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## Neuroimaging Insights into the Role of Cortical GABA Systems and the Influence of Nicotine on the Recovery from Alcohol Dependence

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### Abstract

This paper reviews evidence suggesting that nicotine and tobacco smoke profoundly modulate the effects of alcohol on  $\gamma$ -aminobutyric acid (GABA) neuronal function, specifically at the GABA<sub>A</sub>-benzodiazepine receptor (GABA<sub>A</sub>-BZR). The focus of this paper is on recent neuroimaging evidence in preclinical models as well as clinical experiments. First, we review findings implicating the role of alcohol at the GABA<sub>A</sub>-BZR and discuss the changes in GABA<sub>A</sub>-BZR availability during acute and prolonged alcohol withdrawal. Second, we discuss preclinical evidence that suggests nicotine affects GABA neuronal function indirectly by a primary action at neuronal nicotinic acetylcholine receptors. Third, we show how this evidence converges in studies that examine GABA levels and GABA<sub>A</sub>-BZRs in alcohol-dependent smokers and nonsmokers, suggesting that tobacco smoking attenuates the chemical changes that occur during alcohol withdrawal. Based on a comprehensive review of literature, we hypothesize that tobacco smoking minimizes the changes in GABA levels that typically occur during the acute cycles of drinking in alcohol-dependent individuals. Thus, during alcohol withdrawal, the continued tobacco smoking decreases the severity of the withdrawal-related changes in GABA chemistry.

### Keywords

GABA; alcohol dependence; tobacco smoking; brain imaging

### Introduction

$\gamma$ -aminobutyric acid (GABA) neuronal function is modulated by ethanol and nicotine (Kumar et al., 2009; Markou, 2008). Neuroadaptations within GABA neurons contribute to the development of both ethanol and nicotine dependence, as well as complications associated with the abrupt discontinuation of ethanol or nicotine in dependent individuals (Hughes, 2009). Similarly, GABA systems have been implicated in the recovery from ethanol and nicotine dependence (Mason et al., 2006; Staley et al., 2005a). Given the high rate of alcohol and nicotine dependence comorbidity (Grant et al., 2004), studying the convergent effects of acute and chronic exposure to these substances might provide

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clinically relevant insights about the underlying mechanistic interactions of ethanol and nicotine. In this review we examine evidence suggesting that GABA neuronal function, and specifically the GABA<sub>A</sub>-benzodiazepine receptor (GABA<sub>A</sub>-BZR), is a major neurochemical target mediating the comorbidity of alcohol and nicotine dependence. We hypothesize that nicotine profoundly alters both the acute and chronic effects of ethanol on GABA<sub>A</sub> receptors and thus increases the risk for both alcoholism (by suppressing ethanol neuroadaptations and withdrawal) and nicotine dependence (because the increased tolerability of ethanol and ethanol withdrawal serves as a negative reinforcer for continued smoking). Important contributions of neuroadaptations associated with GABA<sub>A</sub> receptors to ethanol dependence have been reviewed previously (Krystal et al., 2006) and we refer readers to other recently published reviews on the interaction of nicotine and alcohol such as genetic components (Flatscher-Bader and Wilce, 2009; Schlaepfer et al., 2008) and reward mechanisms (Schlaepfer et al., 2008) that contribute to the comorbidity.

## Comorbidity of Alcohol and Nicotine Dependence

The high rate of comorbidity between alcohol and nicotine dependence is well established. While in the general population it is estimated that 25% of people are current cigarette smokers, up to 45% of alcohol-dependent individuals smoke, and as many as 14% of smokers are also alcohol-dependent (Grant et al., 2004). Smokers consume twice as much alcohol as nonsmokers (Carmody et al., 1985), alcohol problems are approximately 10 times more prevalent in smokers vs. nonsmokers (DiFranza and Guerrera, 1990), and alcohol-dependent smokers use more cigarettes per day than non-alcohol-dependent smokers (Dawson, 2000). Unfortunately, individuals with a current or past alcohol problem have a more difficult time quitting smoking than non-alcoholics (Bobo et al., 1987; Kahler et al., 2010a; Romberger and Grant, 2004) and they are more likely to lapse to smoking while they are drinking (Kahler et al., 2010b). However, quitting smoking does not appear to affect rates of heavy drinking (Kahler et al., 2010a), suggesting that quitting smoking and drinking must both be targeted in vulnerable individuals. There are several factors that may underlie the high comorbidity of smoking and drinking (reviewed in (Meyerhoff et al., 2006). First, the effects of the drugs, when used together, may be additive or synergistic with regard to the reinforcing properties. Second, pharmacological effects or interactions of the drugs, such as changes in metabolism or cross-tolerance, may lead to co-abuse. Third, genetic factors likely contribute to this comorbidity.

