CONTRIBUTION OF $GABA_B$ RECEPTORS TO ACUTE ETHANOL SENSITIVITY AND AS A PHARMACOTHERAPEUTIC TARGET

by

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CERTIFICATE OF APPROVAL

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LIST OF ABBREVIATIONS

ACh – acetylcholine

aCSF – artificial cerebral spinal fluid

AHP – afterhyperpolarization

ALLO - allopregnanolone

Amy – amygdala

ANOVA – analysis of variance

aVTA – anterior ventral tegmental area

B6 – C57BL/6J inbred mouse strain

BEC – blood ethanol concentration

BLA – basolateral nucleus of the amygdala

CeA – central nucleus of the amygdala

CPM – counts per minute

CPP – conditioned place preference

CPu – caudate-putamen

CTA – conditioned taste aversion

D2 – DBA/2J inbred mouse strain

DAT – dopamine transporter

EC_{50} – concentration of agonist that produces a half-maximal stimulation of the response

E_{max} – maximal percent stimulation of response

GABA – γ-aminobutyric acid

$GABA_A$ Receptor – γ-aminobutyric acid, subtype A, receptor

$GABA_B$ Receptor – γ-aminobutyric acid, subtype B, receptor

GAT1 – γ-aminobutyric acid transporter, subtype 1

GDP – guanosine 5'-diphosphate

GIRK – G-protein inwardly rectifying potassium channel

GP – globus pallidus

GPCR – G-protein coupled receptor

GTP – guanosine-5'-triphosphate

HAD – high alcohol drinking rat line

HAP – high alcohol preference mouse line

HPC – hippocampus

HPLC – high performance liquid chromatography

ICV – intracerebroventricular

i.p. – intraperitoneal

i.v. – intravenous

KCNQ – potassium channel

LAD – low alcohol drinking rat line

LAP – low alcohol preference mouse line

LDTg – laterodorsal tegmentum

LORR – loss of righting reflex

LS – long-sleep selected mouse line

MSNs – medium spiny neurons

NAc – nucleus accumbens

NMDA – *N*-methyl-D-aspartate

NP – alcohol non-preferring rat line

MD Thal – mediodorsal thalamus

P – alcohol preferring rat line

PfC – prefrontal cortex

PPTg – pedunculopontine tegmental nucleus

rFAST – reverse-selected FAST line

rSLOW – reverse-selected SLOW line

[^{35}S]GTPγS – guanosine 5′-*O*-(3-[^{35}S]thiotriphosphate)

SN – substantia nigra

SNc – substantia nigra pars compacta

sP – Sardinian alcohol-preferring rat line

sNP – Sardinian alcohol-nonpreferring rat line

SS – short-sleep selected mouse line

VGCC – voltage-gated calcium channel

VP – ventral pallidum

VTA – ventral tegmental area

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ABSTRACT

Increased sensitivity to the behavioral stimulant effects of ethanol may be a risk factor for alcohol use disorders, and treatments that reduce this stimulant response may be potential pharmacotherapies. In examining the neurochemical substrates underlying the stimulant response to ethanol in mice selectively bred for extreme sensitivity (FAST) and insensitivity (SLOW) to this response, the γ-aminobutyric acid (GABA) system has repeatedly been implicated. These lines differed in sensitivity to a wide array of $GABA_A$ receptor modulators and to the $GABA_B$ receptor agonist baclofen, and baclofen attenuated the stimulant response to ethanol in FAST mice. Therefore, GABA systems are likely a critical component involved in the stimulant response to ethanol; however, the exact contribution of this system is not known.

The main purpose of this dissertation was to examine how selective breeding of the FAST and SLOW lines had altered GABA systems, and $GABA_B$ receptors in particular, as well as to examine potential mechanisms by which GABAergic drugs attenuate the stimulant response to ethanol. The contribution of the GABA transporter was first examined, and the FAST and SLOW lines were found to differ in acute locomotor sensitivity to the transporter inhibitor NO-711, supporting a difference in GABA system function between the two lines. Further, NO-711 attenuated the locomotor stimulant response to ethanol in FAST mice; however this reduction in stimulation was accompanied by an enhancement of the motor incoordinating effects of a low dose of ethanol. Similar results were observed with the $GABA_A$ receptor agonist muscimol, suggesting that a preferential activation of $GABA_A$ receptors additively

interacts with ethanol to shift the behavioral response towards greater motor impairment and intoxication. Activation of $GABA_B$ receptors by baclofen, however, did not enhance ethanol-induced motor incoordination, suggesting that baclofen may reduce the stimulant response to ethanol by reducing a neurochemical response relevant to ethanol-induced stimulation rather than an enhancement of intoxication.

To further examine how $GABA_B$ receptors may contribute to the stimulant response to ethanol, receptor function and microdialysis assays were performed. FAST and SLOW mice differed in $GABA_B$ receptor function, measured as baclofen-stimulated [^{35}S]GTPγS binding, in the striatum, with increased efficacy of baclofen in FAST mice, and in the ventral midbrain, with increased potency of baclofen in SLOW mice. $GABA_B$ receptors in the ventral midbrain are hypothesized to attenuate the stimulant response to ethanol by attenuating an ethanol-induced stimulation of dopamine signaling. To determine if this was occurring in FAST mice, the effect of baclofen on ethanol-induced locomotor stimulation and ethanol-induced increases in extracellular dopamine in the nucleus accumbens was examined. While there was an elevation of extracellular dopamine levels by ethanol, this effect was not altered by baclofen despite a baclofen-induced attenuation of the stimulant response to ethanol.

These data add to the evidence supporting a contribution of GABA systems to acute ethanol sensitivity. The effect of baclofen on ethanol-stimulated behavior may occur independent of alterations in mesolimbic dopamine signaling, suggesting that other pathways should be explored. In addition, these results suggest that the $GABA_B$ receptor may be an attractive candidate as a pharmacotherapy for alcohol use disorders, because it may block some effects of alcohol without producing undesirable side effects.

CHAPTER 1. General Introduction

Alcohol use disorders are defined by the Diagnostic and Statistical Manual of Mental Disorders-IV-TR (DSM-IV-TR; American Psychiatric Association, 2000) as maladaptive patterns of alcohol consumption that lead to clinically significant impairment and/or distress. These alcohol use disorders are further subdivided into alcohol abuse, with hazardous drinking despite continued social or legal problems, and alcohol dependence, which is marked by the development of physical tolerance to continued consumption and the manifestation of a withdrawal syndrome upon cessation of drinking. In the United States, the prevalence of alcohol disorders is 8.5% of the American population, or 17.6 million adults 18 and over (Grant et al., 2004). Therefore, alcohol use disorders remain a serious health problem in the United States.

Findings from the National Epidemiological Survey on Alcohol and Related Conditions suggest that of these individuals, only a small percentage actually seek treatment, including self-help and 12 step treatment programs (approximately 15% of individuals with a lifetime alcohol use disorder) (Cohen et al., 2007). For those individuals who do attempt recovery, approximately 38% abstain from alcohol use; however, an overwhelming 62% of individuals continue drinking. The majority of these individuals are considered low-risk drinkers, though substantial proportions continue drinking at high levels (Dawson et al., 2007).

In determining treatment options for individuals with alcohol use disorders, it is important to determine whether abstinence is a viable treatment goal. This is especially a concern if only one-third of individuals in remission actually abstain from alcohol use

(Dawson et al., 2007). Some clinicians suggest that, for certain patients, moderation may be a more feasible treatment plan (Finney and Moos, 2006; Marlatt, 1983; Marlatt and Witkiewitz, 2002). When pharmacological management is combined with behavioral therapy for a moderation treatment plan, one concern that arises is that the pharmacotherapy will potentially be co-administered with alcohol. This is even a concern for pharmacotherapies that are designed to be administered with alcohol to curtail drinking. Potential medication interactions with alcohol, particularly interactions which may enhance the intoxicating and sedative properties of alcohol, must be considered. Therefore, it is critical to define potential mechanisms by which alcohol (ethanol) has its effects in the central nervous system, particularly when developing pharmacotherapies for alcohol use disorders. A pharmacotherapy that has actions similar to ethanol could pose serious problems; such a drug may give the appearance of blocking an effect of ethanol by reducing an ethanol-associated behavior, when the pharmacotherapy in fact potentiates a competing effect.

Currently, there are only three approved pharmacotherapies for the treatment of alcohol use disorders: disulfiram (Antabuse®), naltrexone (Depade®, ReVia®), and acamprosate (Campral®). Disulfiram reduces ethanol consumption by inducing an adverse physiological reaction when combined with ethanol. Specifically, it blocks the metabolism of ethanol and leads to an accumulation of acetaldehyde, which results in flushing, headache, nausea, tachycardia, and other adverse, and potentially fatal, physiological reactions with higher ethanol concentrations (Suh et al., 2006). However, due to these health risks associated with the interaction of disulfiram and ethanol, as well as its low treatment adherence by patients, this treatment is not often used (Miller, 2008;

Schuckit, 1996; Suh et al., 2006). Acamprosate and naltrexone are more commonly prescribed (Miller, 2008). Acamprosate is primarily a weak glutamate receptor antagonist which may be useful in the treatment of ethanol withdrawal and relapse, though it may not be effective at curbing ethanol consumption (Mann et al., 2008; Rösner et al., 2008). Naltrexone is a non-specific opioid receptor antagonist and is hypothesized to reduce the rewarding effects of ethanol consumption. Treatment success requires the co-administration of naltrexone with ethanol to experience reduced ethanol reward, and naltrexone has been effective at reducing ethanol consumption (O'Malley et al., 1996; Sinclair, 2001; Volpicelli et al., 1992). Swift and colleagues (1994), however, report that naltrexone enhanced subjective intoxication ratings, including sedation, when combined with ethanol in non-dependent individuals. This may suggest that naltrexone is reducing the number of drinks consumed by enhancing the intoxicating and sedative subjective and physiological effects of ethanol.

There are a variety of other pharmacotherapies that are being considered as potential treatments for alcohol use disorders, including compounds which target serotonin, glutamate, acetylcholine (ACh), and γ-aminobutyric acid (GABA) systems (Miller, 2008). I will focus here briefly on those drugs that potentially inhibit ethanol consumption by activating GABA signaling. Many of these GABAergic pharmacotherapies are potent antiepileptics, and have found success at reducing ethanol consumption in rodent models (Anstrom et al., 2003; Colombo et al., 2000; Nguyen et al., 2005; Wegelius et al., 1993). In clinical studies, the antiepileptic topiramate decreased ethanol consumption and facilitated moderate drinking or abstinence (Johnson et al., 2003, 2007). Topiramate decreased glutamatergic signaling and increased GABAergic

signaling by potentiating the effect of GABA at $GABA_A$ receptors, thereby acting as a $GABA_A$ receptor positive modulator (White et al., 2000). As will be discussed in later sections, ethanol is also characterized as a $GABA_A$ receptor positive modulator; therefore, it might seem perplexing that topiramate, with actions similar to ethanol, would inhibit a behavioral effect of ethanol. Similar questions arise with other potential pharmacotherapies, including GABA transporter inhibitors, which increase extracellular concentrations of GABA, similar to ethanol, and are proposed as potential new pharmacotherapies for alcohol use disorders (Fink-Jensen et al., 1992; Malcolm, 2003). Finally, a few studies have been conducted to examine the effect of the $GABA_B$ receptor agonist baclofen on ethanol consumption. These studies report a reduction in ethanol consumption and the number of heavy drinking days, though an enhancement of sedation has also been reported (Addolorato et al., 2000, 2002; Flannery et al., 2004). Although there is less evidence for an ethanol-mediated enhancement of $GABA_B$ receptor function (Ariwodola and Weiner, 2004), this increase in sedation may suggest that baclofen also attenuated ethanol consumption by enhancing the intoxicating and sedative properties of ethanol.

Therefore, when considering GABA mimetic compounds, or compounds that have actions similar to GABA, as potential pharmacotherapies for alcohol use disorders, it is critical to determine whether these compounds interact with ethanol behaviorally to enhance a potentially negative side effect of ethanol or whether they block a neurochemical mechanism activated by ethanol. Determining whether GABA mimetic compounds attenuate the behavioral stimulant response, a potential risk factor for alcohol

use disorders, by enhancing the acute intoxicant effects of ethanol provides a simple mechanism by which to investigate this possibility.

Behavioral Stimulation to Ethanol

Ethanol produces biphasic effects in humans. Increases in motor activity and subjective measures of behavioral stimulation, including increased talkativeness, as well as feeling high, elated, or energized, rise rapidly and in close proximity to increases in blood and breath ethanol concentrations (Addicott et al., 2007; Ahlenius et al., 1973; Ekman et al., 1963, 1964; Holdstock and de Wit, 1998; King et al., 2002). Conversely, increases in sedation (feeling tired, down, inactive, or sluggish) occur at later time points after blood and breath ethanol concentrations start to decrease (Addicott et al., 2007; Ekman et al., 1963; Holdstock and de Wit, 1998).

Heightened sensitivity to the behavioral stimulant effects of ethanol in humans is linked to both ethanol consumption history (Holdstock et al., 2000; King et al., 2002) and family history of alcoholism. More specifically, low doses of ethanol preferentially increased the subjective and motor stimulant responses in nonalcoholic individuals with a family history of alcoholism (Morzorati et al., 2002; Newlin and Thomson, 1991, 1999; but see Erblich et al., 2003). This suggests a strong genetic component to acute sensitivity to the behavioral or motor stimulant effects of ethanol. And as these individuals are at greater risk for the initiation of alcohol use and transition to alcohol dependence (Heiman et al., 2008), this may implicate the behavioral stimulant response to ethanol as a risk factor for alcohol use disorders.

In addition, decreased sensitivity to the motor incoordinating and sedative effects of ethanol may also be a marker of increased risk for alcohol use disorders. Individuals with a family history of alcoholism were less sensitive to the motor incoordinating effects of ethanol (Schuckit and Smith, 2000, 2001). Further, individuals with reduced sensitivity to these motor incoordinating effects were more likely to develop problem alcohol use than were those with higher sensitivity, regardless of family history (Schuckit, 1994). Combined, these studies support increased sensitivity to the stimulant effects and decreased sensitivity to the motor incoordinating effects of ethanol as predictive factors for alcohol use disorders.

Measuring Acute Ethanol Sensitivity in Rodents

Similar to findings in humans, acute ethanol exposure also elicits biphasic effects in animals, effects that have been most extensively studied in rodents and especially mice (Pohorecky, 1977). There are many ways to examine the genetic and mechanistic contributions to acute ethanol sensitivity experimentally in mice, including the use of heterogeneous stocks, gene knockouts or transgenics, inbred strains, and selectively bred animals. These models have been discussed in several excellent reviews (Bailey et al., 2006; Crabbe, 1999; Crabbe et al., 2006; McClearn, 1991; Phillips and Crabbe, 1991; Phillips et al., 2002a), and the advantages and disadvantages of these models will be briefly discussed here. Many studies have used heterogeneous stocks, including outbred strains and inbred strain crosses, to examine stimulant and sedative sensitivity to ethanol. Due to their genetic heterogeneity, they can be useful for mapping genetic loci that contribute to the phenotypic variance. However, in their more common usage to examine

the relationship between two traits (e.g., a behavioral trait and a neurochemical trait), without determining genotype, only phenotypic correlations can be established. Because both genetic differences and different environmental exposures among individuals (and the possible interaction between these two factors) can contribute to variation in the measured traits, correlations cannot be assumed to be predominantly of genetic origin (i.e., the correlation may or may not be due to common genetic influence).

Gene knockouts, however, can be useful for elucidating the contribution of specific genes to a behavioral trait of interest. Studies with knockout animals can also determine if multiple phenotypic effects are associated with a single mutation, and thus, pleiotropic genetic influence. However, several limitations exist with the use of knockouts for behavioral research. First, behavior, including sensitivity to the stimulant and sedative effects of ethanol, is a polygenic trait – multiple genes contribute to the manifestation of this behavioral response (Downing et al., 2006; Hitzemann et al., 1998; Phillips et al., 1995; Radcliffe et al., 2000). Most gene knockout studies only alter one gene at a time and therefore the polygenic contribution to a trait cannot be assessed. In addition, as a polygenic trait, each gene alone may only have a small influence on the behavioral response. Therefore, robust effects may be difficult to observe when examining only one gene at a time. Additionally, developmental compensation may occur due to the permanent loss of the gene, resulting in the reorganization of neuronal networks and other genes compensating for the functional loss of the mutant gene. Behavioral changes in mutant mice therefore could be due to this reorganization or compensation. Problems also can arise due to genes linked to the targeted mutation (which arise from the ES cell line that the mutation was created on) that are not present in

the genetic background of the control strain, and which may alter the trait of interest and wrongly be attributed to the mutated gene.

One manner by which to examine genetic correlations more thoroughly is to use inbred strain comparisons. Each animal within a strain is genetically identical (with the exception of the X and Y chromosomes), and therefore phenotypic differences within a strain can be attributable to environmental variables. Multiple inbred strains can also be evaluated to compare the distributions of strain means for a variety of behaviors or physiological measures (differences among strains maintained in a similar environment are explicitly genetic in origin, when within strain variation serves as a measure of non-genetic influence). Problematically, some have attempted to infer a genetic relationship by simply comparing two inbred strains (a particular problem in the literature for determining the relationship between ethanol-induced activation of the mesolimbic dopamine system and the behavioral response to ethanol, as will be discussed in later sections). For instance, the DBA/2J (D2) and C57BL/6J (B6) inbred mouse strains differ dramatically in ethanol consumption, with high levels of drinking in B6 mice and low levels of drinking in D2 mice (Belknap et al., 1977; McClearn and Rodgers, 1959; Yoneyama et al., 2008). These strains also differ in sensitivity to the locomotor stimulant effects of ethanol (Crabbe, 1986; Crabbe et al., 1980; Cunningham et al., 1992; Phillips et al., 1995), but in the opposite direction. If only comparing these two strains, it could be inferred that ethanol consumption and stimulant sensitivity are inversely related, a finding that does not support the human literature. The problem in attempting to establish a genetic correlation using only two inbred strains is that there is insufficient power to detect a correlation. In fact, the degrees of freedom required for a correlational analysis

is n-2, where n in this case would be the number of strains. Therefore, for a comparison of two inbred strains, the resultant degrees of freedom is 0, which is insufficient to determine a genetic correlation. Moreover, inbred strains vary for a large number of phenotypes and spurious correlations can arise when only a small number of strains are compared. This problem can be overcome by comparing multiple inbred strains, which provides reasonable power for the detection of genetic relationships. However, inbred strains differ by chance for a trait of interest - frequencies of alleles that influence a trait of interest were not intentionally manipulated to create differences among inbred strains. Selected lines, discussed next, bypass this potential problem as selection specifically alters the frequencies of those alleles that contribute to the selection response while theoretically maintaining variation in the population for non-selection trait relevant alleles. Genetic correlations can then be found, allowing for the detection of genes that pleiotropically influence the selection response and the correlated response. These lines can also be used to establish the neurochemical mechanisms underlying the selection response.

The FAST and SLOW Selected Lines

Multiple selectively bred lines have been created for high and low ethanol consumption and preference, and for high and low sensitivity to the incoordinating and sedative effects of ethanol. However, the FAST and SLOW selected mouse lines are unique in that they are the only lines selectively bred for high and low sensitivity, respectively, to the acute locomotor stimulant effects of ethanol (Crabbe et al., 1987). These lines were bred in replicate, and originated from the HS/Ibg heterogeneous stock,

an 8-way cross of inbred strains, consisting of the A, AK, B6, BALB/c, C3H, D2, Is/Bi, and RIII strains (McClearn et al., 1970). The HS/Ibg stock is a genetically well-defined, segregating population and there are potentially 8 different alleles at each locus with the potential to influence a given trait. Nine families (half of those tested) were randomly selected to form the replicate 1 FAST and SLOW lines, and the other 9 families were selected to form the replicate 2 FAST and SLOW lines. Thus, the replicates were selected and maintained as independent breeding populations. The advantage of replicate lines is that genetically correlated traits found in one set of lines can be examined for confirmation in the other set; a correlated line difference found in both replicates provides strong evidence that the two traits are genetically correlated and did not arise due to the random fixation of selection trait-irrelevant genes or unintentional inbreeding (Crabbe et al., 1991). However, a correlated line difference that is found in one replicate set of lines but not the other does not necessarily imply that the correlation is spurious. Rather, allelic polymorphisms could be lost during the process of selection and therefore no longer influence the selection response or the putative genetically correlated trait. But as the stimulant response to ethanol is a polymorphic trait, it is likely mediated by several parallel mechanisms.

The selection trait originally was defined as the locomotor response (2-6 min after injection) to saline on day 1 subtracted from the locomotor response to 1.5 g/kg ethanol on day 2. Subjects with high activity scores were selected as breeders for the FAST line, whereas subjects with low activity scores, including negative scores, were selected as breeders for the SLOW line. Details specific to the selection experiment can be found in Crabbe et al. (1987). The response to selection is shown in Fig 1.

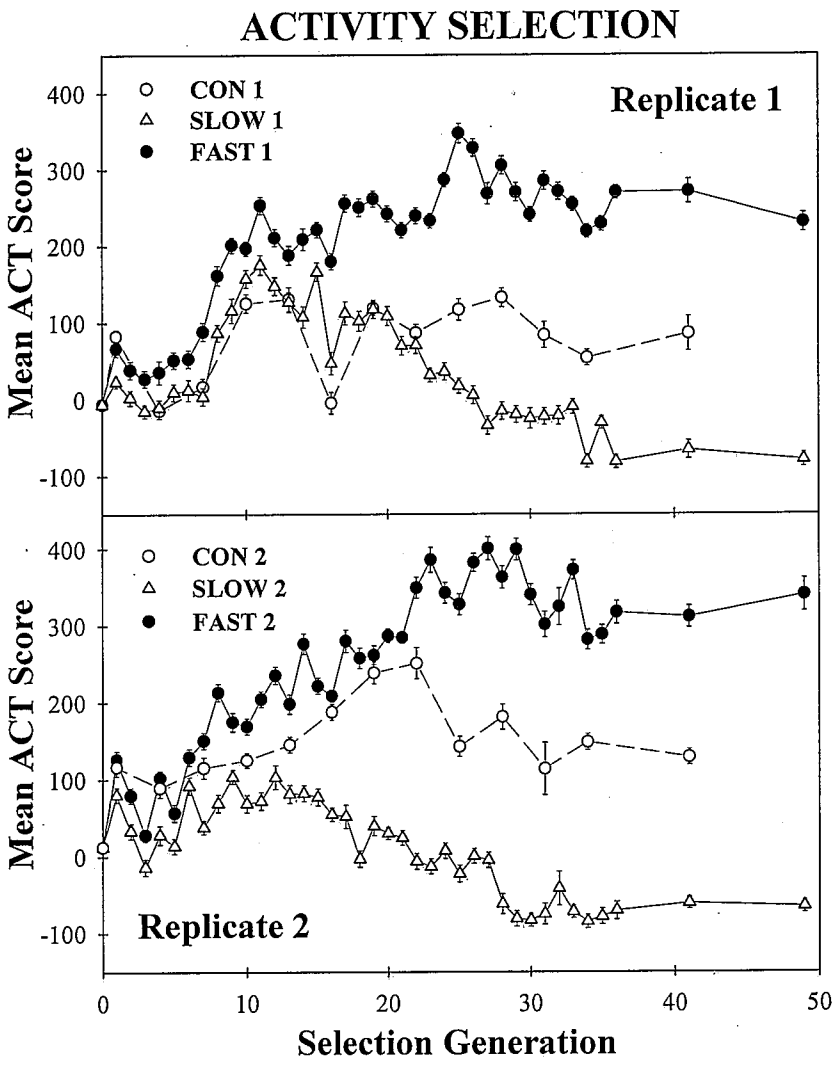


FIGURE 1. Response to selection in the FAST and SLOW mouse lines for acute ethanol sensitivity.

Shown is the mean activity score ± SEM, which was defined as the locomotor response to saline subtracted from the locomotor response to ethanol. CON 1, CON 2 represent the non-selected control lines for replicates 1 and 2, respectively. Selection was stopped at selection generation 37 (S_{37}), after which the lines were maintained by randomly selecting breeders from each line. See text for details specific to the selection procedure.

[Reproduced with permission from Phillips et al., 2002b]

The lines were found to rapidly diverge in the first generation of selection (although the magnitude of this significant line difference was small compared to the magnitude of the line difference at much later selection generations), suggesting a major gene effect. The heritabilities calculated for the two replicates were 0.25 and 0.36, respectively, after the first generation of selection, supporting a significant genetic component to acute ethanol sensitivity (Phillips et al., 1991b). After this first generation, the divergence of the lines was little increased after several generations, and two changes in the selection procedure were undertaken at the 6th selection generation (S_6), with the intention of improving the selection response. These included increasing the dose of ethanol to 2 g/kg and switching the order of ethanol and saline administration (ethanol on day 1). Pilot studies in outbred mice revealed an enhanced magnitude of stimulation to ethanol with a 2 g/kg ethanol dose and with ethanol administered to non-habituated mice (Crabbe et al., 1988). After these changes were made, another jump in the selection response was observed, and the lines have since continued to diverge, and the magnitude of this line difference has dramatically increased (Phillips et al., 1991b; Shen et al., 1995). Selection was discontinued at generation 37, after which selection was relaxed, with breeders selected randomly within line. Inspection of the selection response at later generations (see Fig 1) revealed that the lines remained divergent for the selection response.

The FAST and SLOW lines remained divergent for the selection response when last tested in generation $S_{37}G_{49}$ (Phillips et al., 2002b). Additionally, these lines remained divergent when tested in selection generations $S_{37}G_{58\text{-}64}$ and $S_{37}G_{72\text{-}77}$, with data collected in the first 15 min after a range of ethanol doses, including 2 g/kg ethanol (Holstein et al.,

2005; Palmer et al., 2002a). After selection was relaxed, a reverse selection experiment was undertaken, with the least stimulated of the FAST mice bred together (rFAST) and the most stimulated of the SLOW mice bred together (rSLOW). Surprisingly, the reverse-selected lines returned to non-selected control values (though rFAST-2 mice were more resistant to this effect), suggesting that different genes likely mediate the stimulant and sedative responses to ethanol, and that the lines were homozygously fixed for alleles relevant to their specific selection trait, but remained fully or partially segregating for the opposite trait. As seen in Table 1, the FAST and SLOW lines differ to an array of other drugs and behavioral responses, and many of these correlated line differences were eliminated or reversed in the reverse selected lines. This further supports common genetic influences on ethanol stimulation and the correlated trait (Bergstrom et al., 2003; Meyer and Phillips, 2003b, Palmer et al., 2002a; Palmer and Phillips, 2002; Phillips et al., 2002b).

Behaviorally, the FAST and SLOW lines differ not only to the stimulant response to ethanol, but also to the motor incoordinating and sedative effects of ethanol, with FAST mice less sensitive to these latter effects (Table 1; Phillips et al., 2002b; Shen et al., 1996). These findings are very similar to results in mice selectively bred for differences in sensitivity to the sedative-hypnotic effects of ethanol (the short-sleep, SS, and long-sleep, LS, lines). Mice selected for decreased sensitivity to the sedative effects of ethanol (SS) also show a more prominent stimulant response to ethanol and decreased sensitivity to the ataxic effects of ethanol (Dudek et al., 1984; McClearn and Kakihana, 1981; Phillips and Dudek, 1991; Stinchcomb et al., 1989). Therefore, enhanced sensitivity to the acute stimulant effects of ethanol and decreased sensitivity to the

TABLE 1. Correlated responses in the FAST and SLOW selected lines.

This table summarizes the correlated behavioral traits that have been assessed to date in the FAST and SLOW selected lines. These correlated responses are summarized by replicate lines, and where applicable, the forward- and reverse-selected lines are compared. The abbreviations used in this table are as follows: aVTA – anterior ventral tegmental area; BEC – blood ethanol concentration; CPP – conditioned place preference (for ethanol); CTA – conditioned taste aversion (for ethanol); LORR – loss of righting reflex; rFAST – reverse-selected FAST line; rSLOW – reverse-selected SLOW line; $S_{xx}G_{yy}$ – S_{xx} refers to the selection generation at which these animals were tested, G_{yy} refers to the number of generations that had elapsed since the beginning of selection when these mice were tested.

References:

[1] Bergstrom et al., 2003
[2] Boehm et al., 2002a
[3] Boehm et al., 2002b
[4] Holstein et al., 2005
[5] Meyer et al., submitted
[6] Meyer and Phillips, 2003b
[7] Palmer et al., 2002a
[8] Palmer et al., 2002b
[9] Palmer and Phillips, 2002
[10] Phillips et al., 1989
[11] Phillips et al., 1991a
[12] Phillips et al., 1992
[13] Phillips et al., 2002b
[14] Phillips and Shen, 1996
[15] Risinger et al., 1994
[16] Shen et al., 1995
[17] Shen et al., 1996
[18] Shen et al., 1998
[19] Shen and Phillips, 1998
[20] unpublished results

Trait	Generation	Replicate 1, Forward Selected Line	Replicate 1, Reverse Selected Line	Replicate 2, Forward Selected Line	Replicate 2, Reverse Selected Line	Ref.
CORRELATED LOCOMOTOR RESPONSE – ETHANOL AND OTHER ALCOHOLS						
Ethanol (& ethanol dose-response)	$S_{37}G_{49,58-64}$ $R_{17}G_{17-23}$	FAST-1 > SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2	rFAST-2 > rSLOW-2	[7], [13]
Methanol	S_{13-14} $S_{37}G_{58-64}$ $R_{17}G_{17-23}$	FAST-1 > SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2	rFAST-2 < rSLOW-2 (2 g/kg) rFAST-2 > rSLOW-2 (3.5 g/kg)	[7], [12]
n-Propanol	S_{20}; $S_{37}G_{58-64}$ $R_{17}G_{17-23}$	FAST-1 > SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2	rFAST-2 < rSLOW-2	[7], [12]
t-Butanol	S_{13}; $S_{37}G_{58-64}$ $R_{17}G_{17-23}$	FAST-1 > SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2	rFAST-2 = rSLOW-2	[7], [12]
CORRELATED LOCOMOTOR RESPONSE – GABA AGONISTS AND ANTAGONISTS						
Allopregnanolone	$S_{37}G_{60}$ $R_{17}G_{17-23}$	FAST-1 > SLOW-1	rFAST-1 > rSLOW-1	FAST-2 > SLOW-2	rFAST-2 > rSLOW-2	[7], [8]
Baclofen	S_{33}	FAST-1 > SLOW-1		FAST-2 = SLOW-2		[18]
Baclofen (aVTA)	$S_{37}G_{59-64}$	FAST-1 > SLOW-1		FAST-2 > SLOW-2		[3]
Bicuculline	S_{27-28}	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[18]
Diazepam	$S_{15,\ 24-25}$ $S_{37}G_{58-64}$ $R_{17}G_{17-23}$	FAST-1 > SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2	rFAST-2 < rSLOW-2 (1 mg/kg) rFAST-2 > rSLOW-2 (8 mg/kg)	[7], [12], [18]
Midazolam	S_{23}	FAST-1 > SLOW-1		FAST-2 > SLOW-2		[18]
Muscimol	S_{22}	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[18]
Pentobarbital	S_{12}	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[12]
	S_{17-18} $S_{37}G_{58-64}$ $R_{17}G_{17-23}$	FAST-1 > SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2	rFAST-2 < rSLOW-2	[7], [12]
Phenobarbital	S_{21}	FAST-1 > SLOW-1		FAST-2 > SLOW-2		[12]
Picrotoxin	S_{23}	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[18]
CORRELATED CONVULSANT SENSITIVITY - $GABA_A$ RECEPTOR INVERSE AGONISTS AND ANTAGONISTS						
Bicuculline	$S_{37}G_{39-49}$	FAST-1 > SLOW-1		FAST-2 = SLOW-2		[18]
DMCM	$S_{37}G_{39-49}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[18]
Picrotoxin	$S_{37}G_{39-49}$	FAST-1 > SLOW-1		FAST-2 > SLOW-2		[18]

Trait	Generation	Replicate 1, Forward Selected Line	Replicate 1, Reverse Selected Line	Replicate 2, Forward Selected Line	Replicate 2, Reverse Selected Line	Ref.
PTZ	$S_{37}G_{39\text{-}49}$	FAST-1 > SLOW-1		FAST-2 > SLOW-2		[18]
TBPS	$S_{37}G_{39\text{-}49}$	FAST-1 > SLOW-1		FAST-2 > SLOW-2		[18]
CORRELATED LOCOMOTOR RESPONSE – OTHER DRUGS						
Caffeine	S_{12}	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[12]
Cocaine	$S_{24\text{-}25}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[14]
	$S_{37}G_{52\text{-}54}$	FAST-1 > SLOW-1		FAST-2 = SLOW-2		[1]
	$S_{37}G_{65\text{-}68,\ 74\text{-}80}$ $R_{17}G_{26\text{-}27}$	FAST-1 > SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2	rFAST-2 < rSLOW-2	[1], [5]
D-Amphetamine	S_{11}	FAST-1 < SLOW-1 (2.5 mg/kg) FAST-1 > SLOW-1 (5, 10 mg/kg)		FAST-2 < SLOW-2 (2.5 mg/kg) FAST-2 > SLOW-2 (5, 10 mg/kg)		[12]
	$S_{14\text{-}15}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[12]
	$S_{37}G_{41\text{-}42}$	FAST-1 > SLOW-1		FAST-2 = SLOW-2		[20]
Ketamine	$S_{37}G_{63\text{-}67}$ $R_{17}G_{25\text{-}27}$	FAST-1 > SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2 (10 mg/kg) FAST-2 < SLOW-2 (60 mg/kg)	rFAST-2 = rSLOW-2	[6]
Methamphetamine	$S_{37}G_{41,\ 66\text{-}70}$ $R_{17}G_{26\text{-}27}$	FAST-1 > SLOW-1	rFAST-1 > rSLOW-1	FAST-2 = SLOW-2	rFAST-2 < rSLOW-2	[1]
MK-801	$S_{37}G_{37\text{-}40}$	FAST-1 > SLOW-1		FAST-2 > SLOW-2		[19]
Morphine	$S_{14\text{-}15,\ 20}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[12]
	$S_{37}G_{47\text{-}49,\ 66\text{-}68,\ 72\text{-}77}$ $R_{17}G_{24\text{-}26}$	FAST-1 > SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2	rFAST-2 = rSLOW-2	[1], [4]
Nicotine	S_{17}	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[12]
	$S_{37}G_{40\text{-}42,\ 57\text{-}60}$ $R_{17}G_{17\text{-}20}$	FAST-1 > SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2	rFAST-2 < rSLOW-2	[1]
Raclopride	S_{26}	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[16]
SCH-23390	$S_{27\text{-}28}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[16]
Scopolamine	$S_{37}G_{68\text{-}70}$ $R_{17}G_{27\text{-}29}$	FAST-1 = SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2	rFAST-2 < rSLOW-2	[1]

Trait	Generation	Replicate 1, Forward Selected Line	Replicate 1, Reverse Selected Line	Replicate 2, Forward Selected Line	Replicate 2, Reverse Selected Line	Ref.
CORRELATED ETHANOL RESPONSES						
Acute Withdrawal	$S_{37}G_{39}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[17]
Anxiolysis (Ethanol)	$S_{37}G_{57\text{-}61}$	FAST-1 < SLOW-1		FAST-2 < SLOW-2		[2]
Chronic Withdrawal	$S_{37}G_{39,\ 58\text{-}64}$ $R_{17}G_{17\text{-}23}$	FAST-1 > SLOW-1	rFAST-1 < rSLOW-1	FAST-2 > SLOW-2	rFAST-2 > rSLOW-2	[9], [17]
CPP	$S_{14\text{-}17}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[15]
CTA	$S_{14\text{-}17}$	FAST-1 < SLOW-1		FAST-2 < SLOW-2		[15]
Ethanol Drinking	$S_{14\text{-}17}$	FAST-1 > SLOW-1		FAST-2 > SLOW-2		[15]
Grid Test Ataxia	$S_{7\text{-}8}$	FAST-1 > SLOW-1		FAST-2 > SLOW-2		[10]
	$S_{36\text{-}37}$	FAST-1 < SLOW-1		FAST-2 = SLOW-2		[17]
Hypothermia	$S_{7\text{-}8}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[10]
	$S_{37}G_{38,\ 58\text{-}64}$ $R_{17}G_{17\text{-}23}$	FAST-1 < SLOW-1	rFAST-1 < rSLOW-1	FAST-2 < SLOW-2	rFAST-2 < rSLOW-2	[9], [17]
LORR (latency)	$S_{7\text{-}8}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[10]
	$S_{13,\ 18}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[13]
	$S_{37}G_{38}$	FAST-1 > SLOW-1		FAST-2 > SLOW-2		[17]
	$S_{37}G_{57}$ R_{16}	FAST-1 = SLOW-1	rFAST-1 = rSLOW-1	FAST-2 = SLOW-2	rFAST-2 = rSLOW-2	[13]
LORR (duration)	$S_{7\text{-}8}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[10]
	S_{13}	FAST-1 = SLOW-1		FAST-2 < SLOW-2		[13]
	S_{18}	FAST-1 < SLOW-1		FAST-2 < SLOW-2		[13]
	$S_{37}G_{38,\ 57}$ R_{16}	FAST-1 < SLOW-1	rFAST-1 = rSLOW-1	FAST-2 < SLOW-2	rFAST-2 = rSLOW-2	[13], [17]
LORR (BEC)	$S_{7\text{-}8}$	FAST-1 < SLOW-1		FAST-2 < SLOW-2		[10]
	$S_{13,\ 18}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[13]
	$S_{37}G_{38,\ 57}$ R_{16}	FAST-1 > SLOW-1	rFAST-1 = rSLOW-1	FAST-2 > SLOW-2	rFAST-2 > rSLOW-2	[13], [17]
Repeated Ethanol (Activity)	$S_{13\text{-}14}$	FAST-1 = SLOW-1		FAST-2 = SLOW-2		[11]
Tolerance (Ataxia)	$S_{36\text{-}37}$	FAST-1 < SLOW-1		FAST-2 < SLOW-2		[17]

incoordinating and sedative effects of ethanol are genetically correlated, a finding which mirrors the human data.

Whereas there is some question in the rodent literature as to whether acute stimulant sensitivity to ethanol and ethanol consumption or reward are genetically correlated, there appears to be a stronger link between these phenotypes in the selection literature. FAST mice exhibit increased ethanol consumption compared to SLOW mice, consuming approximately 4-5 g/kg more ethanol per day (Risinger et al., 1994). SS mice, which are more sensitive to the stimulant effects of ethanol (Dudek et al., 1984; Phillips and Dudek, 1991), also appear to consume more ethanol than LS mice (Church et al., 1979; but see Elmer et al., 1990). Similarly, lines that were bred for differences in ethanol consumption (alcohol preferring P rats and alcohol non-preferring NP rats, high alcohol drinking HAD and low alcohol drinking LAD rats, Sardinian alcohol-preferring sP and Sardinian alcohol non-preferring sNP rats) differ in response to the stimulant effects of ethanol, with heightened ethanol consumption positively correlated with increased stimulant sensitivity (Agabio et al., 2001; Krimmer and Schechter, 1992; Päivärinta and Korpi, 1993; Krimmer and Schechter, 1992). These results are striking, as outbred rat lines have generally not been found to stimulate to ethanol (Chuck et al., 2006; Erickson and Kochhar, 1985; Frye and Breese, 1981), though this may be dependent on the protocol (Pastor and Aragon, 2008) and strain used (i.e. certain selectively bred rat lines, as shown above, appear to be more sensitive to the stimulatory effects of ethanol; Criswell et al., 1994). However, the results are not always consistent; the high alcohol preference (HAP) and low alcohol preference (LAP) selected mouse lines did not differ to the locomotor stimulant or depressant effects of ethanol (Grahame

et al., 2000), though this was over a 20-min test, which may miss the most significant stimulant effects of ethanol occurring in the first 5-10 min after ethanol injection (Shen et al., 1995). In addition, short-term selected lines bred for high (STDRHI) and low (STDRLO) ethanol drinking were not found to differ in the acute locomotor stimulant response to ethanol using a larger locomotor activity chamber, but did differ when tested in a smaller conditioned place preference chamber, with the low drinking STDRLO line showing greater locomotor stimulation (Phillips et al., 2005). Overall, several selective breeding experiments support a relationship between heightened stimulant sensitivity to ethanol and heightened ethanol consumption, although this result has not been universal. The FAST lines, however, model the high stimulant sensitivity, low sedative sensitivity and higher drinking reminiscent of at least some individuals who are prone to alcoholism. This makes these lines a strong model to use for the current investigation into the role of GABA systems in acute ethanol sensitivity, and for determining the means by which GABA mimetics reduce the behavioral stimulant response to ethanol (a finding that may have direct relevance for pharmacotherapeutic management of alcohol use disorders).

Of the multiple neurotransmitter systems implicated in acute ethanol sensitivity, the evidence for a significant contribution of GABA systems to the selection response is especially strong. Genetically correlated line differences between sensitivity to the stimulant and depressant effects of ethanol and sensitivity to the stimulant and depressant effects of $GABA_A$ receptor positive modulators occurred early in selection (Phillips et al., 1992; Shen et al., 1998). Other systems, however, are also involved, including glutamatergic, cholinergic, and dopaminergic systems. This conclusion is based on the ability of several pharmacological agents to alter the stimulant response to ethanol in

FAST mice (Table 2). The majority of these manipulations are hypothesized to have their effects on locomotion via alterations in mesolimbic dopamine signaling, and therefore I will focus first on the contribution of this system to the manifestation of stimulation to ethanol.

The Mesolimbic Dopamine Pathway

The mesolimbic dopamine pathway is a key neural circuit involved in the rewarding and stimulant effects of drugs of abuse, including ethanol (Di Chiara and Imperato, 1988; Gallegos et al., 1999; Ikemoto and Wise, 2004; Kalivas et al., 1990; Phillips and Shen, 1996; Tzschentke and Schmidt, 2000; Wise and Bozarth, 1987). A simplified circuit diagram of this pathway and associated pathways is shown in Fig 2. Briefly, the mesolimbic pathway originates in the ventral tegmental area (VTA), with dopamine neurons projecting rostrally to GABAergic medium spiny neurons (MSNs) in the nucleus accumbens (NAc – also referred to as the ventral striatum) (Albanese and Minciacchi, 1983; Ford et al., 2006; Pickel and Chan, 1990). Additionally, the associated mesocortical dopamine pathway is comprised of dopamine neurons from the VTA projecting to the medial prefrontal cortex (PfC). The VTA also sends dopaminergic projections to the ventral pallidum (VP), hippocampus (HPC), and amygdala (Amy) (Albanese and Minciacchi, 1983; Fallon and Moore, 1978; Ford et al., 2006; Klitenick et al., 1992; Scatton et al., 1980). Therefore, VTA dopamine neurons project to a wide variety of limbic, motor, and cortical regions of the midbrain and forebrain. These VTA efferents have been primarily characterized as dopaminergic; however, approximately one-third of the projections from the VTA to the NAc are GABAergic, whereas up to

TABLE 2. Pharmacological systems implicated in the stimulant response to ethanol in FAST mice.

Agent	Mechanism	Effect on ethanol-induced activity in FAST mice	Ref
Acetylcholine			
Dihydro-β-erythroidine	Nicotinic $\alpha_4\beta_2$ receptor antagonist	No effect, 0.5–2 mg/kg	[3]
Hexamethonium	Nonspecific nicotinic receptor antagonist, does not cross BBB	No effect, 2–8 mg/kg	[3]
Mecamylamine	Nonspecific nicotinic receptor antagonist	Decreased stimulation @ 3-6 mg/kg (FAST-1), 1-6 mg/kg (FAST-2)	[3]
Methyllycaconitine	Nicotinic α_7 receptor antagonist	No effect, 1-4 mg/kg	[3]
Scopolamine	Nonspecific muscarinic receptor antagonist	Increased stimulation @ 0.125–0.5 mg/kg	[7]
Dopamine			
Haloperidol	Mixed / D_2-like receptor antagonist	Decreased stimulation @ 0.16 mg/kg	[9]
Quinpirole	D_2 receptor agonist	No effect, 0.005 mg/kg	[6]
Raclopride	D_2 receptor antagonist	Decreased stimulation @ 0.25–0.5 mg/kg	[9]
SCH-23390	D_1 receptor antagonist	Decreased stimulation @ 0.03mg/kg (FAST-1); No effect (FAST-2)	[9]
SCH-23390 + Raclopride	D_1 + D_2 receptor antagonist	Decreased stimulation @ 0.03 mg/kg SCH + 0.25 mg/kg Rac (FAST-2)	[9]
SKF-38393	D_1 agonist	Increased stimulation @ 10 mg/kg (FAST-1); No effect (FAST-2)	[6]
SKF-38393 + Quinpirole	D_1 + D_2 agonist	Increased stimulation @ 10 mg/kg SKF + 0.005 mg/kg quinpirole (FAST-1); No effect (FAST-2)	[6]
GABA			
Baclofen (i.p., ICV, aVTA)	$GABA_B$ receptor agonist	Decreased stimulation @ 1.25–5 mg/kg (i.p.), 1.39–2.77 μg (ICV), 0.01– 0.02 μg/side (aVTA)	[1], [10]
Baclofen (pVTA)	$GABA_B$ receptor agonist	Increased stimulation @ 0.02 μg/side	[1]
Bicuculline	$GABA_A$ receptor antagonist	No specific effect, 0.75–3 mg/kg	[10]
Picrotoxin	$GABA_A$ receptor channel blocker	No specific effect, 0.25–2 mg/kg	[10]
CGP-35348	$GABA_B$ receptor antagonist	No effect, 50-100 mg/kg	[10]
Glutamate			
Ketamine	NMDA receptor antagonist	Increased stimulation to 1 g/kg ethanol @ 10 mg/kg, decreased stimulation to 2 g/kg ethanol @ 5–60 mg/kg	[4]
MK-801	NMDA receptor antagonist	Decreased stimulation @ 0.2 mg/kg	[8]
Opioid			
Estradiol Valerate	B-endorphin lesion	No effect	[5]
Naloxone	Nonspecific opioid receptor antagonist	No effect, 0.3 – 6 mg/kg	[2]

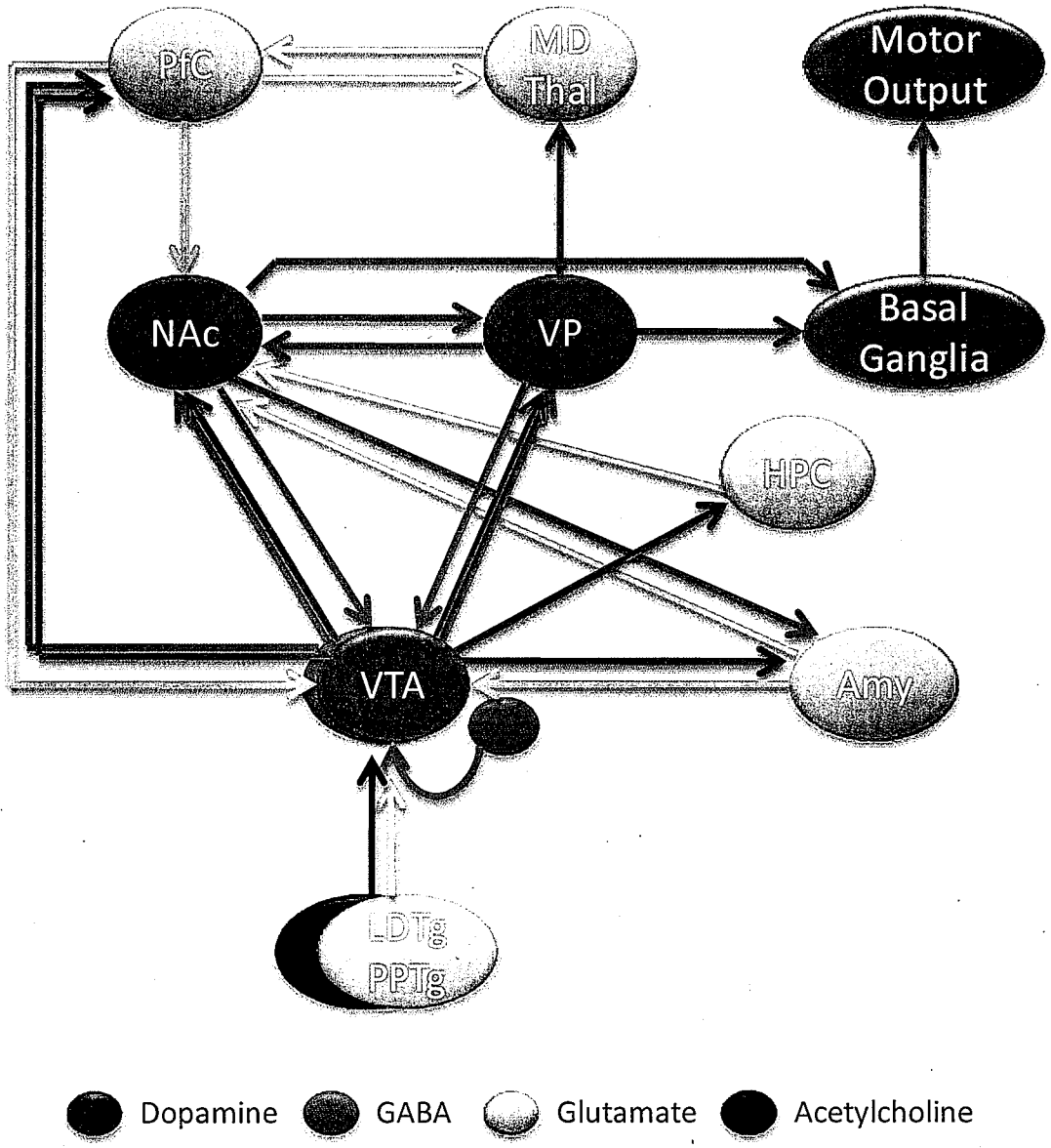
References:

[1] Boehm et al., 2002a
[2] Holstein et al., 2005
[3] Kamens et al., 2008
[4] Meyer and Phillips, 2003b
[5] Meyer et al., submitted
[6] Phillips and Shen, 1996
[7] Scibelli and Phillips, accepted
[8] Shen and Phillips, 1998
[9] Shen et al., 1995
[10] Shen et al., 1998

FIGURE 2. A simplified circuit diagram of the mesolimbic dopamine pathway.

The mesolimbic dopamine pathway originates in the VTA and projects to a variety of limbic (NAc, Amy), cortical (PfC), and motor (VP) regions. Shown for simplicity are the predominant neurotransmitter systems present in each region.

Amy – amygdala (basolateral and central nucleus); HPC – hippocampus; LDTg – laterodorsal tegmental nucleus; MD Thal – mediodorsal thalamus; NAc – nucleus accumbens (also referred to as the ventral striatum); PfC – prefrontal cortex (encompassing anterior cingulate, prelimbic, and infralimbic cortices, among other regions); PPTg – pedunculopontine tegmental nucleus; VP – ventral pallidum; VTA – ventral tegmental area


PfC
MD Thal
Motor Output
NAc
VP
Basal Ganglia
HPC
VTA
Amy
LDTg
PPTg
Dopamine
GABA
Glutamate
Acetylcholine

two-thirds of the projections to the PfC and VP are GABAergic (Carr and Sesack, 2000a; Fields et al., 2007; Klitenick et al., 1992; Van Bockstaele and Pickel, 1995).

The VTA receives reciprocal GABAergic input from the NAc and VP, and reciprocal glutamatergic input from the Amy and PfC (Fudge and Haber, 2000; Gabbott et al., 2005; Horvitz, 2002; Phillipson, 1979; Sesack and Pickel, 1992). In addition, the pedunculopontine and laterodorsal tegmental nuclei (PPTg and LDTg) send excitatory glutamatergic and cholinergic input to the VTA (Oakman et al., 1995; Omelchenko and Sesack, 2005, 2006). Finally, local GABA interneurons within the VTA contribute to the regulation of dopamine cell firing (Fallon and Moore, 1978; Johnson and North, 1992a,b; Kalivas, 1993).

The nucleus accumbens: a limbic-motor integrator

Increases in dopamine release in the NAc are hypothesized to be critical for the induction of the locomotor stimulant and hedonic effects of drugs of abuse, including ethanol (Grace et al., 2007; Ikemoto, 2002; Phillips and Shen, 1996; Tzschentke and Schmidt, 2000; Wise and Bozarth, 1987). Infusion of dopamine and dopamine receptor agonists into the NAc increased locomotor activity (Brudzynski et al., 1993; Ikemoto, 2002; Jones et al., 1981), and inhibition of dopamine signaling in the NAc decreased locomotor activity, both basally and in response to a variety of stimulants (Adams et al., 2001; Chandler et al., 1990; Hoffman and Beninger, 1985; Koob et al., 1981; Louis and Clarke, 1998; Wu et al., 1993).

Based on the interface of input from, and reciprocal output to, the limbic and motor systems, the NAc has been proposed to be a limbic-motor integrator, transducing limbic input to motor output (Mogenson et al., 1980, 1988). The NAc receives excitatory

input from the Amy, HPC, and PfC (Gabbott et al., 2005; Kelley et al., 1982; Lisman and Grace, 2005), and motor input from the VP (Phillipson and Griffiths, 1985). The NAc also receives dopaminergic input not only from the VTA but also from the medial substantia nigra pars compacta (SNc), thereby establishing an integration of the mesolimbic and nigrostriatal pathways (the nigrostriatal pathway is a key pathway for motor behavior) (Albanse and Minciacchi, 1983; Fallon and Moore, 1978; Haber et al., 2000). The nigrostriatal pathway (also a part of the basal ganglia) is a parallel pathway to the mesolimbic pathway which is involved in the control and sequencing of motor programs, initiation and execution of complex motor responses, and in the coordination of movement (Albin et al., 1989; Amalric and Koob, 1987; Hauber et al., 1998; Lalonde and Strazielle, 2007). Briefly, this pathway is comprised of dopaminergic projections from the SNc to the caudate-putamen (CPu, or dorsal striatum). Similar to the NAc, the CPu also sends GABAergic projections to the pallidum, but more specifically to dorsal aspects of this region, which is more commonly referred to as the globus pallidus (GP). The projection from the CPu to the GP is composed of a direct and an indirect pathway. Specifically, the indirect pathway is comprised of a projection from the CPu to the GP (this structure in rodents corresponds to the lateral GP in primates), which then projects to the subthalamic nucleus and then to the entopeduncular nucleus (which corresponds to the medial GP in primates) and the substantia nigra pars reticulata (SNr). The direct pathway, however, projects directly from the CPu to the entopeduncular nucleus and SNr. These regions comprise the output of the nigrostriatal pathway and the basal ganglia, sending projections to the thalamus, which then projects to the cerebral cortex, including the PfC and supplementary motor area (Albin et al., 1989; Hauber et al., 1998).

In addition to receiving input from the SNc, the NAc in turn sends inhibitory projections to various aspects of the basal ganglia/nigrostriatal pathway, including the VP, SN, and GP (Groenewegen et al., 1999; Phillipson and Griffiths, 1985). This convergent input from limbic and motor structures in the NAc, along with strong efferent projections to basal ganglia and motor output structures, suggests that activation of the NAc may integrate limbic inputs and alter motor outputs. Alterations in dopamine input into the NAc may then alter the influence that limbic input has on motor processes (Floresco, 2007; Heimer et al., 1982; Mogenson et al., 1980, 1988). Therefore, activation of mesolimbic dopamine systems by ethanol, and concurrent alterations in GABAergic signaling that will be discussed in later sections, may not only elicit a hedonic response due to the variety of limbic relays, but may also stimulate forward locomotion due to the recruitment of the VP and basal ganglia by the NAc.

