



Theses and Dissertations

---

2014-12-01

## Alcohol Modulation of Dopamine Release

Nathan Dan Schilaty  
*Brigham Young University - Provo*

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



Part of the [Cell and Developmental Biology Commons](#), and the [Physiology Commons](#)

---

### BYU ScholarsArchive Citation

Schilaty, Nathan Dan, "Alcohol Modulation of Dopamine Release" (2014). *Theses and Dissertations*. 5826.  
<https://scholarsarchive.byu.edu/etd/5826>

This Dissertation is brought to you for free and open access by BYU ScholarsArchive. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of BYU ScholarsArchive. For more information, please contact [scholarsarchive@byu.edu](mailto:scholarsarchive@byu.edu), [ellen\\_amatangelo@byu.edu](mailto:ellen_amatangelo@byu.edu).

# Alcohol Modulation of Dopamine Release

Nathan Dan Schilaty

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

Doctor of Philosophy  
Neuroscience

Scott C. Steffensen, Chair  
David D. Busath  
Sterling N. Sudweeks  
Jeffrey G. Edwards  
Richard K. Watt

Department of Physiology and Developmental Biology

Brigham Young University

December 2014

Copyright © 2014 Nathan Dan Schilaty

All Rights Reserved

## ABSTRACT

### Alcohol Modulation of Dopamine Release

Nathan Dan Schilaty

Department of Physiology and Developmental Biology, BYU

Doctor of Philosophy

The mesolimbic dopamine (DA) system projects from the ventral tegmental area (VTA) to structures associated with the limbic system, primarily the nucleus accumbens (NAc). This system has been implicated in the rewarding effects of drugs of abuse. Many drugs of abuse act in the VTA, the NAc, or both. Dopamine neurons in the VTA that project to the NAc, and the GABA neurons that inhibit DA neurons locally in the VTA or project to the NAc, play an important role in mediating addiction to various drugs of abuse, in particular alcohol. There is a growing body of evidence of co-dependence of nicotine and ethanol drug abuse. Given this evidence, it is possible that both ethanol and nicotine target similar receptors in the NAc. The GABA<sub>A</sub> and GABA<sub>B</sub> receptors have also been implicated in the modulation of ethanol's reinforcing properties (Anstrom, Cromwell, Markowski, & Woodward, 2003; Besheer, Lepoutre, & Hodge, 2004; Colombo et al., 2000; Moore & Boehm, 2009; Stromberg, 2004; Walker & Koob, 2007). Thus, there is a growing literature suggesting that GABA receptors are implicated in ethanol reward. In these studies, we evaluated the possibility of co-dependence of nicotine and ethanol by activity on a similar receptor in the NAc. In addition, we evaluated the role of GABA modulation of DA release, in particular GABA<sub>A</sub> receptors and GABA<sub>B</sub> receptors, in modulating DA release in the NAc with acute ethanol exposure. The rationale for this study was predicated on the belief that advancement in the understanding of the brain mechanisms underlying the recreational use and abuse potential of alcohol will pave the way for more effective treatment strategies that could reverse alcohol dependence and co-dependence and save lives and resources throughout the world.

Keywords: dopamine release, ethanol, GABA, nicotine, nucleus accumbens, voltammetry

## ACKNOWLEDGEMENTS

My beautiful and loving wife Angie has been by my side every step of the way. I appreciate her faith and willingness to go wherever our journey takes us and for being my inspiration and strength. As the greatest blessing in my life, she has laughed with me, cried with me, and grown with me. My enrollment in this PhD program was due to her inspiration and her belief in my potential as provider of our family. She deserves more recognition than I do for all of her hard work with our family and home. In addition, I thank my children for their love and support. I hope that my educational journey will serve as an example and inspiration to them of what we can aspire to and accomplish.

I will always be indebted to Scott C. Steffensen for his friendship and mentoring. I honestly could not have selected a better mentor – he has nourished my education in more than just academia. He has been an example of kindness, understanding, generosity and has guided my future career and development. I thank all Steffensen Lab members that have contributed to my project and have become dear friends. My experience here has been awesome!

I extend gratitude of support of many individuals, family and friends. My deepest gratitude is extended to each of them, even though it would take pages to list them by name.

I thank Brigham Young University for providing the platform to conduct this research and acknowledge the important financial support of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA).

## TABLE OF CONTENTS

TITLE PAGE .....	i
ABSTRACT .....	ii
ACKNOWLEDGEMENTS .....	iii
TABLE OF CONTENTS .....	iv
LIST OF FIGURES .....	vii
CHAPTER 1: Introduction .....	1
The Economic and Social Costs of Alcohol Abuse .....	1
The Mesolimbic Dopamine System .....	1
The Cycle of Addiction and Current Treatment Strategies .....	3
Clinical Therapeutics for Addiction .....	4
GABA and Glutamate Synaptic Transmission and Alcohol Reward .....	5
GABA <sub>A</sub> and GABA <sub>B</sub> Receptor Characteristics .....	6
A Role for Dopamine in Alcohol Reward .....	7
Ethanol Modulation of Dopamine Release: Microdialysis Versus Voltammetry .....	8
Mechanism of Action of Ethanol Effects on Dopamine Release .....	10
Rationale for Proposed Studies .....	12
Specific Aim 1 .....	14
Specific Aim 2 .....	14
Specific Aim 3 .....	15
Outcomes .....	15
CHAPTER 2: Materials and Methods .....	17
Animal Subjects and Surgical Procedure .....	17
Preparation of Brain Slices .....	17
Carbon Fiber Electrodes, Calibration, and Fast Scan Cyclic Voltammetry .....	18

Drug Preparation and Administration .....	19
Cerebral Lesion and Verification of Electrode Placement.....	20
Immunohistochemistry .....	20
Statistical Analyses .....	21
CHAPTER 3: Results .....	22
Nicotine Dose-Response, Frequency-Response and Pharmacology in the Shell Region of the Nucleus Accumbens.....	22
Nicotine Dose-Response, Frequency-Response and Pharmacology in the Core Region of the Nucleus Accumbens.....	23
Ethanol Inhibition of Dopamine Release in the Nucleus Accumbens: Role of $\alpha 6^*$ - nAChRs .....	25
Effects of Nicotine In Vivo In Both the Shell and Core of the Nucleus Accumbens .....	27
Role of nAChRs In Ethanol Inhibition of Dopamine Release In Vivo .....	28
Effects of NMDA Antagonist APV on Dopamine Release in the Nucleus Accumbens Ex Vivo .....	29
Effects of GABA <sub>A</sub> Typical Agonists and Antagonists on Dopamine Release in the Nucleus Accumbens Ex Vivo .....	30
Effects of Chlordiazepoxide and Pentobarbital on Dopamine Release in the Nucleus Accumbens Ex Vivo.....	31
Effects of GABA <sub>A</sub> $\delta$ -subunit Agonists and Antagonists and GABA <sub>A</sub> $\delta$ -subunit Knock Out Mice on Dopamine Release in the Nucleus Accumbens Ex Vivo .....	33
Effects of Neurosteroids on Dopamine Release in the Nucleus Accumbens Ex Vivo .....	34
Effects of Ro15-4513 on Dopamine Release in the Nucleus Accumbens In Vivo.....	35
Effects of GABA <sub>A</sub> $\rho$ -subunit Antagonist TPMPA on Dopamine Release in the Nucleus Accumbens .....	36
Immunohistochemistry of GABA <sub>A</sub> $\rho 1$ -subunit in the Nucleus Accumbens.....	36
Effects of Baicalein on Dopamine Release in the Nucleus Accumbens Ex Vivo.....	37
Effects of Baicalein on Dopamine Release in the Nucleus Accumbens In Vivo.....	39
CHAPTER 4: Discussion.....	40

Region Dependent Nicotine Modulation of Dopamine Release .....	40
Ethanol Inhibits Dopamine Release Through nAChR Interactions .....	42
Ethanol Modulation of DA Release .....	45
GABA <sub>A</sub> Receptor Pharmacology in the Nucleus Accumbens .....	46
GABA <sub>B</sub> Receptor Pharmacology in the Nucleus Accumbens .....	47
Effectiveness of Ethanol on the $\delta$ -subunit of the GABA <sub>A</sub> Receptor .....	48
Effects of Neurosteroids in Attenuating the Effects of Ethanol at GABA <sub>A</sub> Receptors in the Nucleus Accumbens .....	49
Confirmation of an Atypical GABA <sub>A</sub> Receptor in the Nucleus Accumbens .....	50
Baicalein .....	52
CHAPTER 5: Conclusions .....	54
Future Directions .....	56
REFERENCES .....	59
CURRICULUM VITAE .....	75

## LIST OF FIGURES

Figure 1: Theoretical Framework for Proposed Studies on Ethanol Effects on Dopamine Release in the Nucleus Accumbens. ....	13
Figure 2: Pharmacology of Nicotine Inhibition of Evoked DA Release in the Shell of the NAc Ex Vivo.....	22
Figure 3: Pharmacology of Nicotine Inhibition of Evoked DA Release in the Core of the NAc Ex Vivo.....	24
Figure 4: Nicotinic Receptor Pharmacology of Ethanol Inhibition of Evoked DA Release in the Core of the NAc Ex Vivo.....	26
Figure 5: Pharmacology of Nicotine Effects of Evoked DA Release in the Shell and Core of the NAc In Vivo. ....	27
Figure 6. Nicotinic Receptor Pharmacology of Ethanol Inhibition of Evoked DA Release in the Core of the NAc In Vivo.....	28
Figure 7: Effects of APV on Phasic DA Release Ex Vivo. ....	29
Figure 8: Effects of GABA <sub>A</sub> Agonists and Antagonists on Phasic DA Release Ex Vivo. ....	30
Figure 9: Effects of Benzodiazepines and Barbiturates on DA Release Ex Vivo. ....	31
Figure 10: Effects of GABA <sub>B</sub> Agonists and Antagonists on Phasic DA Release Ex Vivo.....	32
Figure 11. Effects on GABA Delta-subunit Pharmacology Ex Vivo and Delta Knock-out Mouse with Acute Ethanol.....	33
Figure 12. Effects of Neurosteroids on Phasic DA Release Ex Vivo.....	34
Figure 13. Ro15-4513 Reduces Ethanol Inhibition of DA Release In Vivo. ....	35
Figure 14. Effect of $\rho_1$ -subunit Antagonist TPMPA on Phasic DA Release Ex Vivo.....	36
Figure 15. Expression of GABA <sub>A</sub> $\rho_1$ -subunit in Nucleus Accumbens of CD-1 Mice.....	37
Figure 16. Baicalein Prevents Ethanol Inhibition of DA Release In Vivo. ....	38
Figure 17. Baicalein Attenuates Ethanol Inhibition of DA Release Both Ex Vivo and In Vivo.....	39
Figure 18: Proposed Hodology of GABA Modulation of DA Release in the Nucleus Accumbens. ....	55



## CHAPTER 1: Introduction

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

Widespread illicit and legal drug abuse takes an enormous toll on individual human suffering and society. The consequences of drug abuse include: lost job productivity; squandered earnings; rising healthcare costs; incarcerations; investigations; vehicular accidents; domestic and non-domestic violence; premature death; and the breakdown of the family unit (“National Survey on Drug Use and Health”). The economic and societal implications arising as direct or indirect result of drug abuse consumption are staggering. For example, in 2006 it was determined that there were at least 15.3 million people in the U.S. that met the criteria for alcohol abuse or addiction (Gordis), and that the direct costs alone related to alcohol abuse range as high as \$185 billion in the U.S. annually.

Often taken in concert (Miller & Gold, 1998), the use of alcohol and tobacco are leading causes of preventable death in the United States (Danaei et al., 2009). Approximately 80% of alcoholics are also smokers (Hendrickson, Guildford, & Tapper, 2013; Taylor, Steffensen, & Wu, 2013), and heavy smokers are ten times more likely to be alcoholics (DiFranza & Guerrera, 1990). While smoking rates in the general population have decreased in recent decades, the use of nicotine has remained high (approximately 70-75%) in individuals who regularly use alcohol (Hendrickson et al., 2013; Meyerhoff et al., 2006).

