2014-03-18

# Role of α6 nAChRs in Ethanol Modulation of VTA Neurons

Samuel Injae Shin
*Brigham Young University - Provo*

# Role of α6 nAChRs in Ethanol Modulation of VTA Neurons

Samuel I. Shin

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science in Neuroscience

Scott C. Steffensen, Chair
Sterling N. Sudweeks
Jeff G. Edwards

Department of Physiology and Development Biology

Brigham Young University

March 2014

ABSTRACT

Role of α6 nAChRs in Ethanol Modulation of VTA Neurons

Samuel I. Shin
Department of Physiological and Developmental Biology, BYU
Master of Science in Neuroscience

The prevailing view is that enhancement of dopamine (DA) transmission in the mesolimbic system leads to the rewarding properties of alcohol and nicotine (NIC). The mesolimbic DA system consists of DA neurons in the midbrain ventral tegmental area (VTA) that innervate the nucleus accumbens (NAc). DA neurotransmission is regulated by inhibitory VTA GABA neurons, whose excitability is a net effect of glutamate (GLU) and GABA neurotransmission that are modulated by NIC cholinergic receptors (nAChRs) on afferent terminals. We have previously demonstrated that VTA GABA neurons are excited by low-dose ethanol but are inhibited by moderate to high-dose ethanol, and they adapt to chronic ethanol, evincing marked hyperexcitability during withdrawal.

The aim of this study was to evaluate the role of α6 nAChRs in ethanol effects on VTA GABA and DA neurons. In order to more conclusively demonstrate the role of α6 nAChRs in alcohol modulation in the VTA, we profiled the pharmacological interactions between ethanol and α6 nAChRs using recombinant α6 nAChRs in human epithelia (SH-EP1) cells and evaluated the effects of α6 nAChR antagonists on ethanol inhibition of GABA-mediated synaptic responses in dissociated GABA neurons of the VTA by recording mIPSCs; and assessed the effects of α6 nAChR antagonists on ethanol inhibition of VTA neurons, via eIPSCs on GABA neurons, sIPSCs on GABA neurons, and firing rate of DA neurons. We found that ethanol enhanced NIC currents in SH-EP1 cells via α6 nAChRs. Electrophysiology studies showed that superfusion of ethanol (5-30 mM) enhanced the frequency and amplitude of mIPSCs recorded in acutely dissociated VTA GABA neurons from GAD-GFP mice. Furthermore, the α6 nAChR antagonist α-conotoxin P1A (10 nM) prevented the ethanol-induced changes in mIPSC. In support, eIPSC experiments demonstrated that low doses of ethanol (1-5 mM) enhanced eIPSC peaks and decreased paired-pulse ratio, suggesting a presynaptic effect with ethanol. Alpha-conotoxin MII (α-CTX MII) blocked ethanol's effects on eIPSCs. This effect on VTA GABA neurons was also seen in sIPSCs, as ethanol decreased GABA firing rate. Similarly, the inhibition caused by ethanol was prevented by α-conotoxin P1A (10 nM). Additionally, CPP studies showed that α6 KO mice and WT mice treated with MEC, a non-competitive, non-α7 antagonist, did not show a preference for EtOH compartments that was found in WT mice.

Taken together, these studies indicate that ethanol is acting through α6 nAChRs on GABA terminals to enhance GABA release, suggesting a possible mechanism of action of alcohol and nicotine co-dependence. Through these studies conducted to understand the role of α6 nAChRs in ethanol modulation, we hope to further outline how alcohol alters brain activity so that we can ultimately facilitate the development of therapies/medications for the treatment of alcoholism.

Keywords: ethanol, nicotine, VTA, GABA, α6 nAChRs

ACKNOWLEDGMENTS

I would like to thank all those who helped me with this project from Dr. Steffensen's lab, especially Jennifer Mabey for her help in carrying out this project; Ashley Nelson and Taylor Woodward for performing patching experiments; and Nathan Schilaty and David Hedges for performing FSCV experiments. I would also like to thank our collaborators, Dr. Jie Wu and Devin Taylor from the Barrow Neurological Institute for dissociated studies and CPP experiments, and Dr. Michael McIntosh for providing us with conotoxin drugs for our studies. I would also like to thank my committee, Drs. Sterling Sudweeks and Jeff Edwards for their support, and Dr. Scott Steffensen for his mentorship throughout this project.

TABLE OF CONTENTS

LIST OF FIGURES

# INTRODUCTION

## *The Economic and Social Costs of Alcohol Abuse*

Alcoholism is a disorder pervasive throughout the world. Indeed, alcohol has been rated as one of the most harmful drugs in society (Nutt, King et al. 2010). Alcoholism not only has a negative impact on the user's life but is also substantially detrimental to the people associated with the user (Nutt, King et al. 2010). The consequences of such drug abuse include a decrease in job productivity, squandered earnings, rising healthcare costs, incarcerations, investigations, vehicular accidents, domestic and non-domestic violence, premature death, and breakdown of the family unit (SAMHSA 2006). Alcohol abuse has huge societal and economic implications: it afflicts at least 15.3 million individuals and the costs directly related to alcohol abuse add up to approximately $185 billion annually in the US (Gordis 2000). Despite the negative impacts of alcoholism, very little is understood about it neuromechanistically. Through our endeavors, we hope to be able to understand the mechanism of how alcohol hijacks the brain in order to help alleviate its symptoms and adverse consequences. Through improving our understanding of how alcohol leads to addiction, dependence could be reversed through therapies and/or medication in order to save lives.

## *The Mesolimbic Dopamine System*

Many addictive substances are known to work through the mesolimbic dopaminergic system (Wise and Bozarth 1987). In this system, it is believed that dopamine (DA) neurons originating in the ventral tegmental area (VTA) of the midbrain project to the nucleus accumbens (NAc) and the prefrontal cortex (PFC) (Wise 1996). The release of DA in the NAc is associated with feelings of pleasure that leads to the physiological need that drives addiction (Wise 2008). DA release in the mesolimbic DA system has been shown to have a scalar index of reward (Wise

2008), with greater DA release being directly associated with higher feelings of pleasure. While the release of DA in the NAc leads to symptoms of addiction, it has been suggested that the changes in DA are mediated by VTA γ-amino butyric acid (GABA) neurons (Nugent and Kauer 2008). VTA GABA neurons provide an inhibitory input to DA neurons to regulate their activity level (Tepper, Paladini et al. 1998). Optogenetic studies have shown that selective activation of VTA GABA neurons influences conditioned placed aversion (Tan, Yvon et al. 2012) and can manipulate reward consumption (van Zessen, Phillips et al. 2012). Furthermore, evidence for GABA's role in reward has been demonstrated by multiple labs, including our own, as substances such as nicotine (NIC) and alcohol can alter GABA activity (Yin and French 2000, Erhardt, Schwieler et al. 2002, Mansvelder, Keath et al. 2002) to influence DA activity (Brodie and Appel 1998). This suggests that GABA is a major regulator of DA neurotransmission and therefore plays a critical role in the rewarding properties of drugs such as ethanol (EtOH) and NIC. Figure 1 portrays the synaptic hodology of VTA GABA neurons, including probable receptors involved in EtOH and NIC mechanisms.

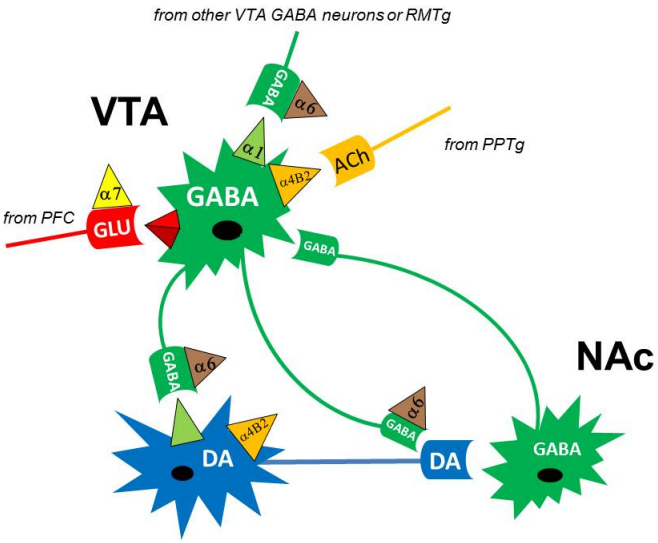


Figure 1: Theoretical Framework for Proposed Studies. Dopamine neurons in the VTA project to the NAc via the medial forebrain bundle (MFB). Interestingly, although considerable attention has been placed on the role of the DA projection from the VTA to the NAc in drug reward and abuse, VTA GABA neurons also project to the NAc (Carr and Sesack 2000). Moreover, a subset of GABAergic NAc neurons sends a reciprocal projection back to the VTA (Tripathi, Prensa et al. 2010). This reciprocal loop between GABA neurons in the VTA and GABA neurons in the NAc is believed to provide inhibitory feedback to regulate DA neuron activity (Rahman and McBride 2000) via VTA GABA neurons (Xia, Driscoll et al. 2011). They may also be inhibited by local circuit GABA neurons and GABAergic input from the RMTg. Cholinergic (ACh) input from the PPTg excites GABA and DA neurons via α4β2 nAChR.

*Ethanol-Nicotine Interactions*

Alcohol and tobacco are some of the most prevalent drugs in society and often are co-abused. Because of this observation, many have suspected that alcohol and NIC (the addictive component of tobacco) may have some form of synergistic interaction. This hypothesis is promoted, as studies have shown that the amount of NIC intake through tobacco is positively correlated with the amount of alcohol consumed and the degree of alcohol dependence (John, Meyer et al. 2003). Additionally, studies measuring NIC and EtOH at the level of discriminative stimulus effects found that smoking promotes greater alcohol consumption (Ford, McCracken et al. 2012). Systemic co-administration of low doses of alcohol and NIC in the VTA also resulted in an additive effect on DA release (Tizabi, Copeland et al. 2002). These findings are further substantiated as alcohol consumption increased pleasure and desire for smoking (Harrison, Hinson et al. 2009). This cumulated evidence suggests that NIC and EtOH could have a shared mechanism that explains how one drug may influence the other and facilitate addiction. The data shows behavioral and cellular support for the interactive properties between alcohol and NIC and advocates the need for further research to understand the possibly shared circuitry.

While alcohol's effects are often misunderstood and can be controversial, NIC's method of action has been outlined more clearly and definitively. NIC addiction has been known to be mediated by nicotinic acetylcholine receptor (nAChR) desensitization of neurons from the VTA projecting to limbic structures (Mansvelder and McGehee 2000, Mansvelder, Keath et al. 2002). Neurons within the VTA express a wide variety of nAChRs (Wooltorton, Pidoplichko et al. 2003), and NIC can activate both DA and GABA neurons (Yin and French 2000, Mansvelder, Keath et al. 2002), with the majority of endogenous cholinergic inputs contacting GABA in the VTA (Garzon, Vaughan et al. 1999, Fiorillo and Williams 2000).

The majority of the GABA neurons in the VTA express α4 and β2 nAChR subunits, which can be blocked by the non-competitive, non-α7 antagonist mecamylamine (MEC) or by the competitive antagonist dihydro-β-erythroidine hydrobromide (DHβE) (Mansvelder, Keath et al. 2002). In addition to the transient actions of NIC on DA and GABA neurons via α4β2 nAChRs, considerable evidence indicates that the actions of NIC within the VTA may be mediated by glutamatergic (GLUergic) transmission. Blockade of GLU NMDA receptors (NMDARs) and α7-nAChRs in the VTA reduces the spike in DA release that is normally induced by NIC (Schilstrom, Svensson et al. 1998, Schilstrom, Fagerquist et al. 2000), and systemically- or locally-injected NMDA antagonists into the VTA can block the rewarding effects of NIC (Papp, Gruca et al. 2002, Laviolette and van der Kooy 2003). The VTA receives GLUergic input from the PFC, providing the major excitatory control of the VTA, influencing DA release in the NAc (Johnson, Seutin et al. 1992, Sesack and Pickel 1992, Suaud-Chagny, Chergui et al. 1992, Kalivas, Churchill et al. 1993, Taber and Fibiger 1995).