Regulation of the mesolimbic dopamine pathway

Recordings from dopamine neurons in the VTA highlight a diversity of functional states of the cell. While many dopamine cells are in a silent, hyperpolarized state, there are also some spontaneously firing dopamine neurons. Of these active cells, the majority show a slow, irregular firing pattern (often termed tonic firing), whereas a smaller percentage show burst or phasic firing, with a train of two or more action potentials that occur in close proximity. Burst firing can elicit large and transient increases in synaptic dopamine levels in the NAc, termed phasic dopamine release. This phasic release activates postsynaptic dopamine receptors, but is rapidly terminated by the efficient reuptake of dopamine by the dopamine transporter (DAT). Behaviorally, burst firing of dopamine cells is found with the delivery of unexpected rewards and with the

presentation of reward-predictive stimuli (Schultz, 1986, 2007; Tobler et al., 2005). Tonic dopamine release, unlike phasic release, can be observed as a general increase in extracellular concentrations in the NAc. This increase could be due to spontaneous or tonic dopamine neural activity, or due to the direct excitation of release by excitatory *N*-methyl-D-aspartate (NMDA) receptors on dopamine terminals in the NAc. This tonic dopamine release occurs much more slowly, yet has an extended duration (Grace, 1991; Grace and Bunney, 1983, 1984a,b; Grace et al., 2007; Heien and Wightman, 2006).

The activity of mesolimbic dopamine neurons appears to be regulated by a variety of sources. Local and afferent GABAergic input maintains a tonic inhibition of dopamine cell firing (Johnson and North, 1992a,b; Kalivas, 1993; Mogenson and Nielsen, 1984; Olpe et al., 1977). Much of this input arises from the VP, but stimulation of the striatum (CPu, NAc) can also lead to an inhibition of dopamine cell firing in the VTA and SN (Floresco et al., 2001; Grace and Bunney, 1985; Grace et al., 2007; Johnson and North, 1992a). Drugs of abuse, including ethanol, may stimulate dopamine cell firing by inactivating this tonic inhibition (the effect of ethanol on inhibitory GABAergic signaling will be discussed in greater detail in later sections). $GABA_A$ receptor agonists in the VTA suppress extracellular dopamine output in the NAc, whereas $GABA_A$ receptor antagonists increase NAc dopamine levels (Ikemoto et al., 1997; Westerink et al., 1996). However, opposite results have also been found (Grace and Bunney, 1979, 1985; Kalivas et al., 1990; Xi and Stein, 1998). This paradoxical activation of dopamine cell firing may be explained by the finding that GABA neurons in the VTA and SN are significantly more sensitive to the inhibitory effects of GABA input than dopamine neurons. Stimulation of dopamine cell firing, which is also seen with repeated trains of stimulation

of the striatum, may occur due to the preferential inhibition of GABA neurons, leading to a disinhibition of dopamine cell firing and release (Grace and Bunney, 1979, 1985; Kalivas et al., 1990).

Changes in VTA dopamine cell firing may also occur via excitatory input from a range of cortical, limbic and hindbrain structures. Stimulation of the medial PfC was found to excite dopamine cell firing, with many cells showing burst firing patterns (Gariano and Groves, 1988; Tong et al., 1996). Stimulation of the HPC (which activated the NAc and inhibited the VP) also increased dopamine cell firing by increasing the number of spontaneously active neurons in the absence of increases in burst firing rate (Floresco et al., 2001, 2003). A similar NAc-induced inhibition of VP firing was found with stimulation of the Amy (Yim and Mogenson, 1983). In addition, excitatory (cholinergic and glutamatergic) input from the PPTg stimulated burst firing of dopamine cells, but did not increase population activity (Floresco et al., 2003). The authors hypothesized that this was due to PPTg input only being able to stimulate already active cells. Inactive dopamine neurons would be at a more hyperpolarized resting membrane potential and would not be activated by glutamatergic input due to the voltage-dependent Mg^{2+} block of NMDA receptors (Floresco et al., 2003; Grace and Bunney, 1984a; Grace et al., 2007). Excitatory input from the LDTg may also regulate dopamine cell firing in the VTA. Although the PPTg sends excitatory inputs to both the VTA and SN, neuroanatomical evidence suggests that the predominant (cholinergic) input to the SN arises from the PPTg, whereas the predominant input to the VTA arises from the LDTg (Oakman et al., 1995). Activation of afferents from the LDTg appear to be critical to the induction of dopamine cell burst firing by the PPTg. Whereas activation of the LDTg

increased the number of active dopamine neurons, its inactivation prevented the stimulatory actions of the PPTg, suggesting that the LDTg may act as a gatekeeper, allowing for the transition of dopamine neurons from spontaneously active to burst firing (Grace et al., 2007; Lodge et al., 2006b).

Dopamine release in the NAc may directly alter the activity of MSNs, or it may modulate the excitatory input onto MSNs. Brady and O'Donnell (2004) reported that stimulation of the PfC stimulated NAc neurons, but this effect was attenuated by stimulation of the VTA; similar effects were found with the HPC and Amy input to the NAc (Mogenson et al., 1988). This suggests that dopamine signaling limits glutamate release into NAc from cortical and limbic regions. Moreover, dopamine release within the NAc may function to select, coordinate, and integrate cortical and limbic input, thus influencing output to the VP and basal ganglia and altering motivated behaviors and actions (Brady and O'Donnell, 2004; Mogenson et al., 1988).

Ethanol activates the mesolimbic dopamine pathway

The vast majority of studies presented here, and throughout the remainder of this thesis, relate to the acute effects of ethanol on various behavioral and neurochemical responses. Acute ethanol exposure stimulates the firing of dopamine neurons within the ventral midbrain (SN and VTA). Similar to the biphasic behavioral effects of ethanol, low to moderate doses of intravenous (i.v.) ethanol (0.5–2 g/kg) rapidly stimulated burst firing of dopamine neurons in the SNc, whereas behaviorally sedative doses of ethanol (4 g/kg and higher) induced a rapid inhibition of dopamine cell firing (Mereu et al., 1984). Intraperitoneally administered ethanol (1-2 g/kg) also stimulated dopamine cell firing in

the VTA and SNc, though the effect was greater in the VTA (Gessa et al., 1985; Mereu et al., 1984).

Similarly, *in vitro* recordings from both mouse and rat midbrain dopamine neurons have documented an ethanol-induced accentuation of dopamine firing rate (Appel et al., 2003; Brodie and Appel, 1998; Brodie et al., 1990, 1999; Okamoto et al., 2006). Recent data from our laboratory suggest that this effect may be involved in acute sensitivity to the locomotor stimulant effects of ethanol, as acute ethanol exposure (50-80 mM) increased the firing rate of dopamine cells in slice recordings from the SNc, an effect that was greater in FAST mice compared to SLOW mice (Beckstead and Phillips, submitted).

Similar to the effect of ethanol on dopamine cell firing, ethanol increased extracellular levels of dopamine in the NAc, an effect also observed with ethanol in humans (Boileau et al., 2003). In rodent studies, ethanol (0.5-3 g/kg) stimulated small increases of approximately 20-50% in extracellular dopamine levels in the NAc as measured by *in vivo* microdialysis (Di Chiara and Imperato, 1988; Howard et al., 2008; Imperato and Di Chiara, 1986; Job et al., 2007; Larsson et al., 2002; Yim et al., 1998; Yoshimoto et al., 1991; Zocchi et al., 2003). Unlike the NAc, there are more mixed results as to whether ethanol stimulates increases in extracellular dopamine levels in the PfC. One study reported an increase in PfC extracellular dopamine levels with very low doses of ethanol in rats, whereas a higher dose reduced extracellular dopamine levels (Dazzi et al., 2002). However, a recent study reported no change in extracellular dopamine levels with a moderate dose of ethanol in either outbred rats or in the alcohol-preferring P rat line (Engleman et al., 2006). Therefore, a preferential activation of the

mesolimbic pathway (VTA-NAc), versus the mesocortical pathway (VTA-PfC), may be the more predominant means by which acute ethanol exposure alters dopamine signaling.

Although multiple groups have found an elevation of NAc dopamine levels after ethanol administration, there is some question regarding the magnitude of the dopamine response to ethanol and the behavioral effects of ethanol, as the magnitude of change in dopamine does not necessarily correlate with the behavioral response to ethanol (Liljequist and Ossowska, 1994; Zapata et al., 2006; but see Kapasova and Szumlinski, 2008). However, ethanol-induced elevations in NAc dopamine were found to be significantly greater in multiple rat lines selectively bred for heightened ethanol consumption relative to their reciprocal line (Katner and Weiss, 2001; Tuomainen et al., 2003). In the FAST and SLOW selected lines, a recent study found that 2 g/kg ethanol increased extracellular dopamine levels in the NAc, an effect which was significantly greater in FAST mice compared to SLOW mice (Meyer et al., submitted). Combined, these data support a relationship between mesolimbic dopamine systems and the acute stimulatory and rewarding effects of ethanol.

There are several possible ways that ethanol may alter the activity of the mesolimbic dopamine pathway. Several studies suggest that this stimulation occurs via a direct effect of ethanol on dopamine neurons. For instance, ethanol attenuated the dopamine cell afterhyperpolarization (AHP, which occurs after an action potential) via an ethanol-induced enhancement of the I_h cation current, which facilitates returning the cell to resting state and contributes to the pacemaker firing pattern of the cell (Okamoto et al. 2006). Others have attributed a stimulatory effect of ethanol on dopamine cell firing to an enhancement of delayed rectifier K^+ channels, which also limit the AHP, or to an

inhibition of M-currents (I_M) through KCNQ channels, which may contribute to overall cell excitability (Appel et al., 2003; Koyama et al. 2007). In the FAST and SLOW selected lines, a line difference was found in basal (pacemaker) dopamine cell firing within the SNc, which may be attributed to a line difference in I_h current. The amount of I_h current in the SNc was greater in FAST mice compared to SLOW mice, and pharmacological inhibition of this I_h current eliminated the line difference in basal dopamine cell firing. As ethanol increased dopamine cell firing to a greater extent in FAST mice relative to SLOW mice, and I_h current was greater in FAST mice, this may suggest that ethanol has a greater stimulatory effect on dopamine cell firing in FAST mice due to a greater ethanol-induced activation of I_h current, thereby limiting the AHP and readying the cell faster for firing (Beckstead and Phillips, submitted). Ethanol may also stimulate dopamine cell firing through an indirect route by inhibiting tonic GABAergic input onto dopamine neurons. This possibility will be discussed at greater length in the section on ethanol and GABA signaling.

Behavioral evidence for an interaction of ethanol and dopamine

In addition to the stimulatory action of ethanol on dopamine signaling in FAST mice, the FAST and SLOW lines were found to differ in response to a wide range of drugs of abuse which stimulate dopamine systems. As shown in Table 1, the FAST and SLOW lines differed in their locomotor stimulant response to the indirect dopamine receptor agonists amphetamine, cocaine, and methamphetamine. In addition, these lines differed in the locomotor stimulant response to the NMDA receptor antagonists ketamine and MK-801, the nonspecific opioid receptor agonist morphine, and the nicotinic acetylcholine receptor agonist nicotine. In all cases, FAST mice were more sensitive to

the locomotor stimulant effects of these compounds, whereas SLOW mice demonstrated an attenuated stimulant response, no change in activity, or the onset of locomotor depression with these compounds (Bergstrom et al., 2003; Holstein et al., 2005; Meyer et al., 2003b; Phillips et al., 1992; Shen et al., 1998). As these compounds, in addition to ethanol, share the ability to stimulate the mesolimbic dopamine pathway or increase extracellular dopamine levels in target areas of this pathway (Camp et al., 1994; Di Chiara and Imperato, 1988; Hamcock and Stamford, 1999; Kretschmer, 1999; Nisell et al., 1994; Phillips and Shen, 1996; Rougé-Pont et al., 2002; Sharp et al., 1987; Yoshida et al., 1993), this supports a common dopaminergic pathway influencing the stimulant response to drugs of abuse (Wise and Bozarth, 1987). The finding that the FAST and SLOW lines did not differ in locomotor response to caffeine (an adenosine A2A antagonist), a compound which does not increase extracellular dopamine levels in the NAc, further supports this hypothesis (De Luca et al., 2007; Phillips et al., 1992). However, it should be noted that line differences to some dopaminergic drugs (i.e., cocaine, amphetamines) were either found exclusively in replicate 1 mice (i.e., amphetamine, methamphetamine) or were larger in magnitude in replicate 1 compared to replicate 2 mice (Bergstrom et al., 2003; Phillips et al., 1992). Therefore, while the lines differ in response to indirect dopamine receptor agonists, the fact that this was predominantly found in replicate 1 mice provides modest evidence of a selection-induced alteration to dopamine systems. In addition, the line differences in response to dopaminergic compounds did not emerge, or did not emerge in both replicates in the expected direction, until the lines were tested after selection had been relaxed (S_{37}), whereas a significant line difference in the stimulant response to ethanol, other alcohols,

and several $GABA_A$ receptor positive modulators, emerged much earlier in selection (Bergstrom et al., 2003; Crabbe et al., 1987; Phillips and Shen, 1996; Phillips et al., 1991b, 1992). This implies that the genes that influence locomotor stimulant sensitivity to psychostimulants (amphetamine, cocaine, methamphetamine) were recruited later in the process of selective breeding, and thus have a smaller impact on the locomotor response to ethanol.

There is still considerable evidence to suggest that mesolimbic dopaminergic systems are involved in acute ethanol sensitivity. First, Meyer and Phillips (unpublished results) found that partial electrolytic lesions of the VTA significantly reduced the locomotor stimulant response to ethanol in FAST mice, but not the locomotor depressant response to ethanol in SLOW mice. Additionally, haloperidol, a mixed dopamine receptor antagonist with greater affinity for D2-like receptors (D2-D4), attenuated the stimulant response to ethanol in FAST mice at doses that did not alter saline activity. The D1 receptor antagonist SCH-23390 also reduced ethanol-induced locomotor stimulation, but only in FAST-1 mice. The D2 receptor antagonist raclopride, however, attenuated ethanol-induced locomotor stimulation in both FAST-1 and FAST-2 mice, though a small reduction was seen in saline activity with raclopride in FAST-1 mice. Combined administration of SCH-23390 and raclopride produced a greater attenuation of ethanol stimulation than either compound alone even when effects on basal activity were considered. This may highlight a crucial role of both receptor subtypes in the stimulant response to ethanol in these mice. Dopamine receptor antagonists did not alter the locomotor depressant response to ethanol in SLOW mice (Shen et al., 1995).

Multiple studies in other rodent lines have also found a reduction of the stimulant

response to ethanol with alterations to dopamine systems. Similar to data in FAST mice, the mixed dopamine receptor antagonists haloperidol and pimozide attenuated the stimulant response to ethanol in mice and rats. Effects were ethanol dose-dependent and these antagonists were not without negative side effects, including reductions in basal activity and an enhancement of the sedative effects of ethanol (Cohen et al., 1997; Koechling et al., 1990; Liljequist et al., 1981). More specific D2-like receptor antagonists (spiperone, sulpiride, tiapride) attenuated the stimulant response to ethanol at doses that did not affect saline activity and did not enhance the sedative effects of ethanol (Cohen et al., 1997; Lê et al., 1997; Pastor et al., 2005). Paradoxically, activation of dopamine D2-like receptors by quinpirole and aripiprazole also attenuated the stimulant response to ethanol in some studies, but not in FAST mice (Cohen et al., 1997; Jerlhag, 2008; Phillips and Shen, 1996), while constitutive absence of the D2 or D4 receptors in mice increased the stimulant response to ethanol (Palmer et al., 2003; Rubinstein et al., 1997). D2-like inhibitory receptors are found both presynaptically, as autoreceptors, and postsynaptically within the mesolimbic dopaminergic pathway (Booth et al., 1990). In addition, D2 receptor agonists may preferentially activate D2 autoreceptors (Elsworth and Roth, 1997; Mercier et al., 2001), which would limit postsynaptic receptor activation similar to a D2 receptor antagonist, and may explain the similar actions of D2-like receptor agonists and antagonists on ethanol-induced locomotion. Similarly, D1-like receptor antagonists attenuated the stimulant response to ethanol in mice, but usually at doses that also reduced the locomotor response to saline (Koechling et al., 1990; Pastor et al, 2005; but see Lê et al., 1997). Conversely, D1-like receptor agonists increased the stimulant response to ethanol in FAST-1, but not FAST-2, mice (Phillips and Shen,

1996). As D1-like agonists and antagonists alter basal activity (Chandler et al., 1990; Desai et al., 2005), this may support a more general role of dopamine D1-like receptors in locomotion.

Interestingly, decreases in dopamine signaling also attenuated the behavioral stimulant effects of ethanol in humans. Ahlenius and colleagues (1973) reported that the tyrosine hydroxylase inhibitor α-methyltyrosine attenuated ethanol-induced increases in talkativeness and alertness, which can be interpreted as a reduction in the low dose behavioral stimulant effects of ethanol in humans. In addition, haloperidol reduced ethanol-induced subjective stimulant ratings in social drinkers (Enggasser and de Wit, 2001). Combined, these studies support a contribution of dopaminergic systems to the low dose stimulant effects of ethanol, with an increased ethanol-induced activation of mesolimbic dopamine systems in subjects with a heightened sensitivity to the behavioral stimulant effects of ethanol. However, as will be described below, a significant contribution of GABA systems to the locomotor stimulant and depressant responses to ethanol is also observed, and GABAergic systems play a significant role in the onset of the sedative actions of ethanol (Engel and Liljequist, 1983).

GABA Release and Reuptake

GABA is the major inhibitory neurotransmitter in brain (Tappaz et al., 1976), and regulation of GABAergic signaling appears to be another mechanism critical to the rewarding, stimulant, and sedative properties of ethanol. Release of GABA into the synaptic cleft and postsynaptic GABA receptor activation is regulated by the GABA transporter. In mammals, there are four main GABA transporter subtypes expressed in

brain, though with varying nomenclature (rat and human: GAT-1, BGT-1, GAT-2, GAT-3, mouse: GAT1, GAT2, GAT3, GAT4, respectively) (Borden, 1996; Gadea and López-Colomé, 2001; Héja et al., 2006). The mouse nomenclature for GABA transporters will be used from here on. GAT1 and GAT4 are the predominant GATs in brain and are localized to both neuronal and glial processes (Conti et al., 1998; Minelli et al., 1995, 2003; Pow et al., 2005), although GAT1 may be primarily localized to neuronal processes, whereas GAT4 may be primarilily localized to astrocytic processes (Bernstein and Quick, 1999; Minelli et al., 1996; Pow et al., 2005). Expression of GAT4 also appears to be more restricted, with the highest expression levels in the thalamus, hypothalamus, and brainstem (Allen Brain Atlas; Lein et al., 2007; Minelli et al., 1996; Pow et al., 2005). Due to the preferential localization of GAT1 to neurons, combined with the breadth of research focused on GAT1, including in drug abuse research, the remainder of this section will be devoted to this transporter subtype.

Expression of GAT1 is dispersed throughout the brain, though there is some discrepancy in reported expression patterns. Pow and colleagues (2005) reported that the greatest density of GAT1 was in rat forebrain structures (cerebral cortex, HPC, striatum) and the cerebellum, with a dramatically reduced density in mid- and hindbrain structures. Others, however, reported a more modest expression of GAT in striatum and a moderate density of transporters in mid- and hindbrain structures, including the ventral midbrain (Allen Brain Atlas; Chiu et al., 2002; Lein et al., 2007; Minelli et al., 1995). Inhibition of GAT1 reduced GABA uptake and consequently increased extracellular GABA concentrations (Bernstein and Quick, 1999; Fink-Jensen et al., 1992). However, similar to other transporter inhibitors (see Yan et al., 2003), increases in extracellular GABA

levels by a GAT1 inhibitor are impulse-dependent, with an elevation of GABA levels dependent on presynaptic GABAergic neuronal impulse flow and the active release of GABA from presynaptic terminals or release sites. As a result of clearing GABA from the extracellular space, GAT1 can regulate phasic and tonic signaling. Phasic GABA release occurs in a very spatially and temporally restricted manner, producing transient increases in synaptic GABA concentrations that are rapidly terminated by GAT1. However, spillover from the GABAergic synapse can occur, and inactivation or deletion of GAT1 enhanced this effect (Jensen et al., 2003; Semyanov et al., 2003; however, inhibition of GAT3/4 may also be required, Keros and Hablitz, 2005). As will be discussed later, phasic and tonic GABA release can specifically activate synaptic and extrasynaptic GABA receptors, respectively.

Ethanol alters GABAergic signaling

A plethora of evidence supports the assertion that ethanol alters GABA signaling, potentially in a multitude of ways. First, acute ethanol (44-100 mM) was found to putatively increase the release of GABA into synaptic and extrasynaptic sites in a variety of brain regions, including the Amy, cerebellum, and HPC, to name a few (Ariwodola and Weiner, 2004; Criswell and Breese, 2005; Criswell et al., 2008; Kelm et al., 2007; Ming et al., 2006; Roberto et al., 2003; Siggins et al., 2005; Zhu and Lovinger, 2006). Within the ventral midbrain, this enhancement of GABA signaling by ethanol has not always been observed. For instance, several studies reported a reduction in GABA cell firing (and presumably GABA release) within the VTA with ethanol administration. This inhibition of firing was observed with both peripheral (0.25-2 g/kg, i.p.) and local ethanol exposure (20-80 mM), and facilitated a disinhibition of dopamine cell firing (Gallegos et

al., 1999; Stobbs et al., 2004; Xiao et al., 2007). Due to the finding that ethanol acts as a $GABA_A$ receptor positive modulator and increases $GABA_A$ receptor function (a topic that will be discussed at greater length below), this ethanol-induced reduction in GABA cell firing may be due to a preferential inhibition of GABA neuron activity in the ventral midbrain (Grace and Bunney, 1979). However, ethanol has also been reported to increase presynaptic GABA release in the SN and VTA (Criswell et al., 2008; Theile et al., 2008), and therefore the exact interaction of ethanol and GABA systems within the VTA is unclear.

Pharmacologically, GAT1 is potently inhibited by the selective and specific GAT1 inhibitors tiagabine and NO-711 (also referred to as NNC-711) (Schousboe et al., 2004; Suzdak et al., 1992). Tiagabine reduced ethanol seeking and consumption in mice (Nguyen et al., 2005); however, this effect was not observed when tiagabine was chronically administered prior to ethanol consumption in rats (Schmitt et al., 2002). Upregulation of the GAT1 gene in mice, leading to heightened transporter function and clearing of GABA from the synapse, enhanced the acute locomotor stimulant response to ethanol. Inhibition of GAT1 by NO-711, conversely, increased the sedative response to ethanol (Hu et al., 2004). These data suggest that alterations in synaptic GABA concentrations can alter the motor stimulant, sedative, and rewarding properties of ethanol. This may occur through a reduction in mesolimbic dopamine signaling, as NO-711 attenuated cocaine-induced increases in extracellular dopamine levels in the NAc (Gerasimov et al., 2000). However, this reduction in the motor and rewarding effects of ethanol could be occurring through an alternative mechanism, such as an enhancement of

the intoxicating and sedative effects of ethanol. This is particularly of concern as ethanol also has GABA mimetic properties similar to the GABA transporter inhibitors.

The $GABA_A$ Receptor

The $GABA_A$ receptor is a ligand-gated Cl^- channel composed of five subunits, and activation of this receptor leads to a rapid influx of Cl^- ions into the cell, resulting in hyperpolarization. $GABA_A$ receptors are widely distributed throughout the rodent brain, with perhaps the highest density in the cerebellum and the olfactory bulb, followed by moderate to high expression in thalamus, cerebral cortex, and HPC, to name just a few regions. $GABA_A$ receptors also exhibit moderate expression levels in other key areas of the mesolimbic and nigrostriatal dopamine pathways, including the CPu, NAc, VP, and Amy. Within the ventral midbrain, $GABA_A$ receptors are expressed, but at a considerably reduced level as compared to other regions. Within this region, $GABA_A$ receptors are also found on both dopaminergic and GABAergic cells (Chu et al., 1990; de Blas et al., 1988; Okada et al., 2004; Schwarzer et al., 2001).

Much of the molecular and functional diversity of the $GABA_A$ receptor arises from there being at least 21 different $GABA_A$ receptor subunits that can be divided into seven classes ($\alpha_{1\text{-}6}$, $\beta_{1\text{-}3}$, $\gamma_{1\text{-}3}$, δ, ε, $\theta_{1\text{-}3}$, π, $\rho_{1\text{-}3}$). In addition, alternative splice variants exist for many of the subunits; for instance, the γ_2 subunit is alternatively spliced into a short (γ_{2S}) and long (γ_{2L}) variant. Despite this plethora of subunit diversity, the most common subunit composition is two α, two β, and one γ subunit, with the $\alpha_1\beta_{2/3}\gamma_2$ subunit conformation being the most widely expressed in rodent brain tissue (Enna, 2007; Farb et

al., 2007; Fritschy et al., 1992; Harris and Allan, 1985; Hevers and Lüddens, 1998; Sieghart and Sperk, 2002).

$GABA_A$ receptor function can be altered by a wide variety of agonists, antagonists, and allosteric modulators. Direct agonists include GABA and muscimol, while the most common competitive antagonist is bicuculline; these drugs bind to the interface of the α and β subunits of the $GABA_A$ receptor (Ebert et al., 1997; Sieghart and Sperk, 2002; Smith and Olsen, 1994). Picrotoxin, which also potently inhibits $GABA_A$ receptor function, binds within the $GABA_A$ receptor pore to block Cl^- uptake and inhibit the receptor (Erkkila et al., 2008). One unique aspect of the $GABA_A$ receptor is its sensitivity to a wide array of allosteric modulators. For instance, benzodiazepines, barbiturates, and endogenous neurosteroids all potentiate agonist-stimulated receptor function. In addition, higher concentrations of barbiturates and neurosteroids can stimulate $GABA_A$ receptor function in the absence of agonist (Belelli and Lambert, 2005; Buck and Harris, 1990; Harris and Allan, 1985; Morrow et al., 1990; Paul and Purdy, 1992; Study and Barker, 1981; Yu et al., 1988).

Finally, in discussing $GABA_A$ receptors, it is critical to discuss the role of synaptic and extrasynaptic receptors to phasic and tonic GABAergic signaling. Synaptic $GABA_A$ receptors, which contribute to phasic GABAergic signaling, are predominantly benzodiazepine-sensitive receptors, composed primarily of $\alpha_{1\text{-}3,5}\beta_x\gamma_2$ subunits. The γ_2 subunit appears to be required for the clustering of $GABA_A$ receptors to postsynaptic locations, and this subunit interacts with a variety of cytoskeletal and trafficking proteins localized to inhibitory synapses (Farrant and Nusser, 2005; Jacob et al., 2008; Leil et al., 2004; Nusser et al., 1998). Extrasynaptic receptors are predominantly composed of

$\alpha_{4,6}\beta_x\delta$ receptors, though $\alpha_{5\text{-}6}\beta_x\gamma_2$ receptors are also found in extrasynaptic locations. These receptors have a higher affinity for GABA, are slower to desensitize (therefore, maintaining tonic inhibition), and are more sensitive to neurosteroid modulation (Farrant and Nusser, 2005; Nusser et al., 1998; Saxena and Macdonald, 1994; Serwanski et al., 2006; Wallner et al., 2003). As will be discussed, extrasynaptic $GABA_A$ receptors may also be more sensitive to ethanol modulation.

Evidence for a direct interaction of acute ethanol with the $GABA_A$ receptor

Perhaps the best evidence for neurochemical effects of ethanol comes from its acute modulatory effects at the $GABA_A$ receptor. Ethanol, even at low concentrations (1-44 mM), was found to potentiate GABA- and muscimol-stimulated $^{36}Cl^-$ uptake, as well as GABA-mediated chloride currents, in Amy, cortex, cerebellum, striatum, and spinal cord in mouse and rat, an effect blocked by bicuculline and picrotoxin (Aguayo, 1990; Allan and Harris, 1987; Allan et al., 1991; Mehta and Ticku, 1988, 1994; Meng et al., 1997; Proctor et al., 1992; Reynolds et al., 1992; Roberto et al., 2003; Suzdak et al., 1986a,b; Ticku et al., 1992; Tsujiyama et al., 1997). This potentiation of agonist-stimulated $GABA_A$ receptor function (an effect similar to that of benzodiazepines and barbiturates) suggests that ethanol acts as a $GABA_A$ receptor positive modulator.

There is some debate, however, as to whether acute ethanol can directly stimulate $GABA_A$ receptor function. Some have reported an increase in $GABA_A$-receptor mediated $^{36}Cl^-$ flux by ethanol (20-50 mM) in the absence of agonist (Mehta and Ticku, 1988, 1994; Meng et al., 1997; Suzdak et al., 1986b), whereas others have reported no direct effect of ethanol at $GABA_A$ receptors (Aguayo, 1990; Allan et al., 1991). Further, an ethanol-induced potentiation of $GABA_A$ receptor function is not always found. For

instance, ethanol did not potentiate GABA-mediated Cl^- currents in neurons cultured from rat dorsal root ganglion even at concentrations as high as 100 mM (White et al., 1990; Zhai et al., 1998), whereas ethanol concentrations above 60 mM were required to see even a small potentiation of GABA-mediated Cl^- currents in *Xenopus* oocytes (Sigel et al., 1993). Finally, Proctor and colleagues (1992) observed an ethanol-induced potentiation of agonist-stimulated $^{36}Cl^-$ flux in rat cerebral cortex and cerebellum, but did not observe a potentiation of $^{36}Cl^-$ flux in the hippocampus. It is likely that subunit composition (which varies among different brain regions), among other variables, may influence the acute stimulatory effect of ethanol on $GABA_A$ receptor function (see Aguayo et al., 2002; Leidenheimer and Harris, 1992 for review). For instance, it was hypothesized that $GABA_A$ receptors required the γ_{2L} subunit to confer ethanol sensitivity (Harris et al., 1995; Sigel et al., 1993; Wafford et al., 1991); however, this was not supported in later studies (Harris et al., 1997; Mihic, 1999; Sigel et al., 1993). More recently, several groups have suggested that receptors containing the δ subunit confer sensitivity to low, pharmacologically relevant, doses of ethanol (1-3 mM) (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003). Extrasynaptic δ-containing receptors are extremely sensitive to agonist stimulation, yet the agonist has low efficacy; therefore, GABA may act as a partial agonist at these receptors. Wallner and colleagues (2003) suggested that ethanol and other positive modulators may potentiate receptor function at such low doses because they convert GABA from a partial to a full agonist and increase the channel opening probability of these receptors. These results were supported by later studies from the same group (Hanchar et al., 2006; Wallner et al., 2006), which proposed a potential low dose ethanol binding site on $\alpha_{4,6}\beta_3\delta$ $GABA_A$ receptors. However, these

results are controversial, as several other groups have not been able to replicate the low dose ethanol effect in δ subunit-containing $GABA_A$ receptors (Borghese and Harris, 2007; Borghese et al., 2006; Korpi et al., 2007; Mehta et al., 2007; for rebuttal, see Olsen et al., 2007). Overall, it can be concluded that acute ethanol exposure, at low, pharmacologically relevant doses (near the legal intoxication threshold of 0.08% w/v or 17.4 mM in humans), potentiates $GABA_A$ receptor function. Receptor subtype conformation and specific experimental parameters, however, may influence this effect of ethanol.

Additionally, ethanol may enhance $GABA_A$ receptor function by increasing circulating levels of endogenous neurosteroids, including the progesterone metabolite allopregnanolone (ALLO) (VanDoren et al., 2000). This ethanol-induced increase in circulating ALLO, may be of peripheral origin, or may be due to ethanol-induced increases in *de novo* synthesis in the brain (O'Dell et al., 2004; Sanna et al., 2004). The potentiation of $GABA_A$ receptor function by ethanol has also been found to be dependent on endogenous neurosteroid tone, as ethanol did not alter $GABA_A$ receptor function in the absence of 5α-tetrahydrodeoxycorticosterone (5α-THDOC), a deoxycorticosterone metabolite (Criswell et al., 1999). However, it has been reported that endogenous neurosteroids may be involved in the maintenance of prolonged ethanol effects on $GABA_A$ receptor function, but not in the acute potentiation of $GABA_A$ receptors by ethanol (Sanna et al., 2004). Therefore, the initial actions of ethanol may be due to a direct effect of ethanol on $GABA_A$ receptor function, while the prolonged effects of ethanol at $GABA_A$ receptors may be related to the synthesis and release of neurosteroids.

Behavioral evidence for an interaction of ethanol with the $GABA_A$ receptor.

There is clear evidence that $GABA_A$ receptors contribute to acute ethanol sensitivity, particularly in the FAST and SLOW selected lines (see Table 1). Early in selection (starting at S_{15}), the FAST and SLOW lines were found to differ in response to an array of $GABA_A$ receptor positive modulators, including the benzodiazepines diazepam and midazolam, and the barbiturates pentobarbital and phenobarbital (Phillips et al., 1992; Shen et al., 1998). Line differences to these compounds were again confirmed after selection was relaxed (Palmer et al., 2002a), along with a line difference in sensitivity to the neurosteroid ALLO (Palmer et al., 2002b). Despite a line difference in basal $GABA_A$ receptor function in the SNc, with greater frequency and larger amplitude of $GABA_A$ receptor-mediated inhibitory currents in SLOW mice compared to FAST mice (Beckstead and Phillips, submitted), these lines have not been found to differ in their locomotor response to $GABA_A$ receptor agonists (muscimol), antagonists (bicuculline), or channel blockers (picrotoxin) (Shen et al., 1998). This discrepancy in line differences to receptor agonists vs. positive modulators suggests a potentially complex selection-induced alteration in $GABA_A$ receptors. For instance, alterations in receptor subunit composition, regional expression, and receptor phosphorylation state, or conversely line differences in downstream targets (i.e. dopamine signaling) might influence responsivity to $GABA_A$ receptor positive modulators differently between the two lines, but not influence responsivity to $GABA_A$ receptor direct agonists. Another possibility is that the doses used in the locomotor studies, particularly of bicuculline and picrotoxin, as necessitated by their convulsant properties, were too low to reveal line differences. In fact, use of higher doses revealed that FAST mice were more sensitive than SLOW mice to the convulsant effects of $GABA_A$ receptor antagonists (including

bicuculline and picrotoxin; see Table 1). This lends additional support for a line difference in GABAergic signaling, with enhanced neuronal excitability in FAST mice when GABA signaling is reduced (Shen et al., 1998).

The above behavioral evidence in the FAST and SLOW selected lines indicates that acute sensitivity to ethanol and acute sensitivity to $GABA_A$ receptor positive modulators are genetically correlated. Moreover, this may implicate the $GABA_A$ receptor in the mediation of the behavioral stimulant response to ethanol. Since SLOW mice are more sensitive to the motor incoordinating and sedative effects of ethanol, as well as the motor depressant effects of $GABA_A$ receptor positive modulators, it is possible that $GABA_A$ receptor function in response to these drugs is greater in SLOW mice compared to FAST mice. This supposition is supported by results found in other lines selectively bred for differences in acute ethanol sensitivity. In lines selectively bred for extreme sensitivity (LS mice and HAS rats) and insensitivity (SS mice and LAS rats) to the sedative effects of ethanol, $GABA_A$ receptor function in response to ethanol and other positive modulators was greater in the more sensitive lines (Harris and Allan, 1989; Liu and Deitrich, 1998; Zahniser et al., 1992). These lines also differed in sensitivity to the locomotor stimulant and depressant effects of ethanol, with enhanced sensitivity to the stimulant effects of ethanol in SS mice, and enhanced sensitivity to the depressant effects of ethanol in HAS rats (Dudek et al., 1984; Phillips and Dudek, 1991; Schechter and Krimmer, 1992). Since the FAST and SLOW mice differ in the sedative response to ethanol, with SLOW mice more sensitive to this effect (Phillips et al., 2002b; Shen et al., 1996), it is a logical extension of these results to predict that $GABA_A$ receptor function would differ between the FAST and SLOW selected lines. In addition, line differences in

$GABA_A$ receptor density and subunit composition may also explain the different behavioral responses between FAST and SLOW mice. These data have not been generated.

Similar to the evidence in FAST and SLOW mice, studies in other rodent lines suggest a role of $GABA_A$ receptors in acute ethanol sensitivity. Whereas $GABA_A$ receptor antagonists and inverse agonists generally did not decrease the locomotor stimulant effects of ethanol in mice (Becker, 1988; June and Lewis, 1994; Liljequist and Engel, 1982; Shen et al., 1998, but see Chester and Cunningham, 1999b; Koechling et al., 1991), activation of $GABA_A$ receptors attenuated ethanol-induced locomotor stimulation (Biswas and Carlsson, 1978; Broadbent and Harless, 1999; Cott et al., 1976; Liljequist and Engel, 1982). However, if a $GABA_A$ receptor activation-induced reduction of the stimulant effects of ethanol, which is itself a $GABA_A$ receptor positive modulator, seems more likely to be occurring through an accentuation of intoxicant and sedative effects that ethanol shares in common with drugs that activate $GABA_A$ receptors, rather than via a blockade of stimulatory actions of ethanol in the brain.

There is supportive evidence for this claim. Multiple studies have found that $GABA_A$ receptor agonists accentuated the motor incoordinating effects of ethanol, a trait genetically correlated with ethanol stimulation (Dar, 2006; Martz et al., 1983; Shen et al., 1996). $GABA_A$ receptor antagonists and the inverse agonist Ro15-4513 (often characterized as an ethanol antagonist) attenuated ethanol-induced ataxia (Dar, 1995; Hoffman et al., 1987; Martz et al., 1983; Meng et al., 1997; Meng and Dar, 1994; Suzdak et al., 1988). Ro15-4513 also antagonized the locomotor depressant effects of ethanol, even inducing a locomotor stimulant response to ethanol, both at higher ethanol doses

and in the B6 mouse strain which is generally found to be insensitive to the locomotor stimulatory effects of ethanol (Becker, 1988; Becker and Hale, 1989).

Finally, it should be noted that alterations in $GABA_A$ receptor function alter the motivational properties of ethanol (for review, see Chester and Cunningham, 2002; Koob, 2004). $GABA_A$ receptor agonists and inverse agonists attenuated ethanol consumption (Janak and Gill, 2003; Samson et al., 1989), while $GABA_A$ receptor positive modulators substituted for the discriminative stimulus effects of ethanol (Bowen et al., 1999). Activation of $GABA_A$ receptors by muscimol in the NAc also substituted for ethanol (Besheer et al., 2003; Hodge et al., 2001), suggesting that the subjective effects of ethanol are mediated, in part, through the $GABA_A$ receptor.

The $GABA_B$ Receptor

The $GABA_B$ receptor is a heterodimeric G-protein coupled receptor ($G\alpha_{i/o}$-coupled) that is composed of the $GABA_{B1}$ and $GABA_{B2}$ subunits (Bettler and Tiao, 2006; Bowery et al., 2002; Kaupmann et al., 1998). Both subunits are required for normal receptor function; the GABA binding site is located at the $GABA_{B1}$ subunit, and the $GABA_{B2}$ subunit couples with G-proteins for cell signaling (Bettler and Tiao, 2006; Gassmann et al., 2004; Kaupmann et al., 1998; Margeta-Mitrovic et al., 2001; Schuler et al., 2001). As a $G\alpha_{i/o}$-coupled receptor, activation of $GABA_B$ receptors results in an inhibition of adenylate cyclase, which decreases production of cAMP and inhibits this intracellular signaling pathway (Bowery et al., 2002; Knight and Bowery, 1996). Presynaptically, activation of this receptor inhibits voltage-gated calcium channels (VGCCs) and inhibits neurotransmitter release; postsynaptic receptor activation

stimulates G-protein coupled inwardly rectifying potassium channels (GIRKs) and induces a hyperpolarization of the cell (Andrade et al., 1986; Bowery and Enna, 2000; Cruz et al., 2004; Lacey et al., 1988; Lüscher et al., 1997; Margeta-Mitrovic et al., 2001; Ulrich and Bettler, 2007; Zhu and Chuang, 1987). At the subcellular level, $GABA_B$ receptors are found both presynaptically and postsynaptically, though studies suggest they are localized more predominantly at postsynaptic sites (Bettler and Tiao, 2006; Kulik et al., 2002). Presynaptically, $GABA_B$ receptors are localized both to GABAergic and non-GABAergic axon terminals, including glutamatergic terminals, and therefore act as both autoreceptors and heteroreceptors. Postsynaptically, $GABA_B$ receptors are localized primarily to extrasynaptic sites. $GABA_B$ receptors are not always located in and adjacent to inhibitory synapses (i.e., at dendritic shafts); in cerebellar Purkinje cells and hippocampal pyramidal cells, the majority of postsynaptic $GABA_B$ receptors are located at putative glutamatergic synapses (i.e., on dendritic spines). These findings suggest that activation of $GABA_B$ receptors not only serves to regulate GABAergic signaling, but may also provide a tight regulation of excitatory glutamatergic signaling. $GABA_B$ receptors at presynaptic and postsynaptic glutamatergic sites may be activated by GABA spillover from nearby GABAergic release sites, thereby decreasing glutamate release and inhibiting postsynaptic actions (Bettler and Tiao, 2006; Boyes and Bolam, 2003; Kulik et al., 2002, 2003; Lei and McBain, 2003; López-Bendito et al., 2004).

The $GABA_B$ receptor and its constituent subunits are widely expressed throughout the rodent central nervous system, with the highest receptor density in the olfactory bulbs, cerebral cortex, Amy, HPC, thalamus, and cerebellum (Chu et al., 1990; Kulik et al., 2002). In these regions and others, there is a considerable overlap in the expression

patterns of the $GABA_{B1}$ and $GABA_{B2}$ subunits; however, $GABA_{B1}$ receptor subunit expression is much higher than $GABA_{B2}$ expression in the striatum (Allen Brain Atlas; Kulik et al., 2002; Lein et al., 2007). Since both subunits are required for receptor function, this may suggest a relatively low expression of functional $GABA_B$ receptors within the striatum, a hypothesis confirmed by $GABA_B$ receptor autoradiography (Chu et al., 1990). Within the ventral midbrain, $GABA_B$ receptor expression is relatively low, though receptor density appears to be greatest on dopaminergic neurons (Chu et al., 1990; Ng and Yung, 2000, 2001; Wirtshafter and Sheppard, 2001). The $GABA_B$ receptor agonist baclofen dose-dependently inhibited dopamine cell firing, including burst firing, in the SN and VTA; baclofen also reduced extracellular dopamine levels in the NAc, both basally and in response to amphetamine, cocaine, morphine, nicotine, and the μ opioid receptor agonist DAMGO (Brebner et al., 2005; Chen et al., 2005; Cruz et al., 2004; Engberg, 1993; Erhardt et al., 2002; Fadda et al., 2003; Grace and Bunney, 1980; Kalivas et al., 1990; Klitenick et al., 1992; Lacey et al., 1988; Olpe et al., 1977; Westerink et al., 1996). The effect of baclofen on extracellular dopamine levels after ethanol has not been examined. In addition, deletion of the $GABA_{B1}$ subunit resulted in a hyperdopaminergic state, while deletion of this subunit or the $GABA_{B2}$ subunit led to a profound hyperactive state, suggesting that $GABA_B$ receptor activation facilitates a tonic inhibition of dopamine neurons and a concomitant reduction in basal activity (Gassmann et al., 2004; Schuler et al., 2001; Vacher et al. 2006).

Contribution of $GABA_B$ receptors to acute ethanol sensitivity and preference.

In recent years, considerable evidence has suggested that activation of $GABA_B$ receptors may be a successful pharmacotherapy for alcohol use disorders. For instance,

baclofen attenuated ethanol consumption and seeking in rodents (Besheer et al., 2004; Daoust et al., 1987; Janak and Gill, 2003; Stromberg, 2004; Walker and Koob, 2007), an effect that was also observed in rat lines (iP, sP) selectively bred for increased ethanol consumption and preference (Colombo et al., 2000, 2002, 2006; Liang et al., 2006, Maccioni et al., 2005). This reduction in ethanol consumption, however, has not always been found, as baclofen, at both a low (1 mg/kg) and high (10 mg/kg) dose, was found to increase ethanol consumption (Czachowski et al., 2006; Moore et al., 2007; Smith et al., 1999). Similar to data with other components of the GABA system, this suggests a complex relationship of the $GABA_B$ receptor with the rewarding effects of ethanol.

Perhaps more consistent is the interaction of the $GABA_B$ receptor with various motor effects of ethanol. Multiple studies have reported a reduction in ethanol-induced locomotor stimulation in mice with baclofen (Broadbent and Harless, 1999; Chester and Cunningham, 1999a; Cott et al., 1976; Humeniuk et al., 1993). Baclofen also attenuated ethanol-induced locomotor stimulation in FAST mice, both when given peripherally and centrally (Boehm et al., 2002a; Shen et al., 1998). As baclofen attenuates the locomotor and NAc dopamine response to psychostimulants (Brebner et al., 2005; Fadda et al., 2003; Lhuillier et al., 2007; Phillis et al., 2001), it is possible that baclofen is attenuating the stimulant effects of ethanol by attenuating the dopamine response to ethanol. This supposition has not been directly tested, but the VTA has been identified as a critical region for this effect of baclofen. In FAST mice, intra-anterior VTA (aVTA) baclofen attenuated the locomotor stimulant response to ethanol in FAST mice. However, intra-posterior VTA (pVTA) baclofen actually enhanced the locomotor stimulant response to ethanol. This may suggest a regional division of the VTA, with potential anterior-

posterior differences in $GABA_B$ receptor location and function, or differences in the regional projections from the aVTA and pVTA (Boehm et al., 2002a). No matter the regional variation in baclofen's effects, it does highlight that activation of $GABA_B$ receptors in the VTA alters the stimulant response to ethanol in FAST mice. Baclofen, however, did not attenuate the locomotor depressant response to ethanol in SLOW mice (Boehm et al., 2002a), but it did have non-specific locomotor depressant effects in these lines, significantly reducing locomotor behavior in both saline and ethanol treated mice. SLOW mice are more sensitive to this motor depressant effect of baclofen as compared to FAST mice (Boehm et al., 2002a; Shen et al., 1998). These data, overall, suggest a significant contribution of $GABA_B$ receptors to acute ethanol sensitivity. However, within the ventral midbrain, there is no significant difference between the lines in $GABA_B$ receptor density (Boehm et al., 2002a). Rather, selection-induced alterations in $GABA_B$ receptor function may be more critical. In rat lines selected for differences in ethanol consumption, $GABA_B$ receptor function was significantly greater in limbic (olfactory tubercle, NAc, and septal nuclei) and cortical tissue from the low drinking sNP line compared to the high drinking sP line (Castelli et al., 2005). Since these lines also differ modestly in the locomotor response to ethanol, with increased locomotor stimulation in sP rats (Agabio et al., 2001), and FAST and SLOW mice differ in ethanol consumption, with heightened ethanol drinking in FAST mice (Risinger et al., 1994), these data may suggest a correlation between increased stimulant sensitivity and decreased $GABA_B$ receptor function.

The speculation that $GABA_A$ receptor-acting drugs could be attenuating the stimulant response to ethanol by accentuating the intoxicating and sedative effects of

ethanol applies to baclofen as well. Some studies suggest that ethanol may potentiate presynaptic, but not postsynaptic, $GABA_B$ receptor function (Ariwodola and Weiner, 2004; Frye and Fincher, 1996; but see Lewohl et al., 1999). Behavioral studies have also reported an enhancement of ethanol-induced motor incoordination and sedation with baclofen (Besheer et al., 2004; Dar, 1996; Martz et al., 1983), and, at higher doses, baclofen acts as a muscle relaxant (Nevins et al., 1993). These results may support the hypothesis that baclofen may be reducing stimulation by shifting the behavioral response to ethanol towards greater intoxication.

Dissertation Goals

From the above-stated evidence, it is clear that mesolimbic dopamine and central GABAergic systems influence acute ethanol sensitivity in the FAST and SLOW selected mouse lines. The manner in which these systems alter the locomotor stimulant response to ethanol, and how these systems have been altered through the process of selection, remain incompletely defined. Therefore, the purpose of this dissertation is to determine potential mechanisms by which selective breeding for extreme sensitivity (FAST mice) and insensitivity (SLOW mice) to the locomotor stimulant effects of ethanol has altered GABA systems, including receptor function. In addition, the purpose of this dissertation is to determine whether activation of GABA systems may attenuate the extreme locomotor stimulant response to ethanol in FAST mice, and potential means by which this may be occurring, including through an alteration to dopamine systems.

In the first series of experiments, the effect of the GABA transporter inhibitor NO-711 was examined in FAST and SLOW mice. Although previous experiments have

identified a role for $GABA_A$ and $GABA_B$ receptors in the selection response (Boehm et al., 2002a; Phillips et al., 1992; Shen et al., 1998), the contribution of the GABA transporter has not been examined. Due to the line differences in locomotor response to GABA receptor agonists and positive modulators, I hypothesized that the FAST and SLOW lines would also differ in their basal locomotor response to NO-711, with an enhanced depressant response in SLOW mice.

Additionally, the effect of NO-711 on ethanol-induced locomotor stimulation and depression was examined to determine whether alterations in GABA signaling attenuated stimulation by blocking a neurochemical mechanism activated by ethanol, and/or by accentuating the behavioral incoordinating and locomotor depressant effects of ethanol, essentially masking locomotor stimulation. Since both ethanol and NO-711 are GABA mimetics, I predicted that NO-711 would attenuate the stimulant response to ethanol in FAST mice by acting additively with ethanol to enhance a competing behavior, namely motor incoordination. I also hypothesized that NO-711 would act additively with ethanol in SLOW mice to accentuate locomotor depression.

In order to determine the relative contributions of the $GABA_A$ and $GABA_B$ receptors to this effect of NO-711, the NO-711 experiments were replicated only in FAST mice, with the $GABA_A$ receptor agonist muscimol and the $GABA_B$ receptor agonist baclofen. Similar to NO-711, I hypothesized that muscimol would attenuate the locomotor stimulant response to ethanol, while accentuating the motor incoordinating effects of ethanol in FAST mice. Since baclofen has potent muscle relaxant properties similar to ethanol (Nevins et al., 1993), I postulated that baclofen would both attenuate the locomotor response to ethanol and accentuate ethanol-induced motor incoordination.

If these GABAergic drugs reduce stimulation by increasing motor incoordination, this would suggest that these pharmacological agents reduce the hedonic effects of ethanol and ethanol consumption at least in part by enhancing behavioral intoxicant and sedative effects of ethanol.

In the second series of experiments, the contribution of $GABA_B$ receptors to the locomotor stimulant effects of ethanol was further examined. Originally, examination of the contribution of $GABA_A$ receptors to the stimulant phenotype was also planned. This was to be accomplished by determining whether the FAST and SLOW selected lines differed in $GABA_A$ receptor function. However, the unavailability of $^{36}Cl^-$ from any manufacturer for the functional assay prevented the performance of this experiment. $GABA_B$ receptor function was examined though, both basally and in response to ethanol. Due to the enhanced sensitivity of SLOW mice to the sedative effects of baclofen and ethanol (Boehm et al., 2002a; Shen et al., 1998), I hypothesized that $GABA_B$ receptor function would be greater in SLOW compared to FAST mice, particularly in regions critical to the locomotor response to ethanol (ventral midbrain and striatum).

Finally, the possible role of dopamine in the ventral striatum, or NAc, in the baclofen-induced attenuation of the locomotor stimulant response to ethanol was examined in FAST mice using *in vivo* microdialysis. Baclofen was hypothesized to attenuate the stimulant effects of ethanol by limiting ethanol-induced increases in dopamine release in the NAc, a supposition supported by studies with other drugs of abuse (Brebner et al., 2005; Fadda et al., 2003).

CHAPTER 2: Attenuation of the stimulant response to ethanol is associated with enhanced ataxia for a $GABA_A$, but not a $GABA_B$, receptor agonist

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Abstract

Background: The γ-aminobutyric acid (GABA) system is implicated in the neurobiological actions of ethanol, and pharmacological agents that increase the activity of this system have been proposed as potential treatments for alcohol use disorders. As ethanol has its own GABA mimetic properties, it is critical to determine the mechanism by which GABAergic drugs may reduce the response to ethanol (i.e., via an inhibition or an accentuation of the neurobiological effects of ethanol). ***Methods:*** In the present study, we examined the ability of three different types of GABAergic compounds, the GABA reuptake inhibitor NO-711, the $GABA_A$ receptor agonist muscimol, and the $GABA_B$ receptor agonist baclofen, to attenuate the locomotor stimulant response to ethanol in FAST mice, which were selectively bred for extreme sensitivity to ethanol-induced locomotor stimulation. In order to determine whether these compounds produced a specific reduction in stimulation, their effects on ethanol-induced motor incoordination were also examined. ***Results:*** NO-711, muscimol, and baclofen were all found to potently attenuate the locomotor stimulant response to ethanol in FAST mice. However, both NO-711 and muscimol markedly increased ethanol-induced ataxia, whereas baclofen did not accentuate this response. ***Conclusions:*** These results suggest that pharmacological agents that increase extracellular concentrations of GABA and $GABA_A$ receptor activity may attenuate the stimulant effects of ethanol by accentuating its intoxicating and sedative properties. However, selective activation of the $GABA_B$ receptor appears to produce a specific attenuation of ethanol-induced stimulation, suggesting that $GABA_B$ receptor agonists may hold greater promise as potential pharmacotherapies for alcohol use disorders.

Introduction

Alcoholism remains a serious health problem in the United States; however, few drugs are approved for its treatment (Miller, 2008). While abstinence is often the standard for treatment success, there is increased debate as to whether moderation should be an alternative treatment goal (Finney and Moos, 2006; Marlatt and Witkiewitz, 2002). Some approved pharmacotherapies are targeted to reduce alcohol (ethanol) consumption by a direct pairing with ethanol, whereas others target craving during abstinence. For example, disulfiram reduces drinking by inducing adverse physiological symptoms when combined with ethanol (Suh et al., 2006). Naltrexone is prescribed partly with the intent of increasing number of days abstinent, but when combined with ethanol, it has been shown to reduce the number of drinks consumed, possibly by decreasing the positive, rewarding effects of ethanol (O'Malley et al., 1996; Sinclair, 2001; Volpicelli et al., 1992). However, Swift and colleagues (1994) reported an enhancement of subjective intoxication ratings, including sedation, when naltrexone was combined with ethanol. This provides an alternative explanation for reduced consumption, namely increased intoxication and sedation, effects which might be seen after consumption of higher ethanol doses.

