2015

A Role for Kv7 Channels in Alcohol Consumption: Genetics, Pharmacology, and Neuroadaptations

Natalie Maurisa Straight-McGuier
Medical University of South Carolina

A Role for Kv7 Channels in Alcohol Consumption: Genetics, Pharmacology, and Neuroadaptations

by

Natalie Maurisa Straight McGuier

A dissertation submitted to the faculty of the Medical University of South Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Graduate Studies

Department of Neurosciences

2015

Approved by:

Chair _____
Patrick J. Mulholland, PhD

Advisory Committee _____
Howard C. Becker, PhD

_____
L. Judson Chandler, PhD

_____
William C. Griffin III, PhD

_____
Antonieta Lavin, PhD

_____
Arthur C. Riegel, PhD

ACKNOWLEDGEMENTS

To Andrew: Thank you for supporting me on this adventure. I could not ask for a better friend, husband, or a more perfect partner. I simply could not have done this without you.

Thank you to my family: Mom, Dad, Leslie, and Lindsay. Thank you for demanding my very best from the beginning and setting the bar high. Without you I would not be able to have accomplished a fraction of what I have. Also, my extended family: Kelley, Joel, Devin and Ian. Thank you for welcoming me into your family, and sharing in the joys and easing the woes of grad school. Having you all truly makes my life great.

To all my friends from coast to coast: Thank you for commiserating, for studying with me, for the happy hours, for allowing me to share in your successes and failures, for answering your phone late at night, for your personal expertise, for being here, and for getting coffee in the Charleston summertime heat.

To the past and present members of the Mulholland, Chandler, Trantham-Davidson, and Becker Labs: Thank for your advice, for lab supplies both freely given and stolen, and for your support. I especially thank Diana Fulmer and Julia Moss who helped with multiple studies associated with this project. I would also like to thank Dr. Justin Gass and Dr. Bailey Glen for their help in executing key experiments to my dissertation.

To those from beyond IOP: Many thanks to Dr. Steve Mahler for providing the perfect VTA coordinates. Thank you to Dr. Elissa Chesler from the Jackson Laboratory for her expertise in bioinformatics and her critical role in the genetics analysis.

To Dr. Peter Berget and Dr. Carrie Doonan: Thank you for initiating and fostering my love of research and teaching. I am extremely grateful for your continued mentoring and friendship.

To Patrick: I am truly grateful to have had you as my mentor. In the last five years I have grown more as a researcher than I had hoped and it because of your mentorship. So, thank you for taking me on as your first grad student, for taking snark and giving it back, for supporting me during crises, and for teaching me how to be a “real scientist”.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

NATALIE MAURISA STRAIGHT MCGUIER. A Role for Kv7 Channels in Alcohol Consumption: Genetics, Drinking Behavior, and Neuroadaptations. (Under the direction of PATRICK J. MULHOLLAND)

Alcohol use disorder (AUD) is a major public health issue that produces enormous societal and economic burdens. Current FDA-approved pharmacotherapies for treating AUD suffer from deleterious side effects and are only effective in a subset of individuals, representing a need for improved medications for the management of AUD. The experiments described in this dissertation provide evidence for a complex relationship between Kv7 channels and alcohol-related behaviors that spans genetics, behavioral pharmacology, and biochemical adaptations suggesting these channels are a target for treating AUD. We first examined the genetic relationship between *Kcnq* genes and alcohol-related behaviors, showing that these channels contribute to an alcohol drinking phenotype. Behavioral pharmacology studies strengthened this relationship by showing that systemic administration and microinjections of retigabine, an FDA-approved anticonvulsant and Kv7 channel positive modulator, to components of the addiction neurocircuitry reduced voluntary consumption in a long-term intermittent access model in an alcohol-specific manner. Finally, we investigated alcohol-induced neuroadaptations in the nucleus accumbens (NAc). Specifically, we observed that long-term drinking enhanced sensitivity to the pro-convulsant effects of Kv7 channel blockade, altered surface trafficking of Kv7.2 channels between detergent resistant and soluble membranes, and reduced Kv7.2 channel SUMOylation in the NAc. To our knowledge, these data are the first to show evidence for post-translation modification by SUMOylation in a model of alcohol or drug exposure. Altogether the work presented in this dissertation indicates that retigabine may be a promising treatment for AUD, and that Kv7 channels are a target of alcohol-induced neuroadaptations.

CHAPTER 1: INTRODUCTION

Millennia of Alcohol Use in Global Cultures

The purposeful creation and consumption of alcohol is as ancient as the first known civilization. Our Stone Age ancestors left behind jugs intentionally meant for fermentation dating back to 10,000 BC (Patrick, 1970). This time period, the Neolithic Revolution, represents a shift from nomadic wandering to an agricultural-based lifestyle. Some anthropologists speculate that the demand for beer inspired farming in the Near East as mass beer consumption preceded the baking of bread (Braidwood, 1953; Katz, 1987). Throughout ancient history alcohol consumption was integrated into nearly every growing civilization. Between 4000 and 3500 BC wine is clearly indicated in Egyptian hieroglyphics, and alcohol use was an important part of societal exchange as well as religion (Cherrington, 1930; Darby, 1977; King, 1947). By 3000 BC alcohol consumption was a mainstay in the Greek, Roman, and East Asian cultures as well; it was engrained throughout the entirety of the known world.

Due in no small part to the psychoactive effects of alcohol, by 700 BC it was a central part of nearly every major religion, although in the centuries that followed most religions and governments would condemn excessive use and drunkenness (Dietler, 2006). Greeks and Romans worshiped gods dedicated to wine and merriment (Sournia, 1990), and when old deities gave way to new in the first and second centuries BC and AD, sacramental wine and beer brewing were at the heart of Jewish and Christian traditions (Raymond, 1927). In a time span that held the birth of Christianity, the Dark Ages of Europe, and its Renaissance many clergy considered beer and wine a necessity

to worship. These traditions were maintained until the 1800s when a social and economic shift spurred Protestant Christians in both Western Europe and America to demand complete abstinence from alcohol (Esteicher, 2006).

In the 19th century, industrialization took hold of the economy of the United States. The increased number of factory positions lead to an influx of formerly rural families to cities. The isolation and cyclical farming work schedule allowed for excessive drinking in rural culture. In contrast, city living was more regimented, which lead to unease with routine drunkenness among factory workers (Dietler, 2006). In the years following the Civil War saloons became increasingly popular in urban areas and synonymous with prostitution, gambling, and other amoral acts (Rorabaugh, 1979). Additionally rural and urban women began to seek safety for themselves and their children from alcohol-induced domestic violence, wage loss, and child neglect (Blocker, 2006). These are the first recorded examples of what would now be diagnosed as alcohol use disorder (AUD). Soon the Protestant church joined the outcry against drinking, and, as a moral and religious crusade, the Temperance Movement was born (Hill, 2004). By 1919 most states in the US had independently banned the sale and consumption of alcoholic substances, however prohibition was formalized at a federal level with the ratification of the 18th amendment, banning the sale of intoxicating beverages with an alcohol content greater than 0.5%.

During the Temperance Movement and prohibition, alcohol consumption per capita was reduced by an estimated 20% in the US (Blocker, 2006). This moderate reduction represents almost the entirety of the success of prohibition. The illegality of alcohol lead to an increase in the consumption and sale of unregulated homemade liquor. Underground bars known as speakeasies replaced saloons, and arguably the most disastrous consequence of prohibition was the formation of organized crime

syndicates that continue to exist today. Prohibition was repealed by the 21st amendment in 1933 as a means of stimulating the economy during the Great Depression. While most regard Prohibition as a misadventure, its failure to induce national abstinence exemplifies how engrained alcohol consumption is in our way of life (Blocker, 2006). Furthermore, the social outcry against alcohol abuse that spurred the Temperance Movement revealed the necessity of understanding and treating alcohol addiction as a disease.

Lawmakers have discarded the idea of banning alcohol sales and consumption, however, the substance is heavily regulated at both the state and federal levels, including laws ranging from the regulation of sales to minimum drinking ages. In the 1940s National Council on Alcoholism was created to promote the disease theory of alcoholism (NCADD, 2015), and in 1968 the National Institute on Alcohol Abuse and Alcoholism (NIAAA) was formed with the mission statement "to promote, direct and support biomedical and behavioral research on the causes, consequences, treatment, and prevention of alcoholism and alcohol-related problems" (NIAAA, 2012). Numerous other organizations have since formed to alleviate the personal and social consequences of alcoholism. In the present day, clinicians and researchers recognize alcohol abuse as an addictive brain disorder that should and can be medically treated.

Prevalence of Alcohol Use Disorder

Alcohol consumption is pervasive in the United States. 51.3% of adults self-report as regular drinkers (>12 drinks in the previous year) and an additional 12.9% report as infrequent drinkers (1-11 drinks in the past year) (Blackwell DL, 2012). 12% of adults will face alcohol use disorder (AUD) at some point in their lives, which the NIAAA defines as a diagnosable medical condition wherein an individual's drinking causes

distress or harm (NIAAA, 2012). The condition is characterized by unhealthy or dangerous drinking habits (alcohol abuse), and by symptoms of alcohol dependence including a forfeit of normal life function in favor of the pursuit of alcohol. AUD comes at a tremendous societal and personal cost. Annually, 15,990 individuals die from alcoholic liver disease, 25,682 die from alcohol-induced deaths (excluding accidents and homicides), and 10,228 people die in alcohol-impaired driving crashes (Corrao G, 2004; LA, 1998; Rehm and Gmel, 2003). Furthermore, in 2006 the Center for Disease Control estimated that excessive alcohol consumption in the United States cost approximately $223.5 billion in expenses related to losses in workplace productivity, healthcare, criminal justice, and motor vehicle accidents (Bouchery EE, 2011). Considering these extreme costs it is obvious that effective, accessible, and inexpensive treatment options for AUD are desperately needed.

Past and Present Treatment Options

There are four key points of treatment when approaching any drug of abuse: 1) intervention to decrease the rewarding value of drugs, 2) increase the value of endogenous rewards while establishing alternative behaviors, 3) to weaken drug-associated responses through behavioral treatments such as extinction learning, and 4) to strengthen executive control through cognitive therapy (Volkow et al., 2003). Several accepted treatment options for AUD exist that target multiple points of intervention. Cognitive and behavioral psychological therapy are perhaps two of the most utilized, and aim to establish healthier behaviors and avoid trigger recognition. Group therapy (e.g. Alcoholics Anonymous; AA) is often the first form of intervention for treatment-seeking patients with AUD. Interestingly, a Cochrane review found no experimental studies that unequivocally demonstrated the effectiveness of AA or similar twelve-step approaches

(Ferri et al., 2006). In addition, individuals may hesitate to initiate twelve-step programs because they require immediate cessation from alcohol, which can result in the debilitating symptoms of alcohol withdrawal syndrome. One-on-one psychological counseling in the form of motivational interviewing (MI) and motivational enhancement therapy (MET) developed as a direct result of this hesitation by addicts (Woody, 2003). While beneficial for the subset of individuals who would otherwise resist group programs, MI and MET on their own are not as efficacious as twelve-step programs for the majority of patients. Psychological interventions are frequently used in concert with pharmacological interventions to ease the physiological symptoms of addiction. Indeed, multi-faceted approaches to treatment are shown to be the most efficacious (Anton et al., 2006; Donovan et al., 2008). However, the risks and benefits of pharmacotherapies must also be taken into account.

Pharmaceutical treatments for AUD act at two discrete points in the addiction process: to prevent relapse or to aid in drinking cessation. Presently there are four major FDA-approved pharmaceuticals for AUD: benzodiazepines, naltrexone, disulfiram, and acamprosate.

Benzodiazepines

Benzodiazepine refers to a class of drugs that contain a benzene ring fused to a diazepine ring. Presently, the two most commonly prescribed are diazepam and chlordiazepoxide. These compounds enhance $GABA_A$ receptor activity resulting in sedative, anxiolytic, and anticonvulsant properties (Browne and Penry, 1973). Benzodiazepines are used to prevent relapse by reducing withdrawal symptoms, which can include headache, nausea, tremors, seizures, hallucinations, and death (Bayard et al., 2004; Mayo-Smith and Bernard, 1995; Myrick et al., 2000; Myrick et al., 2009).

However, like chronic alcohol, prolonged exposure to benzodiazepines can lead to a hyperexcitable state and subsequent seizures (Allison and Pratt, 2003). Consequently, if treated with this class of drug, relapse is often more severe and detrimental than if pharmacological intervention had not been utilized (Malcolm et al., 1993; Malcolm, 2003). Additionally benzodiazepines are addictive and have a variety of adverse side effects that include hypotension, decreased cognitive function, and anhedonia (Vgontzas et al., 1995). Thus, while effective at reducing acute withdrawal symptoms, benzodiazepines have serious long-term consequences making them a non-ideal choice for treating AUD.

Naltrexone

In 1994 the FDA approved naltrexone for the treatment of alcoholism. This mu and kappa opioid receptor antagonist has had the most clinical success; being most effective in individuals who have already ceased drinking and seek to prevent a slip of alcohol use from becoming full-blown relapse. While beneficial, naltrexone has been found to be most efficacious in carriers of the A118G polymorphism on the μ-opioid receptor gene OPRM1 (Anton, 2008; Anton et al., 2012; Schacht et al., 2013). These studies suggest that genetic factors contribute to alcohol and drug abuse, and that naltrexone is not necessarily beneficial to the entire population of AUD patients. Furthermore, this drug hosts a myriad of side effects including anhedonia, nausea/vomiting, and muscle pain (Streeton and Whelan, 2001). Although it is considered the best available pharmaceutical treatment for aiding alcohol abstinence, it leaves much to be desired.

Disulfiram

Disulfiram is another FDA-approved treatment for AUD, and acts by inhibiting a portion of the alcohol and dopaminergic metabolic pathways (Goldstein et al., 1964; Sauter et al., 1977; Sauter and Wartburg, 1977). Alcohol is catabolized by alcohol dehydrogenase to acetaldehyde. Acetaldehyde is a toxin that induces nausea, dizziness and headaches before it is further metabolized into acetate. Disulfiram inhibits aldehyde dehydrogenase, causing a build up of acetaldehyde and subsequent illness when alcohol is consumed. Unsurprisingly, patient compliance is a tremendous obstacle in the efficacy of this treatment. One study (Fuller et al., 1986) estimated that only 20% of subjects followed the prescribed drug regimen. Consequently, disulfiram leaves much to be desired in an AUD pharmacotherapy.

Acamprosate

The fourth major pharmacological treatment for alcoholism is acamprosate (Campral). This drug is believed to enhance GABAergic reception and transmission while reducing glutamate signaling at NMDA receptors, which are both oppositely affected by chronic ethanol exposure (Reilly et al., 2008). Acamprosate likely counteracts the hyperexcitable state in the central nervous system produced by repeated bouts of alcohol exposure and withdrawal (Becker, 1996, 1998; Becker and Hale, 1993). Acamprosate has been efficacious in preventing relapse, but yields small effect sizes. In addition, this drug appears to be most useful to patients who have already withdrawn from alcohol, and consequently does not aid with immediate abstinence (Bouza et al., 2004). A recent publication using multiple rodent models of alcohol exposure suggests that the calcium cation in the salt form of acamprosate is the

active moiety (Spanagel et al., 2014). While the results from this study need to be replicated, it suggests that acamprosate may not be a suitable treatment for AUD.

Together, these pharmaceuticals leave an opening for drugs that will have high patient compliance, non-deleterious side effects, and that will help patients abstain immediately from alcohol.

Advent of Anticonvulsants as Pharmacotherapies

Chronic alcohol consumption with episodes of withdrawal leads to an imbalance of excitatory regulation in the central nervous system. Simply, chronic intermittent alcohol exposure increases excitatory mechanisms (i.e. glutamate receptors, Ca^{2+} channels) and decreases inhibitory mechanisms (i.e. GABA receptors) (Alele and Devaud, 2007; Gass and Olive, 2008; Szumlinski et al., 2007). This allostatic state of brain function is believed to be a compensatory mechanism to counterbalance the depressive effects of alcohol (Mulholland and Chandler, 2007). These effects are visually perceived in alcohol withdrawal syndrome (AWS) where seizures and delirium tremens are common characteristics. Given the similarity of alcohol withdrawal and its biochemical effects to epilepsy, it is unsurprising that since 1976 clinicians have been using anticonvulsants to treat AWS. In the past 10 years, there has been a significant amount of interest in the use of anticonvulsants for the reduction of alcohol-seeking behavior (Ait-Daoud et al., 2006; Book and Myrick, 2005; Malcolm et al., 2001). The following sections highlight some of the preclinical and clinical evidence regarding the more recent anticonvulsants to emerge as candidates to aid in drinking cessation.

Topiramate

Topiramate is perhaps the most widely studied anticonvulsant drug in rodent models of alcohol drinking. It is thought to have a variety of molecular targets including voltage gated sodium channels, high voltage gated calcium channels, and $GABA_A$ receptors (Porter et al., 2012). Regimes of systemic and acute dosing effectively reduce drinking in alcohol-preferring rodent strains (Farook et al., 2009; Gabriel and Cunningham, 2005; Nguyen et al., 2007). However, evidence suggests that low doses of topiramate can increase drinking (Gabriel and Cunningham, 2005), and that the drug is ineffective at reducing drinking in rat strains not specifically bred for high levels of alcohol consumption (Hargreaves and McGregor, 2007).

Clinically, topiramate has been the subject several studies investigating its ability to aid in alcohol abstinence after cessation (for a complete review see (Kenna et al., 2009)). In an open-label trial in India, topiramate was significantly more effective at maintaining abstinence than Disulfiram (De Sousa et al., 2008). Two double blind, placebo-controlled studies indicate that topiramate is effective at decreasing the number of heavy drinking days and the number of drinks per day in AUD patients (Johnson et al., 2003; Johnson et al., 2007). However patients have experienced substantial negative side effects including difficulty concentrating, taste perversion, and anorexia (Johnson et al., 2007). In addition, topiramate appears to be most efficacious in individuals with a specific GRIK1 polymorphism (Kranzler et al., 2014). Together the preclinical and clinical data indicate that topiramate may be useful to help heavy-drinking individuals abstain from alcohol, but there are potentially severe negative side effects.

GABA Analogues

Gabapentin and pregabalin have a similar structure to the amino acid γ-aminobutyric acid (GABA), but have potent anticonvulsants actions mediated through voltage-sensitive Ca^{2+} channels (Stephen and Brodie, 2011). Recent evidence in a variety of rat drinking models indicates that gabapentin can reduce voluntary alcohol consumption, operant responding for alcohol, and cue-induced reinstatement (Roberto et al., 2008). Treatment with pregabalin also can reduce cue-induced reinstatement of alcohol-seeking behavior (Stopponi et al., 2012). Similar to gabapentin and pregabalin, vigabatrin (gamma-vinyl-GABA) is an analogue of GABA, however, vigabatrin influences excitability by inhibiting GABA transaminase (Rogawski and Loscher, 2004). Two studies that used a standard choice model demonstrated that vigabatrin reduced alcohol consumption in alcohol-preferring rats. More recently Griffin et al reported that vigabatrin decreased operant responding for alcohol as well as home cage drinking in mice (Griffin et al., 2012; Stromberg et al., 2001; Wegelius et al., 1993).

Several small, open-label studies indicate that gabapentin can reduce withdrawal symptoms (Bonnet et al., 2007; Myrick et al., 1998). Double blind, placebo-controlled studies investigating the anticonvulsant's ability to reduce drinking have contradicting results. A study investigating consumption in moderate-drinking patients found no effect of gabapentin (Myrick et al., 2007) whereas one investigating the same effect in heavy-drinking individuals found gabapentin to be efficacious (Furieri and Nakamura-Palacios, 2007). Pregabalin helped detoxified patients remain abstinent and reduced craving and withdrawal symptoms in small open-label trials (Di Nicola et al., 2010; Martinotti et al., 2008). However, a double blind trial comparing naltrexone to pregabalin indicated no difference in efficacy between the two (Martinotti et al., 2010). Finally, little is known clinically of vigabatrin, but one open-label study suggests that it may be beneficial in

treating the symptoms of alcohol withdrawal (Stuppaeck et al., 1996). In summary the GABA analogues need further investigation to assess their clinical efficacy.

Levetiracetam

The exact mechanism through which levetiracetam acts is unknown, however there is evidence to suggest that it inhibits neurotransmitter release by interacting with the synaptic vesicle glycoprotein SV2A (Lynch et al., 2004). Only two preclinical studies have examined the effects of levetiracetam on alcohol-related behaviors. In a voluntary alcohol consumption model, repeated doses of levetiracetam significantly reduced alcohol intake and preference for alcohol in rats (Zalewska-Kaszubska et al., 2011). In the second study, levetiracetam blocked the ability of alcohol to reduce intracranial self-stimulation in mice (Robinson et al., 2013).

While preclinical studies suggest that levetiracetam is a strong candidate for treating AUD, there is little clinical evidence to support the use of this anticonvulsant. Three double-blind placebo-controlled studies in medium to high drinking patients showed no effect of the levetiracetam on withdrawal symptoms, an inability to reduce the number of drinks consumed per day, and an inability to reduce relapse (Fertig et al., 2012; Richter et al., 2012; Richter et al., 2010). Together these studies indicate that levetiracetam may not be a suitable treatment for AUD.

Zonisamide

Zonisamide is approved for the treatment of partial onset seizures, although its mechanism of action is not well characterized. The drug is believed to block sodium and T-type calcium channels, and is known to modulate glutamatergic and GABAergic neurotransmission (Leppik, 2004; Mimaki et al., 1990; Ueda et al., 2003). One study has

shown that a relatively high dose of zonisamide (50 mg/kg) was able to reduce alcohol consumption, but only when the drug was actively on board (Knapp et al., 2007). Furthermore, chronic treatments with zonisamide lead to weight loss in rodents.

There have been few clinical trials investigating the effectiveness of zonisamide on preventing alcohol consumption. In open-label trials, zonisamide reduced craving and consumption of alcohol-dependent patients (Knapp et al., 2010; Rubio et al., 2010). In one placebo-controlled study the drug also reduced alcohol consumption and the urge drink, but failed to increase the number of completely abstinent days (Arias et al., 2010). Zonisamide also suffers from several deleterious side effects including anorexia, depression, memory impairment, and dizziness (Services, 2013) suggesting that it may not be advantageous for patients with AUD.

While many of these anticonvulsants show promise as therapeutics for aiding in alcohol abstinence, most have side effects so adverse as to raise concern over patient compliance and quality of life during treatment, or are simply not performing well in large clinical trials. Most anticonvulsants primarily function to dampen glutamatergic signaling, potentiate GABAergic signaling or block voltage-gated sodium channels (White, 1997). Often when treating epilepsy, medical professionals take a multi-hit approach and prescribe multiple drugs that act through different mechanisms (Bianchi et al., 2009; LaFrance and Devinsky, 2004). Thus, there is a constant demand for new anticonvulsants that act at novel targets. One such drug, retigabine, functions to open Kv7 channels, a voltage-gated class of K^+ channels (Tatulian et al., 2001; Wickenden et al., 2000). Neither retigabine nor Kv7 channels are well understood in the context of alcohol exposure; however, recent studies and the pharmacokinetics of both retigabine and Kv7 channels provide exciting implications for alcohol research.

Retigabine: Anticonvulsant and Kv7 Channel Positive Modulator

The FDA approved retigabine in 2011 for the treatment of partial onset seizures. This anticonvulsant primarily acts as a Kv7 channel positive modulator, leading to an increase in K^+ current (Tatulian et al., 2001; Wickenden et al., 2000), and subsequent neuronal inhibition. Positive modulation of Kv7 channels effectively reduces seizures both preclinically and clinically (Armand et al., 1999; Brodie et al., 2010; French et al., 2011; Porter et al., 2007). Retigabine has a mean terminal half-life of approximately 8 hours in humans, with a maximal plasma availability of 420 ng/mL 2 hours after oral administration. In rats, the half-life is also approximately 2 hours after oral dosing (Review: (Mazarati et al., 2008)). A 20 mg/kg systemic dose of retigabine in mice results in a brain concentration of 2.7898 μM (Zhou et al., 2015). The side effects of retigabine are relatively minimal and include dizziness, confusion, fatigue, dysarthria, vision impairment and nausea. These effects are only experienced by 1-12% of patients taking 600 mg of the drug per day, and are reported as tolerable (Crean and Tompson, 2013; Mazarati et al., 2008). 350 mg is effective at reducing seizures in humans whereas 2.5 - 30 mg/kg reduces convulsant activity in a variety of rodent models of epilepsy (Large et al., 2012).

Pharmacokinetic studies of retigabine and Kv7 channels indicate that it has an IC_{50} of 0.6 ± 0.3 μM to 5.2 ± 0.9 μM depending on the Kv7 channel subunit or subunit combination being examined (Tatulian et al., 2001). Importantly, this anticonvulsant only modulates Kv7 channel subunits expressed in the CNS (Kv7.2-Kv7.5). Retigabine produces a hyperpolarizing shift of the activation curve of Kv7 channels by up to 40 mV, greatly increasing the open probability of the channel around the resting membrane potential (Tatulian et al., 2001). This suggests that Kv7 channel positive modulators may reduce drinking and alcohol withdrawal-associated seizures in a manner similar to other

anticonvulsants. Additionally, activation of Kv7 channels can influence DA neurotransmission in the VTA and NAc. Retigabine has been shown to inhibit basal DA synthesis, reduce extracellular DA levels when striatal DA uptake is inhibited, and block depolarization-induced DA release from striatal terminals (Hansen et al., 2008). Given the importance of DA release in addiction (Kalivas, 2002), these studies suggest that retigabine and Kv7 channel activation could influence addictive behaviors.

Kinetics and Regulation of Kv7 Channels

The Kv7 family of voltage-gated potassium channels consists of 5 subunits, Kv7.1-Kv7.5, encoded by the genes *Kcnq1-5*. Four subunits come together to form the channels responsible for M-current in peripheral organs and brain (Jentsch, 2000; Selyanko et al., 2002; Wang et al., 1998). Kv7.1-containing channels are exclusively expressed in the heart, whereas Kv7.2-Kv7.5 are found abundantly in the CNS. Kv7.2 and Kv7.3 subunits are the most widely distributed and are found in the cortex, basal ganglia circuitry, hippocampus, cerebellum, thalamus, hypothalamus, and amygdala. Kv7.3 has the next highest expression level, having been reported in the cortex, striatum, and hippocampus (Kharkovets et al., 2000; Wang et al., 1998). In contrast, Kv7.4 channels appear to be restricted to the VTA and dopaminergic neurons in the SN_C (Kharkovets et al., 2000). Typically, the subunits come together in dimer pairs of Kv7.2/Kv7.3 and Kv7.3/Kv7.4, however current research suggests that channels in the VTA are composed of Kv7.4 homotetramers. All subtypes of Kv7 channels are slowly activated (~100-300 ms at -30mV; (Miceli et al., 2009)), and start opening near resting membrane potential (-60mV). M-current becomes more strongly activated at depolarized potentials. Accordingly, M-current activation is important for repolarizing the cell, fine-tuning the resting membrane potential, and controlling action potential generation and

frequency. Because Kv7 channels are slowly activated, M-current provides a stronger inhibitory input during sustained or repetitive firing. Thus, M-current activation functions as a brake on neuronal excitability.

M-current was named because it is blocked by muscarinic acetylcholine receptor agonists (mAChRs) (Brown and Adams, 1980). However, Kv7 channels are inhibited by the activation of any G_q coupled receptor (Marrion, 1997). In the last 10 years it was established that Kv7 channels require PIP_2 to enter an open state and similarly close when PIP_2 is depleted (Suh and Hille, 2007). Thus, it has become clear that phospholipase C (PLC) hydrolysis of PIP_2 into IP_3 and DAG is responsible for blocking M-current. A-kinase binding protein (AKAP79/150) directly interacts with the Kv7.2 subunit and recruits protein kinase C (PKC) where it can phosphorylate the channel leading to inhibition of M-current (Bal et al., 2010; Hoshi et al., 2003). Kv7 channels are also sensitive to slight increases in Ca^{2+} concentration (Gamper et al., 2003), and calmodulin (CaM) interferes with the AKAP-Kv7.2 protein-protein interaction either by prevention of the physical interaction, by blockade of PKC phosphorylation, or both (Bal et al., 2010). Recent work as also implicated the peptide hormone ghrelin as an inhibitor of Kv7 channels in the SNc, where it can increase the excitability of dopaminergic neurons (Shi et al., 2013). These regulatory interactions highlight mechanisms that open or close Kv7 channels, but not how the channels are trafficked within the neuron.

The trafficking of Kv7 channels is presently not well understood. As previously mentioned, CaM is known to directly interact with the channel, but initial studies with mutants lacking a CaM binding site indicated that Kv7 channels remained at the membrane surface (Wen and Levitan, 2002). One group has suggested that the same mutant is retained in the endoplasmic reticulum (Etxeberria et al., 2008). A recent study showed that constitutive CaM tethering is not required for Kv7 channel function (Gomez-

Posada et al., 2011). In short, CaM's role in Kv7 trafficking is not well defined. In the axon initial segment (AIS) co-assembly of the Kv7.2 and Kv7.3 subunits and interaction with ankyrin-G are required for efficient localization (Rasmussen et al., 2007). Evidence suggests that the protein serum-glucocorticoid-regulated kinase 1 (SGK1) increases surface expression of Kv7 channels leading to an increase in M-current. However whether this is due to a direct interaction of SGK1 with a Kv7 subunit or through direct interactions with Nedd4-2 ubiquitin ligase has yet to be defined (Miranda et al., 2013; Schuetz et al., 2008; Seebohm et al., 2005). In summary, Kv7 channel function and trafficking are regulated through a myriad of protein-protein interactions, but exact mechanisms are currently unclear.

Evidence for Kv7 Channels in Alcohol Use Disorders

Several recent studies have linked Kv7 channel function to drinking and other symptoms of AUDs. Kv7 channels are linked to acute alcohol tolerance and acute alcohol-induced memory impairments in *Drosophila* models (Cavaliere et al., 2012; Cavaliere et al., 2013). Kv7 channel expression has also been more directly related to alcohol consumption. Both alcohol self-administration and home cage drinking decrease *Kcnq3* gene transcripts in the NAc of P rats (Bell et al., 2009; Rodd et al., 2008), suggesting a potential compensatory modulation of expression. Moreover, Metten and colleagues (2014) reported differential expression of *Kcnq2* in the ventral striatum of mice selectively bred for high alcohol consumption/low withdrawal severity versus mice bred for low consumption/high withdrawal severity. These authors also reported a quantitative trait locus (QTL) on distal Chr 2, and alignment of the QTL with differential expression data identified *Kcnq2* as a candidate cis-eQTL for alcohol consumption and withdrawal. Chromosomes 2 and 15 (locations of *Kcnq2/3* have been previously linked

to alcohol consumption as well (Drews et al., 2010). Together these data implicate a genetic component of *Kcnq* genes to a drinking phenotype and a role for M-current in the alcohol addiction process.

Statement of Problem

Pharmacological intervention is a critical component in the treatment of individuals with AUD. Presently, few options are available to AUD patients. Novel pharmaceuticals and targets for intervention are integral to treatment process.

Over the last several decades, it has become clear that numerous environmental and genetic factors contribute to addiction disorders. Research indicates that some pharmacotherapies, such as naltrexone, are more efficacious for individuals with specific SNPs (Anton, 2008; Anton et al., 2008; Schacht et al., 2013). To better investigate the genetic components of addiction, massive databases have been compiled and made available to all research groups through *in silico* techniques. Recent evidence suggests that *Kcnq2* and *Kcnq3* are associated with alcohol-related phenotypes (Bell et al., 2009; Metten et al., 2014; Rodd et al., 2008). Therefore, the first study in this dissertation utilized *in silico* approaches to identify an association of the *Kcnq* family of genes with alcohol-related phenotypes. **It was hypothesized that *Kcnq* genes occur in gene sets accompanying alcohol phenotypes.**

Several studies indicate a relationship between Kv7 channels and alcohol addiction. Some of these include evidence that the K7 channel opener retigabine can reduce DA release from the VTA (Hansen et al., 2008; Jensen et al., 2011; Koyama and Appel, 2006; Koyama et al., 2007; Martire et al., 2007; Sotty et al., 2009), an association of Kv7

channels and the effects of acute alcohol on memory and tolerance (Cavaliere et al., 2012; Cavaliere et al., 2013), and that retigabine can reduce consumption in a limited access, forced-drinking model (Knapp et al., 2014). Altogether these studies provide evidence that positive modulators of Kv7 channels can alter voluntary drinking in rodents. The second study in this dissertation sought to provide clear evidence that retigabine can reduce drinking in a model of voluntary alcohol-consumption that has high face validity. **It was hypothesized that retigabine would reduce voluntary drinking in an alcohol-specific manner.**

The addiction neurocircuitry consists of multiple brain regions that are altered during the addiction process. In a small, but critical part of the circuitry, the ventral tegmental area (VTA) sends dopaminergic projections to the NAc (Kalivas, 2009). Studies indicate that there are alcohol-related adaptations in *Kcnq2/3* mRNA expression in the nucleus accumbens (NAc) in rodent models (Bell et al., 2009; Metten et al., 2014; Rodd et al., 2008), and modulation of M-current in the VTA alters firing and DA release (Hansen et al., 2008; Jensen et al., 2011; Martire et al., 2007; Sotty et al., 2009). These studies suggest that Kv7 channels in both the NAc and VTA may be key regulators in alcohol consumption. Therefore the third study in this dissertation sought to identify if Kv7 channel function in the NAc and VTA affects alcohol-drinking behaviors. **It was hypothesized that increased Kv7 channel function in the NAc and VTA would reduce drinking.**

Repeated episodes of drinking followed by withdrawal are known to induce adaptations in the expression of select proteins throughout CNS (Becker, 1999; Breese et al., 2011; Fadda and Rossetti, 1998). Recent genetic evidence indicates that there are alcohol-

related adaptations in the expression of *Kcnq2/3* in the NAc of rodents (Bell et al., 2009; Metten et al., 2014; Rodd et al., 2008). We found that microinjection of retigabine to the NAc is sufficient to reduce alcohol consumption and that alcohol-exposed rats are sensitive to seizures induced by blocking Kv7 channels. Together these studies indicate that Kv7 channel expression and/or function is altered in the NAc after long-term alcohol exposure. The final experiments of this dissertation sought to characterize alcohol-induced adaptations in Kv7 channel expression in the NAc and identify a mechanism mediating the adaptation. **It was hypothesized that prolonged alcohol consumption causes dysregulation in Kv7 channel expression and trafficking in the NAc.**

In summary, the work presented in this dissertation attempts to achieve the following goals related to the identification and characterization of a novel pharmacological therapeutic and target for the reduction of alcohol consumption:

1. **Identify Kv7 channels as a target for the reduction of alcohol drinking and further explore the role of *Kcnq* genes in alcohol-related behaviors.**
2. **Characterize the effects of the anticonvulsant and Kv7 channel-opener retigabine on alcohol consumption.**
3. **Identify regions of the addiction neurocircuitry sensitive to the effects of retigabine on alcohol consumption.**
4. **Explore a biochemical basis for the pharmacological effects of retigabine in alcohol-exposed rats.**

This dissertation provides a narrative describing a complex interaction between Kv7 channels and alcohol consumption from *in silico* genetics to behavioral pharmacology to alcohol-induced neuroadaptations. We identify the FDA-approved anticonvulsant

retigabine as a potential therapeutic for the reduction of voluntary alcohol consumption. We further provide evidence that Kv7 channels are targets for alcohol-induced plasticity, and that membrane localization of these channels is sensitive to alcohol exposure and withdrawal.