We suggest that there is a critical influence of nicotine on alcohol's actions at the GABA<sub>A</sub> receptor, as an additional factor that may contribute to the comorbidity of these disorders. The cycle of addiction begins with acquisition or initiation of drug taking, which escalates to a steady-state maintenance phase and then cycles of withdrawal and relapse typically occur. It is likely that during these phases, discrete brain areas and neurochemical systems are recruited. Traditionally, the initiation phase is characterized by positive reinforcement, which is critically tied to the mesolimbic dopamine system (Di Chiara and Imperato, 1988). The shift from controlled to uncontrolled use can be associated with a shift from positive to negative reinforcement, i.e., when addicted individuals switch from taking drugs for the euphoric effects to taking drugs to prevent withdrawal symptoms. At that point it becomes critical to maintain homeostasis between the inhibitory (GABAergic) and excitatory (glutamatergic) systems. During alcohol withdrawal, the drug-maintained homeostasis is severely disrupted, most clearly evidenced by seizures, and it is at this critical juncture that nicotine may be conferring protection against changes in neurochemicals and in alcohol withdrawal symptoms. Specifically, the profound effects of nicotine on alcohol-induced alterations of GABA<sub>A</sub> receptors may drive the continued use of both substances.

## Effects of Alcohol and Alcohol Withdrawal on GABA<sub>A</sub> Receptors

GABA<sub>A</sub> receptors are ligand-gated ion channels that are the primary mechanism for modulating inhibitory synaptic transmission in the brain and have a central role in modulating the effects of alcohol in the central nervous system (Davies, 2003; Krystal et al., 2006; Kumar et al., 2004). GABA plays a number of important roles in the brain, including maintaining homeostatic balance between excitation and inhibition (Sivilotti and Nistri, 1991), tuning the activity of glutamate neurons (Sesack et al., 2003), and entraining the coherent oscillatory interactions within cortical networks (Mann and Paulsen, 2007; Wang and Buzsaki, 1996). The GABA<sub>A</sub> receptor is a member of a family of homologous transmitter-gated ion channels along with the nicotinic acetylcholine receptor (nAChR), glycine and 5-HT<sub>3</sub> receptors (Sigel and Buhr, 1997). GABA<sub>A</sub> receptors are pentamers typically comprised of two  $\alpha_{1-6}$ , two  $\beta_{1-3}$ , and one  $\gamma_{1-3}$  subunit. Benzodiazepines bind to a distinct site at the interface between an  $\alpha$  and  $\gamma$  subunit on the GABA<sub>A</sub> receptor, a site commonly referred to as the GABA<sub>A</sub>-benzodiazepine receptor (GABA<sub>A</sub>-BZR).

Although acute ethanol exposure has been reported to potentiate GABA-gated currents (Suzdak and Paul, 1987; Tatebayashi et al., 1998), direct ethanol effects on synaptic GABA<sub>A</sub> receptors only occur at ethanol levels that are lethal in humans (Koski et al., 2002; Koski et al., 2005). Potent effects of ethanol *in vivo* likely reflect direct actions of ethanol at extrasynaptic GABA<sub>A</sub> receptors (Krystal et al., 2006; Sundstrom-Poromaa et al., 2002; Wallner et al., 2003). **Acute** ethanol may also act indirectly at synaptic GABA<sub>A</sub> receptors (via increased glutamate release), thereby increasing the synaptic release of GABA (Ariwodola and Weiner, 2004; Carta et al., 2004; Moghaddam and Bolinao, 1994). Of interest is the capacity of ethanol to raise levels of neurosteroids that interact with GABA<sub>A</sub> receptors (Barbaccia et al., 1999; Morrow et al., 1999) in a manner similar to other GABA<sub>A</sub>-receptor positive modulators that may influence tolerance to alcohol (Morrow et al., 2006). Importantly, GABA-ergic neurosteroids do not exhibit cross-tolerance like benzodiazepines, and have been shown to be protective during ethanol withdrawal (Devaud et al., 1996; Devaud et al., 1995). Neurosteroids act at extrasynaptic GABA<sub>A</sub> receptors that mediate tonic inhibition, rather than at synaptic GABA<sub>A</sub> receptors that mediate phasic inhibition (Stell et al., 2003; Wohlfarth et al., 2002). This is important because as previously mentioned the extrasynaptic GABA<sub>A</sub> receptors have been proposed as a high-affinity target for ethanol (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003). Long-term ethanol exposure stimulates changes in GABA<sub>A</sub> receptor subunit composition (Charlton et al., 1997; Devaud et al., 1997; Petrie et al., 2001), and the consequence of this adaptation is that GABA<sub>A</sub> receptors show higher affinity for GABA, but disrupted chloride channel conductance (Liang et al., 2007; Morrow et al., 1988; Sanna and Harris, 1993). When ethanol is removed, the lowered functionality of the GABA<sub>A</sub> receptors contributes to the heightened excitatory tone (Kang et al., 1996), which is clearly evidenced by irritability, sympathetic activation, seizure activity, and neurotoxicity associated with the ethanol withdrawal syndrome (Hoffman, 1995; Kokka et al., 1993).