Ethanol, among its many effects, has modulatory actions on GABA systems, including alterations in GABA cell firing and synaptic release (Gallegos et al., 1999; Roberto et al., 2003; Siggins et al., 2005; Xiao et al., 2007; Zhu and Lovinger, 2006) and increases in $GABA_A$ receptor function (Allan and Harris, 1987; Suzdak et al., 1986; Wallner et al., 2003). Many of the behavioral effects of ethanol, including sensitivity to

its locomotor stimulant, sedative, incoordinating, and rewarding effects can be attributed, in part, to alterations in GABA systems (Boehm et al., 2006; Chester and Cunningham, 2002; Dar, 1996; Hoffman et al., 1987; Koob, 2004; Martz et al., 1983; Phillips and Shen, 1996; Shen et al., 1998). Benzodiazepines, which share with ethanol the ability to positively modulate $GABA_A$ receptors, are efficacious in the treatment of ethanol withdrawal. However, recent attention has focused on the use of antiepileptics, including topiramate, γ-vinyl GABA, and tiagabine for the treatment of alcoholism. These drugs, among other actions, enhance $GABA_A$ receptor function or increase extracellular GABA concentrations (Davies, 1995; Fink-Jensen et al., 1992; White et al., 2000). In addition, the use of drugs that target the $GABA_B$ receptor, such as baclofen, is being explored. Both $GABA_A$ and $GABA_B$ receptor related drugs have been shown to reduce ethanol consumption in preclinical and clinical studies (Addolorato et al., 2000; Anstrom et al., 2003; Colombo et al., 2000; Johnson et al., 2003; Nguyen et al., 2005; Wegelius et al., 1993). However, reduced consumption could be due to the accentuation of a competing behavior, such as increased intoxication or motor incoordination, rather than an attenuation of the rewarding effects of ethanol experienced when ethanol is consumed. Such accentuated motor side effects would pose significant patient safety concerns.

To explore the possibility that compounds with GABA mimetic actions attenuate alcohol effects by inducing competing motor behaviors, we examined the effects of GABAergic compounds on ethanol-induced locomotor activation and motor coordination. We hypothesized that the GABA mimetic drugs would attenuate ethanol-induced stimulation, but that they would also enhance ataxia. This would suggest that the reduced stimulation was due to the additive effect of the GABAergic drugs with ethanol,

leading to the expression of competing behaviors, namely ataxia. Sensitivity to the behaviorally stimulating and incoordinating effects of ethanol are of additional interest because increased sensitivity to ethanol-induced stimulation and decreased sensitivity to motor incoordination are correlates of ethanol consumption history (Holdstock et al., 2000; King et al., 2002) and familial risk for alcoholism (Newlin and Thomson, 1991, 1999; Schuckit and Smith, 2000, 2001).

We chose the FAST and SLOW mouse lines for these studies. These mice were created by selectively breeding for extreme sensitivity and insensitivity, respectively, to the locomotor stimulant effects of ethanol (Crabbe et al., 1987; Phillips et al., 1991). They therefore represent a genetic model of differential sensitivity to the stimulant effects of ethanol. However, they also differ dramatically in sensitivity to the motor incoordinating and sedative effects of ethanol (with FAST mice less sensitive to these effects; Phillips et al., 2002; Shen et al., 1996), a difference that grew larger over the course of selection (Phillips et al., 2002), providing strong evidence for common genetic regulation of these ethanol effects. Further, FAST mice have been found to consume larger amounts of ethanol (about 4-5 g/kg/day) than SLOW mice (Risinger et al., 1994). Thus, the FAST lines, which exist as two replicates (FAST-1 and FAST-2) appear to model the high stimulant sensitivity, low sedative sensitivity and higher drinking reminiscent of at least some individuals who are prone to alcoholism.

We first examined the effect of the GABA transporter inhibitor NO-711, which increases extracellular GABA concentrations (Fink-Jensen et al., 1992), on ethanol-induced locomotion in FAST and SLOW mice. We predicted that GABA receptor activation induced by NO-711 would attenuate ethanol stimulation in FAST mice by

acting additively with ethanol to accentuate GABAergic activity. We also predicted that NO-711 would enhance the depressant response to ethanol normally seen in SLOW mice. The relative contributions of $GABA_A$ versus $GABA_B$ receptors to attenuation of stimulation were then considered by using drugs that specifically interact with these receptor subtypes. Only FAST mice were used in these studies, due to the desire to see possible effects on both stimulation and motor incoordination (SLOW mice do not exhibit ethanol-induced stimulation at any dose; Palmer et al., 2002a). We predicted that the $GABA_A$ receptor agonist muscimol would attenuate the stimulant response to ethanol in FAST mice, but would also enhance the motor incoordinating effects of ethanol. We also examined the effect of baclofen, a $GABA_B$ receptor agonist, which has been previously shown to attenuate the stimulant response to ethanol in FAST mice (Boehm et al., 2002; Shen et al., 1998). Because the $GABA_B$ receptor agonist baclofen has muscle relaxant properties like ethanol (Nevins et al., 1993), we postulated that baclofen would also accentuate the incoordinating effects of ethanol.

Methods

Subjects

The FAST and SLOW selected lines were selectively bred, in replicate, for extreme sensitivity (FAST-1, FAST-2) and insensitivity (SLOW-1, SLOW-2) to the locomotor stimulant effects of 2 g/kg ethanol. Details specific to the selection process have been published (Crabbe et al., 1987, 1988; Phillips et al., 1991, 2002; Shen et al., 1995). The most recent test of the ethanol dose-response function in these mice (~20 generations since selection was relaxed) showed that FAST mice were significantly

different from SLOW mice in their locomotor response to multiple ethanol doses (i.e., 1.5, 2, 2.5, and 3 g/kg) (Palmer et al., 2002a). Mice used in these experiments were reared with the dam and sire until 20-22 days of age, when they were isosexually housed in polycarbonate [28 X 18 X 13 cm (l X w X h)] cages, 2-4 per cage, with littermates or with non-littermates (to avoid single housing) of the same genotype and age range. Subjects were maintained on a 12:12-h light-dark cycle (lights on at 0600) at 21 ± 2° C with food (Purina Laboratory Rodent Chow #5001; Purina Mills, St. Louis, MO) and water available ad libitum except during behavioral testing. The age, number and generations of mice used for each study are presented in the figure legends.

Apparatus

Locomotor Activity Monitors. Eight locomotor activity detection monitors measuring 40 X 40 X 30 cm (l X w X h) (AccuScan Instruments, Inc., Columbus, OH) were each housed in light-proof, sound-attenuating cabinets (Flair Plastics, Portland, OR). Each cabinet was illuminated by an 8-W fluorescent white light and a fan was mounted on the inside back wall, providing ventilation and background noise. Movement within the monitor was recorded by two sets of eight infrared beams mounted 2 cm above the test chamber floor at right angles to one another, with detectors mounted on the opposite sides. Beam interruptions were automatically recorded and translated to horizontal distance traveled (in cm) by AccuScan software.

Rotarod. Motor incoordination was measured on two AccuRotor Rota Rods (AccuScan Instruments, Columbus, OH). The rotarod consisted of a 6.3 cm diameter dowel covered with 320 grit wet/dry sandpaper that was divided into four lanes by white

plexiglass round discs. The dowel was located 63 cm above a thick layer of sawdust bedding, with a photobeam detector that recorded latency to fall from the dowel in sec.

Drugs

All drugs were dissolved in 0.9% physiological saline (Baxter Healthcare Corporation, Deerfield, IL) on the day of testing. (±)-Baclofen, muscimol, and NO-711 HCl were obtained from Sigma (St. Louis, MO) and injected i.p. at a volume of 10 ml/kg. Ethanol was obtained from Pharmco Products (Brookfield, CT) and was diluted from a 100% stock to a 20% v/v solution in saline and injected i.p.

Procedure

All testing occurred between 0800 and 1600 h. At the end of each experiment, all subjects were euthanized by carbon dioxide asphyxiation. These procedures were approved by the Portland Veterans Affairs Medical Center Institutional Animal Care and Use Committee and complied with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals* (1986).

Locomotor Activity. Mice were moved in their home cages to the procedure room and left undisturbed to acclimate for 45-60 min prior to testing. Subjects were not habituated to the activity monitor or to injections prior to locomotor testing, consistent with the selection procedure (Crabbe et al., 1988; Phillips et al., 1991), as well as previous experiments using GABA mimetic compounds in FAST mice (Palmer et al., 2002a,b; Phillips et al., 1992; Shen et al., 1998). Mice were weighed, injected with the pretreatment drug (saline, NO-711, muscimol, or baclofen), and placed individually into holding cages. At the conclusion of the pretreatment interval (specified below), each subject was injected with saline or ethanol and placed into the activity monitor. Activity

was recorded for 30 min in 5-min time bins. At the conclusion of testing, blood samples (20 µl) were collected from the peri-orbital sinus of ethanol-treated mice for the determination of blood ethanol concentration (BEC), and processed as described by Boehm et al. (2000). BECs were determined using gas chromatography (Agilent 6890) with flame ionization detection.

Fixed-Speed Rotarod. This procedure was adapted from Rustay and colleagues (2003a,b). Mice were moved to the procedure room, weighed, and left undisturbed for approximately 30-45 min. Each mouse was then placed onto the dowel, which rotated at a fixed speed of 3 RPM, and up to five criterion trials were conducted. Subjects met criterion if they successfully remained on the rod for 180-sec. Only one successful trial was required to meet criterion. Afterwards, subjects were returned to their home cages to await testing (for a minimum of 30-45 min), at which time they were pretreated (saline, NO-711, muscimol, or baclofen) and placed in individual holding cages. After the pretreatment interval, subjects were injected with saline or ethanol and placed immediately on the rotating dowel. Three trials were conducted at the T0 time point (immediately after ethanol administration) and at the T10 time point (10 min after ethanol administration), with a 180 sec maximum duration upon the rotarod per trial. Latency to fall from the dowel in sec was automatically recorded when a photocell beam was interrupted.

Experiment 1: Effect of a GABA transporter inhibitor on ethanol-induced locomotion. FAST and SLOW (replicate 1 and 2) male mice were injected with saline or NO-711 (1.25, 2.5, 5, or 7.5 mg/kg) 30 min prior to injection with saline or 2 g/kg ethanol. The NO-711 doses and pretreatment interval were adapted from Dalvi and

Rodgers (1996), while the 2 g/kg ethanol dose was the dose used for the majority of selective breeding generations of the FAST and SLOW lines (Crabbe et al., 1988; Phillips et al., 1991). A follow-up experiment used the same procedures and doses of NO-711 to assess the effects of NO-711 on the locomotor response to a lower dose of ethanol (1 g/kg) which was previously found to elicit only a modest stimulant response in FAST mice (Palmer et al., 2002a).

Experiment 2: NO-711 and ethanol-induced motor incoordination. The effect of NO-711 on the incoordinating effects of ethanol was examined in male FAST-2 mice administered saline or NO-711 (5 and 7.5 mg/kg) 30 min prior to an injection of saline or ethanol (1.2 g/kg). These doses of NO-711 were the most effective at reducing ethanol-induced stimulation in FAST-2 mice in Experiment 1. The dose of ethanol chosen for this experiment was one known to have only minor ataxic effects, thereby allowing for increased ataxia to be observed when ethanol was given in combination with NO-711 (JC Crabbe, personal communication; Rustay et al., 2003 a,b). Only FAST-2 mice were used for this study due to their greater availability, and because comparable results were obtained in the replicate lines in Experiment 1.

Experiment 3: Contribution of the $GABA_A$ receptor to ethanol-induced stimulation. To determine whether $GABA_A$ receptor activation alone would have effects on ethanol-induced locomotor stimulation similar to the effects of NO-711, male FAST-2 mice were injected with saline or muscimol (0.5, 1, 1.5, or 2 mg/kg) 10 min prior to an injection of saline or 2 g/kg ethanol (doses and pretreatment interval adapted from Shen et al., 1998). Due to their greater stimulant response to ethanol (Palmer et al., 2002a) and their greater availability, only FAST-2 mice were used for this study.

Experiment 4: The $GABA_A$ receptor and ethanol-induced motor incoordination. To determine whether muscimol also accentuated ethanol-induced ataxia, male FAST-2 mice were administered saline or muscimol (1 and 1.5 mg/kg) 10 min prior to saline or 1.2 g/kg ethanol. The muscimol doses used were the most effective at reducing ethanol-induced stimulation in Experiment 3 in the absence of gross motor side-effects.

Experiment 5: The $GABA_B$ receptor and ethanol-induced motor incoordination. In a previous study with FAST mice, the $GABA_B$ receptor agonist baclofen was found to attenuate the locomotor stimulant response to 2 g/kg ethanol (Shen et al., 1998). The purpose of this experiment was to examine the possibility that baclofen reduced stimulation by accentuating ethanol-induced motor incoordination. In the initial study, male FAST-2 mice were tested following saline or baclofen (1.25, 2.5, or 5 mg/kg) administered 15 min prior to saline or 1.2 g/kg ethanol. This study was then replicated in male FAST-1 mice.

Experiment 6: Contribution of the $GABA_B$ receptor to ethanol-induced stimulation. To enhance interpretation of the effects of baclofen on ethanol-induced rotarod ataxia, the effect of baclofen on stimulation to a low, sub-stimulating dose of ethanol was examined in FAST mice. FAST-1 and -2 mice were administered saline or baclofen (0.625, 1.25, 2.5, or 5 mg/kg) 15 min prior to an injection of saline or ethanol (1 g/kg).

Data Analysis

For the locomotor activity studies, repeated measures analyses of variance (ANOVAs) were performed to determine whether there was an interaction with time, prompting separate analyses to determine the time period in which the drug effects were

most robust. We determined from these initial analyses that the first 10-min test period best described the drug effects in these studies. Further, this is the time when stimulation to ethanol is most profound (Shen et al., 1995). Data for this time period were then analyzed by factorial ANOVA. Across the multiple studies, several different categorical variables were included in the analysis, including line, replicate, pretreatment dose, and ethanol dose. Interactions of three or more factors were followed up by two-factor ANOVAs as appropriate to further parse the data, and by simple main effects analyses and Newman-Keuls mean comparisons to examine significant two-way interactions and main effects, respectively. For the rotarod ataxia experiments, nonparametric analyses were conducted because the data were not normally distributed (due to a ceiling criterion). Individual Kruskal-Wallis tests were performed to examine the effect of drug pretreatment on saline- and ethanol-responding. Significant effects of pretreatment, as well as analyses of ethanol effects at each pretreatment dose, were examined by Bonferroni-corrected Mann-Whitney U comparisons. Significance levels were set at $\alpha \leq 0.05$. All analyses were conducted with the Statistica 6.1 software package (StatSoft, Inc., Tulsa, OK).

Results

Experiment 1: Sensitivity to NO-711 is correlated with sensitivity to ethanol-induced locomotor stimulation, but NO-711 attenuates the stimulant response to ethanol.

Acute Sensitivity to NO-711 in FAST and SLOW mice. In a separate analysis of data from FAST and SLOW mice that had been pretreated with NO-711 prior to saline administration, FAST and SLOW mice were found to differ in their locomotor response

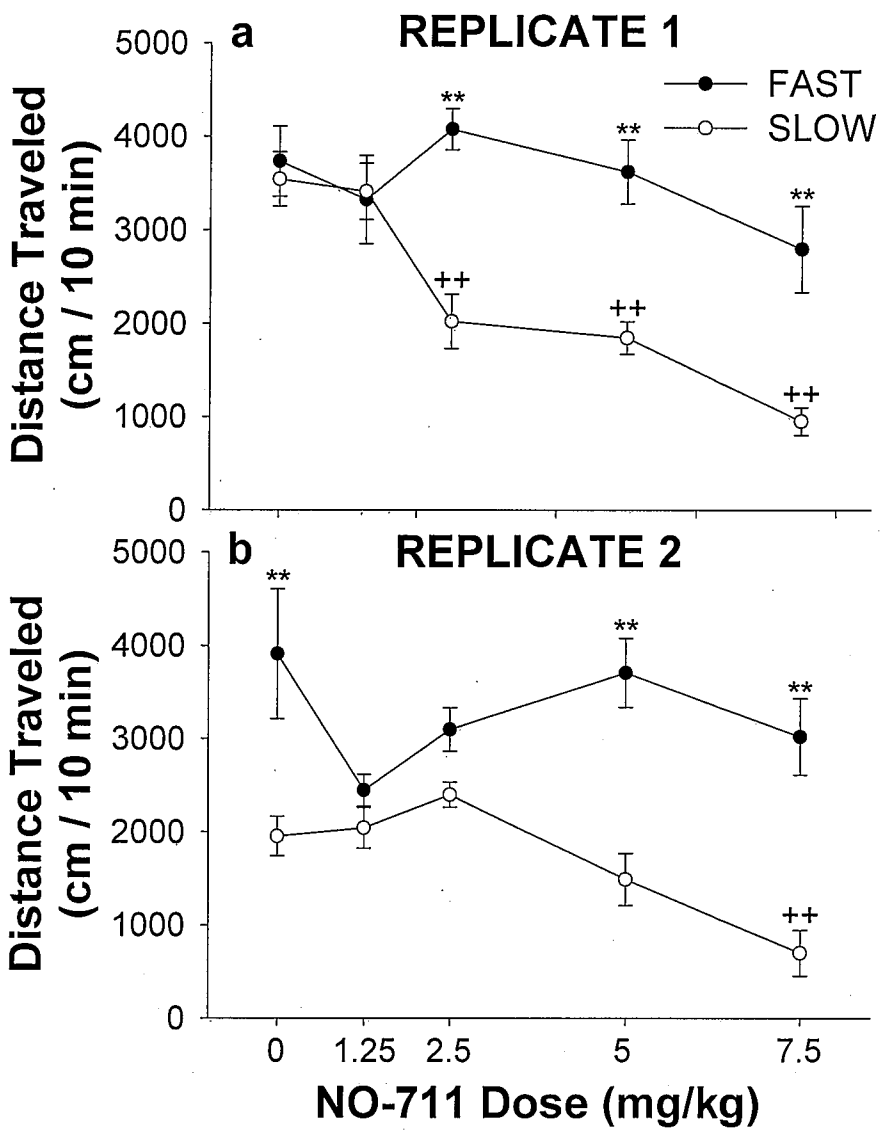
to NO-711 alone (Fig 3). This genetic correlation indicates that some common genes, and therefore mechanism(s), influence sensitivity to ethanol and to this GABA transporter inhibitor. NO-711 produced a significant decrease in the locomotor activity of SLOW mice while not significantly altering the locomotor activity of FAST mice, compared to their activity after saline injection (Fig 3).

Results of repeated measures ANOVA supported these characterizations. Because replicate interacted with time and NO-711 dose [$F_{4, 190}$=2.6, p<0.05], separate analyses were performed on data from each replicate. For replicate 1 data, there was a significant line X NO-711 dose interaction [$F_{4, 92}$=4.8, p<0.01], and post-hoc analyses identified significant differences in locomotor response to 2.5, 5, and 7.5 mg/kg NO-711 between FAST-1 and SLOW-1 mice. Moreover, NO-711 (2.5, 5, and 7.5 mg/kg) significantly reduced the activity of SLOW-1 mice, while having no effect on the activity of FAST-1 mice. For replicate 2 data, there was also a significant line X NO-711 dose interaction [$F_{4, 98}$=3.2, p<0.05]. While interpretation is complicated by a line difference between FAST-2 and SLOW-2 mice in spontaneous activity, the lines were found to differ in response to 5 and 7.5 mg/kg NO-711. Moreover, the activity of SLOW-2 mice was significantly reduced by the 7.5 mg/kg dose; however, NO-711 did not significantly reduce activity in FAST-2 mice.

NO-711 effects on activity after ethanol treatment. In FAST mice, pretreatment with NO-711 dose-dependently abolished the locomotor stimulant response to the selection-dose of ethanol (2 g/kg), while attenuating stimulation to 1 g/kg ethanol in FAST-1, but not FAST-2, mice. Conversely, NO-711 accentuated the locomotor depressant response to 2 g/kg ethanol in SLOW-1 mice (Fig 4). The following statistical

FIGURE 3. The FAST and SLOW selected lines differ in their locomotor response to the GABA transporter inhibitor NO-711.

Shown is the effect of NO-711 on the locomotor response of male FAST and SLOW (replicate 1 and 2) mice subsequently treated with saline (n = 10-12 per group); these data are the saline control data for Experiment 1 in which the effect of NO-711 on saline- and ethanol (2 g/kg)-induced locomotion in FAST and SLOW mice was assessed (see Fig 4). The data are shown separately here to highlight the effect of NO-711 alone on the locomotor activity of FAST and SLOW mice. Data are presented as mean ± SEM and summed over the first 10 min of the activity test. ** Significant line difference in the locomotor response to NO-711 between FAST and SLOW mice, $p<0.01$. ++ Significant reduction in locomotor activity with NO-711 pretreatment, $p<0.01$.


a
REPLICATE 1
FAST
SLOW
Distance Traveled (cm / 10 min)
5000
4000
3000
2000
1000
0
**
++
b
REPLICATE 2
0
1.25
2.5
5
7.5
NO-711 Dose (mg/kg)

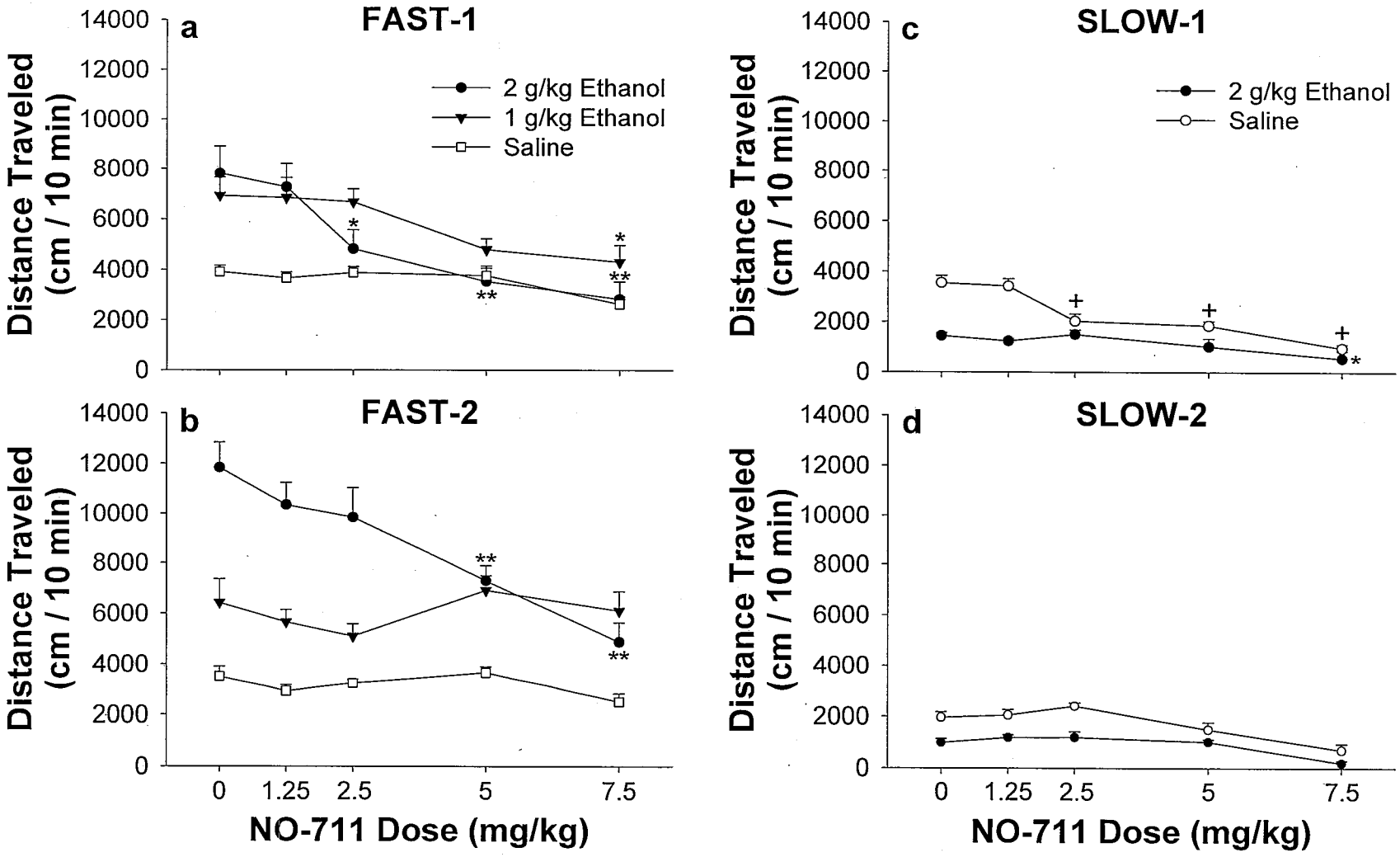
analyses supported the above-stated characterizations.

Data for the 1 and 2 g/kg ethanol doses were collected in separate studies. Our initial study examined the effect of NO-711 on activity after saline or 2 g/kg ethanol in both FAST and SLOW mice. To further test the hypothesis that ethanol and NO-711 have additive effects, a separate study was then performed examining the effect of NO-711 on activity after saline or 1 g/kg ethanol, a sub-maximally stimulating dose, in FAST mice only; there is no dose of ethanol that induces stimulation in SLOW mice. For the purpose of probing the effect of NO-711 across the two ethanol doses, data for FAST mice from these two studies were combined in a single analysis. Activity levels of animals treated with NO-711 prior to saline were not found to significantly differ between the two studies, thus data from the two control groups were combined for this analysis (open symbols in Fig 4, panels a and b).

FAST mice. Because there was an interaction of replicate with NO-711 dose and ethanol dose [$F_{8, 417}$=2.3, p<0.05], data from the two replicate FAST lines were considered in separate analyses. For FAST-1 mice (Fig 4a), there was a significant NO-711 dose X ethanol dose interaction [$F_{8, 203}$=3.8, p<0.001]. The 1 and 2 g/kg ethanol doses unexpectedly induced significant stimulant responses of similar magnitude in this line of mice. While NO-711 pretreatment reduced the stimulant response to both ethanol doses, only the highest dose of NO-711 attenuated the stimulant response to 1 g/kg ethanol, while moderate to high doses of NO-711 (2.5-7.5 mg/kg) attenuated stimulation to 2 g/kg ethanol. There was no effect of NO-711 on saline activity.

For FAST-2 mice (Fig 4b), there was also a NO-711 dose X ethanol dose interaction [$F_{8, 214}$=7.3, p<0.001]. The 1 and 2 g/kg ethanol doses significantly increased

FIGURE 4. NO-711 attenuates the locomotor stimulant response to ethanol in FAST mice and accentuates the locomotor depressant response to ethanol in SLOW mice. Male FAST and SLOW (replicate 1 and 2) mice, 50-88 days of age, from selection generations $S_{37}G_{81\text{-}83}$ and $S_{37}G_{89\text{-}92}$ were used for this study. The effect of NO-711 on activity after saline and after a low (1 g/kg) and moderate (2 g/kg) dose of ethanol was examined in FAST mice (panels a and b). The group size was 10-12 mice. However, saline group data are shown here as combined group means from the two different ethanol dose experiments, resulting in a final group size of 21-25 mice per NO-711 dose group (the saline data were collapsed across the two separate experiments because no statistically significant differences were found between the experiments). The effect of NO-711 on activity after saline and 2 g/kg (but not 1 g/kg) ethanol was also examined in SLOW mice (n = 10 per group; panels c and d – note that the saline data are the same as those presented in Fig 3, but are presented again here for comparison to the ethanol data). Data are presented as mean ± SEM and summed over the first 10 min of the activity test. * $p<0.05$, ** $p<0.01$ for the reduction in stimulation from the ethanol control group (0 mg/kg NO-711). + Significant reduction in activity from the saline control group, $p<0.001$.


a
FAST-1
2 g/kg Ethanol
1 g/kg Ethanol
Saline
Distance Traveled (cm / 10 min)
14000
12000
10000
8000
6000
4000
2000
0
b
FAST-2
c
SLOW-1
d
SLOW-2
0
1.25
2.5
5
7.5
NO-711 Dose (mg/kg)

activity; however, the stimulant response was dose-dependent in this mouse line. NO-711 pretreatment (5 and 7.5 mg/kg) attenuated the stimulant response to 2 g/kg, whereas NO-711 pretreatment did not alter the more modest stimulant response to 1 g/kg ethanol. There was no effect of NO-711 on locomotor activity after saline treatment.

SLOW mice. SLOW mice were examined only at the 2 g/kg ethanol dose. Replicate interacted with NO-711 dose and ethanol dose [$F_{4, 182}$=3.7, p<0.01]. For SLOW-1 mice (Fig 4c), there was a NO-711 dose X ethanol dose interaction [$F_{4, 92}$=6.4, p<0.001]. Consistent with their selection response, 2 g/kg ethanol induced a locomotor depressant response in these mice. There was a significant effect of NO-711 on activity after both saline and ethanol treatment, with dose-dependent effects in both cases (see Fig 4). For SLOW-2 mice (Fig 4d), there were main effects of NO-711 dose [$F_{4, 90}$=15.6, p<0.001] and ethanol dose [$F_{1, 90}$=45.8, p<0.001], but no significant interaction of these two factors, indicating that NO-711 had similar effects on the activity of saline- and ethanol-treated mice. Thus, in general, the effect of both ethanol and NO-711 in SLOW mice was to reduce locomotor behavior.

Analysis of BEC data obtained from samples taken after the 30-min activity test revealed no effect of NO-711, and no line or replicate differences. The mean (± SEM) BEC for the genotypes and NO-711 dose groups combined was 0.68 ± 0.02 mg/ml after 1 g/kg ethanol and 1.32 ± 0.03 mg/ml after 2 g/kg ethanol at the 30-min time point.

Experiment 2: NO-711 accentuates the ataxic effects of ethanol.

To examine whether the attenuation of ethanol-induced locomotor stimulation in FAST-2 mice by NO-711 could be due to an accentuation of ethanol-induced motor incoordination, rotarod ataxia data were collected. As shown in Fig 5, NO-711

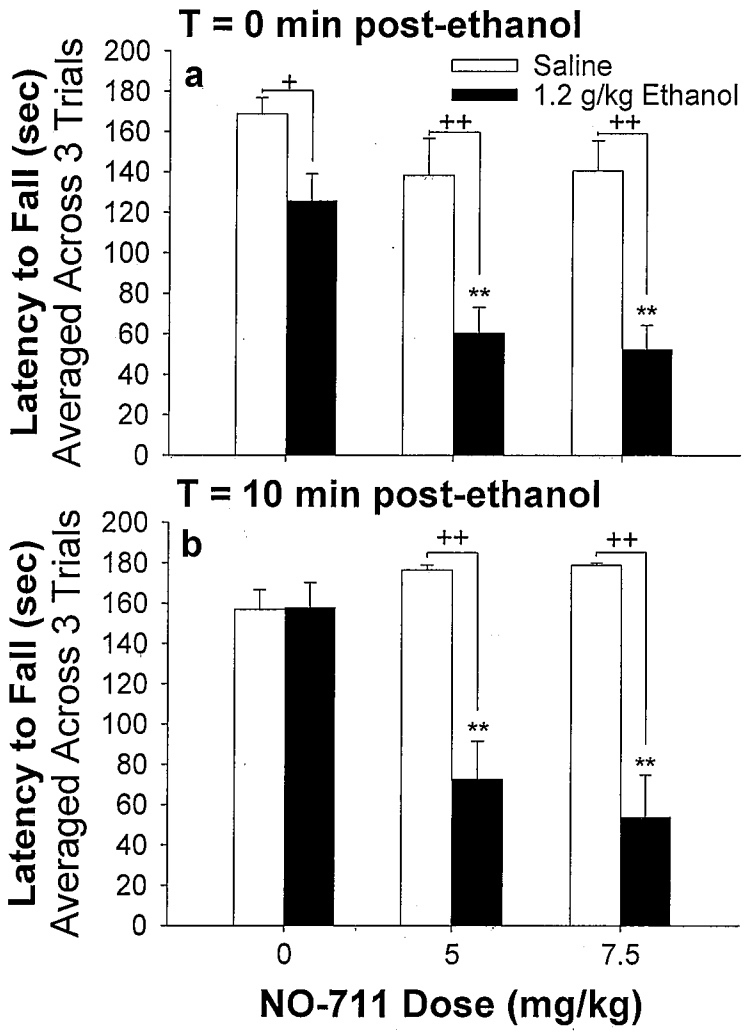


FIGURE 5. NO-711 enhances ethanol-induced motor incoordination in FAST-2 mice.

Male FAST-2 mice, aged 53-87 days, from the $S_{37}G_{83\text{-}85}$ generations were used for this study; n = 10-11 per group. The top panel depicts average ± SEM latency to fall in sec (mean of three, 180-sec trials) from the rotarod immediately after ethanol administration, while the bottom panel depicts average latency ± SEM to fall 10 min after ethanol administration. ** Significant reduction in latency to fall after ethanol, $p<0.01$. + $p<0.05$, ++ $p<0.01$ for the difference between saline and ethanol groups at the indicated dose of NO-711.

significantly accentuated the ataxic effects of ethanol. Data from four mice (5.8 % of all mice tested) were excluded from the study because the mice did not meet the 180-sec baseline rotarod performance criterion. Analyses of latency to fall from the rod across the three trials at T0 (immediately after ethanol administration) and T10 (10 min post-ethanol administration) revealed no significant three-way interaction of trial, NO-711 dose, and ethanol dose; therefore an average of the 3 trials at each time point was used as the dependent variable.

For the T0 time point (Fig 5a), Kruskal-Wallis analyses found no significant effect of NO-711 pretreatment on latency to fall after saline; however, NO-711 did significantly decrease latency to fall after ethanol [$H_{2, N=33}=12.8, p<0.01$], with increased ataxia at 5 and 7.5 mg/kg NO-711 (combined with ethanol) as compared to ethanol alone. In Bonferroni-corrected pairwise comparisons, ethanol was found to significantly decrease latency to fall from the rod at all NO-711 doses (including 0 mg/kg).

By the T10 time point, motor incoordinating effects of ethanol and NO-711 alone had abated, yet there remained significant enhancement of motor incoordination when these two drugs were combined (Fig 5b). Again, there was a significant effect of NO-711 on latency to fall after ethanol [$H_{2, N=33}=12.7, p<0.01$], with a significant decrease in latency scores after treatment with both 5 and 7.5 mg/kg NO-711. NO-711 pretreatment did not affect latency to fall after saline. Furthermore, Bonferroni-corrected pairwise comparisons revealed significant differences between ethanol- and saline-treated mice after treatment with 5 and 7.5 mg/kg NO-711, but not 0 mg/kg NO-711. Overall, these data indicate increased ataxia with the combination of NO-711 and ethanol.

Experiment 3: The $GABA_A$ receptor agonist muscimol attenuates ethanol-induced stimulation.

As shown in Fig 6, pretreatment with the $GABA_A$ receptor agonist muscimol significantly attenuated the locomotor stimulant response to ethanol in FAST-2 mice, an effect similar to that observed for NO-711 in Experiment 1. A significant muscimol dose X ethanol dose interaction was found [$F_{4,109}$=25.2, p<0.001]. Muscimol altered the locomotor response to both saline and ethanol, with a modest locomotor stimulant response induced by 1 and 1.5 mg/kg muscimol in saline-treated mice. However, in ethanol-treated mice, the stimulant response to ethanol was significantly reduced by the three highest doses of muscimol. There was no effect of muscimol pretreatment on BEC determined from samples collected after the 30 min activity test; mean BEC (± SEM) for the combined dose groups was 1.61 ± 0.05 mg/ml.

Experiment 4: Muscimol accentuates the ataxic effects of ethanol.

Similar to NO-711, muscimol had a dramatic motor-incoordinating effect when combined with a low dose of ethanol in FAST-2 mice (Fig 7). Data from four mice (5.3 %) were excluded from the study because the mice were unable to meet the baseline performance criterion. Analyses at both the T0 and T10 time points revealed no interaction of trial, muscimol dose, and ethanol dose, and therefore an average of the 3 trials at each time point was used as the dependent variable.

Immediately after ethanol administration (T0), muscimol significantly decreased latency to fall from the rod after ethanol [$H_{2, N=36}$=17.5, p<0.001], with an increase in ataxia at both 1 and 1.5 mg/kg muscimol (Fig 7a). While muscimol did affect latency to fall after saline [$H_{2, N=35}$=8.2, p<0.05], a significant decrease in latency was only observed

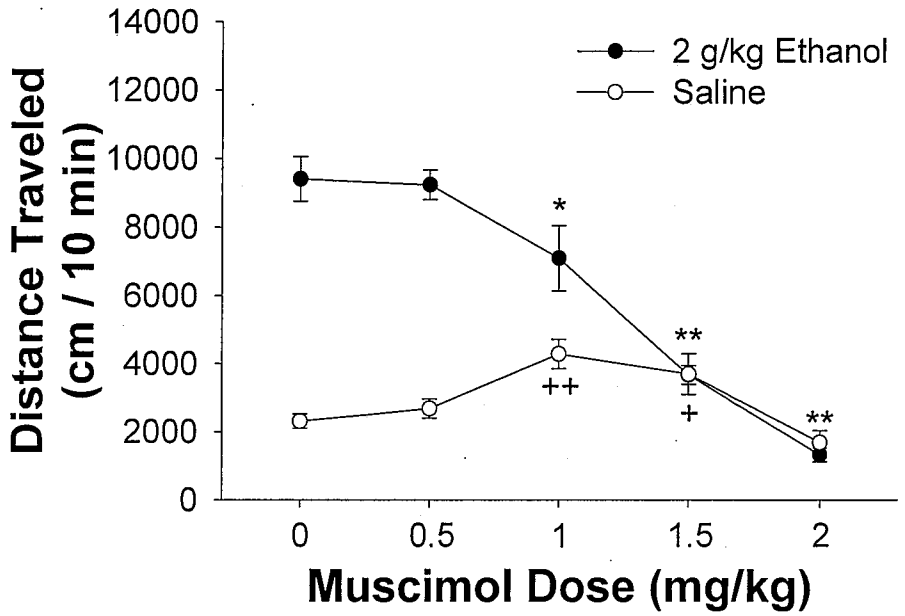


FIGURE 6. The $GABA_A$ receptor agonist muscimol attenuates the locomotor stimulant response to ethanol in FAST-2 mice while stimulating activity on its own at low doses.

Male FAST-2 mice, aged 54-76 days, from selection generations $S_{37}G_{87\text{-}88}$ were used for this study; n = 11-12 per group. Data presented are summed over the first 10-min of the activity test and are presented as mean ± SEM. * $p<0.05$, ** $p<0.01$ for the reduction in ethanol-induced locomotor stimulation by muscimol. + $p<0.05$, ++ $p<0.01$ for the increase in activity above saline control values with the indicated dose of muscimol.

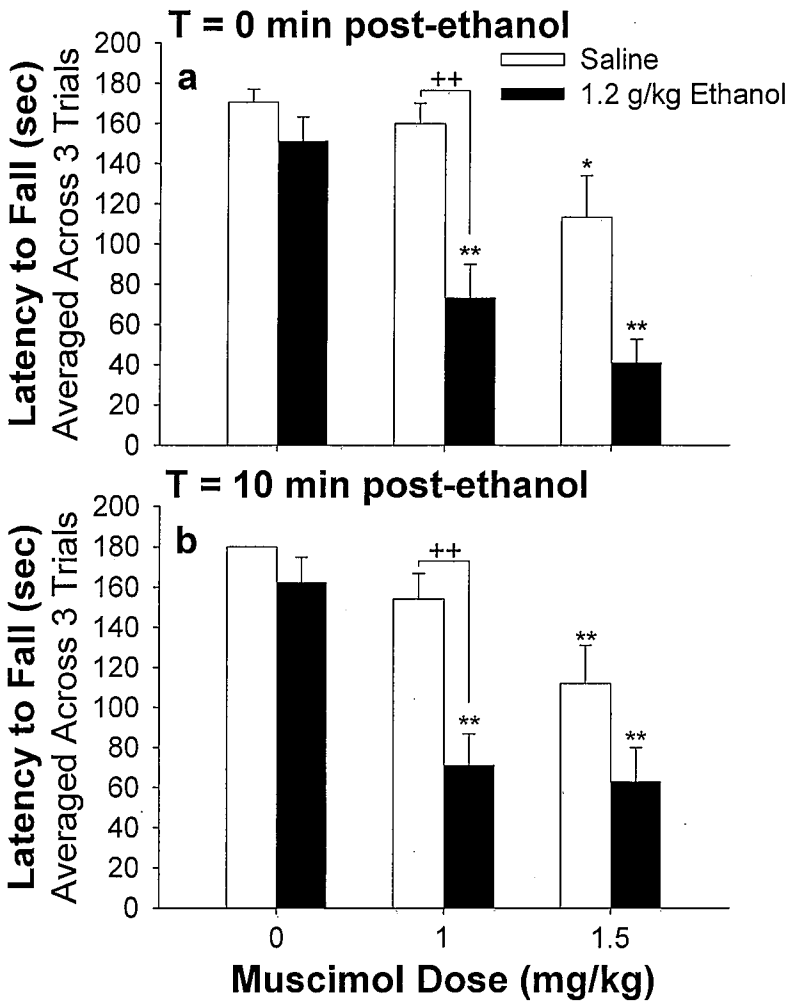


FIGURE 7. Muscimol accentuates ethanol-induced motor incoordination in FAST-2 mice. Male FAST-2 mice ($S_{37}G_{86\text{-}88}$), ages 50-71 days, were used for this study; n = 11-12 per group. The top panel depicts average ± SEM latency to fall in sec (mean of three, 180-sec trials) from the rotarod immediately after ethanol administration, while the bottom panel depicts average latency ± SEM to fall 10 min after ethanol administration. * $p<0.05$, ** $p<0.01$ for the reduction in latency to fall from the respective control group (0 mg/kg muscimol). ++ Significant difference between saline and ethanol groups at the indicated dose of muscimol, $p<0.01$.

at the highest muscimol dose (1.5 mg/kg). With a muscimol-induced effect on motor coordination, there was only a significant difference in latency to fall between ethanol- and saline-treated mice at 1 mg/kg muscimol; ethanol alone did not significantly alter latency to fall.

As seen in Fig 7b, a similar pattern of results was found 10-min post-ethanol treatment (T10). Kruskal-Wallis analyses revealed significant effects of muscimol on latency to fall after saline [$H_{2, N=35}=15.1, p<0.001$] and ethanol [$H_{2, N=36}=15.3, p<0.001$]. While both 1 and 1.5 mg/kg muscimol, in combination with ethanol, increased motor incoordination as compared to ethanol alone, it was only the 1.5 mg/kg dose of muscimol that had ataxic effects on its own. Bonferroni-corrected pairwise comparisons highlighted a significant difference in latency scores between saline- and ethanol-treated mice after 1 mg/kg muscimol, but not after 0 or 1.5 mg/kg muscimol.

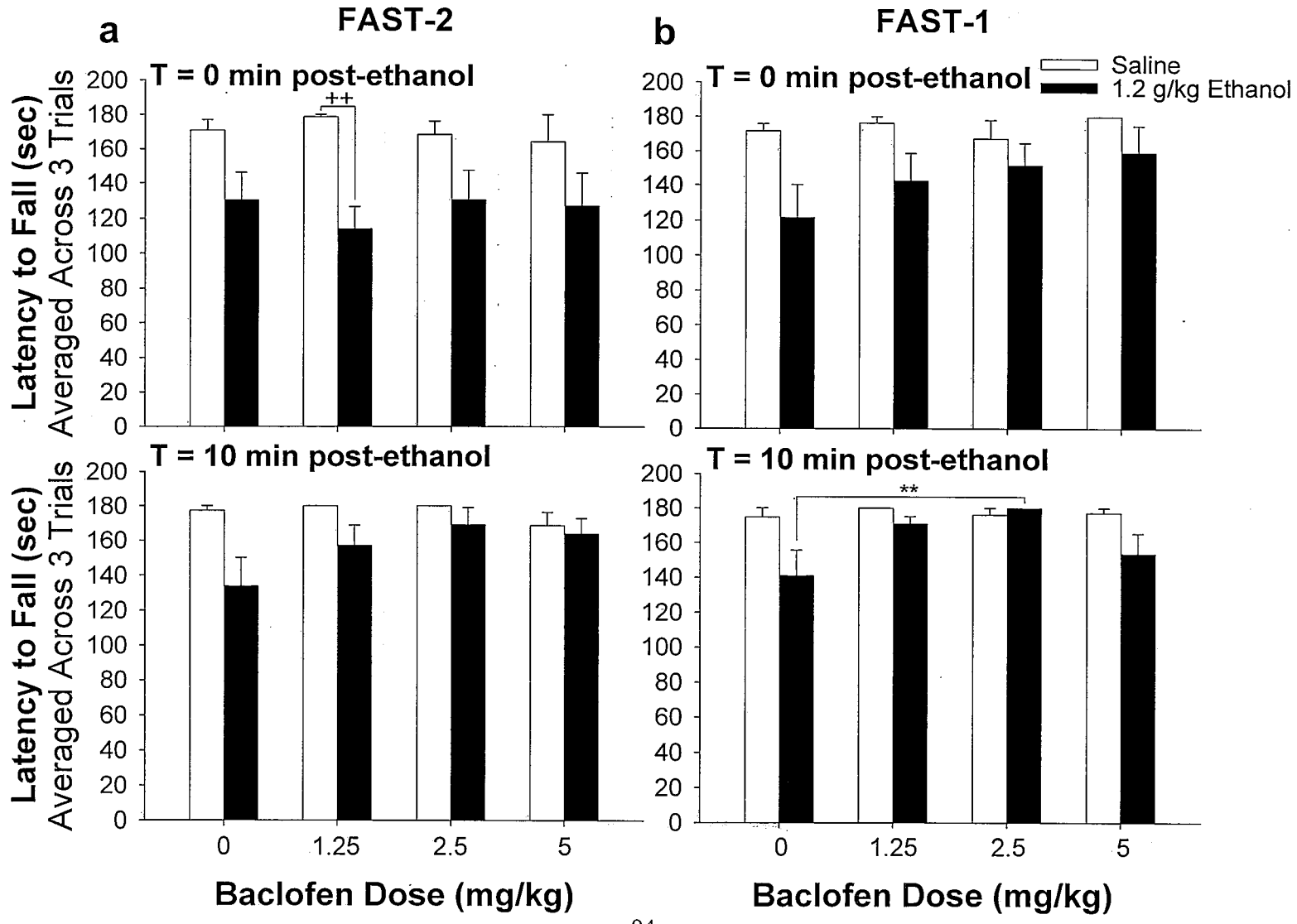
Experiment 5: The $GABA_B$ receptor agonist does not accentuate the ataxic effects of ethanol.

As shown in Fig 8a, overall pretreatment with baclofen, especially at higher doses, did not accentuate ethanol-induced motor incoordination in FAST-2 mice, a result which is in stark contrast to the effects of NO-711 and muscimol. Data from six mice (7.2 %) were excluded from the study because the mice were unable to meet the baseline performance criterion. Analyses did not reveal any interaction of baclofen dose and ethanol dose with trial, so the data were analyzed as a T0 average and a T10 average.

At the T0 time point, Kruskal-Wallis tests revealed no effect of baclofen pretreatment on latency to fall from the rod after saline or ethanol. Additionally, saline- and ethanol-treated FAST-2 mice only differed in latency to fall at the 1.25 mg/kg dose

FIGURE 8. Baclofen does not accentuate ethanol-induced motor incoordination in FAST mice.

(a) Male FAST-2 mice ($S_{37}G_{85\text{-}86}$), ages 50-81 days, were used in this experiment; n = 9-10 per group. The top panel depicts average ± SEM latency to fall in sec (mean of three, 180-sec trials) from the rotarod immediately after ethanol administration, while the bottom panel depicts average latency ± SEM to fall 10 min after ethanol administration. (b) In order to confirm the results found with FAST-2 mice, the same study examining the effect of baclofen on ethanol-induced motor incoordination was conducted in male FAST-1 mice. The subjects were 50-88 days of age and from selection generations $S_{37}G_{90\text{-}91}$; n = 9-10 per dose group. ++ Significant difference between saline and ethanol groups at the indicated dose of baclofen, $p<0.01$. ** Significant difference between ethanol groups at 0 and 2.5 mg/kg baclofen, $p<0.01$.


a
FAST-2
b
FAST-1
Saline
1.2 g/kg Ethanol
T = 0 min post-ethanol
T = 10 min post-ethanol
Latency to Fall (sec)
Averaged Across 3 Trials
++
**
0
20
40
60
80
100
120
140
160
180
200
0
1.25
2.5
5
Baclofen Dose (mg/kg)

of baclofen. At the T10 time point, there was no significant effect of baclofen on the responses of the ethanol or saline groups.

We had predicted that baclofen would accentuate ethanol-induced ataxia; it did not. To confirm these results, the study was repeated using FAST-1 mice, and as shown in Fig 8b, the results were replicated–baclofen did not accentuate ethanol-induced motor incoordination in this line either. Data from one mouse (1.3 %) were excluded from the study because the mouse did not meet the performance criterion. Immediately after ethanol administration (T0), there was again no effect of baclofen pretreatment on latency to fall after saline or ethanol in FAST-1 mice. At the T10 time point, while there was no effect of baclofen on latency to fall after saline treatment, there was a significant effect of baclofen on latency to fall after ethanol [$H_{3, N=38}=9.2$, $p<0.05$]. Bonferroni-corrected post-hoc comparisons revealed that there was actually a significant increase in latency to fall after ethanol with 2.5 mg/kg baclofen as compared to 0 mg/kg baclofen. These results confirmed those obtained using FAST-2 mice that baclofen, in this procedure, does not accentuate ethanol-induced motor incoordination.

Experiment 6: Effects of baclofen on the locomotor stimulant response to a low dose of ethanol.

The striking contrast in motor coordination results seen for NO-711 and muscimol versus baclofen was not predicted. While our laboratory has previously found that peripherally administered baclofen attenuates the locomotor stimulant response to ethanol in FAST mice (Shen et al., 1998), the ethanol dose used was 2 g/kg, a dose higher than that used for the rotarod study (1.2 g/kg). Experiment 6 was designed to determine whether baclofen would reduce stimulation to a lower dose of ethanol, one more

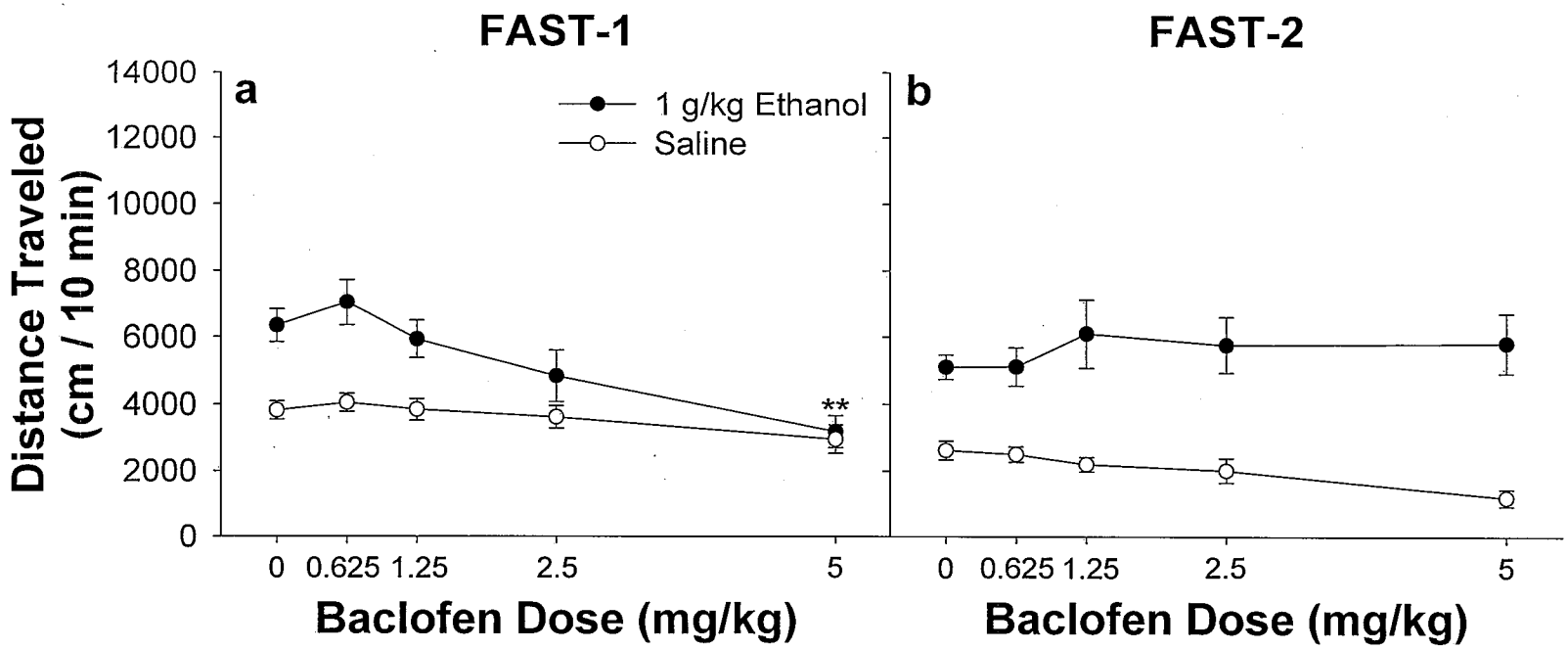
comparable to that used to measure combined drug effects on ataxia. Since baclofen did not accentuate ataxia produced by a relatively low dose of ethanol, a reduction by baclofen of the stimulation produced by this low dose of ethanol would not likely be due to altered coordination. As shown in Fig 9, baclofen dose-dependently reduced the locomotor stimulant response to 1 g/kg ethanol in FAST-1 mice, but did not reduce the stimulant response to this dose of ethanol in FAST-2 mice. A replicate X baclofen dose X ethanol dose interaction [$F_{4, 254}$=3.2, p<0.05] prompted a separate analysis of the data for each replicate line. For FAST-1 mice, there was a significant interaction of baclofen dose and ethanol dose [$F_{4, 139}$=2.6, p<0.05]. A stimulant response to 1 g/kg ethanol was observed in these mice, which was significantly reduced by the highest dose of baclofen (5 mg/kg). In contrast, ethanol had stimulant effects in FAST-2 mice [$F_{1, 115}$=81.5, p<0.001], but there was no effect of baclofen or significant interaction of baclofen dose and ethanol dose. BECs were comparable across baclofen pretreatment and replicate line groups in samples collected after the 30 min activity test. Mean BEC (± SEM) for the combined replicate line and dose groups was 0.55 ± 0.04 mg/ml.

Discussion

FAST and SLOW mice differed in sensitivity to the effects of the GABA transporter inhibitor NO-711 on locomotor activity, adding evidence for common genetic regulation of sensitivity to GABA mimetic drugs and ethanol. While activation of GABA systems, either via an indirect agonist (NO-711) or via receptor specific agonists (muscimol or baclofen), virtually eliminated the locomotor stimulant response to ethanol, there appears to be a receptor subtype-specific behavioral mechanism for this effect. The

FIGURE 9. Baclofen attenuates the locomotor stimulant response to a low dose of ethanol in FAST-1, but not FAST-2, mice.

In this study, male FAST-1 and -2 mice were tested for the effect of baclofen on the stimulant response to a low dose of ethanol. Subjects were 60-97 days of age at the time of testing, from selection generations $S_{37}G_{77\text{-}79}$ and $S_{37}G_{89\text{-}92}$; n = 11-15 per dose group. ** Significant reduction in ethanol-induced locomotor stimulation by baclofen, $p<0.01$.