CHAPTER 2: *KCNQ* GENES AND DRINKING PHENOTYPES

Introduction

In Chapter 1 we discussed the alarming prevalence of AUD in our society and a decided lack of effective pharmacotherapies with minimal side effects. This is not a simple problem as addiction disorders result from complex interactions of environmental and genetic factors. Meta-analyses and twin studies have estimated that approximately 50% of the variance associated with drug and alcohol dependence is heritable (Agrawal et al., 2012). Furthermore, there is evidence indicating that matching genetic variations to specific therapeutics can improve outcomes when treating AUDs (Heilig et al., 2011; Kranzler and McKay, 2012; Sturgess et al., 2011). Such an example is observed in clinical studies where naltrexone is more efficacious in carriers of the A118G polymorphism on the μ-opioid receptor gene *OPRM1* (Anton, 2008; Anton et al., 2012; Schacht et al., 2013). Therefore, identifying genetic factors that contribute to alcohol and drug abuse is paramount to the development of new pharmacotherapies.

The Kv7 family of voltage-gated potassium channels consists of 5 subunits, Kv7.1-Kv7.5, encoded by the genes *Kcnq1-5*. Four subunits come together to form the channels responsible for M-current in peripheral organs and the brain (Jentsch, 2000; Selyanko et al., 2002; Wang et al., 1998). Kv7.2-Kv7.5 are found abundantly in the CNS, and emerging evidence suggests that *KCNQ* genes may be influenced by or influence alcohol-related behaviors. At the genetic level, two studies have found that *Kcnq3* transcriptional levels are decreased in the nucleus accumbens (NAc) following both

alcohol self-administration and voluntary drinking paradigms in rodents (Bell et al., 2006; Rodd et al., 2008). A recent study has associated differential expression of *Kcnq2* as a candidate cis-eQTL for alcohol consumption and withdrawal symptoms (Metten et al., 2014). There is also evidence that *KCNQ* knockout *drosophila* are less sensitive to the effects of alcohol compared to wildtype controls (Cavaliere et al., 2012; Cavaliere et al., 2013). While exciting, these studies only provide a small picture of any potential association between the *KCNQ* genes and alcohol consumption. We sought to further explore this connection through *in silico* means.

As the –omics fields advance, researchers are able to collect vast amounts of data with single experiments. Massive, comprehensive databases are generated and made available to other groups through *in silico* approaches. We capitalized on this form of analysis to examine the association of the *Kcnq* family and alcohol-related phenotypes before pursuing behavioral approaches. The GeneWeaver software system (GeneWeaver.org) (Baker et al., 2012) consists of a curated database of functional genomics experimental results across 9 species and many types of experimental data including gene expression analyses, quantitative trait locus (QTL) positional candidate sets, curated literature annotations for gene functions, chemical interactions, and mutation screens. Using this software system we were able to query every available genetic study associated with an alcohol-phenotype in rodents. In this way we were able to identify a relationship between *Kcnq* genes and alcohol phenotypes as well as two compelling candidate SNPs implicating *Kcnq2* with alcohol-related behaviors. These data provide compelling preliminary evidence to suggest that matching variations in genes to therapeutics can improve treatment outcomes in AUD patients, and that *KCNQ* genes and their products are targets for treating AUD.

Materials and Methods

GeneWeaver Bioinformatics

The GeneWeaver database was queried for '*KcnqX* alcohol' to retrieve experiments that implicate *Kcnq2-5* in alcohol-related behaviors. Results were refined to exclude studies in which alcohol-related behavior was not an explicit characteristic in the experiment. Two analyses were performed: the 'gene set graph' to display a bi-partite graph of genes and the studies to which they are connected, and 'hierarchical similarity graph' which generates a bootstrapped graph of all populated intersections among gene sets, arranged such that the highest order intersections are in the root or uppermost nodes of the graph, and individual gene sets are in the leaves, or lowest level nodes in the graph. Single nucleotide polymorphism (SNP) analyses were performed using the Sanger Mouse Genomes Project SNP viewer (Keane et al., 2011).

Results

Integrative Functional Genomics of Kcnq *Genes*

The nature and extent of the relationship between the genes that encode Kv7 channels and the behavioral effects of alcohol are largely unknown, thus we performed a GeneWeaver database search to identify previous whole genome studies in which *Kcnq* genes have been associated with alcohol. *Kcnq2* and *Kcnq3* (**Table 2.1**) are differentially expressed in a several alcohol-related experimental studies. Specifically, 12 gene sets were found to be associated with *Kcnq2* and alcohol, and 14 gene sets were associated with *Kcnq3* and alcohol. GeneWeaver analysis indicated *Kcnq5* was associated with 5 gene sets, and *Kcnq4* was only found in 1 alcohol-related gene set (**Table 2.2**).

Table 2.1 | Gene sets associated with alcohol phenotypes containing *Kcnq2/3*

GeneWeaver GSID	Description	Number of Genes	Species	Strains	PMID
Kcnq2					
GS1140	Differential expression in 22TNJ EtOH induced locomotor activation mutant	398	Mouse	22TNJ	17663592
GS216898	List of genes associated with EtOH phenotypes from microarray and KO data developed by INIA	70	Mouse	various	23550792
GS135282	Positional candidates for EtOH preference locus, female specific, Alcp6	380	Mouse	AXB/BXA	9880655
GS84137	Positional candidates for high-dose EtOH actions (LORR and neurotensin receptor densities)	559	Mouse	LSXSS	9023306
GS35466	Cerebellum gene expression correlates of EtOH induced locomotor activation	26	Mouse	BXD	19958391
GS84141	Positional candidates for EtOH consumption QTL 1, Vacq1	80	Mouse	AXB/BXA	9880655
GS3647	Differential expression meta-analysis in high vs. low EtOH consuming lines	3298	Mouse	various	16618939
GS35071	Whole brain gene expression correlates of EtOH induced ataxia	49	Mouse	BXD	19958391
GS84140	Positional candidates for EtOH consumption locus	296	Mouse	BALB/cJ vs C57BL/6ByJ	11109025
GS135281	Positional candidates for EtOH preference locus, male specific, Alcp5	380	Mouse	AXB/BXA	9880655
GS84142	Positional candidates for EtOH preference locus	282	Mouse	AXB/BXA	9880655
GS84143	Positional candidates for EtOH consumption locus	282	Mouse	BALB/cJ vs C57BL/6ByJ	11109025
Kcnq3					
GS135133	Differentially expressed in hippocampus of high and low reactivity selected rat lines	3441	Rat	bHR vs bLR	21864320
GS135132	Differentially expressed in nucleus accumbens of high and low reactivity selected rat lines	3441	Rat	bHR vs bLR	21864320
GS84286	Positional candidates for alcohol consumption locus	620	Mouse	BALB/cJ vs C57BL/6ByJ	11109025
GS14929	Differentially expressed following chronic alcohol in nucleus accumbens	179	Rat	iP	18405950
GS84284	Positional candidates for alcohol acceptance locus	432	Mouse	(C57BL/6J x DBA/2J)F2	9399693
GS37147	Differentially expressed genes in the NAc following continuous alcohol drinking in P rats	350	Rat	P	19666046
GS135223	Positional candidates for alcohol acceptance locus	196	Mouse	(C57BL/6J x DBA/2J)F2 RI	9880665
GS115112	Positional candidates for mean ethanol consumption locus, Mec6	1007	Mouse	(C57BL/6J x C3H/HeJ)F2	19691874
GS115114	Positional candidates for alcohol preference locus	843	Mouse	(C57BL/6J x DBA/2J)F2	9145544
GS115115	Positional candidates for voluntary ethanol consumption	659	Mouse	BXD RI	9581646
GS115116	Positional candidates for alcohol acceptance locus, Aaq1	626	Mouse	(C57BL/6J x DBA/2J)F2	9399693
GS115117	Positional candidates for ethanol free choice consumption	843	Mouse	BXD RI	9880656
GS115121	Positional candidates for Ethanol Lore5 locus	309	Mouse	ILS x ISS	12436047
GS75567	Differentially expressed following chronic alcohol in amygdala.	190	Rat	iP	18405950

Table 2.2 | Gene sets associated with alcohol phenotypes containing *Kcnq4/5*

GeneWeaver GSID	Description	Number of Genes	Species	Strains	PMID
Kcnq4					
GS84162	Positional candidates for chronic EtOH withdrawal severity	386	Mouse	WSP WSR	12925894
Kcnq5					
GS84096	Positional candidates for EtOH consumption on Chr1	371	Mouse	B6.C vs B6.I	11109025
GS135279	QTL associated with EtOH preference locus 25, male specific	115	Mouse	C57BL/6J x DBA/2	10443995
GS84098	Positional candidates for EtOH preference locus; Alcp1, Alcp5	475	Mouse	C57BL/6 x DBA/2	10443995
GS84097	Positional candidates for EtOH preference locus; Alcp2, Alcp3, Alcp4	370	Mouse	DBA/2J	9880657
GS84101	Positional candidates for EtOH withdrawal on Chr1	305	Mouse	C57BL/6J DBA/2J BXD	9655868

Hierarchical similarity analysis indicated that *Kcnq2* (**Figure A.1**) and *Kcnq3* (**Figure A.2**) lie in multiple alcohol-related QTLs on mouse chromosomes (Chr) 2 and 15, respectively. In both cases, only a few other genes were also in the same multi-QTL intersections, including *Ppdpf* on Chr *2* (**Figure 2.1a**) and *Ptk2* or *Adcy8* on Chr 15 (**Figure 2.1b**). Analysis of *Kcnq5* (**Figure A.3**) also indicates that this gene also lies in several alcohol-related QTLs, however, at the highest level of the hierarchy, *Kcnq5* was found with 40 other genes. Altogether, these analyses indicate that *Kcnq2* and *Kcnq3* are highly associated with alcohol-related phenotypes, and that while the *Kcnq4* and *Kcnq5* genes are also related to these phenotypes, it is to a much lesser extent. Because of the relatively low number of associated gene sets, subsequent analysis and discussion will focus on *Kcnq2* and *Kcnq3*.

Using the WTSI mouse genomes data, we performed a search for SNPs in the region of *Kcnq2* and *Kcnq3* with the goal of identifying SNPs that could account for QTL effects, which were mapped from different crosses. Therefore, we were looking for loci that differed between C57BL/6 versus both A and DBA/2 strains. These searches

yielded two compelling candidate SNPs in *Kcnq2*, rs27642425 (missense variant) and rs2971971 (splice variant).

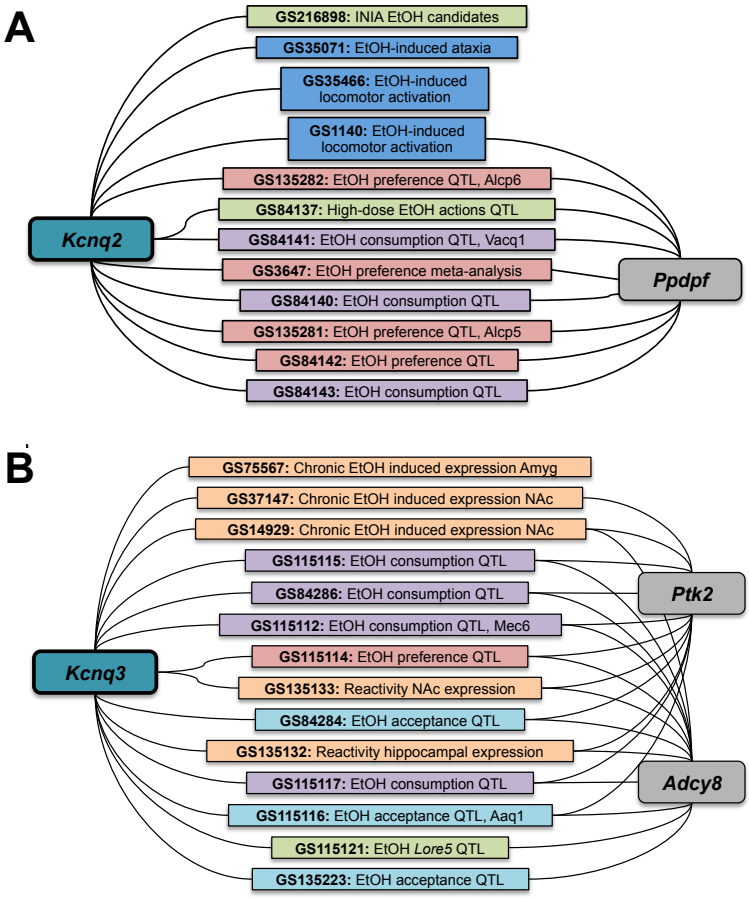

Figure 2.1 | Gene sets containing *Kcnq2* and *Kcnq3* that are associated with multiple alcohol-related phenotypes . (A) *Kcnq2* was found to lie in alcohol-related QTLs on mouse Chr 2. *Ppdpf* was also identified in a subset (9 of 12 gene sets) of the multi-QTL intersections on mouse Chr 2. **(B)** *Kcnq3* was found to lie in alcohol-related QTLs on mouse Chr 15 in rats and mice. *Ptk2* and *Adcy8* in rodents were also identified in some of the multi-QTL intersections. Phenotypes are grouped similarity. Dark blue = motility, red = preference QTLs, purple = consumption QTLs, orange = gene expression, light blue = acceptance QTLs, green = misc. QTLs.

Discussion

Integrative functional genomic analyses using GeneWeaver software demonstrated that *Kcnq2* and *Kcnq3* are included in the support interval for replicated QTLs for alcohol consumption and alcohol-related behaviors on mouse Chrs 2 and 15.

This suggests that *Kcnq2* and *Kcnq3* are candidates for genetic diversity in alcohol-related behavior found in multiple inbred mouse crosses, selected line studies, and mutant studies. Differential expression of *Kcnq2* and *Kcnq3* in these populations is indicated as a mechanism of action of the polymorphisms in the region. Our results indicate that no other positional candidate in the intersection of the QTL loci is as highly connected to alcohol-related phenotypes as *Kcnq2* or *Kcnq3*.

The GeneWeaver analysis identified 3 studies investigating alcohol-associated genetic adaptations explicitly in the NAc. The NAc is a critical region to the addiction neurocircuitry, acting as a gateway to motor output (Kalivas, 2009). *Kcnq* genes are differentially expressed in the NAc of bHR (high drug-responding) and bLR (low drug-responding) rats (Clinton et al., 2011). Two studies using RT-qPCR observed decreased *Kcnq*3 mRNA expression in the NAc of P rats after alcohol consumption (Bell et al., 2009; Rodd et al., 2008). Finally, a recent study not included in the GeneWeaver analysis, has reported differential expression of *Kcnq2* in the ventral striatum (NAc) of mice selectively bred for high alcohol consumption/low withdrawal severity versus mice bred for low consumption/high withdrawal severity (Metten et al., 2014). The authors also reported a quantitative trait locus (QTL) on distal Chr 2, and alignment of the QTL with differential expression data identified *Kcnq2* as a candidate cis-eQTL for alcohol consumption and withdrawal. Together these studies suggest that *KCNQ* genes specifically in the NAc are associated with alcohol phenotypes.

Several polymorphisms were identified in *Kcnq2* that could account for the genetic effects observed among the various mouse crosses, most of which involved deviation from the C57BL/6 phenotype. The candidate SNPs identified in these analyses, rs27642425 and rs2972972, vary between the C57BL/6 and the A and DBA/2 strains, and are not known to be clinically relevant. Alcohol consumption has not been

extensively characterized in the A strains of mice, however there are profound differences in the drinking patterns of C57BL/6 and DBA mice. C57BL/6 mice are an alcohol-preferring strain whereas the DBA mice avoid alcohol. Studies indicate that C57BL/6 mice drink more in 2-bottle choice paradigms (Meliska et al., 1995; Rhodes et al., 2007) and short-access models (Belknap et al., 1997; Le et al., 1994), and that DBA mice are less sensitive to alcohol-induced locomotor sensitization (Kiianmaa and Tabakoff, 1983). Numerous recombinant inbred (RI) strains have been developed from interbreeding the C57BL/6J and DBA/2J strains. These mice, referred to as "BXD" strains, represent continuum of alcohol phenotypes that strongly correlate to their genetic composition (DuBose et al., 2013; Mulligan et al., 2006; Padula et al., 2015; Vanderlinden et al., 2013). Given these differences in alcohol phenotypes, it would be of interest to the alcohol field to test the effects of the SNPs identified in our study on Kv7 channel currents, gene products, and alcohol-related behaviors.

Although preliminary, these data compelling pharmacogenetic evidence that suggests that matching variations in genes to therapeutics can improve treatment outcomes in individuals with AUDs (Anton et al., 2008; Anton et al., 2012; Kranzler and McKay, 2012; Schacht et al., 2013). Thus, *KCNQ2* and *KCNQ3* and their expressed proteins may be useful pharmacogenetic targets to treat AUDs. In the following experiments we directly tested the role of Kv7 channels as a pharmaceutical target to decrease alcohol consumption using the Kv7 channel opener retigabine in a home cage drinking in a long-term access, escalation of drinking model.

CHAPTER 3: SYSTEMIC RETIGABINE AND VOLUNTARY DRINKING

Introduction

In previous chapters we discussed the need for new pharmaceutical interventions for treating alcohol use disorder (AUD), and the high correlation of *Kcnq* genes with alcohol phenotypes across multiple rodent models of alcohol exposure. The *in silico* analysis from Chapter 2 provides evidence that Kv7 channels are involved with regulating alcohol-related behaviors and are a candidate target for treating AUD. As such, the FDA-approved anticonvulsant and positive modulator of Kv7 channels, retigabine may be a useful pharmacotherapy.

There is emerging evidence that Kv7 channels are a target of the actions of alcohol on neuronal function and behavior. Acute alcohol attenuated M-current recorded from human embryonic kidney cells expressing mammalian *KCNQ2/3*, ventral tegmental area (VTA) dopamine (DA) neurons, and pyramidal neurons in hippocampus (Koyama et al., 2007; Moore et al., 1990). Studies in *Drosophila* have shown that Kv7 channels regulate acute alcohol tolerance and acute alcohol-induced memory impairments (Cavaliere et al., 2012; Cavaliere et al., 2013). A recent study using a short-term limited-access (30 min) drinking paradigm showed that retigabine reduced voluntary alcohol consumption in rats (Knapp et al., 2014). Moreover, Metten et al. (2014) recently reported differential expression of *Kcnq2* in the ventral striatum of mice selectively bred for high alcohol consumption/low withdrawal severity versus mice bred for low consumption/high withdrawal severity. These authors also reported a quantitative trait locus (QTL) on distal chromosome (Chr) 2, and alignment of the QTL with differential

expression data identified *Kcnq2* as a candidate cis-eQTL for alcohol consumption and withdrawal. Altogether these studies suggest that positive modulators of Kv7 channels may reduce voluntary drinking in rodents.

There are multiple commonly used rodent models of alcohol consumption (Becker, 2013; Spanagel et al., 2014), however for the purposes of this project, we utilized the 2-bottle choice intermittent alcohol access (IAA) model in Wistar rats. The IAA model was first introduced in the 1970s (Amit et al., 1970; Wayner and Greenberg, 1972; Wise, 1973), and simply requires that rats have intermittent 24-hour access to a choice of water or 20% alcohol. No initiation procedure (e.g. sucrose fadeout, alcohol vapor chamber exposure, water deprivation) is necessary to induce or maintain drinking. Rats will escalate their daily alcohol intake over the first 6-9 drinking sessions and reach and maintain a stable baseline of high consumption (~5-6 g/kg/24 hrs) for several months (Simms et al., 2008; Wise, 1973). At baseline, outbred rat strains (e.g. Long-Evans and Wistar) consume enough alcohol to reach pharmacologically relevant blood ethanol concentrations (BECs) (Bell et al., 2006; Simms et al., 2008) and drink as much as strains specifically bred to prefer drinking alcohol (e.g. P rats) (Bell et al., 2006; Spanagel et al., 2014). This drinking model is quite versatile having been implemented in microinjection studies (Carnicella et al., 2009), and is known to induce neuroadaptations during the maintenance period of drinking (Bell et al., 2006; Camp et al., 2006; Hopf et al., 2011). In summary, the IAA model induces outbred strains to consume high amounts of alcohol without any initiation procedure and represents voluntary, choice drinking in the rodents' home cage.

In the experiments described in this and subsequent chapters, we used the 2-bottle choice IAA paradigm to investigate the potential of retigabine as a pharmacotherapeutic for the treatment of AUD and drinking-induced neuroadaptations in

Kv7 channels. In the following experiments we measured the effect of systemic retigabine on drinking behaviors and investigated the specificity of retigabine on alcohol (ethanol; EtOH) consumption by testing its effects on tastant preference and locomotor activity. Finally, because retigabine is known to have off-target activity at $GABA_A$ receptors (Otto et al., 2002; Rundfeldt, 1997; Rundfeldt and Netzer, 2000), we investigated the mechanism of action through which retigabine may reduce drinking.

Materials and Methods

Animals and Housing

Male Wistar rats (150g; P39-45) were purchased from Harlan (Indianapolis, IN) and housed individually in standard home cages in temperature and humidity controlled environments. In all experiments, rats were exposed to 12 hr light/dark cycles with food and water available *ad libitum* during all procedures. Animals used throughout this project were given at least 1 wk to acclimate to their respective colony rooms. All procedures were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee and were in accordance with the NIH guidelines for the humane care and use of laboratory animals (2011).

Two-bottle Choice 24-hr Intermittent Access & Systemic Drug Treatment

Intermittent access to alcohol (20% *v/v*) was performed as previously described (Simms et al., 2008; Wise, 1973). Briefly, rats had 2 bottles of water available during habitation, and were allowed 24 hr access to one bottle of 20% alcohol (*v/v* tap water) and one bottle of tap water beginning just prior to the start of the dark cycle on Monday, Wednesday and Friday with 24 or 48 hrs of deprivation between drinking sessions. Placement of the alcohol solution (left or right side of the cage) was alternated between

sessions to control for side preference. Leakage from the sipper tubes was accounted for when weighing the bottles. Once rats reached a stable baseline alcohol consumption, drugs were administered via intraperitoneal (IP) injections in a final volume of 3 mL/kg 30 minutes prior to alcohol availability once per week on Wednesdays following a within-subjects design. Drug doses were as follows: 0 – 7.5 mg/kg retigabine (Axon MedChem, The Netherlands), 0-20 mg/kg ML-213 (Tocris Bioscience, Bristol, UK), 0 – 20 mg/kg ICA-069673 (Tocris Bioscience), and 0 – 1 mg/kg muscimol (Tocris Bioscience). Vehicle for all drug injections was 10% Tween80 *v/v* saline. For combined muscimol and ICA-069673 injections, drugs were made up as a single solution. Bottle weights were taken 6 and 24 hrs after alcohol availability.

Blood Ethanol Concentration

Once rats reached a stable baseline of alcohol consumption (3-4 wks), blood was taken from the tail vein 30 min, 1 hr, and 3 hr into a drinking session. Only one time point was collected per day with at least 1 wk between collections. Samples were collected from multiple cohorts and the data were collapsed for analysis (n = 27 samples total). BECs were determined using an Analox Instrument analyzer (Lunenburg, MA, USA).

Intermittent Tastant Access

Two separate cohorts of alcohol-naïve Wistar rats were placed in the 2-bottle choice intermittent access paradigm and were given access to either 0.02% saccharin (*v/v* tap water) or 15 μM quinine (Sigma-Aldrich, St. Louis, MO). Once stable consumption was established (2-3 wks), rats were treated with IP retigabine (7.5 mg/kg) or vehicle (10% Tween80 v/v saline) on Wednesdays using a within-subjects design.

Locomotor Activity Assay

Two cohorts of alcohol-naïve Wistar rats were habituated to a 57 cm x 58 cm x 63 cm opaque acrylic box for 1 hr per day for 2 consecutive days. Over the next 3 consecutive days, rats received a vehicle (10% Tween80 v/v saline) or 7.5 mg/kg IP retigabine injection 1 hr prior to a 1 hr locomotor session. Drug was administered in a pseudo-randomized, repeated measures fashion. Activity was digitally recorded with an overhead video camera under red light. Total movement was automatically scored using EthoVision XT software (Noldus Information Technology, the Netherlands).

Statistical Analysis

A mixed-model analysis of variance procedure was used in the statistical software language SAS to analyze all drinking and locomotor data using repeated measures factors. A between-within subjects method was used to determine degrees of freedom. To follow up significant interactions and treatment effects, Fisher LSD post-hoc tests were used. Significant correlations between BEC and alcohol consumption were calculated using a linear regression analysis. Finally, all data are reported as mean ± SEM and statistical significance was established with $p < 0.05$.

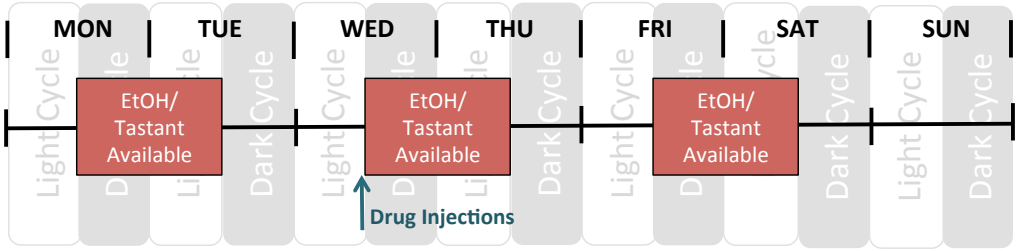

Figure 3.1 | The intermittent alcohol access paradigm. In the IAA paradigm 20% EtOH or tastant solution and water are made available for 24 hrs at the start of the dark cycle on Monday, Wednesday, and Friday. Drugs were administered 30 min prior to EtOH availability (blue arrow) on Wednesdays in a repeated measures fashion.

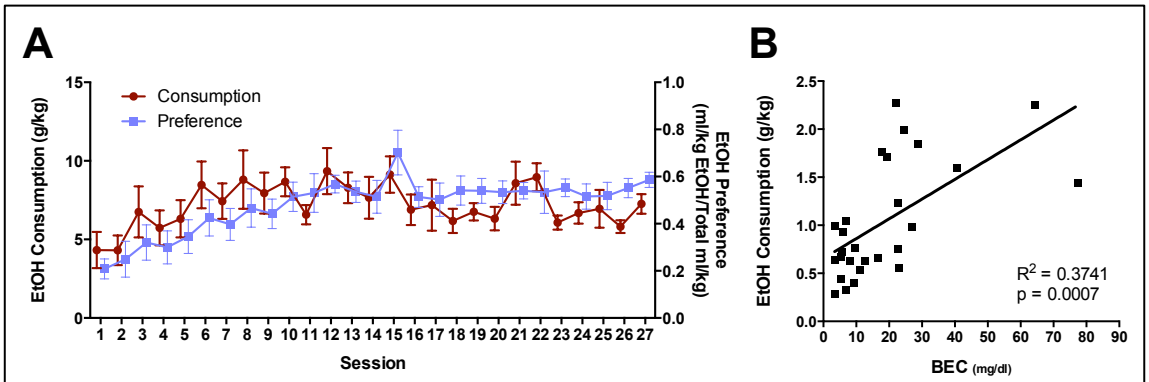

Figure 3.2 | Drinking in the IAA paradigm. **(A)** In the IAA model rats escalate their drinking (red line) and preference (purple line) over the first 6 drinking sessions, after which they maintain a stable baseline for an extended period of time. **(B)** EtOH consumption (g/kg) significantly correlated with blood EtOH concentration (BEC; mg/dL) in the first 30 – 180 min of EtOH availability.

Results

Effect of Retigabine on Voluntary Alcohol Consumption

Given the association of *Kcnq* genes with alcohol phenotypes and recent evidence showing that retigabine reduces drinking a short-access model (Knapp et al., 2014), we hypothesized that retigabine would reduce drinking in the 2-bottle choice intermittent alcohol access drinking paradigm (**Figure 3.1**). In this model, rats regularly consumed ≥3g EtOH/kg/24 hrs (range 3.01 - 11.85 g/kg/24 hrs; average 5.43 ± 0.351 g/kg/24 hrs; **Figure 3.2a**). Alcohol consumption (g/kg) significantly correlated with BECs during the first 30-180 mins of a given drinking session (R^2 = 0.3741, p = 0.0007; **Figure 3.2b**). BECs were 20.39 ± 7.504 mg/dL after 30 min of alcohol availability. After 9 wks of drinking, rats (n = 8-14) received systemic injections of retigabine prior to alcohol availability. Retigabine reduced alcohol consumption (**Figure 3.3a**) and preference **(Figure 3.3b**) as indicated by significant main effects of treatment (consumption: F(3,27) = 8.04; p = 0.0005; preference: F(3,27) = 11.21; p < 0.0001). Post-hoc analysis revealed that 7.5 mg/kg dose reduced drinking compared to vehicle (p < 0.0001), 2.5 mg/kg retigabine (p = 0.0055), and 5 mg/kg retigabine (p = 0.0048). 7.5 mg/kg similarly reduced

preference compared to vehicle ($p < 0.0001$), the 2.5 mg/kg dose ($p = 0.0060$), and the 5 mg/kg dose ($p = 0.0165$). There was no effect of retigabine on total liquid consumption ($F(3,25) = 1.25$; $p = 0.3127$; **Figure 3.3c**).

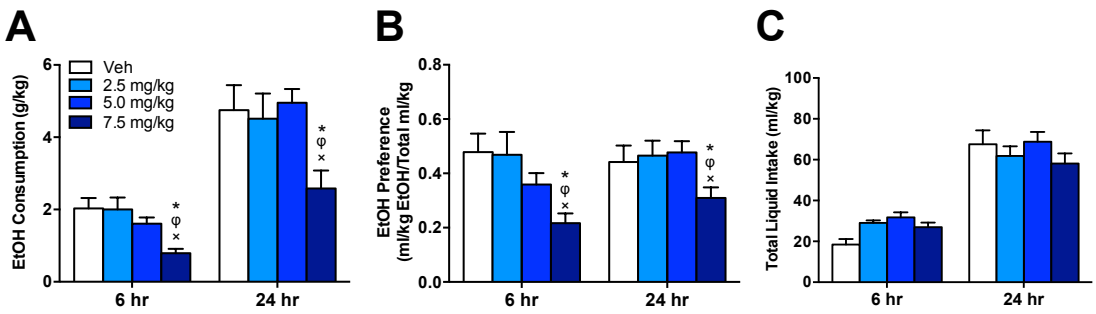

Figure 3.3 | Retigabine reduces voluntary drinking and alcohol preference. 7.5 mg/kg retigabine reduced **(A)** voluntary drinking and **(B)** preference. **(C)** Total liquid intake were not altered by retigabine. (* $p < 0.05$ vs veh; φ $p < 0.05$ vs 2.5 mg/kg; × $p < 0.05$ vs 5.0 mg/kg)

Studies investigating the ability of anticonvulsants to reduce drinking indicate that some are effective only in high-consuming patients and high-preferring rodent lines (Breslin et al., 2010; Chen and Holmes, 2009; Mitchell et al., 2012). Given that there are individual differences in alcohol intake levels in Wistar rats (Blomqvist et al., 1996; Momeni and Roman, 2014), we divided the rats into "high drinking" (HD; n = 4-7) or "low drinking" (LD; n = 4-7) rats using a median split. At the 24 hr time point, HD rats drank significantly more than LD rats after vehicle injections ($t(12) = 4.750$; $p = 0.0005$; mean HD: 6.771 ± 0.7586 g/kg/24 hrs; mean LD: 2.726 ± 0.3867 g/kg/24 hrs). Systemic retigabine had differential effects on the HD and LD groups. In HD rats, retigabine again reduced drinking as indicated by a significant main effect of drug treatment ($F(3,12) = 6.45$; $p = 0.0076$; **Figure 3.4a**). Post-hoc analysis indicated that both 5.0 and 7.5 mg/kg retigabine significantly reduced drinking compared to vehicle ($p = 0.0012$ and $p = 0.0239$, respectively). We also observed a main effect of treatment on alcohol preference ($F(3,12) = 3.20$; $p = 0.0421$; **Figure 3.3d**). Post-hoc analysis showed that 7.5

mg/kg retigabine reduces preference compared to vehicle ($p = 0.0134$). Retigabine had no effect on total liquid intake in HD rats ($F(3,10) = 1.60$; $p = 0.2498$; **Figure 3.4c**).

In the LD group of rats, there was a significant interaction of treatment and time on alcohol consumption ($F(3,12) = 4.21$; $p = 0.0300$; **Figure 3.4d**). At the 6 and 24 hr time points, 7.5 mg/kg retigabine reduced drinking (post-hoc: $p = 0.0499$ and $p = 0.0177$, respectively). Interestingly, 5.0 mg/kg retigabine increased alcohol consumption compared to vehicle at 24 hrs ($p = 0.0008$). There was a main effect of treatment ($F(3,12) = 9.30$, $p = 0.0019$) on alcohol preference, indicating that 7.5 mg/kg retigabine reduced preference in LD rats ($p = 0.0002$; **Figure 3.4e**). Total fluid consumption was not affected by retigabine ($F(3,12) = 2.59$; $p = 0.1012$; **Figure 3.4f**). Together these data indicate that systemic retigabine can reduce drinking in some populations of rats and that the effect is dependent on dose and the individual's propensity to drink.

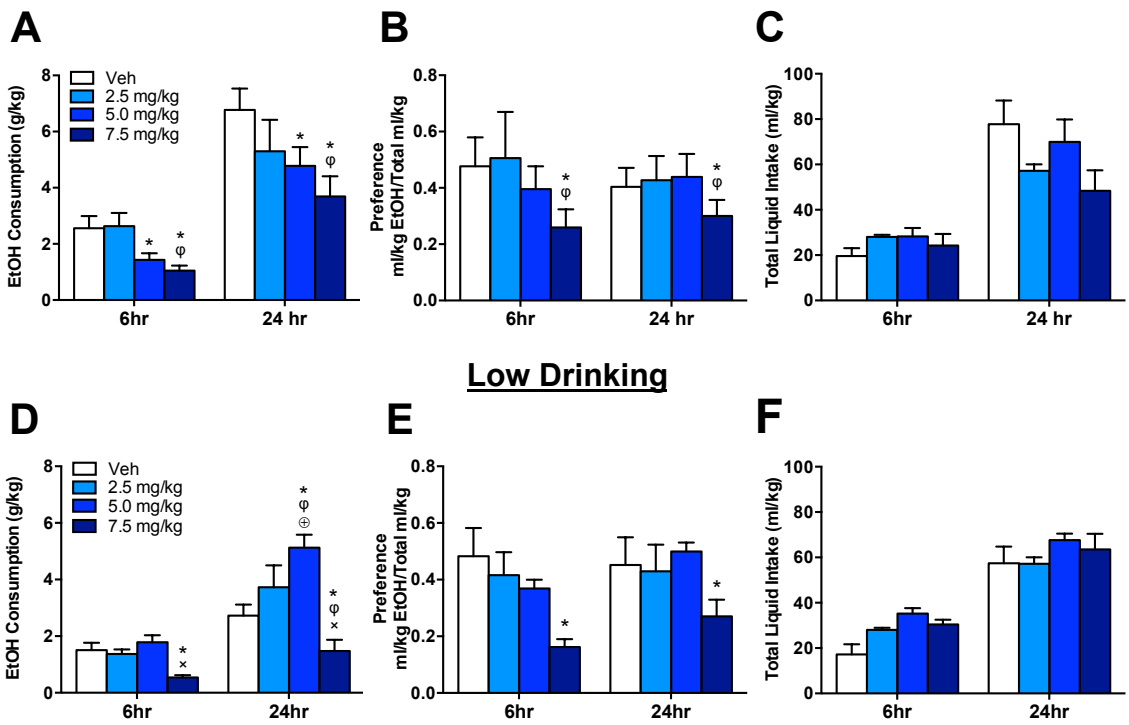

Figure 3.4 | Systemic retigabine differentially affects consumption in high and low drinking populations of rats. Drinking rats were divided into two groups defined by the median level of EtOH consumption. **(A)** In HD rats, 5.0 and 7.5 mg/kg retigabine reduced EtOH consumption. **(B)** 7.5 mg/kg retigabine also reduced EtOH preference. There was not a significant effect of retigabine on **(C)** total fluid intake in high drinking rats. In LD rats **(D)** 7.5 mg/kg retigabine again reduced drinking 6 and 24 hrs after EtOH availability. 5.0 mg/kg increased EtOH consumption at the 24 hr time point. **(E)** 7.5 mg/kg reduced preference, and there was no effect of retigabine on **(F)** total fluid consumption. (* $p < 0.05$ vs veh; φ $p < 0.05$ vs 2.5 mg/kg; × $p < 0.05$ vs 5.0 mg/kg; + $p < 0.05$ vs 7.5 mg/kg)

Effect of Retigabine on Motility and Taste Perception

It is possible that the effect of retigabine on drinking is due to non-alcohol related behaviors such as taste perception and motility. To investigate the effect of systemic retigabine on taste preference, alcohol-naïve rats (n = 8) were given access to either 0.02% saccharin or 15 μM quinine in the IAA model. In these experiments, retigabine (7.5 mg/kg) did not significantly alter consumption of saccharin ($F(1,6) = 0.18$; $p = 0.6828$; **Figure 3.5a)**. However, retigabine did increase consumption of the aversive-

tasting quinine (main effect of treatment: F(1,6) = 0.636; p = 0.0451; **Figure 3.5a**). We again divided the rats into HD and LD groups based on median tastant intake. There was no significant difference in drinking between HD and LD rats in the saccharin cohort (t(6) = 2.264; p = 0.0642; n = 4) or in the quinine cohort (t(5) = 1.917; p = 0.1143; n = 3-4; data not shown), suggesting these cohorts cannot be segregated. In addition, this analysis would be underpowered to make strong conclusions. Subsequent discussion will consider the saccharin and quinine cohorts as single populations of drinkers. Together, these data indicate that retigabine does not simply decrease intake of all drinking solutions, but rather that the reduction appears specific for alcohol.