NIC receptors on GLUergic terminals in the VTA are located presynaptically. Specifically, these receptors are homomeric α7-nAChRs (Mansvelder and McGehee 2000), which enhance the release of GLU on DA and GABA neurons (McGehee, Heath et al. 1995, Girod, Barazangi et al. 2000). They are sensitive to the α7-nAChR antagonist methylylcaconitine (MLA). It has been proposed that the general mechanism of NIC addiction is that all nAChRs become desensitized by NIC (Mansvelder, Keath et al. 2002). NIC is present in the brain of smokers about 10-20 sec after absorption (Oldendorf 1974, Benowitz 1988), reaching blood concentrations of NIC in the range of 250 and 500 nM during and after cigarette smoking (Henningfield, Stapleton et al. 1993). We have recently shown that NIC activates GABA neurons in the VTA via α7 nAChRs on GLU terminals (Taylor, Burman et al. 2013) , probably arising

from the PFC. However, α7-nAChRs take a higher dose in order for desensitization to occur and recover in the order minutes (Fenster, Rains et al. 1997), as opposed to hours for all other nAChRs. It has been proposed that a shift occurs in the activity level of DA neurons relative to GABA cells in the VTA after prolonged exposure to NIC, and this shift would favor increased activity of the mesolimbic DA pathway and DA release in the NAc (Mansvelder, Keath et al. 2002).

In addition to α4β2 and homomeric α7-nAChRs, there is a considerable concentration of heteromeric α6-containing nAChRs (α6*-nAChRs; * denotes α6 subunits combined with other nAChR subunits) in the VTA. Alpha6*-nAChR subunits are functional in recombinant systems when paired with β subunits or hybrids of β subunits. In fact, nAChR α6 subunit mRNA level is 16-fold higher than other subunits in the VTA (Yang, Hu et al. 2009). They have been implicated in DA transmission and NIC dependence (Drenan, Grady et al. 2008, Exley, Clements et al. 2008, Jackson, McIntosh et al. 2009, Drenan, Grady et al. 2010, Gotti, Guiducci et al. 2010).

EtOH is unlikely to have a direct effect on nAChRs. Instead, these changes caused by EtOH could be mediated by plasticity involving other mechanisms. For example, plasticity in GABAergic synapses of the VTA has been discovered with several addictive substances (Nugent and Kauer 2008). To induce LTD and/or LTP, EtOH could activate GABA receptors directly or act on the GLUergic synapses, which promote endocannabinoid-mediated LTD of GABA neurons, as has been observed with other substances (Nugent and Kauer 2008). Additionally, other processes could involve alcohol increasing ACh release in the VTA to potentiate nAChRs (Larsson, Edstrom et al. 2005). These potential pathways exhibit a likelihood of alcohol acting on nAChRs that encourages further examination to explain how alcohol leads to addiction and its interaction with NIC.

## RATIONALE AND HYPOTHESES

As discussed, GABAergic synaptic transmission is still regarded as one of the main factors underlying the intoxicating, rewarding, and withdrawal-related effects of EtOH (Bonci and Williams 1996, Bonci and Williams 1997). The emerging view is that DA neurons in the VTA are under strong, tonic inhibition by GABA innervations. Interestingly, it has been demonstrated by others that the inhibitory drive to GABA neurons exceeds that of DA neurons (Tan, Brown et al. 2010). Consistent with what has been reported, we have found that VTA GABA neurons in horizontal slice preparation have higher spontaneous inhibitory postsynaptic current (IPSC) rates than DA neurons (GABA neurons: 10.4 ± 1.3 Hz; DA neurons: 5.4 ± 0.6 Hz; n=24,16; (Allison, Wilcox et al. 2011)). The inhibitory drive to VTA GABA neurons could be mediated by other local circuit GABA neurons or by GABA inputs from the ventral pallidum, NAc, or RMTg. Spontaneous IPSC (sIPSC) rate in VTA GABA neurons in coronal slices (5.2 ± 0.3 Hz; n=15) is approximately 50% that of horizontal slices. Horizontal slices contain the RMTg, suggesting that the RMTg might be providing this strong inhibitory input to VTA GABA neurons, as GABA input from the NAc is unlikely to have any influence in horizontal slices. Inhibition of VTA GABA neurons is mediated by α1 subunit-containing GABA(A)Rs that are selectively expressed in GABA neurons, but not in DA neurons, in the VTA (Okada, Matsushita et al. 2004, Tan, Brown et al. 2010). Most interestingly, GABA neurons, recorded both *in vivo* and *ex vivo*, have high spike activity even in the presence of pronounced afferent GABAergic drive.

The effects of EtOH on GABA(A)R-mediated inhibition on VTA DA neurons are controversial. For example, one lab has reported that EtOH (20-50 mM) enhances sIPSC frequency on DA neurons (Theile, Morikawa et al. 2008), while another has reported that EtOH

(10-40 mM) reduces sIPSC frequency on DA neurons (Xiao and Ye 2008). We are possibly the only lab that has looked at the effects of EtOH on the pronounced GABA input to unambiguously characterized VTA GABA neurons. Although we have studied scores of cells at 5-60 mM EtOH, we have not seen consistent effects of EtOH on VTA GABA neuron sIPSCs in the slice preparation in the presence of GLU blockers APV and CNQX. However, we aim to show consistent and reproducible preliminary evidence that low doses of EtOH enhance miniIPSC (mIPSCs) in dissociated VTA GABA neurons, which is blocked by α6*-nAChR antagonists.

While α6*-nAChRs have relatively limited expression in the brain, they are markedly expressed in the VTA, as discussed earlier. They are also expressed in the striatum and their activation modulates DA release (Grady, Salminen et al. 2007). Blocking α6*-nAChRs has been shown to modulate EtOH-induced enhancement of DA release as measured by microdialysis (Larsson, Jerlhag et al. 2004). Despite the fact that mice lacking the α6 subunit did not differ in EtOH consumption or EtOH-induced ataxia, they did exhibit more EtOH-induced sedation (Kamens, Hoft et al. 2012). Thus, modulation of GABA inhibition of GABA neurons by α6*-nAChRs may be an important regulator of GABA neuron excitability, which ultimately will govern DA neurotransmission.

Thus, our goal is to define the role of α6*-nAChRs in acute EtOH actions. Our objectives were three-fold: 1) To define the role of α6*-nAChRs in acute EtOH actions on VTA neurons; 2) To outline the role of α6*-nAChRs in acute EtOH actions on DA activity and release; and 3) To identify the role of α6*-nAChRs in mediating EtOH consumption and reward. Through using experimental procedures to address these aims, we expected to help implicate the role α6*-nAChRs in mediating the effects of alcohol and NIC and co-dependence on these substances.

# METHODS

*Animal Subjects*

Black wild-type (WT) male C57BL/6 mice, α6 KO mice, and glutamate-decarboxylase-67 (GAD-67)-green fluorescent protein knock-in (CD-1) mice (Tamamaki, Yanagawa et al. 2003) were bred and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. For each methodology to be employed, animals were treated in strict accordance with the Brigham Young University Animal Research Committee (IACUC) guidelines, which incorporate and exceed current NIH guidelines. The BYU IACUC has reviewed and approved the procedures detailed herein. Once weaned at PND 21, all mice were housed in maximum groups of four and given ad libitum access to solid food and water and placed on a reverse light/dark cycle with lights ON from 8 p.m. to 8 a.m.

*Preparation of Brain Slices*

All brain slice preparations were performed in P18-45 day old C57BL/6 and GAD-GFP knock-in mice. Brains were extracted via under isoflurane (5%) anesthesia and by intraperitoneal (IP) injection with ketamine (60 mg/kg). Upon extraction, the brain was glued onto a cutting stage. The brain was sectioned in ice-cold cutting solution (in mM: 220 Sucrose, 3 KCl, 1.25 $NaH_2PO_4$, 25 $NaH_2CO_3$, 12 $MgSO_4$, 10 Glucose, 0.2 $CaCl_2$, and 0.4 Ketamine) and was perfused with 95% $O_2$ / 5% $CO_2$. Horizontal slices (targeting the VTA were 210 μM thick; targeting the NAc were 400 μM thick) were placed in an incubation chamber containing artificial cerebral spinal fluid (ACSF; in mM: 124 NaCl, 3 KCl, 1.25 $NaH_2PO_4$, 26 $NaHCO_3$, 12 glucose, 1.5 $MgSO_4$, 2 $CaCl_2$) perfused with 95% $O_2$ / 5% $CO_2$ for at least 30 min. After 30 min, brain slices were placed in a recording tissue chamber with ACSF continuously flowing at physiological temperature (36 °C).

*Characterization of Neuron Types*

GABA neurons were studied in GAD-GFP knock-in mice and C57BL/6 mice. In GAD-GFP knock-in mice, VTA GABA neurons were identified by a characteristic glow under fluorescence illumination. In C57BL/6 mice, VTA GABA neurons were characterized using a GABA spike command waveform [spikes at 200 Hz for 500msec; (Steffensen, Taylor et al. 2008)] as GABA neurons will follow the command waveform. Neurons that did not fluoresce and/or exhibit a non-cation specific inward rectifying current ($I_h$) with low input resistance, not following the waveform are assumed to be DA neurons (Johnson and North 1992, Allison, Ohran et al. 2006, Margolis, Lock et al. 2006, Allison, Wilcox et al. 2011, Steffensen, Bradley et al. 2011).

*Whole-Cell Recordings In Vitro*

Electrodes were pulled from borosilicate glass capillary tubes (1.5 mm o.d.; A-M Systems, Sequim, WA) and filled with a KCl pipette solutions [in mM: 128 KCl, 20 NaCl, 0.3 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, 1 EGTA, 2 Mg-ATP, and 0.25 Na-GTP (pH 7.3)] for eIPSC studies. Pipettes having tip resistances of 2.5 – 5 MΩ, and series resistances typically ranging from 7 to 15 MΩ were used. Voltage clamp recordings were filtered at 2 kHz while current-drive spikes were filtered at 10 kHz with an Axon Instruments Multiclamp 700B amplifier and digitized at 5 to 20 kHz respectively using an Axon 1440A digitizer. Axon Instruments pClamp ver10, Mini Analysis (Synaptsoft: Decatur, GA), and Igor Pro (Wavemetrics: Oswego, OR) software packages were utilized for data collection and analysis. eIPSCs were recorded in the presence of 50 μM APV and 30 μM CNQX or 3 mM kynurenic acid to block NMDA and AMPA mediated synaptic currents. Using a paired-pulse stimulus, the stimulation was adjusted

to a half-max level in order to allow measurements of changes that increase or decrease eIPSC levels.

*Cell-Attached, Voltage-Clamp Recording of Spike Activity in Brain Slices*

Electrodes used for cell-attached, voltage-clamp spike studies of DA neurons were pulled from borosilicate glass capillaries (1.5 mm o.d.; A-M Systems, Sequim, WA) and then filled with 150 mM NaCl (2.5-5 MΩ). Positive pressure was applied to the electrode when approaching the neuron under visual inspection. The electrode was pushed against the cell membrane and suction was applied to create a seal (10 MΩ – 1 GΩ) between the cell membrane and the recording pipette. Spontaneous spike activity was then recorded in voltage-clamp mode with an Axon Instruments Multiclamp 700B amplifier and sampled at 10 kHz using an Axon 1440A digitizer, and collected and analyzed using pClamp10 software. Neurons were voltage-clamped at 0 mV throughout the experiment. A stable baseline recording of firing activity was obtained for 5-10 min before adding any substances.

*Carbon Fiber Electrodes, Calibration, and Fast Scan Cyclic Voltammetry*

For *ex vivo* voltammetry recordings a 7.0 μm diameter carbon fiber was inserted into borosilicate capillary tubing (1.2 mm o.d.; A-M Systems, Sequim, WA) under negative pressure and subsequently pulled on a vertical pipette puller (Narishige, East Meadow, NY). The carbon fiber electrode (CFE) was then cut under microscopic control with 150 μm of bare fiber protruding from the end of the glass micropipette. We then filled the CFE with 3M KCl. The CFEs were calibrated with a known concentration of DA. With the CFE immersed in the solution of ACSF, we perfused a known concentration of DA past the electrode and observed the maximum nA signal produced by DA. All of these calibrations were averaged and gave us a

calibration factor that allowed us to convert a nA signal of DA to μM concentration of DA. Previous studies have revealed that DA current is linearly proportional to DA concentration.

