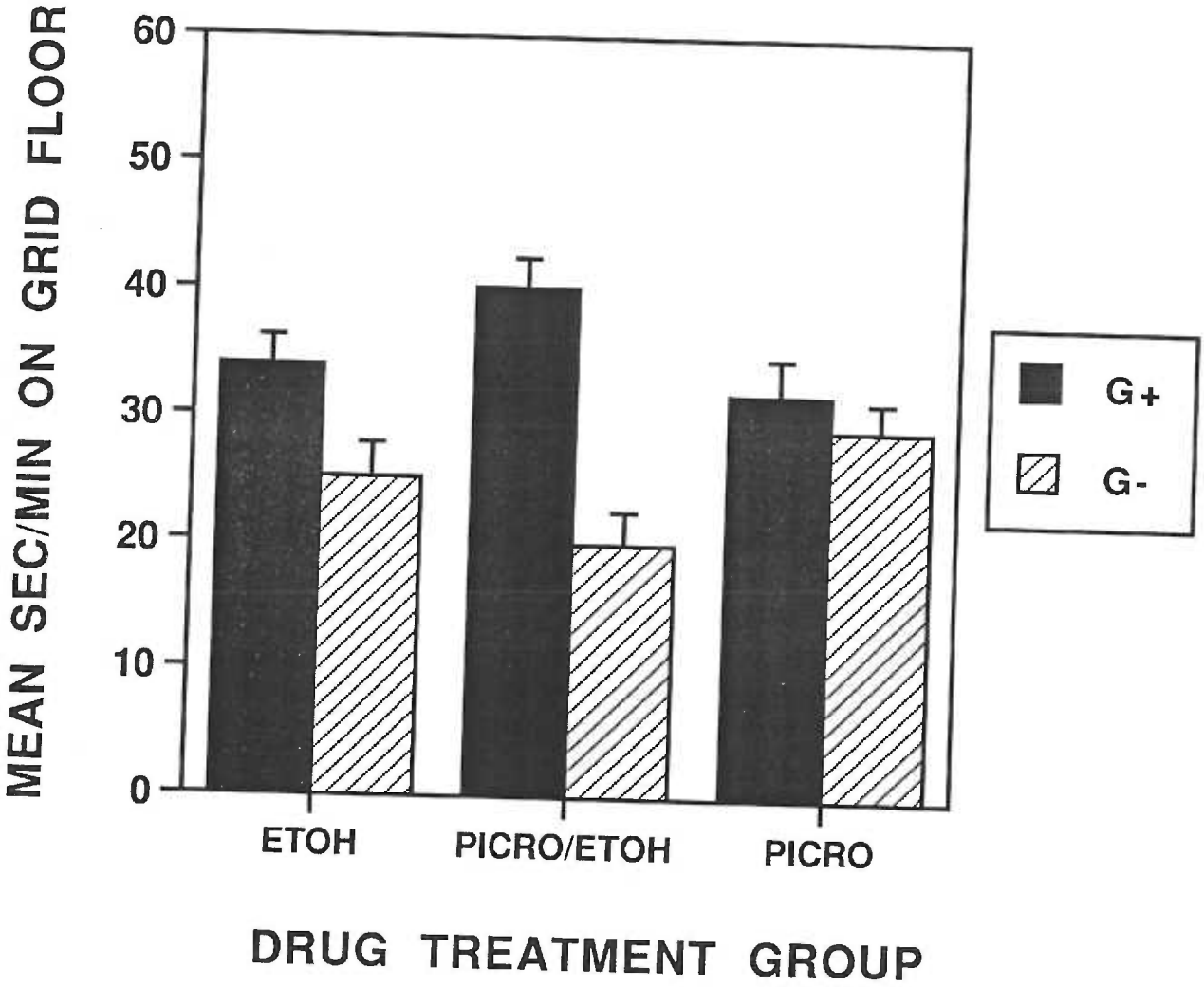
Table 1. Blood Ethanol Concentrations (mg/ml±sem)

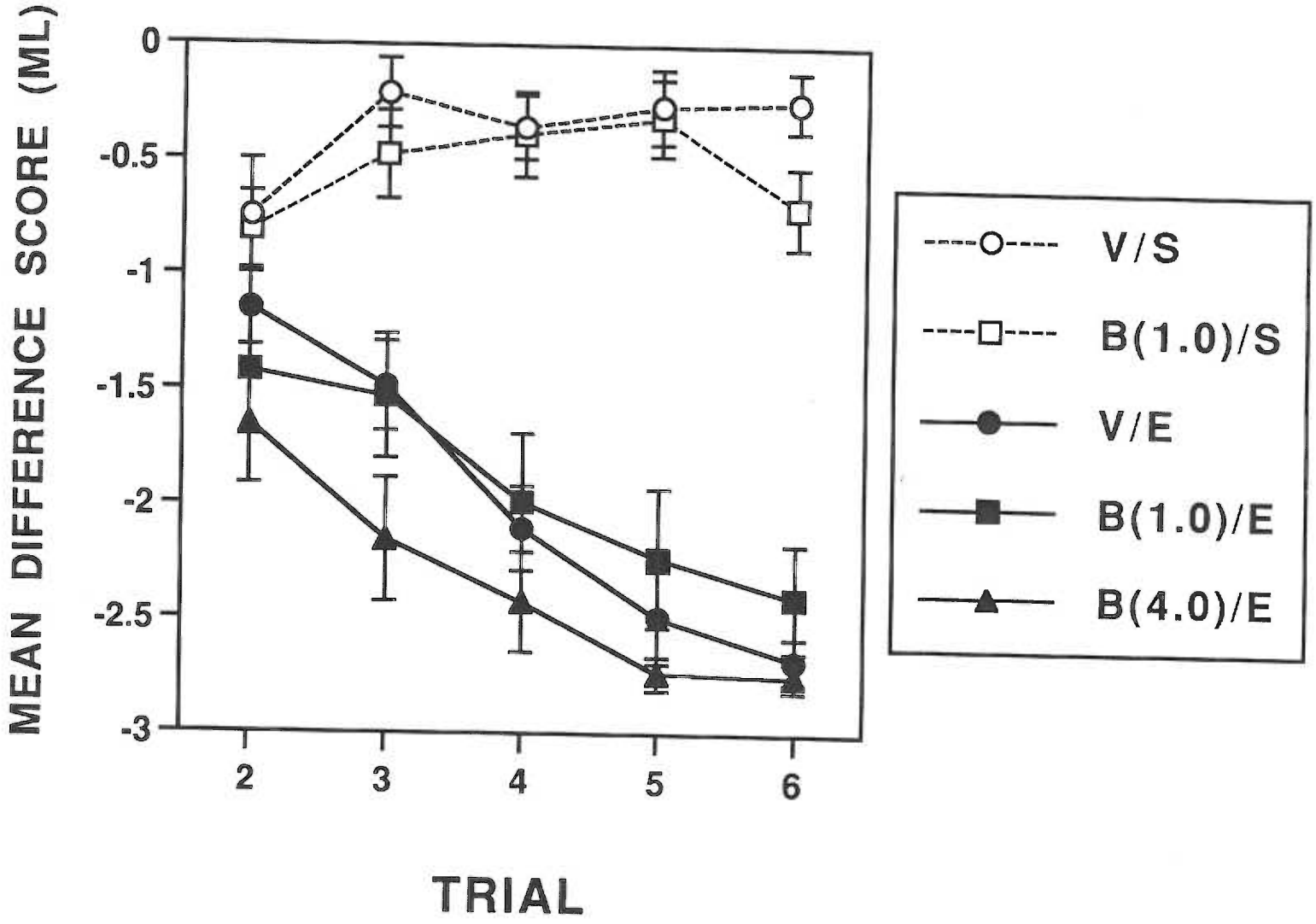
	Time after ethanol (2 g/kg) injection		
Drug Pretreatment	15 min	60 min	120 min
Saline (n=9)	1.09±0.13	1.17±0.14	0.55±0.09
Picrotoxin (0.75 mg/kg; n=8)	1.04±0.11	1.13±0.08	0.74±0.10
Picrotoxin (2.5 mg/kg; n=9)	1.04±0.13	1.33±0.11	0.77±0.07