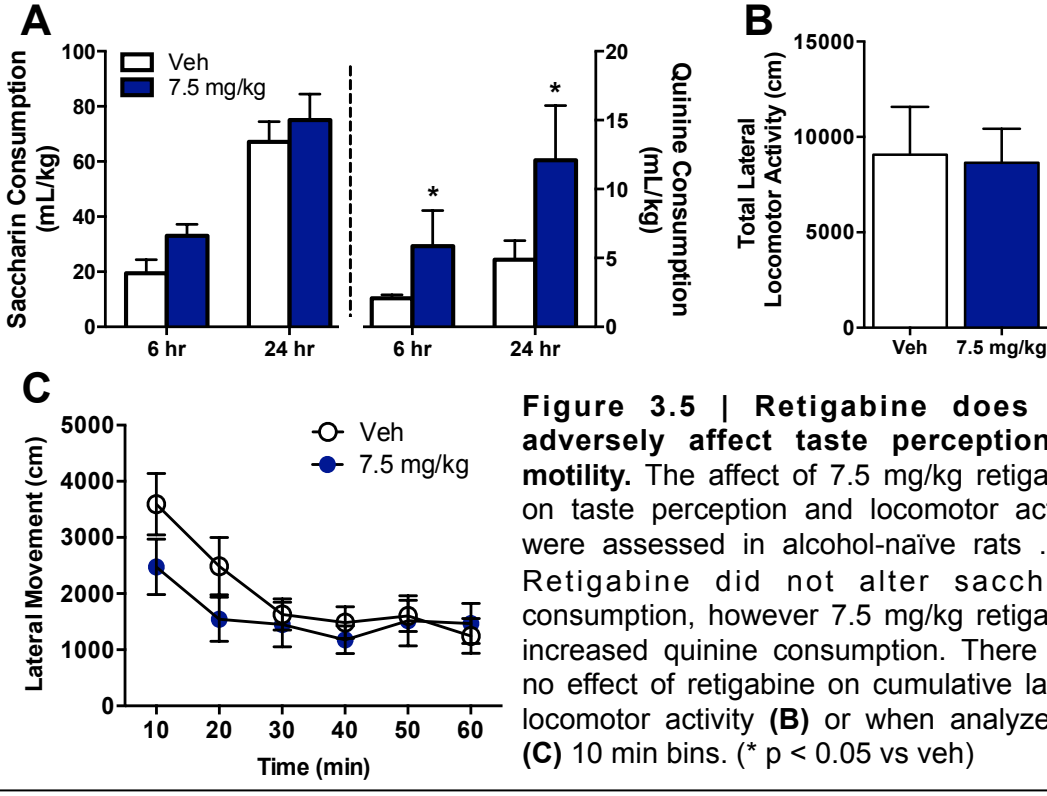

Figure 3.5 | Retigabine does not adversely affect taste perception or motility. The affect of 7.5 mg/kg retigabine on taste perception and locomotor activity were assessed in alcohol-naïve rats . **(A)** Retigabine did not alter saccharin consumption, however 7.5 mg/kg retigabine increased quinine consumption. There was no effect of retigabine on cumulative lateral locomotor activity **(B)** or when analyzed in **(C)** 10 min bins. (* p < 0.05 vs veh)

Retigabine has also been shown to decrease locomotor activity (Hansen et al., 2007), raising the possibility that this drug reduces drinking through motor impairments. To address this, alcohol-naïve rats (n = 8) received systemic retigabine (7.5 mg/kg) or vehicle prior to a 60 min open field exploration task. We found no effect of retigabine on

lateral movement when activity was binned in 10 min periods (F(1,7) = 4.35; *p* = 0.0756; **Figure 3.5b**). There was also no effect of retigabine on cumulative locomotor activity across the entire 60 min test period (t(7) = 0.1850; p = 0.8585; **Figure 3.5c**). These experiments indicate that retigabine reduces voluntary drinking in an alcohol-specific manner.

Determining the mechanism of action of retigabine

Retigabine is a relatively non-selective Kv7 channel positive modulator and increases M-current in homo- and heterotetrameric channels (EC_{50} = 0.6-6.2 μM) (Gunthorpe et al., 2012). Furthermore at high enough concentrations (> 10 μM) (Otto et al., 2002; Rundfeldt, 1997; Rundfeldt and Netzer, 2000), retigabine can activate $GABA_A$ receptors. To determine a mechanism through which retigabine reduces drinking we administered Kv7 channel-specific and subunit-selective drugs to rats in the IAA paradigm (Brueggemann et al., 2014; Yu et al., 2010). First we administered 20 mg/kg ML-213, a Kv7.2/Kv7.4-selective positive modulator to IAA rats (n = 10-11). As shown in **Figure 3.6**, ML-213 had no effect on alcohol consumption (F(1,9) = 2.14; p = 0.1774), preference (F(1,9) = 0.99; p = 0.3469), or total liquid intake (F(1,9) = 2.01; p = 0.1901).

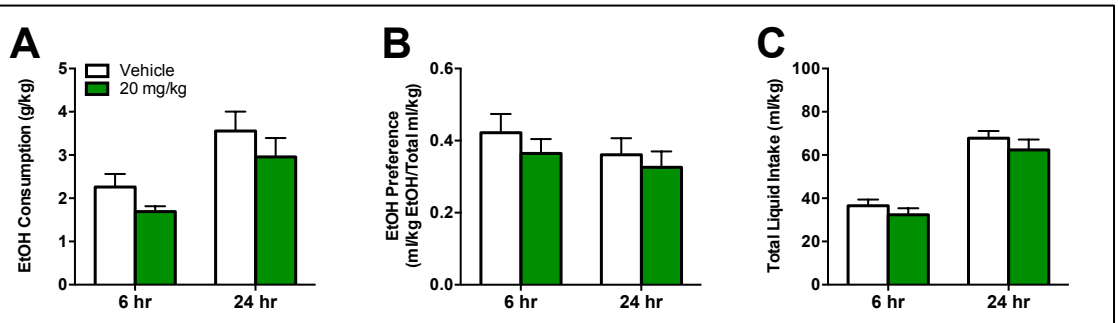

Figure 3.6 | ML-213 does not affect alcohol consumption. Rats in the IAA paradigm received 20 mg/kg IP injections of the Kv7.2/Kv7.4 channel-selective positive modulator ML-213 and drinking was measured 6 and 24 hrs after EtOH availability. In the total population of rats, ML-213 had no affect on **(A)** EtOH consumption, **(B)** preference, or **(C)** total liquid intake.

We again divided the cohort into HD (n = 5) and LD (n = 5-6) groups, where HD rats consumed more alcohol than LD rats ($t(9) = 3.378$, $p = 0.0082$; mean HD: 4.716 ± 0.4605 g/kg/24 hrs; mean LD: 2.591 ± 0.427 g/kg/24 hrs). In the HD group, ML-213 reduced voluntary alcohol consumption ($F(1,4) = 14.39$; $p = 0.0192$; **Figure 3.7a**) and preference ($F(1,4) = 11.63$; $p = 0.0270$; **Figure 3.7b**) as indicated by significant main effects of treatment. There was no effect of ML-213 on total liquid intake ($F(1,4) = 2.71$; $p = 0.1751$; **Figure 3.7c**). In LD rats there was no effect of ML-213 on alcohol consumption ($F(1,4) = 0.69$; $p = 0.4539$; **Figure 3.7d**), preference ($F(1,4) = 1.31$; $p = 0.3155$; **Figure 3.7e**), or total fluid intake ($F(1,4) = 0.37$; $p = 0.5770$; **Figure 3.7f**). Together these data suggest that Kv7.2 and Kv7.4 are important for the regulation of drinking behaviors in HD rats.

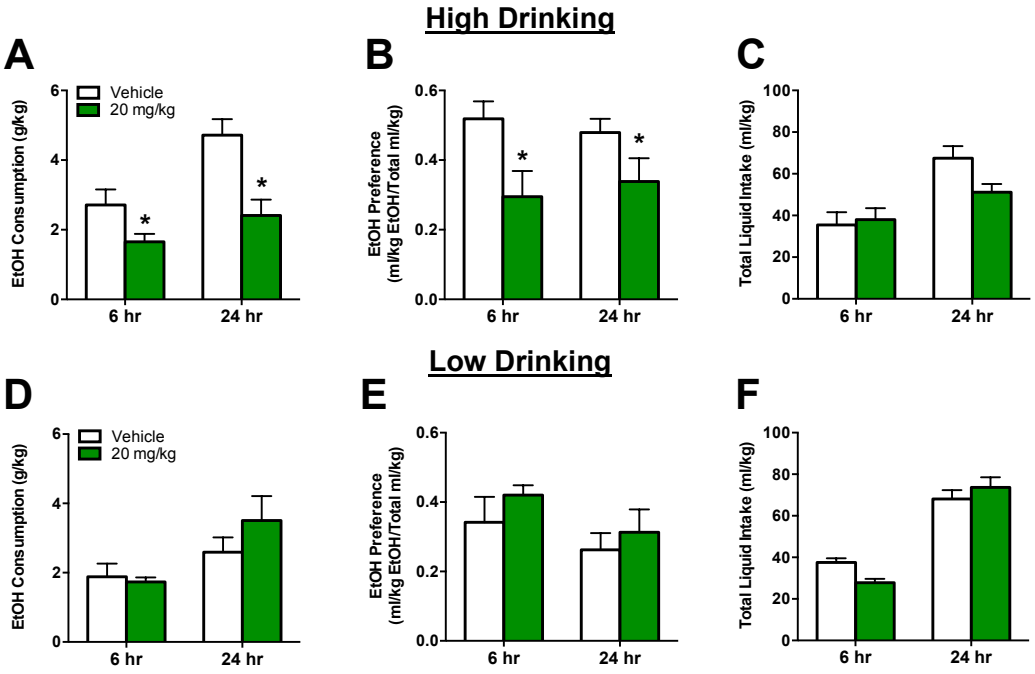

Figure 3.7 | ML-213 decreases voluntary alcohol consumption in HD rats. Rats in the IAA paradigm received 20 mg/kg IP injections of the Kv7.2/Kv7.4 channel-selective positive modulator ML-213. In HD rats ML-213 reduced alcohol **(A)** consumption and **(B)** preference and had no effect on **(C)** total fluid intake. In LD rats there was no effect of ML-213 on alcohol **(D)** consumption, **(E)** preference, or **(F)** total liquid intake. (* $p < 0.05$ vs vehicle)

Next we administered the Kv7.2/Kv7.3-selective positive modulator ICA-069673 to IAA rats (n = 10-15). As shown in **Figure 3.8**, 20 mg/kg of ICA-069673 was unable to reduce alcohol consumption ($F(3,57) = 1.027$; $p = 0.3877$), preference ($F(3,56) = 0.6152$; $p = 0.6080$), or total fluid intake ($F(3,57) = 2.407$; $p = 0.0765$).

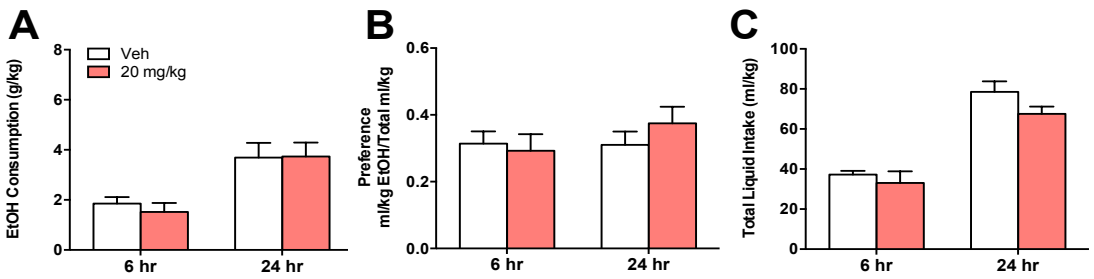

Figure 3.8 | ICA-069673 does not affect voluntary alcohol consumption. Rats in the IAA paradigm received IP injections of the Kv7 channel-specific agonist ICA-069673 (ICA) and drinking was measured 6 and 24 hrs after EtOH availability. In the total population of rats, ICA-069673 had no affect on **(A)** EtOH consumption, **(B)** preference, or **(C)** total liquid intake.

We divided the cohort into HD (n = 4-8) and LD (n = 5-7) rats, where HD rats drank significantly more than the LD group ($t(12) = 3.307$; $p = 0.0063$; mean HD: 5.158 ± 0.823 g/kg/24 hrs; mean LD: 2.218 ± 0.3353 g/kg/24 hrs). There was no effect of ICA-069673 in the HD group on alcohol consumption ($F(1,3) = 2.23$; $p = 0.2321$; **Figure 3.9a**), preference ($F(1,3) = 3.39$; $p = 0.1627$; **Figure 3.9b**), or total liquid intake ($F(1,3) = 2.46$; $p = 0.2150$; **Figure 3.9c**). Similarly, in the LD group, there was no effect of the ICA compound on consumption ($F(1,3) = 4.73$; $p = 0.1180$; **Figure 3.9d**), preference ($F(1,3) = 3.31$; $p = 0.1665$; **Figure 3.9e**), or total fluid intake ($F(1,3) = 2.35$; $p = 0.2520$; **Figure 3.9f**. Due to the lack of effect of ICA-069673 on the total population of rats and when divided into HD and LD groups, subsequent experiments and discussion will consider cohorts treated with ICA-069673 as a whole.

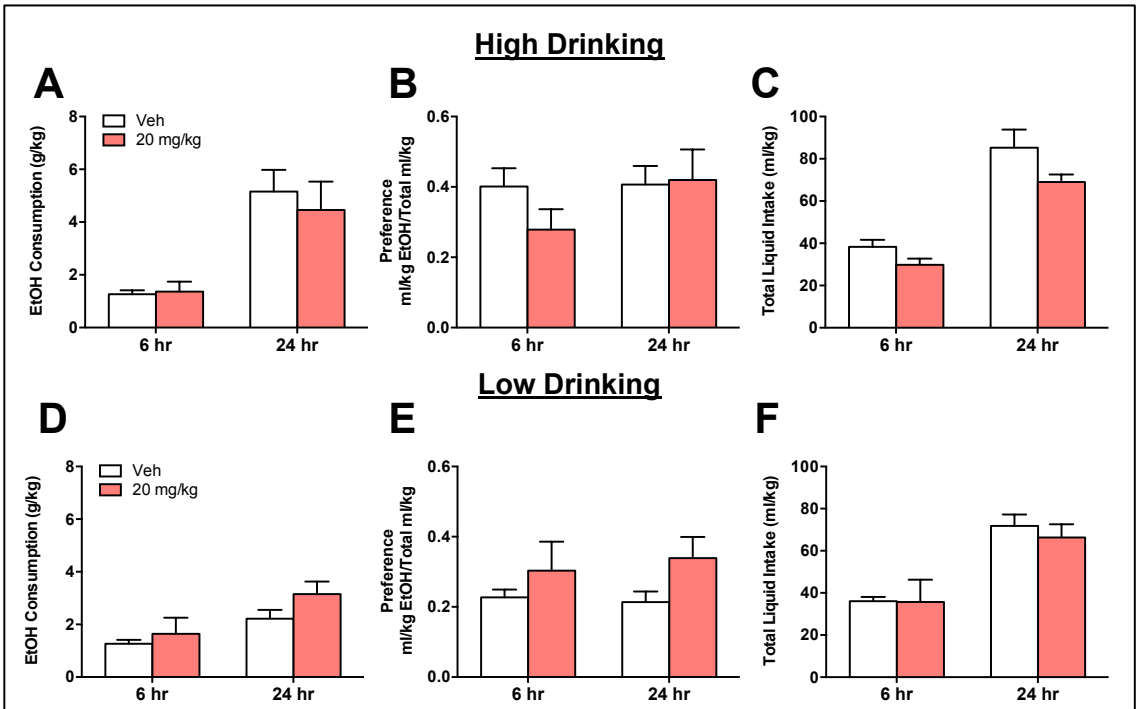

Figure 3.9 | ICA-069673 does not affect voluntary alcohol consumption in HD and LD rats. Rats in the IAA paradigm received IP injections of the Kv7 channel-specific agonist ICA-069673 (ICA). In HD rats, ICA-069673 had no affect on **(A)** EtOH consumption, **(B)** preference, or **(C)** total liquid intake. The ICA compound also had no affect on these parameters when measured in LD rats **(D, E, F)**.

In a separate cohort of rats, we tested the ability of ICA-069673 (20 mg/kg) and muscimol (1 mg/kg), a $GABA_A$-specific agonist, administered both separately and concurrently to reduce drinking. This dose of muscimol was selected because a previous report showed that 3 mg/kg of systemic muscimol reduced alcohol intake in an operant self-administration paradigm, however, a 1 mg/kg dose had no effect (Janak and Michael Gill, 2003). Individually, the ICA compound (20 mg/kg) and muscimol (1 mg/kg) did not alter drinking compared to vehicle (**Figure 3.10a**). As indicated by a main effect of treatment ($F(3,18) = 4.96$; $p = 0.0110$), the combined injection of ICA-069673 with muscimol reduced drinking compared to vehicle ($p = 0.0059$). There was a main effect of treatment on alcohol preference ($F(3,18) = 9.46$; $p = 0.0006$; **Figure 3.10b**), and post-hoc analysis revealed that the combined injection of the ICA compound and muscimol

resulted in decreased preference for alcohol compared to vehicle (p = 0.0009). Individually, ICA-069673 and muscimol did not reduce alcohol preference compared to vehicle. Finally, there was no treatment effect on total fluid consumption ($F(3,18) = 0.52$; $p = 0.6767$; **Figure 3.10c**). Taken together these data indicate opening Kv7.2/Kv7.3-containing channels may be insufficient to alter drinking behaviors on their own but may work synergistically with $GABA_A$ receptors to reduce alcohol consumption.

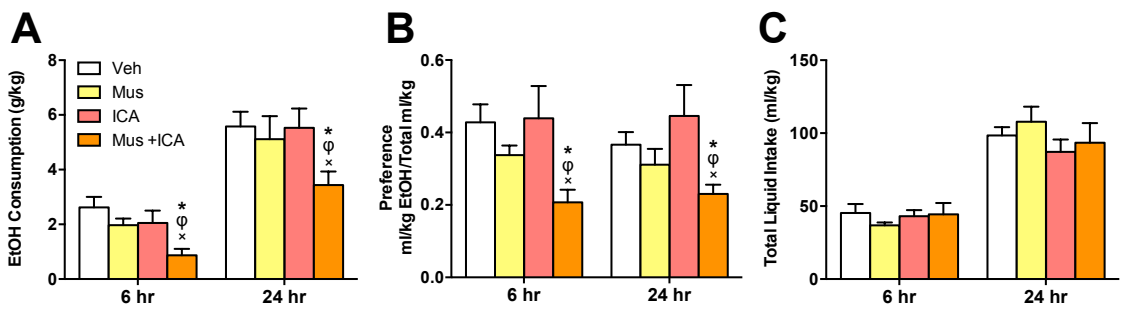

Figure 3.10 | Co-administration of muscimol and ICA-069673 reduce voluntary drinking. Rats in the IAA paradigm received injections of 20 mg/kg ICA-069673 (ICA) and 1 mg/kg muscimol (Mus) both separately and concurrently. Joint injection of Mus and ICA decreased **(A)** EtOH consumption and **(B)** preference. There was no effect of the drugs individually on drinking. There was also no effect of any treatment on **(C)** total liquid intake. (* $p < 0.05$ compared to veh, $^{\Phi}$ $p < 0.05$ compared to Mus, × $p < 0.05$ compared to ICA)

Discussion

In these studies we showed that systemically administered retigabine, a Kv7 channel positive modulator, reduces voluntary alcohol consumption in the IAA model. Analysis of individual drinking indicates that retigabine has differential effects on high and low drinking populations of rats. Furthermore, retigabine did not decrease saccharin or quinine consumption alcohol-naïve rats, and there was no effect of retigabine on motility. Collectively these experiments suggest that retigabine may be a useful treatment for AUD, and that Kv7 channels play a role in alcohol consumption.

A key finding from these experiments is that positive modulation of Kv7 channels decreased voluntary intake and preference using a rodent model of long-term, 24 hr

intermittent alcohol consumption without saccharin fadeout (Simms et al., 2008; Wise, 1973). Our findings are in agreement with a study which found retigabine to reduce drinking in a short-access (30 min) saccharin-induced drinking model (Knapp et al., 2014). In our model, 7.5 mg/kg retigabine reduced consumption by approximately 50% without altering total fluid intake. Using the same IAA rodent model to compare 2 FDA-approved drugs for treating AUDs (naltrexone and acamprosate), the high dose of retigabine was at least equally as effective at reducing drinking. Naltrexone and acamprosate reduced intake by approximately 30% and 10-50% at the 6 hr and 24 hr time points, respectively (Li et al., 2010; Sabino et al., 2013; Simms et al., 2008).

Similar to inconsistent findings reported in clinical and preclinical studies testing anticonvulsants (Padula et al., 2013), we also observed bidirectional effects of retigabine on drinking that were dependent on level of intake and dose. When administered systemically, the two doses of retigabine (5 and 7.5 mg/kg) decreased intake in the HD rats. In the LD rats, the high dose decreased drinking whereas the intermediate dose increased drinking. Although there was no influence on saccharin consumption, we did find that retigabine increased quinine consumption, suggesting that this drug either enhances or antagonizes bitter taste perception. In turn, because alcohol has a bitter taste component (Scinska et al., 2000) and there is a genetic contribution to taste perception in mice (Blizard, 2007), alterations in gustatory processes could be a mechanism to explain increased alcohol consumption at moderate doses of retigabine by LD rats. Interestingly, others have reported similar dissociations between the amounts of alcohol consumed and anticonvulsant effects. In non-treatment seeking alcohol abusers, the anticonvulsant levetiracetam increased consumption in self-reported moderate drinkers (Mitchell et al., 2012). In C57BL/6J mice, administration of 25 mg/kg topiramate increased alcohol preference while 50 mg/kg topiramate reduced

alcohol preference (Gabriel and Cunningham, 2005). In rats, topiramate is efficacious at reducing drinking in alcohol-preferring strains (Breslin et al., 2010; Zalewska-Kaszubska et al., 2013) but fails to reduce drinking in strains not specifically bred for high alcohol preference (Breslin et al., 2010). Together, these studies indicate that while retigabine is able to reduce alcohol consumption, its efficacy may be dependent on individual differences and an underlying genetic disposition to drink.

The mechanism through which systemic retigabine reduces alcohol consumption is not immediately clear. Unlike most other anticonvulsants, altering Kv7 channel function can influence DA transmission in mesencephalic/VTA DA neurons (Hansen et al., 2008; Koyama and Appel, 2006; Sotty et al., 2009), and Kv7 channel positive modulators can attenuate evoked DA efflux and synthesis in the striatum (Hansen et al., 2006; Jensen et al., 2011; Martire et al., 2007; Sotty et al., 2009). Studies investigating differences between strains bred to prefer and avoid alcohol indicate that compared to the avoiding strains, alcohol preferring animals have decreased levels of dopamine receptor 2 (*Drd2)* mRNA expression in the NAc (Bice et al., 2011; Imperato and Di Chiara, 1986; Yim and Gonzales, 2000), decreased basal levels of DA in the NAc (Gongwer et al., 1989; Murphy et al., 1987; Stefanini et al., 1992; Zhou et al., 1995), and a greater DA response to an acute alcohol challenge (Bustamante et al., 2008). Together, these studies indicate that rodents bred for high alcohol consumption have decreased dopaminergic tone in the NAc and an increased dopaminergic response to alcohol challenge compared to their low alcohol-preferring counterparts. Chronic alcohol treatment sensitized VTA DA neurons to alcohol-induced excitation (Brodie, 2002) and enhanced NAc neuron firing after chronic alcohol exposure facilitated alcohol seeking after a period of abstinence (Hopf et al., 2007). Given that retigabine can decrease firing of VTA dopaminergic cells (Koyama and Appel, 2006), it is possible that retigabine

reduces drinking by restoring aberrant dopaminergic tone in high drinking rats. Subsequent studies further investigate role of Kv7 channels in the NAc and VTA.

Although established as a Kv7 channel opener, it not highly selective for specific subunits (Kv7.2/7.3 EC_{50} = 1.6 μM; Kv7.4 EC_{50} = 5.2 μM), and some studies suggest that at high concentrations *in vitro* (10-50 μM), retigabine can increase activity at $GABA_A$ receptors (Otto et al., 2002; Rundfeldt, 1997; Rundfeldt and Netzer, 2000). Here we showed that the Kv7.2/Kv7.4-selective modulator ML-213 reduced voluntary drinking in HD rats, however there was no effect of the Kv7.2/Kv7.3-selevtive positive modulator ICA-069673. Together this suggests that the Kv7.2 and Kv7.4 subunits are particularly important for regulating drinking behaviors. In addition, because Kv7.4 is almost exclusively found in midbrain DA neurons (Hansen et al., 2008), this data indicates that Kv7 channels in the VTA are involved in the regulation of drinking behaviors.

Evidence suggests that systemically increasing GABA function can influence operant responding for alcohol. IP injection of the $GABA_A$ agonist muscimol decreases self-administration (Janak and Michael Gill, 2003). Similarly, IP gamma-vinyl GABA (a GABA transaminase inhibitor) dose-dependently decreases alcohol consumption, suggesting that increased GABA concentration in the CNS leads to decreased drinking (Wegelius et al., 1993). These studies suggest that the ability of retigabine to reduce drinking in the IAA model might be mediated through combined action at Kv7 channels and $GABA_A$ receptors. Here we showed that separate administration of the Kv7 channel-specific positive modulator, ICA-069673 (Amato et al., 2011), and muscimol, a $GABA_A$ receptor agonist did not reduce alcohol consumption in the IAA model. In contrast, a simultaneous injection of the ICA compound and muscimol reduced both alcohol consumption and preference. This data would indicate that systemic retigabine reduces drinking through its activity at both Kv7 channels and $GABA_A$ receptors; however, there

are caveats to this conclusion. 20 mg/kg IP doses of retigabine in mice result in a brain concentration of 2.7898 μM (Zhou et al., 2015). It is therefore unlikely that a 7.5 mg/kg IP injection of retigabine in rats yields the > 10 μM brain concentration required to result in off-target activity at $GABA_A$ receptors in the CNS. In addition, the Kv7 channel-specific positive modulator, ML-213, reduced drinking in HD rats, indicating that modulation of Kv7 channels is sufficient to reduce alcohol consumption. Together these data suggest that the ability of the combined muscimol and ICA injection to reduce alcohol consumption recapitulates the effect of retigabine on drinking, but not necessarily the mechanism. The additive effect of muscimol and the ICA compound does suggest that Kv7 channel positive modulators, including retigabine, may be useful as an adjunct therapy with other anticonvulsants such as the GABA analogues (i.e. gabapentin and vigabatrin) discussed in Chapter 1. While further investigation of this possibility would be interesting, it is outside the scope of this project. Subsequent studies in this dissertation investigate alcohol consumption and the function of Kv7 channels by way of retigabine microinjected into nodes of the addiction neurocircuitry.

In summary, these studies indicate that systemic administration of retigabine can reduce alcohol consumption in a rodent model of voluntary drinking in an alcohol-specific manner. Given that a recent study has shown retigabine to be well tolerated in moderate alcohol drinkers when co-administered with an intoxicating dose of alcohol (Crean and Tompson, 2013), retigabine may be useful as a pharmacotherapy for treating AUD. However, care should be taken clinically to insure that the dose is sufficient as to not increase the desire to drink in some patients. These studies further implicate Kv7 channels in drinking-related behaviors. Subsequent experiments will investigate the effect of retigabine in regions of the addiction neurocircuitry on alcohol consumption.

CHAPTER 4: MICROINFUSION OF RETIGABINE IN THE ADDICTION NEUROCIRCUITRY AND VOLUNTARY DRINKING

Introduction

Studies from this project and previous reports indicate that systemic administration of the Kv7 channel positive modulator, retigabine, reduces voluntary alcohol consumption (Knapp et al., 2014). These results indicate not only that retigabine may be a suitable treatment for alcohol use disorder (AUD), but also that Kv7 channels may play a role in the addictive behaviors. Although the addiction neurocircuitry incorporates nearly the entire brain, certain regions are at the core of the addiction process. It would therefore be interesting to identify specific portions of the circuitry in which activation of Kv7 channels mediates a reduction in voluntary drinking.

The central portion of the addiction neurocircuitry includes the prefrontal cortex (PFC), nucleus accumbens (NAc), the ventral pallidum (VP), and the ventral tegmental area (VTA) (Koob and Volkow, 2010)). These regions are also at the core of any behavior learned in response to salient environmental stimuli (Kalivas, 2002). Both aversive and rewarding (salient) stimuli increase the synaptic release of dopamine (DA) from the VTA onto neurons in every node of corticomesolimbic circuit (Berridge and Robinson, 1998). The PFC and other regions intimately associated with learning and memory (e.g. the hippocampus) send glutamatergic projections to the NAc. The NAc integrates its glutamatergic, dopaminergic, and limbic inputs to initiate an adaptive response through reciprocal GABAergic connections with the VP and downstream motor circuitry (Kalivas, 2002). Over repeated exposure to the same or similar stimuli, the DA

response stabilizes, and the habitual motor circuitry becomes primarily responsible for behavioral output.

Acute exposure to drugs of abuse and alcohol induce a greater release of DA compared to naturally rewarding stimuli (Kalivas, 2009; Kuczenski and Segal, 1999; Marshall et al., 1997). Numerous studies have shown that acute and chronic alcohol exposure stimulates the activation of mesolimbic DA (Brodie, 2002; McBride and Li, 1998; Yoshimoto et al., 2003), inducing a 25-50% increase in DA in the NAc over baseline (Weiss et al., 1996). The dopaminergic response elicited by chronic exposure to drugs of abuse and alcohol results in the formation of strong, salient memories of the drug in question. As a consequence, the individual becomes motivated by the perceived importance of drugs or alcohol, leading to addicted behaviors. Rodent models of chronic alcohol exposure indicate that during withdrawal, the activity of VTA neurons is suppressed, resulting in decreased DA in the NAc (Bailey et al., 1998; Shen, 2003). The NAc can be subdivided into two primary regions: the core and the shell. The NAc shell is primarily associated with drug reward (Rodd-Henricks et al., 2002; Sellings and Clarke, 2003), whereas the core contributes to the initiation of motivated and conditioned behaviors (Cornish and Kalivas, 2000; McFarland et al., 2003). From this perspective, dopaminergic projections from the VTA to the NAc are critical to the learning and initiation of addictive behaviors.

In the following experiments we investigated a role for Kv7 channel function in the NAc core and VTA in drinking behaviors. Here we microinfused retigabine to these regions of rats drinking in the intermittent alcohol access (IAA) paradigm and measured alcohol consumption. Similar to Chapter 3, retigabine reduced alcohol consumption with individual differences in drinking patterns. This study indicates that Kv7 channel

activation in key regions of the addiction neurocircuitry is sufficient to reduce alcohol consumption in high drinking populations of rats.

Materials and Methods

Animals and Housing

Male Wistar rats (150g; P39-45) were purchased from Harlan (Indianapolis, IN) and housed individually in standard home cages in temperature and humidity controlled environments with a 12 hr light/dark cycle (lights out: 1000 hr). Food and water were available *ad libitum* throughout the study. All procedures were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee and were in accordance with NIH guidelines for the humane care and use of laboratory animals (2011).

Two-bottle Choice Drinking

Intermittent access to alcohol (20% *v/v*) was performed as previously described in Chapter 3 (Simms et al., 2008; Wise, 1973). Briefly, rats had 2 bottles of water available during habitation, and were allowed 24 hr access to one bottle of 20% alcohol (*v/v* tap water) and one bottle of tap water beginning just prior to the start of the dark cycle on Monday, Wednesday and Friday. Placement of the alcohol solution (left or right side of the cage) was alternated between sessions to control for side preference. Leakage from the sipper tubes was accounted for when weighing the bottles.

Cannula Implantation and Microinjections

After reaching a stable baseline of alcohol consumption in the IAA drinking paradigm (2-3 wks), separate cohorts of rats were anesthetized with vaporized

isoflurane and placed in a stereotaxic instrument (David Kopf Instruments Tujunga, CA). For NAc microinjections, bilateral microinjection guide cannula (26 ga O.D.; PlasticsOne Roanoke, VA) were aimed to terminate 1 mm dorsal to the NAc core following previously established coordinates (anterior-posterior +1.18 mm, medial-lateral ± 1.2 mm, dorsal-ventral -5 mm) (Gass et al., 2011). For VTA microinjections, bilateral cannula were aimed to terminate 2 mm dorsal to the region following previously established coordinates (anterior-posterior -5.6 mm, medial-lateral ± 2.1 mm, dorsal-ventral -7.1, 10°) (Mahler et al., 2013). The guide cannulas were secured to the skull with stainless steel screws and dental cement (OrthoJet; Lang Dental Manufacturing Co., Inc). 33 ga removable obturators were used to limit the obstruction of the guides by tissue and contamination from external debris. We used topical 2% xylocaine and 2% triple antibiotic ointments to treat the wound and suture it closed. All rats were given carprofen (2.5 mg/kg, SC) for post-operative pain management for 4 consecutive days following the surgery.