### *The Mesolimbic Dopamine System*

The mesolimbic dopamine (DA) system in the mammalian brain projects from the ventral tegmental area (VTA) to structures associated with the limbic system, primarily the nucleus accumbens (NAc). This system has been implicated in the rewarding effects of drugs of abuse

(Blackburn, Phillips, Jakubovic, & Fibiger, 1986; Koob, 1992; McKinzie, Rodd-Henricks, Dagon, Murphy, & McBride, 1999; Pierce & Kumaresan, 2006; Wise, 1996; Wise & Bozarth, 1987). The VTA is a relatively amorphous midbrain structure that contains at least three neuron types: Primary type or DA neurons that project to the NAc; Secondary type or GABA neurons that may participate in local circuitry (acting to inhibit DA neurons) or project to other brain regions: and a population of glutamatergic (GLUergic) neurons (Yamaguchi, Sheen, & Morales, 2007). Dopaminergic neurons represent ~ 65 %, GABAergic neuron ~ 30 %, and GLUergic neurons represent ~ 5 % of the neurons in the VTA (Margolis et al., 2006; Margolis, Toy, Himmels, Morales, & Fields, 2012). The NAc, which is part of the ventral striatum, can be divided into two structures: the core, and the shell; each have different morphology and function. Although both pertain to reward processing, the shell is primarily concerned with *motivation* of reward whereas the core is involved with the *motor aspects* of reward. The shell portion of the NAc appears to be more important than the core for drug reward, as the medial VTA, running rostral to caudal, seems to hold the greatest concentration of DA neurons that project to the NAc (Ikemoto, 2007).

Many drugs of abuse act in both the VTA and the NAc. However, most rats and mice will self-administer cocaine (David et al., 2004, Rodd et al., 2005), ethanol (Gatto et al., 1994, Rodd-Henricks et al., 2000), nicotine (Museo and Wise, 1981, Laviolette and van der Kooy, 2003), cannabinoids (Zangen et al., 2006), and opiates (Bozarth & Wise, 1981; David & Cazala, 1994; Devine & Wise, 1994; Welzl, Kuhn, & Huston, 1989) into the VTA, suggesting that DA neurons in the VTA that project to the shell of the NAc, and the GABA neurons that may inhibit DA neurons locally in the VTA, play an important role in mediating addiction to various drugs of abuse.

Dopamine release in target areas of the mesolimbic system has been implicated in the rewarding properties of drugs of abuse, including alcohol (Blackburn et al., 1986; Koob, 1992; Wise, 1996; Wise & Bozarth, 1987). The emerging view is that the dysregulated homeostasis that accompanies the development of drug addiction may result from experience-dependent neuroadaptations that hijack normal synaptic transmission in the mesolimbic DA system (Hyman & Malenka, 2001; Hyman, Malenka, & Nestler, 2006; Kauer & Malenka, 2007; Nugent & Kauer, 2008).

### *The Cycle of Addiction and Current Treatment Strategies*

A major goal of research on alcoholism is to characterize the critical neural substrates that are most sensitive to alcohol, adapt in association with chronic consumption and drive subsequent alcohol-seeking behavior. A significant need exists for understanding how alcohol is affecting DA transmission in the mesolimbic system. A basis for this study is predicated on the belief that advancement in the understanding of the brain mechanisms underlying the recreational use and abuse potential of alcohol will pave the way for more effective treatment strategies that could reverse alcohol dependence and save lives and resources throughout the world.

Addicts continue their cycle of abuse as a result of low DA levels in pleasure areas of the brain. These low levels of DA result in feelings of anxiety and dysphoria, impelling subsequent drug seeking and taking (Di Chiara, Bassareo, and others). Systemic administration of most drugs of abuse briefly enhances DA levels. It is likely that the short-lived enhancement of DA release in the NAc and other pleasure system structures is what underlies the euphoric aspect of psychoactive drugs and natural rewarding behaviors (eating, drinking, and sex). However, drugs

overwhelm this system and tolerance/dependence result in subsequent drug taking, ultimately causing persistent dysfunctional DA homeostasis.

Conventional therapies for addiction include self-help groups, drug therapy, and substitution therapy. The search for effective medications for substance abuse and addiction has been difficult as these medications produce akinesia, deficits in cognitive performance, and disrupt natural pleasure sensation (Gonzalez, Oliveto, and Kosten). These side-effects result in a lack of patient compliance and poor prognoses.

### *Clinical Therapeutics for Addiction*

Given my clinical background, I am very interested in discovering viable treatments for addiction, in particular alcohol addiction. There are currently no known effective substances that can assist in alleviating the hedonic response of an addict. Replacement therapies exist, but as previously mentioned, they do little good as the addict is still addicted to some form of drug. Nutraceutical therapy would be a viable option if it demonstrated promise in raising DA levels to overcome the hedonic response and was non-addictive. After some research of various compounds and upon a review of the scientific literature, a possible candidate emerged: baicalein. Baicalein is an isoflavone found in the plant *Scutellaria baicalensis* (also known as Chinese Skullcap). Mainly found in the roots of the plant, it has been isolated and tested scientifically with amphetamines (Wu, Shen, Wang, Chi, & Yen, 2006) with successful results on counteracting neurotoxicity and protecting the dopamine transporter (DAT) from destruction (Wu et al., 2006). It is conceivable that this compound could be useful as a clinical method for restoring DA homeostasis in the brains of human drug addicts if the mechanism by which it operates in the brain can be determined.

### *GABA and Glutamate Synaptic Transmission and Alcohol Reward*

A major goal of basic research on alcoholism is to understand the neural basis of alcohol use and the pathological progression to alcohol dependence (Gilpin & Koob, 2008). The compulsion to consume alcohol stems from both its positive reinforcing properties (e.g. anxiolytic and euphoric effects) and its negative reinforcing properties (e.g. aversive withdrawal symptoms that result from abstinence) (Koob, Rassnick, Heinrichs, & Weiss, 1994). The prevailing view is that synaptic transmission in the central nervous system is depressed by acute intoxicating doses of ethanol (Ariwodola et al., 2003; Berry & Pentreath, 1980; Bloom et al., 1984; Deitrich, Dunwiddie, Harris, & Erwin, 1989; Shefner, 1990; Siggins et al., 1987), which might result from either an attenuation of excitatory glutamate (GLU) synaptic transmission (Lovinger, White, & Weight, 1989; D.M. Lovinger, G. White, & F.F. Weight, 1990; Nie, Yuan, Madamba, & Siggins, 1993; Roberto et al., 2004; White, Lovinger, & Weight, 1990) and/or an enhancement of inhibitory  $\gamma$ -aminobutyric acid (GABA) synaptic transmission (Deitrich et al., 1989; Harris & Allan, 1989; Roberto, Madamba, Moore, Tallent, & Siggins, 2003). There is compelling evidence that ethanol can alter GLU N-methyl-D-aspartate (NMDA) receptors (P. Hoffman, Rabe, Moses, & Tabakoff, 1989; P. L. Hoffman & Tabakoff, 1993; Lima-Landman & Albuquerque, 1989; Lovinger et al., 1989; D. M. Lovinger, G. White, & F. F. Weight, 1990; D.M. Lovinger et al., 1990; Roberto et al., 2004), that low doses of ethanol may interact with inhibitory subtype GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) complexes (Allan & Harris, 1986; Liljenquist & Engel, 1982; Mihic & Harris, 1996; Suzdak et al., 1986; Ticku, Lowrimor, & Lehoullier, 1986), and that ethanol can interact with neuromodulators such as neurosteroids (Brot, Akwa, Purdy, Koob, & Britton, 1996; Kumar, Fleming, & Morrow, 2004; Lambert, Peters, & Cottrell, 1987; Simmonds, 1991; Wieland, Belluzzi, Stein, & Lan, 1995). In addition to ethanol acting via

GABA<sub>A</sub>Rs, the GABA<sub>B</sub> receptor (GABA<sub>B</sub>R) has been implicated in the modulation of ethanol's reinforcing properties as well (Anstrom et al., 2003; Besheer et al., 2004; Colombo et al., 2000; Moore & Boehm, 2009; Stromberg, 2004; Walker & Koob, 2007). Thus, there is a growing literature suggesting that both GABA<sub>A</sub> and GABA<sub>B</sub>Rs are implicated in ethanol reward and further understanding of the underlying mechanisms is necessary.

### *GABA<sub>A</sub> and GABA<sub>B</sub> Receptor Characteristics*

Regarding GABA synaptic transmission, GABA activation of GABA<sub>A</sub> and GABA<sub>B</sub> receptors causes hyperpolarization of neuronal membranes and inhibition of neuronal excitability, but their mechanisms differ markedly. GABA receptors are classified into ionotropic and metabotropic subtypes (Chebib & Johnston, 1999). Both the GABA<sub>A</sub>Rs and GABA<sub>C</sub> receptors (GABA<sub>C</sub>Rs) are the ionotropic subtypes while the GABA<sub>B</sub>Rs is the metabotropic subtype (G-coupled protein receptor) (Bormann, 2000). Ionotropic receptors contain a gating mechanism that is activated by specific ligands. When these ligands bind to the GABA receptor subunits, a Cl<sup>-</sup> ion inwardly passes through the channel, causing a fast synaptic inhibition to the respective neuron (Chebib & Johnston, 1999). Metabotropic receptors, when activated by a specific ligand, undergo a morphological change that activates a G-protein and then initiates intracellular cascading mechanisms to occur that can affect many cellular functions such as gene transcription, protein production, phosphorylation, and neurotransmitter release.

GABA<sub>A</sub>Rs are widely distributed throughout the nervous system, both centrally and peripherally, and are pentameric receptors that contain a combination of receptor subunits ( $\alpha_1$ - $\alpha_6$ ,  $\beta_1$ - $\beta_3$ ,  $\gamma_1$ - $\gamma_3$ ,  $\delta$ ,  $\epsilon$ ,  $\theta$ ,  $\rho_1$ - $\rho_3$ , or  $\pi$ ) with the  $\alpha\beta\gamma$  combination being the most typical of the GABA<sub>A</sub>Rs. As GABA<sub>A</sub>Rs are regulatory to the entire nervous system, the pharmacology of GABA<sub>A</sub>Rs has been widely studied and is well known and classified.

GABA<sub>C</sub>Rs, although ionotropic and pentameric like the GABA<sub>A</sub>R, were previously classified separately as they contain only  $\rho$ -subunits (Amin & Weiss, 1994) and were known to be found primarily in the retinal neural structures. The  $\rho$ -subunits homo-oligomerize to form GABA<sub>A</sub> $\rho$  receptors [formerly classified as GABA<sub>C</sub>Rs, (Bormann, 2000)]. Interestingly, although controversial,  $\rho$ -subunits are known to co-assemble with the GABA<sub>A</sub>R subunits producing receptors with unique properties (Milligan, Buckley, Garret, Deuchars, & Deuchars, 2004) and the possibility of  $\rho$ -subunits co-assembling with other GABA<sub>A</sub>R subunits continues to be theorized (Martinez-Delgado, Estrada-Mondragon, Miledi, & Martinez-Torres, 2010). To make matters even more interesting, some have recently theorized that some atypical GABA<sub>A</sub>R characteristics in the nervous system may be due to integration of the  $\rho$ -subunit into the GABA<sub>A</sub>R (Martinez-Delgado et al., 2010; Milligan et al., 2004). Thus, it appears that the classification of the once distinct GABA<sub>C</sub>R is further being merged into the single ionotropic GABA<sub>A</sub>R classification.

GABA<sub>B</sub>Rs are seven transmembrane receptors that are also widely distributed throughout the central and peripheral nervous system where they regulate synaptic transmission and signal propagation by controlling the activity of voltage-gated calcium and inward-rectifier potassium channels (Schwenk et al., 2010). They are heteromeric proteins composed of both B<sub>1</sub> and B<sub>2</sub> subunits. Homomeric possibilities of GABA<sub>B</sub>Rs has not been successfully proven (Benke, Michel, & Mohler, 2002; Bettler, Kaupmann, Mosbacher, & Gassmann, 2004), although theorized (Calver et al., 2000), and atypical characteristics of GABA<sub>B</sub>Rs are not documented.

#### *A Role for Dopamine in Alcohol Reward*

Both *in vivo* (Gessa, Muntoni, Collu, Vargiu, & Mereu, 1985) and *ex vivo* (Brodie & Appel, 1998; Brodie, Shefner, & Dunwiddie, 1990) studies indicate that acute ethanol increases

DA release in limbic structures and/or VTA DA firing rates, and that withdrawal from chronic ethanol reduces DA firing rate and release in the NAc (Diana, Pistis, Carboni, Gessa, & Rossetti, 1993). Furthermore, pharmacological or genetic manipulations that diminish DA activity inhibit ethanol consumption and preference (El-Ghundi et al., 1998; Ikemoto, McBride, Murphy, Lumeng, & Li, 1997; Phillips et al., 1998), conditioned place preference (Cunningham et al., 2000; Risinger, Freeman, Greengard, & Fienberg, 2001), the acquisition of ethanol self-administration (Risinger, Freeman, Rubinstein, Low, & Grandy, 2000), and ethanol preference and sensitivity (Phillips et al., 1998).