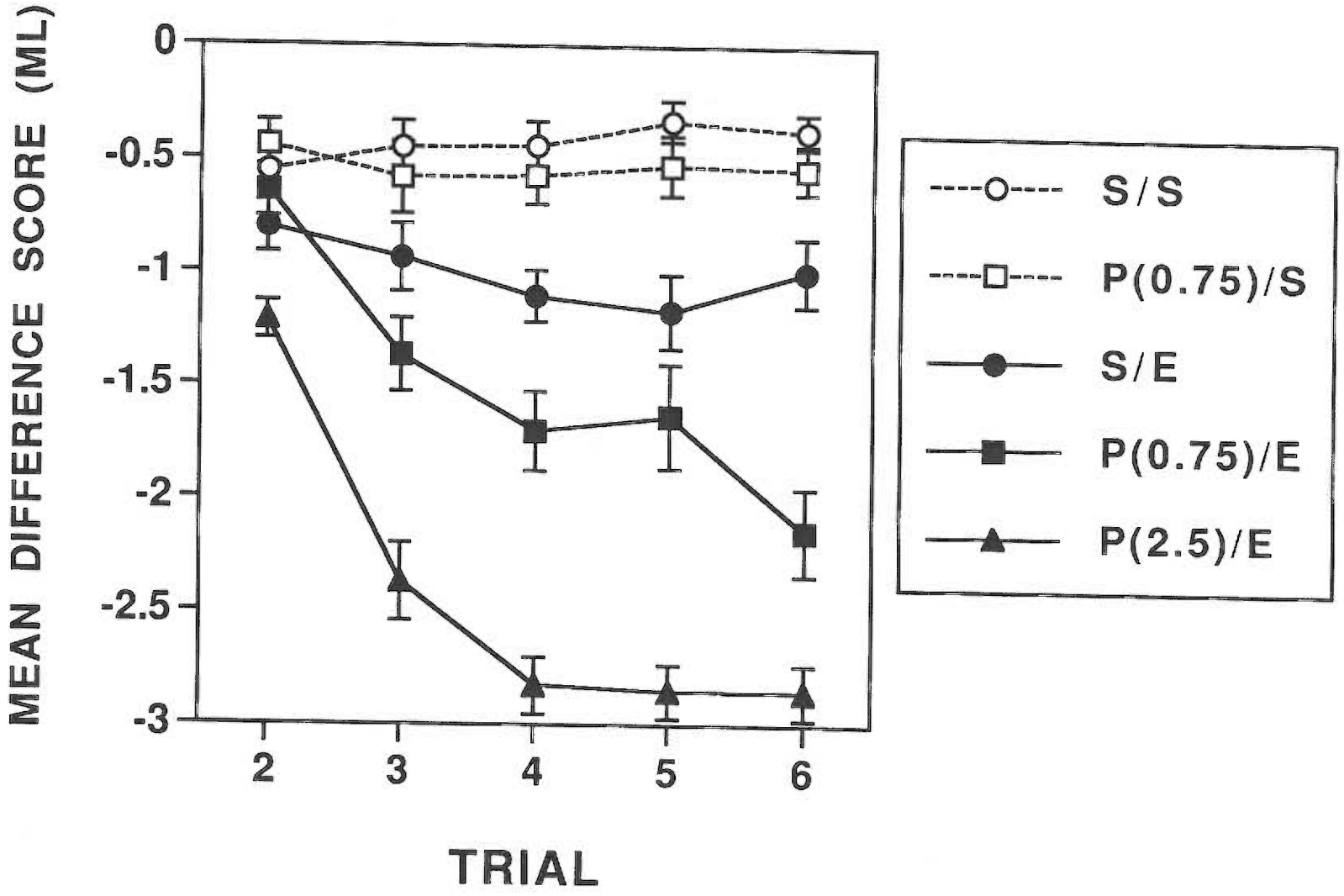

MEAN ACTIVITY COUNTS/MIN
250
200
150
100
50
0
1
2
3
4
CONDITIONING TRIAL
0.0 MG/KG (CS+)
1.0 MG/KG (CS+)
3.0 MG/KG (CS+)
5.0 MG/KG (CS+)
0.0 MG/KG (CS-)
1.0 MG/KG (CS-)
3.0 MG/KG (CS-)
5.0 MG/KG (CS-)


MEAN SEC/MIN ON GRID FLOOR
60
50
40
30
20
10
0
0.0
1.0
3.0
5.0
BICUCULLINE DOSE (MG/KG)
G+
G-


MEAN ACTIVITY COUNTS/MIN
250
200
150
100
50
0
1
2
3
4
CONDITIONING TRIAL
CS+ (ETOH)
CS+ (PICRO/ETOH)
CS+ (PICRO)
CS- (ETOH)
CS- (PICRO/ETOH)
CS- (PICRO)


MEAN SEC/MIN ON GRID FLOOR
60
50
40
30
20
10
0
ETOH
PICRO/ETOH
PICRO
DRUG TREATMENT GROUP
G+
G-


MEAN DIFFERENCE SCORE (ML)
0
-0.5
-1
-1.5
-2
-2.5
-3
2
3
4
5
6
TRIAL
V/S
B(1.0)/S
V/E
B(1.0)/E
B(4.0)/E


MEAN DIFFERENCE SCORE (ML)
0
-0.5
-1
-1.5
-2
-2.5
-3
2
3
4
5
6
TRIAL
S/S
P(0.75)/S
S/E
P(0.75)/E
P(2.5)/E

Baclofen Alters Ethanol-Stimulated Activity but not Conditioned Place Preference or Taste Aversion in Mice

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Running Head: Baclofen and Ethanol Reward and Aversion

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Abstract

The present experiments examined the effects of the $GABA_B$ receptor agonist, baclofen, on the acquisition of ethanol-induced conditioned place preference (CPP) and conditioned taste aversion (CTA) in male DBA/2J mice. Mice in the CPP experiment received four pairings of ethanol (2 g/kg) with a distinctive floor stimulus for a 5-min conditioning session (CS+ sessions). On intervening days (CS- sessions), mice received saline injections paired with a different floor type. On CS+ days, mice also received one of four doses of baclofen (0.0, 2.5, 5.0, or 7.5 mg/kg) 15 min before an injection of ethanol. For the preference test, all mice received saline injections and were placed on a half grid and half hole floor for a 60-min session. Baclofen dose-dependently reduced ethanol-stimulated activity, but did not alter the magnitude of ethanol-induced CPP at any dose. For the CTA experiment, mice were adapted to a 2-hr per day water restriction regimen followed by five conditioning trials every 48 hrs. During conditioning trials, subjects received an injection of saline or baclofen (2.0 and 6.0 mg/kg) 15 min before injection of 2 g/kg ethanol or saline following 1-hr access to a saccharin solution. Baclofen did not alter the magnitude of ethanol-induced CTA at any dose. In addition, baclofen alone did not produce a CTA. The results of the CPP study suggest that ethanol's locomotor-activating effects are not related to its rewarding effects in the CPP paradigm. Overall, these studies show that activation of $GABA_B$ receptors with baclofen does not alter ethanol's rewarding or aversive effects in the CPP and CTA paradigms in DBA/2J mice.

Key Words: Alcohol, DBA/2J, Reward, Aversion, GABA, Locomotor Activity, Place Conditioning, Taste Conditioning

Introduction

Attempts to elucidate the neurochemical substrates involved in ethanol's motivational effects have focused on several neurotransmitter systems, including dopamine, serotonin, opioid, glutamate, and gamma-aminobutyric acid (GABA) (for reviews see Harris, Brodie, & Dunwiddie, 1992; Tabakoff & Hoffman, 1996; Koob et al., 1998). GABA is the primary inhibitory neurotransmitter in the brain, which exerts its actions primarily via two distinct receptor subtypes, $GABA_A$ and $GABA_B$. Several lines of evidence indicate that ethanol exerts many of its pharmacological and behavioral effects through an interaction with the GABA receptor system (see Ticku, 1990; Korpi, 1994; Mihic & Harris, 1996 for reviews). However, relatively few studies have examined the role of GABA receptor subtypes in the motivational effects of ethanol. Of these studies, ethanol drinking and self-administration tasks have been the most commonly used procedures to examine the effect of GABA manipulations on ethanol's motivational properties.

Much of the evidence implicating the GABA receptor system in ethanol's motivational effects comes from studies showing that $GABA_A$ receptor antagonists (Boyle et al., 1993; Rassnick et al., 1993) and benzodiazepine partial inverse agonists (Samson et al., 1987; McBride et al., 1988; Balakleevsky et al., 1990; Wegelius et al., 1994; June et al., 1991;1994;1996; Buczek et al., 1997) consistently reduce ethanol self-administration in rats. However, several studies have also reported a decrease in ethanol self-administration with administration of the non-selective GABA agonists gamma-butyrolactone (Fadda et al., 1983), AOAA, a GABA decarboxylase inhibitor (Daoust et al., 1987), and calcium-acetyl-homotaurine (Boismare et al., 1984). In addition, the specific $GABA_A$ antagonist, bicuculline, attenuated the decrease in ethanol intake observed with calcium-acetyl-homotaurine, suggesting that the $GABA_A$ receptor is involved in mediating the effect of calcium-acetyl-homotaurine on ethanol intake.

The few studies that have examined a role for the $GABA_B$ receptor in modulating ethanol self-administration have provided inconsistent results. In one study, the selective $GABA_B$ agonist, baclofen, increased ethanol intake, but also increased total fluid intake, suggesting the effect of baclofen was not selective for ethanol's motivational effects (Smith et al., 1992). In another study, baclofen decreased ethanol intake without altering total fluid intake (Daoust et al., 1987). The discrepancy between these two studies is possibly due to different doses of baclofen or different procedures used to measure ethanol self-administration. For example, Smith et al. administered baclofen daily during an acquisition phase of self-administration, while Daoust et al. first selected rats that were ethanol-preferring before examining the effect of daily baclofen administration on the maintenance of ethanol self-administration. More recently, Tomkins and Fletcher (1996) showed that direct injections of baclofen into the dorsal raphe nucleus had no effect on ethanol or water consumption. Thus, the role of $GABA_B$ receptors in modulating ethanol self-administration remains unclear.

A potential problem in interpreting self-administration studies is that GABAergic manipulations may be affecting mechanisms involved in consummatory behavior rather that affecting a mechanism modulating ethanol's motivational properties. Indeed, baclofen (Pringle & Ebenezer, 1990; Ebenezer & Pringle, 1992) and $GABA_A$/benzodiazepine receptor agonists have been shown to stimulate feeding in non-deprived rats (Cooper, 1986), while benzodiazepine receptor antagonists reduce food consumption (Cooper, 1986). In addition, ethanol self-administration may be influenced by both rewarding and aversive effects of ethanol, which may be mediated by independent neural mechanisms. Thus, changes in self-administration behavior following pharmacological manipulations may be due to an increase or decrease in ethanol's rewarding or aversive properties. This may account for the discrepancies in the self-administration studies, where a reduction in ethanol self-administration was observed with both GABA agonists and antagonists.

The present experiments used the place and taste conditioning paradigms to examine the effects of the $GABA_B$ receptor agonist, baclofen, on the rewarding and aversive properties of ethanol. One advantage of the place and taste conditioning procedures relative to the oral self-administration paradigm is that they avoid interpretive problems regarding possible non-specific effects of an agonist or antagonist on consummatory behavior, because pharmacological agents are not administered during expression of place or taste conditioning. Another advantage of these paradigms is that they can be used to separately measure both rewarding and aversive effects of ethanol. In this regard, they are also useful for assessing the effects of drugs that may increase or decrease the magnitude of place or taste conditioning, and these drugs can be assessed independently for their own motivational properties as a measure of control.