Rats received microinjections in their home cages beginning 30 min prior to alcohol availability. 33 ga injectors were connected to two Hamilton syringes mounted to a pump (Harvard Apparatus, 11 Plus, Holliston, MA) set to deliver fluids at a rate of 0.5 µl/min. Injectors were inserted bilaterally to a depth of 1-2 mm beyond the tip of the guide cannula. Retigabine (2.5 - 10 ng in 0.01% DMSO *v/v* saline) or vehicle (0.01% DMSO *v/v* saline) was delivered in a final volume of 0.5 µl/side. After the infusion, the injectors were left in the guides for 1 min to allow drug diffusion. Rats were given at least 15 min to rest in their home cages prior to alcohol availability. Drinking was measured at 3, 6, and 24 hrs. Microinjections were completed as a pseudo-random within-subjects design (NAc: n = 13, 4-6 microinjections each; VTA: n = 12, 6 microinjections each). Upon completion of the experiment, rats were euthanized and the brains were Nissl

stained for cannula placement. Rats with cannulas that missed the NAc core (6 total) or VTA (no misses) were excluded from analysis for that brain region.

Statistical Analysis

A mixed-model analysis of variance procedure was used in the statistical software language SAS to analyze all drinking data using repeated measures factors. A between-within method was used to calculate degrees of freedom. Fisher LSD post-hoc tests were used to follow up significant interactions and treatment effects. Finally, all data are reported as mean ± SEM and statistical significance was established with $p < 0.05$.

Results

Effect of Retigabine in the NAc Core on Voluntary Drinking

Evidence indicates that the NAc core regulates alcohol-seeking behaviors (Abrahao et al., 2013; Hopf et al., 2010; Seif et al., 2013), and recent studies suggest that *Kcnq* gene expression is altered in this region after alcohol exposure (Bell et al., 2009; Metten et al., 2014; Rodd et al., 2008). To investigate the role of Kv7 channels in the NAc, we infused retigabine to this region (**Figure 4.1a**) and measured alcohol consumption in the IAA paradigm (n = 13). Intra-accumbal retigabine had no effect on alcohol consumption ($F(2,24) = 2.59$, $p = 0.0962$; **Figure 4.1b**), preference ($F(2,24) = 2.70$, p = 0. 0.0877; **Figure 4.1c**), or total fluid consumption ($F(2,24) = 0.36$; $p = 0.7018$; **Figure 4.1d**).

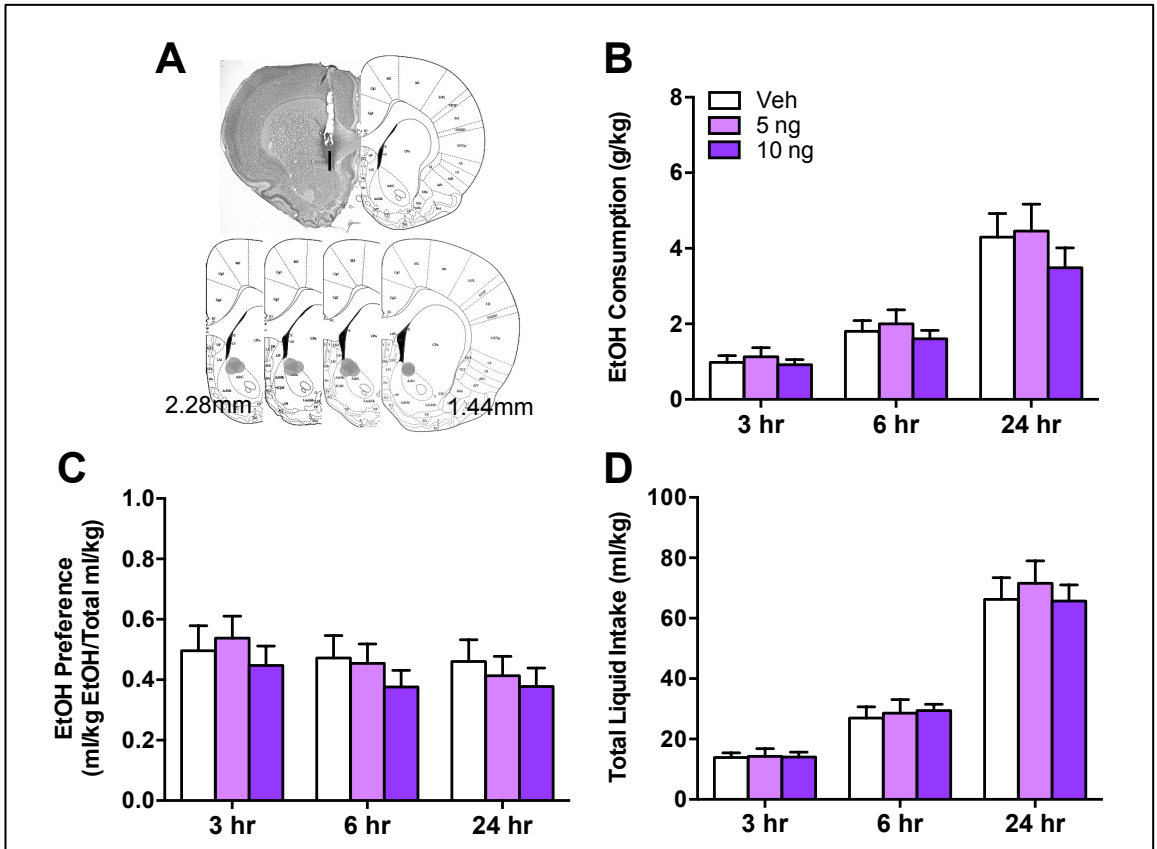

Figure 4.1 | Retigabine in the NAc does not reduce drinking in the total population of rats. (A) Representative images of the placement of bilateral guide cannula targeting the NAc core. Grey circles indicate placements for individual rats. Microinfusion of 5 and 10 ng of retigabine did not affect **(B)** EtOH consumption, **(C)** preference, or **(D)** total liquid intake.

In Chapter 3 we discussed individual differences in the Wistar strain associated with drinking (Blomqvist et al., 1996; Momeni and Roman, 2014), and found that systemic retigabine had differential effects on alcohol consumption that were dependent on the individual's propensity to drink. We again divided the cohort into HD and LD populations based on the median value of alcohol consumption. HD rats (n = 5-6) drank significantly more than LD rats (n = 7) at baseline ($t(11) = 3.385$; $p = 0.0061$; mean HD: 5.963 ± 0.7906 g/kg/24 hrs; mean LD: 2.871 ± 0.5095 g/kg/24 hrs). In HD rats there was a main effect of retigabine on drinking ($F(2,10) = 6.39$; $p = 0.0163$; **Figure 4.2a**) and preference ($F(2,10) = 7.81$; $p = 0.0091$; **Figure 4.2b**). Post-hoc analysis revealed that 10

ng of retigabine reduced consumption (p = 0.0054) and preference (p = 0.0027) compared to vehicle. There was no effect of treatment on total liquid consumption (F(2,10) = 0.07; p = 0.9352; **Figure 4.2c**). In LD rats there was no effect of retigabine on alcohol consumption (F(2,12) = 2.41; p = 0.1317; **Figure 4.2d**), preference (F(2,12) = 1.87; p = 0.1957; **Figure 4.2e**), or total liquid consumption (F(2,12) = 0.75; p = 0.4939; **Figure 4.2f**). Together these data suggest that Kv7 channels in the NAc core are involved with regulating alcohol intake in HD rats.

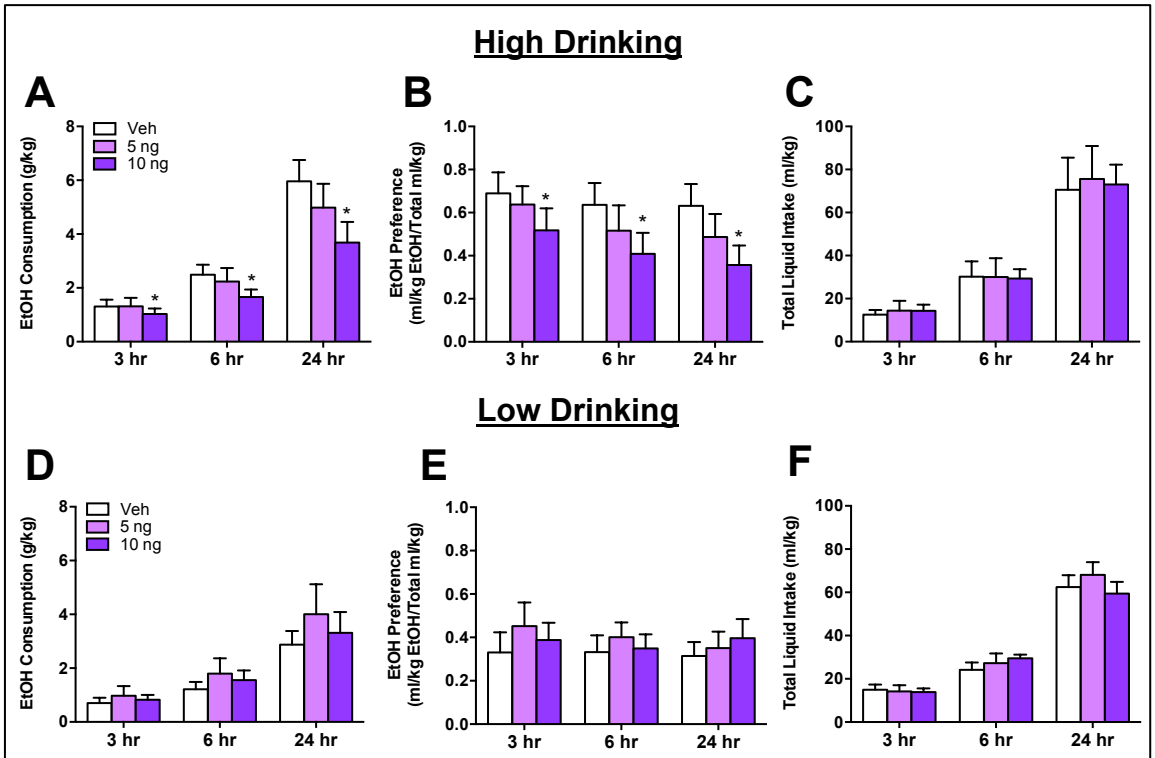

Figure 4.2 | Retigabine in the NAc reduces alcohol consumption in HD rats. Rats were divided into high and low drinking groups based on the median value of EtOH consumption. In the HD population, 10 ng of retigabine reduced **(A)** EtOH consumption and **(B)** preference. There was no effect on **(C)** total fluid intake. In LD rats, retigabine had no effect on **(D)** EtOH consumption, **(E)** preference, or **(F)** total fluid intake. (* p < 0.05 vs veh)

Effect of Retigabine in the VTA on Voluntary Drinking

DA release from the VTA is a central aspect of the addiction process (Koob and Le Moal, 2005), and M-current and retigabine affect DA signaling. Retigabine can

decrease firing of DA VTA neurons (Koyama and Appel, 2006). Altering Kv7 cannel function can also influence DA transmission in mesencephalic/VTA DA neurons (Hansen et al., 2007; Koyama and Appel, 2006; Sotty et al., 2009), and Kv7 channel positive modulators can attenuate evoked DA efflux and synthesis in the striatum (Hansen et al., 2006; Jensen and Yaari, 1997; Martire et al., 2007; Sotty et al., 2009). Thus Kv7 channels in the VTA may regulate drinking behaviors. In IAA drinking rats (n = 11-12), we bilaterally implanted guide cannulas to target the VTA (**Figure 4.3a**). In the total population of drinking rats, retigabine had no effect on alcohol consumption ($F(2,20) = 0.23$, $p = 0.8001$; **Figure 4.3b**), preference ($F(2,20) = 0.29$, $p = 0.7546$; **Figure 4.3c**), or total fluid consumption ($F(2,20) = 3.15$, $p = 0.0649$; **Figure 4.3d**).

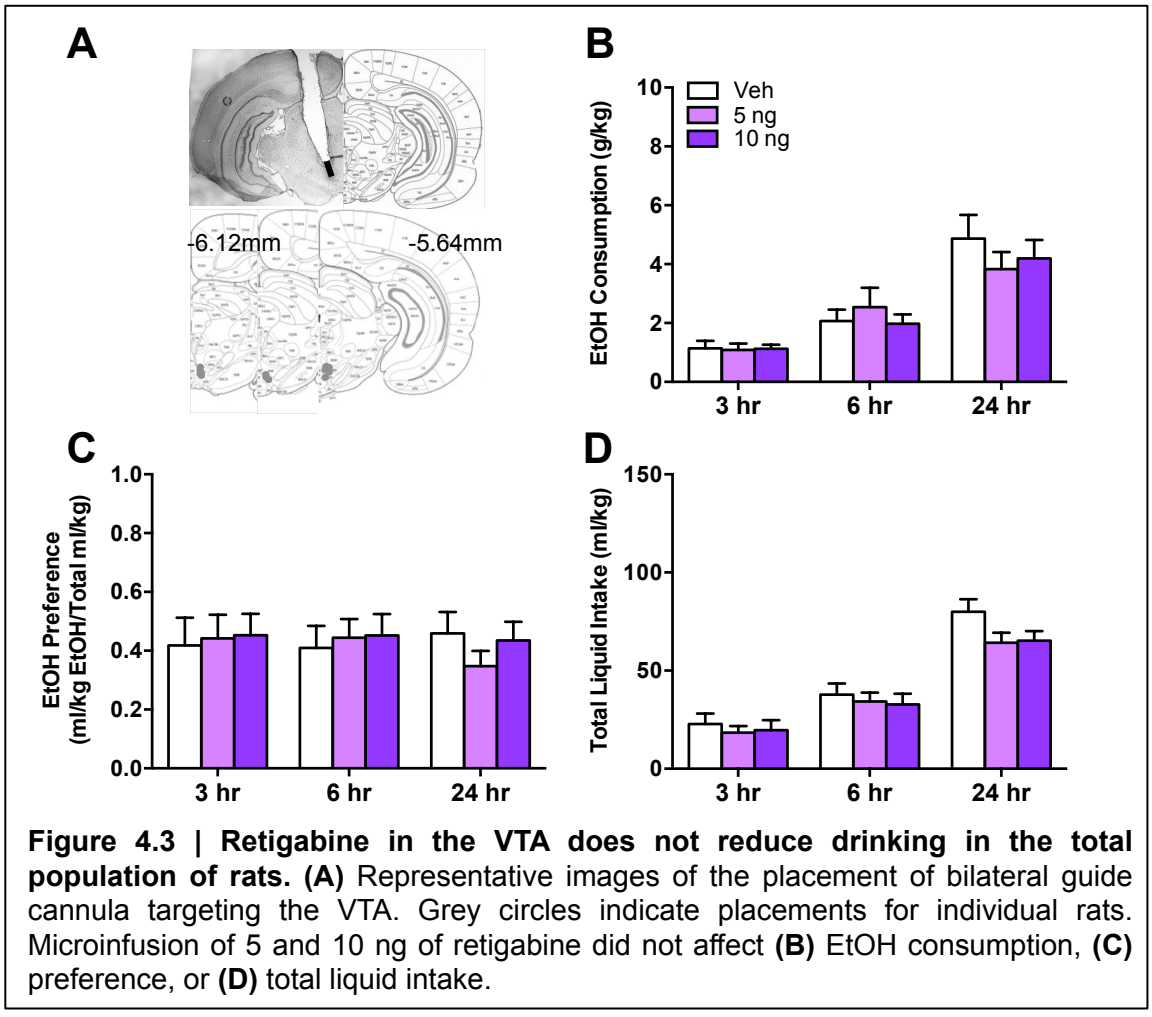

Figure 4.3 | Retigabine in the VTA does not reduce drinking in the total population of rats. (A) Representative images of the placement of bilateral guide cannula targeting the VTA. Grey circles indicate placements for individual rats. Microinfusion of 5 and 10 ng of retigabine did not affect **(B)** EtOH consumption, **(C)** preference, or **(D)** total liquid intake.

We again grouped the rats into HD and LD populations based on median alcohol consumption. As expected, HD rats (n = 5-6) consumed more alcohol than LD rats (n = 5-6) at baseline ($t(9) = 4.379$; $p = 0.0018$; mean HD: 6.787 ± 0.7439 g/kg/24 hrs; mean LD: 2.568 ± 0.5560 g/kg/24 hrs). In the HD group, there was an interaction of time and alcohol consumption ($F(4,20) = 3.04$; $p = 0.0412$; **Figure 4.4a**). Post-hoc analysis indicates that both 5 ng ($p = 0.0004$) and 10 ng ($p = 0.0030$) of retigabine reduced drinking at the 24 hr time point. As evidenced by a main effect of treatment ($F(2,10) = 5.21$; $p = 0.0282$), retigabine also reduced alcohol preference (**Figure 4.4b**). Post-hoc analysis indicates that the 5 ng ($p = 0.0122$) and 10 ng ($p = 0.0328$) dose of retigabine reduced preference compared to vehicle. Finally, intra-VTA retigabine decreased total fluid intake ($F(2,10) = 9.59$, $p = 0.0047$; **Figure 4.4c**). Again, 5 ng ($p = 0.0024$) and 10 ng ($p = 0.0057$) retigabine reduced intake compared to vehicle. In LD rats (n = 5-6), there was no effect of retigabine on alcohol consumption ($F(2,8) = 2.72$, $p = 0.1255$; **Figure 4.4d**). However, retigabine increased alcohol preference ($F(2,8) = 4.86$, $p = 0.0416$; **Figure 4.4e**) and total fluid intake ($F(2,8) = 5.59$, $p = 0.0303$; **Figure 4.4f**). Post-hoc analysis revealed that 5 ng of retigabine increased alcohol preference compared to vehicle ($p = 0.0143$). 5 ng ($p = 0.0248$) and 10 ng ($p = 0.0164$) also reduced total liquid intake compared to vehicle. These data indicate that Kv7 channel function in the VTA play a role in the regulation of drinking behaviors, although the effect may not be specific to alcohol.

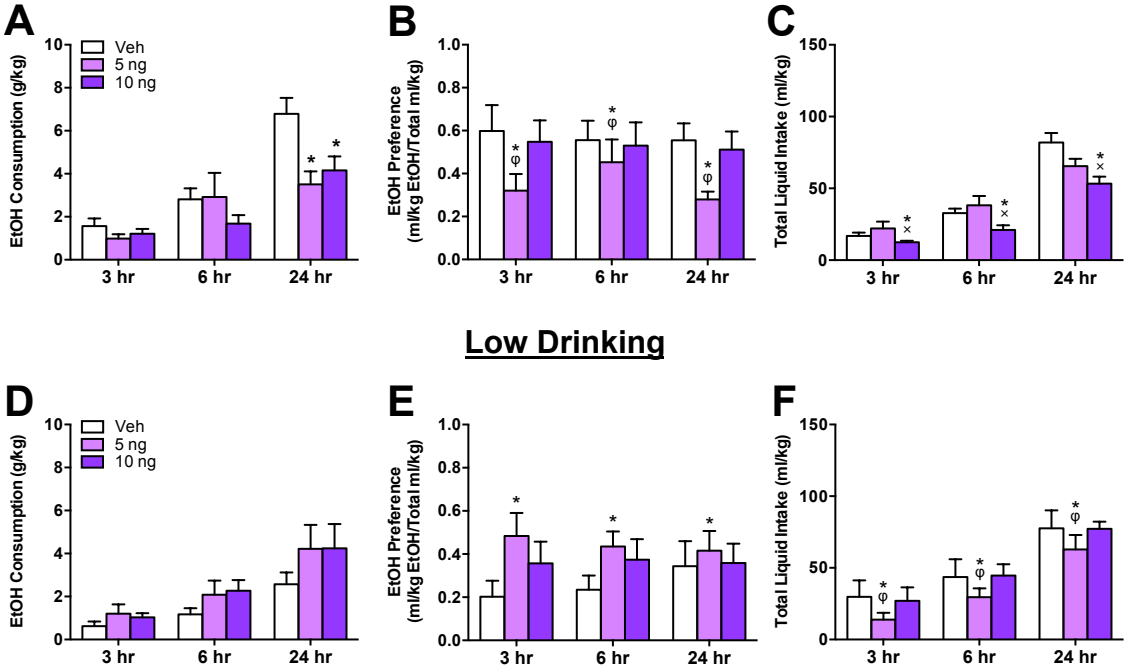

Figure 4.4 | Retigabine in the VTA reduces alcohol consumption HD rats. Rats were divided into high and low drinking groups based on the median value of EtOH consumption. In the HD population, 5 and10 ng of retigabine reduced **(A)** EtOH consumption at the 24 hr time point. **(B)** EtOH preference was reduced by 5 ng retigabine. Finally, **(C)** 10 ng retigabine reduced total fluid intake at 6 and 24 hrs. In LD rats, retigabine had no effect on **(D)** EtOH consumption or **(F)** total fluid intake. 5 ng of retigabine increased **(E)** preference in LD rats. (* $p < 0.05$ vs veh; φ $p < 0.05$ vs 10 ng; × $p < 0.05$ vs 5 ng)

Effect of Retigabine in Missed Cannula Placements

Given the nature of the coordinates used to target the NAc, 5 of the 6 missed cannula placements terminated in the lateral ventricle (**Figure 4.5a**), effectively resulting in an intracranial ventricle (ICV) microinjection. These rats drank more than the average median level of alcohol consumption across the other cohorts (> 4.47 g/kg/24 hrs). Retigabine significantly reduced alcohol consumption ($F(2,8) = 12.09$; $p = 0.0038$; **Figure 4.5b**), preference ($F(2,8) = 12.42$; $p = 0.0035$; **Figure 4.5c**), and total liquid intake ($F(2,8) = 10.30$; $p = 0.0061$; **Figure 4.5d**) as indicated by significant main effects of treatment. Post-hoc analysis indicated that both 5.0 and 10 ng of retigabine reduced

alcohol consumption (p = 0.0020, p = 0.0041), preference (p = 0.0028, p = 0.0024), and liquid intake (p = 0.0076, p = 0.0026). These data indicate that ICV retigabine reduces voluntary alcohol consumption, but that it is it partially mediated through a reduction in total liquid intake.

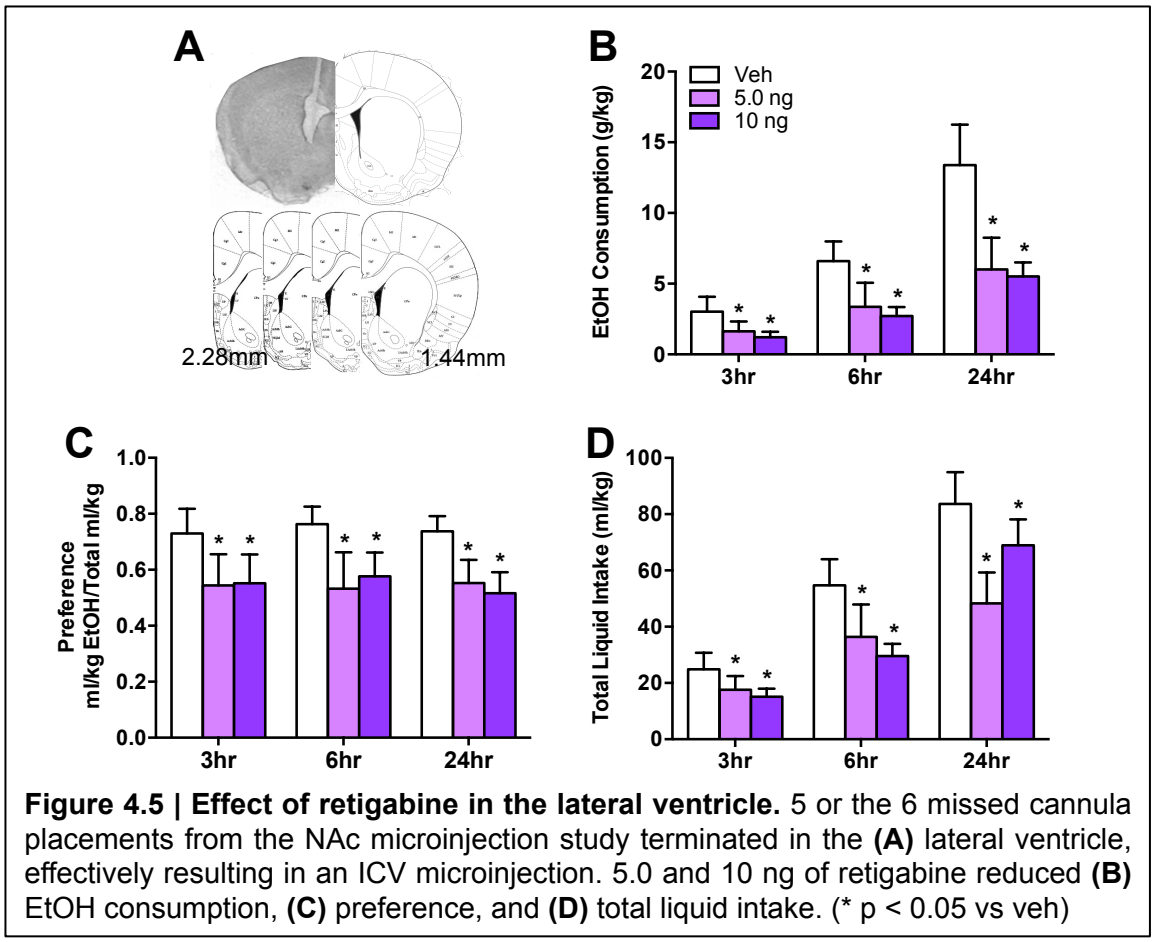

Figure 4.5 | Effect of retigabine in the lateral ventricle. 5 or the 6 missed cannula placements from the NAc microinjection study terminated in the **(A)** lateral ventricle, effectively resulting in an ICV microinjection. 5.0 and 10 ng of retigabine reduced **(B)** EtOH consumption, **(C)** preference, and **(D)** total liquid intake. (* p < 0.05 vs veh)

Discussion

In these experiments we showed that microinfusion of retigabine to the NAc core or the VTA, key nodes of the addiction neurocircuitry, is sufficient to reduce voluntary alcohol consumption in the IAA paradigm. Similar to the findings from Chapter 3, we observed individual differences in the efficacy of microinjected retigabine: the drug was only able to reduce alcohol intake and preference in the high drinking population of rats.

Together these studies suggest that Kv7 channels in the VTA and NAc help regulate alcohol-drinking behaviors.

The key finding from this set of experiments is that microinfusion of retigabine to the NAc core and VTA of high drinking rats reduces voluntary drinking and alcohol preference. Retigabine may accomplish this through the restoration of aberrant DA tone found in high drinking rats. Kv7 channel function can influence DA transmission in mesencephalic/VTA DA neurons (Hansen et al., 2008; Koyama and Appel, 2006; Sotty et al., 2009) and Kv7 channel positive modulators can attenuate evoked DA efflux and synthesis in the striatum (Hansen et al., 2006; Jensen et al., 2011; Martire et al., 2007; Sotty et al., 2009). Decreased levels of dopamine receptor 2 (*Drd2*) mRNA expression are reported in the NAc of high-alcohol preferring mice compared to low-alcohol preferring mice (Bice et al., 2011; Imperato and Di Chiara, 1986; Yim and Gonzales, 2000). Microdialysis studies indicate that high-alcohol drinking (HAD) rats have a lower baseline of DA in the ventral striatum (NAc) compared to low-alcohol drinking (LAD) rats (Gongwer et al., 1989; Murphy et al., 1987; Stefanini et al., 1992; Zhou et al., 1995). Finally alcohol-naïve University of Chile Bibulous rats (derived from Wistar rats) have a greater DA response in the NAc to an acute alcohol challenge compared to abstainer rats (Bustamante et al., 2008). Together these studies indicate that alcohol preferring rats have decreased dopaminergic tone in the NAc, and an increased dopaminergic response to alcohol exposure compared to their low alcohol-preferring counterparts. Thus, intra-accumbal and intra-VTA retigabine could be acting to restore abnormal DA tone in animals predisposed to consume higher amounts of alcohol.

Alternatively, retigabine may reduce consumption through attenuating an alcohol-induced increase in excitability. Studies indicate that chronic alcohol treatment sensitizes VTA DA neurons to alcohol-induced excitation (Brodie, 2002). Also, burst firing in the

VTA encodes the salience of a given environmental stimuli or drug (Overton and Clark, 1997), and alcohol withdrawal increases the probability of burst firing in VTA neurons (Hopf et al., 2007). Furthermore, Kv7 channel positive modulators can prevent burst firing of VTA neurons (Sotty et al., 2009). Given that microinfusion of retigabine to VTA reduced alcohol consumption in HD rats, it is possible that retigabine reduces drinking by suppressing VTA activity. In addition, electrophysiology studies indicate that spike firing is increased in the NAc 3-5 weeks after prolonged alcohol self-administration (Hopf et al., 2010), and that medium spiny neurons (MSNs) in the NAc display increased mini-excitatory postsynaptic currents and increased paired pulse ratios during withdrawal from prolonged alcohol exposure (Marty and Spigelman, 2012a; Spiga et al., 2014). Together these data suggest that the NAc is more excitable during withdrawal. In addition, Kv7 channel activation can prevent presynaptic release of DA in the NAc (Martire et al., 2007). Furthermore, inhibition of the NAc core inhibits the reinstatement of cocaine seeking (Stefanik and Kalivas, 2013), and alcohol self-administration (Hodge et al., 1997). Given that M-current activation is important for repolarizing the cell, fine-tuning the resting membrane potential, and controlling action potential generation and frequency (Miceli et al., 2009), and retigabine potently reduces firing frequency (Dalby-Brown et al., 2006; Tatulian et al., 2001; Wickenden et al., 2000), it is possible that intra-accumbal core retigabine reduces voluntary alcohol consumption through a reduction in NAc core firing and subsequent initiation of drinking behaviors.

In Chapter 3 we showed that a moderate and high dose of IP retigabine reduced alcohol consumption in HD rats, whereas the moderate dose increased consumption in LD rats. Here, we show that intra-accumbal retigabine had no effect on drinking in LD rats, suggesting that Kv7 channels in the NAc are not responsible for the increased drinking caused by moderate systemic doses of retigabine in LD rats. In contrast,

microinfusion of retigabine to the VTA increased alcohol preference in LD rats, and there was a trend towards increased alcohol consumption. This suggests that increased M-current in the VTA mediates the increase in drinking caused by retigabine in LD rats. Given that retigabine alters DA release from the VTA to the NAc (Hansen et al., 2006; Jensen et al., 2011; Martire et al., 2007; Sotty et al., 2009), and the decreased release of DA in response to alcohol observed in alcohol avoiding rat (Bustamante et al., 2008), it is possible that in low drinking rats, retigabine in the VTA alters the rewarding perception of alcohol.

Altogether these studies suggest that retigabine could act through several mechanisms in the NAc and VTA to affect drinking. Specifically, retigabine likely reduces activity in the NAc core, which prevents the initiation of alcohol drinking. Retigabine could also regulate aberrant DA release from the VTA to the NAc to alcohol, preventing its rewarding effects and subsequent consumption. Although outside the scope of this project, additional investigation into the interaction of retigabine, dopamine, and alcohol is necessary and will be an area of focus in future studies.

In these experiments there was not a significant interaction of time and treatment on alcohol consumption when retigabine was infused to the NAc. However, visual inspection of the graphed values suggests that intra-accumbal retigabine does not begin to affect drinking until 6 hrs after presentation of alcohol. Given the instant availability of drug to bind receptors when microinfused, we predicted an immediate effect of retigabine on drinking. The delay in the effect may be contributed to multiple mechanisms. At 3 hrs into a drinking session, rats receiving vehicle infusions drink between 1.5 and 2 g/kg alcohol. Any variability in drinking between rats would prevent an effect of retigabine at such low intake. Another possible explanation for a lack of effect at 3 hrs is that the dopaminergic response to alcohol presentation and drinking in

the IAA model is slow. We argue that retigabine decreases alcohol consumption by stabilizing an aberrant DA response to alcohol. In most rat models of drinking, DA release is measured after an IP injection of an intoxicating amount of alcohol (Feduccia et al., 2014; Soderpalm et al., 2000; Steensland et al., 2012). Operant self-administration procedures with rats suggest a 10-20 min delay in peak DA release during short (30-60 min) sessions where ~2 g/kg of alcohol is consumed (Melendez et al., 2002; Weiss et al., 1993). One study investigating DA release in a choice continuous access model for 6% alcohol, found a 60 min delay in peak DA release (Ericson et al., 1998). How this and an operant self-administration paradigm correlate to our IAA home cage drinking is unclear. It would of great interest to use microdialysis techniques to measure DA release in the NAc during a drinking session in our model.

In the HD population of rats, 5 and 10 ng of retigabine in the VTA reduced voluntary alcohol consumption at the 24 hr time point. However, alcohol preference did not correlate with consumption. 5 ng of retigabine reduced preference at the 3 and 24 hr time points. Retigabine also reduced total fluid consumption 6 and 24 hrs after alcohol availability. Clearly, these data are difficult to interpret; however disruption in naturally rewarding stimuli has been observed after modifying acetylcholine in the VTA. Cholinergic agonists infused to the VTA have been shown to reduce alcohol consumption and increase saccharin consumption in limited access models of drinking (Katner et al., 1997). Microinfusion of atropine, a metabotropic acetylcholine (mACh) receptor antagonist, to the VTA decreases food intake (Rada et al., 2000). Atropine can also prevent the reduction of M current by the mACh receptor agonist muscarine (Pfaffinger, 1988). Therefore in our studies, retigabine may be modifying the behavioral response to the potentially naturally rewarding perception of water. Although outside the

scope of this project, it would be interesting to investigate the role of VTA Kv7 channels in water and saccharin/sucrose consumption in both naïve and alcohol-exposed rats.

The missed placements from the NAc microinjection study provided an opportunity to examine the effect of what were effectively ICV infusions of retigabine on alcohol consumption in HD rats. We found that 5 and 10 ng of retigabine reduced alcohol consumption and preference. These data are in agreement with ours and others' studies showing that systemic retigabine reduces drinking (Knapp et al., 2014). Interestingly, we observed a decrease in total fluid intake, suggesting that the effect of retigabine administered directly to the CNS is not alcohol-specific.

At high concentrations (> 10 μM), retigabine is known to have off target effects at $GABA_A$ receptors (Rundfeldt and Netzer, 2000). It is possible that the reduction in drinking when retigabine is microinfused into the NAc and VTA is mediated, in part, through GABAergic mechanisms. Here we microinfused a 5 – 10 ng of retigabine in a total volume of 0.5 μL, which corresponds to a concentration of 32.97 – 65.94 μM. This would be sufficient to activate $GABA_A$ receptors *in vitro*, however the drug is likely to rapidly diffuse throughout a volume of brain tissue greater than 0.5 μL, reducing the effective concentration. Furthermore, 100 μM retigabine does not alter the amplitude of $GABA_A$ receptor responses in patch clamp recordings from rat entorhinal cortex (Hetka et al., 1999), suggesting a minimal effect of retigabine on GABA signaling. Together, this suggests that the reductions in alcohol consumption by retigabine in the NAc and VTA are likely not mediated by GABAergic mechanisms.

In summary, these studies indicate that retigabine differentially affects voluntary alcohol consumption in high and low drinking populations of outbred rats. Specifically, we showed that microinfusion of retigabine to the NAc core and VTA is sufficient to reduce alcohol consumption in high drinking rats with no effect in low drinking animals.

This suggests that Kv7 channels in key regions of the addiction neurocircuitry play a critical role in drinking behaviors in HD rats. Subsequent experiments will investigate alcohol-induced adaptations in Kv7 channels, which may underlie the effects of retigabine on drinking.