#### *Ethanol Modulation of Dopamine Release: Microdialysis Versus Voltammetry*

Dopamine release, as measured by microdialysis and fast-scan cyclic voltammetry (FSCV), exhibit disparate results with ethanol and nicotine studies in the NAc. These neurochemical studies provide compelling, but complicated, evidence regarding ethanol effects on DA transmission (Brodie & Appel, 1998; Gysling & Wang, 1983; Mereu, K-W., Gessa, Naes, & Westfall, 1987). Microdialysis studies typically demonstrate a rise in DA levels while voltammetry studies show a fall in evoked DA responses by both acute nicotine and ethanol (Budygin et al., 2001; D. L. Robinson, E. C. Howard, S. McConnell, R. A. Gonzales, & R. M. Wightman, 2009; Yorgason, Ferris, Steffensen, & Jones, 2013; Zhang et al., 2009). For example, most microdialysis studies have demonstrated that ethanol enhances DA release at moderate-to-high ethanol concentrations (Blomqvist, Ericson, Engel, & Söderpalm, 1997), though others have reported no effect or DA reductions at high ethanol doses (Blanchard, Steindorf, Wang, & Glick, 1993). Dopamine levels also increase in association with ethanol self-administration (Ericson, Blomqvist, Engel, & Söderpalm, 1998; Weiss, Lorang, Bloom, & Koob, 1993). However, *in vitro* studies have shown that ethanol decreases K<sup>+</sup>-evoked DA



release from synaptosomes (J. J. Woodward, Machu, & Leslie, 1990) and NMDA-stimulated DA release from striatal slices (J. J. Woodward & Gonzales, 1990).

In FSCV studies, wherein both release and uptake of DA can be evaluated, acute ethanol decreases DA release in freely-behaving rats [ $IC_{50}$  of 2.0 g/kg; (Budygin et al., 2001)] and in mouse and rat brain slices (Schilaty et al., 2014), without affecting uptake (S. R. Jones, Mathews, & Budygin, 2006)[but see Robinson, Volz, Schenk, and Wightman (2005)]. Interestingly, while 1-2 g/kg ethanol has enhancing effects on spontaneous DA release, as measured with microdialysis, it has heterogeneous effects on rapid DA release, increasing spontaneous DA transient frequency (Donita L. Robinson, Elaina C. Howard, Scott McConnell, Rueben A. Gonzales, & R. Mark Wightman, 2009), but decreasing electrically stimulated DA amplitude (S. R. Jones et al., 2006), as measured by FSCV, suggesting that the effects of increased tonic firing contribute more to the microdialysis-measured DA levels compared to decreased phasic release of ethanol on DA release in the NAc (Donita L. Robinson et al., 2009). The emerging picture is that ethanol enhances DA neuron activity at moderate to high doses, and spontaneous DA release at moderate to high doses, but exerts an inhibitory effect on phasic DA release at moderate to high doses via its effects on DA terminals.

The disparity between these techniques is due, in part, to differences in measurements of tonic versus phasic DA release (D. L. Robinson et al., 2009). We have recently demonstrated that ethanol preferentially inhibits high-frequency phasic release, but not low-frequency “tonic” release (Yorgason et al., 2013). Similarly, nicotinic mechanisms regulate the frequency dependence of DA release, albeit in a different direction, with greater DA release under phasic-like conditions, and decreased release under tonic-like stimulations (Exley, Clements, Hartung, McIntosh, & Cragg, 2008).

### *Mechanism of Action of Ethanol Effects on Dopamine Release*

One population of GABA neurons in the VTA has projections to the NAc (M. T. Brown et al., 2012b; van Zessen, Phillips, Budygin, & Stuber, 2012). It has recently been theorized that due to the high percentage of co-dependence with nicotine and ethanol, they may act on similar targets in the brain, specifically nicotinic acetylcholine receptors (nAChRs) on presynaptic GABAergic neurons or DAergic terminals (Hendrickson et al., 2013; Taylor, Steffensen, et al., 2013). Ethanol appears to activate DAergic neurons in the VTA through an interaction at nAChRs (Soderpalm, Lof, & Ericson, 2009). To date, there have not been any studies on the possible regulation of nicotine or ethanol on either of these terminals in the NAc via activation of  $\alpha 6^*$ -nAChRs.

Despite the many studies implicating the mesolimbic DA system in ethanol reward, consumption and alcoholism [for recent review see Soderpalm and Ericson (2012)], there are relatively few pharmacological studies elucidating the mechanism of action of ethanol effects on this system. Dopamine release is controlled or modulated by several neurotransmitters including GABA, GLU and glycine (GLY), metabolites including acetaldehyde (Melis, Diana, Enrico, Marinelli, & Brodie, 2009), enzymes including catalase (Zimatkin, Pronko, Vasiliou, Gonzalez, & Deitrich, 2006), receptors including the family of cysteine-loop gated ion channels including nicotinic receptors (Soderpalm, Ericson, Olausson, Blomqvist, & Engel, 2000), GLYRs, GABA<sub>A</sub>Rs and serotonin receptors (5-HT<sub>3</sub>Rs), as well as the DA transporter (DAT) and D<sub>2</sub> receptors, yet the elucidation of the molecular mechanisms by which ethanol affects reward in the context of the DA system remains elusive. As mentioned above, there is fairly compelling evidence based on microdialysis studies that ethanol enhances DA neurotransmission in the mesolimbic system via enhancement of DA neuron activity in the VTA. However, DA neurons

are excited by ethanol only at high-lethal doses of ethanol [*in vivo* (Gessa et al., 1985) and *ex vivo* (Brodie & Appel, 1998; Brodie et al., 1990)].

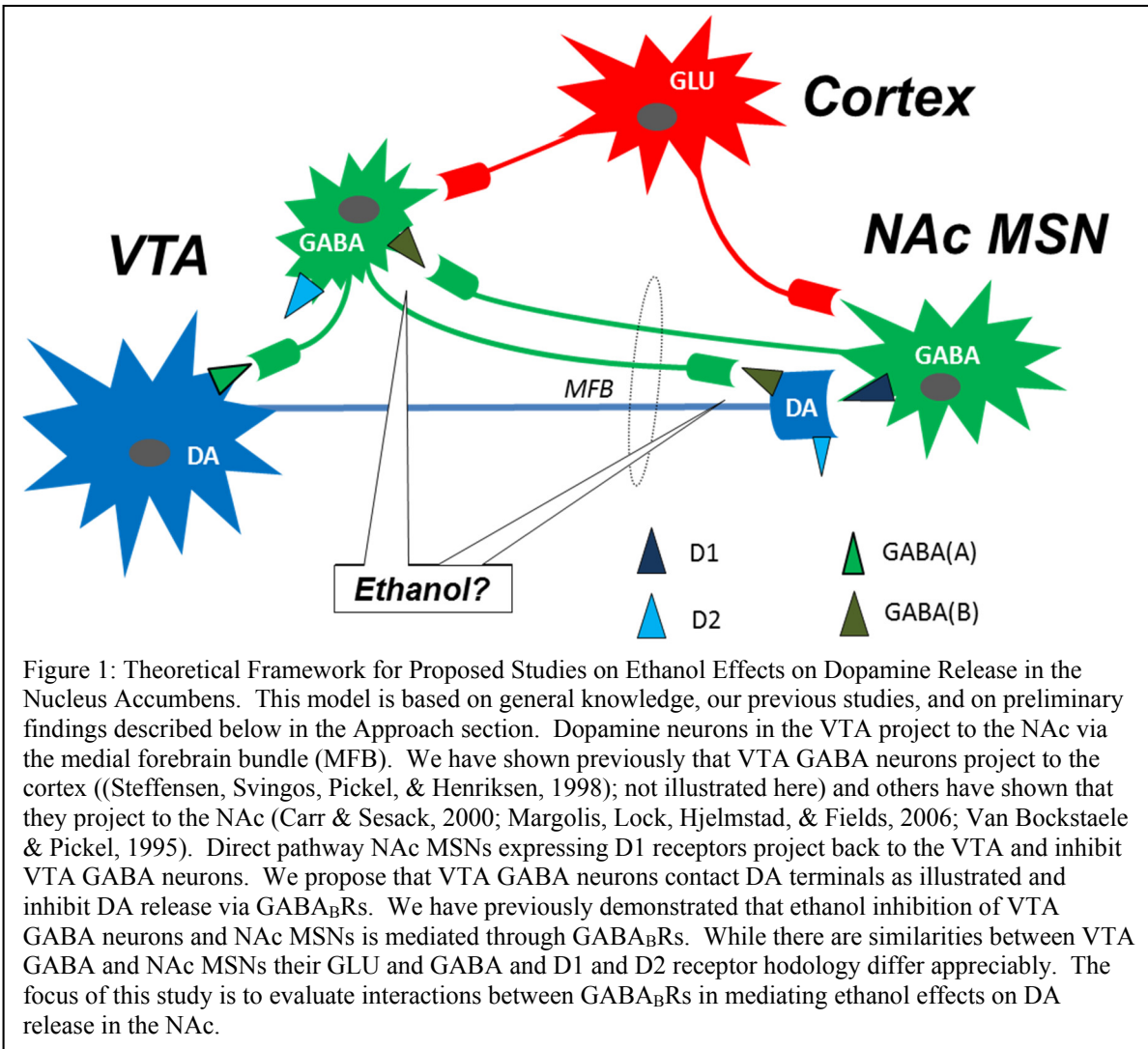
Recently, the Luscher lab has shown evidence that VTA GABA neurons project to the NAc and contact cholinergic interneurons (M. T. Brown et al., 2012a). A subset of GABAergic NAc medium spiny neurons (MSNs), which also express DA D1 receptors, sends a reciprocal projection back to the VTA (Heimer, Zahm, Churchill, Kalivas, & Wohltman, 1991; Kalivas, Sorg, & Hooks, 1993; Nauta, Smith, Faull, & Domesick, 1978; Tripathi, Prensa, Cebrian, & Mengual, 2010; Usuda, Tanaka, & Chiba, 1998). 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 (Einhorn, Johansen, & White, 1988; Rahman & McBride, 2000) via VTA GABA neurons (Xia et al., 2011). Figure 1 shows the synaptic hodology of VTA GABA neurons, including the reciprocal loop between the VTA and NAc, as well as the key receptors involved in ethanol actions on VTA GABA and NAc MSNs. The Steffensen lab has previously demonstrated that both of these GABA neurons are inhibited by physiologically-relevant doses of ethanol, and that ethanol inhibition of NMDA receptor-mediated GLU transmission is blocked by GABA<sub>B</sub>R antagonists (Steffensen et al., 2000). Thus, the mechanism underlying alcohol-induced inhibition of DA transmission is controversial.

Indeed, the neuroadaptions that occur in the brain in association with alcohol dependence may result from plasticity associated with persistent activation of inhibitory GABA and/or excitatory GLU synaptic transmission (Nugent & Kauer, 2008). One speculation for how drugs of abuse might result in dysregulation of mesolimbic DA homeostasis is that repeated exposure results in synaptic plasticity, which is the net effect of potentiation of GLUergic (Bonci & Malenka, 1999; Overton, Richards, Berry, & Clark, 1999) and GABAergic synapses (Nugent &

Kauer, 2008) on DA neurons in the VTA. The Steffensen lab has previously reported in multiple studies that GABA neuron firing rate is inhibited by ethanol in rats *in vivo* with an  $IC_{50}$  of 1.0 g/kg (Gallegos, Criado, Lee, Henriksen, & Steffensen, 1999; K. H. Ludlow et al., 2009; S. C. Steffensen et al., 2009; S.H. Stobbs et al., 2004). Most importantly, they have shown that VTA GABA neurons become tolerant to repeated exposure to ethanol and evince marked hyperexcitability during withdrawal from chronic ethanol, suggesting that these neurons are adaptable substrates to alcohol dependence. The hyperexcitability of VTA GABA neurons evident during withdrawal from chronic ethanol appears to result from cumulative short-term withdrawals from ethanol. Interestingly, ethanol suppression of NMDA receptor-mediated GLU synaptic transmission, which is implicated strongly in most forms of neural plasticity, appears to be mediated by presynaptic  $GABA_B$ Rs on GLU terminals to VTA GABA neurons and NAc MSNs (Steffensen, Nie, Criado, & Siggins, 2000). Thus, ethanol reduction of VTA GABA neuron activity appears to be mediated in part by NMDA receptors, and it is reasonable to assume that they would play a role in short-term plasticity to ethanol exposure and ultimately adaptations in DA release.