The purpose of the current studies was to investigate the role of the $GABA_B$ receptor in modulating the acquisition of ethanol-induced conditioned place preference (CPP) and conditioned taste aversion (CTA) in DBA/2J mice. Based on self-administration studies (Daoust et al., 1987; Smith et al., 1992), it was hypothesized that $GABA_B$ receptor activation modulates ethanol's motivational effects. The present experiments examined the effect of various doses of baclofen on ethanol's rewarding and aversive effects in the CPP and CTA paradigms. Because the existing self-administration data are contradictory, a clear directional prediction for baclofen's effect on ethanol-induced CPP and CTA could not be made.

Method

Subjects

Subjects in both experiments were adult male inbred mice (DBA/2J) obtained from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. For the place conditioning study, mice were housed in polycarbonate cages (27.9 x 9.5 x 12.7 cm) in groups of four.

For the taste conditioning study, mice were housed individually in hanging stainless-steel cages (12 x 18 x 18 cm) with wire mesh fronts and bottoms. Animals were allowed to acclimate to the colony room for 12-14 days before training. During place conditioning, animals were allowed free access to food and water. During taste conditioning, lab chow was continuously available; however, daily access to fluids was restricted according to the procedure described below. Ambient temperature was maintained at 21± 1° C. Experimental procedures were conducted during the light phase of a 12:12 light/dark cycle (lights on at 0700).

Apparatus

Twelve identical acrylic and aluminum boxes (30 x 15 x 15 cm) were separately enclosed in ventilated, light and sound-attenuating chambers (Coulbourn Model E10-20). Six sets of infrared light sources and photodetectors were mounted opposite each other at 5-cm intervals along the length of each box, 2.2 cm above the floor. Occlusion of the infrared light beams was used to measure general activity and location of the animal (left or right) within the box. Total activity counts were recorded every minute by computer (10 msec resolution). The floor of each box consisted of interchangeable halves of one of two distinct textures. "Grid" floors consisted of 2.3 mm stainless steel rods mounted 6.4 mm apart in acrylic rails. "Hole" floors consisted of perforated 16 gauge stainless steel with 6.4 mm round holes on 9.5 mm staggered centers. This combination of floor textures was selected on the basis of previous studies showing that drug-naive DBA/2J mice spend approximately equal time on each floor type during drug-free preference tests (Cunningham, Niehus, et al., 1992; Cunningham & Noble, 1992; Cunningham, 1995). The floors and the inside of the boxes were wiped with a damp sponge and the litter paper beneath the floors was changed between animals.

The taste conditioning experiment was conducted in the home cages. Water and saccharin solutions were presented at room temperature in 25 ml graduated glass cylinders

fitted with stainless-steel drinking spouts inserted through the front of the cage. Consumption was measured to the nearest 0.1 ml and was corrected for evaporation and spillage by subtracting the mean fluid loss measured in two drinking tubes placed on empty cages for an equal amount of time.

Drugs

Ethanol (20% v/v) was prepared from a 95% stock solution using saline as the vehicle. Ethanol was administered intraperitoneally (IP) and the dose was varied by manipulating injection volume. Baclofen (Sigma Chemical Co., St. Louis, MO) was dissolved in saline and administered IP in an injection volume of 10 ml/kg.

Procedure

Place Conditioning. The place conditioning study involved one habituation session, eight conditioning sessions, and one test session. A 2-day weekend break occurred between the first four and last four conditioning sessions. For the habituation session, mice received an injection of saline immediately before being placed in the conditioning box for 5 min on a smooth paper floor.

For conditioning, mice were randomly assigned to one of four baclofen dose groups: 0.0 (saline), 2.5. 5.0, and 7.5 mg/kg (n = 24/dose group). Within each of the four dose groups, mice were randomly assigned to one of two conditioning subgroups (G+ or G-) and exposed to a Pavlovian differential conditioning procedure. On alternating days, mice in the G+ group received an injection of ethanol (2 g/kg; 12.5 ml/kg) immediately before a 5 min session on the grid floor (CS+ sessions). On intervening days, these mice received saline immediately before exposure to the hole floor (CS- sessions). Conversely, mice in the G- group received ethanol paired with the hole floor and saline paired with the grid floor. During conditioning trials, all mice had access to both sides of the apparatus on a homogeneous floor type. All mice received two IP injections before each conditioning session. During CS+ sessions, G+ subjects received an injection of saline, 2.5, 5.0, or

7.5 mg/kg baclofen 15 min before an injection of ethanol and were placed on the grid floor for a 5 min session. During CS- sessions, these mice received two saline injections 15 min apart before a 5 min session on the hole floor. Conversely, G- subjects received saline/ethanol (0.0 mg/kg group) or baclofen/ethanol paired with the hole floor and saline paired with the grid floor. These doses of baclofen were chosen because they are within the range known to alter ethanol's behavioral effects (Cott et al., 1976; Martz et al., 1983). Conditioning groups were matched for overall exposure to CS type (grid or hole) and drug treatment, and the order of drug exposure was counterbalanced within groups. Thus, this procedure provides control over exposure to both the CS (floor type) and the US (ethanol) in both G+ and G- subgroups, with subgroups differing only in the specific floor-ethanol pairing (Cunningham, 1993). The dose of ethanol (2 g/kg) was chosen because it has previously been shown in mice to produce a strong preference for the paired tactile stimuli (e.g., Chester & Cunningham, 1998; Cunningham & Prather, 1992). The 5 min session duration was chosen based on previous studies showing that it produced a stronger conditioned place preference with ethanol in DBA/2J mice than did longer session durations (Cunningham & Prather, 1992).

For the 60-min test session, all mice received two injections of saline 15 min apart to match the cues during conditioning days. The floor of each box was half grid and half hole with left/right position counterbalanced within groups.

Taste Conditioning. Subjects were adapted to a water restriction schedule (2 h water per day) over a 7-day period. At 48-h intervals over the next 10 days, all mice received 1-h access to a solution of saccharin (0.15% w/v sodium saccharin in tap water).

Mice were randomly assigned to one of five drug treatment groups (n=12/group): saline/saline (S/S), baclofen (2.0 mg/kg)/saline [B(2.0)/S], saline/ethanol (S/E), baclofen (2 mg/kg)/ethanol [B(2.0)/E], and baclofen (6 mg/kg)/ethanol [B(6.0)/E]. Immediately after 1-h access to saccharin, mice received injections of saline or baclofen 15 min before

injections of saline or ethanol (2.5 g/kg). All mice also received 30-min access to tap water 5 h after each saccharin access period, in order to prevent dehydration. Two-h access to tap water was given during intervening days.

Results

Place Conditioning

Data were analyzed by analysis of variance (ANOVA) with the alpha level set at 0.05.

Conditioning. Figure 1 shows mean activity counts per min during conditioning trials 1-4 averaged across each baclofen dose group. Ethanol produced significant locomotor activation in the 0.0 mg/kg group during CS+ sessions relative to saline on CS- sessions. Baclofen produced a dose-dependent reduction in ethanol-stimulated locomotor activity during CS+ sessions. As previously observed with DBA/2J mice (e.g., Chester & Cunningham, 1998), activity counts were higher on the last CS+ session compared to the first CS+ session in all baclofen dose groups, suggesting the development of sensitization to ethanol's locomotor-stimulant effects.

Two-way ANOVAs (Dose x Trials) were separately conducted for CS+ and CS- session data. The CS+ ANOVA revealed a significant effect of Dose [$F(3,92) = 29.9$, $p < 0.001$] and Trials [$F(3,276) = 12.4$, $p < 0.001$], but no interaction was found ($F<1$). The effect of Trials indicates that ethanol-induced locomotor sensitization occurred across the four conditioning trials. The lack of interaction signifies that baclofen did not alter the development of sensitization at any dose. Followup comparisons of drug treatment groups showed significant differences between all baclofen dose groups ($ps \leq 0.01$), except between 0.0 and 2.5 mg/kg ($p=0.09$). The CS- ANOVA showed a significant effect of Trials [$F(3,273) = 9.6$, $p<0.001$], indicating habituation to experimental procedures occurred across the four trials. No effect of Dose or interaction was found.

Insert Figure 1 about here

Preference Testing. The amount of time spent on the grid floor in both the G+ and G- subgroups was nearly constant throughout the test session, therefore, the data shown in Figure 2 are collapsed across the 60-min session. Figure 2 shows the mean (±sem) sec per min spent on the grid floor by both conditioning subgroups in the four baclofen dose groups during the preference test. G+ subgroups in each drug treatment group spent significantly more time on the grid floor relative to G- subgroups, indicating the development of ethanol-induced CPP for the grid floor. Baclofen appeared to have little effect on the magnitude of preference.

Overall analysis of the data (Baclofen Dose x Conditioning Group ANOVA) yielded a significant effect of Conditioning Group [$F(1,88) = 81.1$, $p < 0.001$], indicating a conditioned place preference for the ethanol-paired floor. No significant effect of Baclofen Dose or interaction was found. Thus, these data indicate that baclofen did not alter the acquisition of ethanol-induced CPP at any dose.

Insert Figure 2 about here

Mean (±sem) activity counts per min during the 60-min test were 33.6±1.6, 31.2±1.4, 31.0±1.4, and 30.0±1.3, for the 0.0, 2.5, 5.0, and 7.5 mg/kg baclofen groups, respectively. No significant differences in test activity levels were found [$F(3,92)=1.1$, $p=0.4$].

Taste Conditioning

Mean (±sem) consumption of saccharin on trial 1 (before conditioning) for each drug treatment group was 2.92±0.16, 2.91±0.14, 2.82±0.14, 2.87±0.16, and 3.03±0.12 for S/S, B(2.0)/S, S/E, B(2.0)/E and B(6.0)/E, respectively. One-way ANOVA of trial 1 intakes indicated no significant difference between groups in preconditioning consumption of saccharin ($p=0.9$). Nevertheless, to offset minor initial differences in saccharin intake and facilitate presentation of the data, difference scores were calculated for each subject by subtracting the ml of saccharin consumed on trial 1 from the amount consumed on subsequent conditioning trials. Figure 3 shows mean difference scores for each drug treatment group across conditioning trials 2-6.