For *ex vivo* voltammetry recordings, electrodes were positioned ~75 μm below the surface of the slice in the NAc core. Dopamine release was evoked every 2 min by a 1 msec, one to ten-pulse stimulation (biphasic, 350 μA) from a bipolar stimulating electrode (Plastics One, Roanoke, VA) placed 100-200 μm from the CFE. The electrode potential was linearly scanned as a triangular waveform from -0.4 to 1.2 V and back to -0.4 V vs Ag/AgCl using a scan rate of 400 V/s. Cyclic voltammograms were recorded at the carbon fiber electrode every 100 msec (i.e. 10 Hz) by means of a voltage clamp amplifier (ChemClamp, Dagan Corporation, Minneapolis, MN). Voltammetric recordings were performed and analyzed using LabVIEW (National Instruments, Austin, TX)-based customized software (Demon Voltammetry, (Yorgason, Espana et al. 2011)). Stimulations were performed periodically every 2 min. Dopamine levels were monitored for a stabilization period typically lasting 1 hr. Once the stimulated DA response was stable for five successive collections, and did not vary by more than 5%, baseline measurements were taken.

*Conditioned Place Preference*

The conditioned place preference (CPP) apparatus (Med Associates, St. Albans, VT) consisted of two adjacent conditioning compartments (20 x 16 x 21 cm) separated by a manual guillotine-type door. One of the compartments was equipped with vertical striped acrylic walls and a steel mesh floor; the other was equipped with plain acrylic walls and a wire rod floor. Infrared photobeams monitored the animal's position in the apparatus and provided a measure of motor activity. First animals were habituated to the testing apparatus during a single 20 min session with free access to both conditioning compartments. Animals were then subjected to two

20 min pre-conditioning tests in order to determine any initial preference for one of the conditioning compartments. Each animal was then assigned to EtOH (2 g/kg IP) in the initially non-preferred compartment, and saline in the initially preferred compartment. Next, the animals underwent 20 min conditioning sessions twice daily. Saline conditioning sessions were conducted in the morning and EtOH conditioning sessions were conducted in the afternoon. Following 4 sequential conditioning days, animals were tested for place preference by allowing free access to both conditioning compartments for 20 min.

*Drug Preparation and Administration*

Methylylcaconitine (10 nM) was prepared fresh in distilled water. CNQX (30 μM; Abcam) and APV (50 μM; Abcam) were dissolved in distilled water and frozen until used. Kynurenic acid (3 mM; Sigma-Aldrich) was made fresh in distilled water and sonicated to get into solution. α-conotoxin MII (100 nM; provided by Michael McIntosh from the University of Utah) was prepared fresh by dissolving in distilled water. Atropine (10 μM; Sigma-Aldrich) and eticlopride (100 nM; Sigma-Aldrich) were dissolved in distilled water, while CGP55845 (10 μM;Abcam) required sonication to completely dissolve.

While measuring eIPSCs, kynurenic acid or the combination of APV and CNQX was added to the ACSF perfused to the brain slice to block currents from AMPA and NMDA. Afterwards, an EtOH dose response (1 mM, 5 mM, 10 mM, 30 mM, and 50 mM; approximately 5 min at each dose) was administered to the ACSF to observe its effect on eIPSCs. In some experiments, only one dose of EtOH per slice was administered. In other experiments, CGP55845, atropine, and eticlopride was added to kynurenic acid/APV+CNQX to rule out GABA(B)R, D2R, and cholinergic muscarinic effects. After ensuring that EtOH had altered eIPSC peaks, the α6 nAChR antagonist, α-contoxin MII, was added before the EtOH dose

response in order to see if it blocked EtOH's effects. In FSCV studies measuring DA release, methylylcaconitine (MLA), a α7-nAChR antagonist, was perfused to the brain slice preceding exposure to EtOH.

CPP studies were administered a dose of mecamylamine (2 mg/kg), a non-α7 nAChR antagonist. MEC was used *in vivo* to block nAChR, including α6*, as conotoxins (P1A and MII) are impermeable to the blood brain barrier.

*Statistical Analyses*

All results were presented as raw mean values and percent control ± SEM. Results between groups were compared using a two-tailed unpaired *t* test or ANOVA. Experiments relying on variance in time or current were analyzed using mixed models ANOVA with post hoc t-test at individual points. Statistical significance required ≥ 95% level of confidence ($P \leq 0.05$). CPP experiments used a post-hoc Bonferronni Multiple Comparison Test to compare the three groups. Analysis software included Microsoft Excel and Igor Pro (Wavemetrics, Oswego, OR). Significance levels were indicated on graphs with asterisks *,**,*** and correspond to significance levels $P<0.05$, 0.01 and 0.001, respectively. Figures were constructed with Igor Pro software.

# RESULTS

## *The Role of α6*-nAChRs in Acute Ethanol Actions on VTA GABA Neurons*

### Interactions Between Acute EtOH and α6*-nAChRs: Recombinant Studies

Our first goal was to profile pharmacological features of interaction between EtOH and α6*-nAChRs. To accomplish this, we evaluated EtOH effects on the function of heterologously expressed α6*-nAChR-mediated currents in recombinant α6*-nAChRs expressed in an human epithelia (SH-EP1) cell line. Although not as physiologically relevant as native systems, recombinant expression systems have several advantages including: 1) Better control over which subunits are being expressed (e.g., showing the difference between different combinations of subunits); 2) Better access to the cell in order to perform more accurate dose-response curves (e.g., less concern about the concentration of the drug becoming diluted as it diffuses into the tissue, etc.); 3) Better kinetic profiling of the responses (i.e., rise times, decay times, total current, peak amplitudes, desensitization rates); 4) Less artifact than with slices; 5) Simplified pharmacological analysis of the currents; and 6) Facilitation of the study of human nAChR subunits in an expression system. Furthermore, this expression system is extremely valuable as there are currently no available α6*-nAChRs agonists or direct α6*-nAChR-mediated effects in native neurons. Hence, incorporating this expression system was essential for a full understanding of EtOH effects on NIC currents.

Others have shown functional α6*-nAChRs using combinations of α6, α4, and β3 subunits. Therefore, we hypothesized that a combination would yield functional receptors to study EtOH effects on NIC currents. However, NIC currents are small, and interpretations regarding EtOH effects on α6 are put in question because of the addition of α4. Consequently,

we established a functional cell line (SH-EP1) that stably expressed functional α6*-nAChRs including human α6, β4 and mutated β3 ($\beta3^{V9'V}$).

We evaluated the subunit profile necessary to optimize NIC currents and constructed dose-response curves for NIC alone and after EtOH superfusion. NIC was able to induce a typical dose response in NIC currents, indicating that the α6*-nAChRs in SH-EP1 cells were functional (Fig. 2A**)** and sensitive to the highly selective and potent α6*-nAChR antagonist, α-conotoxin P1A (α-CTX P1A) at a concentration of 1 nM (Fig. 2B). Through confirming that the α6*-nAChRs were appropriately responsive, we were able to test the effects of EtOH on these channels. We found that EtOH, at very low concentrations (1 mM) enhanced NIC currents in SH-EPI cells (Fig. 2C, D), suggesting a potent and direct effect on α6*-nAChRs.

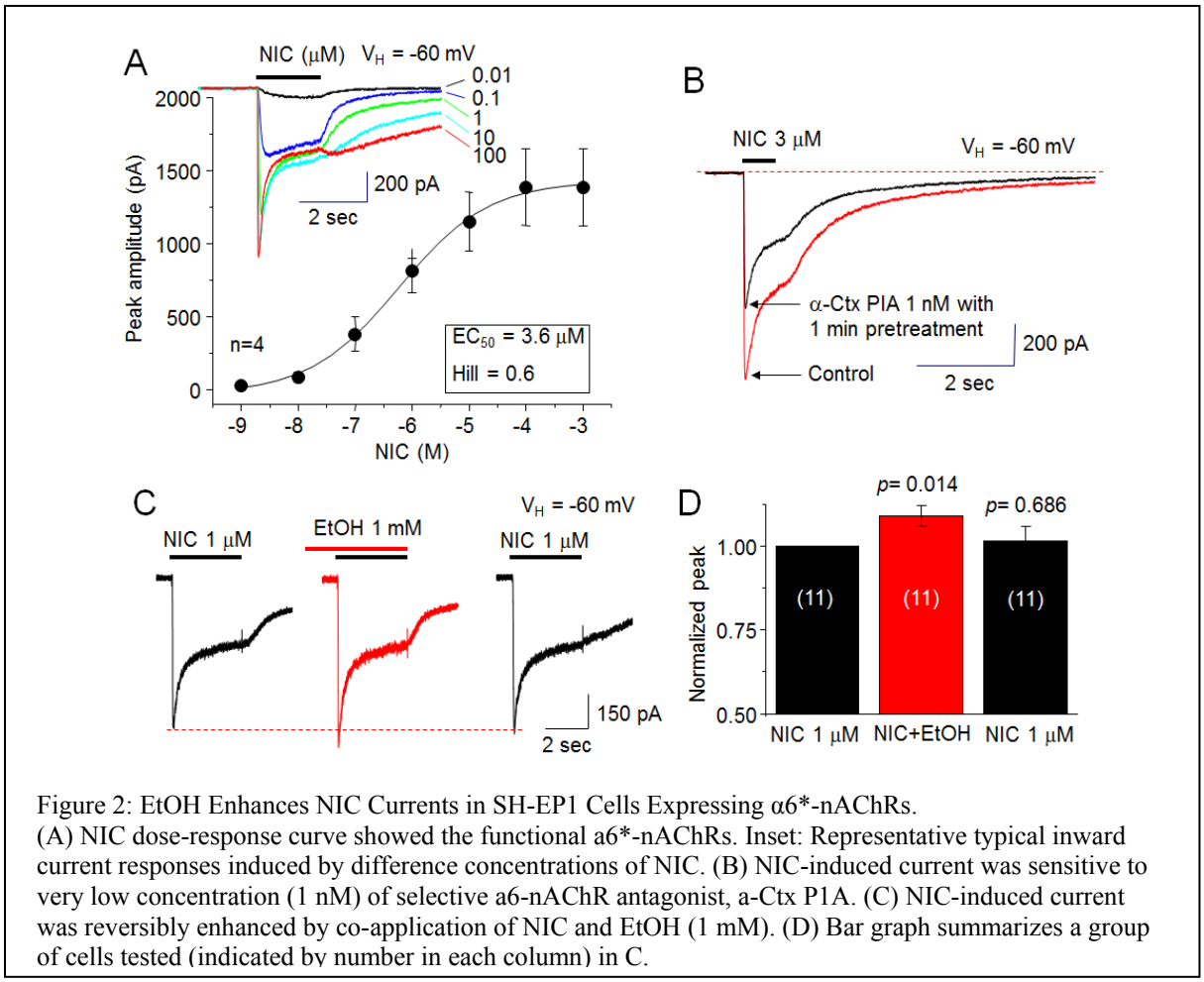


Figure 2: EtOH Enhances NIC Currents in SH-EP1 Cells Expressing α6*-nAChRs.
(A) NIC dose-response curve showed the functional a6*-nAChRs. Inset: Representative typical inward current responses induced by difference concentrations of NIC. (B) NIC-induced current was sensitive to very low concentration (1 nM) of selective a6-nAChR antagonist, a-Ctx P1A. (C) NIC-induced current was reversibly enhanced by co-application of NIC and EtOH (1 mM). (D) Bar graph summarizes a group of cells tested (indicated by number in each column) in C.