Several postmortem studies have been conducted to determine potential differences in numbers of GABA<sub>A</sub>-BZRs between individuals with alcohol dependence and controls; however, the results are conflicting. The studies report decreases in GABA<sub>A</sub>-BZRs of 30% in hippocampus and 25% in frontal cortex (Freund and Ballinger, 1991), no difference in GABA<sub>A</sub>-BZR density in frontal cortex or cerebellum (Korpi and Uusi-Oukari, 1992), and increases in GABA<sub>A</sub> receptor density (Tran et al., 1981) in alcohol-dependent subjects vs. controls. This variability may be due to the lack of control for smoking status, and/or to other caveats associated with the study of postmortem specimens such as postmortem interval, freezer storage time and insufficient information about the chronicity and intensity of drug use over the lifetime.

The use of receptor imaging (single photon emission computed tomography, SPECT, and positron emission tomography, PET) has significantly advanced the field of drug addiction research by allowing us to probe and quantify receptors of interest in the living human brain. SPECT in combination with the radiotracer [ $^{123}\text{I}$ ]iomazenil, a GABA<sub>A</sub>-BZR inverse agonist, and PET with [ $^{11}\text{C}$ ]flumazenil, a GABA<sub>A</sub>-BZR antagonist, provide a way to quantify numbers of GABA<sub>A</sub>-BZRs in human subjects. SPECT and PET studies consistently demonstrate *decreased* GABA<sub>A</sub>-BZRs in alcohol dependent subjects at approximately 1 month (Abi-Dargham et al., 1994), 3 months (Lingford-Hughes et al., 2000; Lingford-Hughes et al., 1998) and 7 months (Lingford-Hughes et al., 2005) of abstinence. The reductions in GABA<sub>A</sub>-BZR availability were consistently reported in the medial frontal cortex (Abi-Dargham et al., 1998; Lingford-Hughes et al., 1998) and cerebellum (in alcoholic women only)(Lingford-Hughes et al., 2000). The most recent study, which was the first to evaluate GABA<sub>A</sub>-BZRs longitudinally within subjects while controlling for smoking status, demonstrated *increased* GABA<sub>A</sub>-BZRs at about 1 week withdrawal in alcohol-dependent nonsmokers (n=8) compared to alcohol-dependent smokers (n=15) and controls (n=15), that normalized to control levels by 4 weeks of abstinence (Staley et al., 2005a). The acute increases were predominantly in the cortical regions (Staley et al., 2005a). These preliminary findings during acute abstinence highlight a temporal change in GABA<sub>A</sub>-BZRs during the recovery from alcohol dependence with increased GABA<sub>A</sub>-BZRs during early withdrawal, which then decreases during extended recovery. It is not yet clear whether GABA<sub>A</sub>-BZR availability normalizes to control levels during prolonged recovery, or whether there may be a sustained, long-lasting decrease (Lingford-Hughes et al., 2005) compared to controls.