### *Rationale for Proposed Studies*

Dr. Steffensen's lab has shown in multiple reports that acute ethanol inhibits the firing rate of VTA GABA neurons in rats with an  $IC_{50}$  of 1.0 g/kg (Gallegos et al., 1999; K. H. Ludlow et al., 2009; S. C. Steffensen et al., 2009; S.H. Stobbs et al., 2004), which is one order of magnitude more sensitive than ethanol effects on DA neurons (Brodie & Appel, 1998; Gysling & Wang, 1983; Mereu et al., 1987). In addition, his lab has reported that VTA GABA neurons are even more sensitive to acute ethanol in mice, characterized by an  $IC_{50}$  of 0.25 g/kg (Steffensen et al., 2010). Most importantly, tolerance accrues to ethanol inhibition of VTA GABA neuron



firing rate and marked hyperexcitability accompanies withdrawal (Gallegos et al., 1999), which is consistent with microdialysis studies demonstrating reductions in DA release during withdrawal (Diana et al., 1993; Diana, Pistis, Muntoni, & Gessa, 1996). Dr. Steffensen's lab reported in 2000 that NAc MSNs were inhibited by physiologically-relevant doses of ethanol and that ethanol inhibition of NMDA responses in these neurons, as well as VTA and hippocampal neurons, involves presynaptic GABA<sub>B</sub>Rs (Steffensen et al., 2000), which is of particular relevance to this study. The main question being addressed in this study is: Does ethanol act in

the VTA to reduce DA neural firing or at DA terminals in the NAc to reduce DA release, or both? The study is organized around three specific aims and hypotheses.

Our proposed studies constituted a focused investigation into the role of VTA GABA neuron projections to the NAc, and in particular GABA<sub>A</sub> and GABA<sub>B</sub>Rs, in mediating acute ethanol effects on DA release in the core under naïve conditions. All the experiments described in this study involved FSCV measurements (single pulse, multiple pulse, paired-pulse, etc...) of DA release in the NAc of C57BL/6 mice *in vivo* and *ex vivo* following acute ethanol exposure.

### Specific Aim 1

To determine the nature of co-dependence of nicotine and ethanol on receptors in the NAc, we evaluated DA release and uptake with an acute challenge of ethanol and nicotine *ex vivo*. We then tested whether nicotinic receptor antagonists block the effects of both nicotine and ethanol *ex vivo*. To establish physiological relevance of our findings, we performed administration of ethanol and nicotine *in vivo*. We hypothesized that nicotine and ethanol would produce co-dependence in the NAc as they would both mediate inhibition of DA release in the NAc through a common receptor binding site.

### Specific Aim 2

To determine the effects of acute ethanol on DA release, we evaluated the effects of acute ethanol (20-160 mM) on peak DA release and uptake in the striatal slice preparation of naïve C57BL/6 mice. To establish the physiological relevancy of ethanol effects on DA release, we evaluated the effects of acute ethanol (1-4 g/kg) on peak DA release and uptake in the NAc core *in vivo* on C57BL/6 mice. To determine the role of GABA receptors on DA terminals, we evaluated the GABA and GLU pharmacology of DA release as well as the pharmacology of

acute ethanol (20-160 mM) inhibition of DA release and uptake in the NAc. We hypothesized that acute ethanol would inhibit DA release in the NAc core via activation of GABA<sub>A</sub> or GABA<sub>B</sub>Rs on DA terminals.

### Specific Aim 3

To determine the effect on DA release of the isoflavone baicalein on acute ethanol exposure, we evaluated the effects of baicalein *ex vivo* (50  $\mu$ M) and *in vivo* (1 mg/kg) on peak DA release of naïve C57BL/6 mice. We hypothesized that acute ethanol inhibition of DA release in the NAc would be attenuated by the isoflavone baicalein.

### *Outcomes*

In this study, we demonstrate that specific  $\alpha 6^*$ -nAChR antagonist  $\alpha$ -conotoxins effectively block inhibition of evoked DA release caused by both nicotine and ethanol, indicating that both drugs of abuse have actions on similar targets in the NAc. Additionally, we show that the  $\alpha 6$ -subunit is functionally operational in the NAc core, but not the NAc shell, for modulating evoked DA release.

Another aspect of this study provides important new insights into the role of GABA receptors regulating DA terminals in the NAc in alcohol reward associated with acute exposure and provides important new insights into the contributory role of VTA GABA neurons in regulating DA neurotransmission and ethanol effects on this system. Results from this study provide a preclinical pharmacologic rationale for considering drugs that act selectively on GABA<sub>A</sub>R and/or GABA<sub>B</sub>R subtypes as putative therapeutic agents for the treatment of alcohol abuse and dependence.

In correlation to GABA modulation of DA release in the NAc, we tested baicalein in combination with acute exposure to ethanol and found that DA levels in the NAc will slightly increase with baicalein administration and that baicalein will attenuate the ethanol inhibition of DA release and help achieve a homeostatic level, possibly leading to counteracting the addictive processes of ethanol.

The main objective of this study was to elucidate the mechanism of action of ethanol inhibition of DA release at terminals in the NAc. We demonstrate that DA release in the NAc is inhibited by physiologically-relevant concentrations of ethanol and that GABA receptors and nAChRs are implicated in ethanol inhibition of DA release.



## CHAPTER 2: Materials and Methods

### *Animal Subjects and Surgical Procedure*

The care and use of mice and experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Brigham Young University which met or exceeded NIH guidelines. Three strains of adult mice (PND 30-120) were used in this study: GABA<sub>A</sub>  $\delta$ -subunit knock-out ( $\delta$ -/-), CD-1 GAD GFP (CD-1), and C57/BL6, with the C57/BL6 mice used as wild-type (WT) controls for all studies involving  $\delta$ -/- mice.  $\delta$ -/- mice used were backcrossed extensively into the C57/BL6 background (Mihalek et al., 2001). Mice in the  $\delta$ -/- colony were genotyped with PCR of ear punches for validation of null mutant genotype (i.e.  $\delta$ -/-) for experimentation. Once weaned at PND 21, all mice were housed in groups of four/cage and placed on a reverse light/dark cycle with lights ON from 8 PM to 8 AM. For DA FSCV recordings *in vivo*, mice were anesthetized using isoflurane (MWI Veterinary Supply, Boise, ID) and placed in a stereotaxic apparatus. Anesthesia levels were maintained at 1 % throughout the experiments. Body temperature was maintained at  $37.4 \pm 0.4^\circ \text{C}$  by a feedback regulated heating pad. With the skull exposed, holes were drilled for placement of stimulating and recording electrodes.

### *Preparation of Brain Slices*

Horizontal brain slices were obtained as previously described (Steffensen et al., 2008). Briefly, mice were anesthetized with Ketamine (60 mg/kg), decapitated, and brains were quickly dissected and sectioned into 400  $\mu\text{m}$  thick horizontal slices in ice-cold artificial cerebrospinal fluid (ACSF), bubbled with 95 % O<sub>2</sub> / 5 % CO<sub>2</sub>. The cutting solution consisted of (in mM): 220 Sucrose, 3 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 25 NaH<sub>2</sub>CO<sub>3</sub>, 12 MgSO<sub>4</sub>, 10 Glucose, and 0.2 CaCl<sub>2</sub>. Slices were immediately placed into an incubation chamber containing normal ACSF bubbled with 95

% O<sub>2</sub> / 5 % CO<sub>2</sub> at 34-35° consisting of (in mM): 124 NaCl, 2 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 24 NaHCO<sub>3</sub>, 12 glucose, 1.2 MgSO<sub>4</sub>, 2 CaCl<sub>2</sub>, pH 7.3. Slices were incubated for at least 30 minutes prior to being transferred to a recording chamber. Once transferred to a recording chamber with continuous normal ACSF flow (2.0 ml/min), the temperature was maintained at 36 °C throughout the experiment. Slices were allowed to stabilize for an additional 15 min before recordings were made. The striatum was visualized with Nikon Diaphot (Nikon, Tokyo, Japan) inverted microscopes in the transmitted mode and the NAc core and shell were visualized by microscopic inspection under low magnification at the level of the anterior commissure. A red light filter was placed in the light path to lower the exposure of the slice to short-wavelength light.

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

For voltammetry recordings both *in vivo* and *ex vivo*, a 7.0 µm diameter carbon fiber was inserted into borosilicate capillary tubing (1.0 mm i.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 200 µm of bare fiber protruding from the end of the glass micropipette. The CFE was back-filled with 3 M KCl. The CFEs were regularly calibrated with a known concentration of DA. With the CFE immersed in the solution of ACSF, we superfused a known concentration of DA at a high flow rate (5 mL/min) past the electrode and observe the maximum nA signal produced by DA. Dopamine calibrations were averaged in order to convert a nA signal of DA to µM concentration of DA.

For *ex vivo* voltammetry recordings, the CFE was positioned in the NAc. Dopamine release was evoked by a 4.0 msec, ten-pulse electrical stimulation (monophasic, 350 µA) from a

bipolar stimulating electrode (Plastics One, Roanoke, VA) placed 100-200  $\mu\text{m}$  from the CFE. The electrode potential was linearly scanned in voltage-clamp mode as a triangular waveform from -0.4 to 1.3 V and back to -0.4 V vs Ag/AgCl using a scan rate of 400 V/s. Cyclic voltammograms were recorded at the CFE every 50 msec (i.e. 20 Hz) by means of a ChemClamp voltage-clamp amplifier (Dagan Corporation, Minneapolis, MN). Voltammetry recordings were performed and analyzed using LabVIEW (National Instruments, Austin, TX)-based customized software. Stimulations were performed periodically every 2 min both *in vivo* and *ex vivo*. Dopamine levels were monitored for a stabilization period typically lasting 1 hr. Once the stimulated DA response produced stable kinetics, baseline measurements were taken.

For *in vivo* recordings, mice were anesthetized with isoflurane and placed in a stereotaxic apparatus (David Kopf Instruments, Tejuanga, CA). Bipolar, coated stainless steel electrodes were stereotaxically implanted into the medial forebrain bundle (MFB;  $-1.3$  P,  $+1.0$  L,  $5.0$ - $5.3$  V), and a capillary glass-based CFE in the NAc core ( $+1.3$  A,  $+0.9$  L,  $-3.8$ - $4.5$  V). The MFB was stimulated with 60 biphasic pulses at 60 Hz (4.0 msec pulse width). Stimulation and recording electrodes were oriented by fine control to optimize the release of DA at 2.0 mA stimulation intensity. Subsequently, the current was adjusted to that stimulus level which evoked DA release at 50 % of the maximum DA current level.

#### *Drug Preparation and Administration*

For *ex vivo* experiments, (+/-)-2-amino-5-phosphonovaleric acid (Abcam), bicuculline (Sigma-Aldrich), chlordiazepoxide (Sigma-Aldrich), muscimol (Sigma-Aldrich), Ro15-4513 (Sigma-Aldrich), picrotoxin (Sigma-Aldrich), CGP55845 hydrochloride (Abcam), baclofen (Sigma-Aldrich), SKF97541 (Abcam), (1,2,5,6-Tetrahydropyridin-4-yl)methylphosphinic acid (Tocris), 5 $\alpha$ -Pregnan-3 $\alpha$ -ol-20-one (Sigma-Aldrich), 5-Androsten-3 $\beta$ -ol-17-one sulfate (Sigma-

Aldrich), pentobarbital (Sigma-Aldrich), (4 $\alpha$ ,5 $\alpha$ ,17 $\beta$ )-3,17-dihydroxy-4,5-epoxyandrost-2-ene-2-carbonitrile (Sigma-Aldrich), 4,5,6,7-Tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol hydrochloride (Tocris), (-)-Nicotine hydrogen tartrate salt (Sigma Aldrich), baicalin hydrate (Sigma Aldrich), baicalein (Caymen Chemicals), dihydro-beta-erythroidine hydrobromide (DH $\beta$ E, Tocris), methyllycaconitine (MLA, Sigma Aldrich),  $\alpha$ -CtxMII (University of Utah) and  $\alpha$ -CtxMII [H9A; L15A] synthesized as previously described (McIntosh et al., 2004), were dissolved in stock solutions and then diluted into ACSF for superfusion onto brain slices at a given molar concentration. For baicalein, we utilized propylene glycol as a solvent.

For *in vivo* experiments, mecamylamine (MEC, Sigma Aldrich), baicalein [in 3 % dimethyl sulfoxide (DMSO)], and Ro15-4513 were dissolved in physiological saline solution and administered intraperitoneally (IP) 30 min prior to ethanol administration. Nicotine was dissolved in physiological saline solution and administered intravenously. Ethanol was administered IP at 16 % w/v in physiological saline solution.

#### *Cerebral Lesion and Verification of Electrode Placement*

Following the *in vivo* experimentation, a current of 2.5 mA was applied through the CFE for 5 seconds with a 5 second interval at both polarities. This current induced an electrolytic lesion in the tissue. The perfused brains were then sliced coronally at 500  $\mu$ m with a vibratome and visualized under light microscope to confirm placement of the electrode in either the NAc shell or core.

