



2016-06-01

Glutamate and GABA Receptor-Mediated Plasticity in the Mesolimbic Dopamine System by Alcohol

Ashley Cerise Nelson
Brigham Young University

Follow this and additional works at: <https://scholarsarchive.byu.edu/etd>

 Part of the [Physiology Commons](#)

BYU ScholarsArchive Citation

Nelson, Ashley Cerise, "Glutamate and GABA Receptor-Mediated Plasticity in the Mesolimbic Dopamine System by Alcohol" (2016). *All Theses and Dissertations*. 6413.
<https://scholarsarchive.byu.edu/etd/6413>

This Thesis is brought to you for free and open access by BYU ScholarsArchive. It has been accepted for inclusion in All Theses and Dissertations by an authorized administrator of BYU ScholarsArchive. For more information, please contact scholarsarchive@byu.edu, ellen_amatangelo@byu.edu.

Glutamate and GABA Receptor-Mediated Plasticity
in the Mesolimbic Dopamine
System by Alcohol

Ashley Cerise Nelson

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

Master of Science
Neuroscience

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

Department of Physiology and Developmental Biology
Brigham Young University

June 2016

Copyright © 2016 Ashley Cerise Nelson

All Rights Reserved

ABSTRACT

Glutamate and GABA Receptor-Mediated Plasticity in the Mesolimbic Dopamine System by Alcohol

Ashley Cerise Nelson

Department of Physiology and Developmental Biology, BYU
Master of Science
Neuroscience

Alcoholism is a devastating chronic relapsing disorder with significant costs to individuals and society. The mesolimbic dopamine (DA) system plays an important role in regulating reward and addiction. GABA neurons located in the ventral tegmental area (VTA) regulate VTA DA neuron activity, and are a relevant target for alcohol in the brain. VTA GABA neurons exhibit marked hyperexcitability during withdrawal from ethanol. Past research has demonstrated that the motivational effects of opiates cause a change in VTA GABA(A) receptors in opiate-dependent animals, which switch from a GABA-induced hyperpolarization of GABA neurons to a GABA-induced depolarization.

The focus of this study was to characterize excitatory and inhibitory synaptic activity in VTA GABA neurons during withdrawal from acute and chronic alcohol, and to evaluate the function of the GABA(A) receptor in the pathway to dependence. Animals were either given injections of ethanol or saline, or were kept in ethanol vapor or air chambers for three weeks. We used standard whole-cell, perforated patch, and cell-attached mode electrophysiological techniques and pharmacology to obtain recordings of cellular activity.

Results for excitatory and inhibitory synaptic events were somewhat mixed and inconclusive. There is evidence for a shift in function of the GABA(A) receptor after exposure to ethanol. We found that after a single injection of ethanol (4.0 g/kg) or a chronic intermittent ethanol vapor exposure, VTA GABA neuron firing rate is less sensitive to muscimol's inhibitory effects. The neural substrates of addiction studied here are important steps in the road to alcohol dependence, and a better understanding of them may lead to novel therapies.

Keywords: alcohol dependence, glutamate, GABA, plasticity, ethanol

ACKNOWLEDGEMENTS

I would like to acknowledge the support of the Steffensen lab, especially Samuel Shin and Jennifer Mabey for training me, and Taylor Woodward, Hyun Jung Park, Stephanie Pistorius, and Stephanie Bair for helping collect patch clamp experiments. I am also indebted to the funding from a grant from the National Institute on Alcohol Abuse and Alcoholism and for my personal financial support from the Department of Physiology and Developmental Biology and the Graduate Research Fellowship Award from BYU Graduate Studies. From the Edwards lab, I am grateful for the help of Teresa Nufer for recording and analyzing AMPA/NMDA ratio and AMPA rectification experiments. I would also like to thank our collaborators Dr. Derek van der Kooy and Hector Vargas-Perez at the University of Toronto for their previous work studying GABA(A)R functionality switch, and Dr. Jie Wu and Dr. Ming Gao of the Barrow Neurological Institute for advice on perforated patch clamp experiments. I am extremely grateful for the support and advice from my committee, Dr. Jeff Edwards, Dr. Sterling Sudweeks, and especially from my chair, Dr. Scott Steffensen. His example of generosity and his mentorship has helped foster a passion for science within me, and has made this project possible. I could not go without thanking my parents for their continual encouragement and love, and that they have always believed in my dreams.

TABLE OF CONTENTS

TITLE PAGE	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF FIGURES	vi
INTRODUCTION	1
The Economic and Social Costs of Alcohol Abuse	1
The Mesolimbic Dopamine System	1
Glutamatergic Synaptic Plasticity in Response to Drugs of Abuse	2
GABA(A) Receptor Functionality Switch	3
RATIONALE AND HYPOTHESES	6
Hypothesis 1: Withdrawal from Acute Ethanol (Non-Dependent Condition) will Enhance GLU NMDA Receptor-Mediated, and Decrease GABA(A) Receptor-Mediated Synaptic Transmission, to VTA GABA Neurons	6
Hypothesis 2: Withdrawal from Chronic Ethanol (Dependent Condition) will Affect GABA(A) Receptor-Mediated, but not NMDA Receptor- Mediated Synaptic Transmission, to VTA GABA Neurons	6
Hypothesis 3: GABA(A) Receptors on VTA GABA Neurons will Switch Their Function with Alcohol Dependence Through a Change in the Chloride Gradient	6
METHODS	7
Animal Subjects	7
Chronic Intermittent Ethanol Exposure	7
Drink-in-the-dark Behavioral Experiments	8
Preparation of Brain Slices	8
Characterization of Neuron Types	8
Whole-cell Recordings in Brain Tissue Slices	9
Cell-attached, Voltage-clamp Recording of Spike Activity in Brain Slices	10
BDNF Protein Determination	10

Perforated Patch Electrophysiology.....	11
Drug Preparation and Administration.....	11
Statistical Analyses.....	12
RESULTS.....	13
Drink-in-the-Dark Behavioral Measure of Ethanol Dependence.....	13
Glutamatergic Spontaneous Events Following Acute and Chronic Ethanol.....	14
AMPA/NMDA Ratio Following Acute and Chronic Ethanol.....	17
GABAergic Spontaneous Events Following Acute and Chronic Ethanol.....	18
Muscimol Inhibition of Firing Rate Following Acute Ethanol.....	20
Muscimol Inhibition of Firing Rate Following Chronic Ethanol.....	21
BDNF Expression in the VTA.....	23
Chloride Reversal Potential with Perforated Patch Clamp.....	24
DISCUSSION.....	26
General Focus of the Study.....	26
Excitatory Plasticity to VTA GABA Neurons by Alcohol.....	27
Inhibitory Plasticity to VTA GABA Neurons by Alcohol.....	28
Functional Shift of GABA(A) Receptor Due to Alcohol.....	28
Overall Conclusions.....	31
REFERENCES.....	33
CURRICULUM VITAE.....	38

LIST OF FIGURES

Figure 1: Theoretical Framework for Proposed Studies.....	2
Figure 2: Proposed Model for Functional Switch of GABA(A)Rs on VTA GABA Neurons.....	4
Figure 3: DID Measurement of Alcohol Dependence.....	13
Figure 4: Spontaneous EPSCs in VTA GABA Neurons Following an Ethanol Exposure	14
Figure 5: Mini EPSCs in VTA GABA Neurons Following an Ethanol Exposure.....	15
Figure 6: Enhancement of AMPA/NMDA Ratio in VTA GABA Neurons Following an Ethanol Exposure.....	16
Figure 7: Rectification Index for AMPA Current in VTA GABA Neurons Following an Ethanol Exposure.....	17
Figure 8: Spontaneous IPSCs in VTA GABA Neurons Following an Ethanol Exposure	18
Figure 9: Mini IPSCs in VTA GABA Neurons Following an Ethanol Exposure.....	19
Figure 10: Acute Ethanol Injection Reduces Muscimol Inhibition.....	20
Figure 11: Chronic Ethanol Injection Reduces Muscimol Inhibition	21
Figure 12: Chronic Ethanol Vapor Reduces Muscimol Inhibition.....	22
Figure 13: Muscimol Inhibition on VTA GABA Firing Rate Seven Days After Ethanol Exposure	23
Figure 14: BDNF Expression Following Ethanol Exposure.....	24

INTRODUCTION

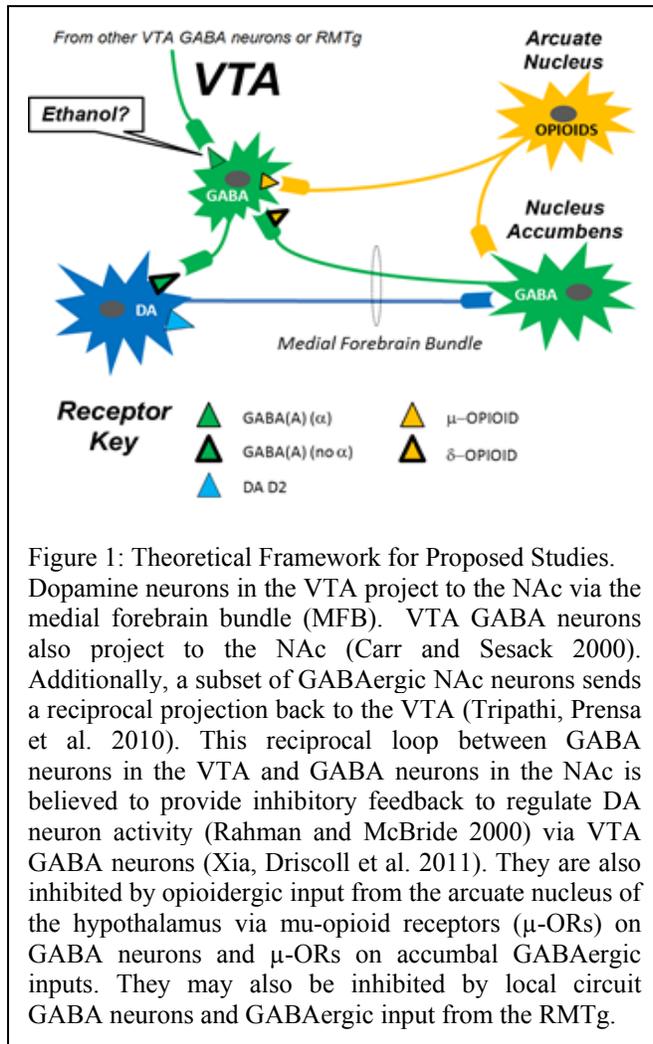
The Economic and Social Costs of Alcohol Abuse

Alcoholism is a chronic relapsing disorder that has enormous impact on society. Excessive alcohol use is the third leading lifestyle-related cause of the death in the United States. Approximately 80,000 deaths each year are attributable to excessive alcohol use (Bouchery, Harwood et al. 2011). The estimated cost of excessive drinking is approximately \$223.5 billion (Bouchery, Harwood et al. 2011). While there is a great societal and economic need to cure alcoholism, there is still little neuro-mechanistic understanding of this pervasive disease. For this project, I endeavored to better understand the neural basis of alcohol use and the pathological progression to alcohol dependence. By understanding the mechanism by which alcohol disrupts normal brain function, treatments and therapies can be developed in order to ameliorate alcohol's devastating effects.

The Mesolimbic Dopamine System

The mesolimbic dopamine (DA) system originates in the midbrain ventral tegmental area (VTA) where DA and γ -amino butyric acid (GABA) cell bodies are located. These neurons project to limbic structures such as the nucleus accumbens (NAc). Dopamine neurons in the VTA are inhibited by GABA inputs. Dopamine release in the mesolimbic DA system has been shown to exhibit a scalar index of reward (Wise 2008). While the release of DA in the NAc leads to pleasure, which positively reinforces drug use, it has been suggested that the changes in DA are strongly regulated by VTA GABA neurons (Nugent and Kauer 2008, Ting and van der Kooy 2012, Bocklisch, Pascoli et al. 2013). VTA GABA neurons provide an inhibitory input to DA neurons to regulate their activity level (Tepper, Paladini et al. 1998). GABA is a major regulator of DA neurotransmission and therefore plays a critical role in the rewarding properties of drugs

of abuse such as ethanol. While current dogma states that the reinforcing properties of alcohol are primarily mediated by its actions on glutamatergic (GLUergic) N-methyl-D-aspartate



receptors (NMDARs) in the VTA, the mechanisms underlying the withdrawal syndrome associated with alcohol dependence remain to be elucidated. We have evidence that repeated alcohol use is associated with neuroadaptations of VTA GABA subtype A receptor [GABA(A)R] complexes on VTA GABA neurons. We have demonstrated previously that VTA GABA neurons are sensitive to ethanol at behaviorally-relevant concentrations, gain tolerance to ethanol inhibition of their firing rate, and become hyperexcitable during withdrawal to chronic ethanol (Gallegos, Criado et al. 1999), which may explain why

DA levels are lowered during withdrawal from chronic ethanol (Diana, Pistis et al. 1993). Figure 1 illustrates the synaptic hodology of VTA GABA neurons, including the probable receptors involved in ethanol pharmacology.