FAST-1
FAST-2
a
b
1 g/kg Ethanol
Saline
Distance Traveled (cm / 10 min)
14000
12000
10000
8000
6000
4000
2000
0
**
0 0.625 1.25 2.5 5
Baclofen Dose (mg/kg)
0 0.625 1.25 2.5 5
Baclofen Dose (mg/kg)

attenuation of ethanol-induced locomotor stimulation by both NO-711 and muscimol was accompanied by a dramatic increase in the motor incoordinating effects of even a low dose of ethanol. In contrast, the $GABA_B$ receptor agonist baclofen attenuated stimulation in the complete absence of an increase in ethanol-induced motor incoordination. These results emphasize that the manner in which GABAergic drugs attenuate ethanol effects should be carefully considered. The most parsimonious explanation for the results described herein is that GABA mimetic drugs that, in part, enhance $GABA_A$ receptor function, act additively with ethanol to shift the behavioral response toward greater intoxication. This may be manifest behaviorally through a reduction in stimulation by the enhancement of a competing behavior, i.e., motor incoordination. Activation of $GABA_B$ receptors, however, may produce a more selective attenuation of the stimulant effects of ethanol, reducing stimulation without severe behavioral side effects. The additive effects of NO-711 or muscimol with ethanol could not be predicted from their effects on saline-treated mice alone; doses of all drugs tested were chosen to have minimal effects in saline-treated mice. Further, comparable mean responses between saline-treated and ethanol-treated mice that have been pretreated with a putative pharmacotherapeutic drug cannot be assumed to indicate the absence of a behavioral effect of the drug combination.

A role for GABA systems in ethanol sensitivity

The FAST and SLOW mouse lines serve as a model to study the genetic and neurobiological mechanisms underlying acute ethanol sensitivity. Previous studies have implicated a role for both $GABA_A$ and $GABA_B$ receptors in the selection phenotype (Palmer et al., 2002a,b; Phillips et al., 1992; Shen et al., 1998). NO-711 (also known as NNC-711) is a highly selective and specific noncompetitive antagonist of the GAT1

GABA transporter subtype, which is generally characterized as a neuronal transporter (Minelli et al., 1995; Suzdak et al., 1992). Inhibition of GAT1 leads to increases in extracellular GABA concentrations (Fink-Jensen et al., 1992); therefore NO-711 acts as an indirect agonist of both $GABA_A$ and $GABA_B$ receptors and likely enhances the tonic inhibition of target neurons. In the current study, SLOW mice were more sensitive to the locomotor depressant effects of NO-711, which is in accord with their enhanced sedative sensitivity to other $GABA_A$ and $GABA_B$ receptor agonists and positive modulators (Palmer et al., 2002a,b; Shen et al., 1998). Ethanol is hypothesized to have GABA mimetic effects (Allan and Harris, 1987; Criswell and Breese, 2005; Roberto et al., 2003; Wallner et al., 2003), and the line differences to a wide array of GABAergic drugs suggest that FAST and SLOW mice differ with regard to some aspect of GABA system function. Our laboratory has preliminary evidence to support this hypothesis, showing that $GABA_A$ receptor inhibitory postsynaptic currents (IPSCs) and $GABA_B$ receptor function in ventral midbrain tissue are greater in SLOW than in FAST mice (unpublished results). However, further studies are required to determine the cellular mechanisms mediating this line difference, and to address the $GABA_A$ receptor subunit-specific nature of ethanol effects that has become somewhat controversial (Borghese and Harris, 2007; Borghese et al., 2006; Hanchar et al., 2005; Korpi et al., 2007; Olsen et al., 2007; Wallner et al., 2003).

Blockade of the GABA transporter and activation of $GABA_A$ receptors attenuates ethanol stimulation by enhancing ataxia

Ethanol has a similar behavioral profile to that of several $GABA_A$ receptor agonists and positive modulators, including the ability to induce behavioral stimulation

(e.g., see Fig 6, showing stimulation to muscimol in FAST mice). Therefore, several groups have hypothesized that ethanol-induced increases in $GABA_A$ receptor function may contribute to ethanol-induced locomotor stimulation (Liljequist and Engel, 1982; Palmer et al., 2002a,b; Phillips et al., 1992; Phillips and Shen, 1996). Given these findings, it is peculiar that activation of $GABA_A$ receptors attenuates ethanol stimulation in FAST mice and other mouse strains (Biswas and Carlsson, 1978; Broadbent and Harless, 1999; Liljequist and Engel, 1982), whereas $GABA_A$ receptor antagonism does not (Liljequist and Engel, 1982; Shen et al., 1998). The attenuation of stimulation by agonists could be due to a pharmacological blockade of the stimulant response to ethanol. Alternatively, it could be due to an additive effect of two GABAergic agonists (i.e., ethanol and the other GABAergic drug), resulting in a shift of the behavioral response towards motor incoordination or intoxication, an effect likely to occur with increased GABA levels. Our data support an additive effect of ethanol and the GABA agonists. Although our data do not directly address mechanism, the genetic correlations in FAST and SLOW mice for responses to ethanol and GABAergic drugs are strongly supportive of a common mechanism involving GABA.

In FAST mice, NO-711 attenuated the locomotor stimulant response to ethanol without significantly altering the activity of saline-treated mice. This lack of an effect of NO-711 on spontaneous activity could suggest that the attenuation of ethanol stimulation was not due to a general reduction in motor activity. Analysis of the activity data in 1-min intervals (data not shown) showed an early reduction in ethanol-stimulated activity in NO-711 pretreated mice that remained reduced throughout the 10-min activity period analyzed. This could have been due to a direct inhibition of the stimulatory effects of

ethanol, or to an enhancement of the motor incoordinating effects of ethanol. Because the locomotor activity procedure is not a sensitive measure of ataxia (i.e., mice can locomote even when moderately ataxic), a rotarod ataxia test was performed. Our rotarod ataxia data showed a large increase in ethanol-induced ataxia with NO-711, as well as ataxic effects of NO-711 alone. Therefore, a reduction in ethanol-induced locomotor stimulation in NO-711 pretreated mice likely occurred because of the accentuation of a competing behavior, namely ataxia. When NO-711 was combined with a lower dose of ethanol (1 g/kg), we observed a reduced ability of NO-711 to attenuate stimulation in FAST-1 mice, and a lack of attenuation of stimulation in FAST-2 mice. This may have occurred because the combination of NO-711 with a lower dose of ethanol (1 g/kg) did not reach a necessary threshold for ataxia, thereby limiting the ability of NO-711 to attenuate stimulation. In SLOW mice, the combination of NO-711 and ethanol further enhanced the locomotor depressant response normally seen to ethanol, as would be predicted if the compounds in combination act to shift the behavior towards greater intoxication.

The results of the experiment with muscimol, but not baclofen, almost entirely mimicked the results for NO-711, suggesting that the predominant mechanism for NO-711's effects is activation of $GABA_A$ receptors. As NO-711 increases extracellular concentrations of GABA (Fink-Jensen et al., 1992), it should be increasing both $GABA_A$ and $GABA_B$ receptor function. Differences in brain distribution for these two receptor subtypes (which are unknown for these mouse lines) or differences in the behavioral effects that occur with the activation of $GABA_A$ vs. $GABA_B$ receptors may explain what appears to be a predominantly $GABA_A$ receptor-mediated effect of NO-711 on ethanol-

induced ataxia. A $GABA_A$ receptor-mediated mechanism by which NO-711 and muscimol, in combination with ethanol, increases ataxia (and thereby decreases stimulation) is consistent with previous work demonstrating that $GABA_A$ receptor agonists accentuate the motor-incoordinating effects of ethanol (Dar, 2006; Martz et al., 1983), while $GABA_A$ receptor blockade results in a reduction of ethanol-induced ataxia (Dar, 2006; Hoffman et al., 1987; Suzdak et al., 1988).

The current data are complementary to recent data in humans showing that the GAT1 inhibitor tiagabine, in combination with ethanol, did not attenuate ethanol-induced activation of limbic regions, but instead produced a large depression of cerebellar activity (possibly related to the increase in ataxia seen in the current study) (Fehr et al., 2007). These data also mimic the effect of NMDA receptor antagonists on ethanol-induced motor behaviors. The NMDA receptor antagonist MK-801 eliminated the stimulant response to ethanol in mice and, at higher doses, attenuated ethanol-induced locomotor sensitization. This effect was accompanied by an increase in ethanol-induced motor incoordination (Meyer and Phillips, 2003a). As ethanol inhibits NMDA receptors (Dildy-Mayfield and Leslie, 1991), these data suggest that MK-801 and ethanol act additively to induce a shift in the behavioral response to ethanol towards greater intoxication. Similar to the current findings, this suggests that pharmacotherapies that act in a manner similar to ethanol may induce their desired behavioral effect via a potentiation of an undesired ethanol effect. However, given in the absence of ethanol, they could be considered replacement therapies. For instance, $GABA_A$ receptor positive modulators, as well as NMDA receptor antagonists, fully substitute for the stimulus

properties of ethanol (Grant, 1999); therefore, drugs that act, in part, to activate $GABA_A$ receptors may reduce ethanol consumption by partial substituting for ethanol.

Activation of $GABA_B$ receptors attenuates stimulation, but in the absence of increases in ataxia

That the $GABA_B$ receptor agonist baclofen had little effect on ethanol-induced motor incoordination was surprising, because baclofen has potent anti-spastic and muscle relaxant properties, a trait it shares with ethanol (Bowery, 2006; Nevins et al., 1993). Our laboratory has previously found that both peripheral and central baclofen administration attenuated ethanol-induced locomotor stimulation in FAST mice (Boehm et al., 2002; Shen et al., 1998), an effect also observed in other mouse strains (Broadbent and Harless, 1999; Chester and Cunningham, 1999; Humeniuk et al., 1993). As central administration would be less likely to induce muscle relaxation, this may suggest a role for $GABA_B$ receptors in ethanol stimulation that is independent of the accentuation of ataxia. This selective reduction in stimulation could plausibly be mediated by several different neuronal mechanisms. First, activation of $GABA_B$ receptors has been found to decrease dopamine cell firing in the ventral midbrain (Erhardt et al., 2002; Mueller and Brodie, 1989), and the dopamine response to several drugs of abuse, including amphetamine, cocaine, morphine, and nicotine (Brebner et al., 2005; Fadda et al., 2003). It is likely that baclofen attenuates ethanol-induced locomotor stimulation through a similar mechanism (a hypothesis that we are currently testing using microdialysis). This would likely result in a reduction in stimulation in the absence of heightened ataxia. Another possibility is that baclofen may inhibit the effect of ethanol at $GABA_A$ receptors. In recordings from rat hippocampus, baclofen blocked the ethanol-induced potentiation of $GABA_A$ receptor

function, likely due to a presynaptic effect of $GABA_B$ receptors (Ariwodola and Weiner, 2004). If this occurs in other brain regions, including the mesolimbic dopamine pathway, it is possible that baclofen would attenuate ethanol-induced locomotor stimulation by blocking the effect of ethanol at the $GABA_A$ receptor, thereby reducing the stimulatory effects of ethanol without accentuating ethanol-induced motor incoordination.

Baclofen was able to attenuate the stimulant effect of a very low dose of ethanol (1 g/kg) in FAST-1 but not FAST-2 mice. This result is in contrast to the ability of baclofen to attenuate the stimulant response to a higher, and more stimulatory, dose of ethanol (2 g/kg) in both FAST-1 and FAST-2 mice (Shen et al., 1998). Although the FAST lines were bred in replicate using identical phenotyping procedures, due to their closed breeding populations and the independence of the two replicate lines, it is unlikely that they are genetically identical, either with regard to genes involved in the selection phenotype or background genes. In fact, this is evidenced by the stronger stimulant response of FAST-2 compared to FAST-1 mice (see Fig. 4), and the fact that FAST-1 mice responded more strongly to reverse selection (Phillips et al., 2002). This may suggest that additional mechanisms, absent in FAST-1 mice, influence the ethanol stimulant response in FAST-2 mice, mechanisms that may be under less control by $GABA_B$ receptors. This mechanism could be more exclusively involved at lower ethanol doses in this line. However, it is clear from the data in FAST-1 mice that the attenuation of the stimulant response to ethanol can occur in the absence of an accentuation of ataxia, an effect dramatically different from that seen with $GABA_A$ receptor agonists.

While accentuation of motor incoordination and sedation by baclofen was not seen in the current studies, previous studies have reported this. For example, high doses

of baclofen potentiated ethanol-induced sedation in C57BL/6J mice (Besheer et al., 2004), and baclofen accentuated ethanol-induced motor incoordination both on the rotarod (Dar, 1996) and on a bar-holding task (Martz et al., 1983). It is possible that our results differ from others due to differences in genotype, dose, and procedure. However, enhancement of ethanol-induced sedation has typically been found with higher baclofen doses than those that were used here (e.g., 10 mg/kg or higher), doses that produce sedative effects on their own (Besheer et al., 2004). In addition, baclofen accentuated ethanol-induced ataxia using a fixed speed rotarod procedure set at 24 RPM (Dar, 1996), a considerably more difficult task than the 3 RPM requirement in the current study. While it is clear that motor incoordination and sedation are effects of $GABA_B$ receptor activation, our results suggest that the severity of these effects, at doses that attenuate ethanol's stimulant effects, is dramatically less than that of $GABA_A$ receptor agonists.

In summary, activation of GABA systems attenuates acute behavioral sensitivity to ethanol; however, $GABA_B$ receptor specific drugs may produce fewer unwanted side effects than $GABA_A$ receptor specific drugs when given in combination with ethanol. Drugs that activate $GABA_A$ receptors, either directly or indirectly, act additively with ethanol and shift the behavioral response to ethanol towards greater intoxication, whereas drugs that activate $GABA_B$ receptors do not appear to enhance ethanol intoxication. These results have significant implications in the consideration of potential pharmacotherapies for alcohol use disorders. Reductions in ethanol consumption, such as seen with the GAT1 inhibitor tiagabine and other drugs which directly or indirectly activate $GABA_A$ receptors, may be due to a significant enhancement of ethanol's negative side effects, including motor incoordination and sedation, rather than a reduction

in ethanol reward. Problematically, tolerance may develop to these side effects, resulting in a return to baseline drinking (see Nguyen et al., 2005). $GABA_B$ receptor agonists, at carefully titrated doses that minimize side effects, may hold greater promise as treatments for alcohol use disorders due to their reduced negative interaction effects with ethanol.

CHAPTER 3: Contribution of the $GABA_B$ receptor to acute ethanol sensitivity

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This manuscript is in publication format and will be submitted for publication in the near future.

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Abstract

Background. The γ-aminobutyric acid$_B$ ($GABA_B$) receptor has been implicated in the locomotor stimulant effects of ethanol in mice selectively bred for extreme sensitivity (FAST) or insensitivity (SLOW) to this trait. $GABA_B$ receptor agonists, such as baclofen, attenuate the stimulant effects of ethanol, both when administered peripherally and when administered into the ventral tegmental area. The exact mechanism by which $GABA_B$ receptors contribute to acute ethanol sensitivity, however, is not known.

Methods. The contribution of $GABA_B$ receptor function to acute ethanol sensitivity, measured as baclofen-stimulated [^{35}S]GTPγS binding, was examined in whole brain and regional (cerebellum, hippocampus, prefrontal cortex, striatum, and ventral midbrain) tissue preparations from FAST and SLOW mice. The effect of baclofen on the stimulant response to ethanol was examined by *in vivo* microdialysis to determine whether this behavioral response was associated with a decrease in extracellular dopamine levels in the nucleus accumbens of FAST mice. ***Results.*** A line difference in $GABA_B$ receptor function was found in the ventral midbrain, with a significant increase in receptor function (agonist potency) in SLOW mice compared to FAST mice. Conversely, $GABA_B$ receptor function, measured as the maximal response to baclofen, was higher in FAST mice in the striatum compared to SLOW mice. Activation of $GABA_B$ receptors by baclofen did attenuate the locomotor stimulant response to ethanol in FAST mice, but did not alter ethanol-induced elevations in extracellular dopamine levels in the NAc.

Conclusions. $GABA_B$ receptors appear to play an integral role in acute ethanol sensitivity, as selection for extreme sensitivity and insensitivity to the locomotor stimulant effects of ethanol resulted in altered $GABA_B$ receptor function in the striatum

and ventral midbrain, two areas hypothesized to be critical to the motor effects of ethanol. However, the attenuation of ethanol-induced locomotor stimulation by the $GABA_B$ receptor agonist baclofen in FAST mice appears to not be associated with decreases in dopamine signaling to the NAc.

Introduction

$GABA_B$ receptor agonists have been proposed as a potential pharmacotherapy for alcohol use disorders, and preliminary studies have reported a reduction in ethanol consumption and an increase in abstinence in alcohol dependent individuals when treated with the $GABA_B$ receptor agonist baclofen (Addolorato et al., 2000, 2002; Flannery et al. 2004; Miller, 2008). Preclinical studies support this result, with baclofen reducing ethanol consumption and seeking (Besheer et al., 2004; Colombo et al., 2000, 2002, 2006; Janak and Gill, 2003; Maccioni et al., 2005; but see Moore et al., 2007; Smith et al., 1999), and the behavioral stimulant effects of ethanol in rodents (Boehm et al., 2002a; Chester and Cunningham, 1999a; Cott et al., 1976; Humeniuk et al., 1993; Shen et al., 1998). This attenuation of ethanol-induced locomotor stimulation appears to be due to a specific inhibition of a neurochemical effect of ethanol, as baclofen, unlike other GABA mimetic drugs, did not potentiate the motor incoordinating effects of ethanol at doses that attenuated stimulation (Holstein et al., 2008). Therefore, $GABA_B$ receptors may be a promising target for the pharmacological treatment of alcohol use disorders, as activation of this system decreases the stimulant, and possibly rewarding, effects of ethanol without shifting the behavioral response to ethanol towards greater intoxication.

Sensitivity to the stimulant effects of ethanol can be simply measured, and appears to be related to both a history of increased ethanol consumption (Holdstock et al., 2000; King et al., 2002), as well as a family history of alcohol use disorders in humans (Newlin and Thomson, 1991, 1999). The FAST and SLOW mouse lines, which were selectively bred for extreme sensitivity (FAST) and insensitivity (SLOW) to this

stimulant effect of ethanol, are a unique animal model of acute ethanol sensitivity which can be used to assess the genetic and neurochemical underpinnings of the stimulant response to ethanol (Crabbe et al., 1987; Phillips et al., 1991b). These lines also differ in sensitivity to the motor incoordinating and sedative effects of ethanol (FAST mice are less sensitive), as well as in ethanol consumption (FAST mice consume approximately 4-5 g/kg/day more ethanol than SLOW mice) (Phillips et al., 2002b; Risinger et al., 1994; Shen et al., 1996); therefore, these lines are unique in that they model the high stimulant sensitivity, low sedative sensitivity and higher drinking reminiscent of individuals who are prone to alcoholism.

One mechanism by which baclofen is proposed to attenuate the stimulant response to ethanol is by reducing the stimulatory actions of ethanol on mesolimbic dopamine neurons. Ethanol has repeatedly been found to increase both dopamine cell firing in the ventral tegmental area (VTA) (Appel et al. 2003; Brodie and Appel 1998; Brodie et al. 1990, 1999; Gessa et al., 1985; Okamoto et al., 2006) and extracellular levels of dopamine in the nucleus accumbens (NAc), a terminal field region of the mesolimbic pathway (Di Chiara and Imperato, 1988; Howard et al., 2008; Imperato and Di Chiara, 1986; Job et al., 2007; Larsson et al., 2002; Szumlinski et al., 2007; Yim et al., 1998; Yoshimoto et al., 1991; Zocchi et al., 2003). Further, ethanol-induced increases in dopamine function have been correlated with acute ethanol sensitivity in some studies (Brodie and Appel, 2000; Imperato and Di Chiara, 1986; Kapasova et al., 2008), including recent results from our laboratory which showed increased dopamine cell firing and extracellular dopamine levels in the NAc in response to ethanol in FAST mice relative to SLOW mice (Beckstead et al., unpublished results; Meyer et al., submitted).

Therefore, evidence supports a role of the mesolimbic dopamine pathway in the locomotor stimulant response to ethanol. Baclofen, conversely, decreased dopamine cell firing in the VTA and decreased extracellular levels of dopamine in the NAc, both basally and in response to amphetamine, cocaine, morphine, and nicotine (Brebner et al., 2005; Chen et al. 2005; Cruz et al., 2004; Erhardt et al., 2002; Fadda et al., 2003; Lacey et al., 1988; Olpe et al., 1977; Westerink et al. 1996). The effect of baclofen on ethanol-induced increases in extracellular dopamine levels in the NAc has not been examined. Combined with evidence that $GABA_B$ receptors are predominantly localized to dopamine neurons in the ventral midbrain (substantia nigra, or SN, and VTA; Wirtshafter and Sheppard, 2001), and that intra-anterior VTA (aVTA) administration of baclofen attenuated the locomotor stimulant response to ethanol in FAST mice (Boehm et al., 2002a), these results support the hypothesis that activation of $GABA_B$ receptors by baclofen inhibits ethanol-induced increases in dopamine signaling, thereby reducing the locomotor stimulant response to ethanol.

The purpose of the current studies was to further examine the contribution of $GABA_B$ receptors, and their interaction with mesolimbic dopamine systems, to acute ethanol sensitivity. The first series of experiments examined whether selection for extreme sensitivity and insensitivity to the stimulant effects of ethanol in the FAST and SLOW mouse lines had altered $GABA_B$ receptor function, a result which would support the contribution of $GABA_B$ receptors to the manifestation of differences in acute ethanol sensitivity. Using baclofen-stimulated guanosine 5′-*O*-(3-[^{35}S]thiotriphosphate) ([^{35}S]GTPγS) binding (a measure of agonist-stimulated G-protein binding and receptor activation; Harrison and Traynor, 2003), $GABA_B$ receptor function in whole brain and

regional tissue preparations was determined. Due to the enhanced sedative sensitivity to ethanol and baclofen in SLOW mice (Boehm et al., 2002a; Shen et al., 1998), I hypothesized that $GABA_B$ receptor function would be significantly higher (as measured by a lower EC_{50} for baclofen or higher E_{max}) in SLOW mice, particularly in regions critical for the locomotor stimulant response to ethanol (i.e., striatum, which encompasses the nucleus accumbens and caudate-putamen, and ventral midbrain, which encompasses the VTA and substantia nigra). In order to examine a potential neurochemical substrate underlying the baclofen-induced attenuation of ethanol stimulation in FAST mice, mesolimbic dopamine signaling was examined by *in vivo* microdialysis. I hypothesized that baclofen would attenuate ethanol-induced increases in extracellular dopamine levels in the NAc, corresponding to an attenuation of ethanol-induced locomotor stimulation, which was measured concurrently with dialysate dopamine levels.

Methods

Subjects

The FAST and SLOW selected lines were derived from an 8-way cross of inbred mouse strains (HS/Ibg) (McClearn et al., 1970), but each line and replicate (FAST-1, FAST-2, SLOW-1, SLOW-2) has been independently maintained since the beginning of selection. Briefly these lines were selectively bred for extreme sensitivity (FAST) and insensitivity (SLOW) to the locomotor stimulant effects of 2 g/kg ethanol. Locomotor activity was measured for 4 min in a circular activity monitor (61 cm diameter; LVE model PAC-001, Lehigh Valley, PA), beginning 2 min following an i.p. injection of saline or ethanol. The selection response was indexed as distance traveled after saline

(day 2) subtracted from distance traveled after ethanol (day 1). Subjects with high activity scores were selected as breeders for the FAST line, whereas subjects with low activity scores, including negative scores, were selected as breeders for the SLOW line. After 37 generations of selection, breeding of the FAST and SLOW lines was performed under relaxed selection conditions, with breeders chosen randomly and bred within line and replicate (but avoiding the pairing of animals with common parents or grandparents to minimize inbreeding) (Crabbe et al. 1987, 1988; Phillips et al. 1991, 2002; Shen et al. 1995).

Experimentally naïve FAST and SLOW mice used in these experiments were reared with the dam and sire until 20-22 days of age, when they were isosexually housed in polycarbonate [28 X 18 X 13 cm (l X w X h)] cages, 2-4 per cage, with littermates or with non-littermates of the same genotype and age range. Subjects were maintained on a 12:12-h light-dark cycle (lights on at 0600) at 21 ± 2° C with food (Purina Laboratory Rodent Chow #5001; Purina Mills, St. Louis, MO) and water available ad libitum except during behavioral testing. All procedures were approved by the Portland Veterans Affairs Medical Center Institutional Animal Care and Use Committee and were consistent with the guidelines set forth by the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals* (1986).

Experiment 1: $GABA_B$ receptor function

Drugs and Reagents

[^{35}S]GTPγS (1250 Ci/mmol) was purchased from PerkinElmer (Waltham, MA). (±)-Baclofen and all other reagents were obtained from Sigma-Aldrich (St. Louis, MO).

Baclofen and guanosine 5'-diphosphate (GDP) were prepared fresh in 50 mM Tris-HCl buffer (pH 7.4) on the day of testing.

Membrane Preparation

Methods for membrane preparation and for the [^{35}S]GTPγS binding assay were modified from Odagaki and Yamauchi (2004).

Whole brain. Male FAST and SLOW (replicate 1 and 2) mice, aged 52-67 days and from selection generations $S_{37}G_{85\text{-}87}$ (where S_{xx} refers to the generation of selection and G_{yy} refers to the number of breeding generations that have elapsed since the beginning of selection), were used for this study. Briefly, subjects were sacrificed by cervical dislocation and brain tissue was rapidly removed. Tissue was then homogenized in 5 ml ice-cold Tris-EDTA-Dithiothreitol (TED) buffer (5 mM Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, 10% w/v sucrose, pH 7.4) using a Polytron Homogenizer (Kinematica, Newark, NJ). All centrifugation procedures occurred at 4°C. Extensive tissue washing, as described next, was performed to aid in removing endogenous GABA from the membrane preparation. Tissue homogenates were centrifuged twice at 1000 x g for 10 min and the resulting supernatants were combined and centrifuged for 20 min at 9000 x g. The tissue pellet was washed in 10 ml TED buffer and centrifuged at 18000 x g for 20 min twice. The resultant tissue pellet was resuspended in 10 ml TED buffer and kept on ice for 30 min, after which the suspension was centrifuged at 35000 x g for 10 min. The final tissue pellet was resuspended in 2 ml of 50 mM Tris-HCl buffer (pH 7.4), aliquoted into microcentrifuge tubes and stored at -80°C until use. An additional aliquot was taken for protein quantification using the Pierce BCA (bicinchoninic acid) protein assay (Rockford, IL).

Microdissections. FAST and SLOW (replicate 1 and 2) male mice from selection generations $S_{37}G_{86\text{-}89}$, aged 51-73 days, were used for this study. Subjects were euthanized by cervical dislocation immediately prior to decapitation, and cerebellum, hippocampus, prefrontal cortex, striatum, and/or ventral midbrain were then rapidly dissected. All dissections were done on an ice-cold platform and stored in ice-cold tubes until homogenized. The cerebellum was isolated from the cerebrum and brain stem. The prefrontal cortex was isolated by placing the brain ventral side up, removing the olfactory bulbs, and isolating approximately the first 1 mm of brain tissue (a coronal slice was made approximately +1.75 mm anterior to bregma). Prefrontal cortex tissue from 2 mice (of the same line and replicate) was pooled for the membrane preparation to provide enough protein for analysis. For striatal tissue, the brain was placed dorsal side up and two coronal slices were made, one at approximately +1.75 mm anterior to bregma and a second at approximately +0.25 mm anterior to bregma. The resultant tissue slice was placed on the platform with the anterior section up and the striatum was visualized and isolated from surrounding cortical tissue. For hippocampal microdissections, the cerebral cortices were peeled away to visualize the hippocampi, which were then dissected out. Ventral midbrain tissue was isolated by peeling away the cerebral cortices and creating two coronal slices, one at -3.25 mm posterior to bregma and a second -4.25 mm posterior to bregma. The resultant tissue slice was placed on the platform, and a horizontal slice was made at the periaqueductal gray. The ventral slice was saved and the cerebral peduncles were removed from the bottom of the slice to isolate the ventral midbrain. Ventral midbrain tissue from 3 mice (of the same line and replicate) was pooled for the membrane preparation to provide enough protein for analysis.

Tissue was homogenized in 1 ml (cerebellum) or 500 µl (remaining brain regions) of ice-cold TED buffer. The methods for membrane preparation matched those used for whole brain, except that a volume of 500 µl – 1 ml TED buffer was used for the initial centrifugation steps (at 1000 x g), followed by 1 ml TED buffer for all remaining steps. The final tissue pellet was resuspended in 50 mM Tris-HCl buffer; the volume of Tris-HCl buffer added was approximately 9 µl/mg wet tissue weight. An aliquot was taken for protein quantification, and the remaining samples were stored at -80°C until use.

In an additional study, the effect of *in vivo* ethanol pre-exposure was examined on $GABA_B$ receptor function. FAST and SLOW (replicate 1 and 2) male mice, aged 53-69 days and from selection generations $S_{37}G_{88\text{-}89}$, were administered 2 g/kg ethanol (i.p.) or an equivalent volume of saline 15 min prior to cervical dislocation. Cerebellar tissue was rapidly dissected on an ice-cold plate and tissue homogenates were prepared as described above. This region was selected for analysis of a potential ethanol effect due to its high density of $GABA_B$ receptors (Chu et al., 1990) and contribution to ethanol sensitivity (Dar, 1995, 1996; Palmer et al., 1984; Seiger et al., 1983).

[^{35}S]GTPγS Binding

Thawed membranes (approximately 10 µg for regional preparations, 20 µg for whole brain preparations, diluted in 50 mM Tris-HCl, pH 7.4) were incubated for 60 min at 30°C in 500 µl of 50 mM Tris-HCl (pH 7.4) containing 5 mM $MgCl_2$, 100 mM NaCl, 0.1 mM EDTA, 0.2 mM EGTA, 0.2 mM dithiothreitol, 20 µM GDP, 0.2 nM [^{35}S]GTPγS and 0.32 µM – 1 mM baclofen. For an analysis of the effect of *in vitro* ethanol exposure on $GABA_B$ receptor function, cerebellar homogenates (10 µg) were incubated under similar conditions, except an approximate EC_{50} dose of baclofen (70 µM) was used and a

range of ethanol concentrations (1 – 316 mM) was included in the assay buffer. Ethanol was added to the tissue first and allowed to incubate for approximately 10 min before the assay buffer was added to facilitate an ethanol effect on $GABA_B$ receptor function prior to [^{35}S]GTPγS binding. All reactions were run in triplicate. The reaction was terminated by rapid filtration through a glass fiber filter (GF/B; PerkinElmer, Waltham, MA) using a 96-well Tomtec cell harvester (Hamden, CT) and washed with ice-cold 50 mM Tris-HCl buffer (pH 7.4). Filters were then allowed to dry and spotted with scintillation fluid (50 μl per side). Bound radioactivity was measured using a Wallac BetaPlate 1205 scintillation counter (Gaithersburg, MD). Non-specific binding was measured in the presence of 100 μM unlabeled GTPγS, with and without 1 mM baclofen. These values did not consistently differ, and therefore non-specific binding in the presence of 100 μM unlabeled GTPγS only was used as the measure of non-specific binding. These values were then subtracted from total binding to define specific [^{35}S]GTPγS binding.

Experiment 2: Microdialysis

Drugs and Reagents

Anesthetic cocktail (1.4 mg/kg acepromazine, 71.4 mg/kg ketamine, and 7.1 mg/kg xylazine in 0.9% physiological saline, Baxter Healthcare Corporation, Deerfield, IL) was obtained from the Portland VA Medical Center research pharmacy. (±)-Baclofen was obtained from Sigma (St. Louis, MO), dissolved in saline on the day of testing, and injected i.p. at a volume of 10 ml/kg. Ethanol was obtained from Pharmco Products (Brookfield, CT) and was diluted from a 100% stock to a 20% v/v solution in saline on the day of testing and injected i.p. All other reagents were obtained from Sigma (St. Louis, MO).

Surgery

Male FAST-1 and FAST-2 mice, aged 49-71 days at the time of surgery and from selection generations $S_{37}G_{89\text{-}94}$ were used in this experiment. Stereotaxic surgery was performed on approximately two-thirds of the subjects using a Cartesion Research Inc. (Sandy, OR) stereotaxic apparatus, while the remaining one-third of surgeries was performed on a David Kopf Instruments (Tujunga, CA) stereotaxic alignment apparatus. On the day of surgery, FAST mice were anesthetized using anesthetic cocktail. The calculation used for anesthetic dosing was:

$$\text{injection volume (ml)} = [(\text{body weight (g)} / 100) - 0.08] * 2$$

Once anesthetized, the mouse was ear-punched for identification and a small area of the dorsal scalp was shaved and a midline incision was made. The exposed cranial surface was cleaned with an ethanol (100%)-soaked cotton swab. Once secured in the stereotaxic apparatus, a magnifying scope (40X) was used to localize bregma, lambda, and the central suture, and using an Anilam micro manipulator (1 μm resolution) with digital display (Jamestown, NY), the distance between bregma and lambda was measured. This distance was then divided by 4.21 mm (the published distance between bregma and lambda for the C57BL/6J mouse in the mouse brain atlas by Paxinos and Franklin, 2001) to account for the size of the individual skull. This adjustment factor was multiplied by each of the stereotactic coordinates to calculate a set of modified coordinates for each subject. One hole was then drilled through the cranium above the nucleus accumbens of the left hemisphere; unilateral placements in the left hemisphere were chosen for consistency with a previous microdialysis experiment in the FAST and SLOW selected lines (Meyer et al., submitted). In initial surgeries, a more lateral NAc placement was

used, with the following coordinates relative to bregma: +1.2 mm anterior, 1.5 mm lateral, 3.4 mm ventral. A more medial placement was later used to better isolate the probe location to medial NAc core and shell: +1.4 mm anterior, 0.6-0.8 mm lateral, 3.8 mm ventral relative to bregma. This change in coordinates was made due to improved basal dopamine levels and a heightened response to ethanol when the probe was localized to more medial aspects of the NAc. A CMA/7 guide cannula, with a shaft length of 7 mm and an approximate outer diameter of 0.38 mm, was implanted 1 mm dorsal to the NAc (CMA/7, CMA Microdialysis, Stockholm, Sweden). A stainless steel obdurator was inserted into the guide cannula to prevent clogging. A second hole was drilled and enlarged above the right hemisphere, midway between bregma and lambda, for the placement of an anchor screw (1/8 inch; Small Parts, Miami Lakes, FL). The guide cannula and anchor screw were secured in place with durelon carboxylate cement (3M, St. Paul, MN), and mice were placed in a heated standard rat cage to allow for recovery from anesthesia. Subjects were returned to the colony room in rat cages with food and water available *ad libitum*; mice were group housed with their original cage mates until the time of testing. A minimum of 4 days elapsed after surgery before the microdialysis probe was implanted.

Microdialysis

Apparatus. Two locomotor activity detection monitors measuring 40 X 40 X 30 cm (l X w X h) (AccuScan Instruments, Inc., Columbus, OH) were each housed in light-proof, sound-attenuating cabinets (Flair Plastics, Portland, OR). Each cabinet was illuminated by an 8-W fluorescent white light and a fan was mounted on the inside back wall, providing ventilation and background noise. Movement within the monitor was

recorded by two sets of eight infrared beams mounted 2 cm above the test chamber floor at right angles to one another, with detectors mounted on the opposite sides. Beam interruptions were automatically recorded and translated to horizontal distance traveled (in cm) by AccuScan software.

Procedure. The evening before testing began (between 1500 and 1800 h), two FAST mice were moved from the colony room to the testing room, weighed, and administered a sub-anesthetic dose of the anesthetic cocktail. The injection volume was calculated with the following formula:

injection volume (ml) = 0.0075 ml/g * body weight (g)

Once lightly anesthetized, obdurators were removed and a CMA/7 microdialysis probe, with a shaft length of 7 mm, 1 mm exposed cuprophane membrane, 0.24 mm outer diameter, and 6 kDa cut-off (CMA Microdialysis, Stockholm, Sweden), was inserted into the guide cannula. While anesthetized, a tethering post (Instech Laboratories, Plymouth Meeting, PA) was cemented to the head mount with dental acrylic. Once dry, mice were tethered to a dual-channel microdialysis swivel (Instech), with a wire from the swivel attached to the tethering post. The swivel was attached to a counterbalanced lever arm (Instech) mounted inside the locomotor activity monitor, allowing free movement of the swivel and the mouse around the activity monitor. The inlet and outlet channels of the swivel were connected to the tubing of the microdialysis probe by FEP tubing (0.12 mm inner diameter, CMA Microdialysis). Once connected, each subject was placed in a rat cage with food and water available; the rat cage was placed inside the activity monitor. Artificial cerebral spinal fluid (aCSF), consisting of 145 mM NaCl, 2.8 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 5.4 mM D-glucose, was then pumped through the tubing and

microdialysis probe via a 2.5 ml glass syringe and a syringe pump (CMA Microdialysis) at a rate of 2.0 µl / min. The tubing output was connected to an automated fraction collector (CMA Microdialysis) and dialysate was collected overnight, though only the last two samples collected before testing began were analyzed. An automated light source maintained lighting conditions similar to those of the colony room, with lights off between 1800 and 0600 h.

The following morning (approximately 12-18 h following probe implantation), the rat cage, along with food and water sources, were removed and subjects were placed directly into the locomotor activity monitoring chambers. Locomotor activity and dialysate samples were then collected in 15 min time bins. Dialysate samples were collected in 0.4 ml glass microvials (Agilent Technologies, Palo Alto, CA) that contained 2 µl of a concentrated antioxidant (20 mM oxalic acid, 2 M glacial acetic acid) to prevent the spontaneous oxidation of dopamine. After one hour of basal activity testing, and thus 4 basal dialysate samples, each mouse was removed from the activity monitor and injected, i.p., with saline. Upon return to the activity monitor, an additional hour of testing occurred, and thus 4 post-saline dialysate samples were collected, after which each mouse was again removed from the activity monitor and injected with saline or one of two doses of baclofen (1.25 or 2.5 mg/kg, i.p.). One 15-min activity and dialysate sample was collected after baclofen administration; each mouse was then removed from the activity monitor and injected i.p. with saline or 2 g/kg ethanol and immediately returned to activity monitoring. Baclofen doses and the 15 min pretreatment interval were chosen from a previous study that showed a dose-dependent reduction in ethanol-stimulated activity with these doses in FAST mice (Shen et al., 1998). A 2 g/kg ethanol

dose was chosen as this was the dose at which the majority of the selection of the FAST and SLOW lines occurred (Crabbe et al., 1988; Phillips et al., 1991b). Four activity and dialysate samples (one hour) were collected immediately after ethanol administration. At the conclusion of the ethanol fraction collections, a high potassium aCSF (50 mM KCl, 98 mM NaCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 5.4 mM glucose) was infused through the probe for 15 min. Normal aCSF was then infused through for another 45 min, at which point the experiment was concluded. For approximately two-thirds of the subjects tested, a small amount of methylene blue (1-2 μl) was infused through the probe prior to sacrifice to aid in probe identification. For the remaining subjects, no dye was infused to allow for re-use of the microdialysis probe; the probe placement was identified by an easily observable probe tract. Subjects were euthanized by cervical dislocation and brain tissue was rapidly removed, flash frozen in ice-cold isopentane, and stored at -80°C. Dialysate samples were stored on dry ice or at -80°C until analyzed.

High Performance Liquid Chromatography (HPLC)

Dialysate concentrations of dopamine were measured by HPLC coupled with electrochemical detection. Briefly, mobile phase (10% acetonitrile, 90 mM NaH_2PO_4, 50 mM citric acid, 1.7 – 2.1 mM octanesulfonic acid, 50 μM EDTA, pH 5.6) was pumped at a rate of 0.35-0.5 ml/min using an ESA 582 isocratic solvent delivery system (ESA Inc., North Chelmsford, MA). Samples (20 μl) were separated using a C18 column (ESA model MD-150, 3 mm inner diameter, 150 mm long, 3 μm particle size) using an ESA 542 autosampler. The column temperature was maintained at 28°C, while the autosampler was refrigerated to 4-6°C. Electrochemical detection of dopamine was conducted using an ESA electrochemical cell (5014B, ESA Inc.), with a reducing

electrode (E1 -100 mV) and an oxidizing electrode (E2 +200 mV). Dialysate concentrations were analyzed by peak area using ESA Coularray for Windows software, and calculated from an external standard curve prepared at the time of analysis (0.15 – 8 nM, or 0.6-30.3 pg/20 µl, dopamine), prepared at the time of analysis. The sensitivity for dopamine was in the femtomole range.

Histology

Each brain was sectioned into 40 µm coronal slices using a cryostat (Leica CM1850; Nussloch, Germany), and thaw mounted on Superfrost Plus slides (VWR, West Chester, PA). Slices were imaged before and after thionin staining using a Leica DMLB light microscope (Model LB30T, Wetzlar, Germany) connected to a SPOT Insight digital camera and software (Diagnostic Instruments, Sterling Heights, MI). Only subjects where the majority of the microdialysis probe was located within the boundaries of the NAc were included in the analysis.

Data Analysis

Percent stimulation of basal [^{35}S]GTPγS binding by baclofen was calculated by nonlinear regression analysis using GraphPad PRISM software (GraphPad 5, San Diego, CA). The concentration of baclofen eliciting a half-maximal stimulation of [^{35}S]GTPγS binding (EC_{50}) and the maximal stimulation of [^{35}S]GTPγS by baclofen (E_{max}) were then calculated for each subject. These values were first analyzed by two-way analysis of variance (ANOVA) for line and replicate effects using Statistica software (StatSoft, Inc., Tulsa, OK); if no interaction with replicate was found, the results were analyzed by one-way ANOVA for line differences. For the *in vivo* ethanol experiment, data were first analyzed by ANOVA for line, replicate, and ethanol dose effects. Again, data were

combined for the two replicates in the absence of interactions with replicate. Significant two-way interactions were analyzed by simple main effect analyses and Newman-Keuls mean comparisons were used to identify group differences as appropriate.

For the microdialysis experiments, locomotor activity data were indexed as distance traveled in cm per 15 min time bin. Data were first analyzed by repeated measures ANOVA (replicate X baclofen dose X ethanol dose X time). Individual ANOVAs were also performed on data for the first and last first 30 min time period after ethanol administration to determine the effect of baclofen on the early and late stimulant response to ethanol. Dialysate dopamine levels were expressed as either percentage of the average saline or baclofen response and analyzed first by repeated measures ANOVA (replicate X baclofen dose X ethanol dose X time) and then by individual ANOVAs on data for the first and last 30 min time period after ethanol administration. For all analyses, interactions were followed up by simple main effect analyses and Newman-Keuls mean comparisons. Significance levels were set *a priori* at $\alpha \leq 0.05$.

Results

Experiment 1. $GABA_B$ receptor function

Basal binding. Basal levels of [^{35}S]GTPγS binding did not significantly differ between the FAST and SLOW lines in whole brain, cerebellum, hippocampus, prefrontal cortex, or ventral midbrain (data not shown). In the striatum, however, [^{35}S]GTPγS binding in the absence of agonist was significantly higher in SLOW mice compared to FAST mice [$F_{1,27}$=8.5; p<0.01]; basal binding was approximately 18% higher in SLOW mice. Preliminary experiments in whole brain tissue demonstrated that baclofen-induced

increases in [^{35}S]GTPγS binding above baseline were specific to the $GABA_B$ receptor, as inclusion of a $GABA_B$ receptor specific antagonist in the incubation buffer (CGP-54626) reduced baclofen-stimulated [^{35}S]GTPγS binding to baseline levels (data not shown).

As summarized in Table 3 , there was a line difference in the potency of baclofen (EC_{50}) between FAST and SLOW mice in the ventral midbrain [$F_{1,44}$=7.0; p<0.05]. The EC_{50} for baclofen was significantly lower in SLOW mice compared to FAST mice, reflecting heightened $GABA_B$ receptor function in the ventral midbrain of SLOW mice (see Fig 10a). While there was no line difference in the EC_{50} for baclofen in striatal homogenates, there was a line difference in percent stimulation of basal [^{35}S]GTPγS binding with baclofen (E_{max}) in this region, with baseline set at 100% [$F_{1,27}$=4.7; p<0.05] (see Fig 10b). The E_{max} for striatal tissue from FAST mice was higher than that for SLOW mice. There were no interactions of line and replicate, and thus the data were analyzed collapsed on replicate for all regions and for whole brain. The lines did not differ in receptor function (EC_{50} or E_{max}) in any other region or in whole brain.

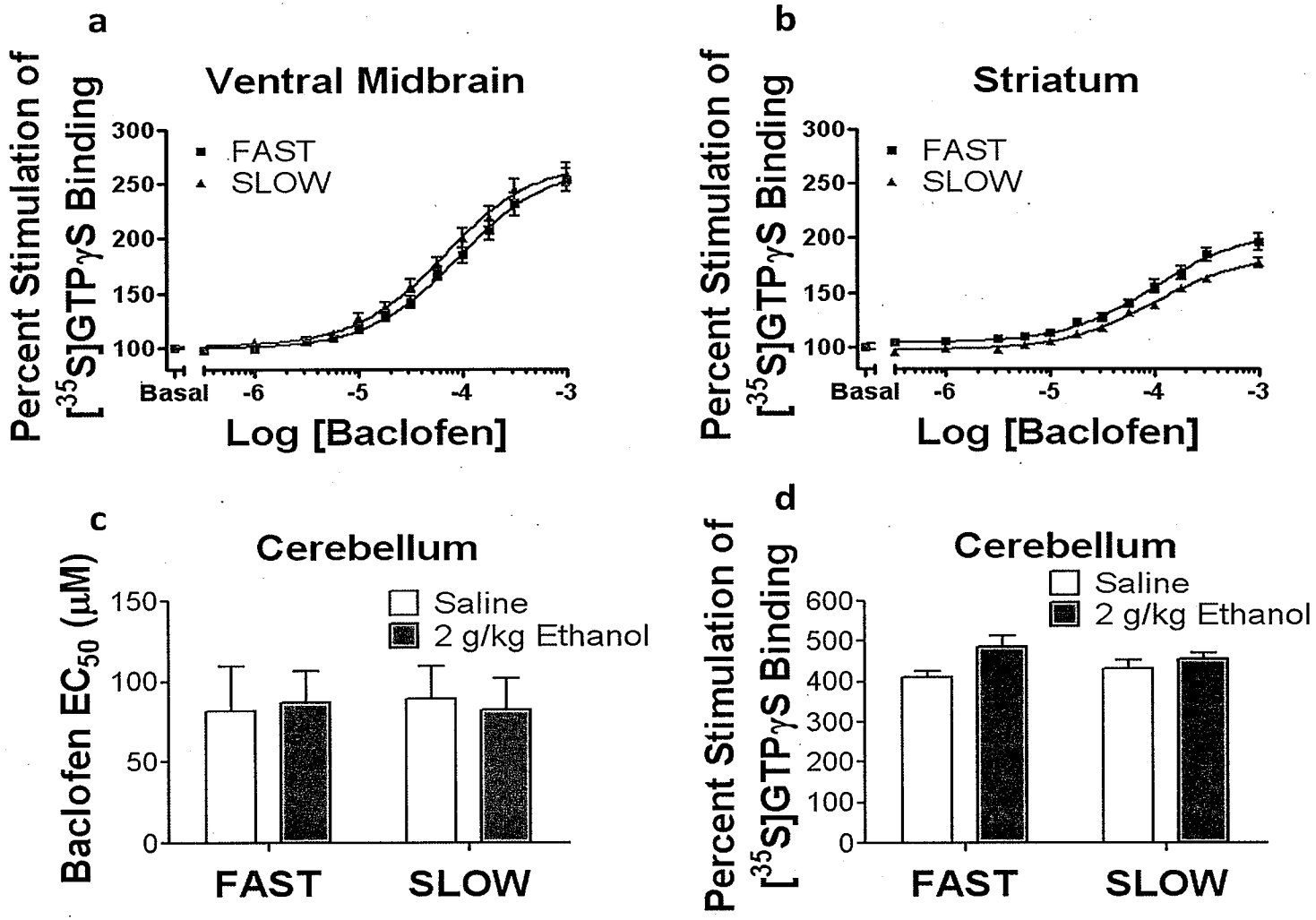
Effect of Ethanol Exposure on $GABA_B$ Receptor Function. As shown in Fig 10 (c and d), there was no effect of *in vivo* ethanol exposure on the EC_{50} for baclofen in the cerebellum, but ethanol did increase E_{max} in this region. However, the FAST and SLOW lines did not differ in this response. The cerebellum was selected for analysis due to its high density of $GABA_B$ receptors and involvement in the motor effects of ethanol (Chu et al., 1990; Dar, 1995, 1996; Palmer et al., 1984; Seiger et al., 1983). Moreover, baclofen was most potent and efficacious in the cerebellum in both FAST and SLOW mice (see Table 3). The initial line X replicate X ethanol dose (0 or 2 g/kg) ANOVA for the EC_{50} for baclofen revealed no main or interaction effects. In contrast to the EC_{50} for baclofen,

TABLE 3. $GABA_B$ receptor function, as measured by baclofen-stimulated [^{35}S]GTPγS binding, in FAST and SLOW mice.

	EC_{50}[a]		E_{max}[b]	
Region	**FAST**	**SLOW**	**FAST**	**SLOW**
Whole Brain	66.7 μM (59.2-75.2 μM)	66.2 μM (59.8-73.2 μM)	259.3 ± 5.9%	260.9 ± 6.5%
Cerebellum	67.8 μM (62.5-73.5 μM)	64.8 μM (60.0-70.1 μM)	458.6 ± 14.3%	439.9 ± 15.4%
Hippocampus	77.2 μM (70.2-84.8 μM)	74.1 μM (65.3-84.1 μM)	348.4 ± 8.9%	340.7 ± 17.4%
Prefrontal Cortex	74.4 μM (65.3-84.8 μM)	72.5 μM (63.3-83.1 μM)	375.2 ± 16.1%	394.6 ± 11.1%
Striatum	96.9 μM (81.1-115.9 μM)	95.7 μM (80.9-113.4 μM)	205.7 ± 8.4% *	184.7 ± 4.4%
Ventral Midbrain	92.7 μM (80.3-107.0 μM) *	70.4 μM (60.0-82.6 μM)	266.6 ± 10.8%	270.3 ± 10.6%

[a] Mean (95% confidence interval) EC_{50} for baclofen
[b] Mean ± S.E.M. maximal percent stimulation of [^{35}S]GTPγS binding above baseline (100%)
n's: Whole Brain = 20 / line, Cerebellum = 26 / line, Hippocampus = 15-16 / line, Prefrontal Cortex = 16 / line, Striatum = 14-15 / line, Ventral Midbrain = 23 / line
* Significantly higher EC_{50} / E_{max} in FAST mice relative to SLOW mice ($p<0.05$)

FIGURE 10. $GABA_B$ receptor function, as measured by baclofen-stimulated [^{35}S]GTPγS binding, is altered in specific brain regions in FAST and SLOW mice. (a) Baclofen dose-response in ventral midbrain tissue; the EC_{50} for baclofen, a measure of agonist potency, was significantly lower in SLOW mice compared to FAST mice in this brain region. Data shown are mean ± SEM; n = 23 / line. Average ± SEM counts per minute (CPM) for [^{35}S]GTPγS in ventral midbrain was 4630 ± 248.8 CPM (basal), 10,367.7 ± 601.2 CPM (maximum binding in the presence of baclofen), and 482.4 ± 25.9 CPM (non-specific; 100 μM unlabeled GTPγS). (b) Baclofen dose-response in striatum. Whereas there was no line difference in baclofen EC_{50} in the striatum, E_{max}, or the maximum percent stimulation of [^{35}S]GTPγS binding by baclofen (expressed as a percentage of baseline), was significantly higher in FAST mice in the striatum compared to SLOW mice. Data shown are mean ± SEM; n = 14-15 / line. Average ± SEM counts per minute (CPM) for [^{35}S]GTPγS in striatum was 7196.1 ± 232.6 (basal), 12,875.3 ± 341.0 (maximum binding in the presence of baclofen), and 422.4 ± 28.1 (non-specific; 100 μM unlabeled GTPγS). (c) Pretreatment of the FAST and SLOW lines 15 min prior to sacrifice with saline or 2 g/kg ethanol (i.p.) did not alter the EC_{50} for baclofen in either FAST or SLOW mice. Data presented are mean and the 95% confidence interval; n = 12 / line (data are collapsed across replicate). (d) Overall, ethanol pretreatment did increase E_{max} ($p<0.05$), but this effect did not interact with line. Data shown are mean ± SEM; n = 12 / line (data are collapsed across replicate).


a
Ventral Midbrain
Percent Stimulation of [35S]GTPγS Binding
300
250
200
150
100
FAST
SLOW
Basal
-6
-5
-4
-3
Log [Baclofen]
b
Striatum
Percent Stimulation of [35S]GTPγS Binding
300
250
200
150
100
FAST
SLOW
Basal
-6
-5
-4
-3
Log [Baclofen]
c
Cerebellum
Baclofen EC50 (μM)
150
100
50
0
Saline
2 g/kg Ethanol
FAST
SLOW
d
Cerebellum
Percent Stimulation of [35S]GTPγS Binding
600
500
400
300
200
100
0
Saline
2 g/kg Ethanol
FAST
SLOW

there was a main effect of ethanol dose on E_{max} [$F_{1,44}$=5.6; p<0.05], with a significantly higher E_{max} in ethanol-treated tissue as compared to saline-treated tissue. However, this effect of ethanol did not vary significantly between the lines.

Unlike the effects of ethanol pretreatment on $GABA_B$ receptor function, inclusion of ethanol (1-316 mM) in the binding assay had only minor effects on baclofen-stimulated [^{35}S]GTPγS binding (at an approximate EC_{50} concentration of baclofen, 70 μM), and only at an extremely high ethanol concentration. There was no significant interaction of line and replicate, and thus the replicate lines were combined for further analysis. This analysis revealed a main effect of line [$F_{1,106}$=14.4; p<0.001] and ethanol dose [$F_{10,106}$=3.0; p<0.01], but no line X ethanol dose interaction. Briefly, [^{35}S]GTPγS binding was higher overall in FAST mice compared to SLOW mice. In addition, ethanol decreased baclofen-stimulated [^{35}S]GTPγS binding (measured as CPM), but only at the highest dose (316 mM), reducing binding by approximately 21% (data not shown). However, this effect did not vary between the FAST and SLOW lines.