A number of studies have also implicated the GABA<sub>A</sub>-BZR in alcohol dependence by demonstrating that alcohol-dependent subjects abstinent at least 2 months have a blunted response to benzodiazepines compared to controls (Bauer et al., 1997; Lingford-Hughes et al., 2005; Volkow et al., 1997). Further, in nonhuman primates that chronically self-administered ethanol, the benzodiazepine sensitivity of GABA<sub>A</sub> receptors in the amygdala was decreased (Anderson et al., 2007). In combination with the neuroreceptor findings, this suggests several main points. First, the upregulation of GABA<sub>A</sub>-BZRs observed in alcohol-dependent nonsmokers (Staley et al., 2005a) seemed to develop over the first week of abstinence and was related to the severity of withdrawal symptoms, suggesting that synaptic GABA<sub>A</sub>-BZRs were recruited in response to alcohol withdrawal. Second, the upregulation/recruitment of receptors was transient, suggesting that the increase represented a transitional stage between two populations of receptors. This is consistent with the preclinical studies suggesting that alcohol potentially facilitates the extrasynaptic, BZ-insensitive GABA<sub>A</sub> receptors (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003), and that the synaptic, BZ-sensitive GABA<sub>A</sub>-receptors reemerge during acute withdrawal. Third, the blunted sensitivity to BZs during prolonged abstinence is consistent with the idea that protracted alcohol withdrawal is associated with the sustained emergence of synaptic GABA<sub>A</sub>-receptors and reduction of extrasynaptic GABA<sub>A</sub>-receptors that are not stimulated by BZ agonists. These findings provide a mechanistic explanation for the cross-tolerance between alcohol and BZs.

One of the caveats to studying neurochemical changes in the brain related to a mental illness, including alcohol dependence, is the high prevalence of persistent brain atrophy in psychiatric populations. Therefore, an observation of a decrease in receptor availability may be due to a reduction in brain volume and not receptor number. Since brain atrophy in alcohol dependence can be widespread and severe (Mechtcheriakov et al., 2007) and compounded by nutritional deficiencies, if not controlled it can introduce a confound to the study. Thus, because differences in GABA<sub>A</sub> receptor density were reported in the absence of changes in gray matter in alcohol-dependent subjects (Lingford-Hughes et al., 1998), it could be speculated that changes in density may not be due to global or regional brain

atrophy but to changes in neurochemistry at the receptor level. Another limitation of measuring the GABA<sub>A</sub>-BZR with SPECT and PET is that changes in GABA<sub>A</sub> receptor density may be due to changes in the composition of the receptor subunits, which cannot be differentiated using existing radiotracers. For example, preclinical (Floyd et al., 2004; Kumar et al., 2003; Luddens and Korpi, 1995; Sarviharju et al., 2006) and postmortem human (Mitsuyama et al., 1998) studies suggest differential changes in subunit expression after chronic ethanol exposure. However, [<sup>123</sup>I]iomazenil SPECT and [<sup>11</sup>C]flumazenil PET measure all GABA<sub>A</sub>-BZRs containing various subunits and we cannot distinguish between them. Thus, continuing translational neuroscience research will be critical to determine specific receptor subunit compositions that may be involved in specific targeted behaviors and whether any respond preferentially to treatment medications (Atack, 2010; Wallner and Olsen, 2008).

Neuroreceptor imaging studies in human alcohol-dependent subjects have yet to control for heritable or developmental (gene by environment interactions) factors that influence ligand binding to GABA<sub>A</sub> receptors. This issue is important because several GABA<sub>A</sub> receptor subunit genes have been reported to contribute to the heritable risk for alcoholism. The GABA<sub>A</sub> α2 subunit gene, GABRA2, is involved in modulating acute alcohol sensitivity (Haughey et al., 2008) and subsequently alcohol dependence (Bierut et al., 2010; Covault et al., 2004; Edenberg et al., 2004; Soyka et al., 2007). Additionally, the GABRG1 polymorphism, which encodes the GABA<sub>A</sub> receptor γ1 subunit and is in linkage disequilibrium with the GABRA2 polymorphism, has been associated with alcohol dependence (Covault et al., 2008). Subsequent studies matching for genotype will be needed to address this issue.