Glutamatergic Synaptic Plasticity in Response to Drugs of Abuse

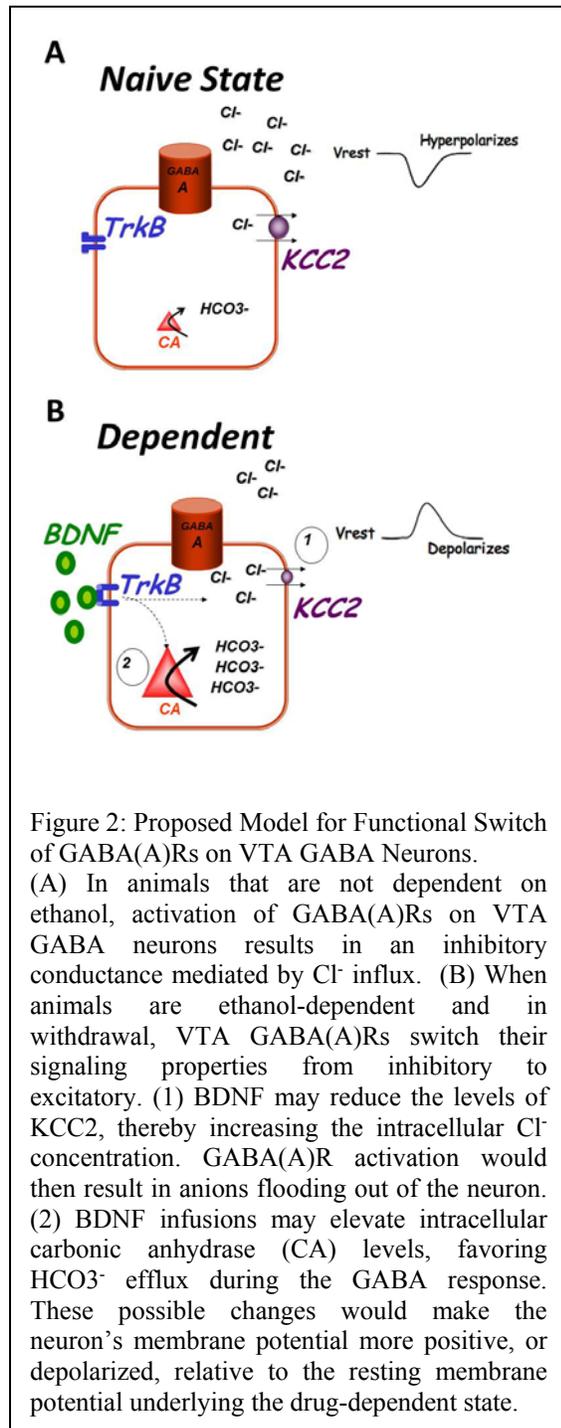
Experience-dependent changes often involve GLUergic synaptic plasticity (Berke and Hyman 2000). It has been well-established that VTA DA neurons undergo NMDA GLUR-

mediated plasticity in response to drugs of abuse, including alcohol (Ungless, Whistler et al. 2001, Saal, Dong et al. 2003, van Huijstee and Mansvelder 2014). This evidence has supported theories regarding the involvement of DA in craving and relapse (Luscher 2013). GLUergic synaptic plasticity on VTA GABA neurons is far less understood. Ungless et al. (2001) showed that an acute *in vivo* exposure to cocaine did not alter the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/NMDA ratio on VTA GABA neurons, only on DA neurons. Additionally, it was shown that VTA GABA neurons do not undergo NMDAR-mediated long-term potentiation (LTP) in response to a high frequency stimulus (Bonci and Malenka 1999, Nugent, Hwong et al. 2008). However, whether or not ethanol induces GLUergic synaptic plasticity in VTA GABA neurons has not been determined. Our lab has repeatedly shown that VTA GABA neurons are a relevant target for alcohol (Steffensen, Walton et al. 2009, Steffensen, Bradley et al. 2011), and we have preliminary evidence that GLUergic activity on VTA GABA neurons is increased during withdrawal from acute ethanol (see below). While the involvement of GLU plasticity in VTA DA neurons during drug withdrawal has received some attention, we have yet to elucidate the role of GLUergic synapses on VTA GABA neurons during alcohol withdrawal.

GABA(A) Receptor Functionality Switch

Considerable evidence suggests that activation of GABA(A)R complexes can produce depolarization in lieu of its more traditional hyperpolarizing response (Kaila, Voipio et al. 1993, Staley, Soldo et al. 1995, Rivera, Voipio et al. 1999, Hubner, Stein et al. 2001, Coull, Boudreau et al. 2003). The switching of GABA(A)R functionality occurs during development and under pathological conditions like epilepsy. One hypothesis to explain this shift involves the neuronal potassium-chloride co-transporter isoform 2 (KCC2), which removes intracellular chloride (Cl⁻)

and thereby maintains an inward-directed hyperpolarizing Cl^- flow (Thompson, Deisz et al. 1988, Rivera, Li et al. 2002, Viitanen, Ruusuvuori et al. 2010). Consequently, blockade of KCC2 should result in a build-up of intracellular Cl^- and a reduction in Cl^- ion influx after long-term



GABA(A)R activation (Fig. 2), allowing other ion flows [such as a bicarbonate (HCO_3^-) efflux] to dominate (Kaila, Voipio et al. 1993, Staley, Soldo et al. 1995, Rivera, Voipio et al. 1999, Sun and Alkon 2001, Coull, Boudreau et al. 2003).

Dr. Steffensen's recent collaboration with Dr. van der Kooy's lab demonstrated that this switch in GABA(A)Rs on VTA GABA neurons is associated with opiate dependence (Vargas-Perez, Kee et al. 2009, Vargas-Perez, Bahi et al. 2014).

The functional switch results from increased levels of brain-derived neurotrophic factor (BDNF), which activates the high-affinity tyrosine kinase B (TrkB) receptor (Vargas-Perez, Kee et al. 2009), which is expressed in VTA GABA neurons (Numan, Lane-Ladd et al. 1998). Ethanol withdrawal produces adaptations in VTA GABA neurons (Gallegos, Criado et al. 1999, Brodie 2002, Hopf, Martin et al. 2007) and GABA(A)R subunit composition in the VTA and the

hippocampus (Charlton, Sweetnam et al. 1997, Cagetti, Liang et al. 2003), and it is reasonable to assume that these changes are important for precipitating this switch in the neurobiological substrates mediating ethanol reinforcement. Functionally, the switch is caused by a change in the ion conductance properties of the GABA(A)Rs themselves (Staley, Soldo et al. 1995, Stein and Nicoll 2003, Laviolette, Gallegos et al. 2004). Figure 2 illustrates the key molecular substrates proposed to mediate the switch in functionality of the GABA(A)R on VTA GABA neurons. This model is based mostly on the work of van der Kooy's lab with opiates, but applied here to ethanol.

RATIONALE AND HYPOTHESES

As previously mentioned, VTA GABA neurons become markedly hyperexcitable during withdrawal from ethanol as measured in single-unit recordings using an *in vivo* system (Gallegos, Criado et al. 1999). Alterations in VTA GABA neurons will in turn alter DA activity and subsequent DA release in the NAc and ultimately lead to drug dependence. VTA GABA neurons are a relevant target for ethanol in the brain (Steffensen, Walton et al. 2009, Steffensen, Bradley et al. 2011). The neuroadaptations of VTA GABA neurons may underlie the behaviors seen in alcohol use disorders. By observing neuronal activity during withdrawal, we can point to mechanisms behind the hedonic drive that leads alcoholics to relapse. I explored the roles of GLU- and GABA-mediated changes in VTA GABA neurons at two levels of ethanol exposure and two different time points of withdrawal. Our original hypotheses are outlined here:

Hypothesis 1: Withdrawal from Acute Ethanol (Non-Dependent Condition) will Enhance GLU NMDA Receptor-Mediated, and Decrease GABA(A) Receptor-Mediated Synaptic Transmission, to VTA GABA Neurons.

Hypothesis 2: Withdrawal from Chronic Ethanol (Dependent Condition) will Affect GABA(A) Receptor-Mediated, but not NMDA Receptor-Mediated Synaptic Transmission, to VTA GABA Neurons.

Hypothesis 3: GABA(A) Receptors on VTA GABA Neurons will Switch Their Function with Alcohol Dependence Through a Change in the Chloride Gradient.

The work from this project dissects the contributions of GLU and GABA inputs on VTA GABA neuron hyperexcitability and suggests a mechanism behind the altered function of the GABA(A) receptor.

METHODS

Animal Subjects

Male C57BL6 (black) and glutamate-decarboxylase-67 (GAD-67)-green fluorescent protein knock-in CD-1 (white albino) mice (Tamamaki, Yanagawa et al. 2003) were bred and cared for in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. For each methodology employed, animals were treated in strict accordance with the Brigham Young University Animal Research Committee (IACUC) guidelines, which incorporated and often exceeded current NIH guidelines. The BYU IACUC reviewed and approved the procedures detailed herein. Once weaned at post-natal day 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 PM to 8 AM. Any mice used in injection experiments were anesthetized with isoflurane (5%), and injected intraperitoneally (IP) with a sterile needle. Animals returned to their home cages 30 minutes following the injection.

Chronic Intermittent Ethanol Exposure

Animals were exposed to chronic intermittent ethanol (CIE) in alcohol vapor chambers where blood alcohol levels (BALs) could be determined. They were exposed to 200 mg% BAL for sixteen hours (1000-0200 hours) beginning in their dark cycle (i.e., reverse cycle light). Three weeks of chronic intermittent exposure were required to produce alcohol dependence (Gallegos, Criado et al. 1999). As a check for dependence, we evaluated DA release in the NAc, which has been shown to be diminished in alcohol dependent animals. We also measured dependence through a behavioral model of alcohol drinking, described below.

Drink-in-the-dark Behavioral Experiments

To observe a behavioral correlate of escalating alcohol dependence, C57BL6 mice were trained and evaluated on a drink-in-the-dark (DID) two-bottle choice alcohol drinking test. Animals were removed from home cages three hours into the dark cycle, and placed individually in cages with bedding and food removed. They were given two sipper tubes, with one containing tap water and the other containing tap water and ethanol (20% v/v). The mice were allowed to drink from the tubes for two hours in the dark, and were then returned to their home cages.

Preparation of Brain Slices

All brain slice preparations were performed in P18-60 day old C57BL/6 and GAD-GFP knock-in mice. Brains were extracted under isoflurane (5 %) anesthesia. Upon extraction, the brain was glued onto a cutting stage. The brain was then sectioned in ice-cold cutting solution (in mM: 194 Sucrose, 30 NaCl, 4.5 KCl, 1 MgCl₂, 26 NaH₂CO₃, 1.2 NaH₂PO₄, 10 Glucose) and perfused with 95 % O₂ / 5 % CO₂. Targeting the VTA, horizontal slices (210 µm thick) were placed in an incubation chamber containing artificial cerebral spinal fluid (ACSF; in mM: 124 NaCl, 3 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 12 glucose, 1.5 MgSO₄, 2 CaCl₂) perfused with 95 % O₂ / 5 % CO₂ for at least 30 minutes. After 30 minutes, brain slices were placed in a recording tissue chamber with ACSF continuously flowing at physiological temperatures (35 °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 follow the command waveform. Neurons that did not fluoresce and/or

exhibited a non-cation specific inward rectifying current (I_h) with low input resistance, and did not follow the voltage command waveform in current clamp mode were 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 Brain Tissue Slices

Electrodes were pulled from borosilicate glass capillary tubes and filled with a KCl pipette solution [in mM: 128 KCl, 20 NaCl, 0.3 CaCl₂, 1.2 MgCl₂, 10 HEPES, 1 EGTA, 2 Mg-ATP, 0.25 Na-GTP, and 4.5 QX-314 (pH 7.3)] for inhibitory post-synaptic current (IPSC) studies. A K-gluconate pipette solution [in mM: 123 K-gluconate, 0.2 EGTA, 10 HEPES, 8 NaCl, 2 Mg-ATP, 2 Na₃-GTP, and 4.5 QX-314 (pH 7.3)] was used for excitatory post-synaptic current (EPSC) studies. QX-314 was included in pipettes to eliminate action potentials interfering with data collection in the cell being recorded. Pipettes having tip resistances of 2.5 – 5 M Ω , and series resistances typically ranging from 7 to 15 M Ω 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. IPSCs were recorded in the presence of 50 μ M (2R)-amino-5-phosphonovaleric acid (APV) and 30 μ M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) or 3 mM kynurenic acid to block NMDA and AMPA mediated synaptic currents. EPSCs were recorded in the presence of 100 μ M picrotoxin to block GABA and glycine mediated synaptic currents. Minis were recorded in the presence of 500 μ M lidocaine to block action potentials. For measurement of AMPA/NMDA ratio and AMPA rectification, evoked EPSCs (eEPSCs) were

recorded at +40 mV to remove the magnesium block from the NMDA receptor. 50 μ M APV was given partway to block NMDA currents, and the NMDA component of the total current was calculated by subtraction. AMPA rectification was calculated by fitting a polynomial curve to the current-voltage plot with current values taken at holding voltages of -70, -40, -20, 0, 20, and 40 mV. The Goldstein rectification index (RI) was calculated according to $RI = [I_{+19}/(19 - aE_{rev})]/[I_{-79}/(-79 - aE_{rev})]$ where aE_{rev} is the apparent reversal potential in standard bath and I_{+19} and I_{-79} are the averaged currents at +19 and -79 mV, respectively (Goldstein, Lee et al. 1995). The Wang RI was calculated according to $RI = (AMPA-EPSC_{+60mV}) / (AMPA-EPSC_{-60mV})$ where AMPA-EPSC is the peak current recorded at the respective holding potentials (Wang and Gao 2012).

Cell-attached, Voltage-clamp Recording of Spike Activity in Brain Slices

Cell-attached mode studies used electrodes pulled from borosilicate glass capillaries (2.5-6M Ω) and then filled with a NaCl solution containing (in mM): 124 NaCl, 2 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 1.2 MgSO₄, 2 CaCl₂ adjusted to pH 7.4 with KOH. Positive pressure was applied to the electrode when approaching the neuron. By applying suction to the electrode, a seal (10 M Ω – 1 G Ω) was created 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, sampled at 10 kHz using an Axon 1440A digitizer, and collected and analyzed using pClamp10 software. Neurons were voltage-clamped at 0 mV throughout these experiments. A stable baseline recording of firing activity was obtained for 5-10 min before adding any substances.