Insert Figure 3 about here

Ethanol-saccharin pairings produced reductions in saccharin intake across trials, indicating the development of CTA in the S/E group. Two-way ANOVA of S/S and S/E groups (Drug Treatment x Trials) showed significant effects of Drug Treatment [$F(1,22)=27.1$, $p<0.001$], Trials [$F(4,88)=20.6$,$p<0.001$], and interaction [$F(4,88)=15.1$, $p<0.001$], signifying the development of ethanol-induced CTA across trials in the S/E group. All ethanol-treated groups (S/E, B(2.0)/E, B(6.0)/E) showed a similar magnitude of CTA across trials, suggesting no effect of baclofen pretreatment (2 or 6 mg/kg) on ethanol-induced CTA. The conclusion was supported by two-way ANOVA of ethanol-treated groups (Drug Treatment x Trials), which showed a significant effect of Trials [$F(4,132)=85.6$, $p<0.001$], but no effect of Drug Treatment or interaction. A separate two-way ANOVA of S/S and B(2.0)/S groups yielded a marginally significant effect of Trials [$F(4,88)=2.5$, $p=0.05$], but no effect of Drug Treatment or interaction ($Fs<1$). This analysis indicates that administration of baclofen alone (2 mg/kg) did not produce a CTA.

Discussion

The present experiments examined a role for the $GABA_B$ receptor in modulating ethanol's rewarding and aversive effects in the CPP and CTA paradigms. The results of the place conditioning study showed that the acquisition of ethanol-induced CPP was not altered by baclofen, the selective $GABA_B$ agonist. The taste conditioning study showed that baclofen did not alter the acquisition of ethanol-induced CTA. In addition, administration of baclofen alone (2.0 mg/kg) did not produce a CTA. This finding is consistent with a previous study showing that baclofen does not produce a CTA in rats (Ebenezer, Houston & Crook, 1992). Overall, these results do not support the hypothesis that the $GABA_B$ receptor is involved in modulating ethanol's motivational effects in the CPP and CTA paradigms.

The finding that baclofen dose-dependently reduced ethanol-stimulated activity in the CPP experiment is consistent with previous studies (Cott et al., 1976; Humeniuk et al., 1993; Shen, Dorow, Harland, Burkhart-Kasch, & Phillips, in press). It is possible that this effect of baclofen is due to a reduction in ethanol-stimulated dopamine release. Baclofen has been shown to decrease the activity of dopamine neurons (Fuxe et al., 1975; Olpe et al, 1977; Lacey et al., 1988) and decrease extracellular dopamine levels in the VTA (Klitenick et al., 1992) and nucleus accumbens (Yoshida et al., 1994). In addition, baclofen has also been shown to reduce the motor-stimulant effect of other drugs known to act through an increase in dopamine levels, such as cocaine or amphetamine (Kuzcenski, 1983; Kalivas et al., 1990). Consistent with previous studies (e.g., Risinger, Dickinson, et al., 1992; Cunningham, 1995; Chester & Cunningham, 1998), these data also suggest a dissociation between ethanol's rewarding and locomotor effects, because baclofen dose-dependently decreased ethanol-stimulated activity, but did not alter the magnitude of ethanol-induced CPP.

The present experiments are the first to examine a role for the $GABA_B$ receptor in modulating ethanol's motivational effects in the CPP and CTA learning paradigms. A few studies have examined the effects of $GABA_B$ receptor activation in modulating ethanol's motivational properties in the self-administration paradigm (Daoust et al., 1987; Smith et al., 1992, Tomkins & Fletcher, 1996). However, these studies were contradictory because baclofen was shown to both reduce established ethanol consumption (Daoust et al.) and facilitate the acquisition of voluntary ethanol consumption (Smith et al.). Moreover, in the latter study, baclofen also increased total fluid intake, suggesting that the facilitatory effect of baclofen was not specific to ethanol consumption. The discrepancy between these studies may be due to different doses of baclofen used or procedural differences in the self-administration paradigm. Regardless, the present data do not support self-administration studies that suggest baclofen alters ethanol's motivational effects. However, the present results are consistent with a recent study that showed no effect on ethanol or water intake when baclofen was administered directly into the dorsal raphe nucleus (Tomkins and Fletcher, 1996), an area where activation of $GABA_A$ receptors has been shown to increase ethanol self-administration (Tomkins, Sellers, & Fletcher, 1994). Taken together, the results of previous self-administration studies and the present experiments suggest that the neural mechanisms modulating ethanol's motivational effects in the self-administration paradigm may be different from those modulating ethanol's motivational effects in the CPP and CTA paradigms.

The present results suggest that $GABA_B$ receptors are not involved in modulating ethanol-induced CPP and CTA. However, we have recently shown that ethanol-induced CPP is increased with administration of the $GABA_A$ antagonists, picrotoxin and bicuculline. In addition, picrotoxin dose-dependently enhanced ethanol-induced CTA (Chester & Cunningham, submitted). Thus, these studies suggest that $GABA_A$ receptor blockade may increase ethanol's rewarding and aversive effects in these paradigms. The

finding that $GABA_A$, but not $GABA_B$ receptors modulate ethanol's motivational properties in these paradigms may be due to their different mechanisms of action in the brain. Although activation of both subtypes produce neuronal inhibition, $GABA_A$ receptors mediate fast synaptic transmission through activation of chloride ion channels (Upton & Blackburn, 1997) whereas $GABA_B$ receptors are responsible for slow synaptic transmission through G-protein coupled mechanisms (Misgeld et al., 1995). It may be that fast synaptic transmission through $GABA_A$ receptor activation in reward-related pathways is important for modulating ethanol's motivational effects in the CPP and CTA paradigms.

In contrast to the present results, $GABA_B$ receptors have been shown to play an important role in modulating the rewarding effects of other drugs of abuse, such as cocaine and morphine. For example, baclofen has been reported to attenuate cocaine self-administration (Roberts, Andrews & Vickers, 1996; Roberts & Andrews, 1997; Shoaib, Swanner, Beyer, Goldberg, & Schindler, 1998) and morphine-induced CPP in rats (Tsuji et al., 1996). It has been suggested this effect of baclofen is due to an effect of $GABA_B$ receptor-mediated inhibition of dopamine neurons (Olpe et al., 1977; Klitenick et al., 1992). It has been hypothesized that dopamine also plays a primary role in the motivational effects of ethanol (Di Chiara & Imperato, 1988, Koob, 1998). However, the present results suggest that ethanol's motivational effects in the CPP and CTA paradigms may not be sensitive to baclofen-induced changes in dopamine transmission. This is consistent with a study that showed no effect of the dopamine antagonist, haloperidol, on the acquisition (Risinger, Dickinson, et al., 1992) or expression (Cunningham, Malott, et al., 1992) of ethanol-induced CPP. However, another study did report a reduction in ethanol-induced CTA with administration of haloperidol and the selective D_2 receptor antagonist, eticlopride (Risinger, 1994).

There are several possible reasons for the difference between baclofen's effect in previous self-administration studies and the present results. For example, it is possible that

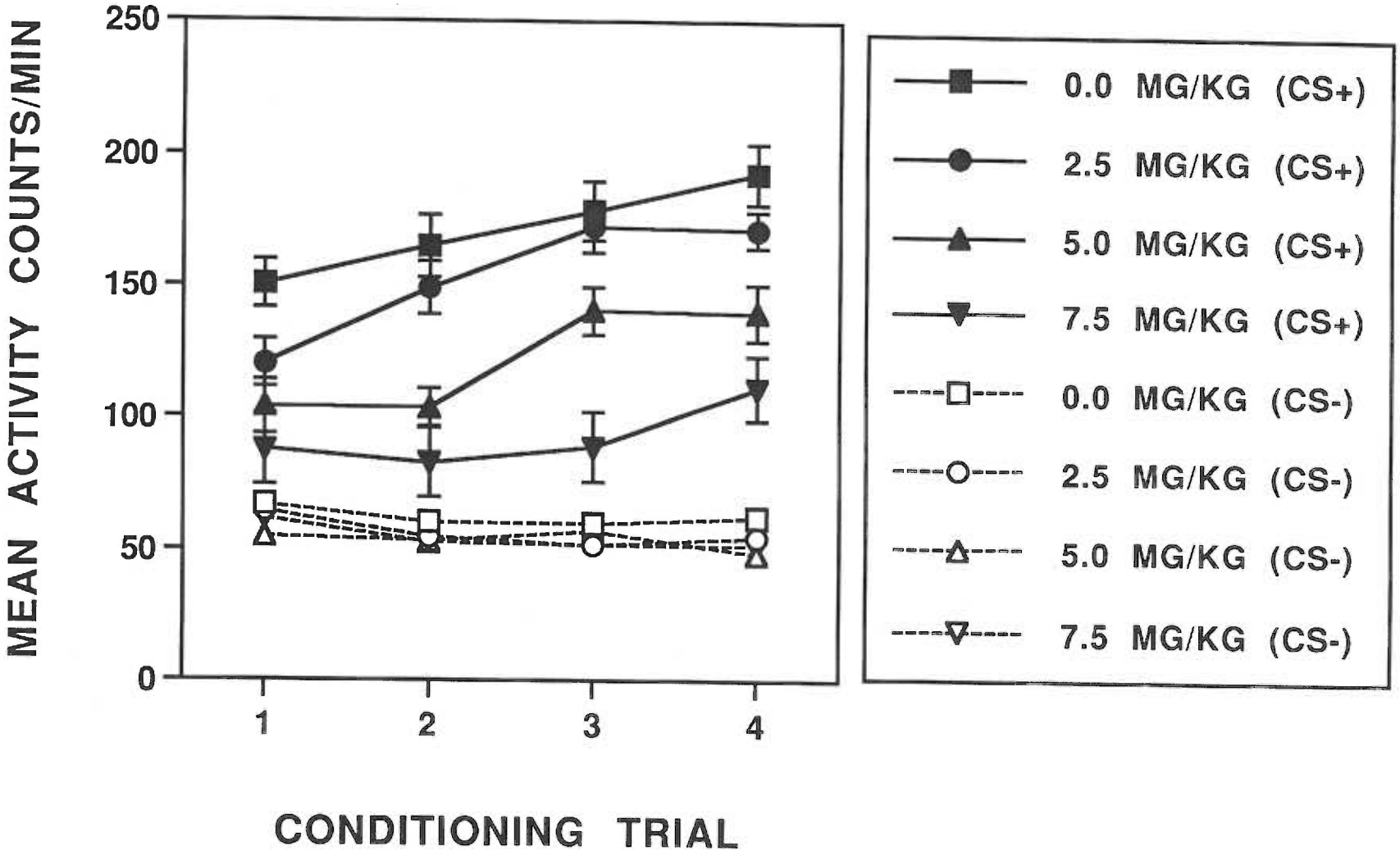
subject vs experimenter control over ethanol exposure is an important factor in determining baclofen's effect in the self-administration paradigm. Thus, baclofen may specifically interact with a neural pathway important for modulating oral ethanol self-administration, and this pathway may be distinct from the pathways modulating ethanol-induced CPP and CTA. In addition, baclofen's effect on ethanol self-administration may be unique to rats. Alternatively, the reported effects of baclofen in the self-administration studies may have been due to a non-specific effect of baclofen on consummatory behavior (Pringle & Ebenezer, 1990; Ebenezer & Pringle, 1992), rather than a selective effect on ethanol's motivational effects. It is also possible that baclofen produced a change in ethanol self-administration by altering the taste or orosensory properties of ethanol. For example, Söderpalm & Hansen (1998) recently showed with taste reactivity tests that $GABA_A$/benzodiazepine agonists increase the palatability of ethanol. Clearly, more studies are needed to determine the effect of baclofen on ethanol self-administration behavior. Nevertheless, the results of the present experiments suggest that $GABA_B$ receptor activation does not modulate ethanol's rewarding or aversive effects in DBA/2J mice in the CPP and CTA paradigms.