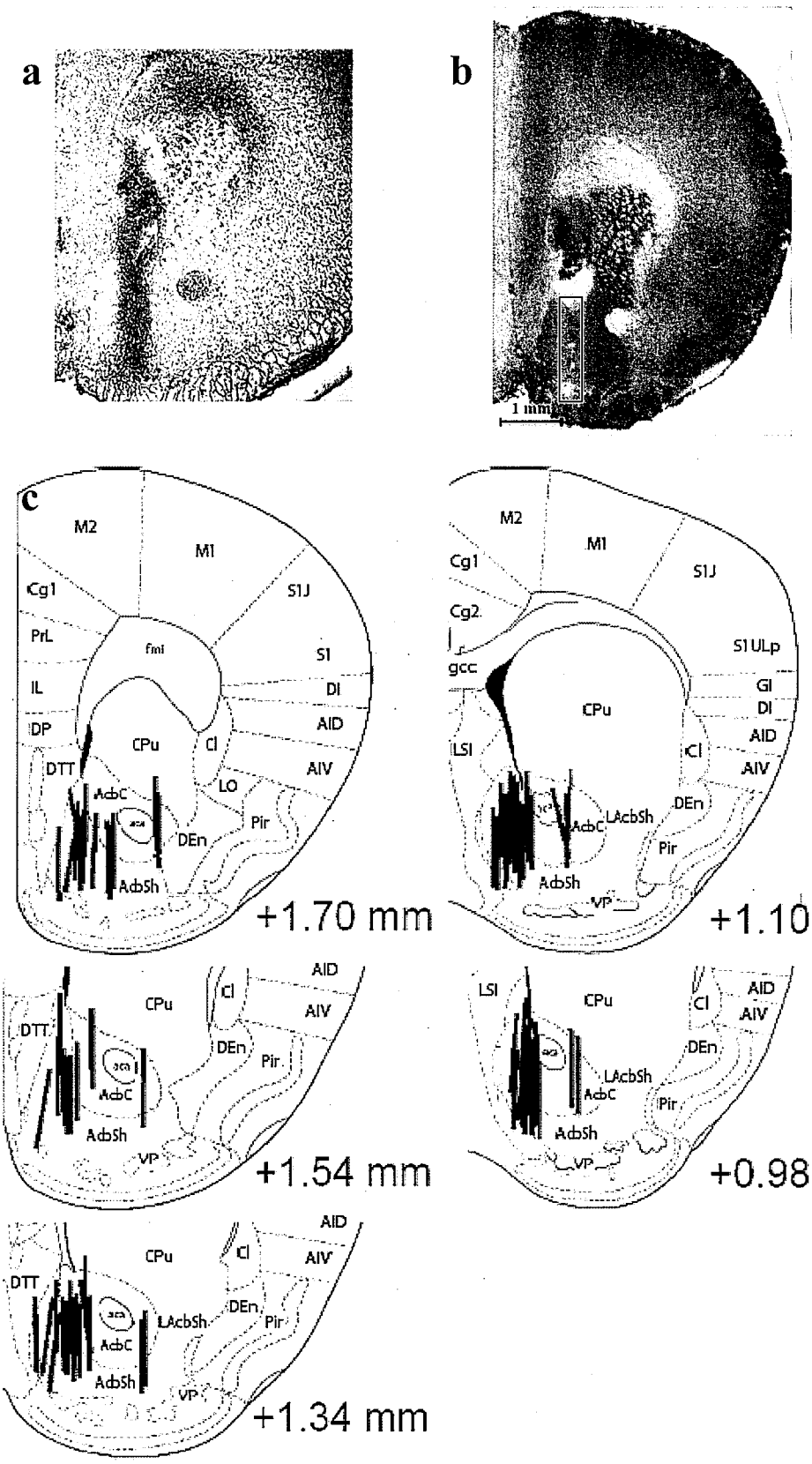
Experiment 2: Microdialysis

Histology. Representative photomicrographs and a summary of microdialysis probe placements are shown in Fig 11. Only animals in which the majority of the probe was located in the NAc were included in the data analysis. Most probe placements were in the anteromedial NAc, corresponding to the medial NAc shell or the interface of the NAc medial shell and core.

Locomotor Activity. As shown in Fig 12, baclofen dose-dependently attenuated the locomotor stimulant response to ethanol in FAST mice. When data from the replicate lines were combined, there was a significant interaction of baclofen dose and ethanol

FIGURE 11. Histology.

Shown are representative photomicrographs of unstained (a) tissue showing the location of the microdialysis probe in the NAc as marked by methylene blue infusion and thionin stained (b) tissue, with the microdialysis probe track highlighted. A diagram of microdialysis probe placements (with only the active membrane represented) is shown in (c).


a
b
1 mm
c
M2
M1
Cg1
S1J
PrL
fmi
S1
IL
DI
DP
CPu
Cl
AID
DTT
AcbC
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AIV
aca
DEn
Pir
AcbSh
+1.70 mm
M2
M1
Cg1
S1J
Cg2
S1ULp
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GI
DI
AID
LSI
Cl
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+0.98 mm
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AIV
DTT
LAcbSh
DEn
Pir
aca
AcbC
AcbSh
VP
+1.34 mm

dose with time [$F_{32,1184}$=1.8; p<0.01]. Follow-up analyses revealed that there was only a baclofen dose X ethanol dose interaction within the 1-hr period after ethanol administration. Therefore the data were analyzed for both the first 30-min time period immediately after ethanol administration, a time when the stimulant response to ethanol is most pronounced (Shen et al., 1995), and for the second 30-min time period after ethanol administration.

In the first 30-min after ethanol administration, there was a main effect of baclofen dose [$F_{2,74}$=3.3; p<0.05] and ethanol dose [$F_{1,74}$=28.9; p<0.0001], as well as an interaction of baclofen dose and ethanol dose [$F_{2,74}$=3.5; p<0.05] (Fig 12a). Simple main effect analyses revealed that ethanol significantly increased locomotor activity in FAST mice, and baclofen (2.5 mg/kg) attenuated this response without affecting saline activity. In the second 30-min after ethanol administration, there was also a main effect of ethanol dose [$F_{1,74}$=4.8; p<0.05], as well as a baclofen dose X ethanol dose interaction [$F_{2,74}$=4.6; p<0.05] (Fig 12c). Similar to the first 30-min, simple main effect analyses revealed a stimulant response to 2 g/kg ethanol in FAST mice during this time period, an effect which was significantly attenuated by both the 1.25 and 2.5 mg/kg doses of baclofen. Baclofen did not alter the locomotor response to saline.

Dopamine. Despite an attenuation of ethanol-induced locomotor stimulation by baclofen in FAST mice, baclofen did not attenuate ethanol-induced increases in NAc dopamine levels in these mice (Fig 12). Mean (± SEM) dopamine levels, expressed as pg per 20 µl sample, are shown in Table 4. When data were expressed as percent change from the average saline response, repeated measures ANOVA found no interactions with time, but the data were analyzed for the first and second 30-min following ethanol

FIGURE 12. The $GABA_B$ receptor agonist baclofen attenuates the stimulant response to ethanol in FAST mice, but does not alter ethanol-induced increases in extracellular dopamine levels in the NAc.

Ethanol (2 g/kg) dramatically increased locomotor activity in FAST mice for the first 30-min (a) and second 30-min (b) time periods following ethanol administration, an effect which was significantly reduced by baclofen. Although there was a main effect of ethanol dose on extracellular dopamine levels in the NAc in FAST mice during both the first (c) and second (d) 30-min time periods after ethanol administration, baclofen pretreatment did not alter this response. Data are presented as mean ± SEM; n = 9-17 / line, baclofen dose, and ethanol dose (data are collapsed across replicate). * Significant reduction in ethanol-induced locomotor stimulation by baclofen, $p < 0.05$.

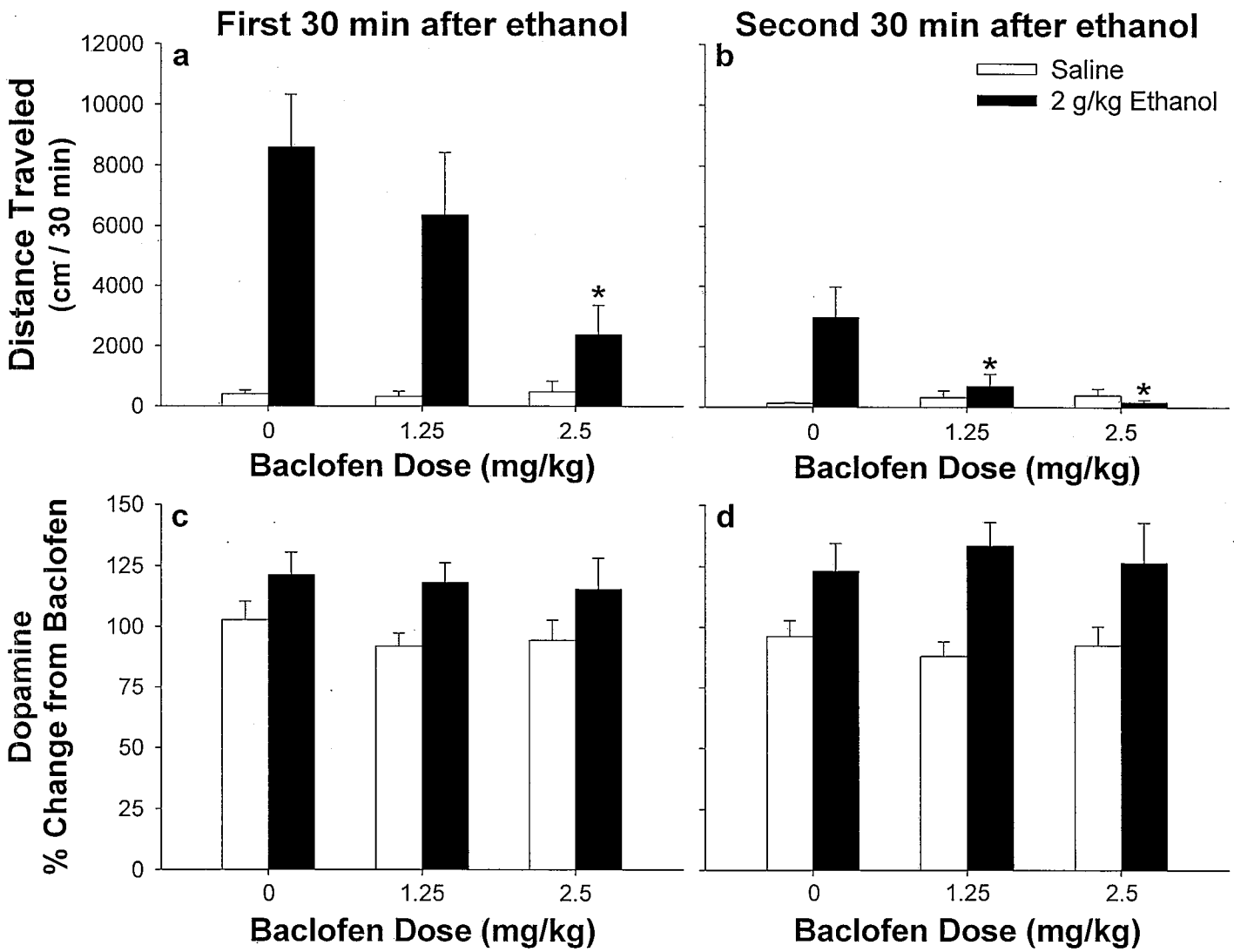

First 30 min after ethanol
Second 30 min after ethanol
a
b
c
d
Distance Traveled
(cm / 30 min)
12000
10000
8000
6000
4000
2000
0
Saline
2 g/kg Ethanol
*
*
*
Dopamine
% Change from Baclofen
150
125
100
75
50
25
0
0
1.25
2.5
Baclofen Dose (mg/kg)

TABLE 4. Summary of average extracellular levels of dopamine after each treatment period

	Average Extracellular Dopamine Levels (pg / 20 µl sample)				
Ethanol Treatment Group	**Baseline**	**Saline**	**Baclofen**	**Ethanol (first 30 min)**	**Ethanol (second 30 min)**
0 g/kg	4.0 ± 0.4	3.6 ± 0.4	3.8 ± 0.4	3.8 ± 0.4	3.6 ± 0.4
2 g/kg	4.4 ± 0.4	4.4 ± 0.4	4.6 ± 0.4	5.5 ± 0.4 †	5.9 ± 0.5 †

Shown are mean ± SEM dopamine levels, n = 35-41 / ethanol treatment group and time point.
† Main effect of ethanol dose at selected time point, $p<0.05$.

administration for comparison to the locomotor activity data. Baclofen treatment alone did not significantly alter extracellular dopamine levels (when compared to the average of the four dialysate samples collected after the first saline injection); however, as seen in Table 5, dopamine levels appeared elevated in the saline control group. When the data were expressed as percent change from the average saline response, there was no main effect of baclofen dose or ethanol dose, nor was there a baclofen dose X ethanol dose interaction in the first 30-min after ethanol administration. However, in the second 30-min time period after ethanol administration, there was a main effect of ethanol [$F_{1,67}$=10.5, p<0.01], but no effect of baclofen dose or an interaction of baclofen dose and ethanol dose. The mean dopamine response to ethanol for each treatment group is shown in Table 5. Due to the heightened dopamine response to saline, which may be a stress response to repeated injections or simply a sampling error, the data were also expressed as percent change from the acute dopamine response to baclofen. This transformation of the data may be optimal because the baclofen injection most immediately preceded the ethanol injection and expressing the data relative to the baclofen response more fully accounts for the potential influence of stress due to multiple injections rather than expressing the data relative to the first injection that occurred 75-min preceding ethanol administration. In addition, by expressing the data as percent change from baclofen, any acute effects of baclofen on extracellular dopamine levels, and the elevated dopamine response in the saline control group (Table 5), would be corrected for, and therefore any ethanol-induced alterations in extracellular dopamine would be originating from the same baseline (100%).

When analyzed as percent change from baclofen, there was a main effect of

TABLE 5. Dopamine response to baclofen and ethanol in FAST mice, expressed as percent of the average saline response.

Treatment Group		Response to Saline	Response to Saline or Ethanol	
Baclofen Dose	**Ethanol Dose**	**or Baclofen**	**First 30 Min**	**Second 30 Min**
0 mg/kg	**0 g/kg**	122.5 ±12.2%	118.6 ± 8.9%	110.6 ± 6.9%
0 mg/kg	**2 g/kg**	105.4 ±12.6%	114.3 ± 7.9%	116.2 ± 9.0%
1.25 mg/kg	**0 g/kg**	108.4 ±4.7%	102.0 ± 6.6%	96.9 ± 6.6%
1.25 mg/kg	**2 g/kg**	109.6 ±3.9%	129.2 ± 10.2%	145.6 ± 14.8%
2.5 mg/kg	**0 g/kg**	111.8 ±4.8%	106.2 ± 11.8%	102.5 ± 10.6%
2.5 mg/kg	**2 g/kg**	114.7 ±10.0%	123.0 ± 11.2%	134.8 ± 15.4%

Shown is mean ± S.E.M. percent increase in extracellular dopamine relative to the average dopamine response to saline.
n = 9-17 / treatment group.

ethanol dose [$F_{1,68}$=9.0; p<0.01] during the first 30-min after ethanol administration (Fig 12b), with ethanol increasing extracellular dopamine levels in FAST mice. However, there was no interaction of baclofen dose and ethanol dose. A similar pattern of results was observed for the second 30-min time period (Fig 12d), with a main effect of ethanol dose [$F_{1,66}$=16.6; p<0.001], but no interaction of baclofen dose and ethanol dose, demonstrating that baclofen pretreatment did not alter the dopamine response to ethanol in the NAc.

Discussion

The current results provide further evidence to support the contribution of $GABA_B$ receptors to acute ethanol sensitivity. In mice selectively bred for extreme sensitivity (FAST) and insensitivity (SLOW) to the locomotor stimulant effects of ethanol, $GABA_B$ receptor function was significantly different in ventral midbrain and striatal tissue. No significant receptor function differences were found between the lines in any other brain region examined. In addition, activation of $GABA_B$ receptors in FAST mice attenuated the locomotor stimulant response to ethanol; however, this attenuation of stimulation was not accompanied by a reduction in ethanol-induced increases in extracellular dopamine in the NAc. These results support (i) a critical role for $GABA_B$ receptors in the acute locomotor response to ethanol, (ii) enhanced receptor function in the ventral midbrain coupled to decreased sensitivity to the stimulant effects of ethanol in SLOW mice, and (iii) enhanced receptor function in the striatum coupled to increased sensitivity to ethanol's stimulant effects in FAST mice. Attenuation of the stimulant response to ethanol by $GABA_B$ receptor activation, however, may not be due to an

inhibition of mesolimbic systems, calling into question whether ethanol-induced elevations in dopamine signaling in the NAc are critical to the induction of the stimulant response to ethanol.

$GABA_B$ receptor function is altered in the FAST and SLOW selected lines

The FAST and SLOW selected lines provide a unique animal model by which the neurochemical systems underlying acute ethanol sensitivity can be assessed. By examining genetically correlated line differences, the systems that were altered through the process of genetic selection, and thereby likely contribute to the selection response, can be inferred (Crabbe et al., 1991). Previous studies have supported a contribution of GABA systems to acute ethanol sensitivity in the FAST and SLOW lines. From early in selection, these lines were found to differ in locomotor sensitivity to a variety of $GABA_A$ receptor positive modulators, including several alcohols, barbiturates, and benzodiazepines (Phillips et al., 1992; Shen et al., 1998). Studies in later generations have shown that they also differ in sensitivity to neurosteroids, the GABA transporter inhibitor NO-711, glutamatergic receptor antagonists, indirect dopaminergic receptor agonists, and baclofen (Bergstrom et al., 2003; Boehm et al., 2002a; Holstein et al., 2008; Meyer et al., 2003b; Palmer et al., 2002b; Shen et al., 1998). The line difference in sensitivity to the locomotor depressant effects of baclofen, with SLOW mice more sensitive than FAST mice, was found with both peripheral and central treatment, including when administered into the VTA (Boehm et al., 2002a). This suggests that some aspect of $GABA_B$ receptor signaling, possibly receptor density, receptor function, or downstream signaling cascades, has been altered through the process of selection. The current data support a selection-induced alteration to $GABA_B$ receptor function in the

ventral midbrain (VTA and substantia nigra), a finding that is particularly interesting as $GABA_B$ receptor density does not differ between the lines in this region (Boehm et al., 2002a).

The [^{35}S]GTPγS binding assay provides a measure of agonist-stimulated G-protein activation predominantly for $G\alpha_{i/o}$-coupled receptors. As such, agonist potency (EC_{50}) and efficacy (E_{max}) can be examined (Harrison and Traynor, 2003). Initial analyses revealed no line difference in basal [^{35}S]GTPγS binding, except in the striatum, where binding was significantly lower in FAST mice compared to SLOW mice. It is difficult to determine the source of this line difference, as this is an effect seen in the absence of agonist. However, certain GPCRs, including the adenosine A_1 receptor, may be tonically active even after membrane preparation and in the absence of agonist in the assay buffer (Laitinen, 1999; Savinainen et al., 2003). Therefore, this line difference may reflect a line difference in the density of constitutively active GPCRs in this brain region.

When [^{35}S]GTPγS binding was examined in the presence of baclofen (and expressed as percent change from basal binding) in whole brain tissue, no line differences in the EC_{50} for baclofen or the maximal percent stimulation of [^{35}S]GTPγS binding (E_{max}) were found. However, small, but significant, line differences restricted to specific brain regions could be difficult to identify in whole brain, and therefore regional analyses were performed. These regions included both limbic and motor areas that are hypothesized to contribute to the motor stimulant and rewarding effects of ethanol (Phillips and Shen, 1996; Tzschentke and Schmidt, 2000; Wise and Bozarth, 1987). In the striatum, a line difference in E_{max} was found, with the maximal percent stimulation of [^{35}S]GTPγS binding by baclofen (a measure of agonist efficacy) being significantly higher in FAST

mice compared to SLOW mice. Functionally, this shift in agonist efficacy at the $GABA_B$ receptor may contribute to the extreme locomotor stimulant response to ethanol observed in FAST mice, as increased receptor function in FAST mice in the striatum could potentiate an ethanol-induced inhibition of the NAc (Criado et al., 1995), leading to increased locomotor activity (Bourdelais and Kalivas, 1992; Mogenson and Nielsen, 1983; Wise, 1998). However, as a measure of the maximal percent stimulation of G-protein activation or turnover, an increase in E_{max} may only be relevant when $GABA_B$ receptors in this region are near saturation with agonist. In these lines, endogenous $GABA_B$ receptor occupancy in the striatum is unknown, as is the effect of baclofen on receptor occupancy. Baclofen doses that alter activity in FAST and SLOW mice likely do not saturate the receptor, as only a small percentage of the baclofen dose may actually cross the blood brain barrier (Deguchi et al., 1995). Moreover, the lines differed in basal [^{35}S]GTPγS binding in this region, with significantly lower basal binding in striatal tissue from FAST mice compared to SLOW mice. As baclofen-stimulated [^{35}S]GTPγS binding is indexed as percent stimulation of basal binding, decreased basal [^{35}S]GTPγS binding in FAST mice could also artificially inflate the baclofen-stimulated potentiation of binding, thereby inflating E_{max} values. Therefore, whether this line difference in striatal receptor function is real and actually contributes to the line difference in acute ethanol and baclofen sensitivity is unknown.

In addition to a line difference in receptor function in the striatum, a line difference in $GABA_B$ receptor function was found in the ventral midbrain. Specifically, the EC_{50} for baclofen, a measure of agonist potency, was significantly lower in SLOW compared to FAST mice, indicating greater $GABA_B$ receptor function in the ventral

midbrain of the SLOW line. This line difference in agonist potency may be more relevant to the line difference in the locomotor responses to baclofen and ethanol as it should be observed at lower agonist concentrations. And as a previous study showed that $GABA_B$ receptor density did not differ between FAST and SLOW lines in the ventral midbrain, line differences in receptor number are not mediating this effect ($GABA_B$ receptor density has not been examined in other brain regions in these mice; Boehm et al., 2002a).

Behaviorally, this line difference in $GABA_B$ receptor function may contribute not only to the enhanced sensitivity of SLOW mice to the locomotor depressant effects of baclofen (an effect also seen with baclofen administration to the VTA; Boehm et al., 2002a), but also of ethanol. In addition, as $GABA_B$ receptors in rat ventral midbrain have been found predominantly on dopaminergic neurons (Wirtshafter and Sheppard, 2001), this line difference in $GABA_B$ receptor function might contribute to differences in dopamine responsivity between the lines. These line differences include alterations to basal dopamine cell firing in the ventral midbrain, with increased dopamine firing frequency in FAST mice (Beckstead and Phillips, submitted), as well as line differences in the locomotor response to drugs of abuse which activate dopamine pathways (Bergstrom et al., 2003). For instance, it could be hypothesized that decreased $GABA_B$ receptor function in FAST mice may contribute to the stimulant response to ethanol and other drugs by limiting the effect of local and afferent inhibitory input to mesolimbic dopamine neurons, promoting dopamine cell firing and release. However, as will be discussed, activation of $GABA_B$ receptors in the ventral midbrain may not alter dopamine signaling, suggesting an alternative contribution of $GABA_B$ receptors in the VTA.

Instead of directly affecting dopamine cell activity in FAST mice, decreased $GABA_B$ receptor function on GABA neurons in the VTA could limit the inhibition of GABAergic output from the VTA, which may contribute to the stimulant response (Cruz et al., 2004; Fields et al., 2007; Van Bockstaele and Pickel, 1995). Alternatively, a more limited activation of $GABA_B$ heteroreceptors in the VTA, including $GABA_B$ receptors on glutamatergic terminals, could facilitate ethanol-induced increases in glutamatergic transmission in the VTA, which may promote locomotion (Boyes and Bolam, 2003; Manzoni and Williams, 1999; Xiao et al., 2008).

Although the functional implication of this line difference in ventral midbrain $GABA_B$ receptor function is unknown, it is worth noting that the sP (Sardinian alcohol-preferring) and sNP (Sardinian alcohol-nonpreferring) rat lines, which were selectively bred for increased and decreased ethanol consumption, respectively, also differed in $GABA_B$ receptor function (measured as the potency of baclofen at the $GABA_B$ receptor), with increased receptor function in the sNP line. However, this occurred in cortical and limbic areas (NAc, olfactory bulb, septal nuclei), regions in which there was no line difference in the EC_{50} for baclofen in the FAST and SLOW lines; ventral midbrain tissue was not tested in the sP and sNP rat lines (Castelli et al., 2005). Despite the potential inconsistency in brain regions, it is intriguing to extend these results to the FAST and SLOW selected lines, as the sP and sNP rat lines also differ in the acute locomotor stimulant response to ethanol, with increased stimulation in the sP line (Agabio et al., 2001). Similarly, the FAST and SLOW lines differ in ethanol consumption (Risinger et al., 1994), and therefore, these results may suggest a correlation between enhanced $GABA_B$ receptor function and insensitivity to the stimulant effects of ethanol, as well as

decreased ethanol consumption and preference.

Finally, an attempt was made to determine whether acute ethanol exposure altered $GABA_B$ receptor function. Receptor function was measured in the cerebellum due to its high density of $GABA_B$ receptors (Chu et al., 1990). Pretreatment of animals with 2 g/kg ethanol 15 min prior to sacrifice did increase E_{max} in cerebellar tissue; however, there was no difference between the lines in this effect, and the magnitude of this effect was very small (approximately 12%). However, this may provide preliminary evidence that ethanol enhances the efficacy of baclofen, potentiating $GABA_B$ receptor function. Contrary to this result, inclusion of ethanol in the incubation buffer significantly decreased [^{35}S]GTPγS binding in response to baclofen; however, this only occurred at the highest ethanol concentration (316 mM), a concentration well above lethal doses in both mouse and human (80-120 mM) (Gable, 2004). This minimal effect of ethanol on $GABA_B$ receptor function is likely due to a limitation of the assay, as relatively long incubation periods are required for the accumulation of [^{35}S]GTPγS binding, factors that may limit the ability to observe acute and transient effects of ethanol on GPCR function. However, ethanol may enhance presynaptic $GABA_B$ receptor function, as suggested by a few electrophysiological studies (Ariwodola and Weiner, 2004; Frye and Fincher, 1996). Further studies will need to be performed in order to determine whether this is occurring in the FAST and SLOW selected lines.

Activation of $GABA_B$ receptors blocks the stimulant response to ethanol, but perhaps not through alterations of mesolimbic dopamine systems.

Perhaps the most surprising result in this series of studies was that baclofen did not reverse ethanol-induced increases in extracellular dopamine in the NAc. In FAST

mice, baclofen has repeatedly been found to attenuate the locomotor stimulant response to ethanol (Holstein et al., 2008; Shen et al., 1998), an effect also observed in other mouse strains (Broadbent and Harless, 1999; Chester and Cunningham, 1999a; Cott et al., 1976; Humeniuk et al., 1993). This effect of baclofen, even when peripherally administered, appears to be due to a selective attenuation of stimulation rather than a shift in the behavioral response to ethanol towards greater intoxication. Baclofen did not accentuate the motor incoordinating effects of a low dose of ethanol, unlike other GABA mimetics, including the GABA transporter inhibitor NO-711 and the $GABA_A$ receptor agonist muscimol (Holstein et al., 2008). This effect may also be localized to the VTA, as administration of baclofen into the anterior, but not posterior, VTA attenuated the stimulant response to ethanol in FAST mice (Boehm et al., 2002a).

The VTA and the associated mesolimbic dopamine pathway have repeatedly been implicated in the motor stimulant and rewarding effects of ethanol and other drugs of abuse (Di Chiara and Imperato 1988; Gallegos et al. 1999; Ikemoto and Wise, 2004; Kalivas et al. 1990; Phillips and Shen 1996; Tzschentke and Schmidt, 2000; Wise and Bozarth 1987). In FAST mice, ethanol increased extracellular dopamine levels in the NAc (a finding confirmed by the current results), an effect which was greater than that seen in SLOW mice (Meyer et al., submitted). Additionally, partial electrolytic lesions of the VTA attenuated the stimulant response to 2 g/kg ethanol in FAST mice (Meyer and Phillips, unpublished results). As activation of $GABA_B$ receptors in the (anterior) VTA attenuated stimulation, and the stimulant effects of ethanol appear to rely on the VTA, I had hypothesized that this baclofen-induced attenuation of ethanol stimulation was occurring through a reduction of the stimulatory effects of ethanol on dopamine

signaling. Baclofen has previously been found to decrease dopamine cell firing in the VTA, and activation of $GABA_B$ receptors in the VTA reduced extracellular dopamine output in the VTA and NAc (Chen et al. 2005; Cruz et al., 2004; Erhardt et al., 2002; Klitenick et al. 1992; Lacey et al., 1988; Olpe et al., 1977; Westerink et al. 1996), supporting the localization of $GABA_B$ receptors to dopamine neurons in this region (Wirtshafter and Sheppard, 2001). Baclofen also attenuated amphetamine-, cocaine-, morphine-, and nicotine-induced increases in extracellular dopamine levels in the NAc in rats (Brebner et al., 2005; Fadda et al., 2003). Therefore, it was surprising that a similar reduction of ethanol-induced increases in extracellular dopamine levels in the NAc was not seen in FAST mice.

There are several potential implications of this finding. First, microdialysis may not be a sensitive enough assay to measure spatially or temporally restricted alterations in dopamine release. While microdialysis allows for the chemical analysis of samples with a high degree of sensitivity, relatively slow perfusion rates and large sample volumes result in dialysate samples being collected every 10-30 min (15 min in the current study). Therefore, rapid and transient changes in dopamine signaling mediated by baclofen may not be observed (Fillenz, 2005; Jones et al., 1999; Robinson et al., 2003, 2008). For instance, baclofen may attenuate ethanol-induced increases in phasic dopamine cell firing in the VTA, either by directly hyperpolarizing dopamine neurons or by a presynaptic inhibition of excitatory input to the VTA (from the prefrontal cortex, amygdala, pedunculopontine/laterodorsal tegmental nuclei, among other sources) which contributes to dopamine burst firing (Floresco et al., 2003; Gabbott et al., 2005; Grace et al., 2007; Manzoni and Williams, 1999; Oakman et al., 1995; Omelchenko and Sesack, 2005,

2006). This inhibition of excitatory input to the VTA may limit an ethanol-induced increase in dopamine cell burst firing and synaptic, or phasic, dopamine release (which produces a large, but transient, increase in dopamine levels; Cheer et al., 2007). However, baclofen may not limit the number of spontaneously active dopamine neurons in the VTA, possibly due to a lack of a direct effect of baclofen on dopamine cell firing or the inability of baclofen to alter an ethanol-induced decrease in inhibitory input to VTA dopamine neurons. Within the VTA, inhibitory input from forebrain regions, including the ventral pallidum, controls the spontaneous, or tonic firing rate of dopamine neurons (Floresco et al., 2003). If baclofen is not limiting ethanol-induced increases to tonic dopamine cell firing and release, measured as changes to extracellular dopamine levels over a longer time course, then a baclofen-induced reduction in dopamine signaling may not be observed.

Alternatively, the NAc may not be critically involved in the locomotor stimulant effects of ethanol. Electrolytic lesions of the NAc did not attenuate the stimulant response to ethanol in DBA/2J (Gremel and Cunningham, 2008) or FAST mice (Meyer and Phillips, unpublished results). In both studies, the lesions encompassed significant portions of both the NAc medial shell and core; however, some cells in these regions may have been preserved, and more lateral aspects of the NAc were not affected. Dopamine signaling to the lateral NAc may be critical to the stimulant response to ethanol and may be preferentially affected by baclofen administration (the majority of microdialysis probes in the current study were localized to the medial shell and core). GABAergic processes, which reportedly compose one-third of the projections from the VTA to NAc

(Fields et al., 2007; Van Bockstaele and Pickel, 1995), may also contribute to the stimulant response to ethanol, and baclofen may inhibit these processes.

Parallel projections from the VTA to other regions may also contribute to the stimulant response to ethanol and may be modulated by both peripheral and intra-anterior VTA administration of baclofen. Efferents from the VTA project to the PfC, amygdala (Amy), ventral pallidum (VP), and caudate-putamen, among other regions (Albanese and Minciacchi, 1983; Fallon and Moore, 1978; Ford et al., 2006; Klitenick et al., 1992). Many of these projections are also GABAergic (Carr and Sesack, 2000; Fields et al., 2007; Klitenick et al., 1992). Ethanol has been found to increase both extracellular dopamine levels (Yoshimoto et al., 2000) and Fos-like immunoreactivity (a marker of neuronal activation) in the Amy. This increase in Fos expression was greater in mice that showed a locomotor stimulant response to ethanol, including FAST mice (Demarest et al., 1998, 1999; Hitzemann and Hitzemann, 1997). While electrolytic lesions of the central nucleus of the Amy did not attenuate the stimulant response to ethanol in FAST mice (Meyer and Phillips, unpublished results), broader lesions of the Amy did reduce stimulation to ethanol in DBA/2J mice (Gremel and Cunningham, 2008).

It is possible that $GABA_B$ receptors are regulating dopamine cell firing and release of Amy-projecting neurons and not NAc-projecting dopamine neurons (dopamine neurons projecting to these two regions are independent of one another; Fallon, 1981; Ford et al., 2006). Ethanol has also been shown to increase GABA release in the Amy (Bajo et al., 2008; Roberto et al., 2003; Siggins et al., 2005; Zhu and Lovinger, 2006), an effect which may be reversed by presynaptic $GABA_B$ receptors. Lesions of the PfC also altered the stimulant response to psychostimulants and glutamatergic antagonists

(Lacroix et al., 1998; Tzschentke and Schmidt, 1998; Ventura et al., 2004), as did lesions of the VP (Gong et al., 1997; Mele et al., 1998). Therefore, baclofen-induced alterations in dopaminergic or GABAergic signaling to these areas may attenuate the stimulant effects of ethanol in FAST mice. Finally, the anterolateral portion of the VTA, a region of the VTA in which baclofen attenuated the stimulant response to ethanol in FAST mice (Boehm et al., 2002a), has been found to send direct projections to the caudate-putamen, or dorsal striatum (Ikemoto et al., 2007); therefore, ethanol may be having its stimulant effects via an activation of the caudate-putamen and associated nigrostriatal pathway, which in turn would affect signaling to the basal ganglia.

Finally, an increase in extracellular dopamine levels in response to ethanol was more pronounced when compared to the dopamine response after the previous baclofen injection rather than to the average saline response. This appears to be due to an elevated dopamine response in the saline control group; for instance, after the second saline injection (0 mg/kg baclofen), extracellular dopamine levels were elevated above those seen in the ethanol control group, despite the fact that they had both received saline injections at this time point (see Table 5). This group difference must be attributed to random experimenter or sampling error, since treatments were constant between groups. When the dopamine response to baclofen was set as the baseline (100%), there was no further elevation in extracellular dopamine levels in response to the third saline injection (0 g/kg ethanol). This allowed an effect of ethanol on dopamine signaling to be observed. Despite this inconsistency among groups, there was clearly no reduction of extracellular dopamine levels by baclofen, no matter how the data were expressed.

Conclusions

$GABA_B$ receptors in the ventral midbrain and striatum appear to contribute significantly to acute ethanol sensitivity, with decreased receptor function in the ventral midbrain and increased receptor function in the striatum coupled with enhanced sensitivity to the stimulant effects of ethanol. This reduction in the behavioral stimulant response to ethanol, however, does not appear to be accompanied by alterations to mesolimbic dopamine signaling. Therefore, while $GABA_B$ receptor function is involved in the acute motor stimulant response to ethanol, a specific interaction of $GABA_B$ receptors and dopamine systems, resulting in a reduction to dopamine signaling in the NAc, was not supported. Analyses in other brain regions, as well as a temporal analysis of changes in dopamine signaling by baclofen and ethanol, are required to determine to what extent the mesolimbic dopamine pathway contributes to the stimulant effects of ethanol, as well as the attenuation of stimulation by baclofen.

CHAPTER 4. General Discussion

The purpose of this dissertation was to examine potential mechanisms by which ethanol may interact with GABA systems to produce its locomotor stimulant and depressant effects, and to determine how GABA agonists reduce the locomotor stimulant response to ethanol. Briefly, in the FAST and SLOW lines, which were selectively bred for extreme sensitivity and insensitivity, respectively, to the locomotor stimulant effects of ethanol (Crabbe et al., 1987; Phillips et al., 1991b), genetically correlated line differences were found for acute sensitivity to a GABA transporter inhibitor, as well as for $GABA_B$ receptor function in both the striatum and the ventral midbrain. These results support the hypothesis that selection for acute ethanol sensitivity has resulted in alterations to GABA systems. In addition, a pharmacological approach was taken to examine possible means by which ethanol elicits the profound locomotor stimulant response seen in FAST mice. In these studies, inhibition of the GABA transporter, as well as activation of the $GABA_A$ and/or $GABA_B$ receptor, attenuated the locomotor stimulant response to ethanol. However, this occurred through divergent and receptor-specific mechanisms; the motor incoordinating effects of ethanol were enhanced by a GABA transporter inhibitor and by a $GABA_A$ receptor agonist, which likely contributed to the reduction in stimulation, but a $GABA_B$ receptor agonist shown previously to attenuate the stimulant response to ethanol in FAST mice (Shen et al., 1998) did not accentuate the motor incoordinating effects of ethanol. I hypothesized that this $GABA_B$ receptor-induced reduction in stimulation occurred through a reduction in the stimulatory effects of ethanol on mesolimbic dopamine function. As will be discussed, this

hypothesis was not supported, questioning the exclusive role of dopaminergic projections to the NAc in the mediation of the acute stimulant response to ethanol.

GABA Systems are Altered in FAST and SLOW Mice

In previous studies of the FAST and SLOW selected lines, genetically correlated line differences to GABAergic agonists and positive modulators have repeatedly been found, including to the $GABA_B$ receptor agonist baclofen, as well as to benzodiazepines, barbiturates, and neuroactive steroids, all of which potentiate agonist-stimulated $GABA_A$ receptor function (Boehm et al., 2002a; Palmer et al., 2002a; Phillips et al., 1992; Shen et al., 1998). Many of these line differences were found early in selection, suggesting that genes that influence the locomotor response to GABAergic drugs have a large impact on the locomotor response to ethanol. Whereas line differences were found for GABA receptor agonists, the contribution of the GABA transporter to the selection response was previously unknown. As shown in Chapter 2, the FAST and SLOW lines differed in acute sensitivity to the GABA transporter inhibitor NO-711, with SLOW mice more sensitive to its locomotor depressant effects. Inhibition of GAT1 results in increased extracellular levels of GABA (Fink-Jensen et al., 1992) and a resultant increase in postsynaptic GABA receptor activation. As such, the increased sensitivity to the locomotor depressant effects of NO-711 in SLOW mice could be due to several different factors, including increased inhibition of GAT1 function by NO-711, increased GAT1 density, elevated extracellular GABA levels, or enhanced postsynaptic GABA receptor function. Further studies are required to determine which of these factors might be involved (as described at the conclusion of this chapter). However, this lends further

1999; Savinainen et al., 2003). In addition, line differences in these measures may indicate selection-induced alterations in receptor function.

Analysis of baclofen-stimulated [^{35}S]GTPγS binding in whole brain tissue revealed no line differences in $GABA_B$ receptor function. However, regional analyses did find a line difference in the EC_{50} for baclofen, or the concentration of baclofen that elicited a half-maximal stimulation of [^{35}S]GTPγS binding (Chapter 3). Specifically, baclofen in the ventral midbrain was significantly more potent in SLOW mice, with the EC_{50} for baclofen being 22 μM lower in SLOW than in FAST mice. Additionally, in striatal tissue, a line difference in E_{max} was found, with the efficacy of the baclofen response, or maximal percent stimulation of [^{35}S]GTPγS binding by baclofen, being significantly higher in FAST compared to SLOW mice. No other significant line differences were found in regional (cerebellum, hippocampus, prefrontal cortex) samples.

The line difference in EC_{50} in ventral midbrain suggests enhanced $GABA_B$ receptor function in SLOW compared to FAST mice, whereas the line difference in E_{max} in the striatum may support enhanced $GABA_B$ receptor function in FAST mice. These line differences in $GABA_B$ receptor function should not reflect line differences in endogenous GABA levels, as GABA should be removed from the tissue preparation with extensive washing. The finding that baclofen is more efficacious in FAST mice in the striatum could reflect a variety of factors, including an increased density of $GABA_B$ receptors in the striatum of FAST mice or more $G\alpha_{i/o}$. Within the striatum, $GABA_B$ receptors are localized to GABAergic medium spiny neurons and striatal interneurons, though they may be more predominantly localized to presynaptic sites, including on glutamatergic terminals, where they regulate glutamate release into the striatum (Lacey et

al., 2005; Seabrook et al., 1991; Yung et al., 1999). As the efficacy of baclofen appears to be greater in FAST mice in this region, this may suggest that baclofen is more effective at inhibiting striatal function in these mice as compared to SLOW mice, either by limiting glutamate release into the striatum, or by directly inhibiting GABAergic medium spiny neurons. Behavioral studies have suggested that drugs of abuse inhibit the NAc, which would then induce increases in locomotor activity and possibly drug reward (Bourdelais and Kalivas, 1992; Mogenson and Nielsen, 1983; Wise, 1998). If this is the case, increased agonist efficacy at $GABA_B$ receptors in FAST mice may contribute to an enhanced ethanol-induced inhibition of the NAc and increased locomotor activity. However, $GABA_B$ receptor density and distribution in the striatum is unknown in the FAST and SLOW lines, so this is a speculation.

As a measure of the maximal percent stimulation of G-protein activation or turnover, an increase in E_{max} may only be relevant when $GABA_B$ receptors in this region are near saturation with agonist. Endogenous striatal $GABA_B$ receptor occupancy is unknown for these mice, as are endogenous and ethanol-induced agonist concentrations. Moreover, when assessing the effect of peripheral baclofen injection on behavioral responses in FAST and SLOW mice and relating this to the line difference in E_{max}, it is critical to consider that very low concentrations of baclofen, which likely do not saturate $GABA_B$ receptors, may actually be reaching the brain. For instance, Deguchi and colleagues (1995) reported that baclofen levels in whole brain, cerebrospinal fluid, and hippocampal interstitial fluid were approximately 30-fold lower than baclofen levels found in plasma after an i.v. infusion of baclofen. Low, peripherally-administered doses of baclofen were effective at reducing locomotor activity in SLOW mice (Shen et al.,

1998) and at attenuating the stimulant response to ethanol in FAST mice (Chapters 2 and 3). The effects of these peripheral administrations were similar to those of centrally administered baclofen (Boehm et al., 2002a). These results may suggest that only very low concentrations of baclofen within the brain are needed to alter behavior, doses which likely would not saturate $GABA_B$ receptors. Moreover, within this region, the FAST and SLOW lines differed in basal [^{35}S]GTPγS binding, with significantly lower basal binding in striatal tissue from FAST mice compared to SLOW mice. This could reflect a line difference in endogenous ligand concentration or density of tonically active receptors, such as the adenosine A_1 receptor, in the striatum. Since baclofen-stimulated [^{35}S]GTPγS binding is indexed as percent stimulation of basal binding, decreased basal [^{35}S]GTPγS binding in FAST mice could artificially inflate E_{max} values. Therefore, whether this line difference in striatal receptor function is real and actually contributes to the line difference in acute ethanol and baclofen sensitivity is unknown.

It is of particular interest that a line difference in baclofen potency, or the EC_{50} for baclofen, was found in the ventral midbrain, as line differences in the locomotor depressant response to baclofen, as well as the baclofen-induced attenuation of ethanol stimulation in FAST mice, have been localized to this region (Boehm et al., 2002a). In addition, the ventral midbrain, and specifically the VTA, appears to be critical to the stimulant response to ethanol, as even partial lesions of this region reduced ethanol-induced locomotor stimulation in FAST mice (Meyer and Phillips, unpublished results). Combined with the finding that FAST and SLOW mice do not differ in $GABA_B$ receptor number in the ventral midbrain (Boehm et al., 2002a), which would confirm that a line difference in the EC_{50} for baclofen is not due to a line difference in receptor density,

these data suggest that alterations in $GABA_B$ receptor function in the ventral midbrain may be a critical factor in the sensitivity difference to acute ethanol stimulation between these lines. A line difference in $GABA_B$ receptor function in this region may signify that decreased $GABA_B$ receptor function is associated with the induction of a stimulant response to ethanol in FAST mice, a response that is completely absent in SLOW mice. In addition, an increased potency of baclofen in SLOW mice in this region may contribute both to their enhanced sensitivity to the locomotor depressant effects of baclofen (Boehm et al., 2002a; Shen et al., 1998), as well as to their enhanced sensitivity to the locomotor depressant, ataxic, and sedative effects of ethanol (Phillips et al., 1991b, 2002b; Shen et al., 1996). Finally, as brain concentrations of peripherally-administered baclofen may be quite low (Deguchi et al., 1995), a line difference in agonist potency may be a more relevant change in $GABA_B$ receptor function for influencing the behavioral response to baclofen.

Selection-induced alterations in receptor function, both in the striatum and ventral midbrain, could be due to a variety of factors. As mentioned previously, this may reflect differences in $GABA_B$ receptor number, though this is not a factor mediating the line difference to baclofen in the ventral midbrain (Boehm et al., 2002a). Alternatively, this measure could reflect line differences in $GABA_B$ receptor subtype expression, homo- vs. heteroligomerization of the receptor, G-protein availability and type, and receptor desensitization, among other factors (Harrison and Traynor, 2003). Other assays are required to determine the source of the line difference. However, many of these factors have previously been found to influence receptor function, or the functional consequence of receptor activation. For instance, the $GABA_{B1}$ subunit has two main subtypes,

$GABA_{B1a}$ and $_{1b}$. Although constitutive absence of either $GABA_{B1}$ subtype does not differentially influence basal receptor function, $GABA_{B1}$ subtype does appear to influence receptor localization and interaction with downstream signaling cascades (Vigot et al., 2006; Waldmeier et al., 2008). G-protein subtype and expression also has been found to alter GPCR function. $GABA_B$ receptors are $G\alpha_{i/o}$-coupled GPCRs (Bettler and Tiao, 2006; Bowery et al., 2002); however, there are at least 6 different $G\alpha$ subtypes in the $G\alpha_{i/o}$ family of G-proteins (Milligan and Kostenis, 2006), and alterations in $G\alpha_{i/o}$ subtype have been found to alter GPCR receptor function (Yang and Lanier, 1999). A similar interaction may be occurring in $GABA_B$ receptors in FAST and SLOW mice.

Another intriguing possibility is that variations in membrane dynamics, such as lipid rafts, may be responsible for differences in $GABA_B$ receptor function between the lines. Briefly, lipid rafts are membrane microdomains that are defined experimentally as being cholesterol-rich and detergent-resistant. These domains are hypothesized to compartmentalize receptors and associated signaling proteins to either promote or inhibit receptor signaling. Localization of the receptor in close proximity to its G-proteins within a lipid raft could enhance receptor function, while physical separation of the receptor and G-proteins could inhibit receptor signaling (Allen et al., 2007). In the cerebellum, a significant proportion of $GABA_B$ receptors are localized to lipid rafts along with their constituent $G\alpha_{i/o}$ proteins (Becher et al., 2001). The enhanced receptor function in the ventral midbrain of SLOW mice, or in the striatum of FAST mice, could be due to an enrichment of $GABA_B$ receptors and their intracellular G-proteins to lipid raft microdomains, thereby promoting $[^{35}S]GTP\gamma S$ binding upon baclofen stimulation due to their close physical proximity.

Initial attempts were also made to determine whether acute ethanol-induced alterations in $GABA_B$ receptor function could be measured in the GTPγS assay. This was examined in cerebellar tissue due to its high density of $GABA_B$ receptors (Chu et al., 1990) and its contribution to the motor incoordinating and sedative effects of ethanol (Dar, 1995, 1996; Palmer et al., 1984; Seiger et al., 1983). The inclusion of various concentrations of ethanol *in vitro* in the binding assay only reduced [^{35}S]GTPγS binding at the highest concentration (316 mM), a concentration significantly above the lethal limit (80-120 mM) (Gable, 2004). The lines did not differ in this response, and binding was not affected by lower, physiologically relevant ethanol concentrations, suggesting that ethanol did not alter $GABA_B$ receptor function. Pretreatment with 2 g/kg ethanol, 15 min prior to sacrifice, did increase E_{max}; however, this effect did not vary by line and although it was a statistically significant effect, it was small in magnitude (an average increase of 12%). This minimal effect of ethanol on E_{max} may be due to a ceiling effect, as baclofen alone (in saline-treated mice) was already producing a 400% increase in [^{35}S]GTPγS binding. Overall, though, these results provide preliminary evidence that *in vivo* ethanol enhances the efficacy of baclofen, at least in cerebellar tissue, the result of which may be an enhancement of the inhibitory effects of $GABA_B$ receptor stimulation. However, the absence of a line difference does not support a role of this factor in the acute ethanol sensitivity difference between FAST and SLOW lines. Whether ethanol alters $GABA_B$ receptor function in other brain regions, and whether the lines differ in this response, is unknown.

Ethanol may be increasing the efficacy of baclofen by increasing neuronal membrane fluidity (Bae et al., 2005; Harris and Schroeder, 1981, 1982). Studies in

hepatocytes and immune cells have suggested that ethanol, by altering membrane fluidity, can alter lipid raft microdomains, either by reducing the recruitment of receptor components into lipid rafts (Dai et al., 2005; Szabo et al., 2007), or by increasing lipid raft clustering, resulting in an accentuation of cell signaling (Nourissat et al., 2008). Therefore, ethanol, by altering membrane fluidity and lipid raft domains, may alter receptor function. However, this is purely speculative and it is critical to temper these results with the fact that the FAST and SLOW lines did not differ for this ethanol-induced enhancement of $GABA_B$ receptor function. This lack of an interaction of ethanol treatment with line implies that this effect of ethanol on receptor function, at least in the cerebellum, does not contribute to the line difference in acute ethanol sensitivity.

The absence of a direct ethanol effect on receptor function, and the small magnitude of an effect of ethanol pretreatment on $GABA_B$ receptor function, may be due to a limitation of the assay, as it requires relatively long incubation times. Acute and transitory effects of ethanol on $GABA_B$ receptor function may be missed during this extended time period. In fact, the most profound behavioral stimulatory effects of ethanol, as well as the loss of righting reflex, occur during the first few minutes after ethanol administration (Crabbe et al., 1988, 2006). Some electrophysiological studies have reported an enhancement of $GABA_B$ receptor function with acute ethanol exposure, though this effect may be limited to presynaptic $GABA_B$ receptors (Ariwodola and Weiner, 2004; Frye and Fincher, 1996). Therefore, more rapid measures may be required to determine whether ethanol differentially alters $GABA_B$ receptor function between the FAST and SLOW lines.

Overall, these studies support a contribution of GABA systems to the selection

response. Increased receptor efficacy in the striatum of FAST mice may potentiate an ethanol-induced inhibition of the striatum, thereby accentuating the stimulant response to ethanol. Heightened $GABA_B$ receptor function in the ventral midbrain may prevent an activational effect of ethanol in SLOW mice, and may enhance the inhibitory and motor depressant responses to GABA mimetics, including ethanol and baclofen, in these mice. Studies in rats suggest that $GABA_B$ receptors are localized primarily to dopaminergic neurons in the ventral midbrain (Wirtshafter and Sheppard, 2001). Increased $GABA_B$ receptor function in SLOW mice in this region may then result in enhanced inhibition of dopamine cell firing in response to ethanol, which would limit the stimulant response to ethanol. Conversely, decreased $GABA_B$ receptor function in FAST mice may limit the consequence of inhibitory input to the ventral midbrain, promoting an ethanol-induced activation of dopamine cell firing. However, the microdialysis data presented in Chapter 3 questions the role of dopamine signaling in the NAc and its modulation by baclofen to the motor effects of ethanol. Therefore, the exact mechanism by which this line difference in $GABA_B$ receptor function contributes to acute ethanol sensitivity remains elusive.

Activation of GABA Systems Attenuates the Stimulant Response to Ethanol

From the above studies, it is clear that GABA systems are involved in acute ethanol sensitivity, supporting the hypothesis that reduced GABA signaling in key regions may contribute to the motor stimulant effects of ethanol, as suggested previously (Engel and Liljequist, 1983). In line with this hypothesis, activation of GABA systems attenuated the stimulant response to ethanol in FAST mice. As reported in Chapter 2, the

GABA transporter inhibitor NO-711, the $GABA_A$ receptor agonist muscimol, and the $GABA_B$ receptor agonist baclofen all attenuated, or even eliminated, the stimulant response to ethanol at doses that did not affect the locomotor activity of vehicle-treated mice (Boehm et al., 2002a; Shen et al., 1998). One concern, however, in interpreting the GABA mimetic-induced attenuation of ethanol stimulation, and in determining whether GABA mimetic compounds hold promise as treatments for alcohol use disorders, is that these drugs could be shifting the behavioral response to ethanol toward greater intoxication. This is especially a concern as ethanol is also a GABAergic compound, increasing GABA release and potentiating $GABA_A$, and possibly $GABA_B$, receptor function (Allan and Harris, 1987; Ariwodola and Weiner, 2004; Criswell and Breese, 2005; Roberto et al., 2003; Suzdak et al., 1986a,b; Zhu and Lovinger, 2006). Therefore, the question becomes, how could two GABAergic agonists have opposing behavioral effects? The results presented in Chapter 2 of this dissertation suggest receptor-specific behavioral mechanisms for this effect. Both blockade of the GABA transporter and activation of $GABA_A$ receptors attenuated the locomotor stimulant response to ethanol in FAST mice; however, this was accompanied by a dramatic increase in ethanol-induced motor incoordination. Activation of $GABA_B$ receptors by baclofen also attenuated the stimulant response to ethanol (Shen et al., 1998), but in the complete absence of any increase in ethanol-induced motor incoordination. The most parsimonious explanation for these results is that GABA transporter inhibitors and $GABA_A$ receptor agonists act additively with ethanol and shift the behavioral response toward greater intoxication, whereas $GABA_B$ receptor activation attenuates a neurochemical response relevant to ethanol-induced locomotor stimulation.

Activation of $GABA_A$ receptors attenuates ethanol stimulation by enhancing ataxia

In FAST mice, the finding that both NO-711 and muscimol increased the ataxic response to ethanol suggests that a reduction in locomotor stimulation likely occurred because of the accentuation of a competing behavior. Therefore, NO-711 may have been less effective at attenuating the stimulant response to a low dose of ethanol (1 g/kg) because the combination of NO-711 and ethanol did not reach the necessary threshold for ataxia. An additive interaction of NO-711 and ethanol to enhance intoxication, and possibly sedation, is also supported by the finding that NO-711 accentuated the motor depressant effects of 2 g/kg ethanol in SLOW mice.

The GABA transporter has recently been identified as a potential target for pharmacotherapies aimed at treating alcohol use disorders (Malcolm, 2003). In preclinical studies, the GABA transporter inhibitor tiagabine (which has a similar binding profile as NO-711) reduced ethanol consumption in mice; however, this effect was transitory, and drinking returned to baseline levels after repeated tiagabine treatment (Nguyen et al., 2005). The current results suggest that this tiagabine-induced reduction in ethanol consumption could have been due to an accentuation of negative side effects of ethanol, namely motor incoordination and intoxication, which may have precluded high levels of drinking. Individuals may become tolerant to this effect of tiagabine, as suggested by a return to baseline drinking with repeated tiagabine exposure, which would limit any potential treatment benefit provided by the drug. An interesting finding that may lend validity to this hypothesis is that in humans, sub-chronic tiagabine exposure did not prevent the activational effects of ethanol in the striatum but did dramatically enhance the inhibitory effects of ethanol in the cerebellum (Fehr et al., 2007). Again, this

suggests that activation of GABA signaling reduces the behavioral response to ethanol, not by reducing its stimulatory actions at the mesolimbic system, but rather by enhancing motor incoordination and intoxication.

Although NO-711 is an indirect GABA receptor agonist, and therefore should increase the activity of both $GABA_A$ and $GABA_B$ receptors, the finding that a $GABA_A$ receptor agonist, but not a $GABA_B$ receptor agonist, mimicked the effect of NO-711 suggests that the predominant mechanism for NO-711 is an activation of $GABA_A$ receptors. This predominant contribution of $GABA_A$ receptors could be due to GABA receptor distribution, with $GABA_A$ receptors more predominantly localized to synaptic regions where GABA transporters would more tightly regulate signaling, whereas $GABA_B$ receptors are more predominantly localized to extrasynaptic regions (Bettler and Tiao, 2006; Kulik et al., 2002; López-Bendito et al., 2004). Alternatively, the predominant contribution of $GABA_A$ receptors could due to a prominent enhancement of ataxia and sedation that occurs with the activation of $GABA_A$ but not $GABA_B$ receptors. The latter possibility may be justified as activation of the $GABA_A$ receptor has previously been found to accentuate the motor-incoordinating effects of ethanol (Dar, 2006; Martz et al., 1983), while inhibition of the $GABA_A$ receptor results in an attenuation of ethanol-induced motor incoordination (Dar, 2006; Hoffman et al., 1987; Suzdak et al., 1988).