BDNF Protein Determination

Tissue punches containing the VTA were excised from 1 mm thick brain slices and immediately placed on dry ice. Samples were stored at -20 $^{\circ}$ C until used in the protein assay. An

enzyme-linked immunosorbent assay (ELISA) kit from Biosensis was used to determine pro-BDNF and mature BDNF concentrations in the tissue.

Perforated Patch Electrophysiology

To determine E_{Cl^-} , perforated patch electrophysiology recordings were performed on VTA GABA neurons. The slice containing the VTA was slowly perfused (~ 1ml/min) with ACSF containing pronase (0.167 mg/ml) for 30 minutes to 1 hour. This step appeared to help open the slice for easier mobility of the pipette in order to obtain perforated patches. The tips of the microelectrodes, with resistances between 4 and 8 M Ω , were prefilled with the pipette solution containing (in mM): 135 KCl, 0.5 CaCl₂, 2 MgCl₂, 10 HEPES, 5 Na-EGTA adjusted to pH 7.2 with KOH and backfilled with the same pipette solution containing 50 μ g/ml of the pore-forming antibiotic gramicidin. Gramicidin creates holes in the membrane of the patch that are permeable to monovalent cations (but not the anion chloride), thus providing electrical access to the cell while maintaining the integrity of the cytoplasm. The access resistance was used to monitor the progression of the perforation. In voltage-clamp mode, currents were elicited by exogenously applying the GABA(A)R agonist muscimol (300 μ M; Sigma) with a gravity-fed solution delivery system via a glass pipette with a tip diameter of ~ 70 μ m. Each cell was held at the following potentials as muscimol was applied: -100mV, -80mV, -60mV, -40mV, -20mV, 0mV.

Drug Preparation and Administration

Drugs used *in vitro* were made fresh in distilled water, added to ACSF, and superfused on brain slices: kynurenic acid (3 mM; Sigma-Aldrich), muscimol (10 nM – 10 μ M; Sigma-Aldrich), APV (50 μ M; Abcam), picrotoxin (100 μ M; Sigma-Aldrich), lidocaine (500 μ M; Sigma-Aldrich). Ethanol (5, 10, 30, 50 mM) is added to ACSF after baseline recordings. Drugs

used for injections were added to sterile 0.9% saline and injected IP: ethanol (16% ethanol v/v), MK-801 (1 mg/kg; Sigma-Aldrich).

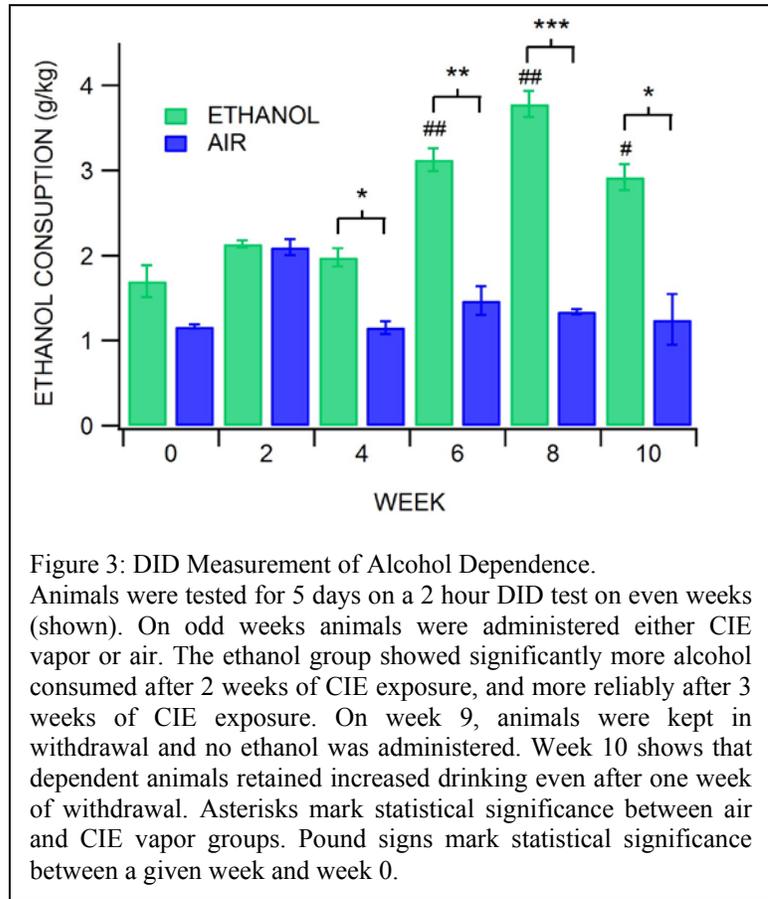
Statistical Analyses

All results are presented as raw mean values and percent control \pm standard error of the mean (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 ($P \leq 0.05$). Analysis software included Minianalysis (Synptosoft), Clampfit (Molecular Devices), Microsoft Excel, and Igor Pro (Wavemetrics, Oswego, OR). Significance levels were indicated on graphs with asterisks *, **, ***, corresponding to significance levels $p < 0.05$, 0.01 and 0.001, respectively. Figures were constructed with Igor Pro software.

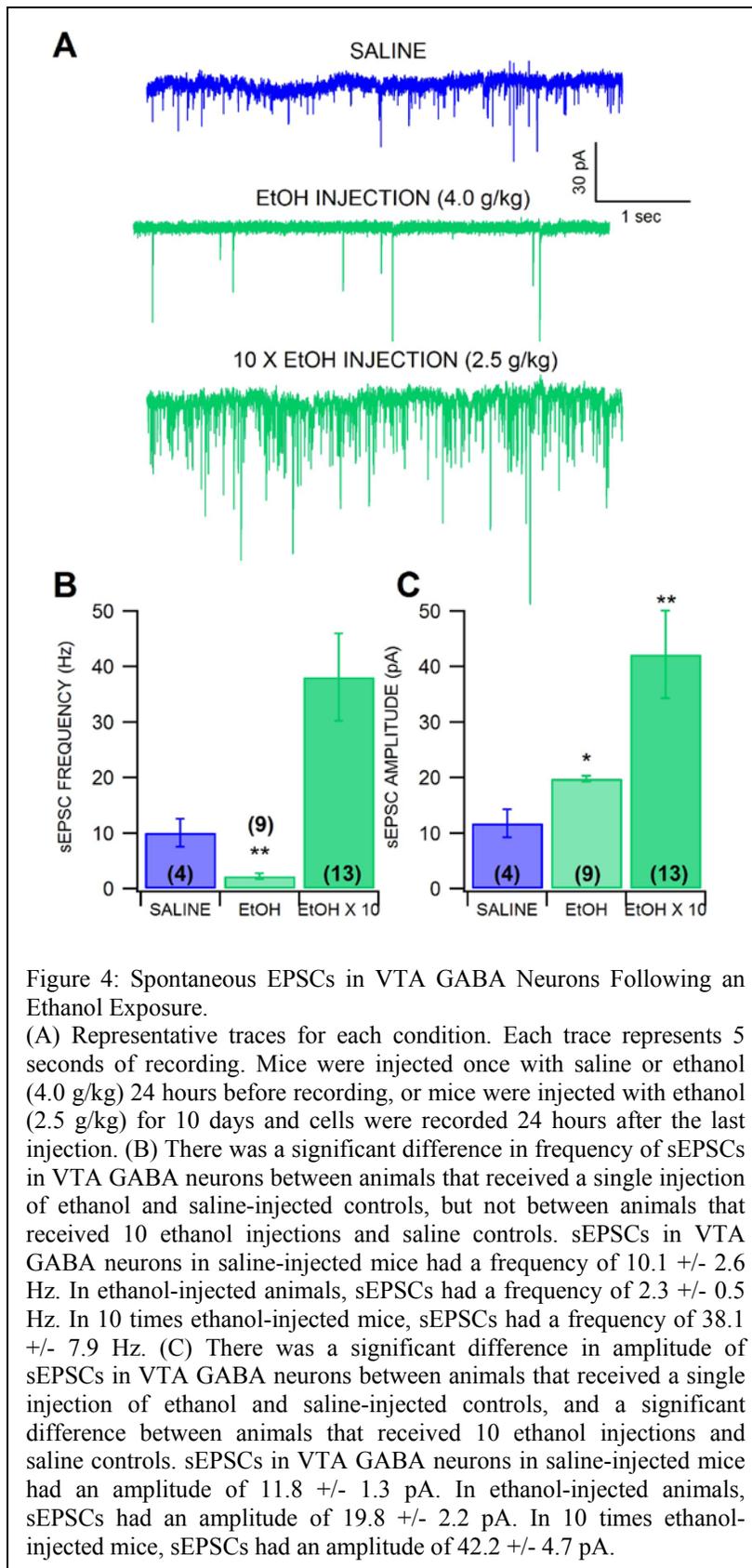
RESULTS

Drink-in-the-Dark Behavioral Measure of Ethanol Dependence

To assess the alcohol dependence of the animals in the CIE vapor chambers, we evaluated alcohol drinking using a DID paradigm. C57BL6 mice underwent a 5 day two-bottle choice alcohol drinking test for 2 hours per day on alternating weeks with bottles containing tap water or tap water plus ethanol (20 % v/v). Week 0 was the baseline alcohol drinking week. DID was then measured on even weeks, and



animals received CIE vapor on odd weeks of 16 hours per day CIE vapor for 4 days and 3 days of withdrawal. Control animals also performed the 5 day DID test on even weeks, but received air on odd weeks. During week 9, mice were kept in withdrawal and did not receive ethanol administration. Figure 3 shows the progression of alcohol consumed by each group. Weeks 4, 6, 8, and 10, show statistical significance between ethanol and air groups, which were analyzed by one-way ANOVA [week 4: $F_{(1,8)}=7.015$ and $p=0.029$; week 6: $F_{(1,8)}=13.757$ and $p=0.006$; week 8: $F_{(1,8)}=35.138$ and $p=0.0004$; week 10: $F_{(1,8)}=8.128$ and $p=0.021$]. In addition, the ethanol group consumed significantly more ethanol than they did during the baseline drinking test (Week



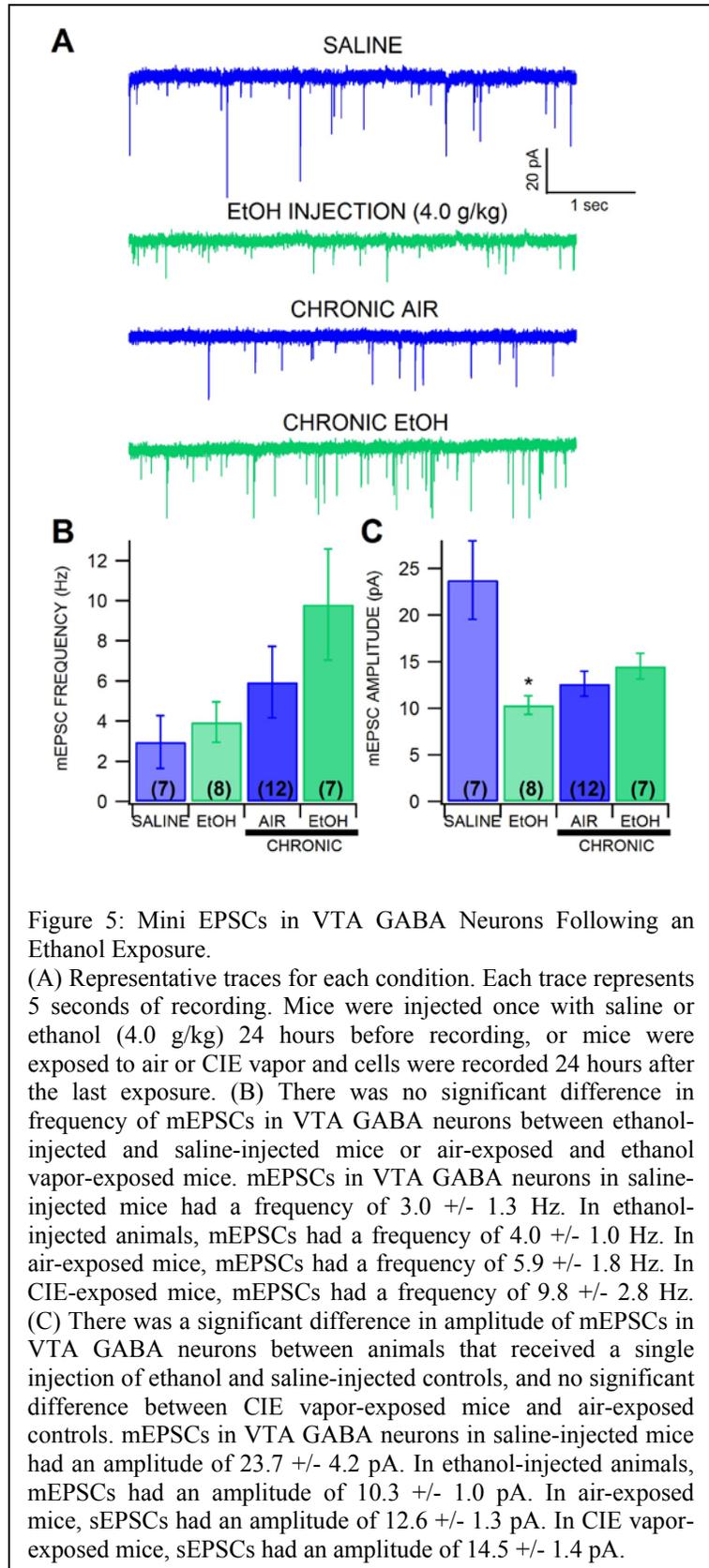
0) on weeks 6, 8, and 10
 [week 6: $F_{(1,8)}=12.087$ and $p=0.008$; week 8: $F_{(1,8)}=18.893$ and $p=0.002$; week 10: $F_{(1,8)}=5.839$ and $p=0.042$].
 There was no significant difference in preference for ethanol between groups (data not shown).