Figure Captions

Figure 1. Mean (±SEM) activity counts per min following ethanol (CS+ sessions) and saline (CS- sessions) for each baclofen dose group (n=24/group) during conditioning trials 1-4. On CS+ days, mice received saline (0.0 mg/kg) or baclofen (2.5, 5.0, or 7.5 mg/kg) 15 min before 2 g/kg ethanol. All mice received saline/saline injections on CS- days. Data are shown collapsed across the 5-min conditioning sessions.

Figure 2. Mean (+SEM) sec per min spent on the grid floor by conditioning subgroups (G+ and G-; n=12/subgroup) of each baclofen dose group during the preference test. During conditioning, mice in the G+ subgroups received saline or baclofen (2.5, 5.0, 7.5 mg/kg) 15 min before ethanol (2 g/kg) paired with the grid floor and saline injections paired with the hole floor. Conversely, mice in the G- subgroups received saline/ethanol (0.0 mg/kg group) or baclofen/ethanol paired with the hole floor and saline paired with the grid floor. Data are shown collapsed across the 60-min test session.

Figure 3. Mean (±SEM) difference scores (ml) during taste conditioning trials 2-6 for each drug treatment group (n=12/group). During conditioning, mice received 1-h access to saccharin followed by injections of saline or baclofen (2.0 or 6.0 mg/kg) 15 min before injections of saline or ethanol (2.5 g/kg). Group abbreviations in legend refer to drug treatment on conditioning trial days: S/S (saline/saline), B(2.0)/S [baclofen (2.0 mg/kg)/saline], S/E (saline/ethanol), B(2.0)/E [baclofen (2.0 mg/kg)/ethanol], and B(6.0)/E [baclofen (6.0 mg/kg)/ethanol]. Difference scores were calculated by subtracting the ml of saccharin consumed on trial 1 from the amount consumed on subsequent conditioning trials.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200
150
100
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1
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CONDITIONING TRIAL
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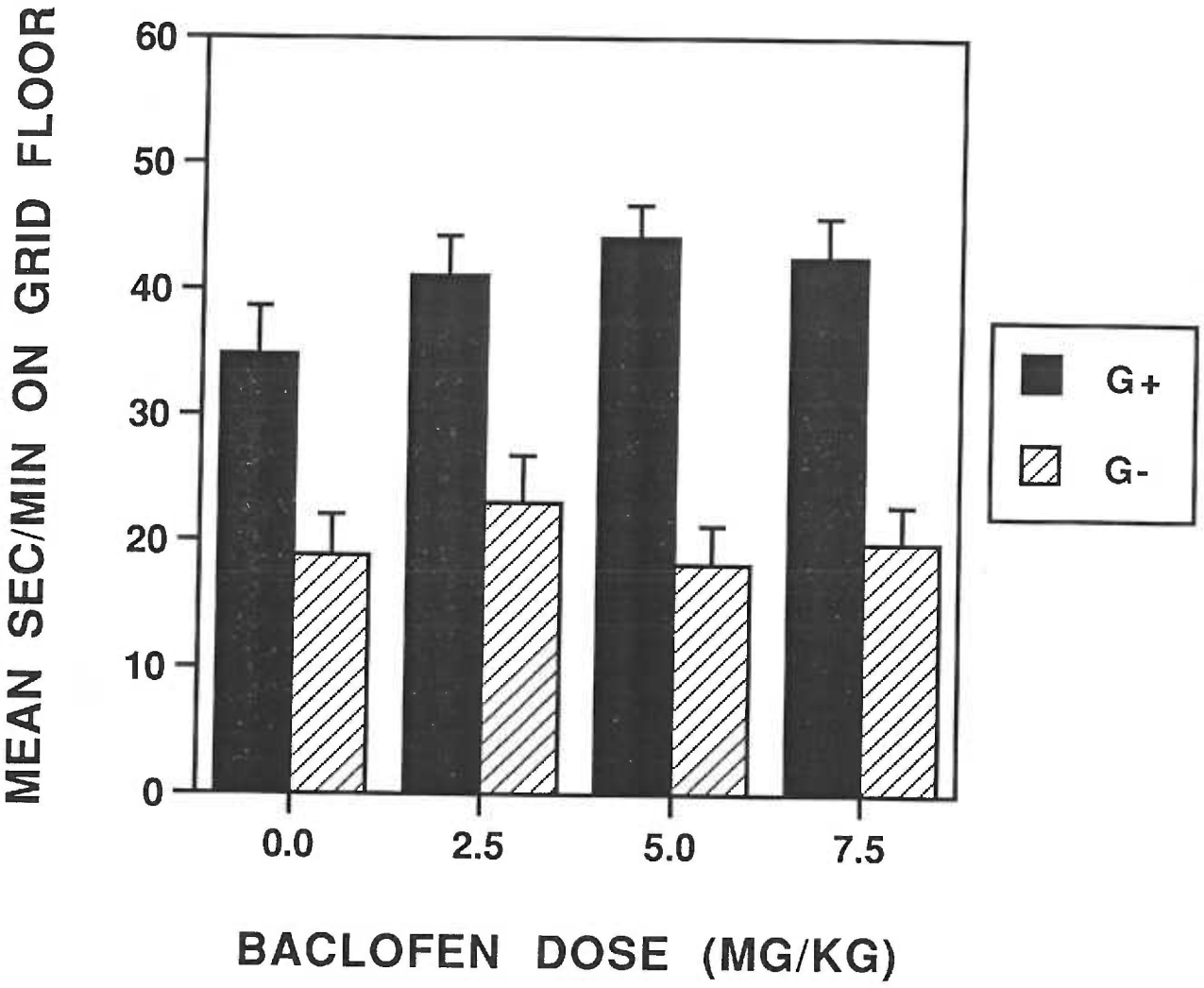
5.0 MG/KG (CS+)
7.5 MG/KG (CS+)
0.0 MG/KG (CS-)
2.5 MG/KG (CS-)
5.0 MG/KG (CS-)
7.5 MG/KG (CS-)


MEAN SEC/MIN ON GRID FLOOR
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BACLOFEN DOSE (MG/KG)
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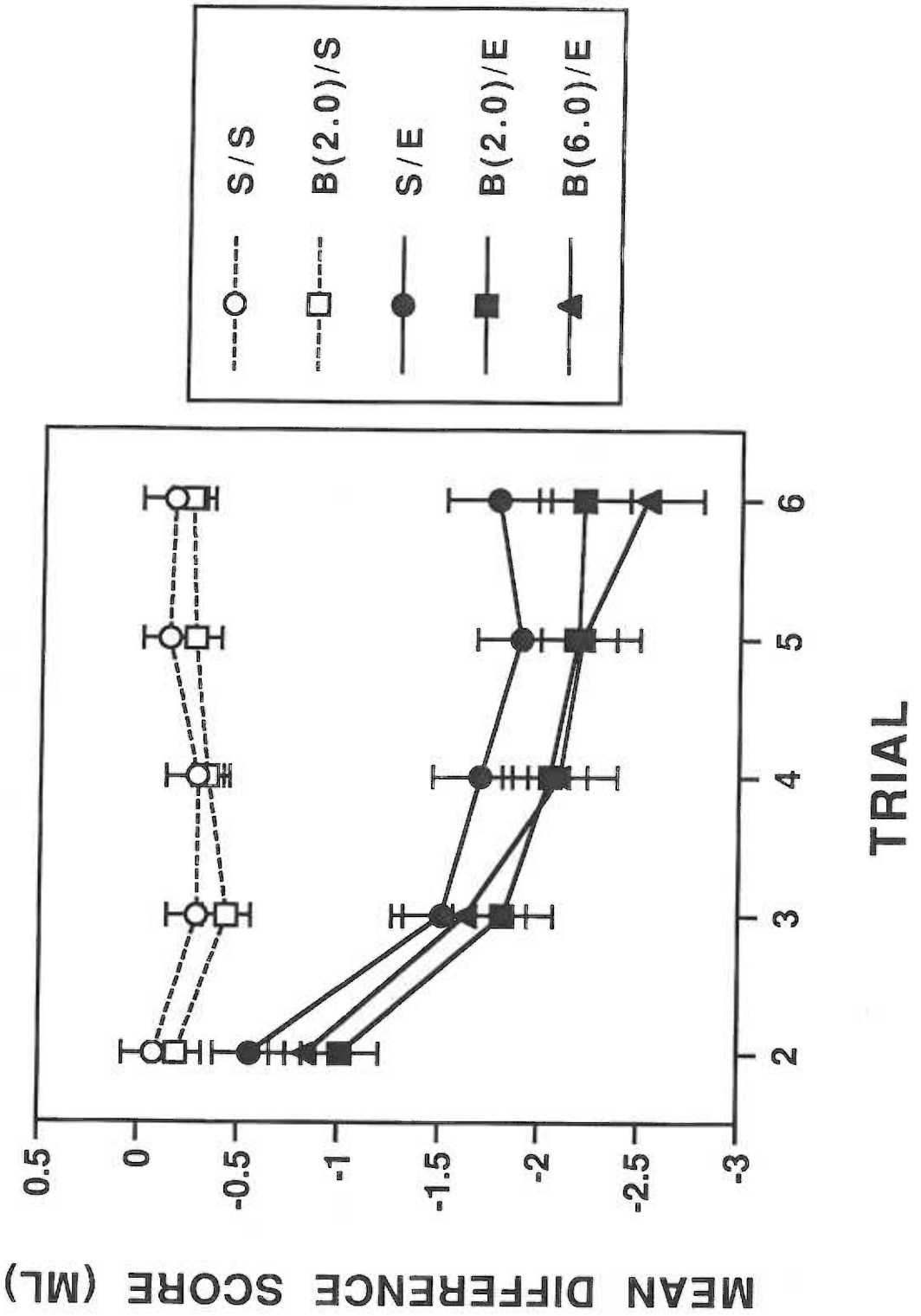
S/S
B(2.0)/S
S/E
B(2.0)/E
B(6.0)/E
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
2
3
4
5
6
TRIAL
MEAN DIFFERENCE SCORE (ML)