CHAPTER 5: DRINKING-INDUCED NEUROADAPTATIONS OF KV7 CHANNELS

Introduction

Studies from the previous chapters indicate that *Kcnq* genes are associated with alcohol phenotypes and that positive modulation of Kv7 channels reduces voluntary alcohol consumption both systemically and when microinjected into the nucleus accumbens (NAc) core or ventral tegmental area (VTA). The ability of retigabine to reduce drinking could be the result of an augmented baseline function of Kv7 channels or a stabilization of an alcohol-induced adaptation in Kv7 channel physiology. The crucial nature of the NAc to addictive behaviors (Kalivas, 2009; Russo et al., 2010) and evidence indicating a high level of alcohol-induced plasticity of excitatory mechanisms and *Kcnq* genes in the NAc, strongly suggest that Kv7 channels undergo adaptations after prolonged alcohol consumption.

In Chapter 4, we discussed the brain regions associated with addiction as a circuit encompassing the prefrontal cortex (PFC), NAc, VTA, and ventral pallidum (VP) (Kalivas, 2002; Koob and Volkow, 2010). The traditional focus of addiction research has been the VTA and its dopaminergic projections, however in the past several decades, there has been a shift to focus on the corticostriatal circuitry in which DA terminals from the VTA are embedded (Kalivas, 2009). These regions are responsible for generating learned and habitual behaviors, and for dictating how an individual responds to environmental stimuli. The circuitry is composed of limbic regions including the NAc, PFC, amygdala, and VTA; and a motor subcircuit including the motor cortex, dorsal striatum, and the substantia nigra. In a simplified interpretation of the corticostriatal

circuit, the NAc acts as a gateway through which information processed in the limbic circuit gains access to the motor circuit (Groenewegen et al., 1996; Yin and Knowlton, 2006). Over time repeated behaviors (such as taking drugs of abuse) reduce the involvement of the limbic regions, and the motor circuitry is more heavily relied upon until a novel stimulus reengages the limbic circuitry (Barnes et al., 2005; Doya, 2008; Yin and Knowlton, 2006). Many drugs of abuse, including alcohol, hijack this circuit reducing the glutamatergic input from the PFC to the NAc, resulting in behaviors mediated by the habitual motor circuitry (Kalivas, 2009). This view of addictive behaviors marks the NAc as a key region of the addiction neurocircuitry for therapeutic intervention.

The NAc is well-researched in alcohol addiction, and studies indicate that prolonged alcohol exposure leads to aberrant glutamate signaling (Gass and Olive, 2008; Griffin et al., 2014; Neasta et al., 2010; Neasta et al., 2011) and altered glutamate receptor expression in the NAc (Cozzoli et al., 2009; Neasta et al., 2011; Obara et al., 2009; Szumlinski et al., 2008; Zhou et al., 2007). Previous research has shown that medium spiny neurons (MSNs) in the NAc become hyperexcitable after chronic alcohol exposure (Marty and Spigelman, 2012a, b; Padula et al., 2015; Spiga et al., 2014). Furthermore, a recent rodent proteomics study found over 50 proteins whose expression was altered in the NAc by alcohol exposure (Uys et al., 2015). Thus, the NAc is a region not only critical to the addition neurocircuitry, but also highly susceptible to alcohol-induced adaptations.

Studies highlighted in the GeneWeaver analysis discussed in Chapter 2 provide genetic evidence that Kv7 channel expression may be altered in the NAc by alcohol exposure. Two studies using RT-qPCR found that *Kcnq*3 mRNA expression in the NAc of P rats was decreased after alcohol consumption (Bell et al., 2009; Rodd et al., 2008). Also, *Kcnq* genes are differentially expressed in the NAc bHR (high drug-responding)

and bLR (low drug-responding) rats (Clinton et al., 2011). Finally a recent study has reported differential expression of *Kcnq2* in the ventral striatum (NAc) of mice selectively bred for high alcohol consumption/low withdrawal severity versus mice bred for low consumption/high withdrawal severity (Metten et al., 2014). The authors also reported a quantitative trait locus (QTL) on distal Chr 2, and alignment of the QTL with differential expression data identified *Kcnq2* as a candidate cis-eQTL for alcohol consumption and withdrawal. These studies indicate that prolonged alcohol consumption leads to adaptations of Kv7 channels in the NAc.

In the following experiments we systemically administered the selective Kv7 channel antagonist XE-991 to long term drinking rats in the IAA model. We observed that blocking these channels induced convulsive activity, suggesting that drinking in the IAA model induces adaptations in the Kv7 channel expression and/or function. We then investigated IAA-induced adaptations in the expression patterns of the Kv7 channel subunit Kv7.2 in the NAc. The Kv7.2 subunit selected for analysis because it has been extensively studied in medium spiny neurons (MSNs) and is a key target for Kv7 channel regulation (Cooper et al., 2001; Delmas and Brown, 2005; Hansen et al., 2008; Martire et al., 2007; Shen et al., 2005). We observed that the expression of Kv7.2 in specific membrane microdomains is altered by alcohol withdrawal. Given that a recent study suggesting that hyper-SUMOylation of Kv7 channels decreases M-current (Qi et al., 2014), we examined SUMOylation as a possible post translation modification that could mediate these expression patterns.

Materials and Methods

Animals and Housing

Male Wistar rats (150g; P39-45) were purchased from Harlan (Indianapolis, IN) and housed individually in standard home cages in temperature and humidity controlled environments with a 12 hr light/dark cycle (lights out: 1000 hr). Food and water were available *ad libitum* throughout the study. All procedures were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee and were in accordance with NIH guidelines for the humane care and use of laboratory animals (2011).

Two-bottle Choice Drinking

Intermittent access to alcohol (20% *v/v*) was performed as previously described in Chapters 3 and 4 (Simms et al., 2008; Wise, 1973). Briefly, rats had 2 bottles of water available during a 1 wk habitation period. Rats were allowed 24 hr access to one bottle of 20% alcohol (*v/v* tap water) and one bottle of tap water beginning just prior to the start of the dark cycle on Monday, Wednesday and Friday with 24 or 48 hrs of deprivation between drinking sessions. Placement of the alcohol solution (left or right side of the cage) was alternated between sessions to control for side preference. Leakage from the bottles was accounted for when weighing the bottles.

XE-991 Sensitivity

While conducting the initial systemic retigabine studies described in Chapter 3, we tried to prevent the effects of retigabine on drinking with pretreatment of the selective Kv7 channel antagonist XE-991. However, XE-991 induced convulsive behaviors (e.g., wild jumping behavior) in all rats within 10 min of the injection and the study was

terminated. This was surprising given that the dose used (2 mg/kg) has been shown to be innocuous and even improve cognitive function (Fontan-Lozano et al., 2011). Although the outcome of this experiment was unexpected, it suggested that prolonged drinking in the IAA model alters Kv7 channel function and/or expression. To formally measure XE-991-induced convulsant activity, Wistar rats that had been drinking for 12 wks in the IAA model received XE-991 (2 mg/kg; IP in sterile saline, 3 ml/kg; Tocris Bioscience; Ellisville, MO) or vehicle (saline) immediately prior to their dark cycle and returned to their home cage. Behavior was digitally recorded under red light for 20 min. A blinded observer manually scored behavioral abnormalities on a modified Rancine seizure scale (Luttjohann et al., 2009). Briefly, this scale uses 10 behavioral categories to assess seizure occurrence and intensity (from relatively least to most severe: MS = motionless staring, FJ = facial jerks, NJ = neck jerks, CS = clonic seizures while sitting, CLB = clonic seizures while lying on its belly, TCLB = tonic–clonic seizures while lying on its belly, T = tonic seizure, CLS = clonic seizures while lying on its side, TCLS = tonic–clonic seizures lying on its side, WJ = wild jumping).

Subcellular Fractionation and Western Blot Analysis

Bilateral tissue punches were taken targeting the NAc core from alcohol-naïve and long-term drinking rats (at least 12 wks of alcohol consumption) 72 hr after the last session. Detergent resistant membrane (DRM) and detergent soluble membrane (DSM) fractions were prepared using Triton X-100 (Mulholland et al., 2011). Briefly, a Dounce homogenate was prepared and centrifuged at 23,000 x g for 30 min. The supernatant or ‘intracellular’ (IC) fraction that contains cytosolic proteins and intracellular light membranes (ILMs) was separated and the pellet was resuspended with buffer containing 0.5% Triton X-100. The suspension was rotated for 15 min at 4^{o}C and then

centrifuged at 12,000 x g for 20 min, generating the soluble (DSM) and insoluble (DRM) fractions. Western blots were performed on these fractions following previous methods (McGuier et al., 2014). Membranes were stained for total protein concentration with amido black, and blots were normalized to the total protein stain (Aldridge et al., 2008). Kv7.2 antibody was purchased from Alomone Labs (#APC-050; Jerusalem, Israel). AKAP150 antibody was purchased from EMD Millipore (#07-210; Billerica, MA). The SGK1 antibody (#S5188) and naphthol blue black were purchased from Sigma-Aldrich.

SUMOylation Assay

To identify possible SUMOylation modification sites on Kv7.2 channels, we performed a bioinformatics search using SUMOplot™ with accession numbers O43526 and O88543 for human and rat Kv7.2, respectively. The SUMOylation assay was completed using the VIVAbind SUMO kit (VIVA Bioscience, Exeter, UK) following the manufacturer's instructions. Briefly, tissue was collected targeting the NAc core of rats and then subfractioned to generate DRM and DSM fractions using the methods described above. The protein concentrations of each DRM and DSM sample were determined using a Bradford assay kit (Pierce Scientific, Rockford, IL). Equal amounts of protein from each sample were rotated with 40 µL of the SUMO matrix overnight at 4°C. SUMOylated proteins were eluted at 95°C in 45 µL of 14% SDS (*v/v* PBS). An equal volume of each eluate was run on a polyacrylamide gel for western blot analysis as described above, and Kv7.2 (Alomone Labs, Jerusalem, Israel) was used for the detection of SUMOylated proteins. To characterize differences in Kv7.2 SUMOylated between fractions, tissue was collected from adult, male, alcohol-naïve Wistar rats. Tissue to quantify alcohol-induced differences in Kv7.2 SUMOylation was collected from

alcohol-naïve and IAA-exposed (at least 12 wks of alcohol consumption), male, rats after 72 hr withdrawal from alcohol in the IAA paradigm.

Statistical Analysis

Due to the non-Gaussian distribution of the behavioral responses in the XE-991 sensitivity study, non-parametric statistical analyses including a Chi-square test for trend and a Mann Whitney test were performed. Western blot data was analyzed using two-tailed t-tests to compare samples from naïve and IAA rats. Finally, all data are reported as mean ± SEM and statistical significance was established with $p < 0.05$.

Results

Sensitivity to XE-991 After Long-term Drinking

As an alternative approached to determining the role of Kv7 channels in alcohol drinking we treated IAA rats with an innocuous dose of the selective Kv7 channel blocker XE-991 in combination with retigabine, hypothesizing that this would block the effects of retigabine on consumption and increase drinking. To test this, we systemically administered 2 mg/kg XE-991 or vehicle 15 min prior to treatment with 7.5 mg/kg retigabine or vehicle. However, in our initial experiment with XE-991, treatment of long-term drinking rats rapidly induced convulsive behaviors (e.g., wild jumping behavior) in all rats within 10 min of administration and the study was discontinued. This finding suggested that rats with a history of long-term alcohol consumption were sensitive to the proconvulsant effects of Kv7 channel blockade. We tested this hypothesis by administering XE-991 (2 mg/kg) or vehicle to a different cohort of alcohol-naïve and long-term drinking rats (n = 4-5/group) immediately prior to a normal drinking session such that the rats were 24 hrs withdrawn from alcohol. Vehicle injections did not produce

any seizure-like activity in naïve or alcohol exposed rats (data not shown). XE-991 administration significantly increased the percentage of IAA rats that experienced more severe seizure activity compared to alcohol-naïve controls (χ^2 for trend (1, n = 9) = 4.908, p = 0.0267; **Figure 5.1a**). Rats with a 12-wk history of drinking also displayed a significant increase in the severity of the worst convulsive activity score compared to naïve rats that were treated with XE-991 (U = 2.000, p = 0.0238; **Figure 5.1b**), indicating that long-term drinking alters Kv7 channel function.

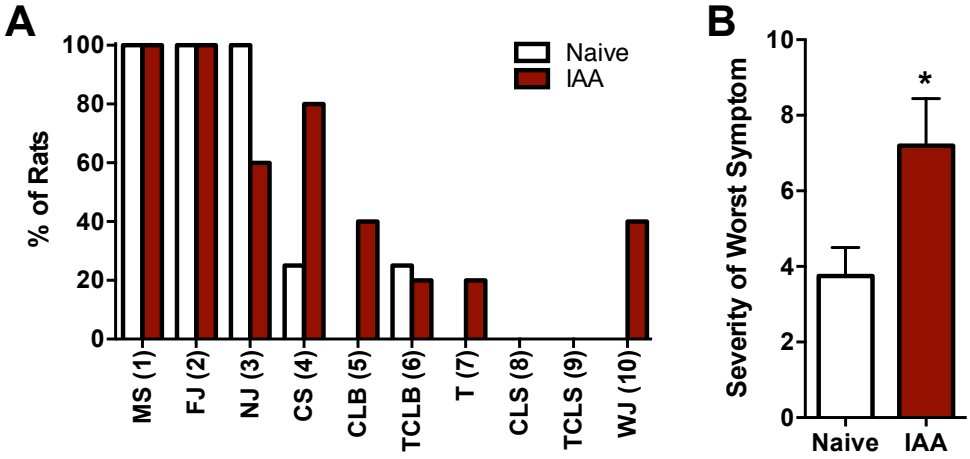

Figure 5.1 | Rats with a history of drinking were more sensitive to Kv7 channel blockade-induced seizures. (A) More rats with a history of alcohol consumption showed enhanced convulsive activity in response to XE-991 (2 mg/kg) compared with alcohol naïve controls (in order of increasing severity MS = motionless staring, FJ = facial jerks, NJ = neck jerks, CS = clonic seizures while sitting, CLB = clonic seizures while lying on its belly, TCLB = tonic–clonic seizures while lying on its belly, T = tonic seizure, CLS = clonic seizures while lying on its side, TCLS = tonic–clonic seizures lying on its side, WJ = wild jumping). **(B)** XE-991 treatment significantly increased the severity of seizure-like activity in long-term drinking rats compared to naïve rats (e.g. wild jumping vs motionless staring). (* $p < 0.05$ compared to naïve)

Alcohol-Induced Adaptations in Kv7.2 Expression

The results from the GeneWeaver analysis and microinjection study indicate that Kv7 channels in the NAc play a role in alcohol drinking. These data, combined with IAA rats being more sensitive to Kv7 blockade compared to naïve rats, suggest that Kv7 channel expression and/or function in the NAc may be altered due to long-term drinking

in the IAA model. Tissue punches targeting the NAc core were obtained after 72-hr withdrawal from the last drinking session. Samples from control and IAA rats (n = 5-6/group) were fractionated into detergent resistant membrane (DRM), detergent soluble membrane (DSM), and intracellular (IC) fractions. **Figure 5.2a** shows the relative expression levels of Kv7.2, AKAP150, and SGK1 in these subfractions. Consistent with previous reports, Kv7.2 was expressed in all 3 fractions, AKAP150 was primarily expressed in the DRM fraction, and SGK was primarily expressed in the IC fraction (Delmas and Brown, 2005; Garcia-Martinez and Alessi, 2008; Hoshi et al., 2003). Western blot analysis indicated that Kv7.2 expression was significantly increased in DRMs in IAA rats compared to naïve rats ($t(10) = 2.250$; $p = 0.0482$; **Figure 5.2b**), whereas Kv7.2 expression was decreased in the DSMs of IAA rats compared to naïve controls ($t(10) = 7.237$; $p < 0.0001$). **Figure 5.3** depicts a cartoon of these expression patterns. There was no effect of alcohol drinking on Kv7.2 channel expression in the IC fraction ($t(9) = 0.425$; $p = 0.6812$). 72 hr withdrawal did not alter expression levels of AKAP150 ($t(10) = 0.3578$; $p = 0.7279$) or SGK1 ($t(9) = 0.5282$; $p = 0.6101$; **Figure 5.2c,d**).

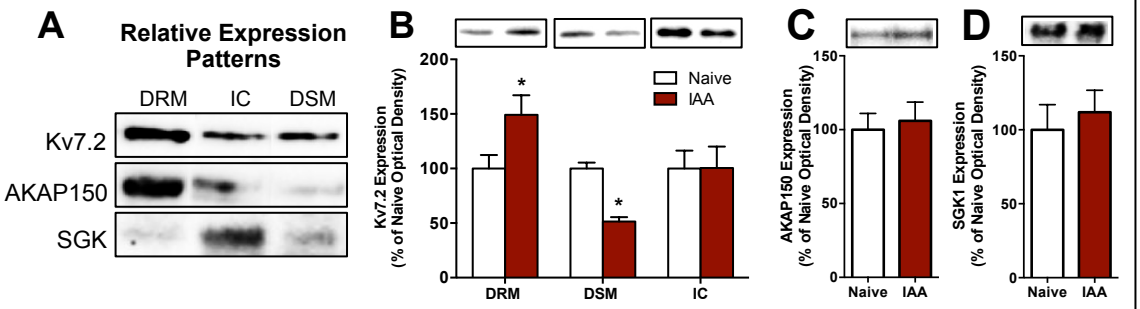

Figure 5.2 | Excessive drinking and abstinence altered surface trafficking of Kv7.2 channels in the NAc. (A) Representative blots showing relative expression patterns of Kv7.2, AKAP150, and SGK1 in detergent resistant membrane (DRM), intracellular (IC), and detergent resistant membrane (DSM) fractions. **(B)** Kv7.2 channel expression is altered in the DRM and DSM fractions after long-term drinking and 72 hr abstinence. A history of drinking did not affect **(C)** AKAP150 or **(D)** SGK1 expression levels. (* $p < 0.05$ compared to naïve)

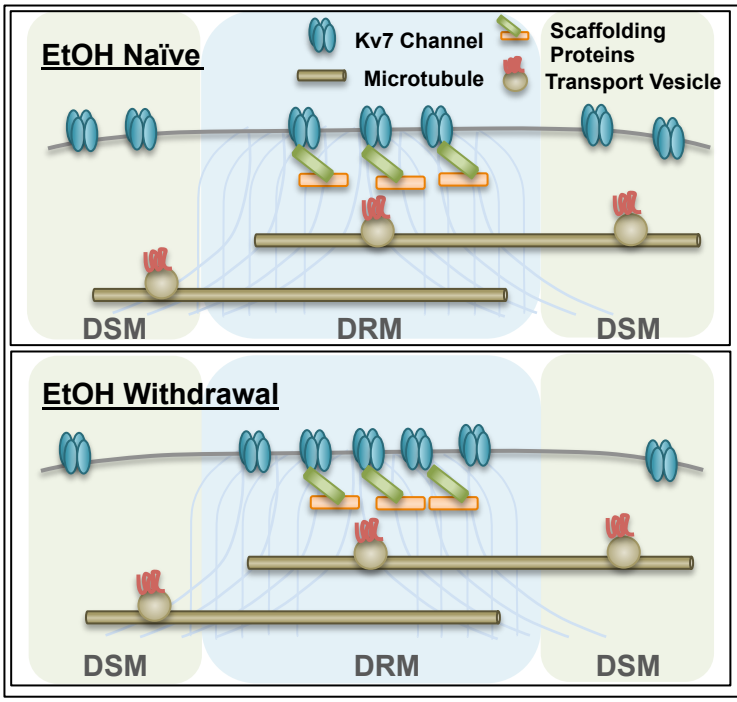

Figure 5.3 | Illustration of the alcohol-induced trafficking pattern of Kv7 channels. (Top) Alcohol-naïve rats have an equal distribution of Kv7.2-containing channels in detergent resistant membrane (DRM; blue background) as detergent soluble membrane (DSM; green background). **(Bottom)** During EtOH withdrawal, expression of Kv7.2-containing channels decreases in the DSM and increases in the DRM.

Alcohol-Induced SUMOylation Pattern of Kv7.2 Channels

Post-translational modifications (PTMs) of proteins can influence their subcellular expression patterns (Perkins, 2006; Reed et al., 2006). SUMOylation is one such PTM that results in the covalent attachment of a small ubiquitin-like modifier (SUMO) protein that has recently been linked to multiple forms of synaptic plasticity and protein function (Luo et al., 2013). A recent report indicated that hyper-SUMOylation of Kv7 channels reduces M-current, suggesting that SUMOylation is be a key regulator of Kv7 channel function (Qi et al., 2014). A search for SUMOylation tetrapartite motifs (Ψ-K-x-D/E; where Ψ is a hydrophobic residue, K is an acceptor lysine, x is any amino acid, and D/E are acidic residues) revealed 12 possible SUMOylation modification sites on human and rat Kv7.2, 3 of which were identified as high probability motifs (human: K21, K625, and K662; rat: K21, K608, and K645). In rat Kv7.2, three consensus motifs were identified on the N-terminus (K21, K49, K66), 1 was cytosolic between transmembrane segments S2

and S3 (K166), 1 was extracellular between S5 and the pore (K255), 1 was in the pore-forming intramembrane (K283), and six were located on the C-terminus (**Figure 5.4**).

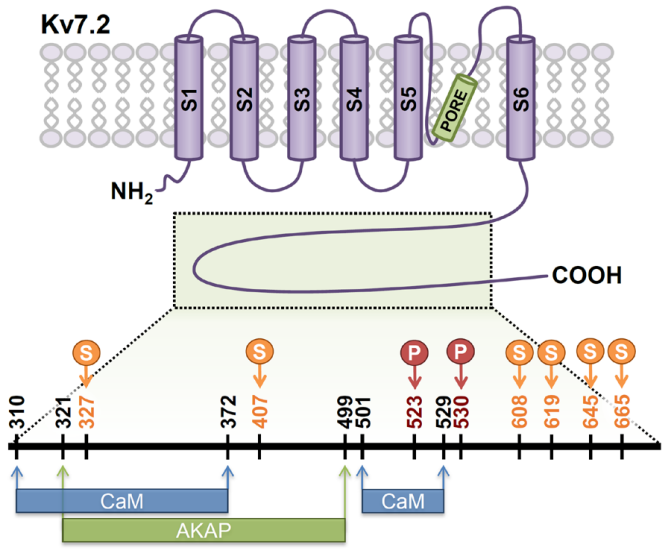

Figure 5.4 | Potential SUMOylation sites on the C-terminus of Kv7.2. A search of the rat Kv7.2 peptide for the SUMOylation consensus sequence ((Ψ-K-x-D/E; where Ψ is a hydrophobic residue, K is an acceptor lysine, x is any amino acid, and D/E are acidic residues) revealed 6 potential sites (orange circles with arrows) on the C-terminus. Two of these sites fall within the CaM and AKAP binding regions.

Using a commercially available kit, we first characterized SUMOylated Kv7.2 protein levels in the NAc of alcohol-naïve rats (n = 4). We observed no difference in the expression of SUMOylated Kv7.2 in the DRM and DSM of the NAc ($t(4) = 1.202$, $p = 0.2955$; **Figure 5.5a**). We then performed a SUMOylation assay on fractionated tissue collected from the NAc of control and IAA rats (n = 6/group). The expression of SUMOylated Kv7.2 in the DSM fraction was significantly decreased in IAA rats compared to naïve rats ($t(9) = 2.427$; $p = 0.0382$; **Figure 5.5b**). There was no difference in SUMOylated Kv7.2 expression in the DRM fraction ($t(10) = 0.9774$; $p = 0.3514$). Finally, the ratio of SUMOylated Kv7.2 to total Kv7.2 was significantly decreased in the DRM after IAA ($t(8) = 3.395$; $p = 0.0094$; **Figure 5.5c**), but not in the DSM ($t(8) = 1.260$; $p = 0.2431$) Combined, these data indicate that long-term alcohol drinking modifies Kv7.2 channel trafficking and SUMOylation levels between DRM and DSM fractions.

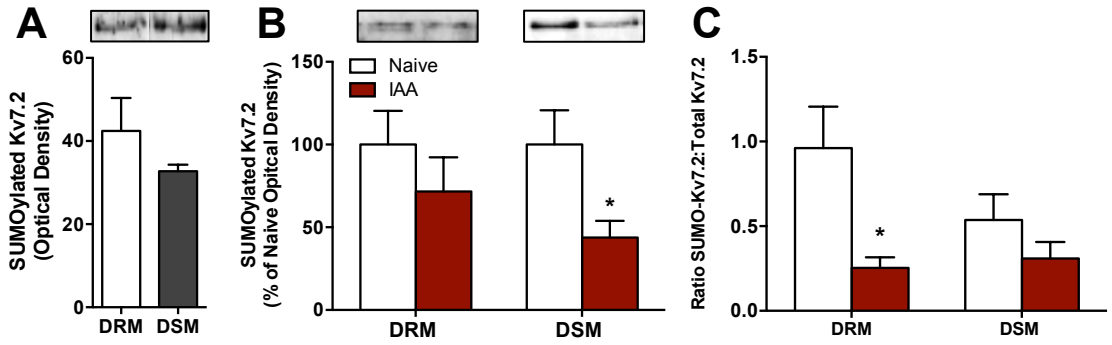

Figure 5.5 | Alcohol withdrawal alters the SUMOylation state of Kv7.2 in the NAc. (A) SUMOylated Kv7.2 is equally expressed in the detergent resistant membrane (DRM) as it is in the detergent soluble membrane (DSM) fractions in the NAc. **(B)** Expression of SUMOylated Kv7.2 in the DSM is decreased in the the NAc of IAA rats compared to alcohol-naïve controls, but not in the DRM. **(C)** The ratio of SUMOylated Kv7.2 to total Kv7.2 was significantly decreased in the DRM after IAA but not in the DSM (* $p < 0.05$ vs naive).

Discussion

Retigabine, a Kv7 channel positive modulator, is able to reduce voluntary alcohol consumption when systemically administered and when microinfused into the NAc core of HD rats in the IAA model. Here we show that long term drinking rats in the IAA paradigm are more sensitive to seizures induced by Kv7 blockade, indicating that prolonged alcohol consumption alters the expression and/or function of Kv7 channels in the CNS. Biochemical assays indicate that drinking and withdrawal alter the surface trafficking of the Kv7.2 subunit within the plasma membrane. Furthermore, this adaptation is associated with alcohol-induced plasticity in SUMOylation state of Kv7.2. Altogether these studies provide the first evidence that prolonged intermittent drinking and withdrawal induce adaptations in Kv7 channels that are likely mediated by changes in SUMOylation of the channel.

A major finding in these studies is that rats with a long history of intermittent alcohol consumption were more sensitive to spontaneous seizures induced by systemic administration of a selective Kv7 channel blocker. Mutations in the *Kcnq2* gene that

cause benign familial neonatal convulsions, type 1 produce a decrease in M-current (Maljevic et al., 2008). Mice expressing mutations in Kv7.2 channels display reduced M-current, decreased threshold for pentylenetetrazole (PTZ)-induced seizures, and hypersensitivity to the pro-convulsant effects of Kv7 channel blockade (Kapfhamer et al., 2010; Otto et al., 2009; Otto et al., 2006; Otto et al., 2004; Watanabe et al., 2000). In contrast, the SGK1.1 transgenic mice with enhanced M-current display diminished sensitivity to kainic acid-induced seizures (Miranda et al., 2013). The dose of XE-991 that produced wild jumping behavior and spontaneous clonic seizures in our long-term alcohol drinking rats has not been reported to cause adverse behaviors in alcohol-naïve rats and mice (Korsgaard et al., 2005; Sander et al., 2012; Xu et al., 2010), though one study reported cognitive enhancing effects in mice at a higher dose than we tested (Fontan-Lozano et al., 2011). While chronic alcohol exposure is known to produce neuroadaptations that alter sensitivity to proconvulsant drugs, this is not a global, non-specific phenomenon (Becker et al., 1998; Finn and Crabbe, 1999; Stephens et al., 2001). M-current is thought to regulate intrinsic excitability and counter afterdepolarizations and sustained NMDA receptor activation required to trigger burst firing in the hippocampus (Gu et al., 2005; Qiu et al., 2007; Yue and Yaari, 2006). Burst firing in CA1 neurons can synchronize population activity and drive seizure initiation (Jensen and Yaari, 1997). Moreover, while the distribution of Kv7.2 channels is widespread, this subunit is concentrated in specific subsets of neurons responsible for controlling neural network oscillations and rhythmic brain activity (Cooper et al., 2001; Leao et al., 2009). Thus, the hypersensitivity to XE-991 suggests that expression or function of Kv7 channels may be decreased by repeated periods of intoxication and withdrawal in brain regions that control motor output and seizure generation, such as the basal ganglia circuitry or hippocampus.

We directly investigated alcohol-induced adaptations in the Kv7.2 subunit and observed that long-term consumption and abstinence produces microdomain-dependent neuroadaptations in Kv7.2 channels in the NAc. The most common pairing of Kv7 channel subunits in the CNS is Kv7.2/Kv7.3 (Delmas and Brown, 2005), and studies indicate that most of the modifications and protein interactions that influence Kv7 channel trafficking and function occur at or on the C-terminus of the Kv7.2 subunit (Bal et al., 2010; Hoshi et al., 2003; Suh and Hille, 2007). Evidence suggests that Kv7.2 channels are expressed in multiple subcellular compartments in neurons, having been reported to localize to the axon initial segment (AIS), lipid rafts, nodes of Ranvier, somatodendritic domains of GABAergic output neurons, dendritic spines, and presynaptic terminals of tyrosine hydroxylase positive axonal projections (Arnsten et al., 2010; Cooper et al., 2000; Devaux et al., 2004; Pan et al., 2006; Roche et al., 2002). Some plasma membrane proteins found in the AIS, lipid rafts, and dendritic spines are insoluble in Triton X-100 (DRMs), whereas some soluble proteins are expressed in extrasynaptic membranes (Abdi and Bennett, 2008; Carlin et al., 1980; Cooper et al., 2000; Cotman et al., 1974; Ferrario et al., 2011a; Ferrario et al., 2011b; Goebel-Goody et al., 2009). Proteins isolated from the AIS, lipid rafts and dendritic spines are often part of the scaffolded signaling networks associated with these microdomains. Thus, it is possible that long-term drinking is shifting Kv7.2 channels from extrasynaptic membranes into specialized signaling domains, such as the AIS or dendritic spines where the channels can influence neuronal excitability.

The possible mechanisms underlying Kv7.2 trafficking between membrane microdomains during abstinence are not immediately clear. As discussed in Chapter 1, there are multiple physical interfaces known to modulate Kv7 channel function. Interaction with AKAP150 and SGK1 standout as the most likely candidates of these to

regulate membrane trafficking (Delmas and Brown, 2005). However when we probed for expression of these proteins in alcohol-exposed rats, we found no difference in their expression compared to naïve animals. This suggests that Kv7.2 membrane trafficking may be mediated by a different mechanism. A recent study suggests that SUMOylation may play an important role in the regulation of Kv7 channel function (Qi et al., 2014). Although SUMOylation was once thought to exclusively regulate nuclear transport, several studies over the last decade indicate that this posttranslational modification plays a key role in synaptic plasticity (Review: (Luo et al., 2013)).

Recent evidence suggests that SUMOylation of Kv2.1 and Kv7 channels decreases K^+-current where as de-SUMOylation increases their function, indicating that SUMOylation state may regulate neuronal excitability (Dai et al., 2009; Plant et al., 2011; Qi et al., 2014). SUMOylation is the covalent attachment of a small ubiquitin-like modifying (SUMO) to a lysine residue via a series of proteins (E1-3; (Ren et al., 2009). The SENP proteases are primarily responsible for the de-SUMOylation process. Studies indicate that SUMOylated proteins and SUMO-related enzymes are enriched in synaptosomes (Feligioni et al., 2009; Martin et al., 2007a; Martin et al., 2007b), and co-localize with pre- and postsynaptic membrane proteins (Jaafari et al., 2013; Martin et al., 2007a; Plant et al., 2011). A recent study suggests that AMPA-induced depolarization of neurons increases SUMOylation (Feligioni et al., 2009), and preventing SUMOylation prevents synaptic insertion of AMPA receptors (Jaafari et al., 2013).

In our study, we searched the rat Kv7.2 peptide for the SUMOylation consensus sequence and found multiple potential SUMOylation sites. Most noteworthy were the 6 sites on the C-terminus and the single site in the pore-forming intramembrane. Modifications at the termini of membrane channels can directly affect protein trafficking (Lu and Roche, 2012; Mao et al., 2011). This concept is true of Kv7.2, which is

influenced by interaction of AKAP150 and CaM at the C-terminus (Delmas and Brown, 2005). Of the 6 SUMOylation sites found on the C-terminus of Kv7.2, two lie within the AKAP150 and CaM binding regions of the terminus (Delmas and Brown, 2005). AKAP150 directly interacts with the Kv7.2 subunit and PKC such that AKAP binds the active site of the kinase, preventing phosphorylation-induced inhibition of M-current (Bal et al., 2010; Hoshi et al., 2003). Thus SUMOylation could prevent the AKAP150-Kv7.2 or Kv7.2-PKC interaction. CaM also directly interacts with the Kv7.2, and although its exact role in Kv7 channel trafficking and function has yet to be defined (Etxeberria et al., 2008; Gomez-Posada et al., 2011; Wen and Levitan, 2002), SUMOylation could alter channel trafficking by preventing this interaction. Posttranslational modifications within or near the Kv7.2 channel pore could potentially block ion conductance, leading to increased excitability. Alternatively, a modification near the pore domain could induce a conformational change in the channel leading to pore closure. Although this may not directly affect channel trafficking, altered Kv7 channel function could initiate other mechanisms to induce activity-dependent trafficking such as those observed with Kv4.2 channels (Kim et al., 2007) and AMPA receptors (Groc et al., 2004; Ju et al., 2004).

Our study showing alcohol-induced increased sensitivity to Kv7 channel blockade suggests that the shift in trafficking patterns has a functional consequence on membrane excitability. A recent study has shown that hyper-SUMOylation of Kv7 channels results in a reduction in M-current not associated with changes in channel expression that leads to deleterious hyperexcitable state (Qi et al., 2014). This suggests that SUMOylation prevents current through Kv7 channels. Altogether, SUMOylation is a likely candidate mechanism for regulating withdrawal-induced trafficking of Kv7.2. Indeed, withdrawal from alcohol decreases the expression of SUMOylated Kv7.2 in DSM, and the ratio of SUMOylated Kv7.2 to total Kv7.2 expression in the DRM. It is interesting to note that

surface expressed Kv2.1 channels also move fluidly in and out of similar microdomains within the plasma membrane (O'Connell et al., 2006), and that current through these channels is affected by SUMOylation state (Dai et al., 2009; Plant et al., 2011; Qi et al., 2014). Chronic alcohol exposure is known to produce activity-dependent homeostatic neuroadaptations to counteract the inhibitory actions of ethanol (Mulholland and Chandler, 2007), and activity-dependent processes alter Kv7.2 channel protein and *Kcnq*2 transcript expression in hippocampus and amygdala (Penschuck et al., 2005; Zhang and Shapiro, 2012). Furthermore, a recent study associated differential expression of *Kcnq2* as a candidate cis-eQTL for alcohol consumption and withdrawal symptoms (Metten et al., 2014). Electrophysiology studies indicate that spike firing is increased in the NAc 3-5 weeks after prolonged alcohol self-administration (Hopf et al., 2010), and that MSNs in the NAc display increased mini-excitatory postsynaptic currents and increased paired pulse ratios during withdrawal from prolonged alcohol exposure (Marty and Spigelman, 2012a; Padula et al., 2015; Spiga et al., 2014). These studies indicate that NAc neurons are more excitable during withdrawal. Although speculative, our experiments suggest that during withdrawal, when cells are in a hyperexcitable state, Kv7 channels in the membrane are de-SUMOylated and recruited to the signaling networks where they can help regulate neuronal excitability.