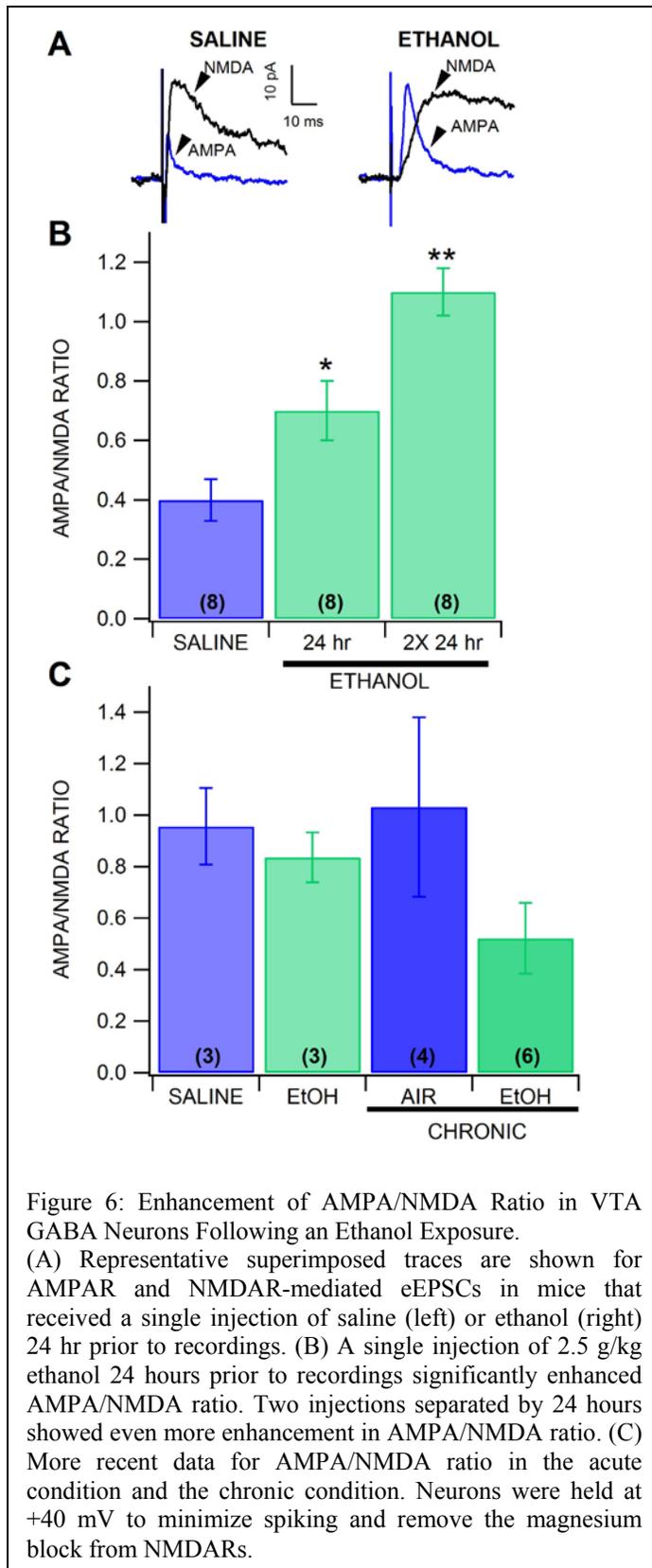
Glutamatergic Spontaneous Events Following Acute and Chronic Ethanol

Spontaneous EPSCs (sEPSCs) were measured as a sign of GLU plasticity. All recordings were taken 24 hours following the last ethanol exposure (Fig. 4). One-way ANOVA was run between conditions. There was a significant difference in sEPSC frequency in VTA GABA neurons between mice that received a single injection of

ethanol (4.0 g/kg) and saline-injected controls ($F_{(1,11)}=18.546$; $p=0.001$). There was also a significant difference in amplitude of sEPSCs between mice that received a single injection of ethanol (4.0 g/kg) and saline-injected controls ($F_{(1,11)}=5.521$; $p=0.039$), and a significant difference between mice that received 10 days of ethanol (2.5 g/kg) injections ($F_{(1,15)}=12.325$; $p=0.003$).

Mini EPSCs (mEPSCs) were also measured as a sign of GLU plasticity (Fig. 5). All minis were recorded in the presence of 500 μ M lidocaine to remove event-related potentials. All recordings were taken 24 hours following the last ethanol exposure. One-way ANOVA was run between conditions. There was no significant





difference in mEPSC frequency in VTA GABA neurons between animals that received a single injection of ethanol (4.0 g/kg) and saline-injected controls or between CIE vapor-exposed mice and air-exposed controls. There was a significant difference in amplitude of mEPSCs between mice that received a single injection of ethanol (4.0 g/kg) and saline-injected controls ($F_{(1,13)}=10.954$; $p=0.006$), but no significant difference between CIE vapor-exposed mice and air-exposed mice. We also have some data from cells that are recorded after a one hour presoak in ethanol, but results are mixed and unremarkable.

Despite statistical significance, I am hesitant about the validity of the data from these sEPSC and mEPSC experiments. Cells demonstrated lots of variability in EPSC rate and amplitude within the same recording and between cells. Further investigation is necessary

to determine if differences are real.

AMPA/NMDA Ratio Following Acute and Chronic Ethanol

As an additional measure of glutamatergic plasticity, we recorded AMPA/NMDA ratio on VTA GABA neurons. Although not published yet, the Steffensen lab had previously shown an increased AMPA/NMDA ratio recorded 24 hours after a single IP injection of ethanol (2.5 g/kg), which becomes even more pronounced after 2 days of ethanol injections (Fig. 6 A,B). However,

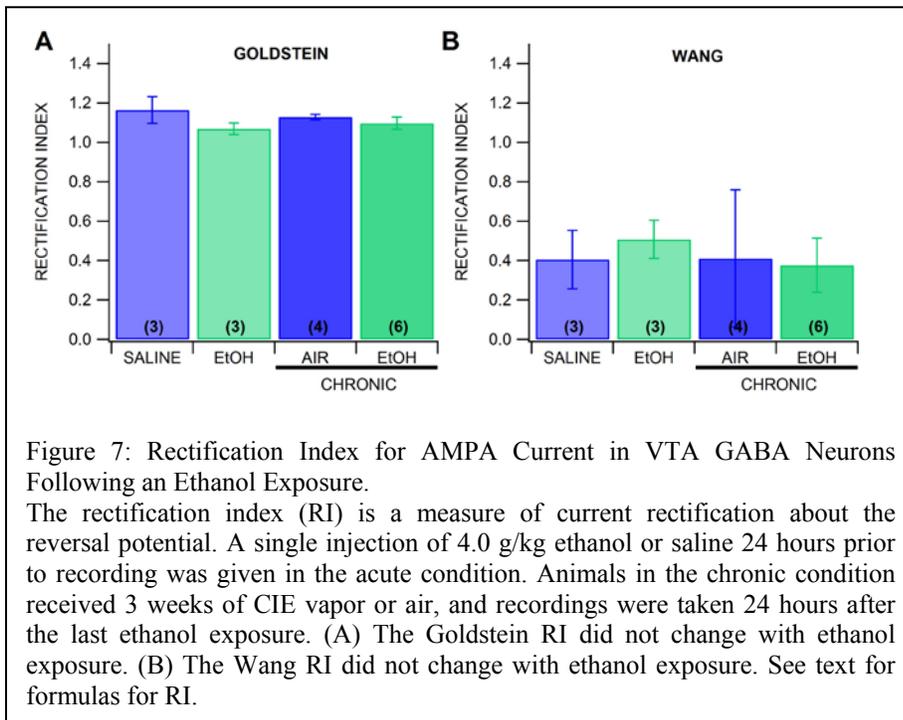


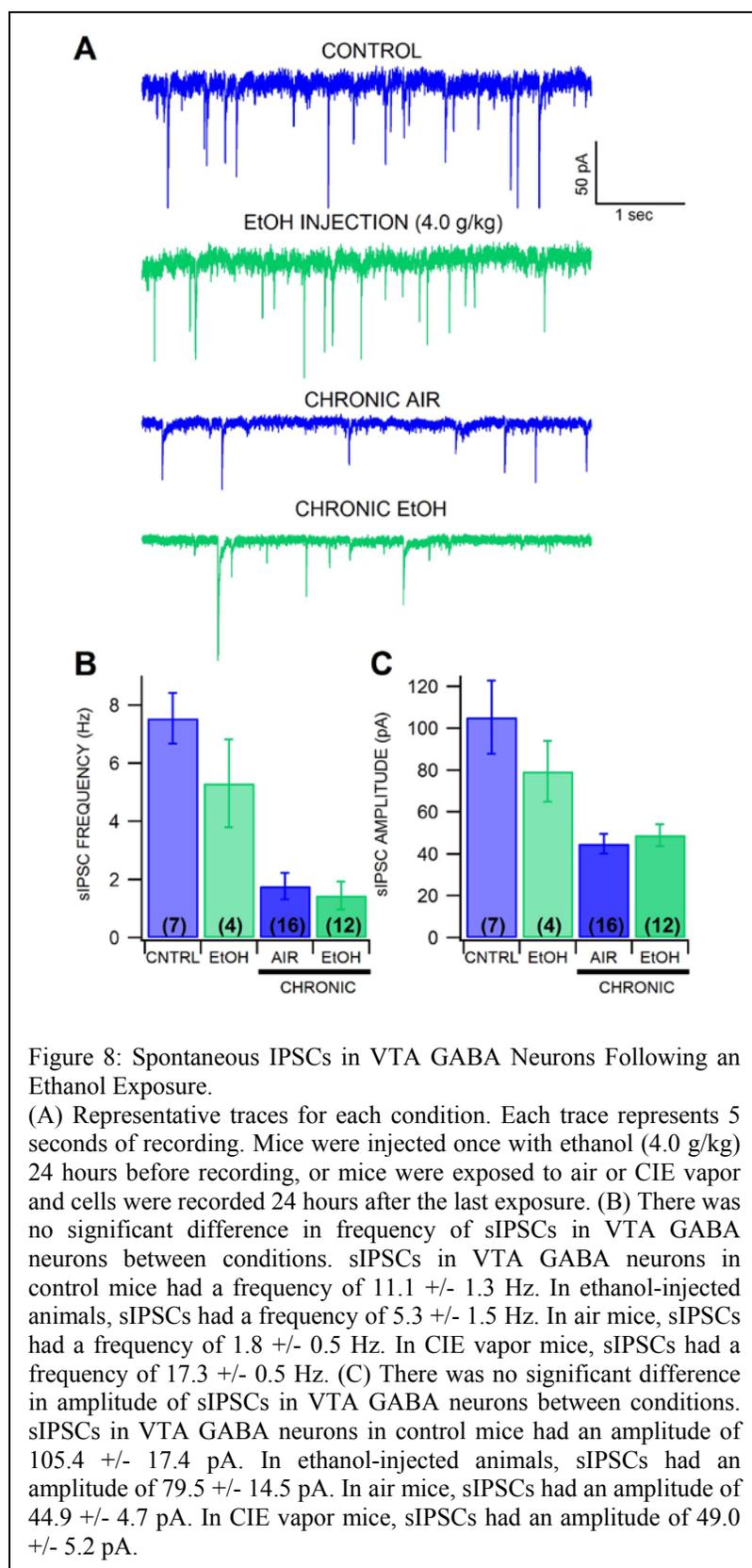
Figure 7: Rectification Index for AMPA Current in VTA GABA Neurons Following an Ethanol Exposure.

The rectification index (RI) is a measure of current rectification about the reversal potential. A single injection of 4.0 g/kg ethanol or saline 24 hours prior to recording was given in the acute condition. Animals in the chronic condition received 3 weeks of CIE vapor or air, and recordings were taken 24 hours after the last ethanol exposure. (A) The Goldstein RI did not change with ethanol exposure. (B) The Wang RI did not change with ethanol exposure. See text for formulas for RI.

we have not been able to replicate this effect more recently (Fig. 6 C). In the latest analysis, there was no significant difference in AMPA/NMDA ratio between any of the groups.

To further

investigate changes in AMPA receptor function, AMPA rectification was measured. As a reflection of AMPA rectification, we calculated a rectification index (RI) using two different methods, labeled as Goldstein RI and Wang RI for the authors of the papers (Goldstein, Lee et al. 1995, Wang and Gao 2012). Both measures of RI compare AMPA current at different voltages about the reversal potential. A Goldstein RI > 1.0 reflects an outward rectification, and a RI < 1.0 reflects an inward rectification. The Goldstein RI takes the reversal potential into account



mathematically, and the Wang RI is a direct comparison of current about the reversal potential. There was no significant difference between groups on either measurement of RI (Fig. 7).