Acute EtOH's Effect on VTA GABA Neurons via α6*-nAChRs: Dissociated Studies

To increase our understanding of α6*-nAChRs involvement in alcohol addiction, we proposed to evaluate the effects of α6*-nAChR antagonists on EtOH effects on GABA-mediated synaptic responses in dissociated GABA neurons of the VTA. We set to determine this through studying GABA-mediated mIPSCs in dissociated, visually-identified VTA GABA neurons from GAD-GFP mice with fluorescent optics. Recently, it has been shown that dissociated DA neurons in the VTA have inhibitory synaptic boutons that contain α6*-nAChRs, and that ACh activation of these receptors leads to enhancement of inhibitory transmission (i.e., mIPSCs) that are GABA(A)R mediated (Yang, Buhlman et al.). The effects of nAChR antagonists on EtOH effects on mIPSCs, but not mEPSCs, can be studied in dissociated neurons.

Although dissociated neuron recordings suffer from the lack of physiological relevancy characteristic of intact circuits in the slice preparation or *in vivo*, its value in our proposed studies was three-fold: 1) Using the U-tube method, EtOH and NIC pharmacology can be accurately and easily studied; 2) While there are viable inhibitory somatic synaptic boutons on dissociated neurons, there are little or no dendritic excitatory synaptic boutons; and 3) Postsynaptic effects can be easily assessed with pharmacological blockers. For example, CGP55845 and atropine was included in the superfusate to assure GABA(A)R-mediated responses. To further confirm this we also superfused the GABA(A)R antagonist bicuculline (10 mM) at the end of some experiments to confirm that the mIPSCs were mediated by GABA.

In our studies, similar to what has been reported previously in dissociated DA neurons (Yang, Buhlman et al. 2011), VTA GABA neurons evinced mIPSCs, which were enhanced by NIC (Fig. 3A). This enhancement could then be prevented by α-conotoxins (Fig. 3B).

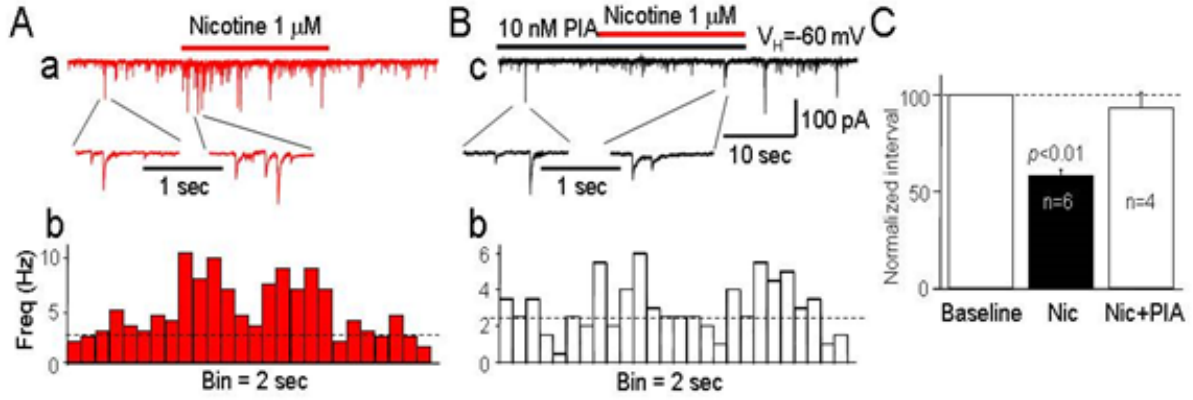


Figure 3: NIC Enhances mIPSCs in VTA GABA Neurons Through α6*-nAChRs.
Bath-application of nicotine (1 mM) significantly increased mIPSC frequency (A), and this effect was prevented by pretreatment with the α6*-nAChR antagonist, α-conotoxin P1A (10 nM, B). (C) Bar graph summarizes the pool data and suggests that nicotine activates α6*-nAChRs on GABAergic presynaptic boutons and increases presynaptic GABA release.

Expounding upon our results from our recombinant system experiments, we found that EtOH enhanced both the frequency and amplitude of GABA mIPSCs at low doses, similar to NIC effects (Fig. 4). This is a critical finding, as it supports the literature demonstrating that EtOH enhances GABA release at physiologically relevant concentrations.

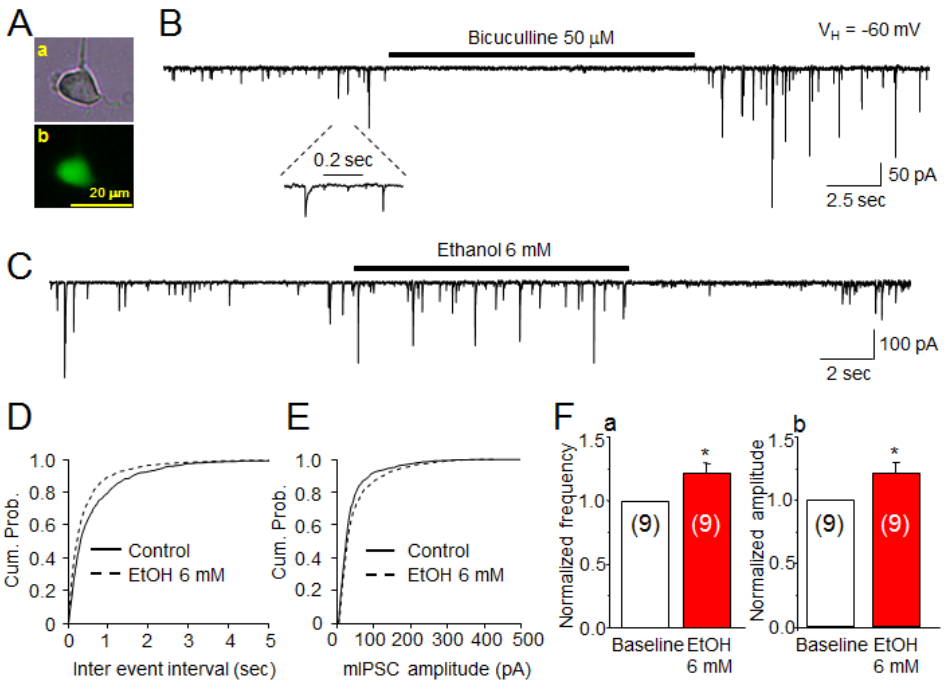


Figure 4: EtOH Enhances mIPSC Frequency and Amplitude in VTA GABA Neurons.
(A) Images show an acutely-dissociated VTA neuron from a GAD-GFP mouse using phase-contrast (Aa) and GPF-filtered (Ab) modes of microscope. (B) A typical trace shows the spontaneous mIPSCs (in the presence of 0.5 µM TTX) since they are sensitive to a selective GABA(A)R blocker, bicuculline. (C) Bath exposure of 6 mM EtOH increased the frequency of spontaneous mIPSCs. Further analysis shows that 6 mM EtOH increases frequency (D) and amplitude (E) of the spontaneous mIPSCs. (F) Bar graphs summarize the effects of EtOH.

Of particular relevance to NIC and EtOH interactions, we found that the α6*-nAChR antagonist α-ctx P1A also blocked EtOH's enhancement of mIPSCs amplitude and frequency (Fig. 5A, B), suggesting that α6*-nAChRs on GABA terminals to VTA GABA neurons are an important link for EtOH and NIC interactions. Moreover, the enhancement of mIPSCs by EtOH in WT mice was not observed in α6 KO mice (Fig. 5C, D), providing further evidence that EtOH acts via presynaptic α6*-nAChRs to increase GABA release. These findings suggest that EtOH is enhancing GABA release at terminals and GABA responses postsynaptically.

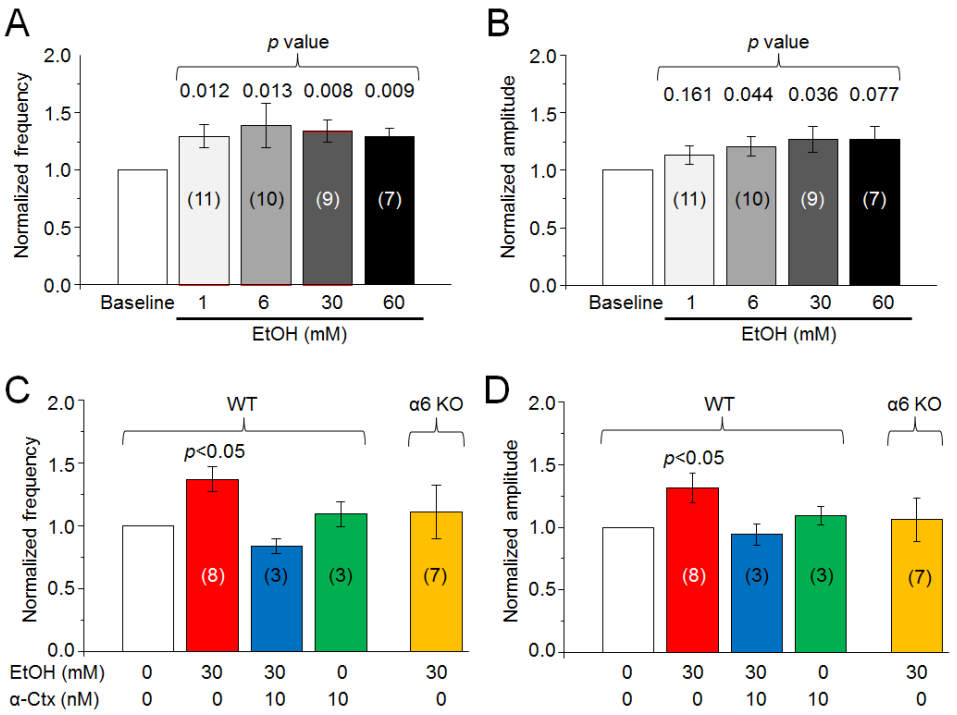


Figure 5: EtOH Enhancement of GABA mIPSCs to VTA GABA Neurons via α6*-nAChRs. Results here are shown at the 30 mM EtOH level. (A) EtOH dose response on mIPSC frequency. (B) EtOH dose response on mIPSC amplitude. (C) The α-conotoxin P1A (10 nM) did not affect mIPSC frequency, but following a 2 min treatment, it prevented EtOH (30 mM)-induced increase in mIPSC frequency in WT mice but not in α6 KO mice. (D) Similarly, P1A did not affect mIPSC frequency, but following a 2 min treatment, it prevented EtOH (30 mM)-induced increase in mIPSC frequency in WT mice but not in α6 KO mice Vertical bars represent means ± SEM.

In further support of a postsynaptic effect, we performed GABA current experiments to study EtOH's influence on GABA. In dissociated VTA GABA neurons from GAD-GFP mice we found that EtOH augmented GABA currents in VTA GABA neurons (Fig. 6).

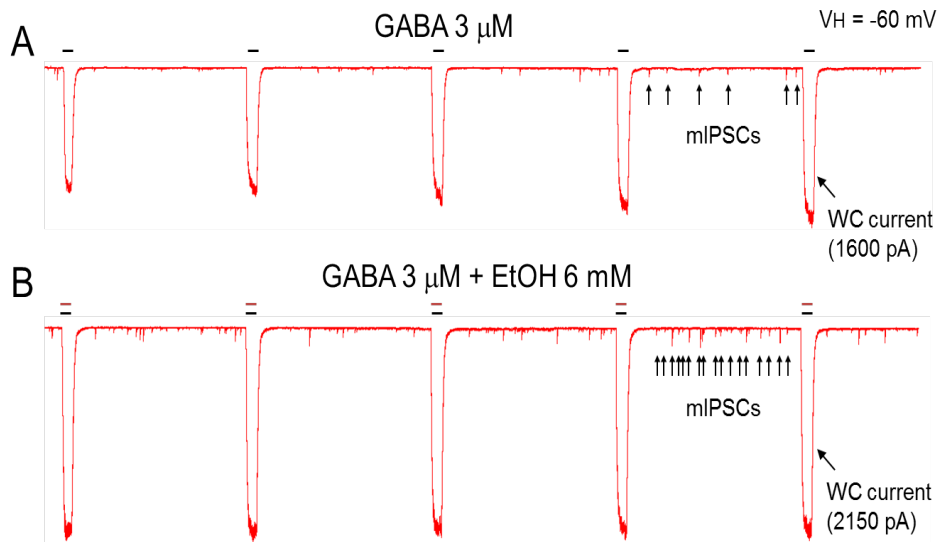


Figure 6: Effects of EtOH on Synaptic GABA Responses in VTA GABA Neurons.
Simultaneous recordings of mIPSCs and GABA-induced whole-cell (WC) currents were made in VTA GABA neurons (A). 6 mM EtOH and 3 mM GABA were repetitively co-applied (1 sec exposure) to a dissociated VTA GABA neuron, which showed an enhancement in mIPSC frequency (arrows) and amplitude and WC current amplitude (B).