Activation of $GABA_B$ receptors selectively attenuates ethanol stimulation

Unlike $GABA_A$ receptor agonists, the $GABA_B$ receptor agonist baclofen did not accentuate ethanol-induced motor incoordination in FAST mice. Peripheral baclofen administration has repeatedly been found to attenuate the locomotor stimulant response to ethanol, both in FAST mice (Shen et al., 1998; Chapter 2; Chapter 3) and in other mouse

strains (Broadbent and Harless, 1999; Chester and Cunningham, 1999a; Cott et al., 1976; Humeniuk et al., 1993). In addition, central administration of baclofen, both ICV and intra-aVTA, has been found to reduce the stimulant response to ethanol in FAST mice (Boehm et al., 2002a), which may suggest that a reduction in locomotor stimulation is not due to the generalized muscle relaxant and anti-spastic properties of baclofen (Bowery, 2006; Nevins et al., 1993). However, several studies have suggested that baclofen may accentuate the motor incoordinating and sedative effects of ethanol. Peripherally administered baclofen potentiated ethanol-induced sedation (Besheer et al., 2004), and accentuated ethanol-induced motor incoordination both peripherally and when administered into the cerebellum (Dar, 1996; Martz et al., 1983). Therefore, it was surprising that baclofen did not affect motor coordination in FAST mice using the fixed-speed rotarod procedure, or accentuate ethanol-induced motor incoordination. In fact, at later time points and higher doses, baclofen appeared to improve performance on the rotarod. This effect could have been due to the reduction in locomotor stimulation to ethanol, thereby improving performance possibly by increasing attention to the task. The rotarod protocol used, however, was fairly simple, utilizing a slow rotation speed (3 RPM), a factor that was intentionally chosen to be equivalent in difficulty to a simple locomotor assay. It is likely that a baclofen-induced increase in motor incoordination seen by others is an effect of $GABA_B$ receptor activation, but this effect may only be seen in more challenging assays of motor function. However, it is also clear that the severity of these effects, at doses that attenuate the stimulant response to ethanol, are dramatically reduced as compared to those of $GABA_A$ receptor activation. Therefore, $GABA_B$

receptor agonists, at carefully titrated doses, may hold greater promise as potential pharmacotherapies for alcohol use disorders.

Contribution of the Mesolimbic Dopamine Pathway to the Locomotor Stimulant Response to Ethanol: Effect of Baclofen

There are several different mechanisms that may explain the ability of baclofen to attenuate the locomotor stimulant response to ethanol in FAST mice without accentuating ethanol-induced motor incoordination. For instance, activation of $GABA_B$ autoreceptors may reduce the effects of ethanol at $GABA_A$ receptors by limiting GABA release and reducing extracellular GABA levels. As GABA is required for an ethanol-induced potentiation of $GABA_A$ receptor function (Aguayo, 1990; Allan et al., 1991), baclofen may reduce stimulation without accentuating motor incoordination by reducing the amount of GABA available for $GABA_A$ receptor binding, thereby limiting an ethanol effect at the receptor (Ariwodola and Weiner, 2004). A more common hypothesis for a $GABA_B$ receptor-mediated attenuation of ethanol stimulation, however, is that baclofen attenuates the ethanol-induced activation of the mesolimbic dopamine pathway. Baclofen has repeatedly been found to reduce dopamine cell firing in the ventral midbrain, as well as to attenuate amphetamine-, cocaine-, DAMGO- (a μ/δ opioid receptor agonist), morphine-, and nicotine-induced increases in extracellular dopamine levels in the NAc (Brebner et al., 2005; Cruz et al., 2004; Fadda et al., 2003; Grace and Bunney, 1980; Kalivas et al., 1990; Lacey et al., 1988; Olpe et al., 1977). Therefore, it was very surprising that in the current results, I did not find a baclofen-induced attenuation of extracellular dopamine levels in response to ethanol (Chapter 3). Ethanol

elicited a small increase in extracellular dopamine in the NAc in FAST mice, of a magnitude consistent with other published reports for ethanol in both mice and rats (Di Chiara and Imperato, 1988; Howard et al., 2008; Imperato and Di Chiara, 1986; Job et al., 2007; Larsson et al., 2002; Yim et al., 1998; Yoshimoto et al., 1991; Zocchi et al., 2003), as well as previous results obtained in FAST mice (Meyer et al., submitted). Ethanol also elicited a large increase in locomotor activity in FAST mice, and consistent with previous studies, baclofen dramatically attenuated this response (Boehm et al., 2002a; Shen et al., 1998). However, the current results suggest that baclofen, at least in FAST mice, may not be having its behavioral effects via an attenuation of dopamine signaling in the NAc. This result may question the contribution of NAc dopamine to ethanol-induced locomotor stimulation.

The contribution of dopamine systems to acute ethanol sensitivity

Although activation of the mesolimbic dopamine pathway is the predominant hypothesized mechanism for the stimulant effects of ethanol (Phillips and Shen, 1996; Tzschentke and Schmidt, 2000; Wise and Bozarth, 1987), the exact contribution of dopamine signaling to this effect is contentious. Previous data have supported a significant dopaminergic component to the locomotor stimulant response of FAST mice to ethanol. Dopamine receptor antagonists attenuated the locomotor stimulant response to ethanol in FAST mice (see Table 2; Shen et al., 1995) and the FAST lines have been found to show a strong locomotor stimulant response to drugs of abuse with dopaminergic mechanisms of action such as amphetamine, cocaine, and methamphetamine (see Table 1; Bergstrom et al., 2003; Meyer et al., submitted; Phillips et al., 1992). However, evidence for a contribution of dopamine signaling to the *line*

difference between FAST and SLOW mice is mixed. Whereas a genetically correlated line difference in the dopamine response to cocaine and ethanol was found in both replicates (with higher extracellular dopamine levels in response to these drugs found in FAST compared to SLOW mice in the NAc; Meyer et al., submitted), the correlated line difference in locomotor response to dopaminergic drugs has consistently only been found, or has been larger in magnitude, in one replicate FAST line (see Table 1; Bergstrom et al., 2003; Phillips et al., 1992; unpublished results). Specifically, data from FAST-1 and SLOW-1 mice indicate a large difference in sensitivity to the stimulant effects of amphetamine, cocaine, and methamphetamine (FAST-1 more sensitive to stimulation than SLOW-1), while FAST-2 and SLOW-2 mice have exhibited differences in sensitivity to cocaine (Bergstrom et al., 2003; Meyer et al., submitted), with increased stimulation in FAST-2 compared to SLOW-2 mice, but have not shown differences in sensitivity to amphetamine and methamphetamine (Bergstrom et al., 2003; Phillips et al., unpublished results). The difference in cocaine response between FAST-2 and SLOW-2 mice was small in magnitude in at least one study, compared to the difference between the replicate 1 lines (Bergstrom et al., 2003).

As discussed earlier, a genetically correlated line difference that is found in only one pair of replicate lines does not necessarily prove that the correlation is spurious. Because several neurochemical systems contribute to acute ethanol sensitivity, it is likely that a locomotor stimulant or locomotor depressant response to ethanol can be achieved through more than one mechanism (i.e., the neurochemical substrates contributing to the selection response in replicate 1 mice may not necessarily be identical to those contributing to the selection response in replicate 2 mice). Results for dopaminergic

drugs suggest that dopaminergic systems contribute more to the line difference in replicate 1 mice than in replicate 2 mice, and that a divergence in acute ethanol sensitivity can occur largely independent of changes in dopamine systems. However, it is also possible that the putative genetic correlation between acute locomotor sensitivity to some dopaminergic drugs (i.e., amphetamines) and ethanol is spurious, and arose through the random fixation of selection trait irrelevant genes in replicate 1 mice over the course of selective breeding. In addition, the finding that the correlated line difference to dopaminergic drugs arose later in selection than did the correlated line difference to GABAergic drugs, suggests that dopaminergic systems play a more minor role in the line difference to ethanol between the FAST and SLOW selected lines.

There is also neurochemical evidence that questions the exact contribution of NAc dopamine signaling to the behavioral effects of ethanol, including stimulation. As seen in Chapter 3, the magnitude of the locomotor stimulant response in FAST mice was large (approximately an 8000 cm increase in distance traveled over the first 30 min, or a 2000% increase in activity); however, the ethanol-induced increase in extracellular dopamine in the NAc was only 21%. This is in contrast to the effects of cocaine on locomotor activity relative to cocaine effects on extracellular dopamine levels in the NAc. Meyer and colleagues (submitted) reported a similarly profound increase in locomotor activity in FAST mice with cocaine, but the average increase in extracellular dopamine levels above baseline was approximately 400%. In addition, as seen in Chapter 3, ethanol induced similar increases in extracellular dopamine levels in FAST mice during both the first 30 min and second 30 min after ethanol administration. The stimulant response to ethanol, however, was significantly elevated during the first 30

min, but had dropped precipitously during the second 30 min, suggesting a mismatch between the time courses of the stimulant effects of ethanol and the dopamine response to ethanol. Finally, some studies have reported no difference in the magnitude of the ethanol-induced increase in dopamine signaling in the NAc in strains of mice that differ widely in ethanol consumption and ethanol-stimulated locomotor activity, among other behavioral traits (Liljequist and Ossowska, 1994; Zapata et al., 2006), although one recent study did report a difference in the same strains (Kapasova and Szumlinski, 2008).

Discrepancies between the magnitude and time course of the stimulant response to ethanol and the magnitude and time course of the dopamine response, as well as the inability of baclofen to attenuate ethanol-induced increases in extracellular dopamine levels (Chapter3), lead to the conclusion that mesolimbic dopaminergic signaling in the NAc is either not involved in the behavioral stimulant response to ethanol, or more likely, is not the sole mediator of this response. Ethanol-induced increases in NAc dopamine signaling may still be involved in the initiation of the neurochemical and locomotor stimulant response to ethanol. As will be discussed in later sections, the lack of temporal resolution is a significant weakness of the *in vivo* microdialysis procedure, and prevents a microscopic analysis of specific aspects of the behavioral stimulant response in which dopamine signaling is involved. For example, acute increases in NAc dopamine signaling may facilitate the initiation of the stimulant response, which may be attenuated by baclofen pretreatment. However, further and more prolonged ethanol-induced increases in extracellular dopamine levels, which appear not to be reversed by baclofen, may not significantly contribute to the behavioral stimulant response in FAST mice. The following sections will examine both dopaminergic and non-dopaminergic pathways,

including parallel pathways to the VTA-NAc projection, which may contribute to the stimulant effects of ethanol.

The ventral tegmental area is critical to the locomotor stimulant response to ethanol

While the current results call into question the contribution of mesolimbic dopamine systems to the stimulant effects of ethanol, there is substantial evidence (albeit unpublished) to support an interaction of ethanol and $GABA_B$ receptors at the level of the ventral midbrain. Ethanol (2 g/kg) significantly increased extracellular dopamine levels in the NAc, an effect which was larger in FAST mice compared to SLOW mice and was attenuated by a partial electrolytic lesion of the VTA. Moreover, these partial electrolytic lesions of the VTA attenuated the stimulant response to ethanol in FAST mice, highlighting that the VTA is critical to the stimulant response to ethanol (Meyer et al., submitted; Meyer and Phillips, unpublished results). Additionally, the modulatory effects of baclofen on ethanol stimulation also appear to occur in the VTA. Microinjection of baclofen into the aVTA attenuated the stimulant response to ethanol, whereas microinjection of baclofen into the pVTA accentuated the stimulant response to ethanol in FAST mice (Boehm et al., 2002a). This apparent contribution of the VTA (or ventral midbrain) to the interaction of ethanol and $GABA_B$ receptors is further supported by the current results revealing a line difference in $GABA_B$ receptor function in the ventral midbrain. The microdialysis results, however, appear to contradict the proposed mechanism by which baclofen and other $GABA_B$ receptor agonists attenuate the stimulant and rewarding properties of ethanol and other drugs of abuse.

A few studies have suggested a VTA-dependent, but dopamine-independent, pathway involved in drug-induced locomotor stimulation and reward (Laviolette and van

der Kooy, 2001; Stinus et al., 1982). For example, the conditioned reinforcing effects of the $GABA_A$ receptor agonist muscimol, when infused into the VTA, were inhibited by dopamine receptor antagonists. However, both the locomotor stimulant effects of the $GABA_A$ receptor channel blocker picrotoxin and the conditioned reinforcing effects of the $GABA_A$ receptor antagonist bicuculline were not inhibited by dopamine receptor antagonists or by a lesion of the dopaminergic projection to the NAc. Due to the fact that ethanol acts as a positive modulator of $GABA_A$ receptors (Allan and Harris, 1987; Allan et al., 1991; Roberto et al., 2003; Suzdak et al., 1986a,b), I would predict that ethanol would be inducing its locomotor stimulant and hedonic effects via a dopamine-dependent pathway, similar to muscimol. However, these results also highlight that VTA signaling can occur independent of dopamine. For instance, the VTA sends GABAergic projections to the PPTg (Semba and Fibiger, 1992; Steininger et al., 1992), and inactivation of this nucleus inhibited the conditioned reinforcing effects of bicuculline (Laviolette and van der Kooy, 2004). The following sections will examine not only the contribution of the NAc to the acute stimulant effects of ethanol, but also of other limbic and motor regions which receive input from the VTA.

Contribution of the nucleus accumbens to acute ethanol sensitivity

Although the NAc is considered a key component of the limbic-motor interface responsible for drug-induced locomotion and the expression of motivated behaviors (Mogenson et al., 1980, 1988), there is some controversy regarding its specific contribution to locomotion. Direct and indirect dopamine receptor agonists injected into the NAc increase locomotor activity (Brudzynski et al., 1993; Boye et al., 2001; Campbell et al., 1997; Ikemoto, 2002; Sellings and Clarke, 2003), supporting a role of

dopamine signaling in the NAc to the mediation of forward locomotion. However, the NAc is not a homogenous structure. Medial aspects of the NAc, including the shell, project to the medial VP, as well as to a variety of limbic structures, including the VTA and the Amy. Conversely, the NAc core preferentially innervates the lateral VP and nigrostriatal/basal ganglia structures, including the CPu, GP, and SN (Heimer et al., 1991; Usuda et al., 1998; Zahm et al., 1999). Structurally, this may suggest a preferential involvement of the NAc core in motor output. However, there is some disagreement as to the role of the core vs. shell in the motor response to drugs of abuse. Dopamine infusion into the core elicited increases in locomotion (Campbell et al., 1997); however, other studies suggested either no difference between dopamine in the core and medial shell in locomotion, or a preferential contribution of the shell to locomotor stimulation (Heidbreder and Feldon, 1998; Ikemoto, 2002). Amphetamine- and cocaine-induced increases in extracellular dopamine were also found to be highest in the medial shell (Heidbreder and Feldon, 1998; Zocchi et al., 2003).

It is critical to highlight the regional diversity of the NAc as a potential confound to the current microdialysis results. The majority of microdialysis probes in the current study were localized to more medial aspects of the NAc. However, critical alterations in dopamine signaling by baclofen may be occurring in more lateral regions. Initial observations suggested that ethanol-induced increases in extracellular levels of dopamine were smaller or non-existent when probes were placed more laterally (data not shown), and therefore the majority of probes were intentionally localized to the medial shell and core. There were not enough samples collected, however, from lateral regions of the NAc to evaluate the data for a possible medial (shell)-lateral (core) difference in

dopamine responsivity. Other studies have found a regional difference in ethanol-induced elevations of extracellular dopamine, with greater elevations in the medial shell (Howard et al., 2008), but similar to the above stated behavioral evidence for NAc core vs. shell effects, these dialysate differences are not always seen (Zocchi et al., 2003). Therefore, ethanol may induce elevations in extracellular dopamine levels preferentially in the medial shell of the NAc, but it is conceivable that baclofen could have heterogeneous effects on dopamine signaling and preferentially reduce extracellular dopamine levels in more lateral regions.

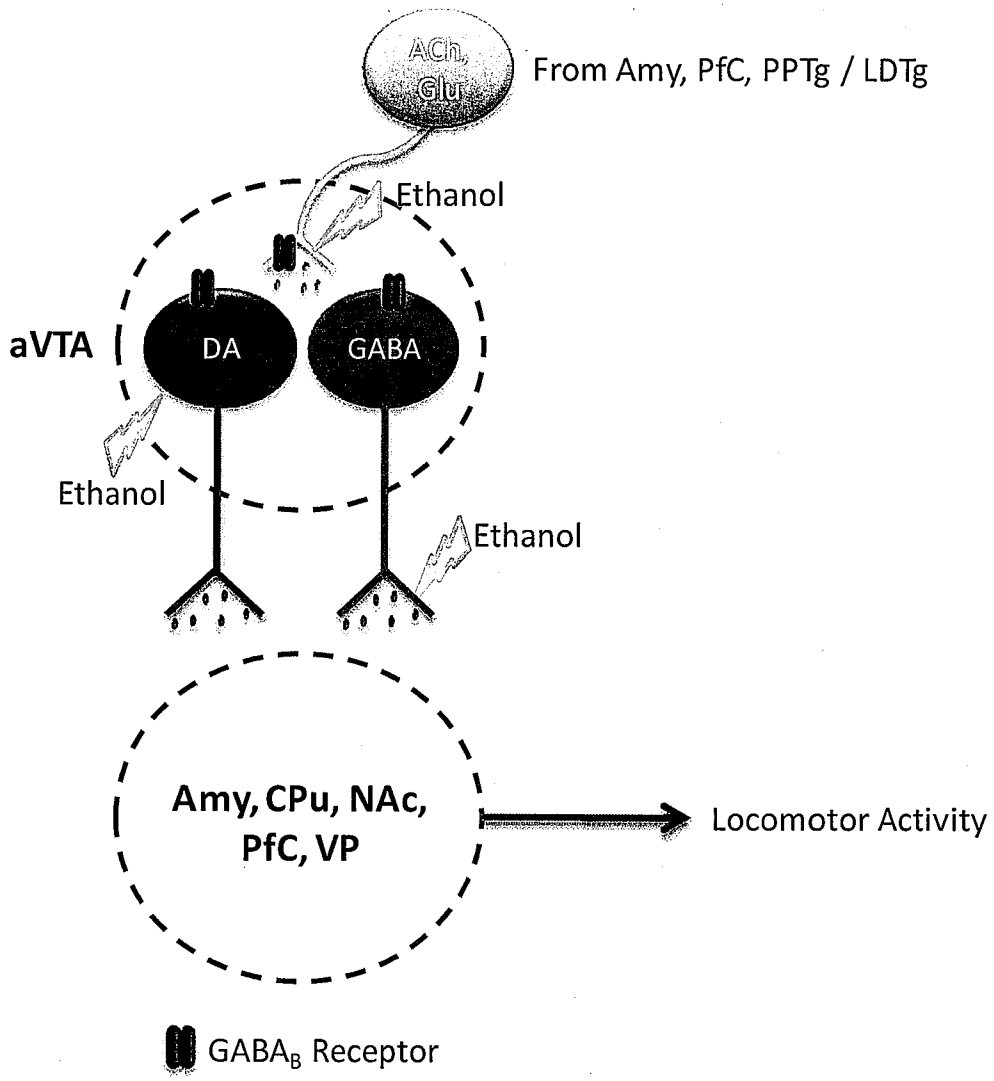
Another possibility is that dopamine signaling within the NAc is being modulated by baclofen, but these alterations cannot be observed by traditional microdialysis procedures since they may not have the necessary temporal sensitivity. The benefits and limitations of *in vivo* microdialysis procedures have been reviewed extensively elsewhere (Fillenz, 2005; Jones et al., 1999; Robinson et al., 2003, 2008). Briefly, microdialysis does allow for a great deal of chemical sensitivity, however, one limitation is that relatively large sample volumes and slow perfusion rates are required, resulting in dialysate samples having to be collected for 10-30 min to obtain a sample of adequate volume. Therefore, rapid and transient changes in dopamine levels are likely not observed unless the DAT has also been inhibited (Floresco et al., 2003; Lu et al., 1998). Obviously, ethanol induces long-term, diffuse increases in dopamine levels, as seen in the current study. It may be possible that baclofen actually attenuated dopamine signaling in our mice, but rather than changes in extracellular concentrations amassed over 15-30 min, which may represent tonic release, baclofen may only attenuate transient or phasic release. Observation of these rapid changes in dopamine signaling require more rapid

sampling techniques, such as fast-scan cyclic voltammetry, which can record sub-second fluctuations in dopamine concentrations directly in tissue. Low doses of ethanol increase the frequency of dopamine transients in the NAc as measured by voltammetry, though this does appear to be site dependent (Cheer et al., 2007); the effect of baclofen on this measure has not been studied.

If baclofen is attenuating phasic dopamine release and decreasing the frequency of dopamine transients in the NAc, this may be occurring through a direct inhibition of dopamine neurons in the VTA, or through alterations of excitatory glutamatergic and cholinergic input to the VTA from the PfC, Amy, or PPTg/LDTg (see Fig 13). Activation of $GABA_B$ receptors within the VTA may inhibit dopamine neurons, reducing burst firing and decreasing dopamine transients in the NAc (and attenuating the stimulant response to ethanol). However, a direct effect of baclofen on dopamine neurons would likely be accompanied by a decrease in extracellular dopamine levels as measured by microdialysis. Baclofen could also limit excitatory input from the PfC, Amy, or LDTg/PPTg onto dopamine neurons within the VTA, which contribute to dopamine cell firing (Floresco et al., 2003; Lodge et al., 2006; Tong et al., 1996). Ethanol has been reported to increase glutamatergic transmission in the VTA (Xiao et al., in press), and activation of $GABA_B$ heteroreceptors on glutamatergic terminals may inhibit this excitatory synaptic transmission onto dopamine neurons (Boyes and Bolam, 2003; Manzoni and Williams, 1999). Inhibition of excitatory input to the VTA by presynaptic $GABA_B$ receptors could reduce ethanol-induced burst firing of dopamine neurons and attenuate phasic dopamine release in the NAc. This effect may preserve the number of spontaneously firing dopamine neurons that maintain tonic firing, resulting in no change

FIGURE 13. $GABA_B$ receptor localization in the VTA and potential interactions with ethanol.

Shown is a simplistic diagram of potential locations of $GABA_B$ receptors in the aVTA and where ethanol may be stimulating neural activity in this circuit. Briefly, the lightning bolts represent areas in which ethanol may be having direct stimulatory effects on cell firing and neurotransmitter release. For instance, ethanol may not only stimulate dopamine cell firing in the VTA, but may also alter glutamate release into the VTA and GABA release in projection fields of the VTA. These dopaminergic and GABAergic projections from the VTA may then activate or inhibit various target regions of the VTA, including the Amy, CPu, NAc, PfC, and VP, all of which may impact ethanol-stimulated locomotion in FAST mice.


ACh, Glu
From Amy, PfC, PPTg / LDTg
Ethanol
aVTA
DA
GABA
Ethanol
Ethanol
Amy, CPu, NAc, PfC, VP
Locomotor Activity
GABA$_B$ Receptor

to extracellular dopamine levels in the NAc.

Another possibility is that baclofen alters a GABAergic effect of ethanol in FAST mice. In rats, a significant portion of the projection from VTA to the NAc is GABAergic (Carr and Sesack, 2000; Fields et al., 2007), though there is some question of whether this is true in mice (Ford et al., 2006). However, determination of a possible GABAergic effect of ethanol, which is modulated by baclofen, is complicated by contradictory results as to whether dopamine and drugs of abuse inhibit (Bourdelais and Kalivas, 1992; Mogenson and Nielsen, 1983; Wise, 1998) or activate (Brady and O'Donnell, 2004; Gonon and Sundstrom, 1996; Grace et al., 2007) the NAc, thereby promoting locomotor activity. Peripheral ethanol administration has been found to inhibit neuronal firing in the NAc in rats; however, in the non-selectively bred rat strains that have been studied, ethanol predominantly elicits motor incoordination and sedation, so the relevance of this finding to stimulation is unknown (Criado et al., 1995). Ethanol could inhibit the NAc and induce locomotor stimulation by activating GABA input to the NAc from the VTA. While ethanol has been reported to decrease GABA cell firing in the VTA (Gallegos et al., 1999; Stobbs et al., 2004; Xiao et al., 2007), it has also been found to increase GABA release in this region (Theile et al., 2008) and may induce small increases in extracellular GABA levels in the NAc (Kapasova and Szumlinski, 2008).

Activation of $GABA_B$ receptors on GABA neurons within the VTA may limit this ethanol-induced increase in extracellular GABA levels in the NAc, thereby limiting the attenuation of NAc cell firing and reducing motor activity. However, ethanol-induced decreases in extracellular GABA levels in the NAc have also been reported (Piepponen et al., 2002), and VTA neurons projecting to the medial shell of the NAc primarily reside in

the posteromedial VTA (Ford et al., 2006; Ikemoto, 2007), an area where baclofen actually potentiated the motor stimulant effects of ethanol in FAST mice (Boehm et al., 2002a).

There is also evidence that the VTA may send a glutamatergic projection to the NAc. However, instead of being a separate projection, several studies have suggested that VTA dopamine neurons may co-release glutamate along with dopamine. For instance, in culture, VTA dopamine neurons form excitatory glutamatergic synaptic connections and are immunopositive for glutamate (Joyce and Rayport, 2000; Sulzer et al., 1998). In the NAc, VTA dopamine neurons form glutamatergic synaptic connections, and stimulation of the VTA leads to a rapid excitatory response. This fast response would be too rapid for dopamine signaling as it would require activation of more slowly activating GPCRs (Chuhma et al., 2004). Additionally, dopamine release in the NAc may modulate concurrent glutamatergic input to the NAc. In the striatum (CPu and NAc), dopamine receptors were found to be primarily located at extrasynaptic sites and away from dopaminergic synapses (Caillé et al., 1996; Hara and Pickel, 2005). Stimulation of the VTA, and consequent increases in extracellular dopamine levels in the NAc, reduced the excitatory response to PfC stimulation in the NAc, an effect which appeared to be due to a dopamine-induced activation of D2 receptors. The authors speculated that a reduction in the excitatory response to PfC stimulation occurred via an activation of dopamine heteroreceptors on glutamatergic corticostriatal neurons, which then inhibited glutamate release into the NAc (Brady and O'Donnell, 2004). Similar effects are seen with other sources of glutamatergic input to the NAc, including from the HPC and PfC (Mogenson et al., 1988). Therefore, dopamine signaling to the NAc may

actually modulate critical glutamatergic inputs into this region, selecting and maintaining strong and/or synchronous glutamatergic input while selectively inhibiting weak, erroneous, and/or irrelevant cortical, and environmental, input (Brady and O'Donnell, 2004; Mogenson et al., 1988).

Therefore, although ethanol may increase dopamine cell firing (Brodie et al., 1990, 1999; Gessa et al., 1985; Okamoto et al., 2006) and extracellular dopamine levels in the NAc (Di Chiara and Imperato, 1988; Howard et al., 2008; Imperato and Di Chiara, 1986; Yim et al., 1998; Yoshimoto et al., 1991), this ethanol effect may actually serve to alter local and efferent glutamatergic signaling. For example, dopamine release in the NAc may selectively facilitate certain glutamatergic inputs or neural circuits which drive forward locomotion. Baclofen, by inhibiting glutamatergic neurons and/or glutamate release (Lacey et al., 2005; Seabrook et al., 1991), or inhibiting phasic dopamine input to the NAc, may limit this selective activation of specific networks, thereby reducing locomotor stimulation. This is, of course, just a speculation, and the effects of ethanol on glutamatergic signaling are mixed. For instance, Kapasova and Szumlinski (2008) reported that acute ethanol exposure in ethanol stimulation-prone D2 mice, but not stimulation-resistant B6 mice, increased extracellular concentrations of both dopamine and glutamate in the NAc. However, another study reported a small reduction in extracellular glutamate levels after acute ethanol administration in rats (Piepponen et al., 2002), while Meyer and colleagues (submitted) found that acute ethanol administration did not alter extracellular glutamate levels in the NAc in FAST or SLOW mice. Therefore, the exact mechanism by which ethanol alters glutamate signaling, or by which

ethanol alters dopamine signaling that then modulates the activity of glutamatergic networks, is unknown.

Finally, it is possible that dopamine signaling to the NAc is not exclusively involved in the mediation of the stimulant response to ethanol. Partial lesions of the NAc did not attenuate the acute locomotor stimulant response to ethanol in FAST mice (Meyer and Phillips, unpublished results) or D2 mice (Gremel and Cunningham, 2008). In addition, elevations in extracellular dopamine levels in the NAc by ethanol do not always correspond with the behavioral stimulant response to ethanol across genotypes (Zapata et al., 2006) or in magnitude (Fig 12 of Chapter 3). Environmental factors that increased the motor response to amphetamine were not found to induce further increases in extracellular dopamine levels in the NAc (Badiani et al., 1998, 2000). Combined with the finding that selection-induced alterations to dopamine systems appear to have arisen later in selection and therefore may only have a minor contribution to the stimulant response to ethanol, these results suggest that dopamine signaling, particularly in the NAc, may not be necessary or exclusively involved in the stimulant response to ethanol. It is likely that parallel circuits contribute to the stimulant and neurochemical effects of ethanol, and a lack of a baclofen-induced attenuation of the (tonic) dopamine response to ethanol in the NAc does not necessarily negate a baclofen-induced alteration to parallel dopaminergic pathways projecting to other limbic and motor regions (see Fig 13). In fact, dopamine, GABA, or even glutamatergic signaling in other regions, including the VP, nigrostriatal pathway, Amy, and PfC may better reflect the magnitude of the stimulant response to ethanol.

Alternatives: the ventral pallidum

Another major output structure of the VTA is the VP (Albanese and Minciacchi, 1983; Fallon and Moore, 1978), a structure which sends projections back to the VTA, as well as to the MD thal, the SN, and the subthalamic nucleus, among other regions (Maurice et al., 1997; Phillipson, 1979; Zahm, 1989). Similar to the NAc, there is some debate as to whether activation of the mesolimbic dopamine pathway inhibits (Grace et al., 2007) or activates the VP. For instance, behavioral studies revealed that inhibition of the VP by GABA agonists reduced locomotor activity (Hooks and Kalivas, 1995; Kretschmer, 2000), whereas activation of the VP by picrotoxin induced a pronounced stimulant response (Jones and Mogenson, 1980). The VP may also modulate the stimulant response to drugs of abuse, as pharmacological inactivation and excitotoxic lesions of the VP attenuated the stimulant response to amphetamine (Mele et al., 1998). The contribution of the VP to forward locomotion is hypothesized to be dependent on an intact VTA-NAc-VP pathway (Hooks and Kalivas, 1995; Jones and Mogenson, 1980; Mogenson et al., 1980), and dopamine signaling in the NAc is proposed to decrease GABA signaling in the VP and increase locomotor activity (Mele et al., 1998; Mogenson and Nielsen, 1983; Wise, 1998). For instance, microinjection of dopamine into the NAc increased motor activity, an effect which was attenuated by an intra-VP microinjection of GABA and accentuated by an intra-VP microinjection of picrotoxin (Jones and Mogenson, 1980; Mogenson and Nielsen, 1983). Therefore, an inhibition of the NAc and disinhibition of the VP may be critical for the locomotor response to drugs of abuse.

However, direct projections from the VTA to the VP may also be critical for the stimulant response, and may explain the behavioral and neurochemical data observed in Chapter 3. Cocaine increased extracellular dopamine levels in the VP, and 6-OHDA

lesions of the VP induced a right-ward shift in the cocaine dose-response curve (Gong et al., 1997). Ethanol, at low to moderate doses, also increased extracellular levels in the VP in rats (Melendez et al., 2003). As dopamine neurons projecting from the VTA to various forebrain regions have been reported to be independent, and not simply collaterals from the same neuron (Fallon, 1981; Ford et al., 2006), ethanol could stimulate dopamine release in the VP in FAST mice, an effect which may be attenuated by the activation of $GABA_B$ receptors that are predominantly localized to VP-, but not NAc-, projecting dopamine neurons. And as dopamine receptor agonists increased motor activity when microinjected into the VP (Gong et al., 1999; Klitenick et al., 1992), a reduction in extracellular dopamine output in the VP by baclofen would likely result in a reduction in locomotor activity.

Despite these findings, temporary inactivation and lesions of the VP did not reverse the stimulant response to the NMDA receptor antagonist MK-801 (Mele et al., 1998). In addition, acute ethanol exposure did not increase extracellular GABA concentrations in the VP of rats (Cowen et al., 1998), suggesting that ethanol-induced alterations in GABA signaling to the VP may not be responsible for the stimulant response to ethanol, and may not be involved in the baclofen-induced attenuation of this response. Therefore, other systems may contribute to the induction and/or magnitude of the locomotor stimulant response to ethanol.

Alternatives: the nigrostriatal pathway

Another aspect of the motor system that may mediate the acute stimulant effects of ethanol, and may be modulated by baclofen pretreatment, is the nigrostriatal pathway. The anterolateral VTA projects not only to the lateral NAc, but also to the CPu in rats

(Ikemoto et al., 2007). Ethanol may be activating dopamine neurons preferentially in the aVTA and contributing to locomotor stimulation by increasing extracellular dopamine levels in the CPu and altering signaling in the nigrostriatal pathway. And as baclofen microinjected into the aVTA, but not pVTA, attenuated the stimulant response to ethanol in FAST mice (Boehm et al., 2002a), and lesions of the aVTA, rather than the pVTA, tended to be more efficacious at reducing the stimulant response to ethanol in FAST mice (Meyer and Phillips, unpublished results), this may support a direct contribution of the nigrostriatal pathway to the stimulant response to ethanol and its modulation by baclofen.

A few studies have supported a contribution of the nigrostriatal pathway to the locomotor stimulant response to psychostimulants, including amphetamine and cocaine. For instance, 6-OHDA lesions of the nigrostriatal pathway dramatically reduced the locomotor stimulant responses to amphetamine and cocaine in rats (Creese and Iversen, 1975; Roberts et al., 1975). A recent study confirmed these results in Pitx3-deficient mice, a genetic knockout which induces an almost complete degradation of the nigrostriatal pathway while maintaining a functional mesolimbic pathway (Beeler et al., 2008). However, few studies have examined the possible contribution of the nigrostriatal pathway and the basal ganglia to the locomotor stimulant effects of ethanol. When ethanol was microinfused into the SNr, an output structure of the nigrostriatal pathway and the basal ganglia, an increase in locomotor activity was found in rats (Arizzi-LaFrance et al., 2006). Beckstead and Phillips (submitted) also showed in an *in vitro* preparation that ethanol increased the firing rate of SNc dopamine neurons, an effect which was much larger in magnitude in FAST mice compared to SLOW mice. Therefore, ethanol, either via direct stimulatory effects on SNc dopamine neurons, or via

an activation of VTA dopamine neurons which project to the CPu (Ikemoto, 2007), may increase extracellular dopamine levels in the CPu and promote locomotor stimulation in FAST mice. This response may be selectively attenuated by baclofen by activating $GABA_B$ receptors on dopamine neurons in the aVTA.

However, there is some question as to whether the nigrostriatal system is significantly involved in the dopamine and locomotor stimulant response to drugs of abuse, including ethanol. Gessa and colleagues (1985) reported that while ethanol did increase the firing rate of SNc dopamine neurons in rats, this effect was significantly reduced as compared to the effect of ethanol on VTA dopamine neurons. Similarly, Imperato and Di Chiara (1986) reported that ethanol increased extracellular dopamine levels in the CPu, but again this effect was significantly smaller than the effect of ethanol on extracellular dopamine levels in the NAc. Finally, others have reported no effect of 6-OHDA lesions of the nigrostriatal pathway on the locomotor stimulant response to amphetamine, while similar lesions of the mesolimbic pathway did block this response (Kelly et al., 1975). Therefore, dopamine signaling in the mesolimbic pathway, rather than the nigrostriatal pathway, may be more important to the locomotor stimulant effects of drugs of abuse, including ethanol. Electrophysiology studies examining VTA dopamine neurons have not been performed in FAST and SLOW mice, which might indicate whether the difference in sensitivity to ethanol-induced increases in firing rate is brain region specific or a characteristic of dopamine neurons in these lines in general.

Alternatives: the amygdala

Dopamine neurons from the aVTA also project to the basolateral (BLA) and central (CeA) nuclei of the Amy (Albanese and Minciacchi, 1983; Fallon and Moore,

1978; Ford et al., 2006). Acute ethanol exposure increased Fos-like immunoreactivity (an immediate early gene that can be used as a precise marker of neuronal activation) in the CeA, an effect which was only observed in mice in which a stimulant response to ethanol was observed, including in FAST mice (Demarest et al., 1998, 1999; Hitzemann and Hitzemann, 1997). Despite this finding, Meyer and Phillips (unpublished results) found no effect of electrolytic lesions of the CeA on the locomotor stimulant response to ethanol in FAST mice. Conversely, Gremel and Cunningham (2008) reported a decrease in the stimulant response to ethanol in D2 mice with electrolytic lesions of the Amy; these lesions, however, expanded beyond the borders of the CeA and included the BLA among other subdivisions of the Amy. Therefore, the Amy may be involved in the mediation of the acute stimulant response to ethanol in FAST mice, but specific regions of the Amy that are involved in the stimulant response to ethanol are currently unclear.

The VTA projections to the Amy in mice may be predominantly dopaminergic (Ford et al., 2006), and inhibition of dopamine signaling in the Amy has been found to increase the locomotor stimulant response to amphetamine (Simon et al., 1988). Conversely, temporary inactivation of the BLA was found to attenuate the stimulant response to amphetamine and the D1-like receptor agonist SKF 38393, but not cocaine (McFarland et al., 2004; Rouillon et al., 2008). Ethanol has been found to increase extracellular dopamine levels in the Amy (Yoshimoto et al., 2000), but the contribution of this effect to stimulation is unknown. It is possible that $GABA_B$ receptors are regulating the firing rate of Amy-projecting dopamine neurons and not NAc-projecting dopamine neurons. However, Ford and colleagues (2006) reported a more prominent

regulation of NAc-projecting dopamine neurons (which appear to originate in the posteromedial VTA) by $GABA_B$ receptors versus BLA-projecting neurons.

Another possibility is that baclofen is altering GABAergic input to the Amy. Ethanol has repeatedly been found to increase GABAergic transmission within the Amy (Bajo et al., 2008; Roberto et al., 2003; Siggins et al., 2005; Zhu and Lovinger, 2006). Activation of presynaptic $GABA_B$ receptors in the Amy, or activation of $GABA_B$ receptors on GABA neurons in the VTA, would attenuate ethanol-induced elevations in GABA release, and consequently, reduce an ethanol-induced potentiation of $GABA_A$ receptor function (Zhu and Lovinger, 2006). Therefore, baclofen may reduce the stimulant response to ethanol by limiting the GABA mimetic effect of ethanol in the Amy. And since the Amy projects to a variety of limbic structures, including the NAc, PfC, and VTA, as well as the SN, alterations in signaling within the Amy by ethanol and baclofen could result in alterations in locomotor activity (Fudge and Haber, 2000; McBride, 2002; Zahm et al., 1999).

Alternatives: prefrontal cortex

Another region of the mesolimbic pathway that receives a significant dopaminergic and GABAergic input from the VTA is the PfC (Albanese and Minciacchi, 1983; Carr and Sesack, 2000; Fallon and Moore, 1978; Fields et al., 2007). The PfC in turn sends glutamatergic efferents back to the VTA, as well as to the Amy, CPu, NAc, MD thal, and the spinal cord (Gabbott et al., 2005). There is some mixed evidence that the PfC may be involved in the mediation of the acute stimulant response to drugs of abuse. For instance, excitotoxic lesions of the PfC attenuated the stimulant response to cocaine and MK-801, but not amphetamine (Lacroix et al., 1998; Sullivan and Gratton,

2002; Tzschentke and Schmidt, 1998). Amphetamine exposure, however, did increase extracellular dopamine concentrations in the PfC, yet 6-OHDA lesions of dopaminergic input to the PfC resulted in an increase in the stimulant response to amphetamine (Ventura et al., 2004). Therefore, the PfC may promote a locomotor stimulant response to some drugs of abuse, although the evidence for this is mixed. As ethanol increased extracellular dopamine levels, an effect which has also been seen at low ethanol doses in the PfC (Dazzi et al., 2002), and like MK-801, ethanol has antagonistic actions at NMDA receptors (Dildy-Mayfield and Leslie, 1991), a similar role of the PfC in ethanol-induced locomotor stimulation would be predicted. In addition, FAST and SLOW mice have been found to differ in sensitivity to MK-801 and to the NMDA receptor antagonist ketamine, suggesting possible alterations in glutamate systems during selection (Meyer and Phillips, 2003b; Shen and Phillips, 1998). However, the exact contribution of the PfC to acute ethanol sensitivity is not known. It is possible that baclofen could attenuate ethanol-induced increases in extracellular dopamine in the PfC (intra-VTA baclofen has previously been found to decrease extracellular dopamine levels in the PfC; Westerink et al., 1998). Conversely, ethanol has been found to inhibit cortical cell firing in the PfC, an effect which could be mediated, in part, by an ethanol-induced increase in GABAergic output to the PfC (Tu et al., 2007). If GABA systems contribute to an ethanol-induced inhibition of the PfC, then activation of $GABA_B$ receptors localized to GABA neurons in the VTA (particularly the aVTA) may limit this inhibitory effect of ethanol in the PfC. Whether this reversal of an ethanol-induced inhibition of the PfC contributes to the locomotor response to ethanol is unknown.

Another possible contribution of the PfC to the stimulant and rewarding effects of ethanol is its proposed actions as an integrator of temporally distinct, and behaviorally relevant, signals arising from the VTA. As stated earlier, there is increasing evidence that VTA dopamine neurons may use glutamate as a co-transmitter, and glutamate release from dopamine neurons may mediate the rapid excitatory effects of VTA stimulation (Chuhma et al., 2004; Joyce and Rayport, 2000; Lapish et al., 2007; Sulzer et al., 1998). Lavin and colleagues (2005) found that stimulation of the VTA in rats elicited a rapid excitatory postsynaptic response in the PfC, an effect which was not altered by dopamine D1- or D2-like receptor antagonists but was blocked by non-NMDA glutamate receptor antagonists. Additionally, this response was blocked by 6-OHDA lesions of the mesocortical dopamine pathway, supporting the hypothesis that this rapid excitatory response in the PfC was mediated by glutamate release from VTA dopamine neurons. Dopamine was not without actions of its own, however. Phasic stimulation of the VTA increased cortical dopamine levels and reduced the spontaneous firing rate of PfC pyramidal cells. Dopamine also accentuated the frequency of depolarization-induced firing in the PfC, an effect which was blocked by dopamine D1 and D2 receptor antagonists.

Dopamine cell firing has been linked not only to the rewarding and stimulatory effects of drugs of abuse, but also to the encoding of reward salience, as well as the encoding of change from expectation (Schultz, 2007; Schultz et al., 1997). These effects may actually be mediated, in part, by glutamatergic signaling to the PfC (among other regions), facilitating executive functioning, decision making, and the generation of discrete motor patterns. Dopamine release in the PfC may then exert a state-dependent

control of PfC networks, limiting the access of weak signals (by reducing spontaneous firing) while facilitating the access of strong, or concurrent, signals to specific PfC networks (Lavin et al., 2005; Lapish et al., 2007). However, as ethanol has been found to inhibit persistent cortical activity (Tu et al., 2007) as well as NMDA receptor-mediated excitatory postsynaptic potentials (Weitlauf and Woodward, 2008), ethanol may instead induce its stimulant response by disregulating the temporal encoding of dopamine and glutamate signals in the PfC.

Overall Conclusions and Implications

In this dissertation, I provide evidence supporting a contribution of GABA systems to acute ethanol sensitivity. Lines selectively bred for extreme sensitivity (FAST) and insensitivity (SLOW) to the locomotor stimulant effects of ethanol also differed in locomotor sensitivity to the GABA transporter inhibitor NO-711 (Chapter 2). Combined with evidence for a line difference in acute sensitivity to $GABA_A$ receptor positive modulators and a $GABA_B$ receptor agonist (Boehm et al., 2002a; Palmer et al., 2002a,b; Phillips et al., 1992; Shen et al., 1998), and the finding that many of these line differences arose early in selection (Phillips et al., 1992), these data provide strong evidence that some aspect of GABA system function was altered through the process of selection. Additionally, this supports the assertion that genes that influence acute sensitivity to GABA mimetic drugs pleiotropically influence acute sensitivity to ethanol. One aspect of GABA systems that does appear to have been altered is $GABA_B$ receptor function. Selection-induced alterations to $GABA_B$ receptor function were found in the striatum and ventral midbrain, but not in other brain regions that were examined because

they have been implicated in the rewarding, stimulant, and sedative effects of ethanol. A small increase in the relative efficacy of baclofen in the striatum of FAST mice may contribute to the stimulant response to ethanol by enhancing an ethanol-induced inhibition of the striatum. However, line differences in basal [^{35}S]GTPγS binding, $GABA_B$ receptor density and relative agonist occupancy of the receptor may also impact this result. In the ventral midbrain, relatively decreased receptor function, as measured by agonist potency, in FAST mice likely also contributes to the stimulant response to ethanol by limiting the effect of local and afferent inhibitory input to mesolimbic neurons. This reduction in inhibitory input would promote an ethanol-induced activation of dopamine, or even GABA, cell firing and release. In addition, a more limited activation of $GABA_B$ heteroreceptors in the VTA, including $GABA_B$ receptors on glutamatergic terminals, would facilitate ethanol-induced increases in glutamatergic transmission in the VTA, further contributing to an enhancement of ventral midbrain activity in FAST mice (Boyes and Bolam, 2003; Manzoni and Williams, 1999; Xiao et al., 2008).

Baclofen, by activating $GABA_B$ receptors and increasing receptor function, might then limit the stimulant response to ethanol in FAST mice. Higher doses of baclofen (likely higher doses than would be required to see alterations in ethanol-induced locomotion in SLOW mice) would increase the functional activation of the $GABA_B$ receptor and inhibit both excitatory transmission into the VTA and dopamine cell firing as a consequence of ethanol administration (Erhardt et al., 2002; Gallegos et al., 1999; Gessa et al., 1985; Olpe et al., 1977). Or at least this is the predominant hypothesis for the effect of baclofen on the neurochemical and behavioral response to ethanol.

Pretreatment with baclofen alone did induce small, but nonsignificant, decreases in extracellular dopamine in the NAc of FAST mice (Chapter 3), which may support a baclofen-induced inhibition of dopamine signaling. Studies in rats support a prominent localization of $GABA_B$ receptors to dopamine neurons in the VTA, as well as in the SN (Boyes and Bolam, 2003; Wirtshafter and Sheppard, 2001). Further studies (as described in greater detail at the end of this chapter) are required to determine the specific cellular location of $GABA_B$ receptors in the VTA in FAST mice. However, microdialysis and electrophysiological studies have supported a tight regulation of dopaminergic activity by $GABA_B$ receptors in the VTA (Chen et al. 2005; Cruz et al., 2004; Erhardt et al., 2002; Klitenick et al. 1992; Olpe et al., 1977; Westerink et al. 1996). Moreover, baclofen attenuated the stimulant and dopamine response to a variety of drugs of abuse (Brebner et al., 2005; Fadda et al., 2003; Kalivas et al., 1990), but did not attenuate the stimulant response to caffeine (De Luca et al., 2007), a drug which is not proposed to activate dopamine systems and a drug for which the FAST and SLOW lines are not differentially sensitive (Phillips et al., 1992). Therefore the finding that baclofen did not reduce ethanol-induced increases in extracellular dopamine levels in the NAc, an effect which is genetically correlated with acute stimulant sensitivity to ethanol (Meyer et al., submitted), is perplexing. Due to this prominent contribution of $GABA_B$ receptors to dopaminergic signaling in the VTA, it is likely that baclofen is altering some aspect of dopamine signaling, but this may be restricted to alterations in phasic signaling which are missed by microdialysis. It is also possible that an examination of the dopamine response to ethanol in FAST and SLOW mice would reveal little regional specificity (i.e., ethanol may increase extracellular dopamine levels in a variety of brain regions which receive input

from the VTA), making the line difference in the NAc more difficult to interpret. However, as $GABA_B$ receptors are also localized to GABAergic and glutamatergic terminals in the ventral midbrain (Boyes and Bolam, 2003; Manzoni and Williams, 1999), and the VTA sends GABAergic projections to an array of limbic and motor structures (Carr and Sesack, 2000; Fields et al., 2007; Klitenick et al., 1992; Van Bockstaele and Pickel, 1995), baclofen-induced alterations to non-dopaminergic systems may also contribute to the attenuation of the stimulant response to ethanol.

No matter the exact neurochemical mechanisms underlying the baclofen-induced attenuation of ethanol stimulation in FAST mice, it is clear from the data presented in this dissertation that baclofen attenuated the stimulant effects of ethanol with fewer undesirable side effects than other GABA mimetics which act, in part, at the $GABA_A$ receptor (Chapter 2). Unlike baclofen, pretreatment with NO-711 or muscimol shifted the behavioral response to ethanol towards greater intoxication. A similar effect may occur if compounds which have actions at $GABA_A$ receptors, many of which are being pursued as pharmacotherapies for alcohol use disorders, are combined with ethanol consumption, either in an attempt to stop drinking or to promote moderate consumption (Finney and Moos, 2006; Johnson et al., 2003, 2007; Marlatt and Witkiewitz, 2002). While this may achieve the desired result of reduced drinking, there are potentially serious health and safety implications if these $GABA_A$ acting compounds are combined with ethanol and enhance motor incoordination and intoxication. In addition, this combination may not elicit a desirable long-term outcome for treatment success. If this pharmacotherapy reduces ethanol consumption due to an enhancement of intoxication and not due to a reduction in the rewarding effects of ethanol or ethanol-withdrawal

symptoms, it might not be effective in promoting abstinence and preventing relapse. For instance, if the combination of a GABAergic agonist, such as a GABA transporter inhibitor, with ethanol enhances ethanol intoxication, it may initially reduce ethanol consumption due to an inability to consume large quantities of ethanol (i.e., due to sedation or the generalized enhancement of negative side effects of ethanol), or due to less ethanol needing to be consumed to achieve the desired state of intoxication. Problematically, tolerance may develop to this effect, and ethanol drinking may return to baseline levels. Moreover, a reduction in the rewarding properties of ethanol may be needed in order to reduce drinking and the risk of relapse. However, $GABA_B$ receptor agonists, at carefully titrated doses that do not promote motor incoordination and sedation, may be more effective at producing a long-term reduction in ethanol consumption, possibly by reducing the rewarding properties of ethanol rather than by shifting the behavioral response to ethanol towards greater intoxication and sedation. In addition, since baclofen may not induce dramatic alterations to tonic dopamine levels, which may prevent an anhedonic response typically seen with reductions to dopamine signaling (Wise, 2006; Wise et al., 1978), these findings further support the use of $GABA_B$ receptor agonists for the treatment of alcohol use disorders. Recently, a $GABA_B$ receptor positive modulator, which binds to an allosteric site on the $GABA_B$ receptor, has been tested for its behavioral side effect profile in rodents. Data suggest that this positive modulator may also reduce ethanol consumption in rats while minimizing side effects normally seen with the baclofen (i.e., generalized decreases in locomotor activity, hypothermia, decreases in cognition) (Cryan et al., 2004; Maccioni et al., 2007),

suggesting that targeting the $GABA_B$ receptor at an allosteric site may be most effective at treating alcohol use disorders due to its reduced side effect profile.

Finally, it is necessary to state that the studies conducted in this thesis were all performed in male mice; moreover, the majority of studies referenced also utilized primarily male mice and rats. Therefore, the effects reported here may be entirely dependent on sex, and a different pattern of results might be observed if females were examined. However, this seems unlikely given the lack of consistent interactions with sex in previous studies in FAST and SLOW mice that examined the stimulant effects of ethanol, correlated responses to GABAergic drugs, and inhibitory effects of baclofen on ethanol-induced locomotor stimulation. For instance, selective breeding of the FAST and SLOW lines was conducted in both sexes, and a sex difference was not found in either line for the stimulant response to ethanol early in selection (Crabbe et al., 1987). Analyses of the first 35 generations of selection identified non-systematic interactions with sex (found in only 3 selection generations) (Shen et al., 1995). No sex effect was found for the stimulant response to ethanol in FAST mice after selection was relaxed (Kamens and Phillips, 2008). As for the $GABA_A$ receptor positive modulator ALLO, in which brain ALLO levels are significantly lower in male vs. female mice (Finn et al., 2004), no line interaction with sex was observed (Palmer et al., 2002b). Finally, the attenuation of the stimulant effects of ethanol by baclofen in FAST mice was not found to differ between males and females (Boehm et al., 2002a), suggesting that similar behavioral, and perhaps neurochemical, effects would have been observed if female mice had been tested. However, additional studies would be needed to conclusively determine whether the current results can be generalized to females.

Future Directions

There are a multitude of future directions that will further clarify or extend the results of the studies presented in this dissertation. I will focus on those that I think are most pertinent to understanding how GABA systems have been altered through the process of selection, as well as those that help to further clarify the neural systems underlying acute ethanol sensitivity.

It is clear from the present results that selection of the FAST and SLOW lines has altered GABA systems; however, more work is necessary to determine how these systems have been changed. A line difference in sensitivity to the locomotor depressant effects of NO-711 suggests potential alterations in GAT1 density, which could be quantified by a GAT1 binding assay or western blot analysis. Alternatively, this difference may reflect line differences in GABA transporter function, which could be tested by [^{3}H]GABA uptake into synaptosomes prepared from FAST and SLOW brain tissue (Hu et al., 2004; Peng and Simantov, 2003). Conversely, this line difference to NO-711 may be due to a line difference in extracellular GABA concentrations as a consequence of blocking reuptake, an hypothesis that could be tested by *in vivo* microdialysis or quantification of GABA levels by HPLC in brain homogenates.

Most likely, this line difference reflects a difference in postsynaptic GABA receptor function. Due to their altered sensitivity to a range of $GABA_A$ receptor positive modulators, it is likely that $GABA_A$ receptor density, function, and/or subunit composition is altered in the FAST and SLOW lines. Moreover, the finding of an additive interaction of ethanol and muscimol in Chapter 2 supports the hypothesis that ethanol is acting, in part, through $GABA_A$ receptors (Allan and Harris, 1987; Suzdak et

al., 1986a,b). To determine whether this is in fact true, $GABA_A$ receptor density could be examined using labeled agonist binding (i.e., [^{3}H]flunitrazepam or [^{3}H]muscimol binding; Churchill et al., 1992; Harris and Allan, 1989). Receptor function, both basally and in response to ethanol and other $GABA_A$ receptor positive modulators, would be assessed by agonist-stimulated $^{36}Cl^-$ flux in microsac preparations from FAST and SLOW mice (Finn et al., 2006; Zahniser et al., 1992). Finally, the contribution of $GABA_A$ receptor subunit composition to the selection response could be assessed by an analysis of subunit mRNA or protein expression. However, there are at least 21 different $GABA_A$ receptor subunits (Enna, 2007; Sieghart and Sperk, 2002), making subunit composition analysis difficult. Using regional analyses, I would first determine whether line differences in expression existed for the α_1, γ_2, and δ subunits, due to their contribution to acute ethanol and benzodiazepine sensitivity (a correlated trait in the FAST and SLOW lines), as well as their involvement in the stimulant, incoordinating, and sedative effects of ethanol (Blednov et al., 2003; Crabbe et al., 2006; Harris et al., 1995; June et al., 2007; Kralic et al., 2003; Sigel et al., 1993; Wafford et al., 1991; Wallner et al., 2003).