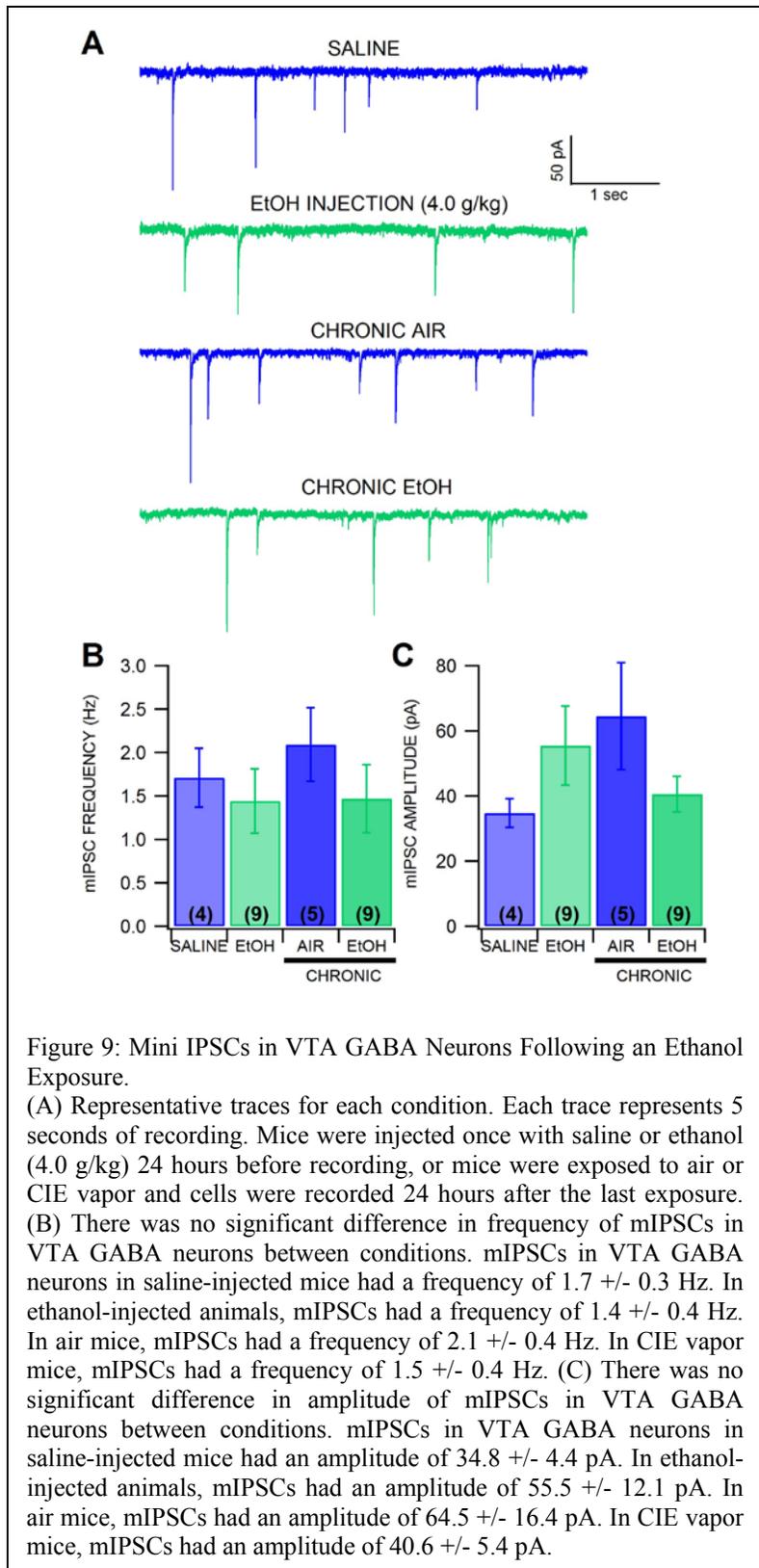
GABAergic Spontaneous Events Following Acute and Chronic Ethanol

Spontaneous IPSCs

(sIPSCs) were measured as a sign of GABA plasticity (Fig. 8). All recordings were taken 24 hours following the last ethanol exposure. One-way ANOVA was run between conditions. There was no significant difference in sIPSC frequency in VTA GABA neurons between mice that received a single injection of ethanol (4.0 g/kg) and controls, or between air-exposed and CIE vapor-exposed

mice in the chronic condition. There was also no significant difference in amplitude of sIPSCs between mice that received a single injection of ethanol (4.0 g/kg) and controls, or between air-exposed and CIE vapor-exposed mice.

Mini IPSCs (mIPSCs) were also measured as a sign of GABA plasticity (Fig. 9). All minis were recorded in the presence of 500 μ M lidocaine to remove event-related potentials. All recordings were taken 24 hours following the last ethanol exposure. One-way ANOVA was run between conditions. There was no significant difference in mIPSC frequency in VTA GABA neurons between animals that received a single injection of ethanol (4.0 g/kg) and saline-

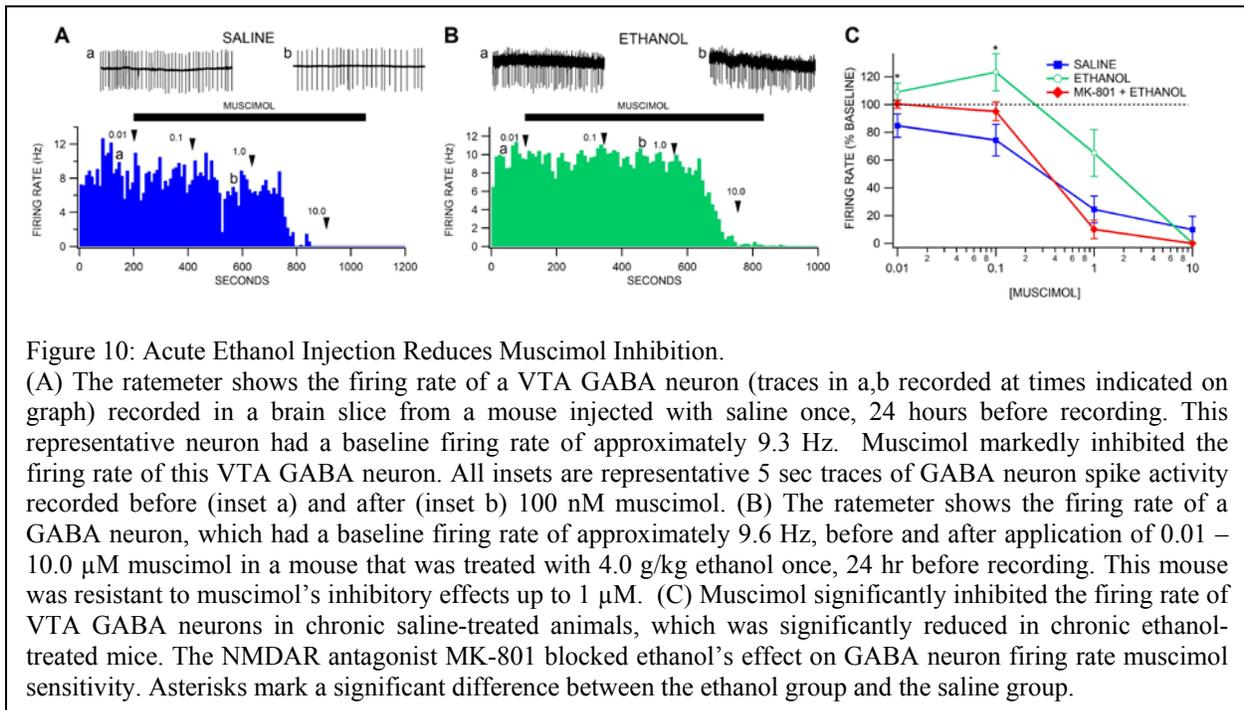


injected controls or between CIE vapor-exposed mice and air-exposed controls. There was no significant difference in amplitude of mIPSCs between mice that received a single injection of ethanol (4.0 g/kg) and saline-injected controls, and no significant difference between CIE vapor-exposed mice and air-exposed mice. We also have some data from cells that are recorded after a one hour presoak in ethanol, but results were mixed and unremarkable.

Muscimol Inhibition of Firing Rate Following Acute Ethanol

GAD GFP mice were given a 4.0 g/kg IP injection of ethanol. While a dose of 2.5 g/kg ethanol did not consistently intoxicate GAD GFP mice, 4.0 g/kg did. Firing rate was recorded in VTA GABA neurons 24 hours after ethanol or saline administration. Figure 10 illustrates that firing rate in VTA GABA neurons was less sensitive to superfusion of the GABA(A) agonist muscimol (0.01, 0.1, 1.0, 10.0 μ M) in animals treated with a single injection of ethanol.

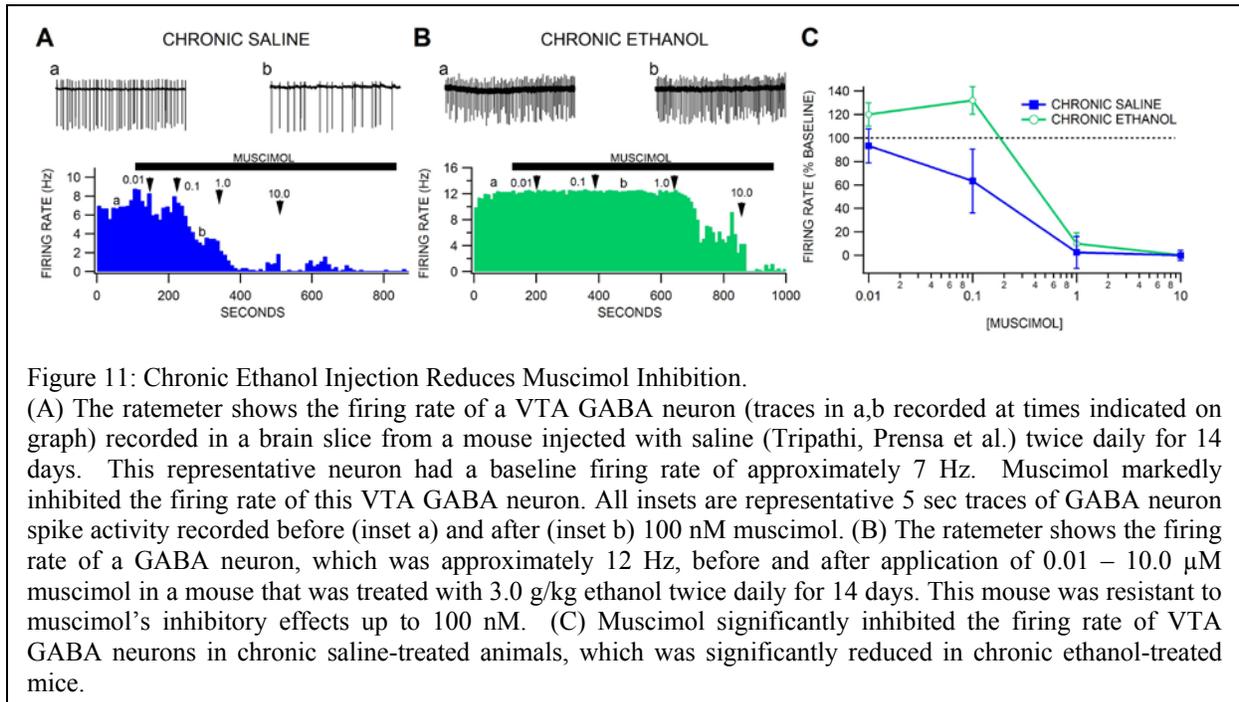
Interestingly, when the NMDAR antagonist MK-801 (0.5 mg/kg) was given as an IP injection 15 minutes before ethanol, this effect was blocked (Fig. 10 C), and VTA GABA neuron firing rate



remained sensitive to muscimol's inhibitory effects. A one-way ANOVA test was run on the normalized firing rate values for each dose of muscimol. The ethanol-injected group is significantly different from saline controls at 0.01 μM muscimol ($F_{(1,16)}=5.214$; $p=0.036$) and at 0.1 μM muscimol ($F_{(1,13)}=7.549$; $p=0.017$). The ethanol group is significantly different from the MK-801 and ethanol group at 1.0 μM muscimol ($F_{(1,7)}=11.05$; $p=0.013$).

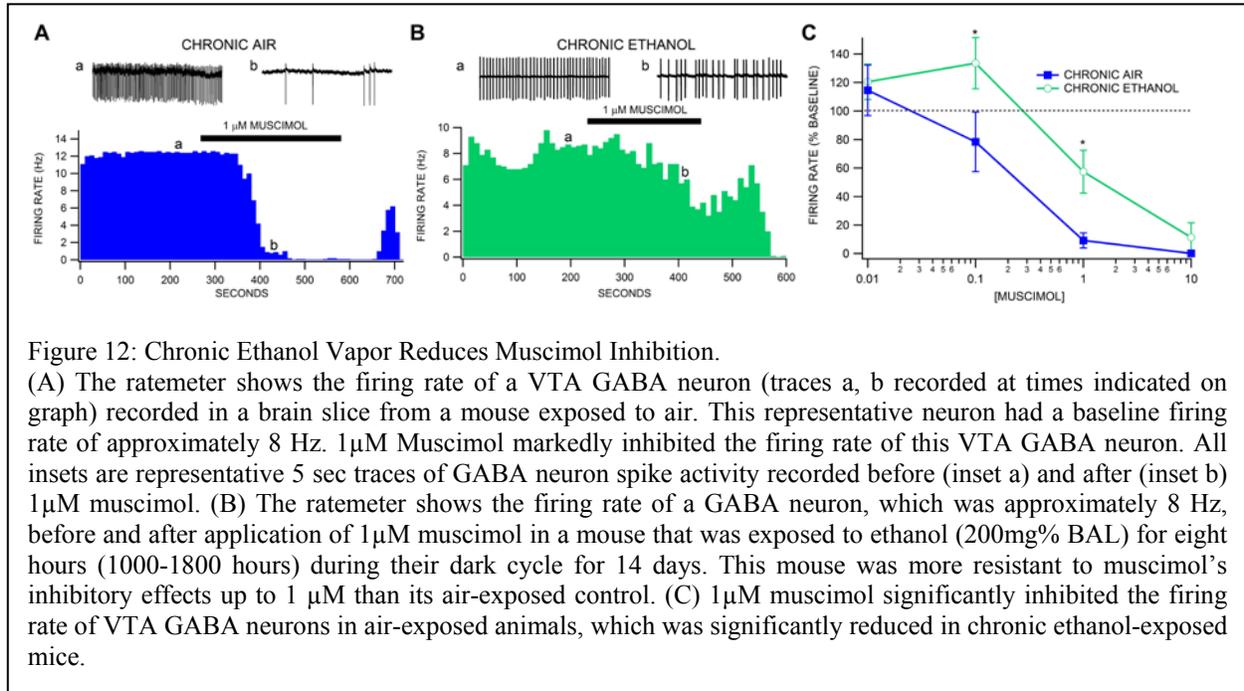
Muscimol Inhibition of Firing Rate Following Chronic Ethanol

After 14 days of twice daily injections of 3.0 g/kg ethanol, muscimol effects were evaluated on VTA GABA neuron firing rate in GAD GFP mice and compared to their saline-injected controls. Firing rate was measured 24 hours after the last administration of ethanol. Figure 11 illustrates that there was less sensitivity to muscimol (0.01, 0.1, 1.0, 10.0 μM) in animals treated with ethanol versus saline-injected controls.