Next, we wanted to confirm that increased inhibitory input to GABA neurons would result in an inhibition of their firing rate. To accomplish this we decided to measure the effects of EtOH on firing rate in dissociated VTA GABA neurons. Using cell-attached, voltage-gated patch clamp studies, we were able to measure the firing rate of these GABA neurons. In our results, we found that EtOH (30 mM) caused a significant decrease in GABA firing rate (Fig. 7). Furthermore, this

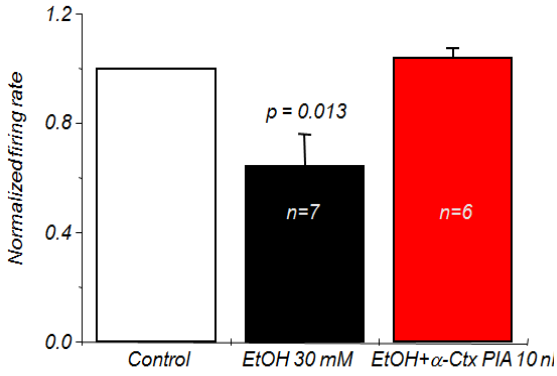


Figure 7: α-Ctx PIA Blocks the EtOH Effect on VTA GABA Neurons.
In dissociated VTA GABA neurons from a GAD-GFP mouse, EtOH (30 mM) significantly decreased firing rate. This decrease in firing rate was blocked by the α6-nAChR antagonist, α-Ctx PIA (10 nM).

decrease in firing rate was prevented by the α6 nAChR antagonist, α-CTX P1A (10 nM; Fig. 7). These results provide further support to our hypothesis that EtOH is enhancing GABA inhibition to VTA GABA neurons EtOH and the role of α6*-nAChR in this process.

Acute EtOH's Effect on VTA GABA Neurons via α6*-nAChRs: Slice Studies

Next, for more physiological relevancy we evaluated the effects of EtOH in the slice preparation. In our slice studies, we utilized GAD-GFP mice to unequivocally identify VTA GABA neurons, similar to the dissociated neuron studies. Additionally, some studies were also performed in C57BL/6 mice, in case of possible EtOH sensitivity differences between the species. For example, GAD-GFP mice only express 50% of the GAD levels of WT mice. For these mice, we employed a variation of the conventional criteria to classify GABA neurons in C57BL/6 mice. Mainly, GABA neurons were subjected to our GABA spike command waveform [spikes at 200 Hz for 500 msec; (Steffensen, Taylor et al. 2008)]. We have found that GABA neurons follow the command waveform while DA neurons do not. Additionally, GABA neuron spike activity is enhanced or not affected by DA (Stobbs, Ohran et al. 2004, Lassen, Brown et al. 2007) while DA neurons are well-known to be inhibited by DA or D2 agonists.

In order to test EtOH's effects on GABA-mediated responses and the role of α6*-nAChR, we performed synaptic evoked IPSC (eIPSC) studies. For these synaptic studies involving GABA(A)R responses, we included APV and CNQX to block excitatory synaptic transmission. In some experiments, we also included 10 mM CGP55845 to rule out GABA(B)R effects, 100 nM eticlopride to rule out D2R effects, and 10 mM atropine to rule out cholinergic muscarinic effects. This served as essential control conditions for nAChR studies in the slice. Notably, a recent study had shown interactions with EtOH and CNQX (Brickley, Farrant et al. 2001,

Maccaferri and Dingledine 2002). Thus, in some studies we used kynurenic acid (3 mM) to block GLU transmission in place of APV and CNQX.

While mIPSCs are best evaluated in dissociated neurons, eIPSCs in the slice preparation provide valuable support to mIPSC studies. Our studies showed that low-dose EtOH (1 and 5 mM) consistently enhanced VTA GABA neuron eIPSC amplitude (Fig. 8A, C; 1 mM; $F_{(1,13)}$=6.65, $p$=0.02 and 5 mM; $F_{(1,15)}$=8.16, $p$=0.01). This effect was blocked by α-conotoxin MII (α-CTX MII), indicating the involvement of α6*-nAChRs (Fig. 8B, D; 1 mM; $F_{(1,11)}$=0.16, $p$=0.691 and 5 mM; $F_{(1,11)}$=0.07, $p$=0.796). However, results were inconsistent with higher doses of EtOH, which also resulted in inconclusive results for the effects of α-CTX MII on eIPSCs in the presence of EtOH. Furthermore, this EtOH effect is likely acting on GABA terminals, as the paired-pulse ratio decreased, suggesting a presynaptic effect (Fig. 8).

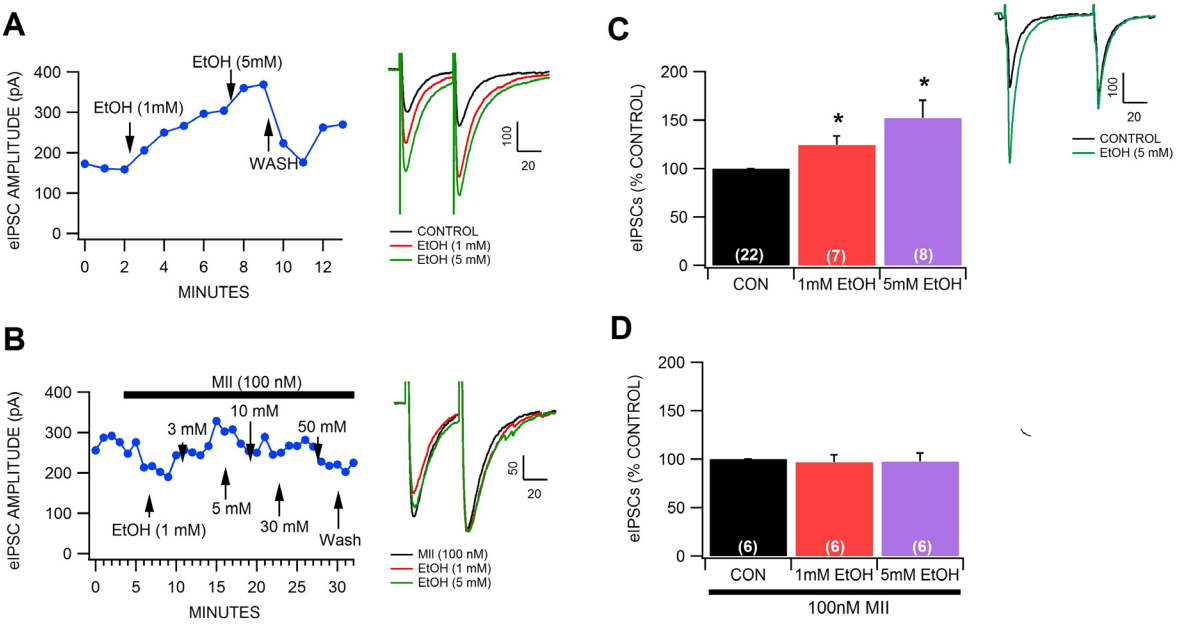


Figure 8: Effects of EtOH and α-CTX MII, on eIPSCs in VTA GABA Neurons.
eIPSCs were recorded in VTA GABA neurons in the horizontal slice in the presence of APV/CNQX. EtOH enhanced eIPSCs at low doses (A), but this effect was blocked in the presence of α-CTX MII (B). (C) Bar graph summarizes the effects of EtOH (1 and 5 mM) and shows that α-CTX MII blocks this effect. Inset shows EtOH enhancement in kynurenic acid + eticlopride, atropine and CGP55845.

*The Role of α6*-nAChRs in Acute Ethanol Actions on VTA DA Neurons*

Acute EtOH's Effect on VTA DA Neuron Firing Rate via α6*-nAChRs

The dogma is that local circuit GABA neurons inhibit DA neurons in the VTA. In order to link the changes we found in VTA GABA neurons to DA neurons, we evaluated the effects of EtOH on VTA DA neuron activity and DA release in the NAc. In addition, we examined the role α6*-nAChRs in this process. Through recording DA activity via firing rate we would be able to determine how EtOH may be acting on VTA GABA neurons to influence DA activity, and ultimately release, a common trait of addiction. In our firing rate studies, we performed cell-attached, voltage-clamped firing rate studies on VTA DA neurons in slice preparation from GAD-GFP mice. VTA DA neurons were identified as non-glowing cells and through their characteristically slow and regular firing rates. We found that low doses of EtOH (5 mM; $F_{(1,21)}$=12.58, $p$=0.002) inhibited VTA DA neuron activity, while high doses (50 mM; $F_{(1,15)}$=4.91, $p$=0.04 and 100 mM; $F_{(1,7)}$=9.98, $p$=0.02) tended to increase activity (Fig. 9). Furthermore, α-CTX MII was only able to block the effects of EtOH at the low dose level ($F_{(1,7)}$=0.70, $p$=0.43) , and did not appear to prevent the increase in firing rate associated with higher doses of EtOH (50 mM; $F_{(1,7)}$=6.39, $p$=0.04 and 100 mM; $F_{(1,8)}$=5.84, $p$=0.04).

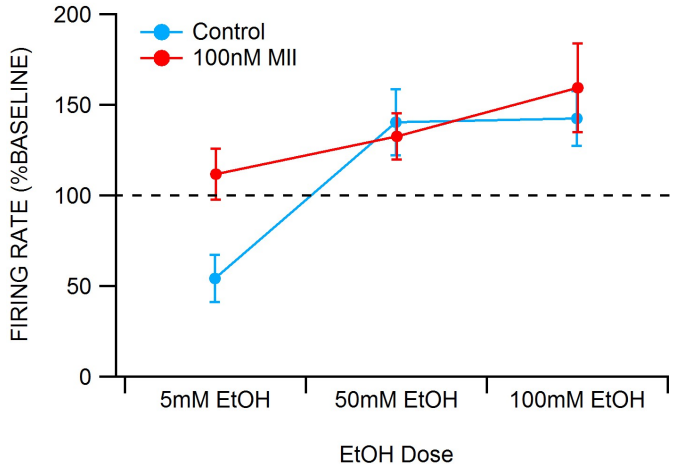


Figure 9: Effects of EtOH and α-CTX MII on VTA DA Neuron Firing Rate.
Using cell-attached mode, spontaneous spike activity was measured in horizontal slices of GAD-GFP mice. Low doses of EtOH inhibited DA activity while higher doses enhanced activity. α-CTX MII was able to block EtOH's low dose effects but not with higher doses.

Acute EtOH's Effect on DA Release in the NAc via α6*-nAChRs

In efforts to expand our understanding concerning α6*-nAChRs in EtOH addiction, we set to determine its role in DA release. In deciding which technique to employ, one concern arose from the fact that microdialysis studies typically show a rise in DA levels while voltammetry studies show a fall in evoked DA responses by both acute NIC and EtOH (Budygin, Phillips et al. 2001, Robinson, Howard et al. 2009, Zhang, Zhang et al. 2009, Yorgason, Ferris et al. 2013). However, this disparity is largely due to the measurements of tonic versus phasic DA release (Robinson, Howard et al. 2009). Recently it has been demonstrated that EtOH preferentially inhibits high-frequency phasic release, but not low-frequency "tonic" release (Yorgason, Ferris et al. 2013). Thus, we determined that fast-scan cyclic voltammetry (FSCV) was the best tool to utilize.