General Discussion

The present experiments examined a role for the GABA receptor system in modulating ethanol's rewarding and aversive effects in DBA/2J mice using the CPP and CTA paradigms. The first set of experiments examined the effects of the $GABA_A$ antagonists, picrotoxin and bicuculline, on the acquisition of ethanol-induced CPP and CTA. The results of these studies showed that both picrotoxin and the lowest dose of bicuculline (1.0 mg/kg) increased the magnitude of CPP. In addition, picrotoxin dose-dependently increased the magnitude of ethanol-induced CTA; however, bicuculline did not have a significant effect on the acquisition of ethanol-induced CTA. The second set of experiments tested the effects of the $GABA_B$ agonist, baclofen, on ethanol-induced CPP and CTA. Although baclofen dose-dependently decreased ethanol-stimulated activity, it did not alter the magnitude of CPP or CTA at any dose. Overall, these findings suggest that blockade of $GABA_A$ receptors may increase the rewarding and aversive effects of ethanol, but that activation of $GABA_B$ receptors does not alter ethanol's motivational effects in the CPP and CTA paradigms.

Taken together, the results with picrotoxin and bicuculline provide mixed evidence for the hypothesis that ethanol-induced CPP and CTA reflect activation of the same neural substrate (Hunt & Amit, 1987). The picrotoxin studies suggest that the $GABA_A$ receptor modulates ethanol's rewarding and aversive effects, because picrotoxin enhanced both CPP and CTA. Whereas the fact that a low dose of bicuculline enhanced ethanol-induced CPP but not CTA suggests that separate neural mechanisms may mediate ethanol's rewarding and aversive effects in these paradigms. However, this suggestion should be interpreted with caution due to several reasons discussed below that may account for the dissociation between bicuculline's effects on ethanol-induced CPP and CTA.

$GABA_A$ Receptor Studies

The $GABA_A$ receptor studies were designed to test the hypothesis that activation of $GABA_A$ receptors during acquisition of ethanol-induced CPP and CTA is an important factor modulating ethanol's rewarding and aversive effects during conditioning. It was predicted that blockade of $GABA_A$ receptors with picrotoxin and bicuculline would attenuate ethanol-induced CPP and CTA. This prediction was based on studies showing that these antagonists reduce many of ethanol's effects, at both a cellular (Allan & Harris, 1986; Suzdak et al., 1986; Mehta & Ticku, 1988) and behavioral (Liljequist & Engel, 1982; Martz et al., 1983) level. Furthermore, picrotoxin has been shown to reduce ethanol self-administration (Boyle et al., 1993) and ethanol-induced CTA in rats (Smith et al., 1989). Opposite to the predicted outcome, picrotoxin enhanced the magnitude of ethanol-induced CPP and CTA. Consistent with the effects of picrotoxin, bicuculline increased ethanol-induced CPP at the lowest dose (1.0 mg/kg). Thus, these data suggest that $GABA_A$ receptor blockade may increase ethanol's rewarding and aversive motivational properties. It is possible that activation of $GABA_A$ receptors during conditioning trials with ethanol may normally have an inhibitory influence on the neuronal substrates mediating ethanol's motivational effects in the CPP and CTA paradigm.

CPP experiments. One interpretation of the enhancement of ethanol-induced CPP with $GABA_A$ antagonists is that these drugs possess rewarding effects in the place conditioning paradigm. Thus, the increase in CPP could be due to a summation of rewarding effects produced separately by the antagonists and ethanol. However, the picrotoxin CPP study showed that picrotoxin alone (2 mg/kg) did not produce place conditioning, suggesting that picrotoxin does not possess motivational properties in DBA/2J mice in the place conditioning paradigm. These data suggest that the effect of $GABA_A$ receptor blockade on ethanol-induced CPP is selective for ethanol's rewarding properties. However, the bicuculline CPP study did not include a control group to assess

the motivational properties of bicuculline in the place conditioning paradigm. Thus, the possibility that the enhancement of CPP with 1.0 mg/kg bicuculline is due to a separate motivational effect of bicuculline cannot be ruled out. Future studies should address this issue. Previous studies have shown that picrotoxin produces a place aversion in rats (Spyraki et al., 1985; Acquas et al., 1989). However, the lack of place conditioning with picrotoxin in the present study may be due to differences in the place conditioning procedure or a species difference in sensitivity to an aversive motivational effect of picrotoxin.

Several explanations may account for the finding that the higher doses of bicuculline (3.0 and 5.0 mg/kg) did not alter the magnitude of ethanol-induced CPP. First, it is possible that higher doses of bicuculline are aversive and produce a separate place aversion that competes with the expression of ethanol preference. Unfortunately, it is not possible to test this idea in the place conditioning procedure because repeated administration of these doses of bicuculline alone produces severe convulsions and death in mice (Freund et al., 1987; Engstrom & Woodbury, 1988; Phillips et al., 1989). In fact, even in combination with ethanol, bicuculline-induced convulsions were noted in some animals in the 5.0 mg/kg dose group (informal observations), suggesting that this dose of bicuculline may have been too high. This may also account for the significant subject attrition in this drug treatment group. Furthermore, the high rate of attrition in this dose group may explain why 5.0 mg/kg bicuculline did not alter the magnitude of ethanol-induced CPP. However, this explanation cannot account for the lack of effect in the 3.0 mg/kg dose group.

Another possible reason for this dose effect is that bicuculline may increase ethanol's rewarding properties only in a low dose range. The mechanism for such an effect is unknown, but may be due to differential sensitivity to bicuculline in certain neuronal populations located in reward-related pathways. For example, several studies have

reported differential sensitivity of dopamine versus non-dopamine (e.g., GABA) cells in the ventral mesencephalon to GABA agonists. Specifically, GABA and muscimol have been shown to preferentially inhibit non-dopamine versus dopamine neurons in both in vivo and in vitro preparations (Grace & Bunney, 1979; Waszczak et al., 1980; Klitenick et al., 1992). Furthermore, Klitenick et al. observed a biphasic dose-response curve for extracellular dopamine with increasing doses of muscimol, possibly due to a greater number of $GABA_A$ receptors on non-dopamine relative to dopamine neurons (Churchill et al., 1992). The observed dose-response relationship for bicuculline's effect on ethanol-induced CPP may be mediated by a similar type of mechanism. Thus, a low dose of bicuculline may produce changes in dopamine release that is counteracted with higher bicuculline doses. This could also be the mechanism by which picrotoxin enhances ethanol-induced CPP; however, a wider dose range of picrotoxin should be tested to determine if a similar dose-response relationship exists for both $GABA_A$ antagonists. Furthermore, lower doses of bicuculline (below 1 mg/kg) should be examined in order to determine the full dose-response pattern for bicuculline's effect on ethanol-induced CPP.

Several studies support the possibility that bicuculline and picrotoxin's effect on CPP may be due to an interaction with the dopamine system. Direct administration of picrotoxin into the VTA has been shown to increase locomotor activity, presumably through direct antagonism of $GABA_A$ receptors on dopamine neurons (Mogenson et al., 1979; Stinus, Herman, & Le Moal, 1982). In addition, bicuculline is self-administered directly into the VTA in mice, and this effect is blocked with administration of the D_2 dopamine receptor antagonist, sulpiride (David et al., 1997). However, the effects of picrotoxin and bicuculline on ethanol-stimulated locomotor activity do not support the idea that these antagonists may produce an increase in dopamine levels, because activity levels were either reduced or unaltered in the CPP experiments. In addition, previous studies in DBA/2J mice have shown that blockade of dopamine receptors with haloperidol does not

reduce the acquisition (Risinger, Dickinson, et al., 1992) or expression (Cunningham, Malott, et al., 1992) of ethanol-induced CPP . It might be useful to test whether the effect of these $GABA_A$ antagonists can be reversed with coadministration of antagonists selective for dopamine receptor subtypes. In addition, because of the ubiquitous distribution of $GABA_A$ receptors in the brain, site-specific injections would be useful to determine the area of the brain that is mediating the effects of these $GABA_A$ antagonists on ethanol-induced CPP.

CTA experiments. The results of the CTA studies showed that picrotoxin, but not bicuculline, dose dependently enhanced the magnitude of ethanol-induced CTA. Thus, unlike the CPP studies, picrotoxin and bicuculline did not have the same effect on ethanol-induced CTA. This finding suggests that $GABA_A$ receptor blockade with picrotoxin increases the aversive properties of ethanol in the CTA paradigm. The mechanism mediating the enhancement of ethanol-induced CTA may also be the same mechanism responsible for the enhancement of ethanol-induced CPP. Thus, blockade of $GABA_A$ receptors with picrotoxin may produce changes in dopamine transmission in the neural pathway(s) that mediate ethanol's motivational effects. Indeed, there is evidence to suggest that dopamine modulates ethanol-induced CTA, because dopamine antagonists have been shown to reduce the acquisition of CTA (Risinger, 1994).