Additionally, we measured VTA GABA neuron firing rate sensitivity to muscimol after 3 weeks of chronic intermittent ethanol in alcohol vapor chambers, compared to air controls.

Figure 12 illustrates that there was less sensitivity to muscimol (0.01, 0.1, 1.0, 10.0 μM) in animals treated with ethanol vapor versus air-exposed controls, similar to what was seen with chronic injected animals and single injected animals. A one-way ANOVA test was run on each dose of muscimol between ethanol and air groups. There is statistical significance at 100 nM muscimol ($F_{(1,26)}=4.470$; $p=0.044$) and 1.0 μM muscimol ($F_{(1,31)}=7.096$; $p=0.012$).



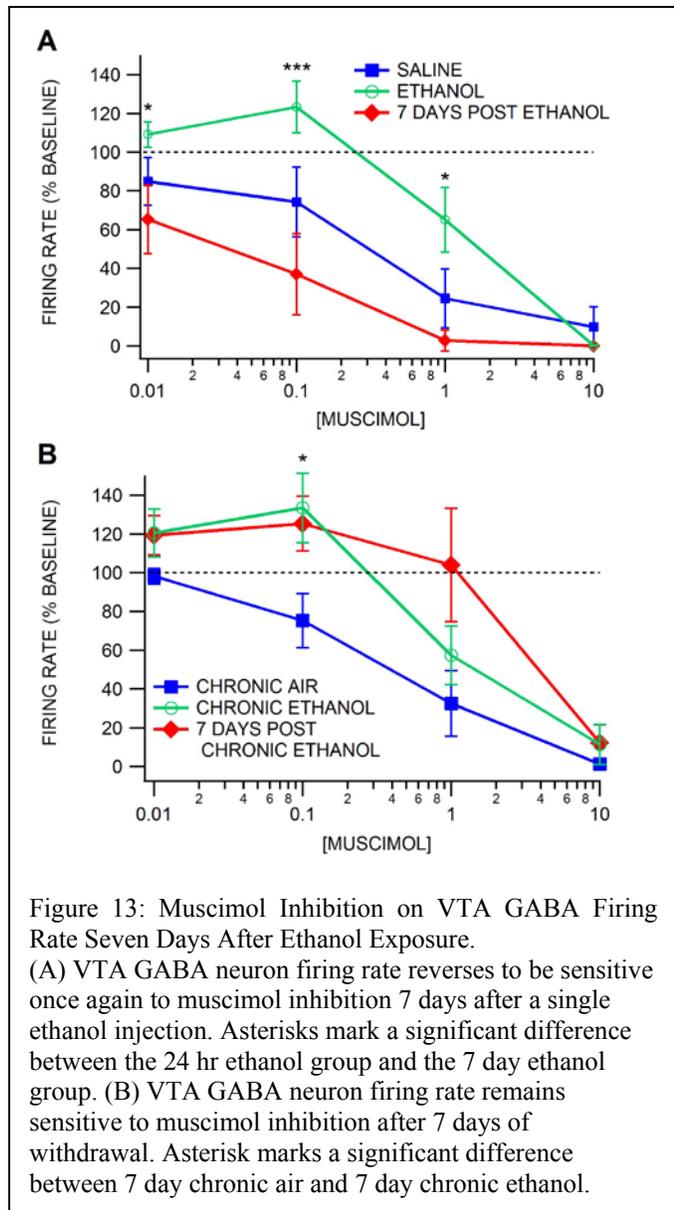
We also measured this phenomenon after 7 days of withdrawal from ethanol. Seven days after an administration of ethanol in the non-dependent condition, VTA GABA neurons were once again sensitive to muscimol's inhibitory effects (Fig. 13 A). The data from figure 10 is repeated in figure 13 as a reference. As measured in a one-way ANOVA, there was statistical significance between the ethanol-injected group measured 24 hours after administration and the ethanol-injected group measured 7 days after ethanol administration at 0.01 μM muscimol ($F_{(1,22)}=4.694$; $p=0.041$), 0.1 μM muscimol ($F_{(1,14)}=19.839$; $p=0.0005$), and 1.0 μM muscimol ($F_{(1,5)}=9.687$; $p=0.026$). There is no statistical significance between the 7 day post-ethanol group

and the saline-injected group. However, in the chronic condition, there was not a reversal after 7 days of withdrawal (Fig. 13 B). The data from the chronic ethanol group that was recorded after 24 hours of withdrawal (Fig. 12) is included as a reference. There is a statistically significant difference between the chronic ethanol group after 7 days of withdrawal and the chronic air group after 7 days of withdrawal at 0.1 μ M muscimol ($F_{(1,20)}=4.725$; $p=0.042$).

BDNF Expression in the VTA

Although not originally proposed in my prospectus, we have investigated BDNF levels in the VTA using an ELISA. Figure 14 illustrates the different levels of

BDNF expressed in a variety of ethanol exposure conditions. In the acute condition, ethanol-injected animals received 2.5 g/kg ethanol 24 hours prior to tissue collection and were compared to saline-injected controls. Animals in the chronic condition received 3 weeks of CIE vapor or air and tissue was taken 24 hours or 7 days after the last ethanol exposure as indicated. A one-way ANOVA test was run to compare groups. We found significantly elevated BDNF in the chronic ethanol group as compared to the chronic air group ($F_{(1,9)}=8.674$; $p=0.016$) and significantly



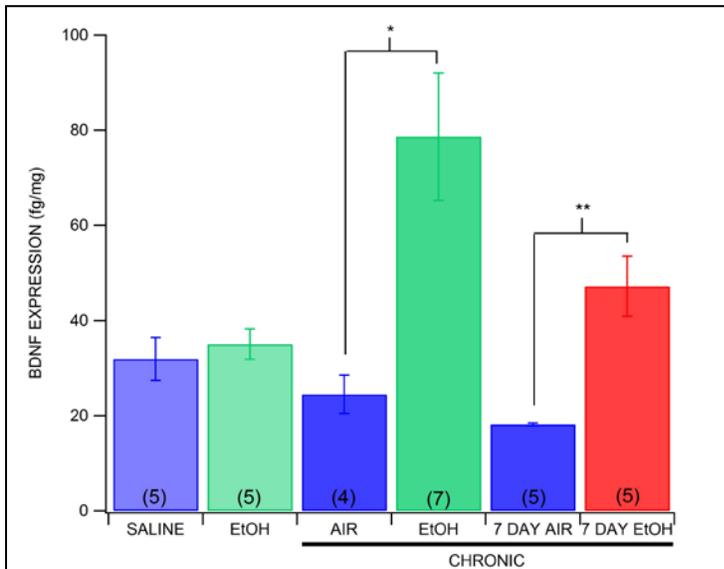


Figure 14: BDNF Expression Following Ethanol Exposure. The graph shows BDNF expression in tissue punches from the VTA in fg of BDNF per mg of tissue. Mice were treated with acute saline or EtOH (2.5 g/kg) 24 hrs prior to tissue collection. Animals in the chronic condition received 3 weeks of CIE vapor or air and tissue was collected 24 hrs or 7 days after the last EtOH exposure. Acute saline group had BDNF levels of 31.9 +/- 4.5 fg/mg. Acute EtOH (2.5 g/kg) had BDNF levels of 35.0 +/- 3.2 fg/mg. Chronic air had BDNF levels of 24.5 +/- 4.0 fg/mg. Chronic EtOH had BDNF levels of 78.6 +/- 13.4 fg/mg. Chronic air group that had 7 days of withdrawal had BDNF levels of 18.2 +/- 0.3 fg/mg. Chronic EtOH group that had 7 days of withdrawal from EtOH had BDNF levels of 47.2 +/- 6.3 fg/mg. Asterisks mark $p < 0.05$.

more BDNF in the chronic ethanol group withdrawn for 7 days as compared to the chronic air group removed from chambers for 7 days ($F_{(1,9)}=21.015$; $p=0.002$).

Chloride Reversal Potential with Perforated Patch Clamp

We have been struggling to get results for this experiment, largely because of difficulties obtaining perforated patches in older animals. The animals are a minimum of seven weeks old after completing a chronic ethanol treatment. We tried dissociating neurons from slices, but

it is difficult and produces about 1-2 neurons per slice in the dish. None of the neurons we observed from dissociating neurons in older animals were healthy enough to withstand a patch, despite changes to the dissociation method. One group was able to measure the chloride reversal potential in older mice using perforated patch in the hippocampus of brain slices (MacKenzie and Maguire 2015). We are still optimistic that we may be able to obtain perforated patches in the VTA in brain slices, even though the VTA can be more difficult than the hippocampus. Currently, we are attempting to pretreat the slice with a one hour perfusion of pronase (1 mg/6 ml ACSF) to open up the tissue and improve visibility. I believe we had one successful

perforated patch, but unfortunately, we lost it before we were able to record any data. We will continue to attempt perforated patch in brain slices through the summer.

As discussed with the committee at our last joint meeting, we may branch out to alternate methods of testing our chloride gradient hypothesis, including intracellular chloride imaging techniques. I think electrophysiology is still the best tool to test our chloride gradient hypothesis, but other methods may be more feasible.

DISCUSSION

General Focus of the Study

The overall aim of this study was to investigate electrophysiological components of the mechanism behind alcohol's effect on the brain. The focus was on VTA GABA neuron hyperexcitability during withdrawal from ethanol (Gallegos, Criado et al. 1999). We describe plasticity in excitatory and inhibitory synapses on VTA GABA neurons, as well as an insensitivity of VTA GABA neuron firing rate to muscimol during withdrawal from ethanol. The results of this study describe alterations in VTA GABA neuron synaptic activity and firing rate that contribute to the mechanism of alcohol in the brain during the withdrawal state. Implications of this study include a molecular mechanism for alcohol's effect on VTA GABA neurons and a cellular correlate to the hedonic drive to seek alcohol during a period of abstinence.

One limitation of the study is the heterogeneity of VTA GABA neurons. The Steffensen lab and others have long held a belief that there are multiple populations of GABA neurons in the VTA that are impossible to distinguish using fluorescence identification in GAD GFP mice. The methods used in the present study do not allow for differentiation of the different GABA populations or the specific inputs seen to those GABA neurons (e.g. rostromedial tegmental nucleus, lateral habenula, reciprocal projections from the striatum, etc.). The VTA is proving to be a diverse area with a variety of functions and responses to novel signals, including reward (Creed, Ntamati et al. 2014, Lammel, Lim et al. 2014, Sanchez-Catalan, Kauffling et al. 2014). In addition to the heterogeneity of VTA GABA neurons, ethanol is known for its variety of binding capabilities in the brain. This study attempted an umbrella investigation of changes in excitatory and inhibitory synaptic events in VTA GABA neurons, but is not completely successful because of the difficulty in isolating effects of ethanol on VTA GABA neurons and their inputs.

Excitatory Plasticity to VTA GABA Neurons by Alcohol

Results from the excitatory synaptic events experiments in the present study are not clear enough to draw a firm conclusion, despite some statistical significance. Undoubtedly, GLU plasticity in the VTA is involved in the path to addiction, but the variability seen in VTA GABA neurons may be masking an alcohol effect in excitatory synapses on VTA GABA neurons. We are interested in increasing sample sizes, especially of sEPSCs in VTA GABA neurons in saline-injected controls, to make more definite conclusions. It may be possible to use a more advanced method, like optogenetics or chemogenetics, which isolates specific GLUergic inputs to VTA GABA neurons to study EPSCs.

The AMPA/NMDA ratio data is currently inconclusive because of the inability to replicate the previous data. Many drugs of abuse have been linked to increases in the AMPA/NMDA ratio in VTA DA neurons (Ungless, Whistler et al. 2001, Saal, Dong et al. 2003), but VTA GABA neurons are far less understood. We are confident that VTA GABA neurons are a relevant target for ethanol in the brain, and that NMDAR mediated plasticity is involved, but it is unclear if AMPA/NMDA ratio is affected by alcohol. AMPA/NMDA ratio is sensitive to many stimuli, so our data may be confounded by random variation in the animal's environment or by the nature of the treatment. We also showed no change in rectification of the current through AMPA receptors. Perhaps the subunit composition of AMPA receptors is not changing, and so channel conductance remains the same. It is possible that the AMPA/NMDA ratio is decreasing after chronic ethanol, as has been seen with chronic cocaine in GABA medium spiny neurons in the NAc shell (Mameli, Halbout et al. 2009, Wolf and Ferrario 2010). To further our efforts, we would like to collect more experiments and reanalyze our data with a different measure of the RI and look at the AMPA/NMDA ratio with the AMPA current measured at a holding potential of -70 mV over the NMDA current at +40 mV. Additional investigation of the

AMPA/NMDA ratio and AMPA rectification is necessary to make a conclusive statement about ethanol's effect on AMPA and NMDA plasticity in VTA GABA neurons.