As shown in Chapter 3, a line difference in ventral midbrain and striatal $GABA_B$ receptor function was found in the FAST and SLOW lines. Follow-up experiments are needed to determine whether the FAST and SLOW lines differ in $GABA_B$ receptor density in the striatum, though this line difference in receptor function in the ventral midbrain is likely not due to a line difference in receptor density (Boehm et al., 2002). Further experiments to determine potential mechanisms by which $GABA_B$ receptor function has been altered in these lines include isolating lipid raft membrane domains in these regions and determining line differences in the relative enrichment of $GABA_B$

receptors and/or G-proteins to these microdomains (Allen et al., 2007). Quantification of G_{α} subtype expression in these brain regions may also elucidate potential mechanisms underlying a selection-induced difference in $GABA_B$ receptor function (Milligan and Kostenis, 2006).

The results presented in this dissertation may call into question the contribution of mesolimbic dopamine systems to the acute stimulant response to ethanol, as baclofen decreased the locomotor stimulant response to ethanol but did not alter ethanol-induced increases in extracellular dopamine levels in the NAc. In order to determine whether baclofen is attenuating ethanol-induced increases in dopamine levels, but only in a temporally and spatially restricted manner that is not readily observed by microdialysis, future studies using fast-scan cyclic voltammetry with carbon electrodes localized to the NAc are required. These experiments would allow a sub-second analysis of changes in extracellular dopamine levels, thereby providing a more thorough analysis as to whether baclofen does alter dopamine signaling in the NAc (Cheer et al., 2007; Robinson et al., 2003, 2008). It would also be useful to determine the localization of $GABA_B$ receptors in the VTA of FAST mice using double immunofluorescence coupled with confocal laser microscopy (López-Bendito et al., 2002). By determining the relative colocalization of the $GABA_B$ receptor with dopaminergic, GABAergic, or even glutamatergic neurons in the VTA, I could determine which neurons baclofen may be predominantly inhibiting.

Another interpretation of the microdialysis results presented in Chapter 3 is that while ethanol does increase extracellular dopamine levels in the NAc, and this effect is correlated with increased sensitivity to the stimulant effects of ethanol (Meyer et al., submitted), it is not critical for the locomotor stimulant response. An ethanol-induced

increase in extracellular dopamine levels may also be occurring in other brain regions, and dopamine signaling in these regions may be more critical to the stimulant effects of ethanol in FAST mice. Further studies are required to determine the neurocircuitry underlying the stimulant response to ethanol and whether the NAc, or other brain regions, contribute to this behavioral response. Electrolytic lesions or temporary inactivation (by a microinjection of muscimol and baclofen) of subregions of the NAc or of other dopaminergic output structures, such as the Amy, subregions of the CPu, PfC, PPTg, and VP, may help determine the neural outputs of VTA activation that contribute to the stimulant response to ethanol.

Finally, additional behavioral studies are required to determine the functional consequence of an enhancement of ethanol-induced motor incoordination and intoxication by GABA mimetic compounds. As NO-711 (or muscimol) enhanced the intoxicant effects of ethanol, it stands to reason that if these drugs reduce ethanol drinking, this may be occurring simply because less ethanol is needed to reach the same state of intoxication, or due to the onset of potentially undesirable side effects. Presumably, tolerance may develop to this interaction of NO-711 and ethanol (see Nguyen et al., 2005); if so, ethanol consumption may return to baseline levels. By tracking the onset of tolerance to the ataxic effects of NO-711 and ethanol and comparing this to changes in ethanol consumption, it could be further determined whether this class of GABA mimetics holds promise as a pharmacotherapy for alcohol use disorders or whether any reductions in drinking are simply due to a shift in the behavioral response to ethanol towards greater intoxication. In addition, chronic pretreatment with NO-711 prior to ethanol consumption may eliminate its effectiveness in reducing ethanol

consumption (see Phillips et al., 1997; Schmitt et al., 2002). It would be particularly interesting to compare this to the effect of baclofen on ethanol consumption, as baclofen did not alter the motor incoordinating effects of ethanol. Therefore, a more thorough examination of any potential clinical implications to these pharmacotherapies could be determined.

REFERENCES

Adams JU, Careri JM, Efferen TR, Rotrosen J (2001) Differential effect of dopamine antagonists on locomotor activity, conditioned activity and conditioned place preference induced by cocaine in rats. Behav Pharmacol 12:603-611.

Addicott MA, Marsh-Richard DM, Mathias CW, Dougherty DM (2007) The biphasic effects of alcohol: comparisons of subjective and objective measures of stimulation, sedation, and physical activity. Alcohol Clin Exp Res 31:1883-1890.

Addolorato G, Caputo F, Capristo E, Colombo G, Gessa GL, Gasbarrini G (2000) Ability of baclofen in reducing alcohol craving and intake: II - preliminary clinical evidence. Alcohol Clin Exp Res 24:67-71.

Addolorato G, Caputo F, Capristo E, Domenicali M, Bernardi M, Janiri L, Agabio R, Colombo G, Gessa GL, Gasbarrini G (2002) Baclofen efficacy in reducing alcohol craving and intake: a preliminary double-blind randomized controlled study. Alcohol Alcohol 37:504-508.

Agabio R, Carai MAM, Lobina C, Pani M, Reali R, Vacca G, Gessa GL, Colombo G (2001) Alcohol stimulates motor activity in selectively bred Sardinian alcohol-preferring (sP), but not in Sardinian alcohol-nonpreferring (sNP), rats. Alcohol 23:123-126.

Aguayo LG (1990) Ethanol potentiates the $GABA_A$-activated Cl^- current in mouse hippocampal and cortical neurons. Eur J Pharmacol 187:127-130.

Aguayo LG, Peoples RW, Yeh HH, Yevenes GE (2002) $GABA_A$ receptors as molecular sites of ethanol action. Direct or indirect actions? Curr Top Med Chem 2:869-885.

Ahlenius S, Carlsson A, Engel J, Svensson T, Södersten P (1973) Antagonism by alpha methyltyrosine of the ethanol-induced stimulation and euphoria in man. Clin Pharmacol Ther 14:586-591.

Albanese A, Minciacchi D (1983) Organization of the ascending projections from the ventral tegmental area: a multiple fluorescent retrograde tracer study in the rat. J Comp Neurol 216:406-420.

Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366-375.

Allan AM, Burnett D, Harris RA (1991) Ethanol-induced changes in chloride flux are mediated by both $GABA_A$ and $GABA_B$ receptors. Alcohol Clin Exp Res 15:233-237.

Allan AM, Harris RA (1987) Acute and chronic ethanol treatments alter GABA receptor-operated chloride channels. Pharmacol Biochem Behav 27:665-670.

Allen Brain Atlas [database online]. Seattle, WA: Allen Institute for Brain Science; 2008. Available at: http://www.brain-map.org.

Allen JA, Halverson-Tamboli RA, Rasenick MM (2007) Lipid raft microdomains and neurotransmitter signaling. Nat Rev Neurosci 8:128-140.

American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders DSM-IV-TR. 4th ed (text revision). American Psychiatric AssoFciation, Washington.

Andrade R, Malenka RC, Nicoll RA (1986) A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus. Science 234:1261-1265.

Anstrom KK, Cromwell HC, Markowski T, Woodward DJ (2003) Effect of baclofen on alcohol and sucrose self-administration in rats. Alcohol Clin Exp Res 27:900-908.

Amalric M, Koob GF (1987) Depletion of dopamine in the caudate nucleus but not in nucleus accumbens impairs reaction-time performance in rats. J Neurosci 7:2129-2134.

Appel SB, Liu Z, McElvain MA, Brodie MS (2003) Ethanol excitation of dopaminergic ventral tegmental area neurons is blocked by quinidine. J Pharmacol Exp Ther 306:437-446.

Ariwodola OJ, Weiner JL (2004) Ethanol potentiation of GABAergic synaptic transmission may be self-limiting: role of presynaptic $GABA_B$ receptors. J Neurosci 24:10679-10686.

Arizzi-LaFrance MN, Correa M, Aragon CM, Salamone JD (2006) Motor stimulant effects of ethanol injected into the substantia nigra pars reticulate: importance of catalase-mediated metabolism and the role of acetaldehyde. Neuropsychopharmacology 31:997-1008.

Badiani A, Oates MM, Day HEW, Watson SJ, Akil H, Robinson TE (1998) Amphetamine-induced behavior, dopamine release, and c-*fos* mRNA expression: modulation by environmental novelty. J Neurosci 18:10579-10593.

Badiani A, Oates MM, Fraioli S, Browman KE, Ostrander MM, Xue C-J, Wolf ME, Robinson TE (2000) Environmental modulation of the response to amphetamine: dissociation between changes in behavior and changes in dopamine and glutamate overflow in the rat striatal complex. Psychopharmacology 151:166-174.

Bae M-K, Jeong D-K, Park N-S, Lee C-H, Cho B-H, Jang H-O, Yun I (2005) The effect of ethanol on the physical properties of neuronal membranes. Mol Cells 19:356-364.

Bailey KR, Rustay NR, Crawley JN (2006) Behavioral phenotyping of transgenic and knockout mice: practical concerns and potential pitfalls. ILAR J 47:124-131.

Bajo M, Cruz MT, Siggins GR, Messing R, Roberto M (2008) Protein kinase C epsilon mediation of CRF- and ethanol-induced GABA release in central amygdala. Proc Natl Acad Sci 105:8410-8415.

Becher A, White JH, McIlhinney RAJ (2001) The γ-aminobutyric acid receptor B, but not the metabotropic glutamate receptor type-1, associates with lipid rafts in the rat cerebellum. J Neurochem 79:787-795.

Becker HC (1988) Effects of the imidazobenzodiazepine Ro15-4513 on the stimulant and depressant actions of ethanol on spontaneous locomotor activity. Life Sci 43:643-650.

Becker HC, Hale RL (1989) Ethanol-induced locomotor stimulation in C57BL/6 mice following RO15-4513 administration. Psychopharmacology 99:333-336.

Beckstead MJ, Phillips TJ (submitted) Mice selectively bred for high or low alcohol-induced locomotion exhibit differences in dopamine neuron function. J Pharmacol Exp Ther.

Beeler JA, Cao ZFH, Kheirbek MA, Zhuang X (2008) Loss of cocaine locomotor response in Pitx3-deficient mice lacking a nigrostriatal pathway. Neuropsychopharmacology: e-pub ahead of print.

Belelli D, Lambert JJ (2005) Neurosteroids: endogenous regulators of the $GABA_A$ receptor. Nat Rev Neurosci 6:565-575.

Belknap JK, Belknap ND, Berg JH, Coleman R (1977) Preabsorptive vs. postabsorptive control of ethanol intake in C57BL/6J and DBA/2J mice. Behav Genet 7:413-425.

Bergstrom HC, Palmer AA, Wood RD, Burkhart-Kasch S, McKinnon CS, Phillips TJ (2003) Reverse selection for differential response to the locomotor stimulant effects of ethanol provides evidence for pleiotropic genetic influence on locomotor response to other drugs of abuse. Alcohol Clin Exp Res 27:1535-1547.

Bernstein EM, Quick MW (1999) Regulation of γ-aminobutyric acid (GABA) transporters by extracellular GABA. J Biol Chem 274:889-895.

Besheer J, Cox AA, Hodge CW (2003) Coregulation of ethanol discrimination by the nucleus accumbens and amygdala. Alcohol Clin Exp Res 27:450-456.

Besheer J, Lepoutre V, Hodge CW (2004) GABA(B) receptor agonists reduce operant ethanol self-administration and enhance ethanol sedation in C57BL/6J mice. Psychopharmacology 174:358-366.

Bettler B, Tiao JY-H (2006) Molecular diversity, trafficking and subcellular localization of $GABA_B$ receptors. Pharmacol Ther 110:533-543.

Biswas B, Carlsson A (1978) Effect of intraperitoneally administered GABA on the locomotor activity of mice. Psychopharmacology 59:91-94.

Blednov YA, Jung S, Alva H, Wallace D, Rosahl T, Whiting PJ, Harris RA (2003a) Deletion of the α_1 or β_2 subunit of $GABA_A$ receptors reduces actions of alcohol and other drugs. J Pharmacol Exp Ther 304:30-36.

Blednov YA, Walker D, Alva H, Creech K, Findlay G, Harris RA (2003b) $GABA_A$ receptor α_1 and β_2 subunit null mutant mice: behavioral responses to ethanol. J Pharamcol Exp Ther 305:854-863.

Boehm II SL, Piercy MM, Bergstrom HC, Phillips TJ (2002a) Ventral tegmental area region governs $GABA_B$ receptor modulation of ethanol-stimulated activity in mice. Neuroscience 115:185-200.

Boehm II SL, Ponomarev I, Blednov YA, Harris RA (2006) From gene to behavior and back again: new perspectives on $GABA_A$ receptor subunit selectivity of alcohol actions. Adv Pharmacol 54:171-203.

Boehm II SL, Reed CL, McKinnon CS, Phillips TJ (2002b) Shared genes influence sensitivity to the effects of ethanol on locomotor and anxiety-like behaviors, and the stress axis. Psychopharmacology 161:54-63.

Boehm II SL, Schafer GL, Phillips TJ, Browman KE, Crabbe JC (2000) Sensitivity to ethanol-induced motor incoordination in 5-HT(1B) receptor null mutant mice is task-dependent: implications for behavioral assessment of genetically altered mice. Behav Neurosci 114:401-409.

Boileau I, Assaad J-M, Pihl RO, Benkelfat C, Leyton M, Diksic M, Tremblay RE, Dagher A (2003) Alcohol promotes dopamine release in the human nucleus accumbens. Synapse 49:226-231.

Booth RG, Baldessarini RJ, Kula NS, Gao Y, Zong R, Neumeyer JL (1990) Presynaptic inhibition of dopamine synthesis in rat striatal tissue by enantiomeric mono- and dihydroxyaporphines. Mol Pharmacol 38:92-101.

Borden LA (1996) GABA transporter heterogeneity: pharmacology and cellular localization. Neurochem Int 29:335-356.

Borghese CM, Harris RA (2007) Studies of ethanol actions on recombinant δ-containing γ-aminobutyric acid type A receptors yield contradictory results. Alcohol 41:155-162.

Borghese CM, Stórustovu SÍ, Ebert B, Herd MB, Belelli D, Lambert JJ, Marshall G, Wafford KA, Harris RA (2006) The δ subunit of γ-aminobutyric acid type A receptors does not confer sensitivity to low concentrations of ethanol. J Pharmacol Exp Ther 316:1360-1368.

Bourdelais A, Kalivas PW (1992) Apomorphine decreases extracellular GABA in the ventral pallidum of rats with 6-OHDA lesions in the nucleus accumbens. Brain Res 577:306-311.

Bowen CA, Purdy RH, Grant KA (1999) Ethanol-like discriminative stimulus effects of endogenous neuroactive steroids: effect of ethanol training dose and dosing procedure. J Pharmacol Exp Ther 289:405-411.

Bowery NG (2006) $GABA_B$ receptor: a site of therapeutic benefit. Curr Opin Pharmacol 6:37-43.

Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M, Bonner TI, Enna SJ (2002) International union of pharmacology. XXXIII. Mammalian γ-aminobutyric $acid_B$ receptors: structure and function. Pharmacol Rev 54:247-264.

Bowery NG, Enna SJ (2000) γ-aminobutyric $acid_B$ receptors: first of the functional metabotropic heterodimers. J Pharmacol Exp Ther 292:2-7.

Boyce JM, Risinger RO (2002) Dopamine D_3 receptor antagonist effects on the motivational effects of ethanol. Alcohol 28:47-55.

Boyce JM, Risinger RO (2000) Enhancement of ethanol reward by dopamine D3 receptor blockade. Brain Res 880:202-206.

Boye SM, Grant RJ, Clarke PBS (2001) Disruption of dopaminergic neurotransmission in nucleus accumbens core inhibits the locomotor stimulant effects of nicotine and D-amphetamine in rats. Neuropharmacology 30:792-805.

Boyes J, Bolam JP (2003) The subcellular localization of $GABA_B$ receptor subunits in the rat substantia nigra. Eur J Neurosci 18:3279-3293.

Brady AM, O'Donnell P (2004) Dopaminergic modulation of prefrontal cortical input to nucleus accumbens neurons *in vivo*. J Neurosci 24:1040-1049.

Brebner K, Ahn S, Phillips AG (2005) Attenuation of *d*-amphetamine self-administration by baclofen in the rat: behavioral and neurochemical correlates. Psychopharmacology 177:409-417.

Broadbent J, Harless WE (1999) Differential effects of $GABA_A$ and $GABA_B$ agonists on sensitization to the locomotor stimulant effects of ethanol in DBA/2J mice. Psychopharmacology 141:197-205.

Brodie MS, Appel SB (2000) Dopaminergic neurons in the ventral tegmental area of C57BL/6J and DBA/2J mice differ in sensitivity to ethanol excitation. Alcohol Clin Exp Res 24:1120-1124.

Brodie MS, Appel SB (1998) The effects of ethanol on dopaminergic neurons of the ventral tegmental area studied with intracellular recording in brain slices. Alcohol Clin Exp Res 22:236-244.

Brodie MS, Pesold C, Appel SB (1999) Ethanol directly excites dopaminergic ventral tegmental area reward neurons. Alcohol Clin Exp Res 23:1848-1852.

Brodie MS, Shefner SA, Dunwiddie TV (1990) Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. Brain Res 508:65-69.

Brudzynski SM, Wu M, Mogenson GJ (1993) Decreases in rat locomotor activity as a result of changes in synaptic transmission to neurons within the mesencephalic locomotor region. Can J Physiol Pharmacol 71:394-406.

Buck KJ, Harris RA (1990) Benzodiazepine agonist and inverse agonist actions on $GABA_A$ receptor-operated chloride channels. I. Acute effects of ethanol. J Pharmacol Exp Ther 253:706-712.

Caillé I, Dumartin B, Bloch B (1996) Ultrastructural localization of D1 dopamine receptor immunoreactivity in rat striatonigral neurons and its relation with dopaminergic innervations. Brain Res 730:17-31.

Camp DM, Browman KE, Robinson TE (1994) The effects of methamphetamine and cocaine on motor behavior and extracellular dopamine in the ventral striatum of Lewis versus Fischer 344 rats. Brain Res 668:180-193.

Campbell A, Villavicencio AT, Yeghiayan SK, Balikian R, Baldessarini RJ (1997) Mapping of locomotor behavioral arousal induced by microinjections of dopamine within nucleus accumbens septi of rat forebrain. Brain Res 771:55-62.

Carr DB, Sesack SR (2000) GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. Synapse 38:114-123.

Castelli MP, Pibiri F, Piras AP, Carboni G, Orrù A, Gessa GL, Carai MAM, Colombo G (2005) Differential G-protein coupling to $GABA_B$ receptor in limbic areas of alcohol-preferring and –nonpreferring rats. Eur J Pharmacol 523:67-70.

Chandler CJ, Starr BS, Starr MS (1990) Differential behavioural interactions between the dopamine D-1 antagonist SCH 23390 and the dopamine D-2 antagonists metoclopramide and sulpiride in nonhabituated mice. Pharmacol Biochem Behav 35:285-289.

Cheer JF, Wassum KM, Sombers LA, Heien MLAV, Ariansen JL, Aragona BJ, Phillips PEM, Wightman RM (2007) Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. J Neurosci 27:791-795.

Chen Y, Phillips K, Minton G, Sher E (2005) $GABA_B$ receptor modulators potentiate baclofen-induced depression of dopamine neuron activity in the rat ventral tegmental area. Br J Pharmacol 144:926-932.

Chester JA, Cunningham CL (1999a) Baclofen alters ethanol-stimulated activity but not conditioned place preference or taste aversion in mice. Pharmacol Biochem Behav 63:325-331.

Chester JA, Cunningham CL (1999b) $GABA_A$ receptors modulate ethanol-induced conditioned place preference and taste aversion in mice. Psychopharmacology 144:363-372.

Chester JA, Cunningham CL (2002) $GABA_A$ receptor modulation of the rewarding and aversive effects of ethanol. Alcohol 26:131-143.

Chiu C-S, Jensen K, Sokolova I, Wang D, Li M, Deshpande P, Davidson N, Mody I, Quick MW, Quake SR, Lester HA (2002) Number, density, and surface/cytoplasmic distribution of GABA transporters at presynaptic structures of knock-in mice carrying GABA transporter subtype 1-green fluorescent protein fusions. J Neurosci 22:10251-10266.

Chu DCM, Albin RL, Young AB, Penney JB (1990) Distribution and kinetics of $GABA_B$ binding sites in rat central nervous system: a quantitative autoradiographic study. Neuroscience 34:341-357.

Chuck TL, McLaughlin PJ, Arizzi-LaFrance MN, Salamone JD, Correa M (2006) Comparison between multiple behavioral effects of peripheral ethanol administration in rats: sedation, ataxia, and bradykinesia. Life Sci 79:154-161.

Chuhma N, Zhang H, Masson J, Zhuang X, Sulzer D, Hen R, Rayport S (2004) Dopamine neurons mediate a fast excitatory signal via their glutamatergic synapses. J Neurosci 24:972-981.

Church AC, Fuller JL, Dann L (1979) Alcohol intake in selected lines of mice. Importance of sex and genotype. J Comp Physiol Psychol 93:242-246.

Churchill L, Dilts RP, Kalivas PW (1992) Autoradiographic localization of γ-aminobutyric acid$_A$ receptors within the ventral tegmental area. Neurochem Res 17:101-106.

Cohen C, Perrault G, Sanger DJ (1997) Evidence for the involvement of dopamine receptors in ethanol-induced hyperactivity in mice. Neuropharmacology 36:1099-1108.

Cohen E, Feinn R, Arias A, Kranzler HR (2007) Alcohol treatment utilization: findings from the National Epidemiologic Survey on Alcohol and Related Conditions. Drug Alcohol Depend 86:214-221.

Colombo G, Agabio R, Carai MA, Lobina C, Pani M, Reali R, Addolorato G, Gessa GL (2000) Ability of baclofen in reducing alcohol intake and withdrawal severity: I-preclinical evidence. Alcohol Clin Exp Res 24:58-66.

Colombo G, Serra S, Brunetti G, Atzori G, Pani M, Vacca G, Addolorato G, Froestl W, Carai MAM, Gessa GL (2002) The GABA$_B$ receptor agonists baclofen and CGP 44532 prevent acquisition of alcohol drinking behavior in alcohol-preferring rats. Alcohol Alcohol 37:499-503.

Colombo G, Serra S, Vacca G, Carai MAM, Gessa GL (2006) Baclofen-induced suppression of alcohol deprivation effect in Sardinian alcohol-preferring (sP) rats exposed to different alcohol concentrations. Eur J Pharmacol 550:123-126.

Conti F, Melone M, De Biasi S, Minelli A, Brecha NC, Ducati A (1998) Neuronal and glial localization of GAT-1, a high-affinity γ-aminobutyric acid plasma membrane transporter, in human cerebral cortex: with a note on its distribution in monkey cortex. J Comp Neurol 396:51-63.

Cott J, Carlsson A, Engel J, Lindqvist M (1976) Suppression of ethanol-induced locomotor stimulation by GABA-like drugs. Naunyn Schmiedebergs Arch Pharmacol 295:203-209.

Cowen M, Chen F, Jarrott B, Lawrence AJ (1998) Effects of acute ethanol on GABA release and GABA$_A$ receptor density in the rat mesolimbic system. Pharmacol Biochem Behav 59:51-57.

Crabbe JC (1986) Genetic differences in locomotor activation in mice. Pharmacol Biochem Behav 25:289-292.

Crabbe JC (1999) Animal models in neurobehavioral genetics: methods for estimating genetic correlation, in Neurobehavioral Genetics: Methods and Applications (Mormede P, Jones BC eds), pp 121-138. CRC Press, Boca Raton, FL.

Crabbe JC, Deutsch CM, Tam BR, Young ER (1988) Environmental variables differentially affect ethanol-stimulated activity in selectively bred mouse lines. Psychopharmacology 95:103-108.

Crabbe JC, Janowsky JS, Young ER, Rigter H (1980) Strain-specific effects of ethanol on open field activity in inbred mice. Subst Alcohol Actions Misuse 1:537-543.

Crabbe JC, Metten P, Ponomarev I, Prescott CA, Wahlsten D (2006) Effects of genetic and procedural variation on measurement of alcohol sensitivity in mouse inbred strains. Behav Genet 36:536-552.

Crabbe JC, Phillips TJ, Harris RA, Arends MA, Koob GF (2006) Alcohol-related genes: contributions from studies with genetically engineered mice. Addict Biol 11:195-269.

Crabbe JC, Phillips TJ, Kosobud A, Belknap JK (1990) Estimation of genetic correlation: interpretation of experiments using selectively bred and inbred animals. Alcohol Clin Exp Res 14:141-151.

Crabbe JC, Young ER, Deutsch CM, Tam BR, Kosobud A (1987) Mice genetically selected for differences in open-field activity after ethanol. Pharmacol Biochem Behav 27:577-581.

Criado JR, Lee R-S, Berg GI, Henriksen SJ (1995) Sensitivity of nucleus accumbens neurons in vivo to intoxicating doses of ethanol. Alcohol Clin Exp Res 19:164-169.

Creese I, Iversen SD (1975) The pharmacological and anatomical substrates of the amphetamine response in the rat. Brain Res 83:419-436.

Criswell HE, Breese GR (2005) A conceptualization of integrated actions of ethanol contributing to its GABAmimetic profile: a commentary. Neuropsychopharmacology 30:1407-1425.

Criswell HE, McCown TJ, Ming Z, Mueller RA, Breese GR (1999) Interactive role for neurosteroids in ethanol enhancement of γ-aminobutyric acid-gated currents from dissociated substantia nigra reticulate neurons. J Pharmacol Exp Ther 291:1054-1059.

Criswell HE, Ming Z, Kelm MK, Breese GR (2008) Brain regional differences in the effect of ethanol on GABA release from presynaptic terminals. J Pharmacol Exp Ther 326:596-603.

Criswell HE, Overstreet DH, Rezvani AH, Johnson KB, Simson PE, Knapp DJ, Moy SS, Breese GR (1994) Effects of ethanol, MK-801, and chlordiazepoxide on locomotor activity in different rat lines: dissociation of locomotor stimulation from ethanol preference. Alcohol Clin Exp Res 18:917-923.

Cruz HG, Ivanova T, Lunn M-L, Stoffel M, Slesinger PA, Lüscher C (2004) Bi-directional effects of $GABA_B$ receptor agonists on the mesolimbic dopamine system. Nat Neurosci 7:153-159.

Cryan JF, Kelly PH, Chaperon F, Gentsch C, Mombereau C, Lingenhoehl K, Froestl W, Bettler B, Kaupmann K, Spooren WPJM (2004) Behavioral characterization of the novel $GABA_B$ receptor-positive modulator GS39783 (*N,N'*-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. J Pharmacol Exp Ther 310:952-963.

Cunningham CL, Niehus DR, Malott DH, Prather LK (1992) Genetic differences in the rewarding and activating effects of morphine and ethanol. Psychopharmacology 107:385-393.

Czachowski CL, Legg BH, Stansfield KH (2006) Ethanol and sucrose seeking and consumption following repeated administration of the $GABA_B$ agonist baclofen in rats. Alcohol Clin Exp Res 30:812-818.

Dai Q, Zhang J, Pruett SB (2005) Ethanol alters cellular activation and CD14 partitioning in lipid rafts. Biochem Biophys Res Commun 332:37-42.

Dalvi A, Rodgers RJ (1996) GABAergic influences on plus-maze behaviour in mice. Psychopharmacology 128:380-397.

Daoust M, Saligaut C, Lhuintre JP, Moore N, Flipo JL, Boismare F (1987) GABA transmission, but not benzodiazepine receptor stimulation, modulates ethanol intake by rats. Alcohol 4:469-472.

Dar MS (1995) Antagonism by intracerebellar Ro15-4513 of acute ethanol-induced motor incoordination in mice. Pharmacol Biochem Behav 52:217-223.

Dar MS (1996) Mouse cerebellar $GABA_B$ participation in the expression of acute ethanol-induced ataxia and in its modulation by the cerebellar adenosinergic A_1 system. Brain Res Bull 41:53-59.

Dar MS (2006) Co-modulation of acute ethanol-induced motor impairment by mouse cerebellar adenosinergic A_1 and $GABA_A$ receptor systems. Brain Res Bull 71:287-295.

Davies JA (1995) Mechanisms of action of antiepileptic drugs. Seizure 4:267-272.

Dawson DA, Goldstein RB, Grant BF (2007) Rates and correlates of relapse among individuals in remission from DSM-IV alcohol dependence: a 3-year follow up. Alcohol Clin Exp Res 31:2036-2045.

Dazzi L, Serra M, Seu E, Cherchi G, Pisu MG, Purdy RH, Biggio G (2002) Progesterone enhances ethanol-induced modulation of mesocortical dopamine neurons: antagonism by finasteride. J Neurochem 83:1103-1109.

De Blas AL, Vitorica J, Friedrich P (1988) Localization of the $GABA_A$ receptor in the rat brain with a monoclonal antibody to the 57,000 M_r peptide of the $GABA_A$ receptor/benzodiazepine receptor/Cl^- channel complex. J Neurosci 8:602-614.

De Luca MA, Bassareo V, Bauer A, Di Chiara G (2007) Caffeine and accumbens shell dopamine. J Neurochem 103:157-163.

Deguchi Y, Inabe K, Tomiyasu K, Nozawa K, Yamada S, Kimura R (1995) Study on brain interstitial fluid distribution and blood-brain barrier transport of baclofen in rats by microdialysis. Pharm Res 12:1838-1844.

Demarest K, Hitzemann B, Mahjubi E, McCaughran Jr. J, Hitzemann R (1998) Further evidence that the central nucleus of the amygdala is associated with the ethanol-induced locomotor response. Alcohol Clin Exp Res 22:1531-1537.

Demarest K, Hitzemann B, Phillips T, Hitzemann R (1999) Ethanol-induced expression of c-Fos differentiates the FAST and SLOW selected lines of mice. Alcohol Clin Exp Res 23:87-95.

Desai RI, Terry P, Katz JL (2005) A comparison of the locomotor stimulant effects of D_1-like receptor agonists in mice. Pharmacol Biochem Behav 81:843-848.

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci 85:5274-5278.

Dildy-Mayfield JE, Leslie SW (1991) Mechanism of inhibition of N-methyl-D-aspartate-stimulated increases in free intracellular Ca^{2+} concentration by ethanol. J Neurochem 56:1536-1543.

Dowining C, Carosone-Link P, Bennett B, Johnson TE (2006) QTL mapping for low-dose ethanol activation in the LXS recombinant inbred strains. Alcohol Clin Exp Res 30:1111-1120.

Dudek BC, Abbott ME, Phillips TJ (1984) Stimulant and depressant properties of sedative-hypnotics in mice selectively bred for differential sensitivity to ethanol. Psychopharmacology 82:46-51.

Ebert B, Thompson SA, Saounatsou K, McKernan R, Krogsgaard-Larsen P, Wafford KA (1997) Differences in agonist/antagonist binding affinity and receptor transduction using recombinant human γ-aminobutyric acid type A receptors. Mol Pharmacol 52:1150-1156.

Ekman G, Frankenhaeuser M, Goldberg L, Bjerver K, Järpe G, Myrsten A-L (1963) Effects of alcohol intake on subjective and objective variables over a five-hour period. Psychopharmacologia 4:28-38.

Ekman G, Frankenhaeuser M, Goldberg L, Hagdahl R, Myrsten A-L (1964) Subjective and objective effects of alcohol as functions of dosage and time. Psychopharmacologia 6:399-409.

Elmer GI, Meisch RA, Goldberg SR, George FR (1990) Ethanol self-administration in long sleep and short sleep mice indicates reinforcement is not inversely related to neurosensitivity. J Pharmacol Exp Ther 254:1054-1062.

Elsworth JD, Roth RH (1997) Dopamine autoreceptor pharmacology and function, in The Dopamine Receptors (Neve KA, Neve RL eds), pp 223-265. Humana Press, Totowa, NJ.

Engberg G, Kling-Petersen T, Nissbrandt H (1993) $GABA_B$-receptor activation alters the firing pattern of dopamine neurons in the rat substantia nigra. Synapse 15:229-238.

Engel J, Liljequist S (1983) The involvement of different central neurotransmitters in mediating stimulatory and sedative effects of ethanol, in Stress and Alcohol Use (Pohorecky LA, Brick J eds), pp 153-168. Elsevier Publishing Co., New York.

Enggasser JL, de Wit H (2001) Haloperidol reduces stimulant and reinforcing effects of ethanol in social drinkers. Alcohol Clin Exp Res 25:1448-1456.

Engleman EA, Ingraham CM, McBride WJ, Lumeng L, Murphy JM (2006) Extracellular dopamine levels are lower in the medial prefrontal cortex of alcohol-preferring rats compared to Wistar rats. Alcohol 38:5-12.

Enna SJ (2007) The GABA receptors, in The GABA Receptors (Enna SJ, Möhler H eds), pp 1-22.Humana Press, Totowa, NJ.

Erblich J, Earleywine M, Erblich B, Bovbjerg DH (2003) Biphasic stimulant and sedative effects of ethanol: are children of alcoholics really different? Addict Behav 28:1129-1139.

Erhardt S, Mathé JM, Chergui K, Engberg G, Svensson TH (2002) $GABA_B$ receptor-mediated modulation of the firing pattern of ventral tegmental area dopamine neurons in vivo. Naunyn-Schmiedeberg's Arch Pharmacol 365:173-180.

Erickson CK, Kochhar A (1985) An animal model of low dose ethanol-induced locomotor stimulation: behavioral characteristics. Alcohol Clin Exp Res 9:310-314.

Erkkila BE, SedeInikova AV, Weiss DS (2008) Stoichiometric pore mutations of the $GABA_AR$ reveal a pattern of hydrogen bonding with picrotoxin. Biophys J 94:4299-4306.

Fadda P, Scherma M, Fresu A, Collu M, Fratta W (2003) Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. Synapse 50:1-6.

Fallon JH (1981) Collateralization of monoamine neurons: mesotelencephalic dopamine projections to caudate, septum, and frontal cortex. J Neurosci 1:1361-1368.

Fallon JH, Moore RY (1978) Catecholamine innervations of the basal forebrain. IV. Topgraphy of the dopamine projection to the basal forebrain and neostriatum. J Comp Neurol 180:545-580.

Farb DH, Steiger JL, Martin SC, Gravielle MC, Gibbs TT, Russek SJ (2007) Mechanisms of $GABA_A$ and $GABA_B$ receptor gene regulation and cell surface expression, in The GABA Receptors (Enna SJ, Möhler H eds), pp 169-238.Humana Press, Totowa, NJ.

Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. Nat Rev Neurosci 6:215-229.

Fehr C, Hohmann N, Grunder G, Dielentheis TF, Buchholz HG, Chechko N, Yakushev I, Landvogt C, Bartenstein P, Urban R, Schreckenberger M (2007) Tiagabine does not attenuate alcohol-induced activation of the human reward system. Psychopharmacology 191:975-983.

Fields HL, Hjelmstad GO, Margolis EB, Nicola SM (2007) Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. Annu Rev Neurosci 30:289-316.

Fillenz M (2005) In vivo neurochemical monitoring and the study of behaviour. Neurosci Biobehav Rev 29:949-962.

Fink-Jensen A, Suzdak PD, Swedberg MDB, Judge ME, Hansen L, Nielsen PG (1992) The γ-aminobutyric acid (GABA) uptake inhibitor, tiagabine, increases extracellular brain levels of GABA in awake rats. Eur J Pharmacol 220:197-201.

Finn DA, Douglass AD, Beadles-Bohling AS, Tanchuck MA, Long SL, Crabbe JC (2006) Selected line difference in sensitivity to a GABAergic neurosteroid during ethanol withdrawal. Genes Brain Behav 5:53-63.

Finn DA, Sinnott RS, Ford MM, Long SL, Tanchuck MA, Phillips TJ (2004) Sex differences in the effect of ethanol injection and consumption on brain allopregnanolone levels in C57BL/6 mice. Neuroscience 123:813-819.

Finney JW, Moos RH (2006) Matching clients' treatment goals with treatment oriented towards abstinence, moderation or harm reduction. Addiction 101:1540-1542.

Flannery BA, Garbutt JC, Cody MW, Renn W, Grace K, Osborne M, Crosby K, Morreale M, Trivette A (2004) Baclofen for alcohol dependence: a preliminary open-label study. Alchol Clin Exp Res 28:1517-1523.

Floresco SB (2007) Dopaminergic regulation of limbic-striatal interplay. J Psychiatry Neurosci 32:400-411.

Floresco SB, Todd CL, Grace AA (2001) Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. J Neurosci 21:4915-4922.

Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nat Neurosci 6:968-973.

Ford CP, Mark GP, Williams JT (2006) Properties and opioid inhibition of mesolimbic dopamine neurons vary according to target location. J Neurosci 26:2788-2797.

Fritschy J-M, Benke D, Mertens S, Oertel WH, Bachi T, Möhler H (1992) Five subtypes of type A γ-aminobutyric acid receptors identified in neurons by double and triple immunofluorescence staining with subunit-specific antibodies. Proc Natl Acad Sci 89:6726-6730.

Frye GD, Breese GR (1981) An evaluation of the locomotor stimulating action of ethanol in rats and mice. Psychopharmacology 75:372-379.

Frye GD, Fincher A (1996) Sensitivity of postsynaptic $GABA_B$ receptors on hippocampal CA1 and CA3 pyramidal neurons to ethanol. Brain Res 735:239-248.

Fudge JL, Haber SN (2000) The central nucleus of the amygdala projection to dopamine subpopulations in primates. Neuroscience 97:479-494.

Gabbott PLA, Warner TA, Jays PRL, Salway P, Busby SJ (2005) Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. J Comp Neurol 492:145-177.

Gable RS (2004) Comparison of acute lethal toxicity of commonly abused psychoactive substances. Addiction 99:686-696.

Gadea A, López-Colomé AM (2001) Glial transporters for glutamate, glycine, and GABA: II. GABA transporters. J Neurosci Res 63:461-468.

Gallegos RA, Lee RS, Criado JR, Henriksen SJ, Steffensen SC (1999) Adaptive responses of γ-aminobutyric acid neurons in the ventral tegmental area to chronic ethanol. J Pharmacol Exp Ther 291:1045-1053.

Gariano RF, Groves PM (1988) Burst firing induced in midbrain dopamine neurons by stimulation of the medial prefrontal and anterior cingulated cortices. Brain Res 462:194-198.

Gassmann M, Shaban H, Vigot R, Sansig G, Haller C, Barbieri S, Humeau Y, Schuler V, Müller M, Kinzel B, Klebs K, Schmutz M, Froestl W, Heid J, Kelly PH, Gentry C, Jaton A-L, Van der Putten H, Mombereau C, Lecourtier L, Mosbacher J, Cryan JF, Fritschy J-M, Lüthi A, Kaupmann K, Bettler B (2004) Redistribution of $GABA_{B\,(1)}$ protein and atypical $GABA_B$ responses in $GABA_{B(2)}$-deficient mice. J Neurosci 24:6086-6097.

Gerasimov MR, Schiffer WK, Brodie JD, Lennon IC, Taylor SJC, Dewey SL (2000) γ-aminobutyric acid mimetic drugs differentially inhibit the dopaminergic response to cocaine. Eur J Pharmacol 395:129-135.

Gessa GL, Muntoni F, Collu M, Vargiu L, Mereu G (1985) Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res 348:201-203.

Gong W, Neill D, Justice Jr. JB (1997) 6-hydroxydopamine lesion of ventral pallidum blocks acquisition of place preference conditioning to cocaine. Brain Res 754:103-112.

Gong W, Neill DB, Lynn M, Justice Jr. JB (1999) Dopamine D_1/D_2 agonists injected into nucleus accumbens and ventral pallidum differentially affect locomotor activity depending on site. Neuroscience 93:1349-1358.

Gonon F, Sundstrom L (1996) Excitatory effects of dopamine released by impulse flow in the rat nucleus accumbens *in vivo*. Neuroscience 75:13-18.

Grace AA (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 41:1-24.

Grace AA, Bunney BS (1979) Paradoxical GABA excitation of nigral dopaminergic cells: indirect mediation through reticulate inhibitory neurons. Eur J Pharmacol 59:211-218.

Grace AA, Bunney BS (1980) Effects of baclofen on nigral dopaminergic cell activity following acute and chronic haloperidol treatment. Brain Res Bull 5(S2):537-543.

Grace AA, Bunney BS (1983) Intracellular and extracellular electrophysiology of nigral dopaminergic neurons – 1. Identification and characterization. Neuroscience 10:301-315.

Grace AA, Bunney BS (1984a) The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci 4:2877-2890.

Grace AA, Bunney BS (1984b) The control of firing pattern in nigral dopamine neurons: single spike firing. J Neurosci 4:2866-2876.

Grace AA, Bunney BS (1985) Opposing effects of striatonigral feedback pathways on midbrain dopamine cell activity. Brain Res 333:271-284.

Grace AA, Floresco SB, Goto Y, Lodge DJ (2007) Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. Trends Neurosci 30:220-227.

Grahame NJ, Rodd-Hendricks K, Li TK, Lumeng L (2000) Ethanol locomotor sensitization, but not tolerance correlates with selection for alcohol preference in high- and low-alcohol preferring mice. Psychopharmacology 151:252-260.

Grant BF, Dawson DA, Stinson FS, Chou SP, Dufour MC, Pickering RP (2004) The 12-month prevalence and trends in DSM-IV alcohol abuse and dependence: United States, 1991-1992 and 2001-2002. Drug Alcohol Depend 74:223-234.

Grant KA (1999) Strategies for understanding the pharmacological effects of ethanol with drug discrimination procedures. Pharmacol Biochem Behav 64:261-267.

Graybiel AM (2005) The basal ganglia: learning new tricks and loving it. Curr Opin Neurobiol 15:638-644.

Gremel CM, Cunningham CL (2008) Roles of the nucleus accumbens and amygdala in the acquisition and expression of ethanol-conditioned behavior in mice. J Neurosci 28:1076-1084.

Groenewegen HJ, Wright CI, Beijer AVJ, Voorn P (1999) Convergence and segregation of ventral striatal inputs and outputs. Ann NY Acad Sci 877:49-63.

Haber SN, Fudge JL, McFarland NR (2000) Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci 20:3269-2382.

Hamcock PJ, Stamford JA (1999) Stereospecific effects of ketamine on dopamine efflux and uptake in the rat nucleus accumbens. Br J Anaesth 82:603-608.

Hanchar HJ, Chutsrinopkun P, Meera P, Supavilai P, Sieghart W, Wallner M, Olsen RW (2006) Ethanol potently and competitively inhibits binding of the alcohol antagonist Ro15-4513 to $\alpha_{4/6}\beta_3\delta$ $GABA_A$ receptors. Proc Natl Acad Sci 103:8546-8551.

Hanchar HJ, Dodson PD, Olsen RW, Otis TS, Wallner M (2005) Alcohol-induced motor impairment caused by increased extrasynaptic $GABA_A$ receptor activity. Nat Neurosci 8:339-345.

Hara Y, Pickel VM (2005) Overlapping intracellular and differential synaptic distributions of dopamine D1 and glutamate N-methyl-D-aspartate receptors in rat nucleus accumbens. J Comp Neurol 492:442-455.

Harris RA, Allan AM (1985) Functional coupling of γ-aminobutyric acid receptors to chloride channels in brain membranes. Science 228:1108-1110.

Harris RA, Allan AM (1989) Genetic differences in coupling of benzodiazepine receptors to chloride channels. Brain Res 490:26-32.

Harris RA, Mihic SJ, Brozowski S, Hadingham K, Whiting PJ (1997) Ethanol, flunitrazepam, and pentobarbital modulation of $GABA_A$ receptors expressed in mammalian cells and *Xenopus* oocytes. Alcohol Clin Exp Res 21:444-451.

Harris RA, Proctor WR, McQuilkin SJ, Klein RL, Mascia MP, Whatley V, Whiting PJ, Dunwiddie TV (1995) Ethanol increases $GABA_A$ responses in cells stably transfected with receptor subunits. Alcohol Clin Exp Res 19:226-232.

Harris RA, Schroeder F (1981) Ethanol and the physical properties of brain membranes: fluorescence studies. Mol Pharmacol 20:128-137.

Harris RA, Schroeder F (1982) Effects of barbiturates and ethanol on the physical properties of brain membranes. J Pharmacol Exp Ther 223:424-431.

Harrison C, Traynor JR (2003) The [^{35}S]GTPγS binding assay: approaches and applications in pharmacology. Life Sci 74:489-508.

Hauber W (1998) Involvement of basal ganglia transmitter systems in movement initiation. Prog Neurobiol 56:507-540.

Heidbreder C, Feldon J (1998) Amphetamine-induced neurochemical and locomotor responses are expressed differentially across the anteroposterior axis of the core and shell subterritories of the nucleus accumbens. Synapse 29:310-322.

Heien MLAV, Wightman RM (2006) Phasic dopamine signaling during behavior, reward, and disease states. CNS Neurol Disord Drug Targets 5:99-108.

Heiman GA, Ogburn E, Gorroochurn P, Keyes KM, Hasin D (2008) Evidence for a two-stage model of dependence using the NESARC and its implications for genetic association studies. Drug Alcohol Depend 92:258-266.

Heimer L, Switzer RD, Van Hoesen GW (1982) Ventral striatum and ventral pallidum: components of the motor system? Trends Neurosci 5:83-87.

Heimer L, Zahm DS, Churchill L, Kalivas PW, Wohltmann C (1991) Specificity in the projection patterns of accumbal core and shell in the rat. Neuroscience 41:89-125.

Héja L, Karacs K, Kardos J (2006) Role for GABA and Glu plasma membrane transporters in the interplay of inhibitory and excitatory neurotransmission. Curr Top Med Chem 6:989-995.

Hevers W, Lüddens H (1998) The diversity of $GABA_A$ receptors. Mol Neurobiol 18:35-86.

Hitzemann R, Cipp L, Demarest K, Mahjubi E, McCaughran Jr. J (1998) Genetics of ethanol-induced locomotor activation: detection of QTLs in a C57BL/6J x DBA/2J F_2 intercross. Mamm Genome 9:956-962.

Hitzemann B, Hitzemann R (1997) Genetics, ethanol and the Fos response: a comparison of the C57BL/6J and DBA/2J inbred mouse strains. Alcohol Clin Exp Res 21:1497-1507.

Hodge CW, Nannini MA, Olive MF, Kelley SP, Mehmert KK (2001) Allopregnanolone and pentobarbital infused into the nucleus accumbens substitute for the discriminative stimulus effects of ethanol. Alcohol Clin Exp Res 25:1441-1447.

Hoffman DC, Beninger RJ (1985) The D1 dopamine receptor antagonist SCH 23390 reduces locomotor activity and rearing in rats. Pharmacol Biochem Behav 22:341-342.

Hoffman PL, Tabakoff B, Szabó G, Suzdak PD, Paul SM (1987) Effect of an imidazobenzodiazepine, Ro15-4513, on the incoordination and hypothermia produced by ethanol and pentobarbital. Life Sci 41:611-619.

Holdstock L, de Wit H (1998) Individual differences in the biphasic effects of ethanol. Alcohol Clin Exp Res 22:1903-1911.

Holdstock L, King AC, de Wit H (2000) Subjective and objective responses to ethanol in moderate/heavy and light social drinkers. Alcohol Clin Exp Res 24:789-794.

Holstein SE, Dobbs L, Phillips TJ (in press) Attenuation of the stimulant response to ethanol is associated with enhanced ataxia for a $GABA_A$ but not a $GABA_B$, receptor agonist. Alcohol Clin Exp Res.

Holstein SE, Pastor R, Meyer PJ, Phillips TJ (2005) Naloxone does not attenuate the locomotor effects of ethanol in FAST, SLOW, or two heterogeneous stocks of mice. Psychopharmacology 182:277-289.

Hooks MS, Kalivas PW (1995) The role of mesoaccumbens-pallidal circuitry in novelty-induced behavioral activation. Neuroscience 64:587-597.

Horvitz JC (2002) Dopamine gating of glutamatergic sensorimotor and incentive motivational input signals to the striatum. Behav Brain Res 137:65-74.

Howard EC, Schier CJ, Wetzel JS, Duvauchelle CL, Gonzales RA (2008) The shell of the nucleus accumbens has a higher dopamine response compared with the core after non-contingent intravenous ethanol administration. Neuroscience 154:1042-1053.

Howland JG, Taepavarapruk P, Phillips AG (2002) Glutamate receptor-dependent modulation of dopamine efflux in the nucleus accumbens by basolateral, but not central, nucleus of the amygdala in rats. J Neurosci 22:1137-1145.

Hu JH, Ma YH, Yang N, Mei ZT, Zhang MH, Fei J, Guo LH (2004) Up-regulation of gamma-aminobutyric acid transporter I mediates ethanol sensitivity in mice. Neuroscience 123:807-812.

Humeniuk RE, White JM, Ong J (1993) The role of $GABA_B$ receptors in mediating the stimulatory effects of ethanol in mice. Psychopharmacology 111:219-224.

Ikemoto S (2007) Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. Brain Res Rev 56:27-78.

Ikemoto S (2002) Ventral striatal anatomy of locomotor activity induced by cocaine, D-amphetamine, dopamine and D_1/D_2 agonists. Neuroscience 113:939-955.

Ikemoto S, Kohl RR, McBride WJ (1997) $GABA_A$ receptor blockade in the anterior ventral tegmental area increases extracellular levels of dopamine in the nucleus accumbens of rats. J Neurochem 69:137-143.

Ikemoto S, Wise RA (2004) Mapping of chemical trigger zones for reward. Neuropharmacology 47:190-201.

Imperato A, Di Chiara G (1986) Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J Pharmacol Exp Ther 239:219-228.

Jacob TC, Moss SJ, Jurd R (2008) $GABA_A$ receptor trafficking and its role in the dynamic modulation of neuronal inhibition. Nat Rev Neurosci 9:331-343.

Janak PH, Gill TM (2003) Comparison of the effects of allopregnanolone with direct GABAergic agonists on ethanol self-administration with and without concurrently available sucrose. Alcohol 30:1-7.

Jensen K, Chiu C-S, Sokolova I, Lester HA, Mody I (2003) GABA transporter-1 (GAT1)-deficient mice: differential tonic activation of $GABA_A$ versus $GABA_B$ receptors in the hippocampus. J Neurophysiol 90:2690-2701.

Jerlhag E (2008) The antipsychotic aripiprazole antagonizes the ethanol- and amphetamine-induced locomotor stimulation in mice. Alcohol 42:123-127.

Job MO, Tang A, Hall FS, Sora I, Uhl GR, Bergeson SE, Gonzales RA (2007) Mu (μ) opioid receptor regulation of ethanol-induced dopamine response in the ventral striatum: evidence of genotype specific sexual dimorphic epistasis. Biol Psychiatry 62:627-634.

Johnson BA, Ait-Daoud N, Bowden CL, DiClemente CC, Roache JD, Lawson K, Javors MA, Ma JZ (2003) Oral topiramate for treatment of alcohol dependence: a randomized controlled trial. Lancet 361:1677-1685.

Johnson BA, Rosenthal N, Capece JA, Wiegand F, Mao L, Beyers K, McKay A, Ait-Daoud N, Anton RF, Ciraulo DA, Kranzler HR, Mann K, O'Malley SS, Swift RM (2007) Topiramate for treating alcohol dependence: a randomized controlled trial. JAMA 298:1641-1651.

Johnson SW, North RA (1992a) Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 12:483-488.

Johnson SW, North RA (1992b) Two types of neurone in the rat ventral tegmental area and their synaptic inputs. J Physiol 450:455-468.

Jones DL, Mogenson GJ (1980) Nucleus accumbens to globus pallidus GABA projection subserving ambulatory activity. Amer J Physiol 238:R65-R69.

Jones DL, Mogenson GJ, Wu M (1981) Injections of dopaminergic, cholinergic, serotonergic and GABAergic drugs into the nucleus accumbens: effects on locomotor activity in the rat. Neuropharmacology 20:29-37.

Jones SR, Gainetdinov RR, Caron MG (1999) Application of microdialysis and voltammetry to assess dopamine functions in genetically altered mice: correlation with locomotor activity. Psychopharmacology 147:30-32.

Joyce MP, Rayport S (2000) Mesoaccumbens dopamine neuron synapses reconstructed in vitro are glutamatergic. Neuroscience 99:445-456.

June HL, Lewis MJ (1994) Interactions of Ro15-4513, Ro15-1788 (flumazenil) and ethanol on measures of exploration and locomotion in rats. Psychopharmacology 116:309-316.

June Sr HL, Foster KL, Eiler II WJA, Goergen J, Cook JB, Johnson N, Mensah-Zoe B, Simmons JO, June Jr HL, Yin W, Cook JM, Homanics GE (2007) Dopamine and benzodiazepine-dependent mechanisms regulate the EtOH-enhanced locomotor stimulation in the $GABA_A$ $\alpha 1$ subunit null mutant mice. Neuropsychopharmacology 32:137-152.

Kalivas PW (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Res Rev 18:75-113.

Kalivas PW, Duffy P, Eberhardt H (1990) Modulation of A10 dopamine neurons by γ-aminobutyric acid agonists. J Pharmacol Exp Ther 253:858-866.

Kamens HM, Phillips TJ (2008) A role for neuronal nicotinic acetylcholine receptors in ethanol-induced stimulation, but not cocaine- or methamphetamine-induced stimulation. Psychopharmacology 196:377-387.

Kapasova Z, Szumlinski KK (2008) Strain differences in alcohol-induced neurochemical plasticity: a role for accumbens glutamate in alcohol intake. Alcohol Clin Exp Res 32:617-631.

Katner SN, Weiss F (2001) Neurochemical characteristics associated with ethanol preference in selected alcohol-preferring and –nonpreferring rats: a quantitative microdialysis study. Alcohol Clin Exp Res 25:198-205.

Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, Beck P, Mosbacher J, Bischoff S, Kulik A, Shigemoto R, Karschin A, Bettler B (1998) $GABA_B$-receptor subtypes assemble into functional heteromeric complexes. Nature 396:683-687.

Kelley AE, Domesick VB, Nauta WJH (1982) The amygdalostriatal projection in the rat – an anatomical study by anterograde and retrograde tracing methods. Neuroscience 7:615-630.

Kelly PH, Seviour PW, Iversen SD (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 94:507-522.

Kelm MK, Criswell HE, Breese GR (2007) Calcium release from presynaptic internal stores is required for ethanol to increase spontaneous γ-aminobutyric acid release onto cerebellum purkinje neurons. J Pharmacol Exp Ther 323:356-364.

Keros S, Hablitz JJ (2005) Subtype-specific GABA transporter antagonists synergistically modulate phasic and tonic $GABA_A$ conductances in rat neocortex. J Neurophysiol 94:2073-2085.

King AC, Houle T, de Wit H, Holdstock L, Schuster A (2002) Biphasic alcohol response differs in heavy versus light drinkers. Alcohol Clin Exp Res 26:827-835.

Klitenick MA, DeWitte P, Kalivas PW (1992) Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an in vivo microdialysis study. J Neurosci 12:2623-2632.