## Effects of Nicotine on GABA<sub>A</sub> Receptors

The primary addictive chemical in tobacco smoke is nicotine, and the majority of work on nicotine's effects on neuronal receptor systems has involved the nicotinic acetylcholine receptor (nAChR) and the catecholaminergic system (Penton and Lester, 2009; Picciotto et al., 1998). However, activation of the nAChR by nicotine or nicotinic agonists has been shown to increase GABA release (Dani and De Biasi, 2001; Lena and Changeux, 1997; Maggi et al., 2001) and influence GABA-mediated inhibition (Freund et al., 1990) within the mesolimbic dopamine system (Balfour et al., 2000; Di Chiara, 2000) and the septohippocampal pathway (Wu et al., 2003). The effects of nicotine on GABA activity likely occur indirectly as a result of nicotine's actions at the nAChRs which are located directly on the soma, preterminal, and presynaptic regions of GABAergic neurons (Colquhoun and Patrick, 1997). Activation of nAChRs enhances GABA activity (Alkondon et al., 1997; Genzen and McGehee, 2005) as well as other neurotransmitter activity (Colquhoun and Patrick, 1997). Acute nicotine administration increases GABA activity (Lena et al., 1993; McMahon et al., 1994a, b), and both acute nicotine (Porcu et al., 2003) and more chronic administration in tobacco smokers (Bjornerem et al., 2004; Field et al., 1994) is associated with increased concentrations of GABA-ergic neurosteroids, which are correlated with negative affect and craving (Marx et al., 2006). The effects of chronic nicotine administration on GABA activity are less clear. While a preclinical study indicated chronic nicotine treatment increased the density of BZ receptors in the cerebral cortex (Magata et al., 2000), a recent neuroimaging study in our laboratory demonstrated no difference in GABA<sub>A</sub>-BZR availability between current healthy smokers and nonsmokers (Esterlis et al., 2009). An important caveat to this study is that smokers were imaged at 7 hours of smoking abstinence. Since tobacco smoke contains over 4000 chemicals in addition to the primary addictive chemical, nicotine, including the harmful alkaloids which are inverse agonists at the GABA<sub>A</sub>-BZR (Rommelspacher et al., 1981; Totsuka et al., 1999), these may have interfered with [<sup>123</sup>I]iomazenil binding to the receptor if they were still



present in the brain. However, a subset of smokers ( $n=4$ ) was imaged again after 5 weeks continued smoking abstinence, and the initial findings of no difference in receptor availability between smokers and nonsmokers were confirmed. Due to the high comorbidity of alcohol dependence and tobacco smoking, it will be important in future studies to determine the effects of nicotine and other components in tobacco smoke on the regulation of the GABA<sub>A</sub> receptor. It is possible that the harmala alkaloids act either alone or in concert with nicotine to oppose the effects of alcohol at the GABA<sub>A</sub> receptor.

## Interactions of Ethanol and Nicotine on GABA<sub>A</sub> Receptors

Preclinical and clinical studies suggest a potential mechanism by which nicotine may modulate alcohol's effects at the GABA<sub>A</sub> receptor. Specifically, modulation of nAChRs by nicotine and a nicotinic antagonist reduced the sensitivity of GABA<sub>A</sub>-receptors to the benzodiazepine diazepam (Lof et al., 2007). The authors suggest that this may translate into the co-abuse of alcohol and tobacco if nicotine acts to decrease the sedative effects of alcohol. This is consistent with findings in humans that nicotine reduces alcohol's sedating effects (Perkins et al., 2000; Perkins et al., 1995) and thus underscores a potential increased risk for both alcohol (the reduced sensitivity to sedation may lead to more drinking) and nicotine dependence (the increased tolerability of alcohol drives continued smoking).

Despite the high comorbidity of tobacco smoking and alcohol dependence, few clinical studies have been conducted on the interaction of alcohol and nicotine on GABA<sub>A</sub> receptors. Our group (Staley et al., 2005a) investigated changes in GABA<sub>A</sub>-BZRs during the recovery from alcohol dependence in tobacco smokers versus nonsmokers using [<sup>123</sup>I]iomazenil and SPECT brain imaging. There was a significantly increased availability of GABA<sub>A</sub>-BZRs in alcohol-dependent nonsmokers versus smokers during acute alcohol withdrawal (1 week) suggesting that tobacco smoke suppressed the alcohol-induced GABA<sub>A</sub>-BZR increase. This acutely increased GABA<sub>A</sub>-BZR availability is likely primarily composed of synaptic GABA<sub>A</sub> receptors that are recruited during early alcohol withdrawal. This elevation was correlated with more severe alcohol withdrawal symptoms in alcohol-dependent nonsmokers, which were not apparent in alcohol-dependent smokers, suggesting that tobacco smoking may block some symptoms of alcohol withdrawal by suppressing the increased availability of GABA<sub>A</sub>-BZRs during acute withdrawal from alcohol.