Inhibitory Plasticity to VTA GABA Neurons by Alcohol

Results from the frequency and amplitude of inhibitory synaptic events are not clear enough to draw a firm conclusion. The present study does not show a statistically significant difference between groups. Perhaps IPSCs are not changing in frequency and amplitude, but only in function, as described below. There is a potential age difference in sIPSCs, as seen in the difference between control groups in the acute and chronic phases. Different stages of development show differences in sIPSC frequency and amplitude in various brain regions, including the prefrontal cortex and the substantia nigra (Chudomel, Herman et al. 2009, Cui, Wang et al. 2010). However, there is little evidence for sIPSCs in GABA neurons in mice older than 30 days, and little evidence about the plasticity over time of sIPSCs in the VTA. Increasing sample size may help solidify some age-related differences in sIPSCs in VTA GABA neurons. We also believe that more advanced techniques that isolate specific inhibitory inputs to VTA GABA neurons may help in investigating inhibitory synaptic events in alcohol dependence.

Functional Shift of GABA(A) Receptor Due to Alcohol

Our hypothesis regarding the function of the GABA(A)R in alcohol dependence revolves around BDNF and intracellular anion concentrations. Results from this phase of my thesis are quite promising, and show some correlation with the work done by the Steffensen and van der Kooy labs on opiate dependence. VTA GABA neurons decrease in sensitivity to the inhibitory effects of the GABA(A)R agonist muscimol due to exposure to alcohol, both acutely and chronically. This measure has been used as evidence of a change in BDNF signaling and the chloride gradient in opiate dependence (Vargas-Perez, Bahi et al. 2014). These results are

complemented by the data showing an increase in BDNF levels in the VTA after a chronic exposure to ethanol vapor, especially since BDNF is even higher after a longer period of withdrawal. BDNF activation of the TrkB receptor leads to downregulation of the KCC2 transporter and an increase in the activity of carbonic anhydrase (CA). These downstream effects result in less Cl^- being transported out of the cell and an increase in HCO_3^- concentration, respectively. The increase in intracellular anions causes a shift in the Cl^- reversal potential. Activating the GABA(A)R, which is a Cl^- channel, during withdrawal would cause a depolarizing response due to efflux of Cl^- , and may contribute to the hyperexcitability of VTA GABA neurons observed during withdrawal from alcohol (Gallegos, Criado et al. 1999). We also want to examine the data for differences within VTA GABA neurons sensitivity to muscimol as a possible differentiation between VTA GABA neuron populations. Future studies can investigate the source of BDNF elevation and the role of the TrkB receptor, along with KCC2 and CA activity, in ethanol dependence and VTA GABA neuron firing rate sensitivity to muscimol. We are particularly interested in pursuing DID or operant self-administration data after a local VTA knockdown of TrkB expression with lentiviral constructs containing interfering ribonucleic acid (siRNA) to see if TrkB receptor activation is required for ethanol-seeking behavior.

We were surprised to find that a single administration of ethanol caused a decrease in sensitivity of VTA GABA neuron firing rate to muscimol's inhibitory effects. As stated in our first hypothesis, we thought acute effects of ethanol in the VTA would be mediated through GLU plasticity, but here we see a contribution from the GABA(A)R. An important point of this study is that the decreased sensitivity of VTA GABA neuron firing rate to muscimol due to acute ethanol was blocked by concomitant administration of the NMDAR antagonist MK-801. This

finding proves that NMDA activation is required for acute ethanol's effect on VTA GABA neuron muscimol sensitivity. Because the path is GLU-dependent, at least at first, there must be some connection between NMDA activation and the altered function of GABA(A)Rs on VTA GABA neurons. Future studies could look at an interaction between GLU and GABA plasticity, or a link between acute NMDA activation and an increase in intracellular chloride concentration. If some NMDA-mediated signaling, perhaps through calcium-dependent pathways, resulted in altered expression or phosphorylation of KCC2 (Friedel, Kahle et al. 2015) and/or carbonic anhydrase, there could be a common end state 24 hours after an alcohol administration in both the non-dependent and ethanol-dependent conditions. Alternatively, blocking NMDARs may result in some upstream hindrance to learning the novel rewarding stimulus with an end result of decreased plasticity in the VTA due to ethanol. We propose that the mechanisms behind this data differ as described between the single injection of ethanol and the CIE vapor exposure, but we remain open that the two could be linked through a common mechanism. Some research has looked at a link between BDNF signaling and NMDA receptor activation with alcohol and amphetamine exposure (Kolb, Trettel et al. 2005, Fuller, Murray et al. 2015). There is a possibility that BDNF or NMDA could be triggering the other, and the downstream effects of such signaling produce the resistance to muscimol inhibition. Future experiments will look at blocking NMDA during the chronic exposure to ethanol.

Another interesting phenomenon in the muscimol firing rate experiments is the reversal of VTA GABA neuron firing rate sensitivity to muscimol seven days after a single ethanol administration and the persistence of the same effect after seven days of withdrawal from chronic ethanol. This finding is very important. Not only does this data suggest a difference between the drive states in non-dependent versus dependent conditions, but it also may signify a difference in

the mechanism of acute versus chronic ethanol. VTA GABA neurons in the dependent state must have longer-lasting functional changes, which could be due to differences in protein expression, transcriptional factors, or regulation of ion concentrations. As described above, we propose the hypothesis for the dependent condition where increased extracellular BDNF signaling and decreased KCC2 expression result in a long-term increase of intracellular anions. We also suggest that a single administration of ethanol causes a change in phosphorylation of KCC2 through calcium-dependent signaling, thereby acutely increasing intracellular Cl⁻ in a reversible manner. Future studies are needed to investigate these claims and determine the mechanism of alcohol in the brain that leads to its habit-forming properties.

In contrast to the theories outlined above regarding the Cl⁻ gradient and the reversal potential of the GABA(A) receptor, the observed differences could be explained by other modifications to GABA(A) receptors. There could be a decrease in number of GABA(A) receptors due to alcohol exposure, decreasing VTA GABA neuron sensitivity to muscimol. There could also be a change in subunit composition of the GABA(A) receptor, changing its affinity for muscimol or its conductance of chloride ions. We are currently in the process of analyzing GABA(A) subunit composition in VTA GABA neurons after an exposure to chronic alcohol.

Overall Conclusions

Although some of our data was inconclusive, some results were robust, and we can make some comments on our original hypotheses. Our first hypothesis stated that GLU plasticity would dominate in the acute phase of alcohol exposure. Previous research has shown GLU involvement in the beginning of alcohol dependence (Saal, Dong et al. 2003, Ding, Engleman et al. 2012), including some data in the present study. However, the muscimol inhibition of firing

rate data shows that the function of the GABA(A) receptor is changing after a single ethanol exposure, so we cannot rule out the parallel involvement of GABA plasticity in the short term.

Second, our next hypothesis stated that GABA plasticity would be the primary factor in an alcohol dependent condition. We cannot conclude that this statement is entirely correct, but we leave open the possibility that GABA plasticity is the main element over GLU plasticity, where both are involved to some degree.

Our final hypothesis stated that a shift in the Cl^- reversal potential caused the change in muscimol sensitivity of VTA GABA neuron firing rate after exposure to ethanol. Our data support this hypothesis, but we need stronger evidence to make a final conclusion. We believe success in the perforated patch clamp experiments on the chloride reversal potential through the GABA(A) receptor will uphold or reject the present hypothesis.

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 **65**(8): 804-813.
- Berke, J. D. and S. E. Hyman (2000). "Addiction, dopamine, and the molecular mechanisms of memory." Neuron **25**(3): 515-532.
- Bocklisch, C., V. Pascoli, J. C. Wong, D. R. House, C. Yvon, M. de Roo, K. R. Tan and C. Luscher (2013). "Cocaine disinhibits dopamine neurons by potentiation of GABA transmission in the ventral tegmental area." Science **341**(6153): 1521-1525.
- Bonci, A. and R. C. Malenka (1999). "Properties and plasticity of excitatory synapses on dopaminergic and GABAergic cells in the ventral tegmental area." J Neurosci **19**(10): 3723-3730.
- Bouchery, E. E., H. J. Harwood, J. J. Sacks, C. J. Simon and R. D. Brewer (2011). "Economic costs of excessive alcohol consumption in the U.S., 2006." Am J Prev Med **41**(5): 516-524.
- Brodie, M. S. (2002). "Increased ethanol excitation of dopaminergic neurons of the ventral tegmental area after chronic ethanol treatment." Alcohol Clin Exp Res **26**(7): 1024-1030.
- Cagetti, E., J. Liang, I. Spigelman and R. W. Olsen (2003). "Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of GABAA receptors." Mol Pharmacol **63**(1): 53-64.
- 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.
- Charlton, M. E., P. M. Sweetnam, L. W. Fitzgerald, R. Z. Terwilliger, E. J. Nestler and R. S. Duman (1997). "Chronic ethanol administration regulates the expression of GABAA receptor alpha 1 and alpha 5 subunits in the ventral tegmental area and hippocampus." J Neurochem **68**(1): 121-127.
- Chudomel, O., H. Herman, K. Nair, S. L. Moshe and A. S. Galanopoulou (2009). "Age- and gender-related differences in GABAA receptor-mediated postsynaptic currents in GABAergic neurons of the substantia nigra reticulata in the rat." Neuroscience **163**(1): 155-167.

- Coull, J. A., D. Boudreau, K. Bachand, S. A. Prescott, F. Nault, A. Sik, P. De Koninck and Y. De Koninck (2003). "Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain." Nature **424**(6951): 938-942.
- Creed, M. C., N. R. Ntamati and K. R. Tan (2014). "VTA GABA neurons modulate specific learning behaviors through the control of dopamine and cholinergic systems." Front Behav Neurosci **8**: 8.
- Cui, J., F. Wang, K. Wang and H. Xiang (2010). "GABAergic signaling increases through the postnatal development to provide the potent inhibitory capability for the maturing demands of the prefrontal cortex." Cell Mol Neurobiol **30**(4): 543-555.
- Diana, M., M. Pistis, S. Carboni, G. L. Gessa and Z. L. Rossetti (1993). "Profound decrement of mesolimbic dopaminergic neuronal activity during ethanol withdrawal syndrome in rats: electrophysiological and biochemical evidence." Proc Natl Acad Sci U S A **90**(17): 7966-7969.
- Ding, Z. M., E. A. Engleman, Z. A. Rodd and W. J. McBride (2012). "Ethanol increases glutamate neurotransmission in the posterior ventral tegmental area of female wistar rats." Alcohol Clin Exp Res **36**(4): 633-640.
- Friedel, P., K. T. Kahle, J. Zhang, N. Hertz, L. I. Pisella, E. Buhler, F. Schaller, J. Duan, A. R. Khanna, P. N. Bishop, K. M. Shokat and I. Medina (2015). "WNK1-regulated inhibitory phosphorylation of the KCC2 cotransporter maintains the depolarizing action of GABA in immature neurons." Sci Signal **8**(383): ra65.
- Fuller, J. J., R. C. Murray and K. A. Horner (2015). "D-Amphetamine withdrawal-induced decreases in brain-derived neurotrophic factor in sprague-dawley rats are reversed by treatment with ketamine." Neuropharmacology **97**: 7-17.
- Gallegos, R. A., J. R. Criado, R. S. Lee, S. J. Henriksen and S. C. Steffensen (1999). "Adaptive responses of GABAergic neurons in the ventral tegmental area to chronic ethanol." J. Pharmacol. Exp. Ther. **291**: 1045-1053.
- Goldstein, P. A., C. J. Lee and A. B. MacDermott (1995). "Variable distributions of Ca(2+)-permeable and Ca(2+)-impermeable AMPA receptors on embryonic rat dorsal horn neurons." J Neurophysiol **73**(6): 2522-2534.
- Hopf, F. W., M. Martin, B. T. Chen, M. S. Bowers, M. M. Mohamedi and A. Bonci (2007). "Withdrawal from intermittent ethanol exposure increases probability of burst firing in VTA neurons in vitro." J Neurophysiol **98**(4): 2297-2310.
- Hubner, C. A., V. Stein, I. Hermans-Borgmeyer, T. Meyer, K. Ballanyi and T. J. Jentsch (2001). "Disruption of KCC2 reveals an essential role of K-Cl cotransport already in early synaptic inhibition." Neuron **30**(2): 515-524.
- 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.