One potential explanation for the enhancement of ethanol-induced CTA with picrotoxin is that picrotoxin slows the metabolism of ethanol. However, determination of blood ethanol concentrations at three time points following 0.75 and 2.5 mg/kg picrotoxin indicated that these doses of picrotoxin do not alter the metabolism of ethanol. This is consistent with another study showing that picrotoxin does not alter ethanol pharmacokinetics in mice (Koechling et al., 1991). In addition, these data indicate that the enhancement of ethanol-induced CPP with 2.0 mg/kg picrotoxin cannot be explained by a change in blood ethanol concentration. Another possible interpretation of picrotoxin's

effect on ethanol-induced CTA is that picrotoxin has aversive motivational effects in the CTA paradigm and produces a separate CTA that summates with the CTA produced by ethanol. However, neither picrotoxin or bicuculline produced a CTA when administered alone at the lowest dose. This is consistent with several studies that failed to find a CTA in rats with picrotoxin (Bures & Buresova, 1989; Smith et al., 1989) or bicuculline (Bures & Buresova, 1989). Thus, these data suggest that picrotoxin selectively enhances ethanol's aversive properties in the taste conditioning paradigm.

The enhancement of ethanol-induced CTA is also inconsistent with a previous study that showed picrotoxin selectively reduced ethanol-induced CTA in rats (Smith et al., 1989). There are several possible explanations that may account for this discrepancy. First, there may be a species difference in sensitivity to ethanol and/or picrotoxin. This could be due to genetic differences in $GABA_A$ receptor function or regional distribution in the CNS. For example, bicuculline has been shown to have opposite effects on ethanol-induced narcosis in two lines of mice selectively bred for differential sensitivity to ethanol's sedative properties (Dudek & Phillips, 1989). Alternatively, the discrepancy could also be due to procedural differences in the CTA paradigm. For example, in the Smith et al. study, picrotoxin was administered 30 min before animals received 20 min access to a saccharin solution that was immediately followed by ethanol. In the present study, picrotoxin was administered immediately after 1-hr access to a saccharin solution and 15 min before ethanol. Thus, in the Smith et al. study, it is possible that picrotoxin altered the stimulus properties of saccharin which may have disrupted conditioning, resulting in the observed attenuation of CTA. In addition, Smith et al. administered only two conditioning trials, whereas five conditioning trials were administered in the present study. Because the effect of picrotoxin on ethanol-induced CTA in the present study developed over the five conditioning trials, it is possible more than two conditioning trials were required in the Smith et al. study to observe an enhancement of ethanol-induced CTA with picrotoxin.

There are several reasons that may explain why bicuculline and picrotoxin did not have the same effect on ethanol-induced CTA. One possibility is that picrotoxin and bicuculline have different pharmacological actions on ethanol's aversive effects in the CTA paradigm. This may be due to the fact that picrotoxin is a non-competitive antagonist that binds within the chloride channel whereas bicuculline is a competitive antagonist that binds to the GABA binding site (see Sivilotti & Nistri, 1991 for review). Bicuculline and picrotoxin have also been shown to differ with respect to blockade of GABA-binding to $GABA_A$ receptors in the brain (Zukin et al., 1974; Simmonds, 1980) and release of $[H^3]$-GABA from brain slices (Johnston & Mitchell, 1971). In addition, bicuculline has been shown to produce pharmacological effects not mediated through the $GABA_A$ receptor, including changes in calcium currents (Heyer et al., 1981; Johnson & Seutin, 1997) and inhibition of acetylcholinesterase production (Olsen et al., 1976). Behavioral studies also show bicuculline and picrotoxin differ with regard to their mechanism of seizure production in mice (Schechter & Tranier, 1977; Engstrom & Woodbury, 1988). For example, bicuculline is a more potent convulsant, with a lower ED_{50} and shorter latency to onset of myoclonus and clonus seizures (Phillips et al., 1989; Engstrom & Woodbury, 1988). Finally, the difference between picrotoxin and bicuculline's effect on ethanol-induced CTA could be due to variations in $GABA_A$ receptor subunit composition throughout the brain. For example, the brain pathway(s) mediating ethanol-induced CTA may contain a higher proportion of $GABA_A$ receptor subunits preferentially sensitive to picrotoxin binding. Indeed, regional differences in $GABA_A$ receptor sensitivities to bicuculline and picrotoxin have been reported (Krnjevic, 1974).

Another possible reason for bicuculline's lack of effect on ethanol-induced CTA is that the dose of ethanol in combination with bicuculline was too high to observe an enhancement of CTA. In order to examine a wide dose range of bicuculline, a dose of 3 g/kg ethanol was chosen in order to avoid bicuculline's proconvulsant effects (Phillips et

al., 1989). However, the greater magnitude of CTA produced by 3 g/kg ethanol (relative to 2 g/kg in the picrotoxin experiment) may have masked any enhancement of ethanol-induced CTA with bicuculline. In addition, a significant number of animals were lost in the high bicuculline dose group (4.0 mg/kg), indicating that this dose combination was detrimental to the health of the subjects during the course of the CTA experiment. Thus, the high subject attrition in the 4.0 mg/kg bicuculline dose group may have precluded detection of an effect of bicuculline on ethanol-induced CTA. A study is currently being conducted to examine a lower dose of ethanol (i.e., 2 g/kg) in combination with lower doses of bicuculline to determine if picrotoxin and bicuculline indeed have different effects on ethanol-induced CTA.

$GABA_A$ Receptors in Learning and Memory

An alternative interpretation of these data should also be considered. Specifically, it is possible that the enhancement of CPP with picrotoxin and bicuculline and CTA with picrotoxin is due to an effect on a mechanism related to learning and memory, rather than a mechanism related to ethanol's motivational properties. Many studies have shown that administration of $GABA_A$ receptor antagonists facilitate the acquisition and retention of learned behavior in several different types of tasks, including appetitively motivated maze learning (Breen & McGaugh, 1961), active (Bovet, McGaugh, & Oliverio, 1966; Yonkov & Georgiev, 1985) and passive avoidance (Brioni & McGaugh, 1988, Castellano & Pavone, 1988, Castellano & McGaugh, 1989), and discrimination learning (Grecksch & Matthies, 1981; Brioni & McGaugh, 1988). Accordingly, administration of picrotoxin and bicuculline during conditioning trials may facilitate the acquisition of CPP and CTA by strengthening the learned association between the conditioned stimuli (floor or flavor) and ethanol's motivational effects. However, this seems unlikely because picrotoxin has been shown to attenuate ethanol induced CTA (Smith et al., 1989) and diazepam-induced CPP in

rats (Spyraki et al., 1985). In addition, administration of picrotoxin and bicuculline in DBA/2 mice has been shown to impair retention of an inhibitory avoidance response (Castellano, Cestari, Cabib, & Puglisi-Allegra, 1993). Thus, the results from these studies do not support the idea that picrotoxin and bicuculline may enhance ethanol-induced CPP and CTA by affecting a learning and memory mechanism.

$GABA_B$ Receptor Studies

The $GABA_B$ studies examined the hypothesis that $GABA_B$ receptor activation is an important factor modulating ethanol's motivational effects. This hypothesis was based on previous studies that suggested $GABA_B$ receptor activation alters ethanol's motivational effect in the oral self-administration paradigm. However, the results of these studies were contradictory, because in one study baclofen reduced established ethanol consumption (Daoust et al., 1987) and in another it facilitated the acquisition of voluntary ethanol consumption (Smith et al., 1992). In the present studies, baclofen (2.5, 5.0, and 7.5 mg/kg) dose-dependently reduced ethanol-stimulated activity in the CPP study; however, it did not alter the magnitude of ethanol-induced CPP at any dose. The CTA study also showed that baclofen (2.0 and 6.0 mg/kg) did not alter the acquisition of ethanol-induced CTA at any dose. In addition, administration of baclofen alone (2.0 mg/kg) did not produce a CTA. This finding is consistent with a study showing that baclofen does not produce a CTA in rats (Ebenezer et al., 1992). Thus, the results of the present experiments do not support a role for the $GABA_B$ receptor in modulating ethanol's rewarding or aversive effects in the CPP and CTA paradigms. The present results are consistent, however, with a recent study that showed no effect of baclofen on ethanol or water intake when it was administered directly into the dorsal raphe nucleus (Tomkins and Fletcher, 1996), an area where activation of $GABA_A$ receptors has been shown to increase ethanol self-administration (Tomkins et al., 1994).

Locomotor Activity

It has previously been suggested that a positive relationship exists between ethanol's motor stimulant effects and its rewarding effects (Wise and Bozarth, 1987). However, consistent with previous studies (Risinger, Dickinson, et al., 1992; Risinger et al., 1994; Cunningham, 1995; Chester & Cunningham, 1998), the CPP experiments demonstrate a clear dissociation between ethanol-stimulated locomotor activity and the rewarding effects of ethanol in the place conditioning paradigm. In the picrotoxin CPP study, picrotoxin significantly reduced ethanol-stimulated but increased ethanol-induced CPP. Furthermore, the bicuculline CPP study showed that 1.0 mg/kg bicuculline did not alter ethanol-stimulated activity, but this dose of bicuculline significantly increased the magnitude of CPP. In addition, 3.0 and 5.0 mg/kg bicuculline dose-dependently decreased ethanol-stimulated activity, but these doses had no effect on ethanol-induced CPP. The reduction in ethanol-stimulated activity with picrotoxin is inconsistent with Liljequist and Engel (1982), who showed a potentiation of ethanol-stimulated activity (3.75 g/kg ethanol) with picrotoxin administration in NMRI mice. However, another study showed that picrotoxin decreased ethanol-stimulated activity with lower ethanol doses (0.8 and 1.2 g/kg) and increased stimulated activity with a higher ethanol dose (1.6 g/kg) in Swiss-Webster mice (Koechling et al., 1991). The discrepancies between the previous and present studies are likely due to differences in ethanol and picrotoxin doses and genetic differences in the mouse strains used.