Knight AR, Bowery NG (1996) The pharmacology of adenylyl cyclase modulation by $GABA_B$ receptors in rat brain slices. Neuropharmacology 35:703-712.

Koechling UM, Smith BR, Amit Z (1990) Differential effects of catecholamine antagonists on ethanol-induced excitation in mice. Psychopharmacology 102:234-238.

Koob GF (2004) A role for GABA mechanisms in the motivational effects of alcohol. Biochem Pharmacol 68:1515-1525.

Koob GF, Stinus L, Le Moal M (1981) Hyperactivity and hypoactivity produced by lesions to the mesolimbic dopamine system. Behav Brain Res 3:341-359.

Korpi ER, Debus F, Linden A-M, Malécot C, Leppä E, Vekovischeva O, Rabe H, Böhme I, Aller MI, Wisden W, Lüddens H (2007) Does ethanol act preferentially via selected brain $GABA_A$ receptor subtypes? The current evidence is ambiguous. Alcohol 41:163-176.

Koyama S, Brodie MS, Appel SB (2007) Ethanol inhibition of M-current and ethanol-induced direct excitation of ventral tegmental area dopamine neurons. J Neurophysiol 97:1977-1985.

Kralic JE, Wheeler M, Renzi K, Ferguson C, O'Buckley TK, Grobin AC, Morrow AL, Homanics GE (2003) Deletion of $GABA_A$ receptor α1 subunit-containing receptors alters responses to ethanol and other anesthetics. J Pharmacol Exp Ther 305:600-607.

Kretschmer BD (1999) Modulation of the mesolimbic dopamine system by glutamate: role of NMDA receptors. J Neurochem 73:839-848.

Kretschmer BD (2000) Functional aspects of the ventral pallidum. Amino Acids 19:201-210.

Krimmer EC, Schechter MD (1992) HAD and LAD rats respond differently to stimulating effect but not discriminative effects of ethanol.

Kulik Á, Nakadate K, Nyíri G, Notomi T, Malitschek B, Bettler B, Shigemoto R (2002) Distinct localization of $GABA_B$ receptors relative to synaptic sites in the rat cerebellum and ventrobasal thalamus. Eur J Neurosci 15:291-307.

Kulik Á, Vida I, Luján R, Haas CA, López-Bendito G, Shigemoto R, Frotscher M (2003) Subcellular localization of metabotropic $GABA_B$ receptor subunits $GABA_{B1a/b}$ and $GABA_{B2}$ in the rat hippocampus. J Neurosci 23:11026-11035.

Lacey CJ, Boyes J, Gerlach O, Chen L, Magill PJ, Bolam JP (2005) $GABA_B$ receptors at glutamatergic synapses in the rat striatum. Neuroscience 136:1083-1095.

Lacey MG, Mercuri NB, North RA (1988) On the potassium conductance increase activated by $GABA_B$ and dopamine D_2 receptors in rat substantia nigra neurones. J Physiol 401:437-453.

Lacroix L, Broersen LM, Weiner I, Feldon J (1998) The effects of excitotoxic lesion of the medial prefrontal cortex on latent inhibition, prepulse inhibition, food

hoarding, elevated plus maze, active avoidance and locomotor activity in the rat. Neuroscience 82:431-442.

Laitinen JT (1999) Selective detection of adenosine A_1 receptor-dependent G-protein activity in basal and stimulated conditions of rat brain [^{35}S]guanosine 5'-(γ-thio)triphosphate autoradiography. Neuroscience 90:1265-1279.

Lalonde R, Strazielle C (2007) Brain regions and genes affecting postural control. Prog Neurobiol 81:45-60.

Lapish CC, Kroener S, Durstewitz D, Lavin A, Seamans JK (2007) The ability of the mesocortical dopamine system to operate in distinct temporal modes. Psychopharmacology 191:609-625.

Larsson A, Svensson L, Söderpalm B, Engel JA (2002) Role of different nicotinic acetylcholine receptors in mediating behavioral and neurochemical effects of ethanol in mice. Alcohol 28:157-167.

Laviolette SR, van der Kooy D (2001) $GABA_A$ receptors in the ventral tegmental area control bidirectional reward signalling between dopaminergic and non-dopaminergic neural motivational systems. Eur J Neurosci 13:1009-1015.

Laviolette SR, van der Kooy D (2004) $GABA_A$ receptors signal bidirectional reward transmission from the ventral tegmental area to the tegmental pedunculopontine nucleus as a function of opiate state. Eur J Neurosci 20:2179-2187.

Lavin A, Nogueira L, Lapish CC, Wightman RM, Phillips PEM, Seamans JK (2005) Mesocortical dopamine neurons operate in distinct temporal domains using multimodal signaling. J Neurosci 25:5013-5023.

Lê AD, Tomkins D, Higgins G, Quan B, Sellers EM (1997) Effects of 5-HT_3, D_1 and D_2 receptor antagonists on ethanol- and cocaine-induced locomotion. Pharmacol Biochem Behav 57:325-332.

Lei S, McBain CJ (2003) $GABA_B$ receptor modulation of excitatory and inhibitory synaptic transmission onto rat CA3 hippocampal interneurons. J Physiol 546:439-453.

Leidenheimer NJ, Harris RA (1992) Acute effects of ethanol on $GABA_A$ receptor function: molecular and physiological determinants, in GABAergic Synaptic Transmission (Biggio G, Concas A, Costa E eds), pp 269-279. Raven Press, New York.

Leil TA, Chen Z-W, Chang C-SS, Olsen RW (2004) $GABA_A$ receptor-associated protein traffics $GABA_A$ receptors to the plasma membrane in neurons. J Neurosci 24:11429-11438.

Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, Boe AF, Boguski MS, Brockway KS, Byrnes EJ, Chen L, Chen L, Chen TM, Chin MC, Chong J, Crook BE, Czaplinska A, Dang CN, Datta S, Dee NR, Desaki AL, Desta T, Diep E, Dolbeare TA, Donelan MJ, Dong HW, Dougherty JG, Duncan BJ, Ebbert AJ, Eichele G, Estin LK, Faber C, Facer BA, Fields R, Fischer SR, Fliss TP, Frensley C, Gates SN, Glattfelder KJ, Halverson KR, Hart MR, Hohmann JG, Howell MP, Jeung DP, Johnson RA, Karr PT, Kawal R, Kidney JM, Knapik RH, Kuan CL, Lake JH, Laramee AR, Larsen KD, Lau C, Lemon TA, Liang AJ, Liu Y, Luong LT, Michales J, Morgan JJ, Morgan RJ, Mortrud MT, Mosqueda NF, Ng LL, Ng R, Orga GJ, Overly CC, Pak TH, Parry SE, Pathak SD, Pearson OC, Puchalski RB, Riley ZL, Rockett HR, Rowland SA, Royall JJ, Ruiz MJ, Sarno NR, Schaffnit K, Shapovalova NV, Sivisay T, Slaughterbeck CR, Smith SC, Smith KA, Smith BI, Sodt AJ, Stewart NN, Stumpf KR, Sunkin SM, Sutram M, Tam A, Teemer CD, Thaller C, Thompson CL, Varnam LR, Visel A, Whitlock RM, Wohnoutka PE, Wolkey CK, Wong VY, Wood M, Yaylaoglu MB, Young RC, Youngstrom BL, Yuan XF, Zhang B, Zwingman TA, Jones AR (2007) Genome-wide atlas of gene expression in the adult mouse brain. Nature 445:168-176.

Lewohl JM, Wilson WR, Mayfield RD, Brozowski SJ, Morrisett RA, Harris RA (1999) G-protein-coupled inwardly rectifying potassium channels are targets of alcohol action. Nat Neurosci 2:1084-1090.

Lhuillier L, Mombereau C, Cryan JF, Kaupmann K (2007) $GABA_B$ receptor positive modulation decreases selective molecular and behavioral effects of cocaine. Neuropsychopharmacology 32:388-398.

Liang J-H, Chen F, Krstew E, Cowen MS, Carroll FY, Crawfod D, Beart PM, Lawrence AJ (2006) The $GABA_B$ receptor allosteric modulator CGP7930, like baclofen, reduces operant self-administration of ethanol in alcohol-preferring rats. Neuropharmacology 50:632-639.

Liljequist S, Berggren U, Engel J (1981) The effect of catecholamine receptor antagonists on ethanol-induced locomotor stimulation. J Neural Transm 50:57-67.

Liljequist S, Engel J (1982) Effects of GABAergic agonists and antagonists on various ethanol-induced behavioral changes. Psychopharmacology 78:71-75.

Liljequist S, Ossowska K (1994) Genotypic differences in locomotor stimulation and dopaminergic activity following acute ethanol administration. Eur Neuropsychopharmacol 4:31-38.

Lisman JE, Grace AA (2005) The hippocampal-VTA loop: controlling the entry of information into long-term memory. Neuron 46:703-713.

Liu Y, Deitrich RA (1998) Role of GABA in the actions of ethanol in rats selectively bred for ethanol sensitivity. Pharmacol Biochem Behav 60:793-801.

Lodge DJ, Grace AA (2006) The laterodorsal tegmentum is essential for burst firing of ventral tegmental area dopamine neurons. Proc Natl Acad Sci 103:5167-5172.

López-Bendito G, Shigemoto R, Kulik Á, Paulsen O, Fairén A, Luján R (2002) Expression and distribution of metabotropic GABA receptor subtypes $GABA_BR1$ and $GABA_BR2$ during rat neocortical development. Eur J Neurosci 15:1766-1778.

López-Bendito G, Shigemoto R, Kulik Á, Vida I, Fairén A, Luján R (2004) Distribution of metabotropic GABA receptor subunits $GABA_{B1a/b}$ and $GABA_{B2}$ in the rat hippocampus during prenatal and postnatal development. Hippocampus 14:836-848.

Louis M, Clarke PBS (1998) Effect of ventral tegmental 6-hydroxydopamine lesions on the locomotor stimulant action of nicotine in rat. Neuropharmacology 37:1503-1513.

Lu Y, Peters JL, Michael AC (1998) Direct comparison of the response of voltammetry and microdialysis to electrically evoked release of striatal dopamine. J Neurochem 70:584-593.

Lüscher C, Jan LY, Stoffel M, Malenka RC, Nicoll RA (1997) G protein-coupled inwardly rectifying K^+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. Neuron 19:687-695.

Maccioni P, Pes D, Orrù A, Froestl W, Gessa GL, Carai MAM, Colombo G (2007) Reducing effect of the positive allosteric modulator of the $GABA_B$ receptor, GS39783, on alcohol self-administration in alcohol-preferring rats. Psychopharmacology 193:171-178.

Maccioni P, Serra S, Vacca G, Orrù A, Pes D, Agabio R, Addolorato G, Carai MAM, Gessa GL, Colombo G (2005) Baclofen-induced reduction of alcohol reinforcement in alcohol-preferring rats. Alcohol 36:161-168.

Mann K, Kiefer F, Spanagel R, Littleton J (2008) Acamprosate: recent findings and future research directions. Alcohol Clin Exp Res 32:1105-1110.

Manzoni OJ, Williams JT (1999) Presynaptic regulation of glutamate release in the ventral tegmental area during morphine withdrawal. J Neurosci 19:6629-6636.

Malcolm RJ (2003) GABA systems, benzodiazepines, and substance dependence. J Clin Psychiatry 64 (Suppl 3):36-40

Margeta-Mitrovic M, Jan YN, Jan LY (2001) Function of GB1 and GB2 subunits in G protein coupling of $GABA_B$ receptors. Proc Natl Acad Sci 98:14649-14654.

Marlatt GA (1983) The controlled-drinking controversy. A commentary. Am Psychol 38:1097-1110.

Marlatt GA, Witkiewitz K (2002) Harm reduction approaches to alcohol use: health promotion, prevention, and treatment. Addict Behav 27:867-886.

Martz A, Deitrich RA, Harris RA (1983) Behavioral evidence for the involvement of γ-aminobutyric acid in the actions of ethanol. Eur J Pharmacol 89:53-62.

Maurice N, Deniau JM, Menetrey A, Glowinski J, Thierry AM (1997) Position of the ventral pallidum in the rat prefrontal cortex-basal ganglia circuit. Neuroscience 80:523-534.

McBride WJ (2002) Central nucleus of the amygdala and the effects of alcohol and alcohol-drinking behavior in rodents. Pharmacol Biochem Behav 71:509-515.

McClearn GE (1991) The tools of pharmacogenetics, in The Genetic Basis of Alcohol and Drug Actions (Crabbe Jr. JC, Harris RA eds), pp 1-24. Plenum Press, New York.

McClearn GE, Kakihana R (1981) Selective breeding for ethanol sensitivity: short-sleep and long-sleep mice, in Development of Animal Models as Pharmacogenetic Tools (McClearn GE, Deitrich RA, Erwin VG eds), pp 147-159. NIAAA Research Monograph, Rockville, MD.

McClearn GE, Rodgers DA (1959) Differences in alcohol preference among inbred strains of mice. QJ Stud Alcohol 20:691-695.

McClearn GE, Wilson SG, Meredith W (1970) The use of isogenic and heterogenic mouse stocks in behavioral research, in Contributions to Behavior-Genetic Analysis: the Mouse as a Prototype (Thiessen DD ed), pp 3-22. Appleton-Century-Crofts, New York.

McFarland K, Davidge SB, Lapish CC, Kalivas PW (2004) Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. J Neurosci 24:1551-1560.

Mehta AK, Ravindran CRM, Ticku MK (2007) Low concentrations of ethanol do not affect radioligand binding to the δ-subunit-containing $GABA_A$ receptors in the rat brain. Brain Res 1165:15-20.

Mehta AK, Ticku MK (1994) Ethanol enhancement of GABA-induced $^{36}Cl^-$ influx does not involve changes in Ca^{2+}. Pharmacol Biochem Behav 47:355-357.

Mehta AK, Ticku MK (1988) Ethanol potentiation of GABAergic transmission in cultured spinal cord neurons involves γ-aminobutyric $acid_A$-gated chloride channels. J Pharmacol Exp Ther 246:558-564.

Mele A, Thomas DN, Pert A (1998) Different neural mechanisms underlie dizocilpine maleate- and dopamine agonist-induced locomotor activity. Neuroscience 82:43-58.

Melendez RI, Rodd-Henricks ZA, McBride WJ, Murphy JM (2003) Alcohol stimulates the release of dopamine in the ventral pallidum but not in the globus pallidus: a dual-probe microdialysis study. Neuropsychopharm 28:939-946.

Meng Z-H, Anwer J, Dar MS (1997) The striatal adenosinergic modulation of ethanol-induced motor incoordination in rats: possible role of chloride flux. Brain Res 776:235-245.

Meng Z-H, Dar MS (1994) Intrastriatal Ro15-4513 functionally antagonizes ethanol-induced motor incoordination and striatal adenosinergic modulation of ethanol-induced motor incoordination in rats. J Pharmacol Exp Ther 271:524-534.

Mercier D, Falardeau P, Lévesque D (2001) Autoreceptor preference of dopamine D_2 receptor agonists correlates with preferential coupling to cyclic AMP. Neuroreport 12:1473-1479.

Mereu G, Fadda F, Gessa GL (1984) Ethanol stimulates the firing rate of nigral dopaminergic neurons in unanesthetized rats. Brain Res 292:63-69.

Meyer PJ, Meshul CK, Phillips TJ (submitted) Ethanol- and cocaine-induced locomotion are genetically related to increases in accumbal dopamine. Genes Brain Behav.

Meyer PJ, Phillips TJ (2003a) Bivalent effects of MK-801 on ethanol-induced sensitization do not parallel its effects on ethanol-induced tolerance. Behav Neurosci 117:641-649.

Meyer PJ, Phillips TJ (2003b) Sensitivity to ketamine, alone or in combination with ethanol, is altered in mice selectively bred for sensitivity to ethanol's locomotor effects. Alcohol Clin Exp Res 27:1701-1709.

Mihic SJ (1999) Acute effects of ethanol on $GABA_A$ and glycine receptor function. Neurochem Int 35:115-123.

Miller G (2008) Tackling alcoholism with drugs. Science 320:168-170.

Milligan G, Kostenis E (2006) Heterotrimeric G-proteins: a short history. Br J Pharmacol 147:S46-S55.

Minelli A, Barbaresi P, Conti F (2003) Postnatal development of high-affinity plasma membrane GABA transporters GAT-2 and GAT-3 in the rat cerebral cortex. Brain Res Dev Brain Res 142:7-18.

Minelli A, Brecha NC, Karschin C, DeBiasi S, Conti F (1995) GAT-1, a high-affinity GABA plasma membrane transporter, is localized to neurons and astroglia in the cerebral cortex. J Neurosci 15:7734-7746.

Minelli A, DeBiasi S, Brecha NC, Zuccarello LV, Conti F (1996) GAT-3, a high-affinity GABA plasma membrane transporter, is localized to astrocytic processes, and is not confined to the vicinity of GABAergic synapses in cerebral cortex. J Neurosci 16:6255-6264.

Ming Z, Criswell HE, Yu G, Breese GR (2006) Competing presynaptic and postsynaptic effects of ethanol on cerebellar purkinje neurons. Alcohol Clin Exp Res 30:1400-1407.

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14:69-97.

Mogenson GJ, Nielsen MA (1983) Evidence that an accumbens to subpallidal GABAergic projection contributes to locomotor activity. Brain Res Bull 11:309-314.

Mogenson GJ, Nielsen M (1984) A study of the contribution of hippocampal-accumbens-subpallidal projections to locomotor activity. Behav Neural Biol 42:38-51.

Mogenson GJ, Yang CR, Yim CY (1988) Influence of dopamine on limbic inputs to the nucleus accumbens. Ann NY Acad Sci 537:86-100.

Moore EM, Serio KM, Goldfarb KJ, Stepanovska S, Linsenbardt DN, Boehm II SL (2007) GABAergic modulation of binge-like ethanol intake in C57BL/6J mice. Pharmacol Biochem Behav 88:105-113.

Morrow AL, Pace JR, Purdy RH, Paul SM (1990) Characterization of steroid interactions with γ-aminobutyric acid receptor-gated chloride ion channels: evidence for multiple steroid recognition sites. Mol Pharmacol 37:263-270.

Morzorati SL, Ramchandani VA, Flury L, Li TK, O'Connor S (2002) Self-reported subjective perception of intoxication reflects family history of alcoholism when breath alcohol levels are constant. Alcohol Clin Exp Res 26:1299-1306.

Mueller AL, Brodie MS (1989) Intracellular recording from putative dopamine-containing neurons in the ventral tegmental area of Tsai in a brain slice preparation. J Neurosci Methods 28:15-22.

National Institutes of Health (1986) Guidelines for the care and use of laboratory animals (DHEW Publication No. 86-203). US Government Printing Office, Washington DC.

Nevins ME, Nash SA, Beardsley PM (1993) Quantitative grip strength assessment as a means of evaluating muscle relaxation in mice. Psychopharmacology 110:92-96.

Newlin DB, Thomson JB (1991) Chronic tolerance and sensitization to alcohol in sons of alcoholics. Alcohol Clin Exp Res 15:399-405.

Newlin DB, Thomson JB (1999) Chronic tolerance and sensitization to alcohol in sons of alcoholics: II. replication and reanalysis. Exp Clin Psychopharmacol 7:234-243.

Ng TKY, Yung KKL (2000) Distinct cellular distribution of $GABA_BR1$ and $GABA_A\alpha1$ receptor immunoreactivity in the rat substantia nigra. Neuroscience 99:65-76.

Ng TKY, Yung KKL (2001) Subpopulations of neurons in rat substantia nigra display $GABA_BR2$ receptor immunoreactivity. Brain Res 920:210-216.

Nguyen SA, Deleon CP, Malcolm RJ, Middaugh LD (2005) Tiagabine reduces ethanol reward in C57BL/6 mice under acute and chronic administration regimens. Synapse 56:135-146.

Nisell M, Nomikos GG, Svensson TH (1994) Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. Synapse 16:36-44.

Nourissat P, Travert M, Chevanne M, Tekpli X, Rebillard A, Le Moigne-Müller G, Rissel M, Cillard J, Dimanche-Boitrel M-T, Lagadic-Gossmann D, Sergent O (2008) Ethanol induces oxidative stress in primary rat hepatocytes through the early involvement of lipid raft clustering. Hepatology 47:59-70.

Nusser Z, Sieghart W, Somogyi P (1998) Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. J Neurosci 18:1693-1703.

O'Dell LE, Alomary AA, Vallée M, Koob GF, Fitzgerald FL, Purdy RH (2004) Ethanol-induced increases in neuroactive steroids in the rat brain and plasma are absent in adrenalectomized and gonadectomized rats. Eur J Pharmacol 484:241-247.

O'Malley SS, Jaffe AJ, Rode S, Rounsaville BJ (1996) Experience of a "slip" among alcoholics treated with naltrexone or placebo. Am J Psychiatry 153:281-283.

Oakman SA, Faris PL, Kerr PE, Cozzari C, Hartman BK (1995) Distribution of pontomesencephalic cholinergic neurons projecting to substantia nigra differs significantly from those projecting to ventral tegmental area. J Neurosci 15:5859-5869.

Odagaki Y, Yamauchi T (2004) γ-hydroxybutyric acid, unlike γ-aminobutyric acid, does not stimulate G_i/G_o proteins in rat brain membranes. Basic Clin Pharmacol Toxicol 94:89-98.

Okada H, Matsushita N, Kobayashi K, Kobayashi K (2004) Identification of $GABA_A$ receptor subunit variants in midbrain dopaminergic neurons. J Neurochem 89:7-14.

Okamoto T, Harnett MT, Morikawa H (2006) Hyperpolarization-activated cation current (I_h) is an ethanol target in midbrain dopamine neurons of mice. J Neurophysiol 95:619-626.

Olpe H-R, Koella WP, Wolf P, Haas HL (1977) The action of baclofen on neurons of the substantia nigra and of the ventral tegmental area. Brain Res 134:577-580.

Olsen RW, Hanchar HJ, Meera P, Wallner M (2007) $GABA_A$ receptor subtypes: the "one glass of wine" receptors. Alcohol 41:201-209.

Omelchenko N, Sesack SR (2006) Cholinergic axons in the rat ventral tegmental area synapse preferentially onto mesoaccumbens dopamine neurons. J Comp Neurol 494:863-875.

Omelchenko N, Sesack SR (2005) Laterodorsal tegmental projections to identified cell populations in the rat ventral tegmental area. J Comp Neurol 483:217-235.

Päivärinta P, Korpi ER (1993) Voluntary ethanol drinking increases locomotor activity in alcohol-preferring AA rats. Pharmacol Biochem Behav 44:127-132.

Palmer AA, Low MJ, Grandy DK, Phillips TJ (2003) Effects of a *Drd2* deletion mutation on ethanol-induced locomotor stimulation and sensitization suggest a role for epistasis. Behav Genet 33:311-324.

Palmer AA, McKinnon CS, Bergstrom HC, Phillips TJ (2002a) Locomotor activity responses to ethanol, other alcohols, and GABA-A acting compounds in forward- and reverse-selected FAST and SLOW mouse lines. Behav Neurosci 116:958-967.

Palmer AA, Miller MN, McKinnon CS, Phillips TJ (2002b) Sensitivity to the locomotor stimulant effects of ethanol and allopregnanolone is influenced by common genes. Behav Neurosci 116:126-137.

Palmer AA, Phillips TJ (2002) Effect of forward and reverse selection for ethanol-induced locomotor response on other measures of ethanol sensitivity. Alcohol Clin Exp Res 26:1322-1329.

Palmer MR, Olson L, Dunwiddie TV, Hoffer BJ, Seiger Å (1984) Neonatal cerebellectomy alters ethanol-induced sleep time of short sleep but not long sleep mice. Pharmacol Biochem Behav 20:153-159.

Pastor R, Aragon CMG (2008) Ethanol injected into the hypothalamic arcuate nucleus induces behavioral stimulation in rats: an effect prevented by catalase inhibition and naltrexone. Behav Pharmacol 19:698-705.

Pastor R, Miquel M, Aragon CMG (2005) Habituation to test procedure modulates the involvement of dopamine D2- but not D1-receptors in ethanol-induced locomotor stimulation in mice. Psychopharmacology

Paul SM, Purdy RH (1992) Neuroactive steroids. FASEB J 6:2311-2322.

Paxinos G, Franklin KBJ (2001) The Mouse Brain in Stereotaxic Coordinates. 2nd ed. Academic Press, San Diego, CA.

Peng W, Simantov R (2003) Altered gene expression in frontal cortex and midbrain of 3,4-methylenedioxymethamphetamine (MDMA) treated mice: differential regulation of GABA transporter subtypes. J Neurosci Res 72:250-258.

Peris J, Wehner JM, Zahniser NR (1989) [^{35}S]TBPS binding sites are decreased in the colliculi of mice with a genetic predisposition to bicuculline-induced seizures. Brain Res 503:288-295.

Phillips TJ, Belknap JK, Hitzemann RJ, Buck KJ, Cunningham CL, Crabbe JC (2002a) Harnessing the mouse to unravel the genetics of human disease. Genes Brain Behav 1:14-26.

Phillips TJ, Broadbent J, Burkhart-Kasch S, Henderson C, Wenger CD, McMullin C, McKinnon CS, Cunningham CL (2005) Genetic correlational analyses of ethanol reward and aversion phenotypes in short-term selected mouse lines bred for

ethanol drinking or ethanol-inudced conditioned taste aversion. Behav Neurosci 119:892-910.

Phillips TJ, Burkhart-Kasch S, Gwiazdon CC, Crabbe JC (1992) Acute sensitivity of FAST and SLOW mice to the effects of abused drugs on locomotor activity. J Pharmacol Exp Ther 261:525-533.

Phillips TJ, Burkhart-Kasch S, Crabbe JC (1991a) Locomotor activity response to chronic ethanol treatment in selectively bred FAST and SLOW mice. Alcohol Alcohol Suppl 1:109-113.

Phillips TJ, Burkhart-Kasch S, Terdal ES, Crabbe JC (1991b) Response to selection for ethanol-induced locomotor activation: genetic analyses and selection response characterization. Psychopharmacology 103:557-566.

Phillips TJ, Crabbe Jr. JC (1991) Behavioral studies of genetic differences in alcohol action, in The Genetic Basis of Alcohol and Drug Actions (Crabbe Jr. JC, Harris RA eds), pp 25-104. Plenum Press, New York.

Phillips TJ, Dudek BC (1989) Modification of ethanol effects by bicuculline: genotype-dependent responses and inheritance. Psychopharmacology 98:549-555.

Phillips TJ, Dudek BC (1991) Locomotor activity responses to ethanol in selectively bred long- and short-sleep mice, two inbred mouse strains, and their F1 hybrids. Alcohol Clin Exp Res 15:255-261.

Phillips TJ, Huson M, Gwiazdon C, Burkhart-Kasch S, Shen EH (1995) Effects of acute and repeated ethanol exposures to the locomotor activity of BXD recombinant inbred mice. Alcohol Clin Exp Res 19:269-278.

Phillips TJ, Limm M, Crabbe JC (1989) Correlated behavioral responses as potential predictors of sensitivity to ethanol's rewarding effects and addiction liability, in Genetic Aspects of Alcoholism, Vol 37 (Kiianmaa K, Tabakoff B, Saito T eds), pp 197-206. The Finnish Foundation for Alcohol Studies, Helsinki, Finland.

Phillips TJ, Shen EH (1996) Neurochemical bases of locomotion and ethanol stimulant effects. Int Rev Neurobiol 39:243-282.

Phillips TJ, Shen EH, McKinnon CS, Burkhart-Kasch S, Lessov CN, Palmer AA (2002b) Forward, relaxed, and reverse selection for reduced and enhanced sensitivity to ethanol's locomotor stimulant effects in mice. Alcohol Clin Exp Res 26:593-602.

Phillips TJ, Wenger CD, Dorow JD (1997) Naltrexone effects on ethanol drinking acquisition and on established ethanol consumption in C57BL/6J mice. Alcohol Clin Exp Res 21:691-702.

Phillipson OT (1979) Afferent projections to the ventral tegmental area of tsai and interfascicular nucleus: a horseradish peroxidase study in the rat. J Comp Neurol 187:117-144.

Phillipson OT, Griffiths AC (1985) The topographic order of inputs to nucleus accumbens in the rat. Neuroscience 16:275-296.

Phillis BD, Ong J, White JM, Bonnielle C (2001) Modification of *d*-amphetamine-induced responses by baclofen in rats. Psychopharmacology 153:277-284.

Pickel VM, Chan J (1990) Spiny neurons lacking choline acetyltransferase immunoreactivity are major targets of cholinergic and catecholaminergic terminals in rat striatum. J Neurosci Res 25:263-280.

Piepponen TP, Kiianmaa K, Ahtee L (2002) Effects of ethanol on the accumbal output of dopamine, GABA and glutamate in alcohol-tolerant and alcohol-nontolerant rats. Pharmacol Biochem Behav 74:21-30.

Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) $GABA_A$ receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101:815-850.

Pohorecky LA (1977) Biphasic action of ethanol. Biobehav Revi 1:231-240.

Pow DV, Sullivan RKP, Williams SM, Scott HL, Dodd PR, Finkelstein D (2005) Differential expression of the GABA transporters GAT-1 and GAT-3 in brains of rats, cats, monkeys and humans. Cell Tissue Res 320:379-392.

Proctor WR, Allan AM, Dunwiddie TV (1992) Brain region-dependent sensitivity of $GABA_A$ receptor-mediated responses to modulation by ethanol. Alcohol Clin Exp Res 16:480-489.

Radcliffe RA, Bohl ML, Lowe MV, Cycowski CS, Wehner JM (2000) Mapping of quantitative trait loci for hypnotic sensitivity to ethanol in crosses derived from the C57BL/6 and DBA/2 mouse strains. Alcohol Clin Exp Res 24:1335-1342.

Reynolds JN, Prasad A, MacDonald JF (1992) Ethanol modulation of GABA receptor-activated Cl^- currents in neurons of the chick, rat and mouse central nervous system. Eur J Pharmacol 224:173-181.

Risinger FO, Malott DH, Prather LK, Niehus DR, Cunningham CL (1994) Motivational properties of ethanol in mice selectively bred for ethanol-induced locomotor differences. Psychopharmacology 116:207-216.

Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR (2003) Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. Proc Natl Acad Sci 100:2053-2058.

Roberts DCS, Zis AP, Fibiger HC (1975) Ascending catecholamine pathways and amphetamine-induced locomotor activity: importance of dopamine and apparent non-involvement of norepinephrine. Brain Res 93:441-454.

Robinson DL, Hermans A, Seipel AT, Wightman RM (2008) Monitoring rapid chemical communication in the brain. Chem Rev 108:2554-2584.

Robinson DL, Venton BJ, Heien MLAV, Wightman RM (2003) Detecting subsecond dopamine release with fast-scan cyclic voltammetry in vivo. Clin Chem 49:1763-1773.

Rodd ZA, Melendez RI, Bell RL, Kuc KA, Zhang Y, Murphy JM, McBride WJ (2004) Intracranial self-administration of ethanol within the ventral tegmental area of male Wistar rats: evidence for involvement of dopamine neurons. J Neurosci 24:1050-1057.

Rodd-Hendricks ZA, McKinzie DL, Crile RS, Murphy JM, McBride WJ (2000) Regional heterogeneity for the intracranial self-administration of ethanol within the ventral tegmental area of female Wistar rats. Psychopharmacology 149:217-224.

Rösner S, Leucht S, Lehert P, Soyka M (2008) Acamprosate supports abstinence, naltrexone prevents excessive drinking: evidence from a meta-analysis with unreported outcomes. J Psychopharmacol 22:11-23.

Rougé-Pont F, Usiello A, Benoit-Marand M, Gonon F, Piazza PV, Borrelli E (2002) Changes in extracellular dopamine induced by morphine and cocaine: crucial control by D2 receptors. J Neurosci 22:3293-3301.

Rouillon C, Abraini JH, David HN (2008) Prefrontal cortex and basolateral amygdala modulation of dopamine-mediated locomotion in the nucleus accumbens core. Exp Neurol 212:213-217.

Rubinstein M, Phillips TJ, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G, Fang Y, Larson JL, McDougall JA, Chester JA, Saez C, Pugsley TA, Gershanik O, Low MJ, Grandy DK (1997) Mice lacking dopamine D4 receptors are supersensitivie to ethanol, cocaine, and methamphetamine. Cell 90:991-1001.

Rustay NR, Wahlsten D, Crabbe JC (2003a) Assessment of genetic susceptibility to ethanol intoxication in mice. Proc Natl Acad Sci 100:2917-2922.

Rustay NR, Wahlsten D, Crabbe JC (2003b) Influence of task parameters on rotarod performance and sensitivity to ethanol in mice. Behav Brain Res 141:237-249.

Samson HH, Haraguchi M, Tolliver GA, Sadeghi KG (1989) Antagonism of ethanol-reinforced behavior by the benzodiazepine inverse agonists Ro15-4513 and FG 7142: relation to sucrose reinforcement. Pharmacol Biochem Behav 33:601-608.

Sanna E, Talani G, Busonero F, Pisu MG, Purdy RH, Serra M, Biggio G (2004) Brain steroidogenesis mediates ethanol modulation of $GABA_A$ receptor activity in rat hippocampus. J Neurosci 24:6521-6530.

Savinainen JR, Saario SM, Niemi R, Järvinen T, Laitinen J (2003) An optimized approach to study endocannabinoid signaling: evidence against constitutive activity of rat brain adenosine A_1 and cannabinoid CB_1 receptors. Br J Pharmacol 140:1451-1459.

Saxena NC, Macdonald RL (1994) Assembly of $GABA_A$ receptor subunits: role of the δ subunit. J Neurosci 14:7077-7086.

Scatton B, Simon H, Le Moal M, Bischoff S (1980) Origin of dopaminergic innervation of the rat hippocampal formation. Neurosci Lett 18:125-131.

Schechter MD, Krimmer EC (1992) Differences in response to the aversive properties and activity effects of low dose ethanol in LAS and HAS selectively bred rats. Psychopharmacology 107:564-568.

Schmitt U, Waldhofer S, Weigelt T, Hiemke C (2002) Free-choice ethanol consumption under the influence of GABAergic drugs in rats. Alcohol Clin Exp Res 26:457-462.

Schousboe A, Sarup A, Larsson OM, White HS (2004) GABA transporters as drug targets for modulation of GABAergic activity. Biochem Pharmacol 68:1557-1563.

Schuckit MA (1994) Low levels of response to alcohol as a predictor of future alcoholism. Am J Psychiatry 151:184-189.

Schuckit MA (1996) Recent developments in the pharmacotherapy of alcohol dependence. J Consult Clin Psychol 64:669-676.

Schuckit MA, Smith TL (2000) The relationships of a family history of alcohol dependence, a low level of response to alcohol and six domains of life functioning to the development of alcohol use disorders. J Stud Alcohol 61:827-835.

Schuckit MA, Smith TL (2001) Correlates of unpredicted outcomes in sons of alcoholics and controls. J Stud Alcohol 62:477-485.

Schuler V, Lüscher C, Blanchet C, Klix N, Sansig G, Klebs K, Schmutz M, Heid J, Gentry C, Urban L, Fox A, Spooren W, Jaton A-L, Vigouret J-M, Pozza M, Kelly PH, Mosbacher J, Froestl W, Käslin E, Korn R, Bischoff S, Kaupmann K, van der Putten H, Bettler B (2001) Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic $GABA_B$ responses in mice lacking $GABA_{B(1)}$. Neuron 31:47-58.

Schultz W (2007) Multiple dopamine functions at different time courses. Annu Rev Neurosci 30:259-288.

Schultz W (1986) Responses of midbrain dopamine neurons to behavioral trigger stimuli in the monkey. J Neurophysiol 56:1439-1461.

Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275:1593-1599.

Schwarzer C, Berresheim U, Pirker S, Wieselthaler A, Fuchs K, Sieghart W, Sperk G (2001) Distribution of the major gamma-aminobutyric $acid_A$ receptor subunits in the basal ganglia and associated limbic brain areas of the adult rat. J Comp Neurol 433:526-549.

Scibelli AC, Phillips TJ (accepted, pending minor revisions) Combined scopolamine and ethanol treatment results in a synergistic locomotor stimulant response not blocked by dopamine receptor antagonists. Alcohol Clin Exp Res.

Seabrook GR, Howson W, Lacey MG (1991) Subpopulations of GABA-mediated synaptic potentials in slices of rat dorsal striatum are differentially modulated by presynaptic $GABA_B$ receptors. Brain Res 562:332-334.

Seiger Å, Sorensen SM, Palmer MR (1983) Cerebellar role in the differential ethanol sensitivity of long sleep and short sleep mice. Pharmacol Biochem Behav 18:495-499.

Sellings LHL, Clarke PBS (2003) Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. J Neurosci 23:6295-6303.

Semba K, Fibiger HC (1992) Afferent connections of the laterodorsal and the pedunculopontine tegmental nuclei in the rat: a retro- and antero-grade transport and immunohistochemical study. J Comp Neurol 323:387-410.

Semyanov A, Walker MC, Kullmann DM (2003) GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. Nat Neuroci 6:484-490.

Serwanski DR, Miralles CP, Christie SB, Mehta AK, Li X, De Blas AL (2006) Synaptic and nonsynaptic localization of $GABA_A$ receptors containing the α5 subunit in the rat brain. J Comp Neurol 499:458-470.

Sesack SR, Pickel VM (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. J Comp Neurol 320:145-160.

Sharp T, Zetterström T, Ljungberg T, Ungerstedt U (1987) A direct comparison of amphetamine-induced behaviours and regional brain dopamine release in the rat using intracerebral dialysis. Brain Res 401:322-330.

Shen EH, Crabbe JC, Phillips TJ (1995) Dopamine antagonist effects on locomotor activity in naïve and ethanol-treated FAST and SLOW selected lines of mice. Psychopharmacology 118:28-36.

Shen EH, Dorow J, Harland R, Burkhart-Kasch S, Phillips TJ (1998) Seizure sensitivity and GABAergic modulation of ethanol sensitivity in selectively bred FAST and SLOW mouse lines. J Pharmacol Exp Ther 287:606-615.

Shen EH, Dorow JD, Huson M, Phillips TJ (1996) Correlated responses to selection in FAST and SLOW mice: effects of ethanol on ataxia, temperature, sedation, and withdrawal. Alcohol Clin Exp Res 20:688-696.

Shen EH, Harland RD, Crabbe JC, Phillips TJ (1995) Bidirectional selective breeding for ethanol effects on locomotor activity: characterization of FAST and SLOW mice through selection generation 35. Alcohol Clin Exp Res 19:1234-1245.

Shen EH, Phillips TJ (1998) MK-801 potentiates ethanol's effects on locomotor activity in mice. Pharmacol Biochem Behav 59:135-143.

Sieghart W, Sperk G (2002) Subunit composition, distribution and function of $GABA_A$ receptor subtypes. Curr Top Med Chem 2:795-816.

Sigel E, Baur R, Malherbe P (1993) Recombinant $GABA_A$ receptor function and ethanol. FEBS Lett 324:140-142.

Siggins GR, Roberto M, Nie Z (2005) The tipsy terminal: presynaptic effects of ethanol. Pharmacol Ther 107:80-98.

Simon H, Taghzouti K, Gozlan H, Studler JM, Louilot A, Herve D, Glowinski J, Tassin JP, Le Moal M (1988) Lesion of dopaminergic terminals in the amygdala

produces enhanced locomotor response to D-amphetamine and opposite changes in dopaminergic activity in prefrontal cortex and nucleus accumbens. Brain Res 447:335-340.

Sinclair JD (2001) Evidence about the use of naltrexone and for different ways of using it in the treatment of alcoholism. Alcohol Alcohol 36:2-10.

Smith BR, Boyle AEL, Amit Z (1999) The effects of the $GABA_B$ agonist baclofen on the temporal and structural characteristics of ethanol intake. Alcohol 17:231-240.

Smith GB, Olsen RW (1994) Identification of a [^{3}H]muscimol photoaffinity substrate in the bovine γ-aminobutyric $acid_A$ receptor α subunit. J Biol Chem 269:20380-20387.

Steininger TL, Rye DB, Wainer BH (1992) Afferent projections to the cholinergic pedunculopontine tegmental nucleus and adjacent midbrain extrapyramidal area in the albino rat. I. Retrograde tracing studies. J Comp Neurol 321:515-543.

Stinchcomb A, Bowers BJ, Wehner JM (1989) The effects of ethanol and Ro 15-4513 on elevated plus-maze and rotarod performance in long-sleep and short-sleep mice. Alcohol 6:369-376.

Stinus L, Herman JP, Le Moal M (1982) GABAergic mechanisms within the ventral tegmental area: involvement of dopaminergic (A10) and non-dopaminergic neurones. Psychopharmacology 77:186-192.

Stobbs SH, Ohran AJ, Lassen MB, Allison DW, Brown JE, Steffensen SC (2004) Ethanol suppression of ventral tegmental area GABA neuron electrical transmission involves *N*-methyl-D-aspartate receptors. J Pharmacol Exp Ther 311:282-289.

Stromberg MF (2004) The effect of baclofen alone and in combination with naltrexone on ethanol consumption in the rat. Pharmacol Biochem Behav 78:743-750.

Study RE, Barker JL (1981) Diazepam and (-)-pentobarbital: fluctuation analysis reveals different mechanisms for potentiation of γ-aminobutyric acid responses in cultured central neurons. Proc Natl Acad Sci 78:7180-7184.

Suh JJ, Pettinati HM, Kampman KM, O'Brien CP (2006) The status of disulfiram: a half of a century later. J Clin Psychopharmacol 26:290-302.

Sullivan RM, Gratton A (2002) Behavioral effects of excitotoxic lesions of ventral medial prefrontal cortex in the rat are hemisphere-dependent. Brain Res 927:69-79.

Sulzer D, Joyce MP, Lin L, Geldwert D, Haber SN, Hattori T, Rayport S (1998) Dopamine neurons make glutamatergic synapses *in vitro*. J Neurosci 18:4588-4602.

Sundstrom-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A, Wiedmann M, Williams K, Smith SS (2002) Hormonally regulated $\alpha_4\beta_2\delta$ $GABA_A$ receptors are a target for alcohol. Nat Neurosci 5:721-722.

Suzdak PD, Frederiksen K, Andersen KE, Sørensen PO, Knutsen LJS, Nielsen EB (1992) NNC-711, a novel potent and selective γ-aminobutyric acid uptake inhibitor: pharmacological characterization. Eur J Pharmacol 223:189-198.

Suzdak PD, Glowa JR, Crawley JN, Schwartz RD, Skolnick P, Paul SM (1986a) A selective imidazobenzodiazepine antagonist of ethanol in the rat. Science 234:1243-1247.

Suzdak PD, Paul SM, Crawley JN (1988) Effects of Ro15-4513 and other benzodiazepine receptor inverse agonists on alcohol-induced intoxication in the rat. J Pharmacol Exp Ther 245:880-886.

Suzdak PD, Schwartz RD, Skolnick P, Paul SM (1986b) Ethanol stimulates γ-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. Proc Natl Acad Sci 83:4071-4075.

Swift RM, Whelihan W, Kuznetsov O, Buongiorno G, Hsuing H (1994) Naltrexone-induced alterations in human ethanol intoxication. Am J Psychiatry 151:1463-1467.

Szabo G, Dolganiuc A, Dai Q, Pruett SB (2007) TLR4, ethanol, and lipid rafts: a new mechanism of ethanol action with implications for other receptor-mediated effects. J Immunol 178:1243-1249.

Szumlinski KK, Diab ME, Friedman R, Henze LM, Lominac KD, Bowers MS (2007) Accumbens neurochemical adaptations produced by binge-like alcohol consumption. Psychopharmacology 190:415-431.

Tappaz ML, Brownstein MJ, Palkovits M (1976) Distribution of glutamate decarboxylase in discrete brain nuclei. Brain Res 108:371-379.

Theile JW, Morikawa H, Gonzales RA, Morrisett RA (2008) Ethanol enhances GABAergic transmission onto dopamine neurons in the ventral tegmental area of the rat. Alcohol Clin Exp Res 32:1040-1048.

Ticku MK, Mhatre M, Mehta AK (1992) Modulation of GABAergic transmission by ethanol, in GABAergic Synaptic Transmission (Biggio G, Concas A, Costa E eds), pp 255-268. Raven Press, New York.

Tobler PN, Fiorillo CD, Schultz W (2005) Adaptive coding of reward value by dopamine neurons. Science 307:1642-1645.

Tong Z-Y, Overton PG, Clark D (1996) Stimulation of the prefrontal cortex in the rat induces patterns of activity in midbrain dopaminergic neurons which resemble natural burst events. Synapse 22:195-208.

Tsujiyama S, Akaike A, Ujihara H, Sasa M (1997) Potentiation by ethanol of GABA-induced current and facilitation of its desensitization in cultured rat cortical neurons. Gen Pharmacol 28:375-380.

Tu Y, Kroener S, Abernathy K, Lapish C, Seamans J, Chandler LJ, Woodward JJ (2007) Ethanol inhibits persistent activity in prefrontal cortical neurons. J Neurosci 27:4765-4775.

Tuomainen P, Patsenka A, Hyytiä P, Grinevich V, Kiianmaa K (2003) Extracellular levels of dopamine in the nucleus accumbens in AA and ANA rats after reverse microdialysis of ethanol into the nucleus accumbens or ventral tegmental area. Alcohol 29:117-124.

Tzschentke TM, Schmidt WJ (1998) Discrete quinolinic acid lesions of the rat prelimbic medial prefrontal cortex affect cocaine- and MK-801-, but not morphine- and amphetamine-induced rewared and psychomotor activation as measured with the place preference conditioning paradigm. Behav Brain Res 97:115-127.

Tzschentke TM, Schmidt WJ (2000) Functional relationship among medial prefrontal cortex, nucleus accumbens, and ventral tegmental area in locomotion and reward. Crit Rev Neurobiol 14:131-142.

Ulrich D, Bettler B (2007) $GABA_B$ receptors: synaptic functions and mechanisms of diversity. Curr Opin Neurobiol 17:298-303.

Usuda I, Tanaka K, Chiba T (1998) Efferent projections of the nucleus accumbens in the rat with special reference to subdivision of the nucleus: biotinylated dextran amine study. Brain Res 797:73-93.

Vacher CM, Gassmann M, Desrayaud S, Challet E, Bradaia A, Hoyer D, Waldmeier P, Kaupmann K, Pévet P, Bettler B (2006) Hyperdopaminergia and altered locomotor activity in $GABA_{B1}$-deficient mice. J Neurochem 97:979-991.

Van Bockstaele EJ, Pickel VM (1995) GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. Brain Res 682:215-221.

VanDoren MJ, Matthews DB, Janis GC, Grobin AC, Devaud LL, Morrow AL (2000) Neuroactive steroid 3α-hydroxy-5α-pregnan-20-one modulates electrophysiological and behavioral actions of ethanol. J Neurosci 20:1982-1989.

Ventura R, Alcaro A, Cabib S, Conversi D, Mandolesi L, Puglisi-Allegra S (2004) Dopamine in the medial prefrontal cortex controls genotype-dependent effects of amphetamine on mesoaccumbens dopamine release and locomotion. Neuropsychopharmacology 29:72-80.

Vigot R, Barbieri S, Bräuner-Osborne H, Turecek R, Shigemoto R, Zhang Y-P, Luján R, Jacobson LH, Biermann B, Fritschy J-M, Vacher C-M, Müller M, Sansig G, Guetg N, Cryan JF, Kaupmann K, Gassmann M, Oertner TG, Bettler B (2006) Differential compartmentalization and distinct functions of $GABA_B$ receptor variants. Neuron 50:589-601.

Volpicelli JR, Alterman AI, Hayashida M, O'Brien CP (1992) Naltrexone in the treatment of alcohol dependence. Arch Gen Psychiatry 49:876-880.

Wafford KA, Burnett DM, Leidenheimer NJ, Burt DR, Wang JB, Kofuji P, Dunwiddie TV, Harris RA, Sikela JM (1991) Ethanol sensitivity of the $GABA_A$ receptor expressed in Xenopus oocytes requires 8 amino acids contained in the γ2L subunit. Neuron 7:27-33.

Waldmeier PC, Kaupmann K, Urwyler S (2008) Roles of $GABA_B$ receptor subtypes in presynaptic auto- and heteroreceptor function regulating GABA and glutamate release. J Neural Transm 115:1401-1411.

Walker BM, Koob GF (2007) The γ-aminobutyric acid-B receptor agonist baclofen attenuates responding for ethanol in ethanol-dependent rats. Alcohol Clin Exp Res 31:11-18.

Wallner M, Hanchar HJ, Olsen RW (2003) Ethanol enhances $\alpha_4\beta_3\delta$ and $\alpha_6\beta_3\delta$ γ-aminobutyric acid type A receptors at low concentrations known to affect humans. Proc Natl Acad Sci 100:15218-15223.

Wallner M, Hanchar HJ, Olsen RW (2006) Low-dose alcohol actions on $\alpha4\beta3\delta$ $GABA_A$ receptors are reversed by the behavioral alcohol antagonist Ro15-4513. Proc Natl Acad Sci 103:8540-8545.

Wegelius K, Halonen T, Korpi ER (1993) Gamma-vinyl GABA decreases voluntary alcohol consumption in alcohol-preferring AA rats. Pharmacol Toxicol 73:150-152.

Weitlauf C, Woodward JJ (2008) Ethanol selectively attenuates NMDAR-mediated synaptic transmission in the prefrontal cortex. Alcohol Clin Exp Res 32:690-698.

Westerink BH, Enrico P, Feimann J, De Vries JB (1996) The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. J Neurosci 16:2605-2611.

Westerink BH, Enrico P, Feimann J, De Vries JB (1998) The pharmacology of mesocortical dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and prefrontal cortex of the rat brain. J Pharmacol Exp Ther 285:143-154.

White HS, Brown SD, Woodhead JH, Skeen GA, Wolf HH (2000) Topiramate modulates GABA-evoked currents in murine cortical neurons by a nonbenzodiazepine mechanism. Epilepsia 41(Suppl 1):S17-S20.

White G, Lovinger DM, Weight FF (1990) Ethanol inhibits NMDA-activated current but does not alter GABA-activated current in an isolated adult mammalian neuron. Brain Res 507:332-336.

Wirtshafter D, Sheppard AC (2001) Localization of $GABA_B$ receptors in midbrain monoamine containing neurons in the rat. Brain Res Bull 56:1-5.

Wise RA (1998) Drug-activation of brain reward pathways. Drug Alc Depend 51:13-22.

Wise RA (2006) Role of brain dopamine in food reward and reinforcement. Phil Trans R Soc B 361:1149-1158.

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469-492.

Wise RA, Spindler J, DeWit H, Gerber GJ (1978) Neuroleptic-induced "anhedonia" in rats: pimozide blocks reward quality of food. Science 201:262-264.

Wu M, Brudzynski SM, Mogenson GJ (1993) Functional interaction of dopamine and glutamate in the nucleus accumbens in the regulation of locomotion. Can J Physiol Pharmacol 71:407-413.

Xi ZX, Stein EA (1998) Nucleus accumbens dopamine release modulation by mesolimbic $GABA_A$ receptors – an in vivo electrochemical study. Brain Res 798:156-165.

Xiao C, Shao XM, Olive MF, Griffin III WC, Li K-Y, Krnjević K, Zhou C, Ye J-H (2008) Ethanol facilitates glutamatergic transmission to dopamine neurons in the ventral tegmental area. Neuropsychopharmacology, e-pub ahead of print.

Xiao C, Zhang J, Krnjević K, Ye JH (2007) Effects of ethanol on midbrain neurons: role of opioid receptors. Alcohol Clin Exp Res 31:1-8.

Yan QS (2003) Involvement of non-exocytotic mechanisms in ethanol-induced in vivo dopamine release: comparisons with cocaine. Eur J Pharmacol 477:37-44.

Yang Q, Lanier SM (1999) Influence of G protein type on agonist efficacy. Mol Pharmacol 56:651-656.

Yim CY, Mogenson GJ (1983) Response of ventral pallidal neurons to amygdala stimulation and its modulation by dopamine projections to nucleus accumbens. J Neurophysiol 50:148-161.

Yim HJ, Schallert T, Randall PK, Gonzales RA (1998) Comparison of local and systemic ethanol effects on extracellular dopamine concentration in rat nucleus accumbens by microdialysis. Alcohol Clin Exp Res 22:367-374.

Yoneyama N, Crabbe JC, Ford MM, Murillo A, Finn DA (2008) Voluntary ethanol consumption in 22 inbred mouse strains. Alcohol 42:149-160.

Yoshida M, Yokoo H, Tanaka T, Mizoguchi K, Emoto H, Ishii H, Tanaka M (1993) Facilitatory modulation of mesolimbic dopamine neuronal activity by a μ-opioid agonist and nicotine as examined with in vivo microdialysis. Brain Res 624:277-280.

Yoshimoto K, McBride WJ, Lumeng L, Li T-K (1991) Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. Alcohol 9:17-22.

Yoshimoto K, Ueda S, Kato B, Takeuchi Y, Kawai Y, Noritake K, Yasuhara M (2000) Alcohol enhances characteristic release of dopamine and serotonin in the central nucleus of the amygdala. Neurochem Int 37:369-376.

Yu O, Chiu TH, Rosenberg HC (1988) Modulation of GABA-gated chloride ion flux in rat brain by acute and chronic benzodiazepine administration. J Pharmacol Exp Ther 246:107-113.

Yung KKL, Ng TKY, Wong CKC (1999) Subpopulations of neurons in the rat neostriatum display $GABA_BR1$ receptor immunoreactivity. Brain Res 830:345-352.

Zahm DS (1989) The ventral striatopallidal parts of the basal ganglia in the rat – II. Compartmentation of ventral pallidal efferents. Neuroscience 30:33-50.

Zahm DS, Jensen SL, Williams ES, Martin III JR (1999) Direct comparison of projections from the central amygdaloid region and nucleus accumbens shell. Eur J Neurosci 11:1119-1126.

Zahniser NR, Buck KJ, Curella P, McQuilkin SJ, Wilson-Shaw D, Miller CL, Klein RL, Heidenreich KA, Keir WJ, Sikela JM, Harris RA (1992) $GABA_A$ receptor function and regional analysis of subunit mRNAs in long-sleep and short-sleep mouse brain. Mol Brain Res 14:196-206.

Zapata A, Gonzales RA, Shippenberg TS (2006) Repeated ethanol intoxication induces behavioral sensitization in the absence of a sensitized accumbens dopamine response in C57BL/6J and DBA/2J mice. Neuropsychopharmacology 31:396-405.

Zeiss CJ (2005) Neuroanatomical phenotyping in the mouse: the dopaminergic system. Vet Pathol 42:753-773.

Zhai J, Stewart RR, Friedberg MW, Li C (1998) Phosphorylation of the $GABA_A$ receptor γ2L subunit in rat sensory neurons may not be necessary for ethanol sensitivity. Brain Res 805:116-122.

Zhu PJ, Lovinger DM (2006) Ethanol potentiates GABAergic synaptic transmission in a postsynaptic neuron/synaptic bouton preparation from basolateral amygdala. J Neurophysiol 96:433-441.

Zhu XZ, Chuang DM (1987) Modulation of calcium uptake and D-aspartate release by $GABA_B$ receptors in cultured cerebellar granule cells. Eur J Pharmacol 141:401-408.

Zocchi A, Girlanda E, Varnier G, Sartori I, Zanetti L, Wildish GA, Lennon M, Mugnaini M, Heidbreder CA (2003) Dopamine responsiveness to drugs of abuse: a shell-core investigation in the nucleus accumbens of the mouse. Synapse 50:293-302.