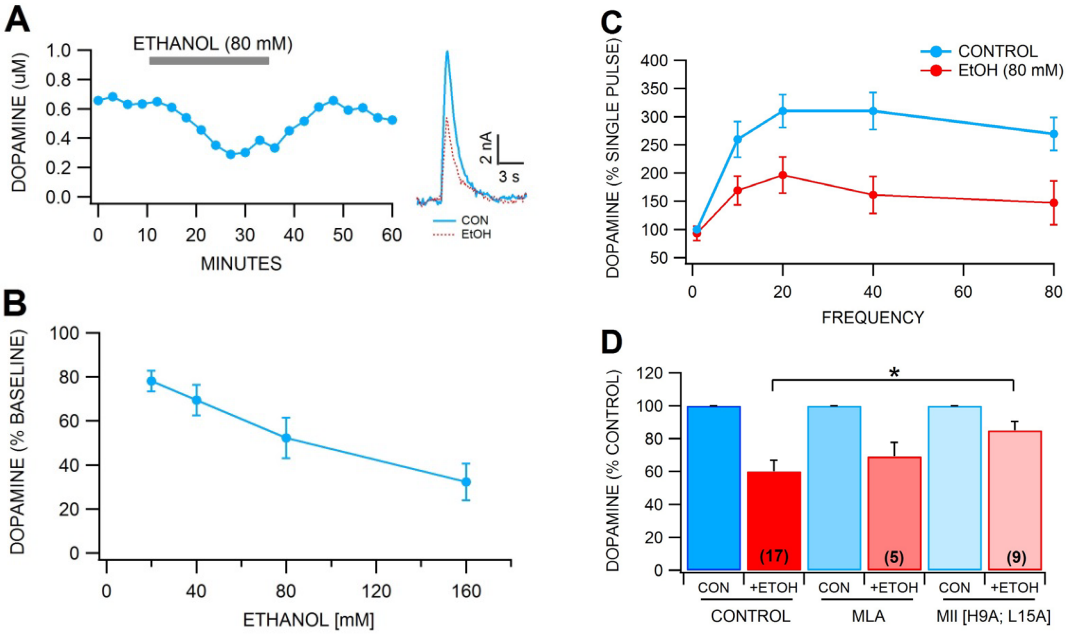


Figure 10: EtOH Inhibits DA Release in the NAc.
(A) This graph shows the time course of 80 mM EtOH effects on a representative recording of DA release in the NAc. (B) This graph shows an EtoH dose response on DA recordings. Increasing doses decreased DA release. (C) This graph shows a frequency response on DA recordings (D) This graph summarizes the effects of EtOH on DA. While MLA was without affect, the a6*-nAChR antagonist a-CTX-MII significantly attenuate EtOH inhibition at 80 mM.

As seen by others with FSCV, EtOH significantly decreased the peak amplitude of the DA signal. We found that it had an $IC_{50}$ of approximately 80 mM (Fig. 10A, B; 20 mM: $p$=0.001, n=11; 40 mM: $p$=0.001, n=11; 80 mM: $p$=0.0002, n=11; 160 mM: $p$<0.0001, n=10). EtOH significantly decreased the amplitude of the DA signal across all frequencies tested with the exception of single pulse stimulation (Fig. 10C). Next, we wanted to measure the amount of DA release in the presence of EtOH with the specific α7 and α6 nAChR antagonists methylylcaconitine (MLA; 100 nM) and α-CTX MII [H9A; L15A] (500 nM), respectively. We found that MLA did not alter EtOH inhibition of evoked DA release in the core at 80 mM EtOH (Fig. 10D; $F_{(1,20)}$=0.478, $p$=0.497). However, superfusion of the specific α6*-nAChR antagonist α-CTX MII was able to significantly block EtOH's inhibition of evoked DA release in the NAc (Fig. 10D; $F_{(1,30)}$=4.296, $p$=0.047).

*The Role of α6*-nAChRs in Mediating Ethanol Consumption and Reward*

Conditioned place preference (CPP) is a procedure used by many labs to study reward. Given the changes in VTA GABA and DA neurons, including DA release in the NAc, that we observed in our EtOH experimental procedures, we attempted to use CPP to show how these neuronal alterations would manifest in affecting behavior in mice. We found that WT mice receiving EtOH injections (2 g/kg IP) showed a higher preference for the EtOH compartment compared to

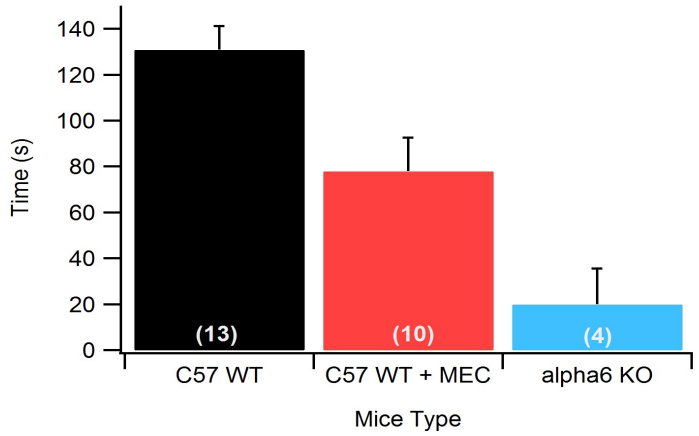


Figure 11: α6*-nAChR Mediate EtOH Consumption and Reward. Results show WT mice have an EtOH preference, which is not exhibited with α6 KO mice and mice treated with MEC, a nonselective nAChR antagonist.

EtOH treated α6 KO (Fig. 11). Furthermore, to ensure that α6 KO did not have any other additional behavioral changes inherent to their genetic modification, we pretreated WT mice with mecamylamine (MEC; 2 mg/kg), a non-α7 nAChR antagonist. The mice treated with MEC showed similar preference to the α6 KO. A one way ANOVA demonstrated significance between groups ($F_{(2,26)}$=12.94, $p$=0.0002), with Bonferroni's Multiple Comparison post-hoc analysis demonstrating significance in C57 WT vs C57 WT + MEC ($p<0.05$) and C57 WT vs α6 KO ($p<0.05$), with no difference in C57WT + MEC vs α6 KO.

# DISCUSSION

In recombinant SH-EP1 cells, NIC currents in functional α6*-nAChRs showed a significant enhancement in the presence of EtOH. Our results confirmed the NIC and EtOH interaction that others have previously observed through population-based samples and general neuron activity in the VTA (Tizabi, Copeland et al. 2002, John, Meyer et al. 2003, Ford, McCracken et al. 2012). While previous research revealed a synergistic relationship between NIC and EtOH, we were able to outline that this mechanism of actions was likely working through nAChRs, specifically α6*-nAChRs. We found that α6*-nAChRs showed a potent response to low doses of NIC (1 μM) and EtOH (1 mM), which effect was blocked by α-CTX P1A suggesting α6*-nAChRs probable direct involvement in mediating addiction in these two drugs.

In order to increase our understanding of the role α6*-nAChRs in modulating alcoholism, we needed to study native GABA neurons. We found that nicotine enhanced mIPSC frequency in dissociated VTA GABA neurons. A change in mIPSC frequency suggests a presynaptic response. As such, an increase in mIPSC frequency characterized greater inhibition to VTA GABA neurons. This effect was blocked by α6*-nAChR antagonists, suggesting that nicotine enhances inhibition to VTA GABA neurons via α6*-nAChRs to modulate addictive changes. Low doses of EtOH (6 mM) also enhanced mIPSC frequency in dissociated VTA GABA neurons. Administration of bicuculline helped determine that this inhibitory input is a result from increased GABA release to VTA GABA neurons. Again, this increase in mIPSC frequency was blocked by α6*-nAChR antagonists and not seen in α6 KO mice, suggesting that EtOH also enhances GABA release on to VTA GABA neurons via α6*-nAChRs. These findings, mirroring

NIC's effects on mISPC, further support the hypothesis that NIC and EtOH, at physiologically relevant levels, may be working through a shared mechanism.

Furthermore, an enhancement in mIPSC amplitude indicated that EtOH also had a postsynaptic effect on VTA GABA neurons. EtOH could be acting directly on GABA(A)Rs in addition to its presynaptic effect on GABA terminals via α6*-nAChRs to augment inhibition to VTA GABA neurons. As with frequency, the increase in mIPSC amplitude was blocked by α6*-nAChR antagonists and not observed in α6 KO mice too. Because α6*-nAChR antagonists prevented the increase in mIPSCs, it is likely that this increased inhibition in VTA GABA neurons is dependent on GABA release through α6*-nAChR. However, as a postsynaptic effect exists, EtOH may have a dual role in enhancing GABA release and amplifying the subsequent response.

Although EtOH increases inhibition to VTA GABA neurons, it was necessary to determine if this increase was enough to alter VTA GABA neuron activity. Our results showed that EtOH markedly lowered firing rate of dissociated VTA GABA neurons. Again, as seen with our previous EtOH studies, this effect was blocked by α6*-nAChR antagonists. This demonstrates that EtOH decreases VTA GABA activity via α6*-nAChRs, signifying the potential role that α6*-nAChRs have in causing alcoholic addiction.

While our results supported a link between α6*-nAChRs and EtOH in the VTA in dissociated neurons, it was pivotal to strengthen and prove the role that α6*-nAChR have with EtOH in a more physiological setting. Using slice preparation as this resource, we found that low doses of EtOH (1 and 5 mM) enhanced eIPSC amplitudes in VTA GABA neurons. Additionally, this enhancement is blocked by α6*-nAChR antagonists, providing further support that presynaptic α6*-nAChRs on GABA terminals modulate GABA release which is sensitive to

EtOH. This change in VTA GABA activity through α6*-nAChR could be the possible means by which EtOH addiction is developed.

However, our DA experiments showed somewhat of a discrepancy. Similar to many reports, we found that high doses of EtOH increased VTA DA firing rate (Brodie, Shefner et al. 1990). Moreover, we were also able to show that low doses of EtOH decreased VTA DA activity. Alpha6*-nAChR antagonists were able to block the effects of low dose EtOH, but were unable to do so in the higher doses. While there is evidence that α6*-nAChR may mediate changes in VTA DA activity, it appears likely that α6*-nAChR may only be involved in lower doses of EtOH.

While the effects of EtOH on GABA and DA may not coincide with what we expected to see based on our theoretical model of the VTA network, there could be some other workings to explain this discrepancy. It may be possible that the population of VTA GABA neurons we studied do not inhibit DA neurons in the VTA. The inhibitory input to DA may be from areas such as the RMTg or ventral pallidum, as there are GABA neurons from the RMTg that innervate the VTA. Another possibility is that EtOH may have an independent effect on VTA DA neurons. This may be plausible as high EtOH doses are unaffected by α6*-nAChR antagonist. In addition, our FSCV studies of DA release in the NAc do not contain VTA GABA neuron cell bodies. However, this is not very likely as α6*-nAChR antagonist block EtOH's effect on DA release, yet α6*-nAChR are not found on DA neurons. As we have hypothesized, α6*-nAChR are likely to be located on GABA terminals, which are present in the NAc, where they could synapse on DA terminals. As such, the VTA GABA neurons we studied might only synapse with DA neurons at their terminals, therefore not having an effect on DA activity, but only impacting DA release in the NAc. The latter theory is supported by our FSCV studies

showing EtOH inhibition of dopamine release at terminals in the nucleus accumbens being blocked by α6*-nAChR antagonists. Thus EtOH's mechanism of action is likely to be through α6*-nAChRs located on these VTA GABA terminals which synapse on DA terminals in the NAc.

Lastly, CPP studies showed that WT mice tend to spend more time in the EtOH compartment than α6 KO mice or mice treated with MEC, suggesting that α6*-nAChRs mediates alcoholic behaviors in mice. This is vital, as it shows that α6*-nAChRs is involved in process of addiction and its accompanying behaviors rather than the many physiological influences that EtOH can have on the brain other than addiction, such as ataxia. As WT mice exhibited more time in the EtOH compartment, it showed evidence that α6*-nAChRs are indeed involved in the process of alcohol addiction. The behavioral differences demonstrate that α6*-nAChRs allow EtOH to influence distinguishable reward-seeking behaviors caused by hijacking the mesolimbic DA system.