#### *Immunohistochemistry*

In order to visualize evidence of  $\rho_1$ -subunits in the NAc, CD-1 mice were perfused as previously described (Jang et al., 2014). Mice brains were removed under pentobarbital

anesthesia (80 mg/kg, IP), post-fixed with 4 % paraformaldehyde, cryoprotected in 30 % sucrose, and cryosectioned into 30  $\mu$ m slices. The sections were incubated in blocking buffer containing 4 % normal goat serum and 0.1 % bovine serum albumin at room temperature for 1 hour. The sections were incubated with primary antibody for rabbit anti-GABRR1 (1:200; Biorbyt, San Francisco, CA) at 4° C overnight. The sections were then processed with secondary antibodies, goat anti-rabbit Alexa Fluor 594 (red; 1:250; Invitrogen, OR) at room temperature for 2 hours. Tissue sections were imaged with a 40X objective using a confocal fluorescent imaging system (Olympus FluoView FV1000, PA).

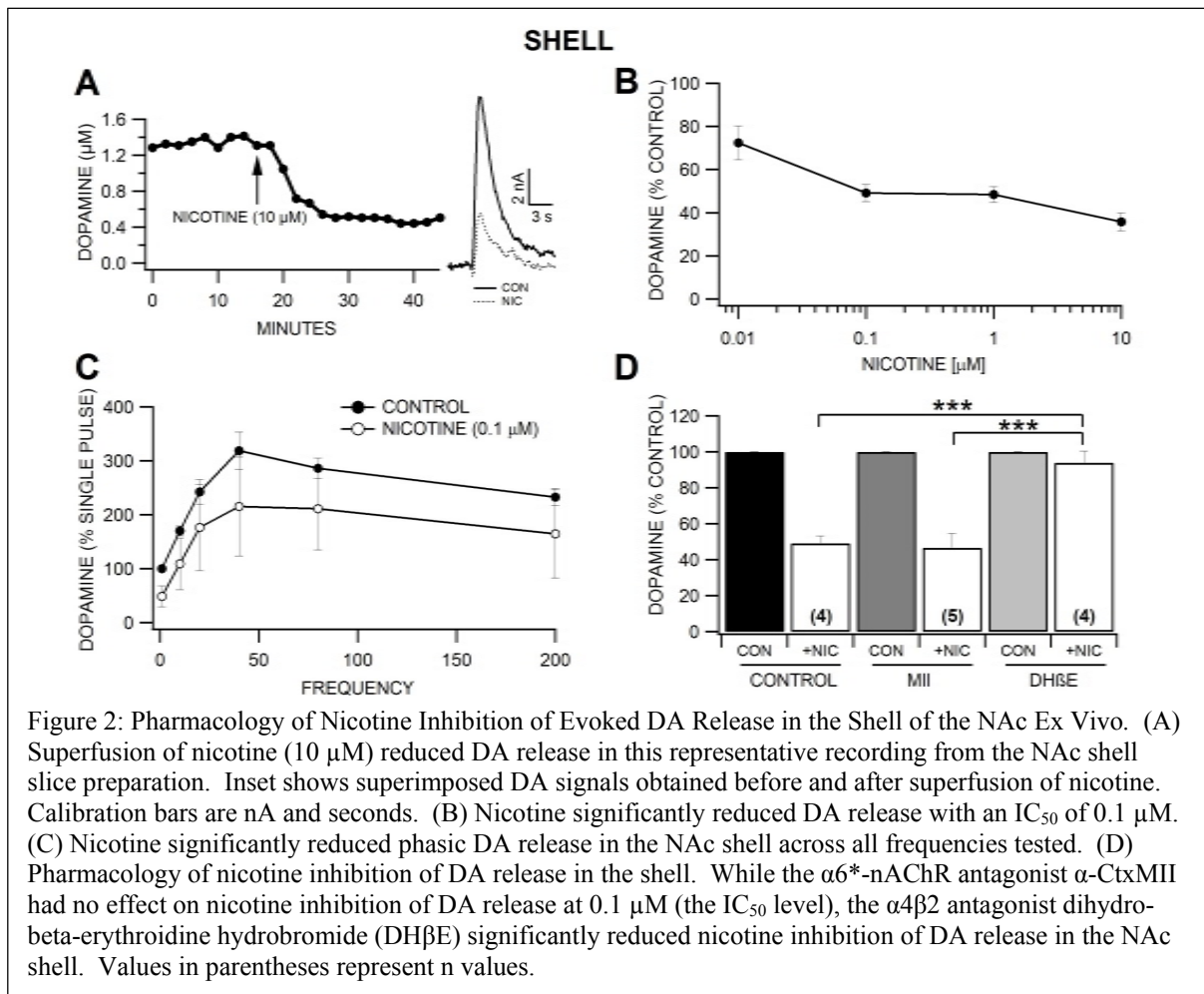
### *Statistical Analyses*

The results for control and drug treatment groups were derived from calculations performed on voltammetry current vs time plots. Peak amplitude was determined by a median filter peak detection algorithm in LabVIEW. Three successive DA peak responses were averaged for control and drug conditions within each experiment. The means were then grand-averaged across animals. Values were expressed as means  $\pm$  SEM for cumulated data. Between-subject group comparisons were analyzed via either t-tests or one-way ANOVAs. Within-subject frequency responses were analyzed with a two-way repeated measures ANOVA, with frequency and drug as the within subject comparisons. Within-subjects comparisons were evaluated via one-way ANOVA and between-subject comparisons were evaluated via one-way ANOVA with Tukey post-hoc analysis where pertinent. For the comparison between  $\delta^{-/-}$  and WT mice, a two-way ANOVA was performed with genotype as the between-subject factor and ethanol concentration as the within-subject factor. The criterion of significance was set at  $p < 0.05$  (\*),  $p < 0.01$  (\*\*), and  $p < 0.001$  (\*\*\*). All statistics were calculated with IBM SPSS Statistics 21 (Armonk, New York).

## CHAPTER 3: Results

### *Nicotine Dose-Response, Frequency-Response and Pharmacology in the Shell Region of the Nucleus Accumbens*

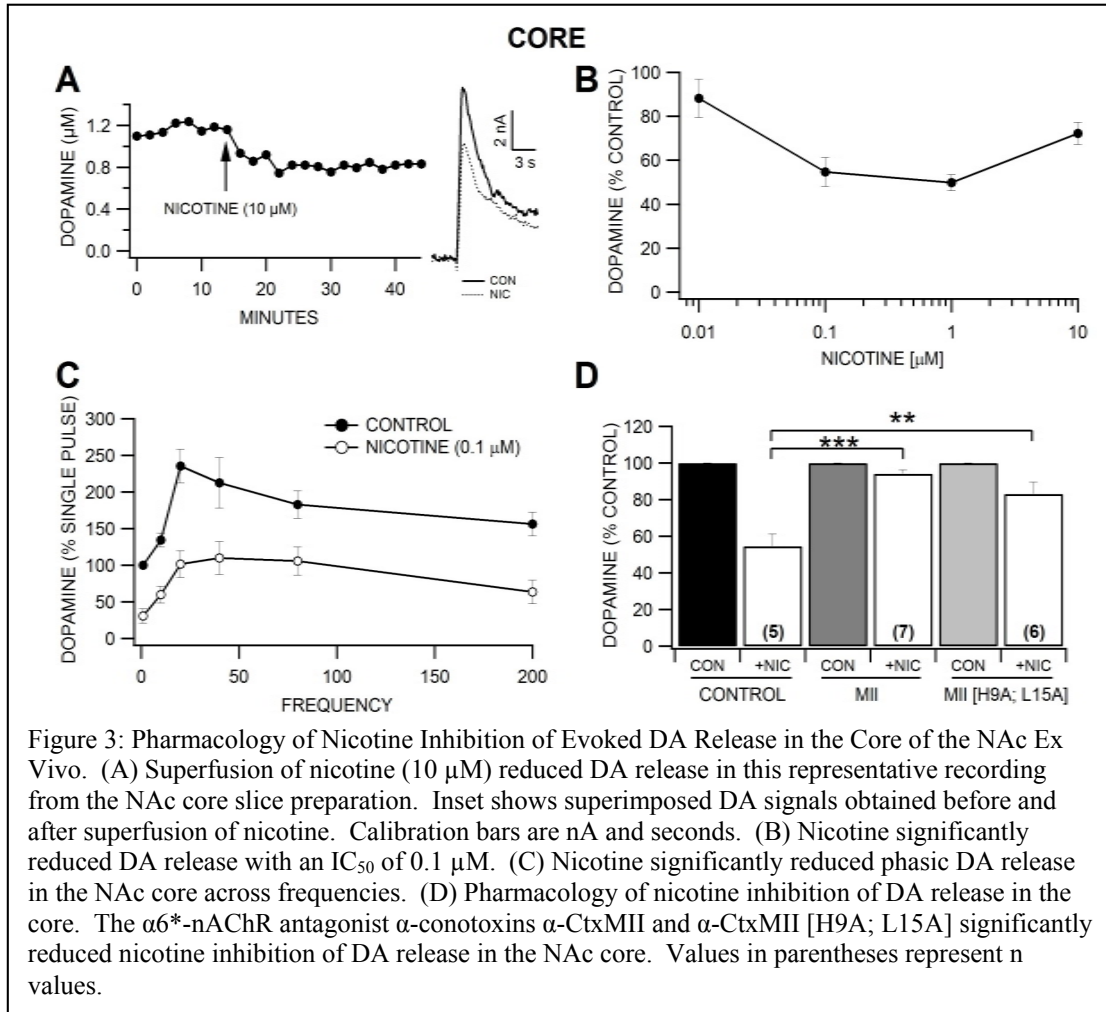
Using FSCV, we evaluated the effects of nicotine (0.01 – 10  $\mu$ M) on evoked DA release in the NAc shell. Nicotine significantly decreased the peak amplitude of the DA signal with an  $IC_{50}$  of approximately 0.1  $\mu$ M (Fig. 2A, B; 0.01  $\mu$ M:  $p=0.39$ ,  $n=4$ ; 0.1  $\mu$ M:  $p=0.001$ ,  $n=4$ ; 1.0  $\mu$ M:  $p=0.001$ ,  $n=4$ ; 10.0  $\mu$ M:  $p<0.0001$ ,  $n=7$ ). We evaluated the frequency dependency for nicotine effects on DA release in the NAc shell. We applied stimulation trains (10 pulses) at varying frequencies ranging from 10 - 200 Hz and compared the response produced by multiple



pulses to that obtained by a single pulse. The frequency response followed an inverted-U relationship, with the maximum release occurring at 40 Hz stimulation in the NAc shell (Fig. 2C). Nicotine significantly decreased the amplitude of the DA signal across all frequencies tested. Two-way repeated measures ANOVA revealed a main effect of frequency for the amplitude of the DA signal ( $F_{5,20}=2.897, p=0.04$ ), and nicotine ( $F_{1,4}=16.367, p=0.016$ ). Next we evaluated the effects of various nAChR antagonists on evoked DA release in the NAc shell. We analyzed evoked DA release in the presence of nicotine with the selective  $\alpha 6^*$  antagonist  $\alpha$ -CtxMII (100 nM) and also the partially selective  $\alpha 4\beta 2^*$  antagonist DH $\beta$ E (50  $\mu$ M). Superfusion of  $\alpha$ -CtxMII did not block the inhibition caused by 0.1  $\mu$ M nicotine in the NAc shell whereas DH $\beta$ E did (Fig. 2D).  $\alpha$ -CtxMII did not significantly affect the DA signal ( $F_{1,7}=0.090, p=0.773$ ). However, superfusion of DH $\beta$ E significantly inhibited the DA signal about 40% from baseline. There was no statistical significance between  $\alpha$ -CtxMII exposed nicotine and control nicotine ( $p>0.05$ ). However, one-way ANOVA comparing effects of DH $\beta$ E + nicotine to nicotine alone on evoked DA in the NAc shell revealed a significant attenuation in nicotine effects on NAc shell DA transmission ( $F_{(1,6)}=35.376, p=0.001$ ). Additionally, DH $\beta$ E blockade of nicotine induced DA reductions was significantly increased when compared to  $\alpha$ -CtxMII experiments ( $F_{1,7}=27.790, p=0.0012$ ).