Preliminary evidence from an ongoing study in our laboratory suggests that even in long-term abstinent alcohol-dependent individuals (abstinent at least 6 months) smoking continues to modify cortical GABA<sub>A</sub>-BZR availability, such that abstinent alcoholics who continued to smoke had lower GABA<sub>A</sub>-BZR availability than abstinent alcoholics who remained nonsmokers (Esterlis et al., 2010). Thus, during both acute and long-term alcohol withdrawal, tobacco smoking profoundly regulates alcohol-induced brain chemistry changes.

In addition to SPECT and PET imaging modalities, proton magnetic resonance spectroscopy (MRS) can be used to measure brain chemicals, such as GABA, *in vivo*. In a preliminary study, Mason and colleagues (2006) measured occipital GABA levels in alcohol-dependent smokers and nonsmokers over the course of recovery from alcohol dependence. Alcohol-dependent nonsmokers at 1 week of alcohol abstinence had higher GABA levels than alcohol-dependent smokers (alcohol-dependent smokers had levels similar to healthy controls), and at 1 month of abstinence GABA levels decreased in nonsmokers and remained unchanged in smokers (Mason et al., 2006). However, there is a limitation to MRS measures of extracellular GABA. MRS measures the total amount of GABA in the volume of tissue, but because the extracellular GABA levels exist in a low concentration, they could

be significantly changed without affecting the total concentration of GABA measured in the tissue volume.

Taken together, these initial studies suggest that during acute withdrawal (1 week of abstinence) there may be increased cortical GABA levels and cortical synaptic GABA<sub>A</sub>-BZRs in nonsmokers, but not smokers, and that by 1 month of abstinence, both GABA levels and GABA<sub>A</sub>-BZRs have “normalized” in the nonsmokers, while remaining constant in the smokers (Mason et al., 2006; Staley et al., 2005b). In long-term abstinent alcoholics (greater than 1 month), there is consistent evidence for a reduction in GABA<sub>A</sub>-BZRs (Abi-Dargham et al., 1998; Gilman et al., 1996; Lingford-Hughes et al., 1998), and preliminary evidence indicates that tobacco smoking may mediate this reduction (Esterlis et al., 2010). Thus, prior to chronic alcohol dependence, available GABA<sub>A</sub>-receptors are BZ sensitive; but during alcohol dependence, the GABA<sub>A</sub>-BZ insensitive receptors, which are extrasynaptic, are recruited. These extrasynaptic GABA<sub>A</sub> receptors have a higher affinity for ethanol, but lower conductance than synaptic GABA<sub>A</sub> receptors, which accounts for alcohol tolerance. With the shift from synaptic to more extrasynaptic GABA<sub>A</sub> receptors, the overall conductance capacity for chloride is reduced leading to a smaller response to alcohol and a vulnerability to withdrawal due to a functional GABA deficiency. When alcohol is removed from the system, during acute recovery, there is a re-emergence of the GABA<sub>A</sub>-BZ sensitive receptors in addition to the insensitive receptors resulting in an overall upregulation of GABA<sub>A</sub>-BZRs as noted in Staley et al., 2005, which resolves and then decreases during extended abstinence.

Nicotine and alcohol may also interact via their actions on glutamic acid decarboxylase (GAD) 67, one of the two enzymes that synthesize GABA in the brain. GAD67 and 65 appear to be responsible for GABA synthesis in terminals and cell bodies, respectively, with GAD67 maintaining levels of GABA (Soghomonian and Martin, 1998). Nicotine has been shown to upregulate GAD67 expression by its actions at nAChRs located on GABAergic interneurons in the cortex and hippocampus (Satta et al., 2008). Chronic alcohol consumption has been linked to a reduction in GAD 67 mRNAs (Falco et al., 2009). Another potential mechanism by which GABA levels could be changed is rapid change of GAD between its inactive apoenzyme to its active holoenzyme. The conversions from the active to inactive forms can occur within minutes (Porter and Martin, 1988; Soghomonian and Martin, 1998). Thus, nicotine may reverse the alcohol-induced suppression in GAD by acute and chronic stimulation of GAD. Indeed, acute nicotine inhalation has been reported to increase the rate of GABA synthesis in the human brain (Mason et al., 2007). It may be by its action on GAD67 that nicotine compensates for alcohol-induced alterations in GABA<sub>A</sub>-BZRs.