In summary these studies indicate that prolonged alcohol consumption leads to increased sensitivity to Kv7 channel blockade possibly resulting from the observed adaptations in the Kv7 channel expression and SUMOylation state during alcohol withdrawal. These studies are the first to show that Kv7 channel trafficking within the plasma membrane can be affected by external stimuli, and provide the first direct evidence that SUMOylation may regulate Kv7 channel expression.

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions

Alcohol use disorders (AUDs) represent a substantial social and economic burden around the world. Current FDA-approved pharmacotherapies for AUDs are efficacious in only a subset of individuals and suffer from deleterious side effects. It is therefore essential to find improved medications for the management of these disorders. The experiments described in this dissertation provide strong evidence for retigabine, an FDA-approved anticonvulsant, as a pharmacotherapy for aiding in drinking cessation to treat AUD. This work further describes a complex relationship between Kv7 channels and alcohol-drinking behaviors that spans genetic variation, behavioral pharmacology, and biochemical adaptations.

Results from the bioinformatics search described in Chapter 2 showed that *Kcnq2/3* contribute to an alcohol drinking phenotype across multiple strains of rodents. The data suggest that *Kcnq2* and *Kcnq3* and their encoded proteins may be useful pharmacogenetic targets to treat AUDs. The behavioral pharmacology studies in Chapters 3 strengthened this relationship by showing that systemic administration retigabine and the Kv7 channel-specific positive modulator ML-213 reduce alcohol consumption in a long-term intermittent access model. Furthermore, in Chapter 4 we showed that microinjections of retigabine to the ventral tegmental area (VTA) and nucleus accumbens (NAc) core also reduce voluntary drinking. Notably, retigabine was more effective at reducing drinking in Wistar rats with a heavy drinking phenotype. An aberrant dopaminergic response is associated with prolonged alcohol exposure (Brodie,

2002) and heavy drinking phenotypes (Bice et al., 2008; Bustamante et al., 2008; Gongwer et al., 1989; Imperato and Di Chiara, 1986; Zhou and Palmiter, 1995). Given that retigabine, unlike most other anticonvulsants, can alter dopamine (DA) neurotransmission (Hansen et al., 2006; Koyama and Appel, 2006; Sotty et al., 2009), these data suggest that retigabine may decrease alcohol consumption through restoration of the DA response. In a clinical context, these experiments indicate both that retigabine may be a promising candidate for treating AUDs and that Kv7 channel function in key nodes of the addiction neurocircuitry regulates drinking behaviors.

In Chapter 5 we described experiments showing that long-term drinking enhanced sensitivity to the pro-convulsant effects of Kv7 channel blockade and altered surface trafficking of Kv7.2 channels between detergent resistant and soluble membranes in the NAc. The surface trafficking was associated with reduced Kv7.2 channel SUMOylation in the DRM fraction. To our knowledge, these data are the first to show evidence for post-translational modification by SUMOylation in a model of alcohol or drug exposure. Repeated exposure to alcohol induces a hyperexcitable state in MSNs of the NAc (Marty and Spigelman, 2012b; Padula et al., 2015; Spiga et al., 2014). Given that hyper-SUMOylation of Kv7 channels reduces M-current (Qi et al., 2014), these biochemical experiments suggest that Kv7 channel function may be dependent both on localization within distinct microdomains in the plasma membrane and SUMOylation state.

In summary the work described in this dissertation provide strong evidence for the use of retigabine as a pharmacotherapy for the treatment of AUDs. Furthermore, these data highlight Kv7 channels as targets of alcohol-induced neuroplasticity.

Future Directions

Detailed discussions and interpretations of the results from the experiments performed during this dissertation are provided within their individual chapters. However some of these explanations warrant additional thought and further experimentation. The following sections highlight future directions and experiments that would provide direct insight to particular conclusions from this dissertation.

Kv7 Channel Trafficking, Alcohol Withdrawal, and Retigabine

One of the most exciting observations from this work is that withdrawal from long-term drinking in the IAA model induces a shift in Kv7.2 expression from detergent soluble membrane (DSM) to detergent resistant membrane (DSM). Some plasma membrane proteins found in the axon initial segment (AIS), lipid rafts, and dendritic spines are insoluble in Triton X-100 (DRMs), whereas some soluble proteins are expressed in extrasynaptic membranes (Abdi and Bennett, 2008; Arnsten et al., 2010; Carlin et al., 1980; Cooper et al., 2000; Cotman et al., 1974; Ferrario et al., 2011a; Ferrario et al., 2011b; Goebel-Goody et al., 2009). Proteins isolated from the AIS, lipid rafts and dendritic spines are often part of the scaffolded signaling networks associated with these microdomains. Thus, it is possible that long-term drinking is shifting Kv7.2 channels from extrasynaptic membranes into specialized signaling domains, such as the AIS or dendritic spines where the channels can influence neuronal excitability. Studies indicate that chronic alcohol exposure induces a hyperexcitable state in MSNs of the NAc (Marty and Spigelman, 2012b; Padula et al., 2015; Spiga et al., 2014). The AIS is a likely candidate microdomain for alcohol-induced neuroplasticity of excitatory mechanisms because it is the point of initiation an action potential (Harty et al., 2013) and is enriched with Kv7 channels (Pan et al., 2006). Moreover, Kv7 channels in the AIS

modulate spike initiation and adaptation (Cooper, 2011), and Kv7.2 co-assembly with Kv7.3 is required for the recruitment of Kv7 channels to this region (Cooper et al., 2000). The AIS is also susceptible to activity-dependent relocation relative to the soma in such a manner as to regulate neuronal excitability in dissociated hippocampal cultures (Grubb and Burrone, 2010). A recent study showed that hyper-SUMOylation of Kv7 channels substantially decreased M-current (Qi et al., 2014). In our experiments, Kv7.2 SUMOylation in the NAc was decreased in the DSM fraction during withdrawal. Although speculative, it is possible that during an alcohol withdrawal-induced hyperexcitable state, Kv7 channels are de-SUMOylated and recruited to the signaling networks within the AIS where they can help control aberrant excitability.

The ability of retigabine to reduce voluntary alcohol consumption could capitalize on withdrawal-induced trafficking adaptations. In Chapter 4 we argued that microinfusion of retigabine to the NAc core could reduce alcohol consumption by suppressing activity and subsequent initiation of alcohol-seeking behaviors. In our experiments, retigabine is administered 10-30 minutes prior to alcohol availability after a 24-hour withdrawal period from the previous session. At this point, Kv7 channels may have already been recruited to the AIS and other DRMs in the NAc to ease withdrawal-induced hyperexcitability (Marty and Spigelman, 2012b; Padula et al., 2015; Spiga et al., 2014), making these membranes particularly sensitive to the inhibitory effects of retigabine. Alternatively, if 24-hour withdrawal is not sufficient to alter Kv7 channel trafficking, retigabine could simply stabilize membrane excitability by augmenting the function of the few channels remaining in the AIS.

These hypotheses could be tested through a series of experiments using immunohistochemistry (IHC) and electrophysiology. Specifically, tissue from the NAc taken at 0, 24 and 72 hours withdrawal from IAA drinking could be fixed and probed for

co-expression of Kv7.2 and ankyrin-G, a necessary component to AIS scaffolding (Kordeli et al., 1995). If Kv7 channels are recruited to the AIS to regulate excitability, we would predict greater co-expression of Kv7.2 and ankyrin-G during withdrawal compared to alcohol-naïve rats. The role SUMOylation may be better visualized using an alternative model of alcohol exposure because it is dependent on active processes within the cell. No SUMO-Kv7 channel antibodies currently exist; therefore to capture SUMOylation-related changes in channel trafficking, the system would need to be treated with a SUMOylating enzyme (i.e. Ubc-9) or a de-SUMOylating enzyme (i.e. SENP2) and then immediately fixed for IHC. However, drinking rats must be perfused with PFA and before IHC, but organotypic hippocampal slice cultures (OHSCs) can be chronically treated with cycles alcohol exposure and withdrawal, recapitulating some of the biochemical adaptations seen in models of alcohol consumption (Mulholland et al., 2011). We could induce the de-SUMOylation using the SENP enzyme process in OHSCs and then immediately fix the tissue. IHC experiments could then be conducted examining the co-expression of Kv7.2 and ankyrin-G in the AIS of pyramidal neurons. We would predict that during withdrawal, de-SUMOylation process would recruit Kv7 channels to the AIS in naïve cultures in a manner similar to alcohol-exposed cultures.

The physiological relevance of alcohol-induced Kv7 channel trafficking could be investigated using patch-clamp recordings of M-current within and immediately adjacent to the AIS of MSNs in NAc tissue from IAA rats during various withdrawal time points. The effects of retigabine on M-current could also be measured at these time points. Furthermore, by treating the tissue with SENP2, we could potentially measure the physiological relevance of SUMOylated Kv7 channels. Here we would predict that M-current is increased in the AIS both compared to adjacent areas and compared to the AIS of alcohol-naïve rats. Also, de-SUMOylating the channels should increase M-current

in membrane adjacent to the AIS, regardless of the withdrawal time point. Both the IHC and electrophysiological experiments were outside the scope of this dissertation, but would provide valuable insight to alcohol-induced adaptations in Kv7 channel expression and function.

Alternative Kv7 Channel Regulation

Experiments described in Chapters 3 and 4 we show that retigabine reduces voluntary alcohol consumption both when administered systemically and when microinfused into nodes of the addiction neurocircuitry. While altered DA transmission likely represents the downstream effect of retigabine on the addiction neurocircuitry, upstream activity is equally noteworthy. Antagonists of the peptide hormone ghrelin are able to reduce alcohol consumption (Jerlhag et al., 2011; Landgren et al., 2008) and there is an established role of ghrelin in multiple addictive substances (review: (Panagopoulos and Ralevski, 2014)). Recent evidence indicates that ghrelin enhances neuronal excitability by inhibiting Kv7 channels (Shi et al., 2013). Given the results of our experiments, these studies suggest that the ability of ghrelin antagonists to reduce drinking may ultimately be through an increase in Kv7 channel function, and that ghrelin may be an upstream target of alcohol's effect on Kv7 channel function.

Kv7 Channels and Alcohol Withdrawal Syndrome

In Chapter 5 we found that rats with a history of alcohol consumption are susceptible to seizure-like activity induced by blocking Kv7 channels, which provided evidence that prolonged drinking leads to changes in Kv7 channel function and/or expression. Although convulsant activity is a symptom of alcohol withdrawal syndrome (AWS) (Bayard et al., 2004), the IAA paradigm has not been previously reported to

induce such symptoms in rats. As previously mentioned, there is indeed a shift in Kv7.2 expression from DSM to DRM in the NAc during withdrawal that has the potential to affect neuronal excitability and behaviors. To date, M-current has only been recorded from dopaminergic neurons in response to acute bath application of alcohol (Koyama and Appel, 2006). We hypothesize that during withdrawal from prolonged IAA drinking, M-current would be elevated compared to naïve controls, however, studies to quantify changes in Kv7 channel expression and M-current in regions critical to seizure generation (i.e. the hippocampus and amygdala) (Engel, 1995) would provide a better understanding of the convulsant activity associated with alcohol withdrawal syndrome.

Individual Differences, Retigabine, and Alcohol

One of the key findings in this dissertation is that retigabine reduces voluntary alcohol consumption when systemically injected and when microinfused to the NAc and VTA of high drinking rats but not low drinking rats (Chapters 3 and 4). Numerous studies have observed quantifiable characteristics associated with drinking behaviors in cohorts of outbred rat strains. Wistar rats in a choice drinking paradigm were divided into high (~ 4.2 g/kg/24 hrs) and low drinking (~ 2.1 g/kg/24 hrs) subpopulations (Momeni and Roman, 2014). In addition, alcohol consumption in Long Evans rats is predictive of anxiety-like behaviors (Hayton et al., 2012). Multiple inbred rodent inbred strains have been generated to either prefer or avoid alcohol. Two strains P (alcohol preferring) and NP (non-preferring) were derived from the outbred Wistar strain (Waller et al., 1983). These strains represent the extreme ends of the continuum of drinking observed in the cohorts used in our studies, and have less genetic variation compared to their outbred parent strain (Funk et al., 2006). Consequently, these strains more definitively represent high and low drinking populations, and using them in the IAA model would provide an

alternative to measuring the effect of retigabine on high and low drinking rats. Given that retigabine decreased alcohol consumption in high drinking Wistar rats and increased consumption in the low drinking population, we would expect similar results of systemic retigabine in P and NP strains. The microinjection studies could also be replicated in P and NP rats to provide further insight into the role of Kv7 channel function in high and low drinking populations.

Using selectively-bred high and low preferring strains to measure the efficacy of retigabine on drinking would provide insight into the two ends of the drinking spectrum, however the BXD strains of mice would provide an opportunity to examine the spectrum of alcohol consumption in mice with different drinking phenotypes. C57BL/6 mice are an alcohol-preferring strain whereas the DBA mice avoid alcohol. Studies indicate that C57BL/6 mice drink more in 2-bottle choice paradigms (Meliska et al., 1995; Rhodes et al., 2007) and short-access models (Belknap et al., 1997; Le et al., 1994), and that DBA mice are less sensitive to alcohol-induced locomotor sensitization (Kiianmaa and Tabakoff, 1983). Numerous recombinant inbred (RI) strains have been developed from interbreeding the C57BL/6J and DBA/2J strains. These mice, referred to as “BXD” strains, represent continuum of alcohol phenotypes that strongly correlate to their genetic composition (DuBose et al., 2013; Mulligan et al., 2006; Padula et al., 2015; Vanderlinden et al., 2013). By exposing the parent strains (C57BL/6 and DBA) and a selection of BXD strains from across the drinking spectrum to the IAA paradigm, we would be able to precisely investigate the effects of retigabine on populations with different dispositions to consume alcohol. We predict that the reduction in drinking by retigabine would be more effective in high drinking BXD strains. Similarly, increased consumption by retigabine would diminish as we move from non-preferring to preferring

strains. In the middle of this spectrum of drinkers we would predict no effect of retigabine on drinking.

Several studies have also identified a strong correlation between individual differences in drinking with differences in anxiety. A recent study grouped Long Evans rats into high anxiety and low anxiety groups, and found that the high anxiety group voluntarily consumed more alcohol compared low anxiety rats from the same cohort (Hayton et al., 2012). Finally, high anxiety in Wistar rats in an elevated plus maze was predictive of high anxiety in other models (Ho et al., 2002). These studies suggest that high drinking rats are prone to higher levels of anxiety, and that alcohol consumption may alleviate that anxiety. Chronic stress can increase anxiety-like behavior in multiple strains of mice (Kim and Han, 2006; Mineur et al., 2006), and repeated exposure to stressors is known to increase alcohol consumption (Becker et al., 2011). This suggests a correlation between anxiety and alcohol consumption, and retigabine is known to have anxiolytic effects. Specifically, retigabine and other Kv7 channel positive modulators reduce anxiety-like behaviors associated with a zero maze in mice (Blackburn-Munro and Jensen, 2003; Korsgaard et al., 2005). Furthermore, newer-generation anticonvulsants (e.g. gabapentin and lamotrigine) are often prescribed as off-label treatments for patients with anxiety disorders, which like epilepsy, are characterized by overactive neuronal activity (Blackburn-Munro et al., 2005). In our drinking model we anecdotally observed some anxiety-like behaviors (i.e. excessive forearm grooming and porphyrin staining), however we did not formally assess these behaviors. It would also be interesting to examine the effects of retigabine on anxiety associated with alcohol-withdrawal. It is possible that the ability of retigabine to reduce drinking is mediated through a combination dopaminergic, anxiolytic, and anticonvulsant mechanisms. Although outside the scope of this project, this possibility warrants further investigation.

Dopamine Release in the IAA Model

As previously mentioned, we observed individual differences in the efficacy of retigabine in high and low drinking rats from the same cohort. Of particular interest was that retigabine reduced alcohol consumption when microinfused to the NAc and VTA in high drinking rats but had no effect on drinking when administered to low drinking rats (Chapter 4). Individual differences such as these likely relate to slight genetic variation within the cohort, and studies investigating inbred rat strains specifically selected to prefer or avoid alcohol have found numerous dopaminergic discrepancies. Compared to alcohol-avoiding strains, alcohol-preferring rats have decreased *Drd2* mRNA expression in the NAc (Bice et al., 2008; Imperato and Di Chiara, 1986), a lower baseline level of DA in the ventral striatum (Gongwer et al., 1989; Zhou and Palmiter, 1995), and an increased dopaminergic response to acute alcohol (Bustamante et al., 2008). These studies indicate that, under basal conditions, alcohol-preferring rats have an aberrant DA tone.

Given that retigabine can alter DA neurotransmission (Hansen et al., 2008; Koyama and Appel, 2006; Sotty et al., 2009), we argue that retigabine reduces alcohol consumption by restoring dopaminergic tone. This could be directly tested through microdialysis experiments. Specifically, dopamine release could be measured in the NAc during the first several hours of drinking in the IAA paradigm after systemic retigabine was administered. Such an experiment would provide two highly interesting pieces of information. The first would be to better elucidate the mechanism through which retigabine reduces alcohol consumption. The second would be to provide insight into the DA response during voluntary drinking in the IAA paradigm. To our knowledge, most microdialysis studies measuring DA response to alcohol in drinking rats have either been completed in operant administration models (Melendez et al., 2002; Weiss et al., 1993)

or after an intoxicating IP dose of alcohol (Feduccia et al., 2014; Soderpalm et al., 2000; Steensland et al., 2012). We found one study measuring accumbal DA in a continuous access choice drinking model for 6% alcohol and DA was only collected for 60 minutes after alcohol availability (Ericson et al., 1998). Such an experiment in our IAA model is beyond the current project, but would provide valuable knowledge. Given the critical nature of DA release from the VTA to the NAc in addiction processes and the ability of retigabine to modulate this release, experiments to directly investigate the relationship between retigabine, drinking, and DA will be a major focal point in future studies.

The work presented in this dissertation illustrates an intricate relationship between Kv7 channels and alcohol consumption spanning genetic variation, pharmacology, and biochemical adaptations. Genetic meta-analysis demonstrated that *Kcnq2/3* contribute to an alcohol drinking phenotype and alcohol-related behaviors in rodents. Behavioral pharmacology experiments indicate that positive modulation of Kv7 channels both systemically and in key nodes of the addiction neurocircuitry can reduce voluntary alcohol consumption. Furthermore, this effect is dependent on individual differences and observed in rats with a heavy drinking phenotype. Finally, Kv7 channels in the NAc are susceptible to alcohol-induced neuroadaptations and posttranslational modification, as evidenced by biochemical assays. Together, these experiments support retigabine as a potential pharmacotherapy for the treatment of AUD, and highlight Kv7 channels as mediators of alcohol-related behaviors as well as targets of alcohol-induced neuroadaptations.

APPENDIX

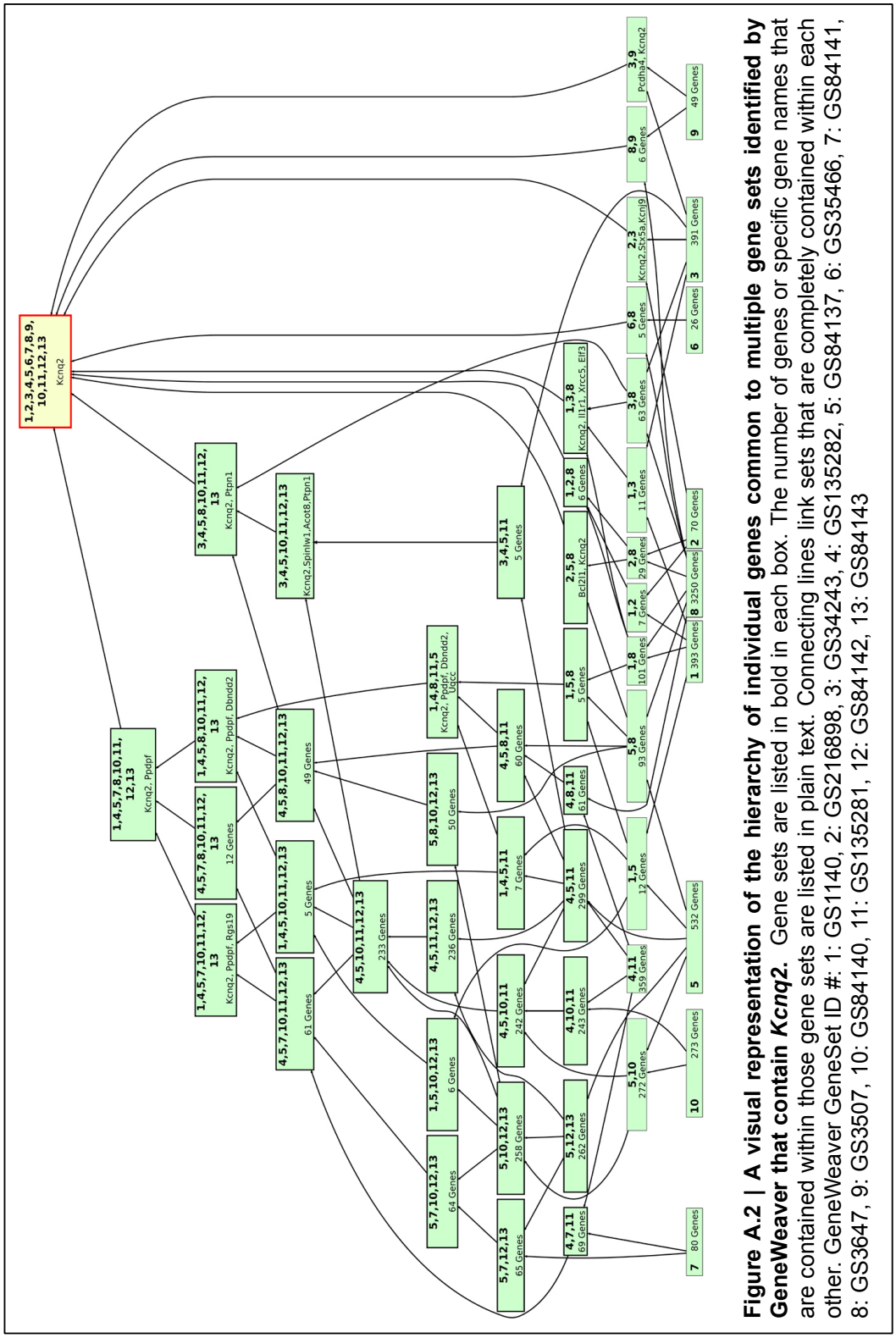

Figure A.2 | A visual representation of the hierarchy of individual genes common to multiple gene sets identified by GeneWeaver that contain *Kcnq2*. Gene sets are listed in bold in each box. The number of genes or specific gene names that are contained within those gene sets are listed in plain text. Connecting lines link sets that are completely contained within each other. GeneWeaver GeneSet ID #: 1: GS1140, 2: GS216898, 3: GS34243, 4: GS135282, 5: GS84137, 6: GS35466, 7: GS84141, 8: GS3647, 9: GS3507, 10: GS84140, 11: GS135281, 12: GS84142, 13: GS84143

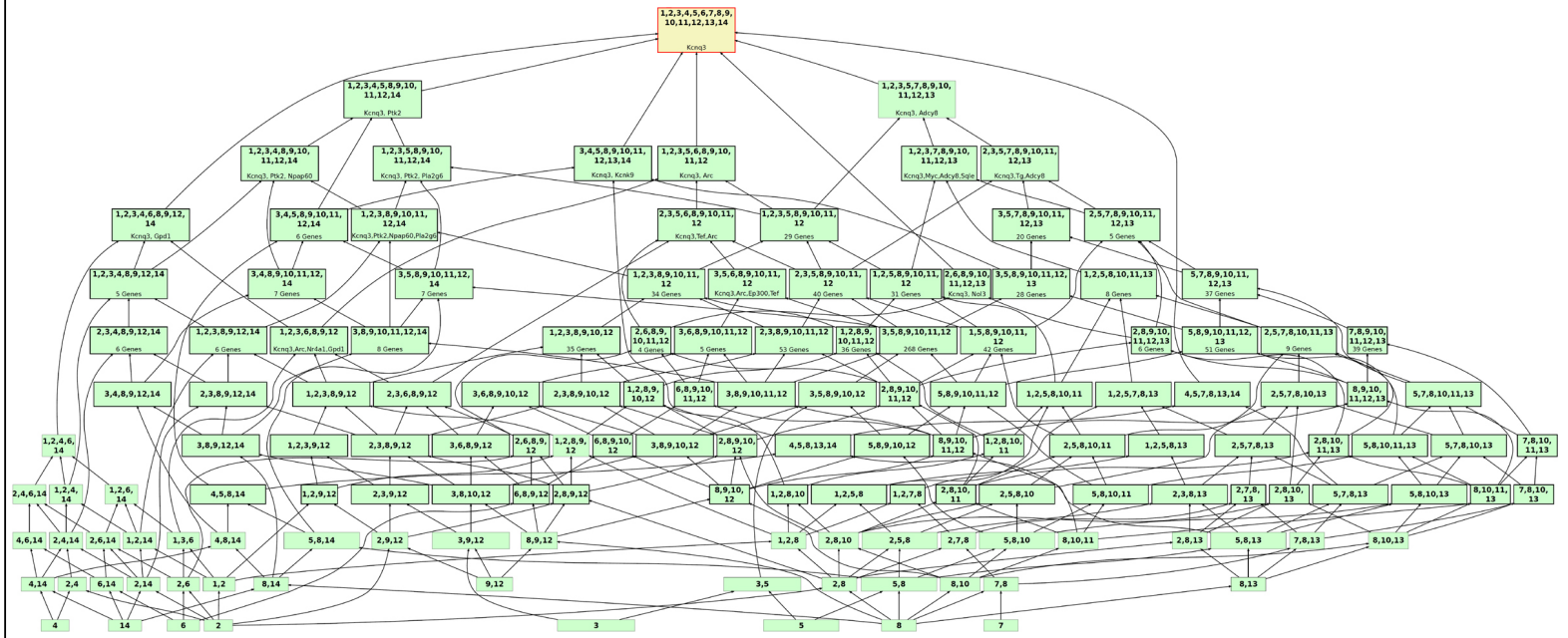

Figure A.2 | A visual representation of the hierarchy of individual genes common to multiple gene sets identified by GeneWeaver that contain *Kcnq3*. Gene sets are listed in bold in each box. The number of genes or specific gene names that are contained within those gene sets are listed in plain text. Connecting lines link sets that are completely contained within each other. GeneWeaver GeneSet ID #: 1: 135133, 2: 135132, 3: 84286, 4:14929, 5: 84284, 6: 37147, 7: 135223, 8: 115112, 9: 115114, 10: 115115, 11: 115116, 12: 11511, 13: 115121, 14: 75567

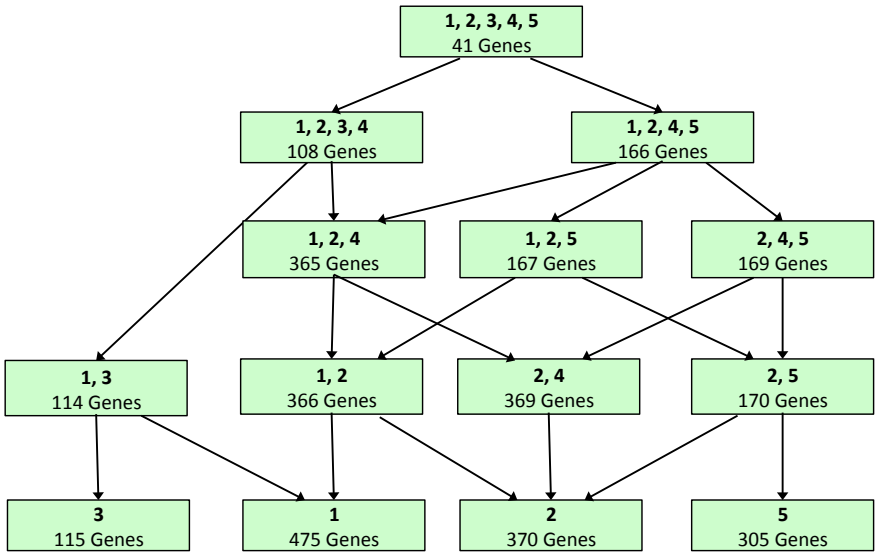

Figure A.3 | A visual representation of the hierarchy of individual genes common to multiple gene sets identified by GeneWeaver that contain *Kcnq3*. Gene sets are listed in bold in each box. The number of unique genes or specific gene names that are contained within those gene sets are listed in plain text. Connecting lines link sets that are completely contained within each other. GeneWeaver GeneSet ID #: 1: 84098, 2: 84097, 3: 135279, 4: 84096, 5: 84101

LIST OF REFERENCES

Abdi, K.M., and Bennett, V. (2008). Adducin promotes micrometer-scale organization of beta2-spectrin in lateral membranes of bronchial epithelial cells. Mol Biol Cell *19*, 536-545.

Abrahao, K.P., Ariwodola, O.J., Butler, T.R., Rau, A.R., Skelly, M.J., Carter, E., Alexander, N.P., McCool, B.A., Souza-Formigoni, M.L., and Weiner, J.L. (2013). Locomotor sensitization to ethanol impairs NMDA receptor-dependent synaptic plasticity in the nucleus accumbens and increases ethanol self-administration. The Journal of neuroscience : the official journal of the Society for Neuroscience *33*, 4834-4842.

Agrawal, A., Freedman, N.D., Cheng, Y.C., Lin, P., Shaffer, J.R., Sun, Q., Taylor, K., Yaspan, B., Cole, J.W., Cornelis, M.C*., et al.* (2012). Measuring alcohol consumption for genomic meta-analyses of alcohol intake: opportunities and challenges. The American journal of clinical nutrition *95*, 539-547.

Ait-Daoud, N., Malcolm, R.J., Jr., and Johnson, B.A. (2006). An overview of medications for the treatment of alcohol withdrawal and alcohol dependence with an emphasis on the use of older and newer anticonvulsants. Addictive behaviors *31*, 1628-1649.

Aldridge, G.M., Podrebarac, D.M., Greenough, W.T., and Weiler, I.J. (2008). The use of total protein stains as loading controls: an alternative to high-abundance single-protein controls in semi-quantitative immunoblotting. J Neurosci Methods *172*, 250-254.

Alele, P.E., and Devaud, L.L. (2007). Sex differences in steroid modulation of ethanol withdrawal in male and female rats. The Journal of pharmacology and experimental therapeutics *320*, 427-436.

Allison, C., and Pratt, J.A. (2003). Neuroadaptive processes in GABAergic and glutamatergic systems in benzodiazepine dependence. Pharmacology & therapeutics *98*, 171-195.

Amato, G., Roeloffs, R., Rigdon, G.C., Antonio, B., Mersch, T., McNaughton-Smith, G., Wickenden, A.D., Fritch, P., and Suto, M.J. (2011). N-Pyridyl and Pyrimidine Benzamides as KCNQ2/Q3 Potassium Channel Openers for the Treatment of Epilepsy. ACS Medicinal Chemistry Letters *2*, 481-484.

Amit, Z., Stern, M.H., and Wise, R.A. (1970). Alcohol preference in the laboratory rat induced by hypothalamic stimulation. Psychopharmacologia *17*, 367-377.

Anton, R.F. (2008). Genetic basis for predicting response to naltrexone in the treatment of alcohol dependence. Pharmacogenomics *9*, 655-658.

Anton, R.F., O'Malley, S.S., Ciraulo, D.A., Cisler, R.A., Couper, D., Donovan, D.M., Gastfriend, D.R., Hosking, J.D., Johnson, B.A., LoCastro, J.S*., et al.* (2006). Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: a randomized controlled trial. Jama *295*, 2003-2017.

Anton, R.F., Oroszi, G., O'Malley, S., Couper, D., Swift, R., Pettinati, H., and Goldman, D. (2008). An evaluation of mu-opioid receptor (OPRM1) as a predictor of naltrexone response in the treatment of alcohol dependence: results from the Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence (COMBINE) study. Arch Gen Psychiatry *65*, 135-144.

Anton, R.F., Voronin, K.K., Randall, P.K., Myrick, H., and Tiffany, A. (2012). Naltrexone modification of drinking effects in a subacute treatment and bar-lab paradigm: influence of OPRM1 and dopamine transporter (SLC6A3) genes. Alcoholism, clinical and experimental research *36*, 2000-2007.

Arias, A.J., Feinn, R., Oncken, C., Covault, J., and Kranzler, H.R. (2010). Placebo-controlled trial of zonisamide for the treatment of alcohol dependence. Journal of clinical psychopharmacology *30*, 318-322.

Armand, V., Rundfeldt, C., and Heinemann, U. (1999). Effects of retigabine (D-23129) on different patterns of epileptiform activity induced by 4-aminopyridine in rat entorhinal cortex hippocampal slices. Naunyn-Schmiedeberg's archives of pharmacology *359*, 33-39.

Arnsten, A.F., Paspalas, C.D., Gamo, N.J., Yang, Y., and Wang, M. (2010). Dynamic Network Connectivity: A new form of neuroplasticity. Trends in cognitive sciences *14*, 365-375.

Bailey, C.P., Manley, S.J., Watson, W.P., Wonnacott, S., Molleman, A., and Little, H.J. (1998). Chronic ethanol administration alters activity in ventral tegmental area neurons after cessation of withdrawal hyperexcitability. Brain research *803*, 144-152.

Baker, E.J., Jay, J.J., Bubier, J.A., Langston, M.A., and Chesler, E.J. (2012). GeneWeaver: a web-based system for integrative functional genomics. Nucleic Acids Res *40*, D1067-1076.

Bal, M., Zhang, J., Hernandez, C.C., Zaika, O., and Shapiro, M.S. (2010). Ca2+/calmodulin disrupts AKAP79/150 interactions with KCNQ (M-Type) K+ channels. The Journal of neuroscience : the official journal of the Society for Neuroscience *30*, 2311-2323.

Barnes, T.D., Kubota, Y., Hu, D., Jin, D.Z., and Graybiel, A.M. (2005). Activity of striatal neurons reflects dynamic encoding and recoding of procedural memories. Nature *437*, 1158-1161.

Bayard, M., McIntyre, J., Hill, K.R., and Woodside, J., Jr. (2004). Alcohol withdrawal syndrome. American family physician *69*, 1443-1450.

Becker, H. (1999). Alcohol Withdrawal: Neuroadaptation and Sensitization. CNS Spectrums *4*, 38-40, 57-65.

Becker, H.C. (1996). The alcohol withdrawal "kindling" phenomenon: clinical and experimental findings. Alcoholism, clinical and experimental research *20*, 121A-124A.

Becker, H.C. (1998). Kindling in alcohol withdrawal. Alcohol health and research world *22*, 25-33.

Becker, H.C. (2013). Animal models of excessive alcohol consumption in rodents. Current topics in behavioral neurosciences *13*, 355-377.

Becker, H.C., and Hale, R.L. (1993). Repeated episodes of ethanol withdrawal potentiate the severity of subsequent withdrawal seizures: an animal model of alcohol withdrawal "kindling". Alcoholism, clinical and experimental research *17*, 94-98.

Becker, H.C., Lopez, M.F., and Doremus-Fitzwater, T.L. (2011). Effects of stress on alcohol drinking: a review of animal studies. Psychopharmacology *218*, 131-156.

Becker, H.C., Veatch, L.M., and Diaz-Granados, J.L. (1998). Repeated ethanol withdrawal experience selectively alters sensitivity to different chemoconvulsant drugs in mice. Psychopharmacology *139*, 145-153.