#### *Nicotine Dose-Response, Frequency-Response and Pharmacology in the Core Region of the Nucleus Accumbens*

Using FSCV, we evaluated the effects of nicotine (0.01 – 10  $\mu$ M) on evoked DA release in the NAc core. Nicotine significantly decreased the peak amplitude of the DA signal with an IC<sub>50</sub> of approximately 0.1  $\mu$ M (Fig. 3A, B; 0.01  $\mu$ M:  $p=0.269, n=4$ ; 0.1  $\mu$ M:  $p=0.006, n=4$ ; 1.0  $\mu$ M:  $p=0.001, n=4$ ; 10.0  $\mu$ M:  $p=0.002, n=7$ ). We noted varying results at higher concentrations



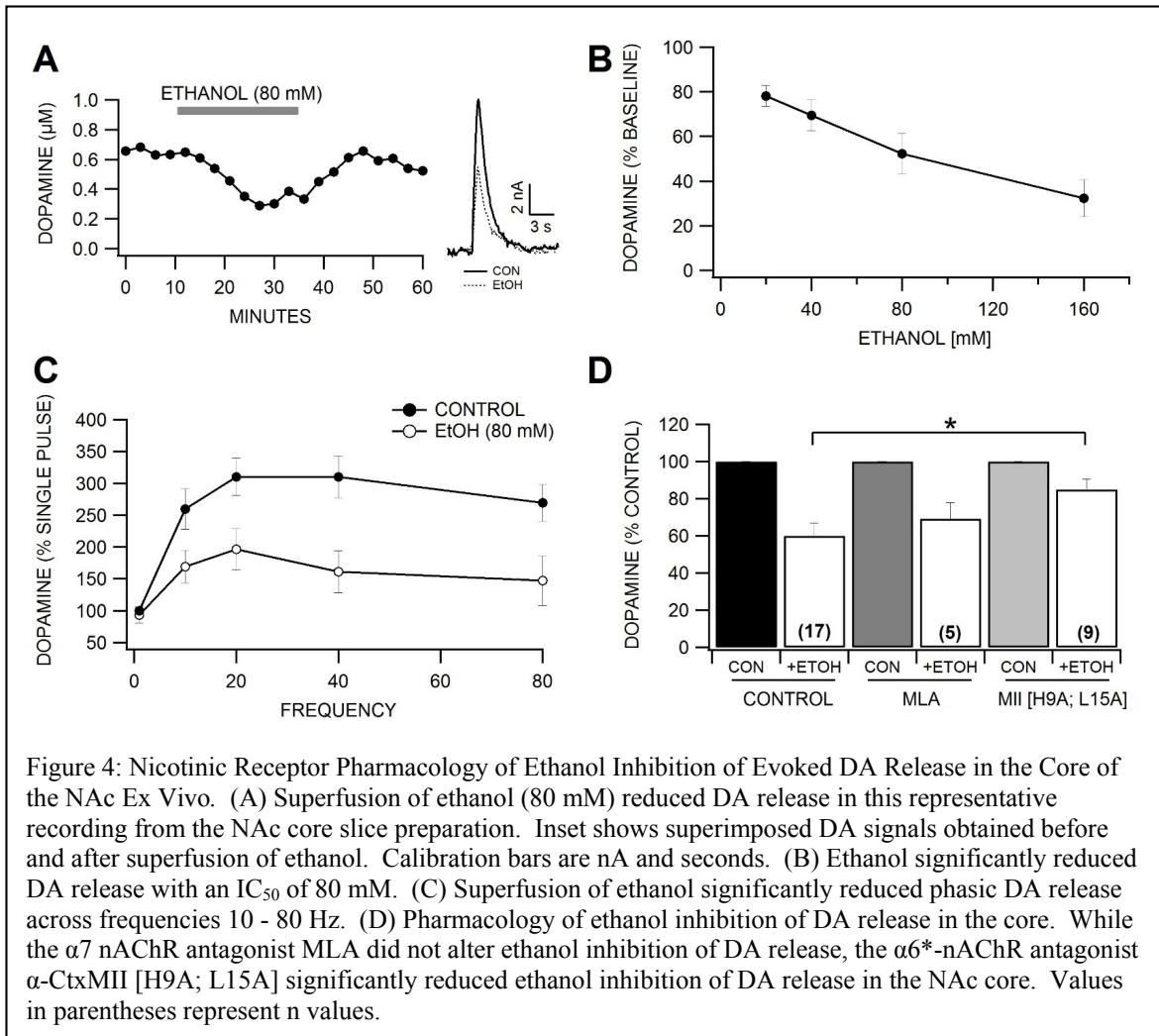
of nicotine (10  $\mu$ M) in the NAc shell (Fig. 2B) vs the NAc core (Fig. 3B). Specifically, there was a significant difference between the shell and the core at the 10  $\mu$ M nicotine concentration ( $F_{1,12}=35.765$ ,  $p<0.0001$ ). The frequency dependency for nicotine effects on DA release in the NAc core (Fig. 3C) was similar to the shell; however, the maximum release occurred at 20 Hz rather than 40 Hz. Nicotine significantly decreased the amplitude of the DA signal across all frequencies tested. Two-way repeated measures ANOVA revealed a main effect of frequency for the amplitude of the DA signal ( $F_{5,15}=16.632$ ,  $p=0.0001$ ), and nicotine ( $F_{1,3}=592.034$ ,  $p=0.0002$ ). We analyzed the effects of the  $\alpha 6$  antagonist  $\alpha$ -conotoxins  $\alpha$ -CtxMII (100 nM) and  $\alpha$ -CtxMII [H9A; L15A] on nicotine inhibition of evoked DA release in the NAc core. We



utilized the more specific  $\alpha$ -CtxMII [H9A; L15A] to compare with  $\alpha$ -CtxMII. However, as  $\alpha$ -CtxMII [H9A; L15A] is less potent (McIntosh et al., 2004), a higher concentration (500 nM) was utilized. Superfusion of  $\alpha$ -CtxMII significantly inhibited the DA signal about 30% from baseline while the more specific  $\alpha 6^*$  antagonist  $\alpha$ -CtxMII [H9A; L15A] only slightly inhibited the DA signal 8% from baseline. Both of the  $\alpha$ -conotoxins blocked the inhibition caused by 0.1  $\mu$ M nicotine in the NAc core (Fig. 3D). One-way ANOVA comparing nicotine and MII + nicotine revealed significance ( $F_{1,8}=48.971, p=0.0001$ ). Given that  $\alpha$ -CtxMII in the NAc core completely attenuated the inhibition of nicotine, we determined not to perform any additional experiments with other nAChR antagonists (as we did in the NAc shell) as nicotine was apparently not acting by any other nAChR subunit in the NAc core. One-way ANOVA comparing nicotine and MII [H9A; L15A] + nicotine revealed significant reductions in nicotine effects in MII pretreated slices ( $F_{1,8}=8.775, p=0.0181$ ).

#### *Ethanol Inhibition of Dopamine Release in the Nucleus Accumbens: Role of $\alpha 6^*$ -nAChRs*

As nicotine inhibition of evoked DA release in the core was blocked by the  $\alpha 6$  antagonist  $\alpha$ -CtxMII (100 nM), and we have previously demonstrated that ethanol similarly decreases evoked DA release in the core (Yorgason et al., 2013), we evaluated the effects of ethanol (20 – 160 mM) on DA release, as well as the effects of  $\alpha$ -CtxMII (100 nM) on ethanol inhibition of DA release in the NAc core. From our previous experimentation, we have noted no disparities of the effects of ethanol in the NAc shell or core as we have with nicotine. Ethanol significantly decreased the peak amplitude of the DA signal with an  $IC_{50}$  of approximately 80 mM (Fig. 4A, B; 20 mM:  $p=0.001, n=11$ ; 40 mM:  $p=0.001, n=11$ ; 80 mM:  $p=0.0002, n=11$ ; 160 mM:  $p<0.0001, n=10$ ). Ethanol significantly decreased the amplitude of the DA signal across all frequencies tested with the exception of single pulse stimulation (Fig. 4C). This confirms the



frequency dependent nature of ethanol in the NAc reported previously (Yorgason et al., 2013).

A two-way Repeated Measures ANOVA revealed a main effect of frequency for the amplitude of the DA signal ( $F_{4,32}=25.703$ ,  $p<0.0001$ ), ethanol ( $F_{1,8}=6.744$ ,  $p=0.032$ ), and interaction of frequency by ethanol ( $F_{1,8}=4.427$ ,  $p=0.006$ ). We analyzed evoked DA release in the presence of ethanol with the specific  $\alpha 7$  and  $\alpha 6$  nAChR antagonists methyllycaconitine (MLA; 100 nM) and  $\alpha$ -CtxMII [H9A; L15A] (500 nM), respectively. MLA did not alter ethanol inhibition of evoked DA release in the core at 80 mM ethanol (Fig. 4D;  $F_{1,20}=0.478$ ,  $p=0.497$ ). However, superfusion of the specific  $\alpha 6^*$ -nAChR antagonist  $\alpha$ -CtxMII revealed a significant effect of  $\alpha$ -CtxMII [H9A; L15A] on ethanol inhibition of evoked DA release in the NAc (Fig. 4D;  $F_{1,30}=4.296$ ,  $p=0.047$ ).

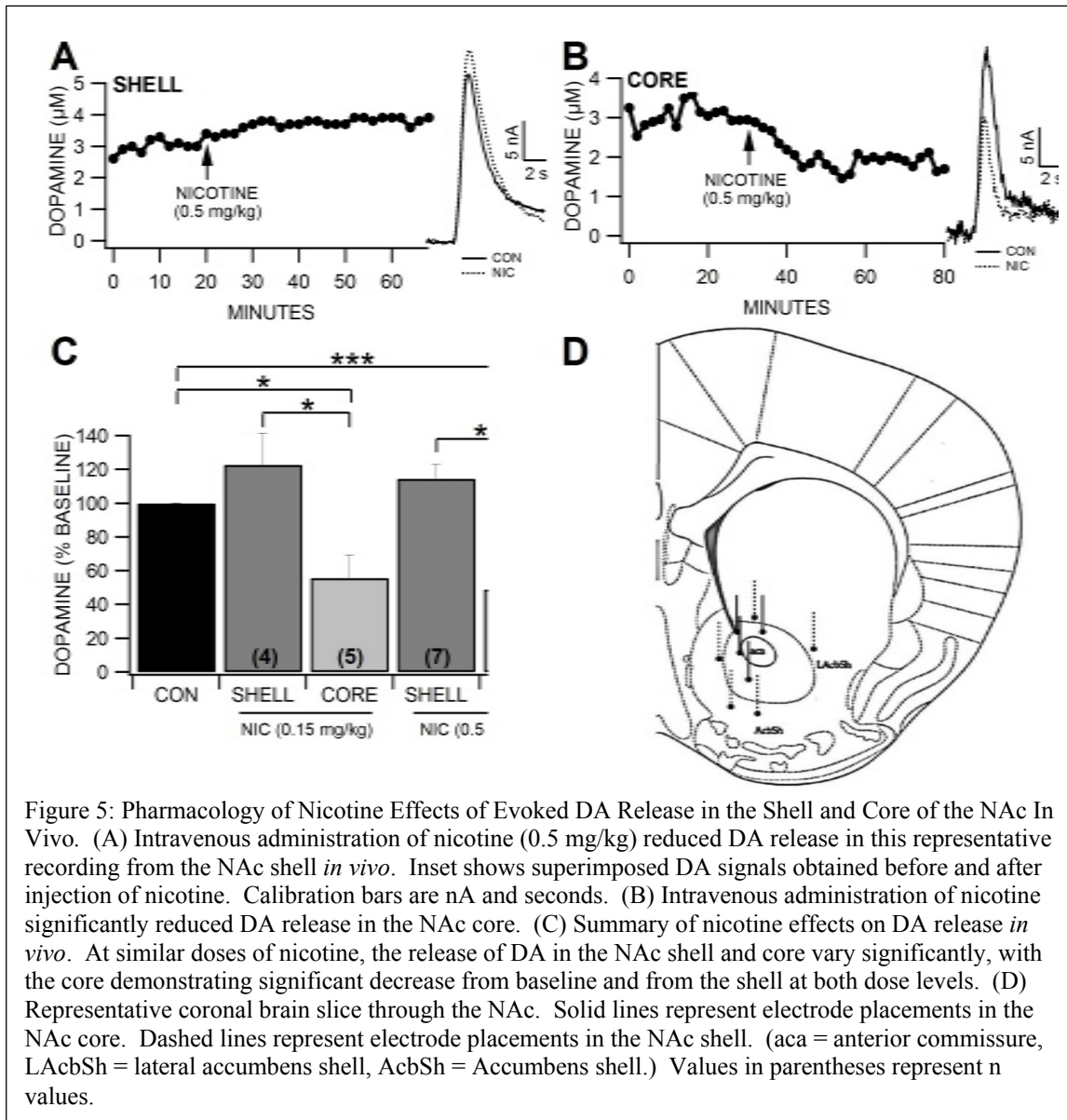


Figure 5: Pharmacology of Nicotine Effects of Evoked DA Release in the Shell and Core of the NAc In Vivo. (A) Intravenous administration of nicotine (0.5 mg/kg) reduced DA release in this representative recording from the NAc shell *in vivo*. Inset shows superimposed DA signals obtained before and after injection of nicotine. Calibration bars are nA and seconds. (B) Intravenous administration of nicotine significantly reduced DA release in the NAc core. (C) Summary of nicotine effects on DA release *in vivo*. At similar doses of nicotine, the release of DA in the NAc shell and core vary significantly, with the core demonstrating significant decrease from baseline and from the shell at both dose levels. (D) Representative coronal brain slice through the NAc. Solid lines represent electrode placements in the NAc core. Dashed lines represent electrode placements in the NAc shell. (aca = anterior commissure, LAcbSh = lateral accumbens shell, AcbSh = Accumbens shell.) Values in parentheses represent n values.