Taken together, our results show that EtOH is acting through α6*-nAChRs on GABA terminals to enhance GABA release. This enhancement of GABA release leads to greater inhibition to VTA GABA neurons which has the potential to influence VTA DA neurons and their release in the NAc. While further studies are necessary to outline the connection between VTA GABA neurons and VTA DA neurons to completely understand the hodology involved in EtOH addiction, our findings demonstrate a shared mechanism of alcohol and nicotine co-dependence. As this indicates some specificity in EtOH's mode of action, we believe we have identified a mechanism of alcohol addiction that could help facilitate the development of future therapies/medications for the treatment of alcoholism and other addictions.

REFERENCES

Allison, D. W., A. J. Ohran, S. H. Stobbs, M. Mameli, C. F. Valenzuela, S. N. Sudweeks, A. P. Ray, S. J. Henriksen and S. C. Steffensen (2006). "Connexin-36 gap junctions mediate electrical coupling between ventral tegmental area GABA neurons." Synapse 60(1): 20-31.

Allison, D. W., R. S. Wilcox, K. L. Ellefsen, C. E. Askew, D. M. Hansen, J. D. Wilcox, S. S. Sandoval, D. L. Eggett, Y. Yanagawa and S. C. Steffensen (2011). "Mefloquine effects on ventral tegmental area dopamine and GABA neuron inhibition: A physiologic role for connexin-36 gap junctions." Synapse.

Allison, D. W., R. S. Wilcox, K. L. Ellefsen, C. E. Askew, D. M. Hansen, J. D. Wilcox, S. S. Sandoval, D. L. Eggett, Y. Yanagawa and S. C. Steffensen (2011). "Mefloquine effects on ventral tegmental area dopamine and GABA neuron inhibition: a physiologic role for connexin-36 GAP junctions." Synapse 65(8): 804-813.

Benowitz, N. L. (1988). "Drug therapy. Pharmacologic aspects of cigarette smoking and nicotine addition." N Engl J Med 319(20): 1318-1330.

Bonci, A. and J. T. Williams (1996). "A common mechanism mediates long-term changes in synaptic transmission after chronic cocaine and morphine." Neuron 16: 631-639.

Bonci, A. and J. T. Williams (1997). "Increased probability of GABA release during withdrawal from morphine." J. Neurosci. 17: 796-803.

Brickley, S. G., M. Farrant, G. T. Swanson and S. G. Cull-Candy (2001). "CNQX increases GABA-mediated synaptic transmission in the cerebellum by an AMPA/kainate receptor-independent mechanism." Neuropharmacology 41(6): 730-736.

Brodie, M. S. and S. B. Appel (1998). "The effects of ethanol on dopaminergic neurons of the ventral tegmental area studied with intracellular recording in brain slices." Alcohol Clin Exp Res 22(1): 236-244.

Brodie, M. S., S. A. Shefner and T. V. Dunwiddie (1990). "Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro." Brain Res 508(1): 65-69.

Budygin, E. A., P. E. Phillips, D. L. Robinson, A. P. Kennedy, R. R. Gainetdinov and R. M. Wightman (2001). "Effect of acute ethanol on striatal dopamine neurotransmission in ambulatory rats." J Pharmacol Exp Ther 297(1): 27-34.

Carr, D. B. and S. R. Sesack (2000). "GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex." Synapse 38(2): 114-123.

Drenan, R. M., S. R. Grady, A. D. Steele, S. McKinney, N. E. Patzlaff, J. M. McIntosh, M. J. Marks, J. M. Miwa and H. A. Lester (2010). "Cholinergic modulation of locomotion and striatal dopamine release is mediated by alpha6alpha4* nicotinic acetylcholine receptors." J Neurosci 30(29): 9877-9889.

Drenan, R. M., S. R. Grady, P. Whiteaker, T. McClure-Begley, S. McKinney, J. M. Miwa, S. Bupp, N. Heintz, J. M. McIntosh, M. Bencherif, M. J. Marks and H. A. Lester (2008). "In vivo activation of midbrain dopamine neurons via sensitized, high-affinity alpha 6 nicotinic acetylcholine receptors." Neuron 60(1): 123-136.

Erhardt, S., L. Schwieler and G. Engberg (2002). "Excitatory and inhibitory responses of dopamine neurons in the ventral tegmental area to nicotine." Synapse 43(4): 227-237.

Exley, R., M. A. Clements, H. Hartung, J. M. McIntosh and S. J. Cragg (2008). "Alpha6-containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine neurotransmission in nucleus accumbens." Neuropsychopharmacology 33(9): 2158-2166.

Fenster, C. P., M. F. Rains, B. Noerager, M. W. Quick and R. A. Lester (1997). "Influence of subunit composition on desensitization of neuronal acetylcholine receptors at low concentrations of nicotine." J Neurosci 17(15): 5747-5759.

Fiorillo, C. D. and J. T. Williams (2000). "Cholinergic inhibition of ventral midbrain dopamine neurons." J Neurosci 20(20): 7855-7860.

Ford, M. M., A. D. McCracken, N. L. Davis, A. E. Ryabinin and K. A. Grant (2012). "Discrimination of ethanol-nicotine drug mixtures in mice: dual interactive mechanisms of overshadowing and potentiation." Psychopharmacology (Berl) 224(4): 537-548.

Garzon, M., R. A. Vaughan, G. R. Uhl, M. J. Kuhar and V. M. Pickel (1999). "Cholinergic axon terminals in the ventral tegmental area target a subpopulation of neurons expressing low levels of the dopamine transporter." J Comp Neurol 410(2): 197-210.

Girod, R., N. Barazangi, D. McGehee and L. W. Role (2000). "Facilitation of glutamatergic neurotransmission by presynaptic nicotinic acetylcholine receptors." Neuropharmacology 39(13): 2715-2725.

Gordis, E. (2000). "Tenth Special Report to the US Congress on Alcohol and Health." 1–463. Print.

Gotti, C., S. Guiducci, V. Tedesco, S. Corbioli, L. Zanetti, M. Moretti, A. Zanardi, R. Rimondini, M. Mugnaini, F. Clementi, C. Chiamulera and M. Zoli (2010). "Nicotinic acetylcholine receptors in the mesolimbic pathway: primary role of ventral tegmental area alpha6beta2* receptors in mediating systemic nicotine effects on dopamine release, locomotion, and reinforcement." J Neurosci 30(15): 5311-5325.

Grady, S. R., O. Salminen, D. C. Laverty, P. Whiteaker, J. M. McIntosh, A. C. Collins and M. J. Marks (2007). "The subtypes of nicotinic acetylcholine receptors on dopaminergic terminals of mouse striatum." Biochem Pharmacol 74(8): 1235-1246.

Harrison, E. L., R. E. Hinson and S. A. McKee (2009). "Experimenting and daily smokers: episodic patterns of alcohol and cigarette use." Addict Behav 34(5): 484-486.

Henningfield, J. E., J. M. Stapleton, N. L. Benowitz, R. F. Grayson and E. D. London (1993). "Higher levels of nicotine in arterial than in venous blood after cigarette smoking." Drug Alcohol Depend 33(1): 23-29.

Jackson, K. J., J. M. McIntosh, D. H. Brunzell, S. S. Sanjakdar and M. I. Damaj (2009). "The role of alpha6-containing nicotinic acetylcholine receptors in nicotine reward and withdrawal." J Pharmacol Exp Ther 331(2): 547-554.

John, U., C. Meyer, H. J. Rumpf, A. Schumann, J. R. Thyrian and U. Hapke (2003). "Strength of the relationship between tobacco smoking, nicotine dependence and the severity of alcohol dependence syndrome criteria in a population-based sample." Alcohol Alcohol 38(6): 606-612.

Johnson, S. W. and R. A. North (1992). "Two types of neurone in the rat ventral tegmental area and their synaptic inputs." J Physiol 450: 455-468.

Johnson, S. W., V. Seutin and R. A. North (1992). "Burst firing in dopamine neurons induced by N-methyl-D-aspartate: role of electrogenic sodium pump." Science 258(5082): 665-667.

Kalivas, P. W., L. Churchill and M. A. Klitenick (1993). "GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area." Neuroscience 57(4): 1047-1060.

Kamens, H. M., N. R. Hoft, R. J. Cox, J. H. Miyamoto and M. A. Ehringer (2012). "The alpha6 nicotinic acetylcholine receptor subunit influences ethanol-induced sedation." Alcohol 46(5): 463-471.

Larsson, A., L. Edstrom, L. Svensson, B. Soderpalm and J. A. Engel (2005). "Voluntary ethanol intake increases extracellular acetylcholine levels in the ventral tegmental area in the rat." Alcohol Alcohol 40(5): 349-358.

Larsson, A., E. Jerlhag, L. Svensson, B. Soderpalm and J. A. Engel (2004). "Is an alpha-conotoxin MII-sensitive mechanism involved in the neurochemical, stimulatory, and rewarding effects of ethanol?" Alcohol 34(2-3): 239-250.

Lassen, M. B., J. E. Brown, S. H. Stobbs, S. H. Gunderson, L. Maes, C. F. Valenzuela, A. P. Ray, S. J. Henriksen and S. C. Steffensen (2007). "Brain stimulation reward is integrated by a network of electrically coupled GABA neurons." Brain Res 1156: 46-58.

Laviolette, S. R. and D. van der Kooy (2003). "The motivational valence of nicotine in the rat ventral tegmental area is switched from rewarding to aversive following blockade of the alpha7-subunit-containing nicotinic acetylcholine receptor." Psychopharmacology (Berl) 166(3): 306-313.

Maccaferri, G. and R. Dingledine (2002). "Complex effects of CNQX on CA1 interneurons of the developing rat hippocampus." Neuropharmacology 43(4): 523-529.

Mansvelder, H. D., J. R. Keath and D. S. McGehee (2002). "Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas." Neuron 33(6): 905-919.

Mansvelder, H. D. and D. S. McGehee (2000). "Long-term potentiation of excitatory inputs to brain reward areas by nicotine." Neuron 27(2): 349-357.

Margolis, E. B., H. Lock, G. O. Hjelmstad and H. L. Fields (2006). "The ventral tegmental area revisited: is there an electrophysiological marker for dopaminergic neurons?" J Physiol 577(Pt 3): 907-924.

McGehee, D. S., M. J. Heath, S. Gelber, P. Devay and L. W. Role (1995). "Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors." Science 269(5231): 1692-1696.

Nugent, F. S. and J. A. Kauer (2008). "LTP of GABAergic synapses in the ventral tegmental area and beyond." J Physiol 586(6): 1487-1493.

Nutt, D. J., L. A. King, L. D. Phillips and D. Independent Scientific Committee on (2010). "Drug harms in the UK: a multicriteria decision analysis." Lancet 376(9752): 1558-1565.

Okada, H., N. Matsushita and K. Kobayashi (2004). "Identification of GABAA receptor subunit variants in midbrain dopaminergic neurons." J Neurochem 89(1): 7-14.

Oldendorf, W. H. (1974). "Lipid solubility and drug penetration of the blood brain barrier." Proc Soc Exp Biol Med 147(3): 813-815.

Papp, M., P. Gruca and P. Willner (2002). "Selective blockade of drug-induced place preference conditioning by ACPC, a functional NDMA-receptor antagonist." Neuropsychopharmacology 27(5): 727-743.

Rahman, S. and W. J. McBride (2000). "Feedback control of mesolimbic somatodendritic dopamine release in rat brain." J Neurochem 74(2): 684-692.

Robinson, D. L., E. C. Howard, S. McConnell, R. A. Gonzales and R. M. Wightman (2009). "Disparity between tonic and phasic ethanol-induced dopamine increases in the nucleus accumbens of rats." Alcohol Clin Exp Res 33(7): 1187-1196.

SAMHSA (2006). "National Survey on Drug Use and Health." n.pag. Print.

Schilstrom, B., M. V. Fagerquist, X. Zhang, P. Hertel, G. Panagis, G. G. Nomikos and T. H. Svensson (2000). "Putative role of presynaptic alpha7* nicotinic receptors in nicotine stimulated increases of extracellular levels of glutamate and aspartate in the ventral tegmental area." Synapse 38(4): 375-383.