It remains unclear at which point in the addiction process tobacco smoking modulates the alcohol-induced changes on GABA neuronal function. We hypothesize that tobacco smoking is functionally changing or dampening GABA tone in the alcohol maintenance phase during the repeated cycles of drinking that occur every few days during chronic episodes of drinking. There are a variety of drinking patterns in alcohol-dependent individuals. While some individuals engage in daily drinking (Danel et al., 2003; Hatton et al., 2009), others engage in several days of drinking followed by acute withdrawal periods (Mello and Mendelson, 1971). Among younger individuals a common pattern is to engage in binge-drinking on weekends (Harrison et al., 2008). Chronic tobacco smokers do engage in daily smoking, so an alcohol-dependent smoker may have either alcohol or nicotine continuously in their system. In alcohol-dependent smokers, during acute alcohol withdrawal, the neuronal excitability may remain attenuated by continued tobacco smoking. In general, studies suggest that people do not change their smoking status during alcohol and substance abuse treatment (Friend and Pagano, 2005; Toneatto et al., 1995), but there is a

lack of research on smoking consumption changes during early alcohol withdrawal. It will be important to determine in future studies whether alcohol-dependent smokers transiently increase their smoking consumption during acute alcohol withdrawal, which may relieve withdrawal symptoms. In summary, during acute withdrawal, there is increased GABA-ergic tone in both alcohol-dependent smokers and nonsmokers, but it is significantly greater in nonsmokers. Over the course of protracted abstinence (greater than 1 month) we hypothesize the GABA oscillations return to baseline conditions in both smokers and nonsmokers (Figure 1).

## Implications

The interaction of ethanol and nicotine at the GABA receptor may contribute to the high rates of the persistence of co-abuse of the drugs resulting in increased intake of alcohol in tobacco smokers and of tobacco in alcohol-dependent individuals. These findings have implications for the treatment of alcohol-dependent smokers and nonsmokers during alcohol withdrawal. Specifically, smokers may be benefiting from a protective effect of nicotine or tobacco smoke on GABA-ergic alcohol-withdrawal symptoms. If nicotine does suppress the alcohol-induced increase in GABA<sub>A</sub>-BZRs, this suggests that nicotine replacement therapies and nicotinic agonist medications may be useful treatment strategies in both smokers and nonsmokers (nicotine replacement is not typically addictive) during alcohol withdrawal. There is recent preclinical (Steenland et al., 2007) and clinical (McKee et al., 2009) evidence to support the use of varenicline, a partial  $\beta_2$ -nAChR agonist, for reducing alcohol consumption. Additionally, the nicotine patch reduced alcohol self-administration in a human laboratory paradigm in heavy drinking daily smokers (McKee et al., 2008). Although studies examining the direct effects of ethanol on  $\beta_2$ -nAChRs are mixed (Gorbounova et al., 1998; Ribeiro-Carvalho et al., 2009; Robles and Sabria, 2008), prolonged abstinence from chronic alcohol consumption was recently associated with decreased  $\beta_2$ -nAChR availability compared to baseline in nonhuman primates (Cosgrove et al., 2010). If this decrease is found to be associated with withdrawal symptoms and increased relapse rates in humans, this suggests that nicotinic agonist drugs may be useful to help keep the  $\beta_2$ -nAChRs at baseline levels. Thus, nicotinic agonist therapy (varenicline, nicotine replacement) may be indicated for both alcohol and nicotine withdrawal symptoms in alcohol-dependent smokers encouraged to quit drinking and smoking at the same time. Because preclinical evidence suggests varenicline is useful in reducing alcohol-seeking behaviors in animals not exposed to nicotine (Hendrickson et al., 2010; Steenland et al., 2007), future research should also evaluate the potential benefit of nicotinic agonist therapy during initial abstinence from alcohol in alcohol-dependent nonsmokers.

Further, both ethanol and nicotine have been shown to increase levels of GABA-ergic neurosteroids that act at the GABA<sub>A</sub> receptor in a manner similar to positive modulators. Thus, an alcohol-dependent smoker who quits drinking may benefit from the protective effects of neurosteroids that are promoted as they continue to smoke. A recent study suggests that neuroactive steroids may be a novel treatment for smoking cessation (Marx et al., 2006); and, they may also prove useful during alcohol withdrawal and for dual alcohol and smoking cessation, especially because neurosteroids do not exhibit cross-tolerance as do benzodiazepines.

In addition to viewing nicotine and nicotinic agonists as potential therapeutics for alcohol dependence, it is possible that GABA-ergic agents may be helpful in tobacco smoking cessation. Preclinical (Corrigall et al., 2001; Paterson et al., 2004) and clinical (Franklin et al., 2009) studies suggest that baclofen attenuates nicotine and cigarette consumption. A clinical limitation of baclofen is that it must be taken every 4-6 hours. Thus, effective



GABA-ergic drugs for smoking cessation will be dependent on new medications development.