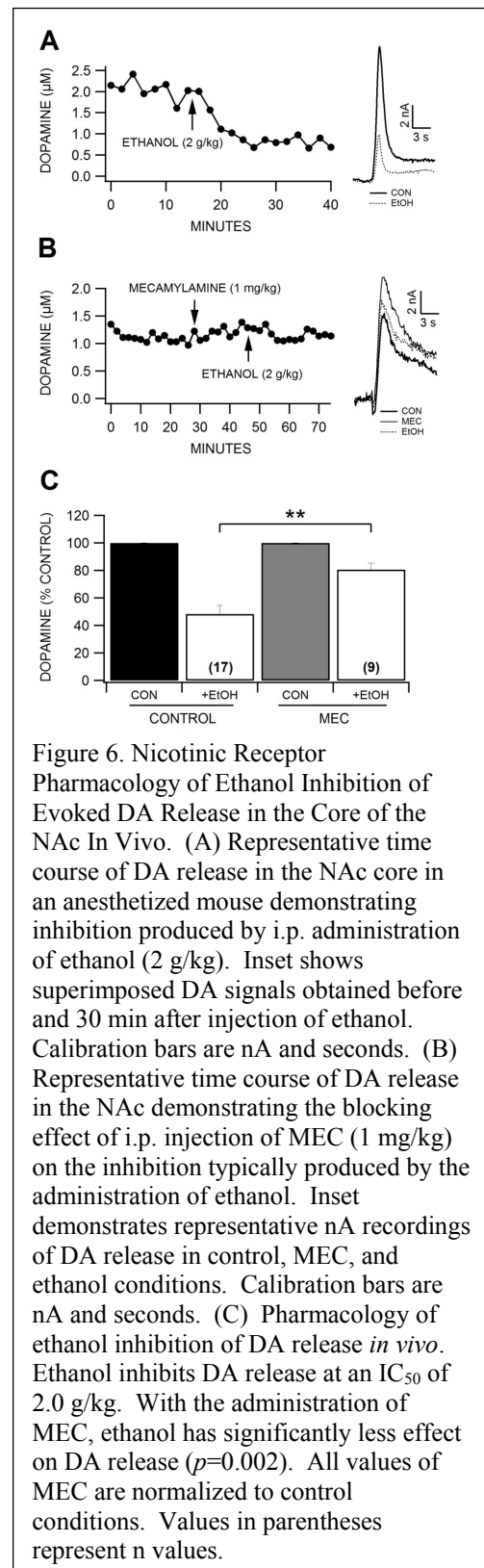
### Effects of Nicotine In Vivo In Both the Shell and Core of the Nucleus Accumbens

Utilizing FSCV, we evaluated the effects of i.v. nicotine (0.15 – 0.5 mg/kg) on evoked DA release in the NAc shell and core *in vivo*. Nicotine had a slightly excitatory effect in the NAc shell (Fig. 5A, C), although it did not prove statistically significant via one-way ANOVA at 0.15 mg/kg ( $F_{(1,7)}=1.508$ ,  $p=0.265$ ) or 0.5 mg/kg ( $F_{(1,13)}=3.008$ ,  $p=0.108$ ). However, nicotine had an inhibitory effect on evoked DA release in the NAc core at both 0.15 mg/kg ( $F_{(1,9)}=10.435$ ,

$p < 0.012$ ) and 0.5 mg/kg (Fig. 5B, C;  $F_{1,15} = 27.766$ ,  $p < 0.0001$ ). Although an  $IC_{50}$  is statistically visualized at 0.15 mg/kg, the signals were more inconsistent at that dose than at 0.5 mg/kg. Thus, we utilized 0.5 mg/kg nicotine as our  $IC_{50}$  dose. As observed with the *ex vivo* experimentation, there was a significant difference between the response of the NAc shell and core with nicotine administration (Fig. 5C). One-way ANOVA revealed significance of 0.15 mg/kg core vs shell ( $F_{1,8} = 8.874$ ,  $p = 0.021$ ) and 0.5 mg/kg core vs shell ( $F_{1,14} = 25.448$ ,  $p = 0.0002$ ). Electrode placements were verified by microscopically visualizing the lesions in coronal slices induced by high current at the tip of the CFE (Fig. 5D).

#### *Role of nAChRs In Ethanol Inhibition of Dopamine Release In Vivo*

Typically, acute ethanol administration (2 g/kg) reduces the DA signal approximately 50% (Fig. 6A) *in vivo*. It has previously been shown that the non-specific nAChR antagonist mecamylamine [(MEC); (Peng et al., 2013)] reduces ethanol consumption and blocks ethanol-induced DA release in the NAc (Blomqvist, Engel, Nissbrandt, & Soderpalm, 1993; Hendrickson et



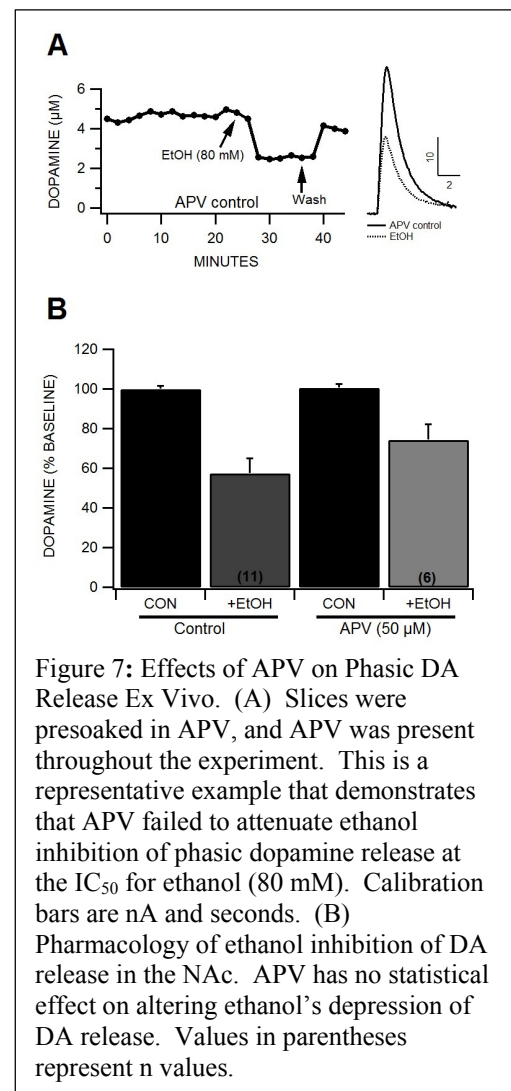
**Figure 6. Nicotinic Receptor Pharmacology of Ethanol Inhibition of Evoked DA Release in the Core of the NAc In Vivo.** (A) Representative time course of DA release in the NAc core in an anesthetized mouse demonstrating inhibition produced by i.p. administration of ethanol (2 g/kg). Inset shows superimposed DA signals obtained before and 30 min after injection of ethanol. Calibration bars are nA and seconds. (B) Representative time course of DA release in the NAc demonstrating the blocking effect of i.p. injection of MEC (1 mg/kg) on the inhibition typically produced by the administration of ethanol. Inset demonstrates representative nA recordings of DA release in control, MEC, and ethanol conditions. Calibration bars are nA and seconds. (C) Pharmacology of ethanol inhibition of DA release *in vivo*. Ethanol inhibits DA release at an  $IC_{50}$  of 2.0 g/kg. With the administration of MEC, ethanol has significantly less effect on DA release ( $p = 0.002$ ). All values of MEC are normalized to control conditions. Values in parentheses represent n values.

al., 2013). In order to understand how nAChRs contribute to ethanol's inhibitory effects on DA in the NAc, we evaluated the effects of MEC on evoked DA signals in anesthetized mice. Mecamylamine did not alter the DA signal on its own, consistent with our ex vivo studies (data not shown). However, MEC (1 mg/kg, i.p.) effectively blocked the inhibitory effects of ethanol in the NAc (Fig. 6B). Normalizing data to control values, MEC + ethanol demonstrated a statistical significance from ethanol alone (Fig. 6C;  $F_{1,24}=12.279$ ,  $p=0.002$ ).

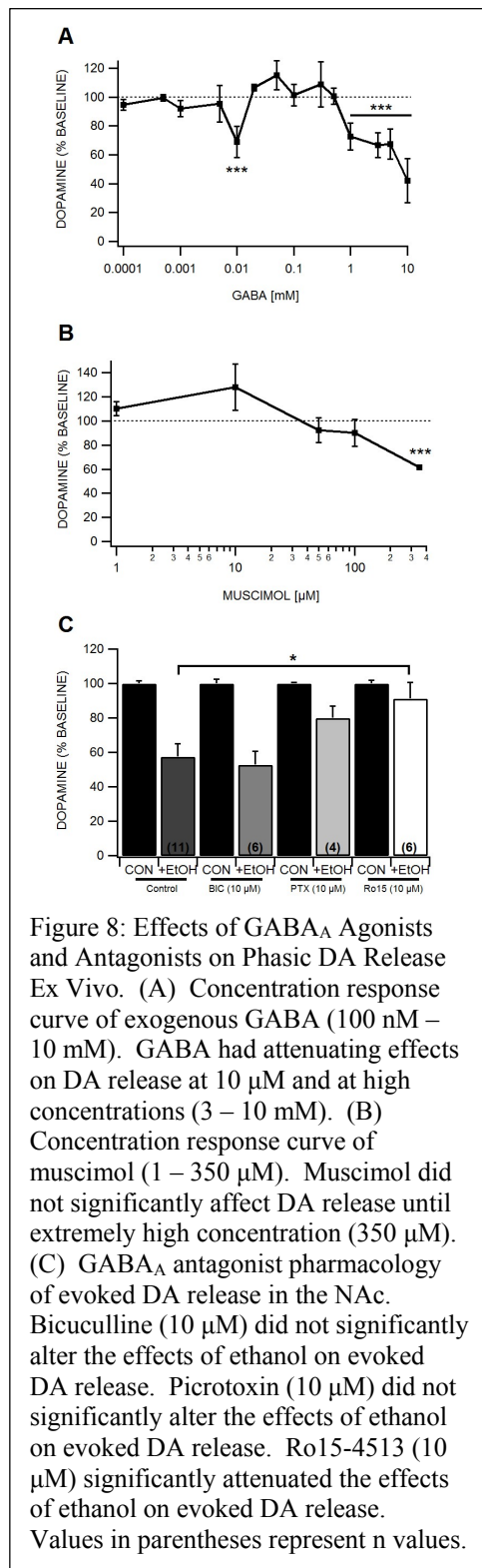
#### *Effects of NMDA Antagonist APV on Dopamine Release in the Nucleus Accumbens Ex Vivo*

Whenever there is a change in neuron firing intensity or quantal neurotransmitter release, it is important to first determine whether changes are due to NMDA related long-term potentiation (LTP). As we have previously demonstrated that ethanol significantly reduces phasic DA release (Schilaty et al., 2014; Yorgason et al., 2013), we tested the effects of the NMDA antagonist D- 2-amino-5-phosphonovaleric acid (APV) on DA release in the NAc. The slices were pre-treated with APV (50  $\mu$ M) for 1 hour prior to experimentation. In the presence of APV, phasic DA release was inhibited by superfusion of 80 mM ethanol (Fig. 7A), the  $IC_{50}$  level of ethanol on phasic DA release (Schilaty et al., 2014; Yorgason et al., 2013).

Two-way ANOVA of control vs control ethanol revealed a main effect of ethanol ( $F_{1,33}=33.6$ ,



$p<0.0001$ ) but no significant effect of APV ( $p>0.05$ ) and no interactions between APV and ethanol (Fig. 7B;  $p>0.05$ ).



### *Effects of GABA<sub>A</sub> Typical Agonists and Antagonists on Dopamine Release in the Nucleus Accumbens Ex Vivo*

To determine the role of GABA in ethanol inhibition of DA release in the NAc, and the pharmacological characteristics of the hypothesized GABA<sub>A</sub>R present in the NAc that is modulating the release of DA, we evaluated the effects of superfusion of GABA and select GABA<sub>A</sub>R agonists and antagonists.