- Kaila, K., J. Voipio, P. Paalasmaa, M. Pasternack and R. A. Deisz (1993). "The role of bicarbonate in GABAA receptor-mediated IPSPs of rat neocortical neurones." J Physiol **464**: 273-289.
- Kolb, J. E., J. Trettel and E. S. Levine (2005). "BDNF enhancement of postsynaptic NMDA receptors is blocked by ethanol." Synapse **55**(1): 52-57.
- Lammel, S., B. K. Lim and R. C. Malenka (2014). "Reward and aversion in a heterogeneous midbrain dopamine system." Neuropharmacology **76 Pt B**: 351-359.
- Laviolette, S. R., R. A. Gallegos, S. J. Henriksen and D. van der Kooy (2004). "Opiate state controls bi-directional reward signaling via GABAA receptors in the ventral tegmental area." Nat Neurosci **7**(2): 160-169.
- Luscher, C. (2013). "Drug-evoked synaptic plasticity causing addictive behavior." J Neurosci **33**(45): 17641-17646.
- MacKenzie, G. and J. Maguire (2015). "Chronic stress shifts the GABA reversal potential in the hippocampus and increases seizure susceptibility." Epilepsy Res **109**: 13-27.
- Mameli, M., B. Halbout, C. Creton, D. Engblom, J. R. Parkitna, R. Spanagel and C. Luscher (2009). "Cocaine-evoked synaptic plasticity: persistence in the VTA triggers adaptations in the NAc." Nat Neurosci **12**(8): 1036-1041.
- 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.
- Nugent, F. S., A. R. Hwong, Y. Udaka and J. A. Kauer (2008). "High-frequency afferent stimulation induces long-term potentiation of field potentials in the ventral tegmental area." Neuropsychopharmacology **33**(7): 1704-1712.
- 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.
- Numan, S., S. B. Lane-Ladd, L. Zhang, K. H. Lundgren, D. S. Russell, K. B. Seroogy and E. J. Nestler (1998). "Differential regulation of neurotrophin and trk receptor mRNAs in catecholaminergic nuclei during chronic opiate treatment and withdrawal." J Neurosci **18**(24): 10700-10708.
- Rahman, S. and W. J. McBride (2000). "Feedback control of mesolimbic somatodendritic dopamine release in rat brain." J Neurochem **74**(2): 684-692.
- Rivera, C., H. Li, J. Thomas-Crusells, H. Lahtinen, T. Viitanen, A. Nanobashvili, Z. Kokaia, M. S. Airaksinen, J. Voipio, K. Kaila and M. Saarma (2002). "BDNF-induced TrkB activation down-regulates the K⁺-Cl⁻ cotransporter KCC2 and impairs neuronal Cl⁻ extrusion." J Cell Biol **159**(5): 747-752.

- Rivera, C., J. Voipio, J. A. Payne, E. Ruusuvuori, H. Lahtinen, K. Lamsa, U. Pirvola, M. Saarma and K. Kaila (1999). "The K⁺/Cl⁻ co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation." Nature **397**(6716): 251-255.
- Saal, D., Y. Dong, A. Bonci and R. C. Malenka (2003). "Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons." Neuron **37**(4): 577-582.
- Sanchez-Catalan, M. J., J. Kaufling, F. Georges, P. Veinante and M. Barrot (2014). "The antero-posterior heterogeneity of the ventral tegmental area." Neuroscience **282C**: 198-216.
- Staley, K. J., B. L. Soldo and W. R. Proctor (1995). "Ionic mechanisms of neuronal excitation by inhibitory GABA_A receptors [see comments]." Science **269**(5226): 977-981.
- 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.
- Steffensen, S. C., C. H. Walton, D. M. Hansen, J. T. Yorgason, R. A. Gallegos and J. R. Criado (2009). "Contingent and non-contingent effects of low-dose ethanol on GABA neuron activity in the ventral tegmental area." Pharmacol Biochem Behav **92**(1): 68-75.
- Stein, V. and R. A. Nicoll (2003). "GABA generates excitement." Neuron **37**(3): 375-378.
- Sun, M. K. and D. L. Alkon (2001). "Pharmacological enhancement of synaptic efficacy, spatial learning, and memory through carbonic anhydrase activation in rats." J Pharmacol Exp Ther **297**(3): 961-967.
- 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.
- 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.
- Thompson, S. M., R. A. Deisz and D. A. Prince (1988). "Relative contributions of passive equilibrium and active transport to the distribution of chloride in mammalian cortical neurons." J Neurophysiol **60**(1): 105-124.
- Ting, A. K. R. and D. van der Kooy (2012). "The neurobiology of opiate motivation." Cold Spring Harb Perspect Med **2**(10).
- 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.

- Ungless, M. A., J. L. Whistler, R. C. Malenka and A. Bonci (2001). "Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons." Nature **411**(6837): 583-587.
- van Huijstee, A. N. and H. D. Mansvelder (2014). "Glutamatergic synaptic plasticity in the mesocorticolimbic system in addiction." Front Cell Neurosci **8**: 466.
- Vargas-Perez, H., A. Bahi, M. R. Bufalino, A. K. R. Ting, G. Maal-Bared, J. Lam, A. Fahmy, L. Clarke, J. K. Blanchard, B. R. Larsen, S. Steffensen, J. Dreyer and D. Van der Kooy (2014). "BDNF signaling in the VTA links the drug dependent state to drug withdrawal aversions." J. Neurosci. **In Press**.
- Vargas-Perez, H., R. T. Kee, C. H. Walton, D. M. Hansen, R. Razavi, L. Clarke, M. R. Bufalino, D. W. Allison, S. C. Steffensen and D. van der Kooy (2009). "Ventral tegmental area BDNF induces an opiate-dependent-like reward state in naive rats." Science **324**(5935): 1732-1734.
- Viitanen, T., E. Ruusuvuori, K. Kaila and J. Voipio (2010). "The K⁺-Cl cotransporter KCC2 promotes GABAergic excitation in the mature rat hippocampus." J Physiol **588**(Pt 9): 1527-1540.
- Wang, H. X. and W. J. Gao (2012). "Prolonged exposure to NMDAR antagonist induces cell-type specific changes of glutamatergic receptors in rat prefrontal cortex." Neuropharmacology **62**(4): 1808-1822.
- Wise, R. A. (2008). "Dopamine and reward: the anhedonia hypothesis 30 years on." Neurotox Res **14**(2-3): 169-183.
- Wolf, M. E. and C. R. Ferrario (2010). "AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine." Neurosci Biobehav Rev **35**(2): 185-211.
- 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.

CURRICULUM VITAE

Ashley Cerise Nelson

Education

- 1/2015—8/2016 MS in Neuroscience Brigham Young University
- Thesis: “Glutamate and GABA Receptor-Mediated Plasticity in the Mesolimbic Dopamine System by Alcohol”
- 8/2011—12/2014 BS in Neuroscience, Chemistry minor Brigham Young University
- GPA 3.95

Research Experience

- 1/2013—present Research Assistant Brigham Young University
- Principal Investigator: Scott Steffensen, PhD (scott_steffensen@byu.edu)
 - Investigate the neural substrates involved in drug addiction
 - Patch clamp electrophysiology in brain tissue slices
 - Fast-scan cyclic voltammetry (FSCV) in brain tissue slices
 - Supervise a team comprised of undergraduates, graduate students, and one post-doctoral fellow
 - Manage and breed a mouse colony
- 5/2011—8/2011 Research Intern Arizona State University
- Principal Investigator: Jeffrey Kleim, PhD (jkleim@asu.edu)
 - Supervisor: Nagheme Thomas (nagheme.thomas@asu.edu)
 - Investigate novel rehabilitation methods for stroke patients
 - Behavioral experiments with rats
 - Immunohistochemistry

Teaching Experience

- 1/2016—2/2016 Neurobiology Teaching Practicum Brigham Young University
- Neuroscience 205, introductory course for Neuroscience majors
 - Lecture on the fundamentals of neuroscience for one-third of the semester
 - Administer on-line and in-class quizzes and one exam for the chapters taught
 - Meet with students during office hours
- 1/2015—4/2015 Teaching Assistant Brigham Young University
- Neuroscience 481, Advanced Neuroscience Laboratory
 - Supervise student lab work, prepare lab reagents and supplies
 - Hold review sessions
 - Grade assignments and tests

4/2012—12/2013 Teaching Assistant Brigham Young University

- MMBio 241, Molecular and Cellular Biology Laboratory
- Give lectures and supervise student lab work
- Prepare lab reagents and supplies
- Hold review sessions
- Grade assignments and tests

Grants, Scholarships, and Awards

2016 3 Minute Thesis University Competition First Place (Presentation Competition; \$5000)

2016 3 Minute Thesis Life Sciences Finalist (Presentation Competition; \$500)

2015 BYU Graduate Research Travel Award (Poster presentation at Neuroscience Meeting)

2015 BYU Hinckley Research Travel Award (Poster presentation at Neuroscience Meeting)

2015—2016 BYU Graduate Research Fellowship Award (Graduate funding; \$15,000)

2014 Magna Cum Laude Graduation Honors

2014 BYU ORCA Grant Recipient (Undergraduate research proposal; \$1500)

2014—2015 BYU Marigold N. Saunders Scholarship

2014 *Chiasm: BYU Undergraduate J. of Neuroscience* Author Award

2013—2014 BYU Abrelia Clarissa Hinckley Academic Scholarship

2013 BYU C.H. Nye Academic Scholarship

2011—2012 BYU Academic Scholarship

2011—2014 Dean's List

Leadership Positions

4/2015—present Executive Director/Head of Delegation BYU Special Olympics Team

- Organize teams of athletes with mental or intellectual disabilities and BYU student volunteers
- Manage practices and competitions in several different sports, coach and encourage athletes
- Administrative duties and service training
- Program director from 1/2014-4/2015, Program Director of the Month 9/2014
- Volunteer since 3/2012

8/2013—4/2014 Activities/Brain Awareness Week Officer BYU Neuro Club

- Plan and execute club activities (dinners with faculty, educational events)
- Organize Brain Awareness Week at BYU
 - K-12 school outreach program in over 30 classrooms
 - Outreach events at BYU
 - Manage volunteers

Community Service

3/2012—present	BYU Special Olympics	450 hours
1/2014—3/2014	Brain Awareness Week 2014	60 hours
3/2014—5/2014	Courtyard at Jamestown Assisted Living Center	35 hours
9/2-12—12/2012	Utah State Hospital	15 hours

Various opportunities with a minor role including Community Outreach Day at BYU 2015 and 2012, Brain Awareness Week 2015, 2013, and 2012, Chemistry Week 2014, BYU Best Buddies activities 2012—present, and church activities

Professional Memberships

2014—present	Research Society on Alcoholism
2013—present	Society for Neuroscience

Publications

Jang, E.Y., Yang, C.H., Hedges, D.M., Kim, S.P., Lee, J.Y., Ekins, T.G., Garcia, B.T., Kim, H.Y., Nelson, A.C., Kim, N.J., and Steffensen, S.C. The role of reactive oxygen species in methamphetamine self-administration and dopamine release in the nucleus accumbens. *Addiction Biology*. Accepted 5-13-2016.

Steffensen, S.C., Shin, S.I., Nelson, A.C., Woodward, T.J., Park, H.J., Friend, L., Taylor, D.H., Gao, M., Gao, F., Olive, M.F., Edwards, J.G., Sudweeks, S., Buhlman, L., McIntosh, J.M., and Wu, J. $\alpha 6$ subunit-containing nicotinic receptors on GABA terminals mediate low-dose ethanol effects on ventral tegmental area GABA neurons and ethanol reward. *(Under review)*

Nelson, A.C. To connectome or not to connectome. *Chiasm: BYU Undergrad. J. of Neurosci.*, 6 (2014) 13-15.

Nelson, A.C. and Steffensen, S.C. Effects of chronic ethanol on GABA(A) receptors in the VTA. *BYU ORCA Grant Proposal* (2014).

Conference Presentations

Nelson, A., Woodward, T.J., Park, H., Shin, S.I., Pistorius, S.S., Bair, S.D., and Steffensen, S.C. NMDA and GABAA receptor-mediated plasticity in the mesolimbic dopamine system by acute and chronic ethanol. *Soc. Neurosci Absts* 40 (2015) 314.13

Park, H.J., Nelson, A.C., Woodward, T.J., Shin, S.I., Pistorius, S.S., Bair, S.D., and Steffensen, S.C. Functional switch in GABA(A) receptors on VTA GABA neurons by acute and chronic ethanol. *Soc. Neurosci Absts* 40 (2015) 314.14

Woodward, T.J., Hedges, D.M., Nelson, A.C., Park, H.J., Schilaty, N.D., and Steffensen, S.C. Neurosteroids modulate ethanol effects on dopamine release in the nucleus accumbens

- via actions on GABA(A) receptors on VTA GABA neurons. *Soc. Neurosci Absts* 40 (2015) 695.12
- Nelson, A.C., Woodward, T.J., Hedges, D.M., Jang, E.Y., Park, H.J., Shin, S.I. and Steffensen, S.C. NMDA and GABAA receptor-mediated plasticity in the mesolimbic dopamine system by acute and chronic ethanol. *Alcoholism: Clin. Exp. Res.* (2015) 39(S1) 129A(202)
- Nelson, A., Woodward, T.J., Mabey, J.K., Shin, S.I., 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 (2014) 53.03
- Woodward, T.J., Shin, S.I., Mabey, J.K., Nelson, A.C., Schilaty, N.D., Taylor, D.H. Wu, J., McIntosh, M. and Steffensen, S.C. Ethanol inhibits GABA neurons in the ventral tegmental area and dopamine release in the nucleus accumbens via presynaptic $\alpha 6$ nicotinic receptors on GABA terminals. *Soc. Neurosci Absts* 39 (2014) 53.01
- Mabey, J.K., Shin, S.I., Nelson, A.C., Woodward, T.J., 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.* (2014) 38(S1) 161A(642)
- Shin, S.I., Mabey, J.K., Nelson, A.C., Woodward, T.J., 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 presynaptic alpha-6 nicotinic receptors on GABA terminals. *Alcoholism: Clin. Exp. Res.* (2014) 38(S1) 161A(641)
- Schilaty, N., Yorgason, J.T., Hedges, D.M., Jang, E.Y., Folsom, R.J., Nelson, A., Vera, A., Hanson, S., Wallner, M., Jones, S., and Steffensen, S.C. Ethanol inhibits dopamine release at terminals in the nucleus accumbens via GABA receptors. *Soc. Neurosci Absts* 38 (2013) 349.10