## Recommendations for Future Research

### 1. Continued development of neuroimaging ligands

It is important to continue development of compounds for *in vivo* imaging. Specifically, ligands that preferentially measure different subunit compositions and differentiate between synaptic and extrasynaptic GABA<sub>A</sub> receptors, based on the evidence that subunit composition of the GABA<sub>A</sub> receptor changes with the development of alcohol dependence (Krystal et al., 2006). The importance of a polymorphism in GABRA2 (Edenberg et al., 2004; Soyka et al., 2007) and GABRG1 (Covault et al., 2008) for alcohol dependence highlights the need for an  $\alpha 2$  and  $\gamma 1$  selective ligands. The GABA<sub>B</sub>-receptor is also a site of interest and a radiotracer for this receptor is currently under development.

### 2. Continued development of imaging methodology

Currently, MRS has been used to measure a specific voxel of interest in the occipital cortex in alcohol-dependent smokers and nonsmokers. With the advancing technology, GABA and other chemicals measurements in other brain regions of interest have been examined in other populations and it will be critical to examine alternative brain regions such as the frontal cortex that are critically involved in alcohol dependence.

### 3. Better characterization of alcohol and tobacco smoking use in imaging studies

Recommendations for assessing patterns of alcohol consumption have been given (Leeman et al., 2007). We would additionally recommend obtaining measures in tobacco smokers that assess tobacco smoking dependence, craving and withdrawal, including the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991), the Tiffany Questionnaire for Smoking Urges (QSU) (Tiffany et al., 1993), and the Nicotine Withdrawal Scale (Hughes and Hatsukami, 1986). Biochemical measures of recent smoking, including carbon monoxide and cotinine levels, should be used for confirmation of smoking and nonsmoking status in addition to self-reports of cigarettes smoked. Time since last cigarette should be reported and standardized in imaging protocols. Obtaining these measures in all subjects will increase cohesion between imaging groups and the interpretation of findings across studies.

### 4. Integration of receptor imaging with other biochemical tools, i.e., multimodal imaging

Because many imaging studies are limited in their findings, e.g., to a difference in receptor availability between two groups, or a change over time, it is important to increase the amount of biochemical data obtained in subjects. Examples include obtaining MRS in the same subjects, measuring functional brain changes, obtaining additional receptor imaging scans to examine changes in more than one receptor system in the same subjects, and correlating promising biomarkers with the brain measures.

## Summary

Despite the high prevalence of comorbid tobacco smoking and alcohol dependence, studies on the neurochemical mechanisms underlying these disorders and their interactions are scarce. Moreover, because of the high rate of comorbidity and the devastating medical consequences of using both drugs, additional studies are needed to address changes that occur during acute withdrawal from alcohol alone, and from dual alcohol and nicotine withdrawal, to delineate the best strategy for abstinence in alcohol-dependent individuals **who** abuse both drugs, i.e., stop alcohol first vs. nicotine first, or both simultaneously. In this review we have described evidence that nicotine profoundly alters both the acute and

chronic effects of ethanol on GABA<sub>A</sub> receptors, which may underlie the high comorbidity of alcohol and nicotine dependence. This relationship has significant implications for our understanding of the recovery from alcohol dependence in both smokers and nonsmokers. Future studies should systematically examine the effects of comorbid tobacco smoking during the recovery from alcohol dependence in order to successfully inform the clinical treatment of alcoholism.

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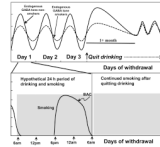


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**Figure 1.**

The top panel illustrates a hypothetical series of several days of drinking in a chronic alcohol-dependent individual followed by acute and prolonged withdrawal. Repeated dosing of alcohol and withdrawal with regular episodes of smoking shown over the course of one day (bottom panel) may cycle endogenous GABAergic tone up protectively during periods of alcohol withdrawal and down during periods of drinking (top panel), when alcohol supplements the endogenous tone. Smoking may compensate for a loss of alcohol during periods of acute withdrawal (top panel), allowing alcohol-dependent smokers to maintain greater stability of GABAergic tone. Specifically, during acute alcohol withdrawal, there is an initial increase in GABAergic tone in alcohol-dependent smokers and nonsmokers, which is more severe in nonsmokers. During prolonged withdrawal there is likely a return to baseline, or normal cycling of GABA.