Belknap, J.K., Richards, S.P., O'Toole, L.A., Helms, M.L., and Phillips, T.J. (1997). Short-term selective breeding as a tool for QTL mapping: ethanol preference drinking in mice. Behavior genetics *27*, 55-66.

Bell, R.L., Kimpel, M.W., McClintick, J.N., Strother, W.N., Carr, L.G., Liang, T., Rodd, Z.A., Mayfield, R.D., Edenberg, H.J., and McBride, W.J. (2009). Gene expression changes in the nucleus accumbens of alcohol-preferring rats following chronic ethanol consumption. Pharmacology, biochemistry, and behavior *94*, 131-147.

Bell, R.L., Rodd, Z.A., Lumeng, L., Murphy, J.M., and McBride, W.J. (2006). The alcohol-preferring P rat and animal models of excessive alcohol drinking. Addiction biology *11*, 270-288.

Berridge, K.C., and Robinson, T.E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain research Brain research reviews *28*, 309-369.

Bianchi, M.T., Pathmanathan, J., and Cash, S.S. (2009). From ion channels to complex networks: magic bullet versus magic shotgun approaches to anticonvulsant pharmacotherapy. Medical hypotheses *72*, 297-305.

Bice, P.J., Lai, D., Zhang, L., and Foroud, T. (2011). Fine mapping quantitative trait loci that influence alcohol preference behavior in the High and Low Alcohol Preferring (HAP and LAP) mice. Behavior genetics *41*, 565-570.

Bice, P.J., Liang, T., Zhang, L., Strother, W.N., and Carr, L.G. (2008). Drd2 expression in the high alcohol-preferring and low alcohol-preferring mice. Mammalian genome : official journal of the International Mammalian Genome Society *19*, 69-76.

Blackburn-Munro, G., Dalby-Brown, W., Mirza, N.R., Mikkelsen, J.D., and Blackburn-Munro, R.E. (2005). Retigabine: chemical synthesis to clinical application. CNS drug reviews *11*, 1-20.

Blackburn-Munro, G., and Jensen, B.S. (2003). The anticonvulsant retigabine attenuates nociceptive behaviours in rat models of persistent and neuropathic pain. European journal of pharmacology *460*, 109-116.

Blackwell DL, L.J., Clarke TC (2012). Summary health statistics for US adults: National Health Interview Survey (National Center for Health Statistics).

Blizard, D.A. (2007). Sweet and bitter taste of ethanol in C57BL/6J and DBA2/J mouse strains. Behavior genetics *37*, 146-159.

Blocker, J.S., Jr. (2006). Did prohibition really work? Alcohol prohibition as a public health innovation. American journal of public health *96*, 233-243.

Blomqvist, O., Ericson, M., Johnson, D.H., Engel, J.A., and Soderpalm, B. (1996). Voluntary ethanol intake in the rat: effects of nicotinic acetylcholine receptor blockade or subchronic nicotine treatment. European journal of pharmacology *314*, 257-267.

Bonnet, U., Specka, M., Leweke, F.M., Nyhuis, P., and Banger, M. (2007). Gabapentin's acute effect on mood profile -- a controlled study on patients with alcohol withdrawal. Progress in neuro-psychopharmacology & biological psychiatry *31*, 434-438.

Book, S.W., and Myrick, H. (2005). Novel anticonvulsants in the treatment of alcoholism. Expert opinion on investigational drugs *14*, 371-376.

Bouchery EE, H.H., Sacks JJ, Simon CJ, Brewer RD (2011). Economic costs of excessive alcohol consumption in the United States. AM J Prev Med *41*, 516-524.

Bouza, C., Angeles, M., Munoz, A., and Amate, J.M. (2004). Efficacy and safety of naltrexone and acamprosate in the treatment of alcohol dependence: a systematic review. Addiction *99*, 811-828.

Braidwood, R. (1953). Symposium: Did man once live by beer alone?, Vol 55 (American Anthropologist).

Breese, G.R., Sinha, R., and Heilig, M. (2011). Chronic alcohol neuroadaptation and stress contribute to susceptibility for alcohol craving and relapse. Pharmacology & therapeutics *129*, 149-171.

Breslin, F.J., Johnson, B.A., and Lynch, W.J. (2010). Effect of topiramate treatment on ethanol consumption in rats. Psychopharmacology *207*, 529-534.

Brodie, M.J., Lerche, H., Gil-Nagel, A., Elger, C., Hall, S., Shin, P., Nohria, V., Mansbach, H., and Group, R.S. (2010). Efficacy and safety of adjunctive ezogabine (retigabine) in refractory partial epilepsy. Neurology *75*, 1817-1824.

Brodie, M.S. (2002). Increased ethanol excitation of dopaminergic neurons of the ventral tegmental area after chronic ethanol treatment. Alcoholism, clinical and experimental research *26*, 1024-1030.

Brown, D.A., and Adams, P.R. (1980). Muscarinic suppression of a novel voltage-sensitive K+ current in a vertebrate neurone. Nature *283*, 673-676.

Browne, T.R., and Penry, J.K. (1973). Benzodiazepines in the treatment of epilepsy. A review. Epilepsia *14*, 277-310.

Brueggemann, L.I., Haick, J.M., Cribbs, L.L., and Byron, K.L. (2014). Differential activation of vascular smooth muscle Kv7.4, Kv7.5, and Kv7.4/7.5 channels by ML213 and ICA-069673. Molecular pharmacology *86*, 330-341.

Bustamante, D., Quintanilla, M.E., Tampier, L., Gonzalez-Lira, V., Israel, Y., and Herrera-Marschitz, M. (2008). Ethanol induces stronger dopamine release in nucleus accumbens (shell) of alcohol-preferring (bibulous) than in alcohol-avoiding (abstainer) rats. European journal of pharmacology *591*, 153-158.

Camp, M.C., Mayfield, R.D., McCracken, M., McCracken, L., and Alcantara, A.A. (2006). Neuroadaptations of Cdk5 in cholinergic interneurons of the nucleus accumbens and prefrontal cortex of inbred alcohol-preferring rats following voluntary alcohol drinking. Alcoholism, clinical and experimental research *30*, 1322-1335.

Carlin, R.K., Grab, D.J., Cohen, R.S., and Siekevitz, P. (1980). Isolation and characterization of postsynaptic densities from various brain regions: enrichment of different types of postsynaptic densities. J Cell Biol *86*, 831-845.

Carnicella, S., Ahmadiantehrani, S., Janak, P.H., and Ron, D. (2009). GDNF is an endogenous negative regulator of ethanol-mediated reward and of ethanol consumption after a period of abstinence. Alcoholism, clinical and experimental research *33*, 1012-1024.

Cavaliere, S., Gillespie, J.M., and Hodge, J.J. (2012). KCNQ channels show conserved ethanol block and function in ethanol behaviour. PloS one *7*, e50279.

Cavaliere, S., Malik, B.R., and Hodge, J.J. (2013). KCNQ channels regulate age-related memory impairment. PloS one *8*, e62445.

Chen, Y.C., and Holmes, A. (2009). Effects of topiramate and other anti-glutamatergic drugs on the acute intoxicating actions of ethanol in mice: modulation by genetic strain and stress. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology *34*, 1454-1466.

Cherrington, E. (1930). Standard Encyclopedia of the Alcohol Problem, Vol 1 (Westerville, OH: American Issue Publishing, Co).

Clinton, S.M., Stead, J.D., Miller, S., Watson, S.J., and Akil, H. (2011). Developmental underpinnings of differences in rodent novelty-seeking and emotional reactivity. Eur J Neurosci *34*, 994-1005.

Cooper, E.C. (2011). Made for "anchorin": Kv7.2/7.3 (KCNQ2/KCNQ3) channels and the modulation of neuronal excitability in vertebrate axons. Seminars in cell & developmental biology *22*, 185-192.

Cooper, E.C., Aldape, K.D., Abosch, A., Barbaro, N.M., Berger, M.S., Peacock, W.S., Jan, Y.N., and Jan, L.Y. (2000). Colocalization and coassembly of two human brain M-type potassium channel subunits that are mutated in epilepsy. Proceedings of the National Academy of Sciences of the United States of America *97*, 4914-4919.

Cooper, E.C., Harrington, E., Jan, Y.N., and Jan, L.Y. (2001). M channel KCNQ2 subunits are localized to key sites for control of neuronal network oscillations and synchronization in mouse brain. The Journal of neuroscience : the official journal of the Society for Neuroscience *21*, 9529-9540.

Cornish, J.L., and Kalivas, P.W. (2000). Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. The Journal of neuroscience : the official journal of the Society for Neuroscience *20*, RC89.

Corrao G, B.V., Zombon A, La Vecchia C (2004). A meta-analysis of alcohol consumption and the risk of 15 diseases. Prev Med *38*, 613-619.

Cotman, C.W., Banker, G., Churchill, L., and Taylor, D. (1974). Isolation of postsynaptic densities from rat brain. J Cell Biol *63*, 441-455.

Cozzoli, D.K., Goulding, S.P., Zhang, P.W., Xiao, B., Hu, J.H., Ary, A.W., Obara, I., Rahn, A., Abou-Ziab, H., Tyrrel, B.*, et al.* (2009). Binge drinking upregulates accumbens mGluR5-Homer2-PI3K signaling: functional implications for alcoholism. The Journal of neuroscience : the official journal of the Society for Neuroscience *29*, 8655-8668.

Crean, C.S., and Tompson, D.J. (2013). The effects of ethanol on the pharmacokinetics, pharmacodynamics, safety, and tolerability of ezogabine (retigabine). Clin Ther *35*, 87-93.

Dai, X.Q., Kolic, J., Marchi, P., Sipione, S., and Macdonald, P.E. (2009). SUMOylation regulates Kv2.1 and modulates pancreatic beta-cell excitability. Journal of cell science *122*, 775-779.

Dalby-Brown, W., Hansen, H.H., Korsgaard, M.P., Mirza, N., and Olesen, S.P. (2006). K(v)7 channels: function, pharmacology and channel modulators. Curr Top Med Chem *6*, 999-1023.

Darby, W. (1977). Food: The Gift of the Osiris, Vol 1 & 2 (London: Academic Press).

De Sousa, A.A., De Sousa, J., and Kapoor, H. (2008). An open randomized trial comparing disulfiram and topiramate in the treatment of alcohol dependence. Journal of substance abuse treatment *34*, 460-463.

Delmas, P., and Brown, D.A. (2005). Pathways modulating neural KCNQ/M (Kv7) potassium channels. Nature reviews Neuroscience *6*, 850-862.

Devaux, J.J., Kleopa, K.A., Cooper, E.C., and Scherer, S.S. (2004). KCNQ2 is a nodal K+ channel. The Journal of neuroscience : the official journal of the Society for Neuroscience *24*, 1236-1244.

Di Nicola, M., Martinotti, G., Tedeschi, D., Frustaci, A., Mazza, M., Sarchiapone, M., Pozzi, G., Bria, P., and Janiri, L. (2010). Pregabalin in outpatient detoxification of subjects with mild-to-moderate alcohol withdrawal syndrome. Human psychopharmacology *25*, 268-275.

Dietler (2006). Alcohol: Anthropological/Archaeological Perspective. Annu Rev Anthropol *25*, 229-249.

Donovan, D.M., Anton, R.F., Miller, W.R., Longabaugh, R., Hosking, J.D., Youngblood, M., and Group, C.S.R. (2008). Combined pharmacotherapies and behavioral interventions for alcohol dependence (The COMBINE Study): examination of posttreatment drinking outcomes. Journal of studies on alcohol and drugs *69*, 5-13.

Doya, K. (2008). Modulators of decision making. Nature neuroscience *11*, 410-416.

Drews, E., Racz, I., Lacava, A.D., Barth, A., Bilkei-Gorzo, A., Wienker, T.F., and Zimmer, A. (2010). Quantitative trait loci contributing to physiological and behavioural ethanol responses after acute and chronic treatment. Int J Neuropsychopharmacol *13*, 155-169.

DuBose, C.S., Chesler, E.J., Goldowitz, D., and Hamre, K.M. (2013). Use of the expanded panel of BXD mice narrow QTL regions in ethanol-induced locomotor activation and motor incoordination. Alcoholism, clinical and experimental research *37*, 170-183.

Engel, J., Jr. (1995). Concepts of epilepsy. Epilepsia *36 Suppl 1*, S23-29.

Ericson, M., Blomqvist, O., Engel, J.A., and Soderpalm, B. (1998). Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. European journal of pharmacology *358*, 189-196.

Esteicher, S. (2006). Wine from Neolithic Times to the 21st Century (New York: Algora Publishing).

Etxeberria, A., Aivar, P., Rodriguez-Alfaro, J.A., Alaimo, A., Villace, P., Gomez-Posada, J.C., Areso, P., and Villarroel, A. (2008). Calmodulin regulates the trafficking of KCNQ2 potassium channels. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *22*, 1135-1143.

Fadda, F., and Rossetti, Z.L. (1998). Chronic ethanol consumption: from neuroadaptation to neurodegeneration. Progress in neurobiology *56*, 385-431.

Farook, J.M., Lewis, B., Littleton, J.M., and Barron, S. (2009). Topiramate attenuates the stress-induced increase in alcohol consumption and preference in male C57BL/6J mice. Physiology & behavior *96*, 189-193.

Feduccia, A.A., Simms, J.A., Mill, D., Yi, H.Y., and Bartlett, S.E. (2014). Varenicline decreases ethanol intake and increases dopamine release via neuronal nicotinic acetylcholine receptors in the nucleus accumbens. British journal of pharmacology *171*, 3420-3431.

Feligioni, M., Nishimune, A., and Henley, J.M. (2009). Protein SUMOylation modulates calcium influx and glutamate release from presynaptic terminals. The European journal of neuroscience *29*, 1348-1356.

Ferrario, C.R., Loweth, J.A., Milovanovic, M., Ford, K.A., Galinanes, G.L., Heng, L.J., Tseng, K.Y., and Wolf, M.E. (2011a). Alterations in AMPA receptor subunits and TARPs in the rat nucleus accumbens related to the formation of Ca(2)(+)-permeable AMPA receptors during the incubation of cocaine craving. Neuropharmacology *61*, 1141-1151.

Ferrario, C.R., Loweth, J.A., Milovanovic, M., Wang, X., and Wolf, M.E. (2011b). Distribution of AMPA receptor subunits and TARPs in synaptic and extrasynaptic membranes of the adult rat nucleus accumbens. Neurosci Lett *490*, 180-184.

Ferri, M., Amato, L., and Davoli, M. (2006). Alcoholics Anonymous and other 12-step programmes for alcohol dependence. The Cochrane database of systematic reviews, CD005032.

Fertig, J.B., Ryan, M.L., Falk, D.E., Litten, R.Z., Mattson, M.E., Ransom, J., Rickman, W.J., Scott, C., Ciraulo, D., Green, A.I.*, et al.* (2012). A double-blind, placebo-controlled trial assessing the efficacy of levetiracetam extended-release in very heavy drinking alcohol-dependent patients. Alcoholism, clinical and experimental research *36*, 1421-1430.

Finn, D.A., and Crabbe, J.C. (1999). Chronic ethanol differentially alters susceptibility to chemically induced convulsions in withdrawal seizure-prone and -resistant mice. The Journal of pharmacology and experimental therapeutics *288*, 782-790.

Fontan-Lozano, A., Suarez-Pereira, I., Delgado-Garcia, J.M., and Carrion, A.M. (2011). The M-current inhibitor XE991 decreases the stimulation threshold for long-term synaptic plasticity in healthy mice and in models of cognitive disease. Hippocampus *21*, 22-32.

French, J.A., Abou-Khalil, B.W., Leroy, R.F., Yacubian, E.M., Shin, P., Hall, S., Mansbach, H., Nohria, V., and Investigators, R.S. (2011). Randomized, double-blind, placebo-controlled trial of ezogabine (retigabine) in partial epilepsy. Neurology *76*, 1555-1563.

Fuller, R.K., Branchey, L., Brightwell, D.R., Derman, R.M., Emrick, C.D., Iber, F.L., James, K.E., Lacoursiere, R.B., Lee, K.K., Lowenstam, I.*, et al.* (1986). Disulfiram treatment of alcoholism. A Veterans Administration cooperative study. Jama *256*, 1449-1455.

Funk, D., Marinelli, P.W., and Le, A.D. (2006). Biological processes underlying co-use of alcohol and nicotine: neuronal mechanisms, cross-tolerance, and genetic factors. Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism *29*, 186-192.

Furieri, F.A., and Nakamura-Palacios, E.M. (2007). Gabapentin reduces alcohol consumption and craving: a randomized, double-blind, placebo-controlled trial. The Journal of clinical psychiatry *68*, 1691-1700.

Gabriel, K.I., and Cunningham, C.L. (2005). Effects of topiramate on ethanol and saccharin consumption and preferences in C57BL/6J mice. Alcoholism, clinical and experimental research *29*, 75-80.

Gamper, N., Stockand, J.D., and Shapiro, M.S. (2003). Subunit-specific modulation of KCNQ potassium channels by Src tyrosine kinase. The Journal of neuroscience : the official journal of the Society for Neuroscience *23*, 84-95.

Garcia-Martinez, J.M., and Alessi, D.R. (2008). mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). The Biochemical journal *416*, 375-385.

Gass, J.T., and Olive, M.F. (2008). Glutamatergic substrates of drug addiction and alcoholism. Biochemical pharmacology *75*, 218-265.

Gass, J.T., Sinclair, C.M., Cleva, R.M., Widholm, J.J., and Olive, M.F. (2011). Alcohol-seeking behavior is associated with increased glutamate transmission in basolateral amygdala and nucleus accumbens as measured by glutamate-oxidase-coated biosensors. Addiction biology *16*, 215-228.

Goebel-Goody, S.M., Davies, K.D., Alvestad Linger, R.M., Freund, R.K., and Browning, M.D. (2009). Phospho-regulation of synaptic and extrasynaptic N-methyl-d-aspartate receptors in adult hippocampal slices. Neuroscience *158*, 1446-1459.

Goldstein, M., Anagnoste, B., Lauber, E., and McKeregham, M.R. (1964). Inhibition of Dopamine-Beta-Hydroxylase by Disulfiram. Life sciences *3*, 763-767.

Gomez-Posada, J.C., Aivar, P., Alberdi, A., Alaimo, A., Etxeberria, A., Fernandez-Orth, J., Zamalloa, T., Roura-Ferrer, M., Villace, P., Areso, P*., et al.* (2011). Kv7 channels can function without constitutive calmodulin tethering. PloS one *6*, e25508.

Gongwer, M.A., Murphy, J.M., McBride, W.J., Lumeng, L., and Li, T.K. (1989). Regional brain contents of serotonin, dopamine and their metabolites in the selectively bred high- and low-alcohol drinking lines of rats. Alcohol *6*, 317-320.

Griffin, K.W., Scheier, L.M., Acevedo, B., Grenard, J.L., and Botvin, G.J. (2012). Long-term effects of self-control on alcohol use and sexual behavior among urban minority young women. International journal of environmental research and public health *9*, 1-23.

Griffin, W.C., 3rd, Haun, H.L., Hazelbaker, C.L., Ramachandra, V.S., and Becker, H.C. (2014). Increased extracellular glutamate in the nucleus accumbens promotes excessive ethanol drinking in ethanol dependent mice. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology *39*, 707-717.

Groc, L., Heine, M., Cognet, L., Brickley, K., Stephenson, F.A., Lounis, B., and Choquet, D. (2004). Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors. Nature neuroscience *7*, 695-696.

Groenewegen, H.J., Wright, C.I., and Beijer, A.V. (1996). The nucleus accumbens: gateway for limbic structures to reach the motor system? Progress in brain research *107*, 485-511.

Grubb, M.S., and Burrone, J. (2010). Activity-dependent relocation of the axon initial segment fine-tunes neuronal excitability. Nature *465*, 1070-1074.

Gu, N., Vervaeke, K., Hu, H., and Storm, J.F. (2005). Kv7/KCNQ/M and HCN/h, but not KCa2/SK channels, contribute to the somatic medium after-hyperpolarization and excitability control in CA1 hippocampal pyramidal cells. J Physiol *566*, 689-715.

Gunthorpe, M.J., Large, C.H., and Sankar, R. (2012). The mechanism of action of retigabine (ezogabine), a first-in-class K+ channel opener for the treatment of epilepsy. Epilepsia *53*, 412-424.

Hansen, H.H., Andreasen, J.T., Weikop, P., Mirza, N., Scheel-Kruger, J., and Mikkelsen, J.D. (2007). The neuronal KCNQ channel opener retigabine inhibits locomotor activity and reduces forebrain excitatory responses to the psychostimulants cocaine, methylphenidate and phencyclidine. European journal of pharmacology *570*, 77-88.

Hansen, H.H., Ebbesen, C., Mathiesen, C., Weikop, P., Ronn, L.C., Waroux, O., Scuvee-Moreau, J., Seutin, V., and Mikkelsen, J.D. (2006). The KCNQ channel opener retigabine inhibits the activity of mesencephalic dopaminergic systems of the rat. The Journal of pharmacology and experimental therapeutics *318*, 1006-1019.

Hansen, H.H., Waroux, O., Seutin, V., Jentsch, T.J., Aznar, S., and Mikkelsen, J.D. (2008). Kv7 channels: interaction with dopaminergic and serotonergic neurotransmission in the CNS. J Physiol *586*, 1823-1832.

Hargreaves, G.A., and McGregor, I.S. (2007). Topiramate moderately reduces the motivation to consume alcohol and has a marked antidepressant effect in rats. Alcoholism, clinical and experimental research *31*, 1900-1907.

Harty, R.C., Kim, T.H., Thomas, E.A., Cardamone, L., Jones, N.C., Petrou, S., and Wimmer, V.C. (2013). Axon initial segment structural plasticity in animal models of genetic and acquired epilepsy. Epilepsy research *105*, 272-279.

Hayton, S.J., Mahoney, M.K., and Olmstead, M.C. (2012). Behavioral traits predicting alcohol drinking in outbred rats: an investigation of anxiety, novelty seeking, and cognitive flexibility. Alcoholism, clinical and experimental research *36*, 594-603.

Heilig, M., Goldman, D., Berrettini, W., and O'Brien, C.P. (2011). Pharmacogenetic approaches to the treatment of alcohol addiction. Nature reviews Neuroscience *12*, 670-684.

Hetka, R., Rundfeldt, C., Heinemann, U., and Schmitz, D. (1999). Retigabine strongly reduces repetitive firing in rat entorhinal cortex. European journal of pharmacology *386*, 165-171.

Hill, J. (2004). Defining Moments: Prohibition. (Detroit, MI: Omnigraphics).

Ho, Y.J., Eichendorff, J., and Schwarting, R.K. (2002). Individual response profiles of male Wistar rats in animal models for anxiety and depression. Behavioural brain research *136*, 1-12.

Hodge, C.W., Samson, H.H., and Chappelle, A.M. (1997). Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens. Alcoholism, clinical and experimental research *21*, 1083-1091.

Hopf, F.W., Bowers, M.S., Chang, S.J., Chen, B.T., Martin, M., Seif, T., Cho, S.L., Tye, K., and Bonci, A. (2010). Reduced nucleus accumbens SK channel activity enhances alcohol seeking during abstinence. Neuron *65*, 682-694.

Hopf, F.W., Martin, M., Chen, B.T., Bowers, M.S., Mohamedi, M.M., and Bonci, A. (2007). Withdrawal from intermittent ethanol exposure increases probability of burst firing in VTA neurons in vitro. Journal of neurophysiology *98*, 2297-2310.

Hopf, F.W., Simms, J.A., Chang, S.J., Seif, T., Bartlett, S.E., and Bonci, A. (2011). Chlorzoxazone, an SK-type potassium channel activator used in humans, reduces excessive alcohol intake in rats. Biological psychiatry *69*, 618-624.

Hoshi, N., Zhang, J.S., Omaki, M., Takeuchi, T., Yokoyama, S., Wanaverbecq, N., Langeberg, L.K., Yoneda, Y., Scott, J.D., Brown, D.A.*, et al.* (2003). AKAP150 signaling complex promotes suppression of the M-current by muscarinic agonists. Nature neuroscience *6*, 564-571.

Imperato, A., and Di Chiara, G. (1986). Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. The Journal of pharmacology and experimental therapeutics *239*, 219-228.

Jaafari, N., Konopacki, F.A., Owen, T.F., Kantamneni, S., Rubin, P., Craig, T.J., Wilkinson, K.A., and Henley, J.M. (2013). SUMOylation is required for glycine-induced increases in AMPA receptor surface expression (ChemLTP) in hippocampal neurons. PloS one *8*, e52345.

Janak, P.H., and Michael Gill, T. (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, M.M., Lange, S.C., Thomsen, M.S., Hansen, H.H., and Mikkelsen, J.D. (2011). The pharmacological effect of positive KCNQ (Kv7) modulators on dopamine release from striatal slices. Basic Clin Pharmacol Toxicol *109*, 339-342.

Jensen, M.S., and Yaari, Y. (1997). Role of intrinsic burst firing, potassium accumulation, and electrical coupling in the elevated potassium model of hippocampal epilepsy. Journal of neurophysiology *77*, 1224-1233.

Jentsch, T.J. (2000). Neuronal KCNQ potassium channels: physiology and role in disease. Nature reviews Neuroscience *1*, 21-30.

Jerlhag, E., Landgren, S., Egecioglu, E., Dickson, S.L., and Engel, J.A. (2011). The alcohol-induced locomotor stimulation and accumbal dopamine release is suppressed in ghrelin knockout mice. Alcohol *45*, 341-347.

Johnson, B.A., Ait-Daoud, N., Bowden, C.L., DiClemente, C.C., Roache, J.D., Lawson, K., Javors, M.A., and Ma, J.Z. (2003). Oral topiramate for treatment of alcohol dependence: a randomised controlled trial. Lancet *361*, 1677-1685.

Johnson, B.A., Roache, J.D., Ait-Daoud, N., Wells, L.T., Wallace, C.L., Dawes, M.A., Liu, L., and Wang, X.Q. (2007). Effects of topiramate on methamphetamine-induced changes in attentional and perceptual-motor skills of cognition in recently abstinent methamphetamine-dependent individuals. Progress in neuro-psychopharmacology & biological psychiatry *31*, 123-130.

Ju, W., Morishita, W., Tsui, J., Gaietta, G., Deerinck, T.J., Adams, S.R., Garner, C.C., Tsien, R.Y., Ellisman, M.H., and Malenka, R.C. (2004). Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. Nature neuroscience *7*, 244-253.

Kalivas, P. (2002). Neurocircuitry of addiction. In Neuropsychopharmacology: the fifth generation of progress (Philadelphia: Lippincott Williams & Wilkins), pp. 1357-1366.

Kalivas, P.W. (2009). The glutamate homeostasis hypothesis of addiction. Nature reviews Neuroscience *10*, 561-572.

Kapfhamer, D., Berger, K.H., Hopf, F.W., Seif, T., Kharazia, V., Bonci, A., and Heberlein, U. (2010). Protein Phosphatase 2a and glycogen synthase kinase 3 signaling modulate prepulse inhibition of the acoustic startle response by altering cortical M-Type potassium channel activity. The Journal of neuroscience : the official journal of the Society for Neuroscience *30*, 8830-8840.

Katner, S.N., McBride, W.J., Lumeng, L., Li, T.K., and Murphy, J.M. (1997). Alcohol intake of P rats is regulated by muscarinic receptors in the pedunculopontine nucleus and VTA. Pharmacology, biochemistry, and behavior *58*, 497-504.

Katz, S.a.V., MM (1987). Bread and beer: The early use of cereals in the human diet, Vol 28 (Expedition).

Keane, T.M., Goodstadt, L., Danecek, P., White, M.A., Wong, K., Yalcin, B., Heger, A., Agam, A., Slater, G., Goodson, M.*, et al.* (2011). Mouse genomic variation and its effect on phenotypes and gene regulation. Nature *477*, 289-294.

Kenna, G.A., Lomastro, T.L., Schiesl, A., Leggio, L., and Swift, R.M. (2009). Review of topiramate: an antiepileptic for the treatment of alcohol dependence. Current drug abuse reviews *2*, 135-142.

Kharkovets, T., Hardelin, J.P., Safieddine, S., Schweizer, M., El-Amraoui, A., Petit, C., and Jentsch, T.J. (2000). KCNQ4, a K+ channel mutated in a form of dominant deafness, is expressed in the inner ear and the central auditory pathway. Proceedings of the National Academy of Sciences of the United States of America *97*, 4333-4338.

Kiianmaa, K., and Tabakoff, B. (1983). Neurochemical correlates of tolerance and strain differences in the neurochemical effects of ethanol. Pharmacology, biochemistry, and behavior *18 Suppl 1*, 383-388.

Kim, J., Jung, S.C., Clemens, A.M., Petralia, R.S., and Hoffman, D.A. (2007). Regulation of dendritic excitability by activity-dependent trafficking of the A-type K+ channel subunit Kv4.2 in hippocampal neurons. Neuron *54*, 933-947.

Kim, K.S., and Han, P.L. (2006). Optimization of chronic stress paradigms using anxiety- and depression-like behavioral parameters. Journal of neuroscience research *83*, 497-507.

King, F. (1947). Beer has a History (London: Hutchinson's Scientific and Technical Publications).

Knapp, C.M., Mercado, M., Markley, T.L., Crosby, S., Ciraulo, D.A., and Kornetsky, C. (2007). Zonisamide decreases ethanol intake in rats and mice. Pharmacology, biochemistry, and behavior *87*, 65-72.

Knapp, C.M., O'Malley, M., Datta, S., and Ciraulo, D.A. (2014). The Kv7 potassium channel activator retigabine decreases alcohol consumption in rats. The American journal of drug and alcohol abuse *40*, 244-250.

Knapp, C.M., Sarid-Segal, O., Richardson, M.A., Colaneri, L.S., Afshar, M., Devine, E., Streeter, C.C., Piechniczek-Buczek, J., and Ciraulo, D.A. (2010). Open label trial of the tolerability and efficacy of zonisamide in the treatment of alcohol dependence. The American journal of drug and alcohol abuse *36*, 102-105.

Koob, G.F., and Le Moal, M. (2005). Plasticity of reward neurocircuitry and the 'dark side' of drug addiction. Nature neuroscience *8*, 1442-1444.

Koob, G.F., and Volkow, N.D. (2010). Neurocircuitry of addiction. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology *35*, 217-238.

Kordeli, E., Lambert, S., and Bennett, V. (1995). AnkyrinG. A new ankyrin gene with neural-specific isoforms localized at the axonal initial segment and node of Ranvier. The Journal of biological chemistry *270*, 2352-2359.

Korsgaard, M.P., Hartz, B.P., Brown, W.D., Ahring, P.K., Strobaek, D., and Mirza, N.R. (2005). Anxiolytic effects of Maxipost (BMS-204352) and retigabine via activation of neuronal Kv7 channels. The Journal of pharmacology and experimental therapeutics *314*, 282-292.

Koyama, S., and Appel, S.B. (2006). Characterization of M-current in ventral tegmental area dopamine neurons. Journal of neurophysiology *96*, 535-543.

Koyama, S., Brodie, M.S., and Appel, S.B. (2007). Ethanol inhibition of m-current and ethanol-induced direct excitation of ventral tegmental area dopamine neurons. Journal of neurophysiology *97*, 1977-1985.

Kranzler, H.R., Covault, J., Feinn, R., Armeli, S., Tennen, H., Arias, A.J., Gelernter, J., Pond, T., Oncken, C., and Kampman, K.M. (2014). Topiramate treatment for heavy drinkers: moderation by a GRIK1 polymorphism. The American journal of psychiatry *171*, 445-452.

Kranzler, H.R., and McKay, J.R. (2012). Personalized treatment of alcohol dependence. Curr Psychiatry Rep *14*, 486-493.

Kuczenski, R., and Segal, D.S. (1999). Sensitization of amphetamine-induced stereotyped behaviors during the acute response. The Journal of pharmacology and experimental therapeutics *288*, 699-709.

LA, G. (1998). Alcohol and Crime: An analysis of national data on the prevalence of alcohol involvement in crime. In Assistant Attorney General's National Symposium on Alcohol Abuse and Crime (Washing, DC: US Department of Justice).

LaFrance, W.C., Jr., and Devinsky, O. (2004). The treatment of nonepileptic seizures: historical perspectives and future directions. Epilepsia *45 Suppl 2*, 15-21.

Landgren, S., Jerlhag, E., Zetterberg, H., Gonzalez-Quintela, A., Campos, J., Olofsson, U., Nilsson, S., Blennow, K., and Engel, J.A. (2008). Association of pro-ghrelin and GHS-R1A gene polymorphisms and haplotypes with heavy alcohol use and body mass. Alcoholism, clinical and experimental research *32*, 2054-2061.

Large, C.H., Sokal, D.M., Nehlig, A., Gunthorpe, M.J., Sankar, R., Crean, C.S., Vanlandingham, K.E., and White, H.S. (2012). The spectrum of anticonvulsant efficacy of retigabine (ezogabine) in animal models: implications for clinical use. Epilepsia *53*, 425-436.

Le, A.D., Ko, J., Chow, S., and Quan, B. (1994). Alcohol consumption by C57BL/6, BALB/c, and DBA/2 mice in a limited access paradigm. Pharmacology, biochemistry, and behavior *47*, 375-378.

Leao, R.N., Tan, H.M., and Fisahn, A. (2009). Kv7/KCNQ channels control action potential phasing of pyramidal neurons during hippocampal gamma oscillations in vitro. The Journal of neuroscience : the official journal of the Society for Neuroscience *29*, 13353-13364.

Leppik, I.E. (2004). Zonisamide: chemistry, mechanism of action, and pharmacokinetics. Seizure *13 Suppl 1*, S5-9; discussion S10.

Li, J., Cheng, Y., Bian, W., Liu, X., Zhang, C., and Ye, J.H. (2010). Region-specific induction of FosB/DeltaFosB by voluntary alcohol intake: effects of naltrexone. Alcoholism, clinical and experimental research *34*, 1742-1750.

Lu, W., and Roche, K.W. (2012). Posttranslational regulation of AMPA receptor trafficking and function. Current opinion in neurobiology *22*, 470-479.

Luo, J., Ashikaga, E., Rubin, P.P., Heimann, M.J., Hildick, K.L., Bishop, P., Girach, F., Josa-Prado, F., Tang, L.T., Carmichael, R.E.*, et al.* (2013). Receptor trafficking and the regulation of synaptic plasticity by SUMO. Neuromolecular medicine *15*, 692-706.

Luttjohann, A., Fabene, P.F., and van Luijtelaar, G. (2009). A revised Racine's scale for PTZ-induced seizures in rats. Physiol Behav *98*, 579-586.

Lynch, B.A., Lambeng, N., Nocka, K., Kensel-Hammes, P., Bajjalieh, S.M., Matagne, A., and Fuks, B. (2004). The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. Proceedings of the National Academy of Sciences of the United States of America *101*, 9861-9866.

Mahler, S.V., Smith, R.J., and Aston-Jones, G. (2013). Interactions between VTA orexin and glutamate in cue-induced reinstatement of cocaine seeking in rats. Psychopharmacology *226*, 687-698.

Malcolm, R., Brady, K.T., Johnston, A.L., and Cunningham, M. (1993). Types of benzodiazepines abused by chemically dependent inpatients. Journal of psychoactive drugs *25*, 315-319.

Malcolm, R., Myrick, H., Brady, K.T., and Ballenger, J.C. (2001). Update on anticonvulsants for the treatment of alcohol withdrawal. The American journal on addictions / American Academy of Psychiatrists in Alcoholism and Addictions *10 Suppl*, 16-23.