The results of the baclofen CPP study also suggest a dissociation between ethanol's rewarding and locomotor effects, because baclofen dose-dependently decreased ethanol-stimulated activity but did not alter the magnitude of ethanol-induced CPP. The finding that baclofen dose-dependently reduced ethanol-stimulated activity is consistent with previous studies (Cott et al., 1976; Humeniuk et al., 1993; Shen et al., in press). It is possible that this effect of baclofen is due to a reduction in ethanol-stimulated dopamine

release. Baclofen has been shown to decrease the activity of dopamine neurons (Fuxe et al., 1975; Olpe et al, 1977; Lacey et al., 1988) and decrease extracellular dopamine levels in the VTA (Klitenick et al., 1992). In addition, baclofen has also been shown to reduce the motor-stimulant effect of other drugs known to act through an increase in dopamine levels, such as cocaine or amphetamine (Kuzcenski, 1983; Kalivas et al., 1990).

Relationship to Self-Administration Studies

Most studies that have examined a role for the GABA receptor system in modulating ethanol's motivational effects have used the oral self-administration paradigm. Many of these studies have consistently reported that $GABA_A$/BZ receptor antagonists reduce ethanol consumption in several different types of self-administration procedures (see Table 1). The results of the CPP studies suggest that this reduction in ethanol-self-administration with $GABA_A$ receptor blockade may be due to an increased sensitivity to ethanol's rewarding effects. An increased sensitivity to ethanol's rewarding effects may allow the animal to consume less ethanol to obtain the same motivational effect. Alternatively, the results of the CTA experiment with picrotoxin suggest that $GABA_A$ receptor blockade may increase ethanol's aversive effects. Thus, an increase in ethanol's aversive effect in the self-administration paradigm might also produce an decrease in ethanol consumption. The CPP and CTA paradigms offer an advantage to the self-administration model in that they avoid potential effects of an agonist or antagonist on ingestive behavior. Indeed, an alternate interpretation of the self-administration data is that the effect of $GABA_A$ antagonists are due to their effects on consummatory behavior, rather than an effect on ethanol's motivational properties. This possibility is supported by many studies reporting that GABA/BZ agents alter taste mechanisms (e.g., Berridge & Treit, 1986), food consumption (see Cooper, 1980; 1986 for reviews), and the palatability of ethanol (Söderpalm & Hansen, 1998). Thus, changes in ethanol-self-administration

reported with GABA receptor ligands may be due to their effects on consummatory or satiety mechanisms, or alterations in the palatability of ethanol.

There are several possible reasons for the difference between baclofen's effect in previous self-administration studies and the present results. For example, it is possible that subject vs experimenter control over ethanol exposure is an important factor in determining baclofen's effect in the self-administration paradigm. Thus, baclofen may specifically interact with a neural pathway important for modulating oral ethanol self-administration, and this pathway may be distinct from the pathways modulating ethanol-induced CPP and CTA. In addition, baclofen's effect on ethanol self-administration may be unique to rats. An alternate interpretation of the self-administration studies with baclofen should also be considered. Specifically, because baclofen has been shown to alter food (Ebenezer & Pringle, 1992; Zarrindast et al., 1989 Stratford & Kelley, 1997) and water (Ebenezer et al., 1992) consumption in rats, changes in ethanol consumption following baclofen administration may be due to its effects on ingestive behavior, rather than a selective effect on ethanol's motivational properties. Furthermore, these reported effects of baclofen on consummatory behavior may help explain the discrepancies reported in the literature regarding the effect of baclofen on ethanol self-administration (Daoust et al., 1987; Smith et al., 1992). Finally, it is important to consider that the effects of GABA receptor compounds on ethanol self-administration may not correlate with their effects on ethanol-induced CPP and CTA, since different neural mechanisms may modulate ethanol's motivational effects in each paradigm. Table 2 summarizes the effects of bicuculline, picrotoxin and baclofen in the present experiments and previous studies that tested these compounds on ethanol reward-related behaviors.

Table 2. Summary of the effects of bicuculline, picrotoxin, and baclofen on ethanol self-administration, CPP and CTA.

Table 2

Compound	Paradigm	Effect	Reference
Bicuculline	Self-administration	No effect	Boismare et al. (1984)
	CPP	Increase (low dose)	Present data
	CTA	No effect	Present data
Picrotoxin	Self-administration	Decrease	Boyle et al. (1993)
	CPP	Increase	Present data
	CTA	Increase	Present data
		Decrease	Smith et al. (1989)
Baclofen	Self-administration	Increase	Smith et al. (1992)
		Decrease	Daoust et al. (1987)
		No effect (DRN)	Tomkins & Fletcher (1996)
	CPP	No effect	Present data
	CTA	No effect	Present data

DRN - Dorsal Raphe Nucleus

Future Directions

Control Groups and Dose-Response Curves

One weakness of the bicuculline CPP study is that it did not include a control group to assess the possible motivational effects of bicuculline alone. Thus, a future study should test whether the enhancement of ethanol-induced CPP with 1.0 mg/kg bicuculline is due to a separate rewarding effect of bicuculline that summates with the preference induced by ethanol. Since ethanol-induced CPP was increased with only the lowest dose of bicuculline tested (1.0 mg/kg), it is possible that this dose is on the descending portion of an inverted U-shaped dose-response curve. It would be useful to examine lower doses of bicuculline (below 1.0 mg/kg) to establish the full dose-response pattern for bicuculline's effect on ethanol-induced CPP. Future studies should also test a wider dose range of picrotoxin to determine if a similar dose-response relationship exists for both $GABA_A$ antagonists. In addition, it might be possible to examine higher doses of bicuculline and picrotoxin alone in the place conditioning paradigm and avoid generalized convulsions if they were administered to regionally specific brain areas.

Characterization of $GABA_A$ Antagonist Effects

Because of the ubiquitous distribution of $GABA_A$ receptors in the brain, site-specific injections would be useful to determine the area of the brain that is mediating the effects of bicuculline and picrotoxin on ethanol-induced CPP and CTA (picrotoxin). In addition, there is evidence to suggest that these effects may be due to alterations in dopaminergic activity. For example, David et al. (1997) showed that mice will self-administer bicuculline directly into the VTA, which is blocked with administration of a D_2 antagonist. If $GABA_A$ antagonists produce an increase in the magnitude of CPP and CTA by increasing dopamine release, it would be predicted that administration of a dopamine antagonist would reverse this effect. Future studies might test whether the effects of

bicuculline and picrotoxin can be blocked with coadministration of antagonists selective for dopamine receptor subtypes.

$GABA_A$ Agonists

It would be interesting to determine the effects of $GABA_A$ agonists, such as muscimol or THIP, on the acquisition of ethanol-induced CPP and CTA. Based on the results of the present studies, $GABA_A$ agonists would be expected to decrease the magnitude of ethanol-induced CPP and CTA. A full parametric study to determine effective dose combinations of $GABA_A$ agonists and ethanol should be conducted.

A recent study (Risinger & Cunningham, in press) found significant genetic correlations between ethanol-induced CTA and severity of acute ethanol withdrawal in BXD recombinant inbred mice. Specifically, a stronger magnitude of ethanol-induced CTA was observed in strains that display greater handling induced convulsions 4-12 hrs after an injection of 4 g/kg ethanol (Buck, Metten, Belknap, & Crabbe, 1997). Given these findings, it is quite possible that aversive motivational effects of ethanol-withdrawal contribute to the development of ethanol-induced CTA. Furthermore, it is tempting to speculate that picrotoxin enhances ethanol-induced CTA by increasing aversive motivational effects of ethanol-withdrawal. One interesting experiment would be to administer anticonvulsants, such as $GABA_A$ agonists, during the period of peak ethanol withdrawal to see whether picrotoxin's effect on ethanol-induced CTA is attenuated.

Species/Strain/Procedural Differences

The enhancement of ethanol-induced CTA with picrotoxin was in contrast to Smith et al. (1989) who showed that picrotoxin selectively reduced ethanol-induced CTA in rats. One possible reason for this discrepancy is that Smith et al. used different taste conditioning procedures/ parameters from the present studies. To examine this issue, an experiment could be conducted that tests the effects of picrotoxin on ethanol-induced CTA in rats using similar taste conditioning parameters used in the present experiments. In

addition, since ethanol also possesses aversive motivational effects in the place conditioning paradigm in rats (Cunningham et al., 1993) and mice (Cunningham et al., 1997), it would be interesting to test the effects of bicuculline and picrotoxin on ethanol-induced CPA in rats and mice. Finally, future studies should examine the effects of GABAergic compounds on ethanol-induced CPP and CTA in other strains of mice to test whether the present results are generalizable to the mouse species as a whole.

Conclusions

The present studies examined a role for the GABA receptor system in modulating ethanol's rewarding and aversive properties in the CPP and CTA paradigms in DBA/2J mice. The first set of experiments examined the effect of two $GABA_A$ antagonists, bicuculline and picrotoxin, on the acquisition of ethanol-induced CPP and CTA. In contrast to the expected outcome, picrotoxin increased the magnitude of both ethanol-induced CPP and CTA. Bicuculline also enhanced ethanol-induced CPP at the lowest dose, but did not alter the magnitude of CTA. These results suggest that $GABA_A$ receptor blockade may increase ethanol's rewarding and aversive properties. The second set of experiments tested the effect of the $GABA_B$ agonist, baclofen, on ethanol-induced CPP and CTA. These studies showed that baclofen dose-dependently reduced ethanol-stimulated activity, but did not alter the magnitude of ethanol-induced CPP or CTA at any dose. In addition, the results of the CPP studies showed a clear dissociation between ethanol's locomotor-stimulant and rewarding effects, because changes in ethanol-stimulated activity with administration of GABA drugs were unrelated to their effects on ethanol-induced CPP. Overall, the results of these studies suggest that $GABA_A$, but not $GABA_B$ receptors modulate ethanol's motivational effects in the CPP and CTA paradigms in DBA/2J mice.

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