Schilstrom, B., H. M. Svensson, T. H. Svensson and G. G. Nomikos (1998). "Nicotine and food induced dopamine release in the nucleus accumbens of the rat: putative role of alpha7 nicotinic receptors in the ventral tegmental area." Neuroscience 85(4): 1005-1009.

Sesack, S. R. and V. M. Pickel (1992). "Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area." J Comp Neurol 320(2): 145-160.

Steffensen, S. C., K. D. Bradley, D. M. Hansen, J. D. Wilcox, R. S. Wilcox, D. W. Allison, C. B. Merrill and J. G. Edwards (2011). "The role of connexin-36 gap junctions in alcohol intoxication and consumption." Synapse 65(8): 695-707.

Steffensen, S. C., S. R. Taylor, M. L. Horton, E. N. Barber, L. T. Lyle, S. H. Stobbs and D. W. Allison (2008). "Cocaine disinhibits dopamine neurons in the ventral tegmental area via use-dependent blockade of GABA neuron voltage-sensitive sodium channels." Eur J Neurosci 28: 2028-2040.

Stobbs, S. H., A. J. Ohran, M. B. Lassen, D. W. Allison, J. E. Brown and S. C. Steffensen (2004). "Ethanol suppression of ventral tegmental area GABA neuron electrical transmission involves N-methyl-D-aspartate receptors." J Pharmacol Exp Ther 311(1): 282-289.

Suaud-Chagny, M. F., K. Chergui, G. Chouvet and F. Gonon (1992). "Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area." Neuroscience 49(1): 63-72.

Taber, M. T. and H. C. Fibiger (1995). "Electrical stimulation of the prefrontal cortex increases dopamine release in the nucleus accumbens of the rat: modulation by metabotropic glutamate receptors." J Neurosci 15(5 Pt 2): 3896-3904.

Tamamaki, N., Y. Yanagawa, R. Tomioka, J. Miyazaki, K. Obata and T. Kaneko (2003). "Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse." J Comp Neurol 467(1): 60-79.

Tan, K. R., M. Brown, G. Labouebe, C. Yvon, C. Creton, J. M. Fritschy, U. Rudolph and C. Luscher (2010). "Neural bases for addictive properties of benzodiazepines." Nature 463(7282): 769-774.

Tan, K. R., C. Yvon, M. Turiault, J. J. Mirzabekov, J. Doehner, G. Labouebe, K. Deisseroth, K. M. Tye and C. Luscher (2012). "GABA neurons of the VTA drive conditioned place aversion." Neuron 73(6): 1173-1183.

Taylor, D. H., P. N. Burman, D. M. Hansen, J. D. Wilcox, B. R. Larsen, J. K. Blanchard, C. B. Merrill, J. G. Edwards, S. N. Sudweeks, J. Wu, H. R. Arias and S. C. Steffensen (2013). "Nicotine enhances the excitability of GABA neurons in the ventral tegmental area via activation of alpha 7 nicotinic receptors on glutamate terminals." Biochem Pharmacol 1(1).

Tepper, J. M., C. A. Paladini and P. Celada (1998). "GABAergic control of the firing pattern of substantia nigra dopaminergic neurons." Adv Pharmacol 42: 694-699.

Theile, J. W., H. Morikawa, R. A. Gonzales and R. A. Morrisett (2008). "Ethanol enhances GABAergic transmission onto dopamine neurons in the ventral tegmental area of the rat." Alcohol Clin Exp Res 32(6): 1040-1048.

Tizabi, Y., R. L. Copeland, Jr., V. A. Louis and R. E. Taylor (2002). "Effects of combined systemic alcohol and central nicotine administration into ventral tegmental area on dopamine release in the nucleus accumbens." Alcohol Clin Exp Res 26(3): 394-399.

Tripathi, A., L. Prensa, C. Cebrian and E. Mengual (2010). "Axonal branching patterns of nucleus accumbens neurons in the rat." J Comp Neurol 518(22): 4649-4673.

van Zessen, R., J. L. Phillips, E. A. Budygin and G. D. Stuber (2012). "Activation of VTA GABA neurons disrupts reward consumption." Neuron 73(6): 1184-1194.

Wise, R. A. (1996). "Addictive drugs and brain stimulation reward." Annu Rev Neurosci 19: 319-340.

Wise, R. A. (2008). "Dopamine and reward: the anhedonia hypothesis 30 years on." Neurotox Res 14(2-3): 169-183.

Wise, R. A. and M. A. Bozarth (1987). "A psychomotor stimulant theory of addiction." Psychol Rev 94(4): 469-492.

Wooltorton, J. R., V. I. Pidoplichko, R. S. Broide and J. A. Dani (2003). "Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas." J Neurosci 23(8): 3176-3185.

Xia, Y., J. R. Driscoll, L. Wilbrecht, E. B. Margolis, H. L. Fields and G. O. Hjelmstad (2011). "Nucleus accumbens medium spiny neurons target non-dopaminergic neurons in the ventral tegmental area." J Neurosci 31(21): 7811-7816.

Xiao, C. and J. H. Ye (2008). "Ethanol dually modulates GABAergic synaptic transmission onto dopaminergic neurons in ventral tegmental area: role of mu-opioid receptors." Neuroscience 153(1): 240-248.

Yang, K., L. Buhlman, G. M. Khan, R. A. Nichols, G. Jin, J. M. McIntosh, P. Whiteaker, R. J. Lukas and J. Wu (2011). "Functional nicotinic acetylcholine receptors containing alpha6 subunits are on GABAergic neuronal boutons adherent to ventral tegmental area dopamine neurons." J Neurosci 31(7): 2537-2548.

Yang, K., J. Hu, L. Lucero, Q. Liu, C. Zheng, X. Zhen, G. Jin, R. J. Lukas and J. Wu (2009). "Distinctive nicotinic acetylcholine receptor functional phenotypes of rat ventral tegmental area dopaminergic neurons." J Physiol 587(Pt 2): 345-361.

Yin, R. and E. D. French (2000). "A comparison of the effects of nicotine on dopamine and non-dopamine neurons in the rat ventral tegmental area: an in vitro electrophysiological study." Brain Res Bull 51(6): 507-514.

Yorgason, J. T., R. A. Espana and S. R. Jones (2011). "Demon voltammetry and analysis software: analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures." J Neurosci Methods 202(2): 158-164.

Yorgason, J. T., M. J. Ferris, S. C. Steffensen and S. R. Jones (2013). "Frequency-Dependent Effects of Ethanol on Dopamine Release in the Nucleus Accumbens." Alcohol Clin Exp Res.

Zhang, T., L. Zhang, Y. Liang, A. G. Siapas, F. M. Zhou and J. A. Dani (2009). "Dopamine signaling differences in the nucleus accumbens and dorsal striatum exploited by nicotine." J Neurosci 29(13): 4035-4043.

CURRICULUM VITAE

Samuel Injae Shin

Education

2014 – MS candidate in Neuroscience, Brigham Young University

2012 – BS in Neuroscience, Brigham Young University

Honors and Awards

| | |
|---|---|
| BYU Neuroscience Fellowship | 2012-2014 |
| BYU RPA Recipient | 2013 |
| RSA Student Merit Award – Travel Award | 2013 |
| Mary Lou Fulton Conference, Neuroscience 1st place. | 2012 |
| ORCA Grant Recipient | 2011 |

Publications

1. Shin, S.I.; Andersen, D.J.; Hansen, D.M.; Yorgason, J.T; Schilaty, N.D.; Busath, D.D.; Steffensen, S.C. Connexin-36 knock-out mice have increased threshold for kindled seizures: role of GABA inhibition. *Biochemistry and Neuropharmacology* (2013).
2. Ting-A-Kee, Ryan; Vargas-Perez, Hector; Mabey, Jennifer; Shin, Samuel; Steffensen, Scott; van der Kooy, Derek. Ventral Tegmental Area GABA Neurons and Opiate Motivation. *Psychopharmacology* (2013).

Published Abstracts/Conference Presentations

1. Shin, S.I., Mabey, J.K., White, D.N., Sandoval, S.S., Nielson, C.A., Schilaty, N.D., Taylor, D.H., Sudweeks, S.N., Edwards, J.G, Wu, J., McIntosh, M. and Steffensen, S.C. Ethanol inhibits GABA neurons in the VTA and dopamine release in the nucleus accumbens via alpha-6 nicotinic receptors on GABA terminals. Soc. Neurosci. Absts 39 (2013) 60.08
2. Mabey, J.K., Shin, S.I., White, D.N., Nielson, C.A., Schilaty, N.D., Ting-A-Kee, R., Vargas-Perez, H., van der Kooy, D. and Steffensen, S.C. Functional switch in GABA(A) receptors on VTA GABA neurons by chronic ethanol. Soc. Neurosci. Absts 39 (2013) 349.12
3. Shin, S.I., Mabey, J.K., White, D.N., Sandoval, S.S., Schilaty, N.D., Taylor, D.H., Sudweeks, S.N., Edwards, J.G, Wu, J., McIntosh, M. and Steffensen, S.C. Ethanol inhibits GABA neurons in the VTA and dopamine release in the nucleus accumbens via alpha-6 nicotinic receptors on GABA terminals. LDS Life Science Research Symposium (2013)
4. Mabey, J.K., Shin, S.I., White, D.N., Nielson, C.A., Schilaty, N.D., Ting-A-Kee, R., Vargas-Perez, H., van der Kooy, D. and Steffensen, S.C. Functional switch in GABA(A) receptors on VTA GABA neurons by chronic ethanol. LDS Life Science Research Symposium (2013)
5. Shin, S.I., Mabey, J.K., White, D.N., Nielson, C.A., Schilaty, N.D., Taylor, D.H., Wu, J., McIntosh, M. and Steffensen, S.C. Ethanol inhibits GABA neurons in the VTA and dopamine release in the nucleus accumbens via alpha-6 nicotinic receptors on GABA terminals. Alcoholism: Clin. Exp. Res. (2013) 37(2) 238A(909)

6. Mabey, J.K., Shin, S.I., White, D.N., Nielson, C.A., Schilaty, N.D., Ting-A-Kee, R., Vargas-Perez, H., van der Kooy, D. and Steffensen, S.C. Functional switch in GABA(A) receptors on VTA GABA neurons by chronic ethanol. Alcoholism: Clin. Exp. Res. (2013) 37(2) 238A(908)
7. Schilaty N.D., Mabey, J.K., Shin, S.I., Hedges D.M., Steffensen, S.C. Biomarkers of addiction. Brigham Young University Grad Expo (2013)
8. Samuel I. Shin, Benjamin T. Carter, Andrew A. Welch, Ronald C. Lopez, Jordan L. Davies, and Steffensen, S.C. Pharmacology of Ethanol-induced Inhibition of Dopamine Release in the Nucleus Accumbens. INS Snowbird Symposium (2012)
9. Mabey, J.K., Shin, S.I, White, D., Nielson, C., Vargas-Perez, H., Ting-A-Kee, R., Bahi, A., Van der Kooy, D., and Steffensen, S.C. Ventral tegmental area GABAergic activity underlies opiate motivation. INS Snowbird Symposium (2012)
10. Steffensen, S.C.; Andersen, D.J.; Hansen, D.M.; Shin, S.I.; Everette, C.J.; Fleming, D.E.; Busath, D.D. Connexin-36 knock-out mice have increased threshold for kindled seizures: role of GABA inhibition. Soc. Neurosci. Absts 37 (2011) 60.07
11. Hansen, D.M.; Yorgason, J.T.; Wilcox, J.D.; Konstantopoulos, J.K.; Hedges, D.M.; Jones, S.R.; Shin, S.I.; Steffensen, S.C. Effects of D2 type autoreceptor antagonists on ethanol-induced inhibition of dopamine release in the nucleus accumbens. Society for Neuroscience 2013.

Teaching/Research Experience

Instructor, Brigham Young University 2014
Advanced Neuroscience Laboratory.

Teaching Assistant, Brigham Young University. 2012 – 2014
Advanced Neuroscience Laboratory.

Research Assistant, Brigham Young University. 2010 – 2014
Neurophysiology research on the mechanism of addiction.