Malcolm, R.J. (2003). GABA systems, benzodiazepines, and substance dependence. The Journal of clinical psychiatry *64 Suppl 3*, 36-40.

Maljevic, S., Wuttke, T.V., and Lerche, H. (2008). Nervous system KV7 disorders: breakdown of a subthreshold brake. J Physiol *586*, 1791-1801.

Mao, L.M., Guo, M.L., Jin, D.Z., Fibuch, E.E., Choe, E.S., and Wang, J.Q. (2011). Post-translational modification biology of glutamate receptors and drug addiction. Frontiers in neuroanatomy *5*, 19.

Marrion, N.V. (1997). Control of M-current. Annual review of physiology *59*, 483-504.

Marshall, D.L., Redfern, P.H., and Wonnacott, S. (1997). Presynaptic nicotinic modulation of dopamine release in the three ascending pathways studied by in vivo microdialysis: comparison of naive and chronic nicotine-treated rats. Journal of neurochemistry *68*, 1511-1519.

Martin, S., Nishimune, A., Mellor, J.R., and Henley, J.M. (2007a). SUMOylation regulates kainate-receptor-mediated synaptic transmission. Nature *447*, 321-325.

Martin, S., Wilkinson, K.A., Nishimune, A., and Henley, J.M. (2007b). Emerging extranuclear roles of protein SUMOylation in neuronal function and dysfunction. Nature reviews Neuroscience *8*, 948-959.

Martinotti, G., Di Nicola, M., Tedeschi, D., Andreoli, S., Reina, D., Pomponi, M., Mazza, M., Romanelli, R., Moroni, N., De Filippis, R.*, et al.* (2010). Pregabalin versus naltrexone in alcohol dependence: a randomised, double-blind, comparison trial. Journal of psychopharmacology *24*, 1367-1374.

Martinotti, G., Di Nicola, M., Tedeschi, D., Mazza, M., Janiri, L., and Bria, P. (2008). Efficacy and safety of pregabalin in alcohol dependence. Advances in therapy *25*, 608-618.

Martire, M., D'Amico, M., Panza, E., Miceli, F., Viggiano, D., Lavergata, F., Iannotti, F.A., Barrese, V., Preziosi, P., Annunziato, L.*, et al.* (2007). Involvement of KCNQ2 subunits in [3H]dopamine release triggered by depolarization and pre-synaptic muscarinic receptor activation from rat striatal synaptosomes. Journal of neurochemistry *102*, 179-193.

Marty, V.N., and Spigelman, I. (2012a). Effects of alcohol on the membrane excitability and synaptic transmission of medium spiny neurons in the nucleus accumbens. Alcohol *46*, 317-327.

Marty, V.N., and Spigelman, I. (2012b). Long-lasting alterations in membrane properties, k(+) currents, and glutamatergic synaptic currents of nucleus accumbens medium spiny neurons in a rat model of alcohol dependence. Front Neurosci *6*, 86.

Mayo-Smith, M.F., and Bernard, D. (1995). Late-onset seizures in alcohol withdrawal. Alcoholism, clinical and experimental research *19*, 656-659.

Mazarati, A., Wu, J., Shin, D., Kwon, Y.S., and Sankar, R. (2008). Antiepileptogenic and antiictogenic effects of retigabine under conditions of rapid kindling: an ontogenic study. Epilepsia *49*, 1777-1786.

McBride, W.J., and Li, T.K. (1998). Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. Critical reviews in neurobiology *12*, 339-369.

McFarland, K., Lapish, C.C., and Kalivas, P.W. (2003). Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience *23*, 3531-3537.

McGuier, N.S., Padula, A.E., Lopez, M.F., Woodward, J.J., and Mulholland, P.J. (2014). Withdrawal from chronic intermittent alcohol exposure increases dendritic spine density in the lateral orbitofrontal cortex of mice. Alcohol.

Melendez, R.I., Rodd-Henricks, Z.A., Engleman, E.A., Li, T.K., McBride, W.J., and Murphy, J.M. (2002). Microdialysis of dopamine in the nucleus accumbens of alcohol-preferring (P) rats during anticipation and operant self-administration of ethanol. Alcoholism, clinical and experimental research *26*, 318-325.

Meliska, C.J., Bartke, A., McGlacken, G., and Jensen, R.A. (1995). Ethanol, nicotine, amphetamine, and aspartame consumption and preferences in C57BL/6 and DBA/2 mice. Pharmacology, biochemistry, and behavior *50*, 619-626.

Metten, P., Iancu, O.D., Spence, S.E., Walter, N.A., Oberbeck, D., Harrington, C.A., Colville, A., McWeeney, S., Phillips, T.J., Buck, K.J.*, et al.* (2014). Dual-trait selection for ethanol consumption and withdrawal: genetic and transcriptional network effects. Alcoholism, clinical and experimental research *38*, 2915-2924.

Miceli, F., Cilio, M.R., Taglialatela, M., and Bezanilla, F. (2009). Gating currents from neuronal K(V)7.4 channels: general features and correlation with the ionic conductance. Channels (Austin) *3*, 274-283.

Mimaki, T., Suzuki, Y., Tagawa, T., Karasawa, T., and Yabuuchi, H. (1990). [3H]zonisamide binding in rat brain. Medical journal of Osaka University *39*, 19-22.

Mineur, Y.S., Belzung, C., and Crusio, W.E. (2006). Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. Behavioural brain research *175*, 43-50.

Miranda, P., Cadaveira-Mosquera, A., Gonzalez-Montelongo, R., Villarroel, A., Gonzalez-Hernandez, T., Lamas, J.A., Alvarez de la Rosa, D., and Giraldez, T. (2013). The neuronal serum- and glucocorticoid-regulated kinase 1.1 reduces neuronal excitability and protects against seizures through upregulation of the M-current. The Journal of neuroscience : the official journal of the Society for Neuroscience *33*, 2684-2696.

Mitchell, J.M., Grossman, L.E., Coker, A.R., and Messing, R.O. (2012). The anticonvulsant levetiracetam potentiates alcohol consumption in non-treatment seeking alcohol abusers. Journal of clinical psychopharmacology *32*, 269-272.

Momeni, S., and Roman, E. (2014). Subgroup-dependent effects of voluntary alcohol intake on behavioral profiles in outbred Wistar rats. Behavioural brain research *275*, 288-296.

Moore, S.D., Madamba, S.G., and Siggins, G.R. (1990). Ethanol diminishes a voltage-dependent K+ current, the M-current, in CA1 hippocampal pyramidal neurons in vitro. Brain research *516*, 222-228.

Mulholland, P.J., Becker, H.C., Woodward, J.J., and Chandler, L.J. (2011). Small conductance calcium-activated potassium type 2 channels regulate alcohol-associated plasticity of glutamatergic synapses. Biological psychiatry *69*, 625-632.

Mulholland, P.J., and Chandler, L.J. (2007). The thorny side of addiction: adaptive plasticity and dendritic spines. ScientificWorldJournal *7*, 9-21.

Mulligan, M.K., Ponomarev, I., Hitzemann, R.J., Belknap, J.K., Tabakoff, B., Harris, R.A., Crabbe, J.C., Blednov, Y.A., Grahame, N.J., Phillips, T.J.*, et al.* (2006). Toward understanding the genetics of alcohol drinking through transcriptome meta-analysis. Proceedings of the National Academy of Sciences of the United States of America *103*, 6368-6373.

Murphy, J.M., McBride, W.J., Lumeng, L., and Li, T.K. (1987). Contents of monoamines in forebrain regions of alcohol-preferring (P) and -nonpreferring (NP) lines of rats. Pharmacology, biochemistry, and behavior *26*, 389-392.

Myrick, H., Anton, R., Voronin, K., Wang, W., and Henderson, S. (2007). A double-blind evaluation of gabapentin on alcohol effects and drinking in a clinical laboratory paradigm. Alcoholism, clinical and experimental research *31*, 221-227.

Myrick, H., Brady, K.T., and Malcolm, R. (2000). Divalproex in the treatment of alcohol withdrawal. The American journal of drug and alcohol abuse *26*, 155-160.

Myrick, H., Malcolm, R., and Brady, K.T. (1998). Gabapentin treatment of alcohol withdrawal. The American journal of psychiatry *155*, 1632.

Myrick, H., Malcolm, R., Randall, P.K., Boyle, E., Anton, R.F., Becker, H.C., and Randall, C.L. (2009). A double-blind trial of gabapentin versus lorazepam in the treatment of alcohol withdrawal. Alcoholism, clinical and experimental research *33*, 1582-1588.

NCADD (2015). Nation Council on Alcoholism and Drug Dependence.

Neasta, J., Ben Hamida, S., Yowell, Q., Carnicella, S., and Ron, D. (2010). Role for mammalian target of rapamycin complex 1 signaling in neuroadaptations underlying alcohol-related disorders. Proceedings of the National Academy of Sciences of the United States of America *107*, 20093-20098.

Neasta, J., Ben Hamida, S., Yowell, Q.V., Carnicella, S., and Ron, D. (2011). AKT signaling pathway in the nucleus accumbens mediates excessive alcohol drinking behaviors. Biological psychiatry *70*, 575-582.

Nguyen, S.A., Malcolm, R., and Middaugh, L.D. (2007). Topiramate reduces ethanol consumption by C57BL/6 mice. Synapse *61*, 150-156.

NIAAA (2012). Alcohol Use Disorder, NIAAA, ed.

O'Connell, K.M., Rolig, A.S., Whitesell, J.D., and Tamkun, M.M. (2006). Kv2.1 potassium channels are retained within dynamic cell surface microdomains that are defined by a perimeter fence. The Journal of neuroscience : the official journal of the Society for Neuroscience *26*, 9609-9618.

Obara, I., Bell, R.L., Goulding, S.P., Reyes, C.M., Larson, L.A., Ary, A.W., Truitt, W.A., and Szumlinski, K.K. (2009). Differential effects of chronic ethanol consumption and withdrawal on homer/glutamate receptor expression in subregions of the accumbens and amygdala of P rats. Alcoholism, clinical and experimental research *33*, 1924-1934.

Otto, J.F., Kimball, M.M., and Wilcox, K.S. (2002). Effects of the anticonvulsant retigabine on cultured cortical neurons: changes in electroresponsive properties and synaptic transmission. Molecular pharmacology *61*, 921-927.

Otto, J.F., Singh, N.A., Dahle, E.J., Leppert, M.F., Pappas, C.M., Pruess, T.H., Wilcox, K.S., and White, H.S. (2009). Electroconvulsive seizure thresholds and kindling

acquisition rates are altered in mouse models of human KCNQ2 and KCNQ3 mutations for benign familial neonatal convulsions. Epilepsia *50*, 1752-1759.

Otto, J.F., Yang, Y., Frankel, W.N., White, H.S., and Wilcox, K.S. (2006). A spontaneous mutation involving Kcnq2 (Kv7.2) reduces M-current density and spike frequency adaptation in mouse CA1 neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience *26*, 2053-2059.

Otto, J.F., Yang, Y., Frankel, W.N., Wilcox, K.S., and White, H.S. (2004). Mice carrying the szt1 mutation exhibit increased seizure susceptibility and altered sensitivity to compounds acting at the m-channel. Epilepsia *45*, 1009-1016.

Overton, P.G., and Clark, D. (1997). Burst firing in midbrain dopaminergic neurons. Brain research Brain research reviews *25*, 312-334.

Padula, A.E., Griffin, W.C., 3rd, Lopez, M.F., Nimitvilai, S., McGuier, N.S., Chesler, E.J., Miles, M.F., Williams, R.W., Randall, P.K., Woodward, J.J.*, et al.* (2015). KCNN genes That Encode Small-Conductance Ca-Activated K Channels Influence Alcohol and Drug Addiction. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.

Padula, A.E., McGuier, N.S., Griffin III, W.C., Lopez, M.F., Becker, H.C., and Mulholland, P.J. (2013). Novel anticonvulsants for reducing alcohol consumption: A review of evidence from preclinical rodent drinking models. OA Alcohol *1*, 2.

Pan, Z., Kao, T., Horvath, Z., Lemos, J., Sul, J.Y., Cranstoun, S.D., Bennett, V., Scherer, S.S., and Cooper, E.C. (2006). A common ankyrin-G-based mechanism retains KCNQ and NaV channels at electrically active domains of the axon. The Journal of neuroscience : the official journal of the Society for Neuroscience *26*, 2599-2613.

Panagopoulos, V.N., and Ralevski, E. (2014). The role of ghrelin in addiction: a review. Psychopharmacology *231*, 2725-2740.

Patrick, C. (1970). Alcohol, Culture and Society (Durham, NC: Duke U. Press).

Penschuck, S., Bastlund, J.F., Jensen, H.S., Stensbol, T.B., Egebjerg, J., and Watson, W.P. (2005). Changes in KCNQ2 immunoreactivity in the amygdala in two rat models of temporal lobe epilepsy. Brain Res Mol Brain Res *141*, 66-73.

Perkins, N.D. (2006). Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. Oncogene *25*, 6717-6730.

Pfaffinger, P. (1988). Muscarine and t-LHRH suppress M-current by activating an IAP-insensitive G-protein. The Journal of neuroscience : the official journal of the Society for Neuroscience *8*, 3343-3353.

Plant, L.D., Dowdell, E.J., Dementieva, I.S., Marks, J.D., and Goldstein, S.A. (2011). SUMO modification of cell surface Kv2.1 potassium channels regulates the activity of rat hippocampal neurons. The Journal of general physiology *137*, 441-454.

Porter, R.J., Dhir, A., Macdonald, R.L., and Rogawski, M.A. (2012). Mechanisms of action of antiseizure drugs. Handbook of clinical neurology *108*, 663-681.

Porter, R.J., Partiot, A., Sachdeo, R., Nohria, V., Alves, W.M., and Study, G. (2007). Randomized, multicenter, dose-ranging trial of retigabine for partial-onset seizures. Neurology *68*, 1197-1204.

Qi, Y., Wang, J., Bomben, V.C., Li, D.P., Chen, S.R., Sun, H., Xi, Y., Reed, J.G., Cheng, J., Pan, H.L.*, et al.* (2014). Hyper-SUMOylation of the Kv7 potassium channel diminishes the M-current leading to seizures and sudden death. Neuron *83*, 1159-1171.

Qiu, C., Johnson, B.N., and Tallent, M.K. (2007). K+ M-current regulates the transition to seizures in immature and adult hippocampus. Epilepsia *48*, 2047-2058.

Rada, P.V., Mar, G.P., Yeomans, J.J., and Hoevel, B.G. (2000). Acetylcholine release in ventral tegmental area by hypothalamic self-stiulation, eating, and drinking. Pharmacology Biochemistry and Behavior *65*, 375-379.

Rasmussen, H.B., Frokjaer-Jensen, C., Jensen, C.S., Jensen, H.S., Jorgensen, N.K., Misonou, H., Trimmer, J.S., Olesen, S.P., and Schmitt, N. (2007). Requirement of subunit co-assembly and ankyrin-G for M-channel localization at the axon initial segment. Journal of cell science *120*, 953-963.

Raymond, I. (1927). The Teaching of the Early Church on the Use of Wine and Strong Drink (New York: Columbia University Press).

Reed, N.A., Cai, D., Blasius, T.L., Jih, G.T., Meyhofer, E., Gaertig, J., and Verhey, K.J. (2006). Microtubule acetylation promotes kinesin-1 binding and transport. Current biology : CB *16*, 2166-2172.

Rehm, J., and Gmel, G. (2003). Alcohol consumption and total mortality/morbidity-definitions and methodological implications. Best practice & research Clinical gastroenterology *17*, 497-505.

Reilly, M.T., Lobo, I.A., McCracken, L.M., Borghese, C.M., Gong, D., Horishita, T., and Harris, R.A. (2008). Effects of acamprosate on neuronal receptors and ion channels expressed in Xenopus oocytes. Alcoholism, clinical and experimental research *32*, 188-196.

Ren, J., Gao, X., Jin, C., Zhu, M., Wang, X., Shaw, A., Wen, L., Yao, X., and Xue, Y. (2009). Systematic study of protein sumoylation: Development of a site-specific predictor of SUMOsp 2.0. Proteomics *9*, 3409-3412.

Rhodes, J.S., Ford, M.M., Yu, C.H., Brown, L.L., Finn, D.A., Garland, T., Jr., and Crabbe, J.C. (2007). Mouse inbred strain differences in ethanol drinking to intoxication. Genes, brain, and behavior *6*, 1-18.

Richter, C., Effenberger, S., Bschor, T., Bonnet, U., Haasen, C., Preuss, U.W., Heinz, A., Forg, A., Volkmar, K., Glauner, T.*, et al.* (2012). Efficacy and safety of levetiracetam

for the prevention of alcohol relapse in recently detoxified alcohol-dependent patients: a randomized trial. Journal of clinical psychopharmacology *32*, 558-562.

Richter, C., Hinzpeter, A., Schmidt, F., Kienast, T., Preuss, U.W., Plenge, T., Heinz, A., and Schaefer, M. (2010). Levetiracetam for the treatment of alcohol withdrawal syndrome: a multicenter, prospective, randomized, placebo-controlled trial. Journal of clinical psychopharmacology *30*, 720-725.

Roberto, M., Gilpin, N.W., O'Dell, L.E., Cruz, M.T., Morse, A.C., Siggins, G.R., and Koob, G.F. (2008). Cellular and behavioral interactions of gabapentin with alcohol dependence. The Journal of neuroscience : the official journal of the Society for Neuroscience *28*, 5762-5771.

Robinson, J.E., Chen, M., Stamatakis, A.M., Krouse, M.C., Howard, E.C., Faccidomo, S., Hodge, C.W., Fish, E.W., and Malanga, C.J. (2013). Levetiracetam has opposite effects on alcohol- and cocaine-related behaviors in C57BL/6J mice. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology *38*, 1322-1333.

Roche, J.P., Westenbroek, R., Sorom, A.J., Hille, B., Mackie, K., and Shapiro, M.S. (2002). Antibodies and a cysteine-modifying reagent show correspondence of M current in neurons to KCNQ2 and KCNQ3 K+ channels. British journal of pharmacology *137*, 1173-1186.

Rodd, Z.A., Kimpel, M.W., Edenberg, H.J., Bell, R.L., Strother, W.N., McClintick, J.N., Carr, L.G., Liang, T., and McBride, W.J. (2008). Differential gene expression in the nucleus accumbens with ethanol self-administration in inbred alcohol-preferring rats. Pharmacology, biochemistry, and behavior *89*, 481-498.

Rodd-Henricks, Z.A., McKinzie, D.L., Li, T.K., Murphy, J.M., and McBride, W.J. (2002). Cocaine is self-administered into the shell but not the core of the nucleus accumbens of Wistar rats. The Journal of pharmacology and experimental therapeutics *303*, 1216-1226.

Rogawski, M.A., and Loscher, W. (2004). The neurobiology of antiepileptic drugs for the treatment of nonepileptic conditions. Nature medicine *10*, 685-692.

Rorabaugh, W. (1979). The Alcoholic Republic: An American Tradition (New York: Oxford University Press).

Rubio, G., Lopez-Munoz, F., Ferre, F., Martinez-Gras, I., Ponce, G., Pascual, J.M., Jimenez-Arriero, M.A., and Alamo, C. (2010). Effects of zonisamide in the treatment of alcohol dependence. Clinical neuropharmacology *33*, 250-253.

Rundfeldt, C. (1997). The new anticonvulsant retigabine (D-23129) acts as an opener of K+ channels in neuronal cells. European journal of pharmacology *336*, 243-249.

Rundfeldt, C., and Netzer, R. (2000). Investigations into the mechanism of action of the new anticonvulsant retigabine. Interaction with GABAergic and glutamatergic

neurotransmission and with voltage gated ion channels. Arzneimittelforschung *50*, 1063-1070.

Russo, S.J., Dietz, D.M., Dumitriu, D., Morrison, J.H., Malenka, R.C., and Nestler, E.J. (2010). The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. Trends in neurosciences *33*, 267-276.

Sabino, V., Kwak, J., Rice, K.C., and Cottone, P. (2013). Pharmacological characterization of the 20% alcohol intermittent access model in Sardinian alcohol-preferring rats: a model of binge-like drinking. Alcoholism, clinical and experimental research *37*, 635-643.

Sander, S.E., Lemm, C., Lange, N., Hamann, M., and Richter, A. (2012). Retigabine, a K(V)7 (KCNQ) potassium channel opener, attenuates L-DOPA-induced dyskinesias in 6-OHDA-lesioned rats. Neuropharmacology *62*, 1052-1061.

Sauter, A.M., Boss, D., and von Wartburg, J.P. (1977). Reevaluation of the disulfiram-alcohol reaction in man. Journal of studies on alcohol *38*, 1680-1695.

Sauter, A.M., and Wartburg, J.P. (1977). Quantitative analysis of disulfiram and its metabolites in human blood by gas-liquid chromatography. Journal of chromatography *133*, 167-172.

Schacht, J.P., Anton, R.F., Voronin, K.E., Randall, P.K., Li, X., Henderson, S., and Myrick, H. (2013). Interacting effects of naltrexone and OPRM1 and DAT1 variation on the neural response to alcohol cues. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology *38*, 414-422.

Schuetz, F., Kumar, S., Poronnik, P., and Adams, D.J. (2008). Regulation of the voltage-gated K(+) channels KCNQ2/3 and KCNQ3/5 by serum- and glucocorticoid-regulated kinase-1. American journal of physiology Cell physiology *295*, C73-80.

Scinska, A., Koros, E., Habrat, B., Kukwa, A., Kostowski, W., and Bienkowski, P. (2000). Bitter and sweet components of ethanol taste in humans. Drug and alcohol dependence *60*, 199-206.

Seebohm, G., Strutz-Seebohm, N., Baltaev, R., Korniychuk, G., Knirsch, M., Engel, J., and Lang, F. (2005). Regulation of KCNQ4 potassium channel prepulse dependence and current amplitude by SGK1 in Xenopus oocytes. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology *16*, 255-262.

Seif, T., Chang, S.J., Simms, J.A., Gibb, S.L., Dadgar, J., Chen, B.T., Harvey, B.K., Ron, D., Messing, R.O., Bonci, A*., et al.* (2013). Cortical activation of accumbens hyperpolarization-active NMDARs mediates aversion-resistant alcohol intake. Nature neuroscience *16*, 1094-1100.

Sellings, L.H., and Clarke, P.B. (2003). Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. The Journal of neuroscience : the official journal of the Society for Neuroscience *23*, 6295-6303.

Selyanko, A.A., Delmas, P., Hadley, J.K., Tatulian, L., Wood, I.C., Mistry, M., London, B., and Brown, D.A. (2002). Dominant-negative subunits reveal potassium channel families that contribute to M-like potassium currents. The Journal of neuroscience : the official journal of the Society for Neuroscience *22*, RC212.

Services, T.e. (2013). Zonerga Product Information (Australia: SciGen).

Shen, R.Y. (2003). Ethanol withdrawal reduces the number of spontaneously active ventral tegmental area dopamine neurons in conscious animals. The Journal of pharmacology and experimental therapeutics *307*, 566-572.

Shen, W., Hamilton, S.E., Nathanson, N.M., and Surmeier, D.J. (2005). Cholinergic suppression of KCNQ channel currents enhances excitability of striatal medium spiny neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience *25*, 7449-7458.

Shi, L., Bian, X., Qu, Z., Ma, Z., Zhou, Y., Wang, K., Jiang, H., and Xie, J. (2013). Peptide hormone ghrelin enhances neuronal excitability by inhibition of Kv7/KCNQ channels. Nat Commun *4*, 1435.

Simms, J.A., Steensland, P., Medina, B., Abernathy, K.E., Chandler, L.J., Wise, R., and Bartlett, S.E. (2008). Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. Alcoholism, clinical and experimental research *32*, 1816-1823.

Soderpalm, B., Ericson, M., Olausson, P., Blomqvist, O., and Engel, J.A. (2000). Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. Behavioural brain research *113*, 85-96.

Sotty, F., Damgaard, T., Montezinho, L.P., Mork, A., Olsen, C.K., Bundgaard, C., and Husum, H. (2009). Antipsychotic-like effect of retigabine [N-(2-Amino-4-(fluorobenzylamino)-phenyl)carbamic acid ester], a KCNQ potassium channel opener, via modulation of mesolimbic dopaminergic neurotransmission. The Journal of pharmacology and experimental therapeutics *328*, 951-962.

Sournia, J. (1990). A History of Alcoholism (Oxford: Basil Blackwell).

Spanagel, R., Vengeliene, V., Jandeleit, B., Fischer, W.N., Grindstaff, K., Zhang, X., Gallop, M.A., Krstew, E.V., Lawrence, A.J., and Kiefer, F. (2014). Acamprosate produces its anti-relapse effects via calcium. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology *39*, 783-791.

Spiga, S., Talani, G., Mulas, G., Licheri, V., Fois, G.R., Muggironi, G., Masala, N., Cannizzaro, C., Biggio, G., Sanna, E.*, et al.* (2014). Hampered long-term depression and thin spine loss in the nucleus accumbens of ethanol-dependent rats. Proceedings of the National Academy of Sciences of the United States of America *111*, E3745-3754.

Steensland, P., Fredriksson, I., Holst, S., Feltmann, K., Franck, J., Schilstrom, B., and Carlsson, A. (2012). The monoamine stabilizer (-)-OSU6162 attenuates voluntary

ethanol intake and ethanol-induced dopamine output in nucleus accumbens. Biological psychiatry *72*, 823-831.

Stefanik, M.T., and Kalivas, P.W. (2013). Optogenetic dissection of basolateral amygdala projections during cue-induced reinstatement of cocaine seeking. Frontiers in behavioral neuroscience *7*, 213.

Stefanini, E., Frau, M., Garau, M.G., Garau, B., Fadda, F., and Gessa, G.L. (1992). Alcohol-preferring rats have fewer dopamine D2 receptors in the limbic system. Alcohol and alcoholism *27*, 127-130.

Stephen, L.J., and Brodie, M.J. (2011). Pharmacotherapy of epilepsy: newly approved and developmental agents. CNS drugs *25*, 89-107.

Stephens, D.N., Brown, G., Duka, T., and Ripley, T.L. (2001). Impaired fear conditioning but enhanced seizure sensitivity in rats given repeated experience of withdrawal from alcohol. Eur J Neurosci *14*, 2023-2031.

Stopponi, S., Somaini, L., Cippitelli, A., de Guglielmo, G., Kallupi, M., Cannella, N., Gerra, G., Massi, M., and Ciccocioppo, R. (2012). Pregabalin reduces alcohol drinking and relapse to alcohol seeking in the rat. Psychopharmacology *220*, 87-96.

Streeton, C., and Whelan, G. (2001). Naltrexone, a relapse prevention maintenance treatment of alcohol dependence: a meta-analysis of randomized controlled trials. Alcohol and alcoholism *36*, 544-552.

Stromberg, M.F., Mackler, S.A., Volpicelli, J.R., O'Brien, C.P., and Dewey, S.L. (2001). The effect of gamma-vinyl-GABA on the consumption of concurrently available oral cocaine and ethanol in the rat. Pharmacology, biochemistry, and behavior *68*, 291-299.

Stuppaeck, C.H., Deisenhammer, E.A., Kurz, M., Whitworth, A.B., and Hinterhuber, H. (1996). The irreversible gamma-aminobutyrate transaminase inhibitor vigabatrin in the treatment of the alcohol withdrawal syndrome. Alcohol and alcoholism *31*, 109-111.

Sturgess, J.E., George, T.P., Kennedy, J.L., Heinz, A., and Muller, D.J. (2011). Pharmacogenetics of alcohol, nicotine and drug addiction treatments. Addiction biology *16*, 357-376.

Suh, B.C., and Hille, B. (2007). Regulation of KCNQ channels by manipulation of phosphoinositides. The Journal of physiology *582*, 911-916.

Szumlinski, K.K., Ary, A.W., Lominac, K.D., Klugmann, M., and Kippin, T.E. (2008). Accumbens Homer2 overexpression facilitates alcohol-induced neuroplasticity in C57BL/6J mice. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology *33*, 1365-1378.

Szumlinski, K.K., Diab, M.E., Friedman, R., Henze, L.M., Lominac, K.D., and Bowers, M.S. (2007). Accumbens neurochemical adaptations produced by binge-like alcohol consumption. Psychopharmacology *190*, 415-431.

Tatulian, L., Delmas, P., Abogadie, F.C., and Brown, D.A. (2001). Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine. The Journal of neuroscience : the official journal of the Society for Neuroscience *21*, 5535-5545.

Ueda, Y., Doi, T., Tokumaru, J., and Willmore, L.J. (2003). Effect of zonisamide on molecular regulation of glutamate and GABA transporter proteins during epileptogenesis in rats with hippocampal seizures. Brain research Molecular brain research *116*, 1-6.

Uys, J.D., McGuier, N.S., Gass, J.T., Griffin, W.C., 3rd, Ball, B.E., and Mulholland, P.J. (2015). Chronic intermittent ethanol exposure and withdrawal leads to adaptations in nucleus accumbens core postsynaptic density proteome and dendritic spines. Addiction biology.

Vanderlinden, L.A., Saba, L.M., Kechris, K., Miles, M.F., Hoffman, P.L., and Tabakoff, B. (2013). Whole brain and brain regional coexpression network interactions associated with predisposition to alcohol consumption. PloS one *8*, e68878.

Vgontzas, A.N., Kales, A., and Bixler, E.O. (1995). Benzodiazepine side effects: role of pharmacokinetics and pharmacodynamics. Pharmacology *51*, 205-223.

Volkow, N.D., Fowler, J.S., and Wang, G.J. (2003). The addicted human brain: insights from imaging studies. The Journal of clinical investigation *111*, 1444-1451.

Waller, M.B., McBride, W.J., Lumeng, L., and Li, T.K. (1983). Initial sensitivity and acute tolerance to ethanol in the P and NP lines of rats. Pharmacology, biochemistry, and behavior *19*, 683-686.

Wang, H.S., Pan, Z., Shi, W., Brown, B.S., Wymore, R.S., Cohen, I.S., Dixon, J.E., and McKinnon, D. (1998). KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. Science *282*, 1890-1893.

Watanabe, H., Nagata, E., Kosakai, A., Nakamura, M., Yokoyama, M., Tanaka, K., and Sasai, H. (2000). Disruption of the epilepsy KCNQ2 gene results in neural hyperexcitability. Journal of neurochemistry *75*, 28-33.

Wayner, M.J., and Greenberg, I. (1972). Effects of hypothalamic stimulation, acclimation and periodic withdrawal on ethanol consumption. Physiology & behavior *9*, 737-740.

Wegelius, K., Halonen, T., and Korpi, E.R. (1993). Gamma-vinyl GABA decreases voluntary alcohol consumption in alcohol-preferring AA rats. Pharmacology & toxicology *73*, 150-152.

Weiss, F., Lorang, M.T., Bloom, F.E., and Koob, G.F. (1993). Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. The Journal of pharmacology and experimental therapeutics *267*, 250-258.

Weiss, F., Parsons, L.H., Schulteis, G., Hyytia, P., Lorang, M.T., Bloom, F.E., and Koob, G.F. (1996). Ethanol self-administration restores withdrawal-associated deficiencies in

accumbal dopamine and 5-hydroxytryptamine release in dependent rats. The Journal of neuroscience : the official journal of the Society for Neuroscience *16*, 3474-3485.

Wen, H., and Levitan, I.B. (2002). Calmodulin is an auxiliary subunit of KCNQ2/3 potassium channels. The Journal of neuroscience : the official journal of the Society for Neuroscience *22*, 7991-8001.

White, H.S. (1997). Clinical significance of animal seizure models and mechanism of action studies of potential antiepileptic drugs. Epilepsia *38 Suppl 1*, S9-17.

Wickenden, A.D., Yu, W., Zou, A., Jegla, T., and Wagoner, P.K. (2000). Retigabine, a novel anti-convulsant, enhances activation of KCNQ2/Q3 potassium channels. Molecular pharmacology *58*, 591-600.

Wise, R.A. (1973). Voluntary ethanol intake in rats following exposure to ethanol on various schedules. Psychopharmacologia *29*, 203-210.

Woody, G.E. (2003). Research findings on psychotherapy of addictive disorders. The American journal on addictions / American Academy of Psychiatrists in Alcoholism and Addictions *12 Suppl 2*, S19-26.

Xu, W., Wu, Y., Bi, Y., Tan, L., Gan, Y., and Wang, K. (2010). Activation of voltage-gated KCNQ/Kv7 channels by anticonvulsant retigabine attenuates mechanical allodynia of inflammatory temporomandibular joint in rats. Mol Pain *6*, 49.

Yim, H.J., and Gonzales, R.A. (2000). Ethanol-induced increases in dopamine extracellular concentration in rat nucleus accumbens are accounted for by increased release and not uptake inhibition. Alcohol *22*, 107-115.

Yin, H.H., and Knowlton, B.J. (2006). The role of the basal ganglia in habit formation. Nature reviews Neuroscience *7*, 464-476.

Yoshimoto, K., Komura, S., Hattori, H., Yamori, Y., Miura, A., Yoshida, T., Hioki, C., Kato, B., Fukuda, F., Tanaka, S*., et al.* (2003). Pharmacological studies of alcohol susceptibility and brain monoamine function in stroke-prone spontaneously hypertensive rats (SHRSP) and stroke-resistant spontaneously hypertensive rats (SHRSR). The Tohoku journal of experimental medicine *201*, 11-22.

Yu, H., Wu, M., Hopkins, C., Engers, J., Townsend, S., Lindsley, C., McManus, O.B., and Li, M. (2010). A small molecule activator of KCNQ2 and KCNQ4 channels. In Probe Reports from the NIH Molecular Libraries Program (Bethesda (MD)).

Yue, C., and Yaari, Y. (2006). Axo-somatic and apical dendritic Kv7/M channels differentially regulate the intrinsic excitability of adult rat CA1 pyramidal cells. Journal of neurophysiology *95*, 3480-3495.

Zalewska-Kaszubska, J., Bajer, B., Czarnecka, E., Dyr, W., and Gorska, D. (2011). Voluntary alcohol consumption and plasma beta-endorphin levels in alcohol preferring rats chronically treated with levetiracetam: a preliminary study. Physiology & behavior *102*, 538-541.

Zalewska-Kaszubska, J., Bajer, B., Gorska, D., Andrzejczak, D., Dyr, W., and Bienkowski, P. (2013). Effect of repeated treatment with topiramate on voluntary alcohol intake and beta-endorphin plasma level in Warsaw alcohol high-preferring rats. Psychopharmacology *225*, 275-281.

Zhang, J., and Shapiro, M.S. (2012). Activity-dependent transcriptional regulation of M-Type (Kv7) K(+) channels by AKAP79/150-mediated NFAT actions. Neuron *76*, 1133-1146.

Zhou, F.C., Anthony, B., Dunn, K.W., Lindquist, W.B., Xu, Z.C., and Deng, P. (2007). Chronic alcohol drinking alters neuronal dendritic spines in the brain reward center nucleus accumbens. Brain research *1134*, 148-161.

Zhou, F.C., Zhang, J.K., Lumeng, L., and Li, T.K. (1995). Mesolimbic dopamine system in alcohol-preferring rats. Alcohol *12*, 403-412.

Zhou, P., Zhang, Y., Xu, H., Chen, F., Chen, X., Li, X., Pi, X., Wang, L., Zhan, L., Nan, F.*, et al.* (2015). P-retigabine: an N-propargyled retigabine with improved brain distribution and enhanced antiepileptic activity. Molecular pharmacology *87*, 31-38.

Zhou, Q.Y., and Palmiter, R.D. (1995). Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell *83*, 1197-1209.