Superfusion of GABA (100 nM – 10 mM) demonstrated a sensitivity at 10 μM via one-way ANOVA (Fig. 8A;  $F_{1,17}=28.85$ ,  $p<0.0001$ ) and at very high concentration levels via one-way ANOVA [1 – 10 mM; at 1 mM (Fig.

8A;  $F_{1,23}=12.302$ ,  $p=0.002$ ]. Superfusion of the potent, selective GABA<sub>A</sub> agonist, muscimol (1 – 350 μM), did not significantly affect phasic DA release at 1 – 100 μM, but significantly reduced phasic DA release at the high concentration (350 μM; Fig. 8B;  $F_{1,10}=132.34$ ,

$p<0.0001$ ). We tested the effects of select GABA antagonists on phasic DA release and ethanol inhibition of phasic DA release. The chloride channel GABA antagonist picrotoxin demonstrated marginal

effectiveness in attenuating ethanol inhibition of DA release at 10  $\mu$ M , a dose typically used by us and others to block GABA inhibition of mesolimbic neuronal activity, however the results were not statistically significant (Fig. 8C;  $F_{1,13}=2.906$ ,  $p=0.112$ ). The GABA<sub>A</sub>R antagonist, bicuculline (10  $\mu$ M), a dose typically used by us and others to block GABA<sub>A</sub>R-mediated inhibition, had no effect on its own, and was completely ineffective at blocking ethanol inhibition of DA release (Fig. 8C;  $F_{1,16}=0.174$ ,  $p=0.682$ ).

#### *Effects of Chlordiazepoxide and Pentobarbital on Dopamine Release in the Nucleus Accumbens Ex Vivo*

Benzodiazepines and barbiturates are well known drugs that act as potent GABA<sub>A</sub> positive allosteric modulators, potentiating the effects of GABA at the GABA<sub>A</sub>R (Carter, Kozuska, & Dunn, 2010). Additionally, the binding sites of barbiturates and benzodiazepines are distinct and separate from one another and from GABA. Superfusion of the benzodiazepine chlordiazepoxide (CDX; 30  $\mu$ M), a dose used by us and others to enhance GABA inhibition (Kasparov et al., 2001), did not significantly affect phasic DA release as seen with 80 mM ethanol superfusion (Fig. 9A, C;  $F_{1,13}=1.269$ ,  $p=0.280$ ). Superfusion of the barbiturate pentobarbital (100  $\mu$ M)

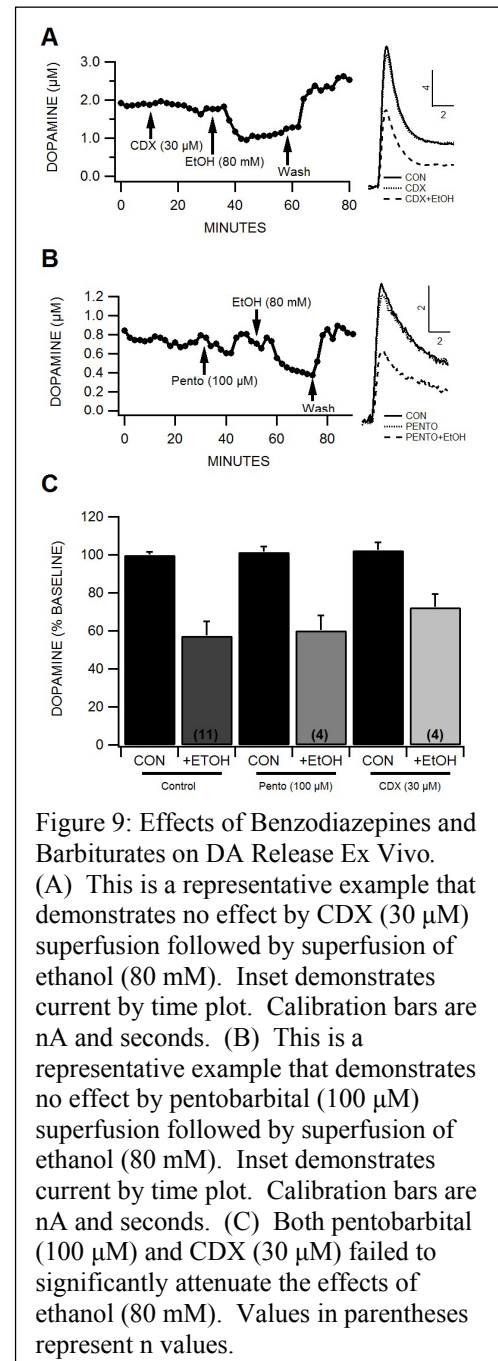
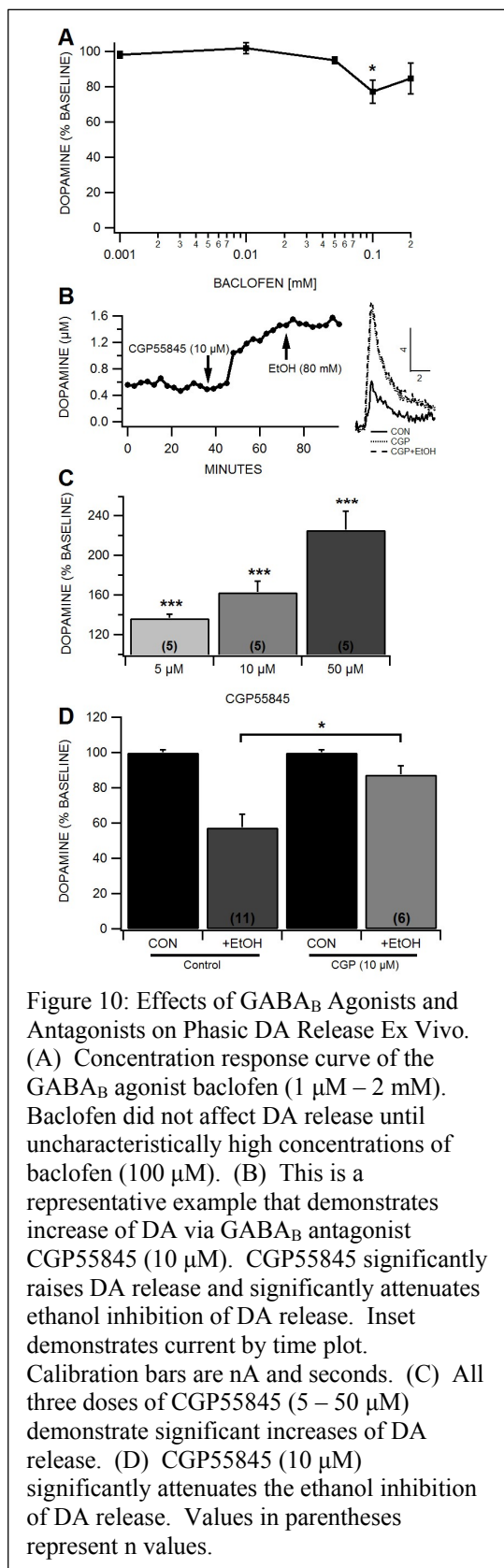


Figure 9: Effects of Benzodiazepines and Barbiturates on DA Release Ex Vivo. (A) This is a representative example that demonstrates no effect by CDX (30  $\mu$ M) superfusion followed by superfusion of ethanol (80 mM). Inset demonstrates current by time plot. Calibration bars are nA and seconds. (B) This is a representative example that demonstrates no effect by pentobarbital (100  $\mu$ M) superfusion followed by superfusion of ethanol (80 mM). Inset demonstrates current by time plot. Calibration bars are nA and seconds. (C) Both pentobarbital (100  $\mu$ M) and CDX (30  $\mu$ M) failed to significantly attenuate the effects of ethanol (80 mM). Values in parentheses represent n values.





had no effect on DA release and did not alter the effects of ethanol (Fig. 9B, C;  $F_{1,13}=0.043$ ,  $p=0.838$ ).

### *Effects of GABA<sub>B</sub> Agonists and Antagonists on Dopamine Release in the Nucleus Accumbens Ex Vivo*

Due to the lack of effects of typical GABA<sub>A</sub> agonists to mimic the effects of the IC<sub>50</sub> of ethanol, as well as the lack of typical GABA antagonists to affect ethanol inhibition of phasic DA release, we evaluated the effects of select GABA<sub>B</sub> receptor agonists and antagonists. Superfusion of the GABA<sub>B</sub> agonist baclofen (0.001 – 2 mM) did not significantly affect phasic DA release until 100 μM (Fig. 10A;  $F_{1,6}=10.555$ ,  $p=0.017$ ), a dose that is 100X higher than the typical IC<sub>50</sub> effect of baclofen on neuronal activity in the mesolimbic neurons. Interestingly, the potent and selective antagonist CGP55845 (5 – 50 μM) remarkably raised DA release on its own and significantly attenuated ethanol inhibition of phasic DA release (Fig. 10B, C;  $F_{1,15}=7.653$ ,  $p=0.014$ ). As the GABA<sub>B</sub> antagonist was effective in altering the response of terminal

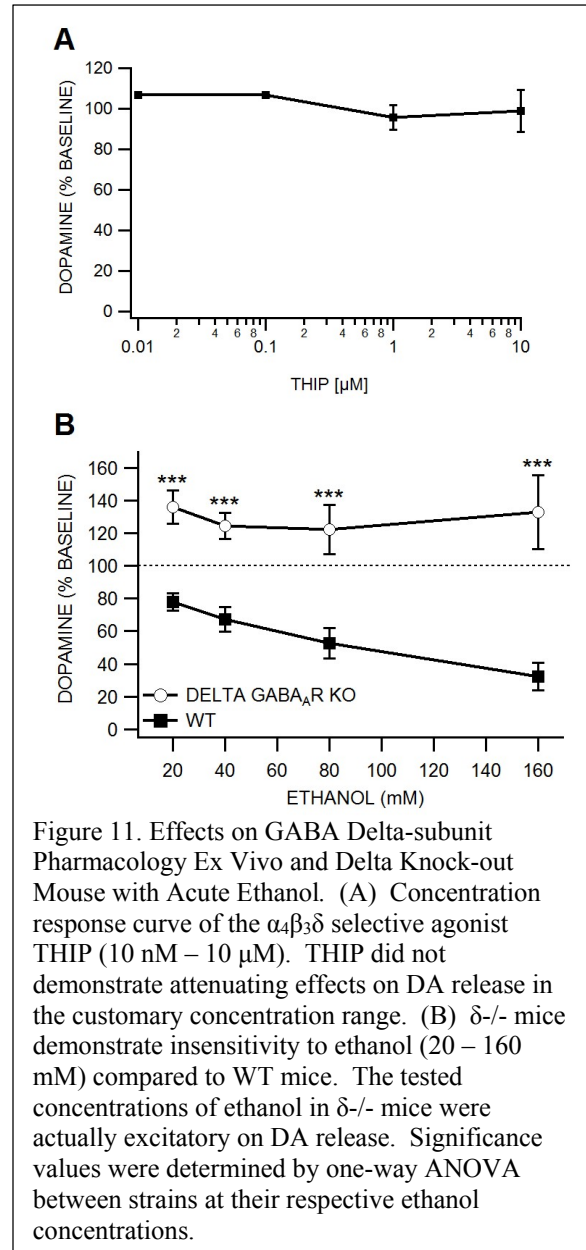


evoked DA release but baclofen was not effective under normal concentrations, we tested the effects of SKF97541, a GABA<sub>B</sub> agonist that is 10X more potent than baclofen. SKF97541 (10  $\mu$ M) did not significantly affect phasic DA release in the NAc (data not shown;  $F_{1,11}=0.460$ ,  $p=0.512$ ), suggesting that CGP55845 may be acting through non-GABA<sub>B</sub> dependent mechanisms to enhance DA release and attenuate ethanol's effects.

*Effects of GABA<sub>A</sub>  $\delta$ -subunit Agonists and Antagonists and GABA<sub>A</sub>  $\delta$ -subunit Knock Out Mice on Dopamine Release in the Nucleus Accumbens Ex Vivo*

Given that the typical GABA<sub>A</sub> agonists and antagonists were ineffective in altering evoked DA release, and that there were mixed results with GABA<sub>B</sub> agonists and antagonists, we considered the involvement of atypical GABA<sub>A</sub>R subunits. Superfusion of the  $\delta$ -subunit selective GABA<sub>A</sub> agonist 4,5,6,7-Tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol hydrochloride (THIP; 10 nM – 10  $\mu$ M) had no effect on phasic DA release (Fig. 11A). Superfusion of the  $\delta$ -subunit selective GABA<sub>A</sub> antagonist Ro15-4513 (10  $\mu$ M) had no effect on phasic DA release, but significantly reduced ethanol inhibition of phasic DA release (Fig. 8C;  $F_{1,15}=7.618$ ,  $p=0.015$ ). Subsequently,

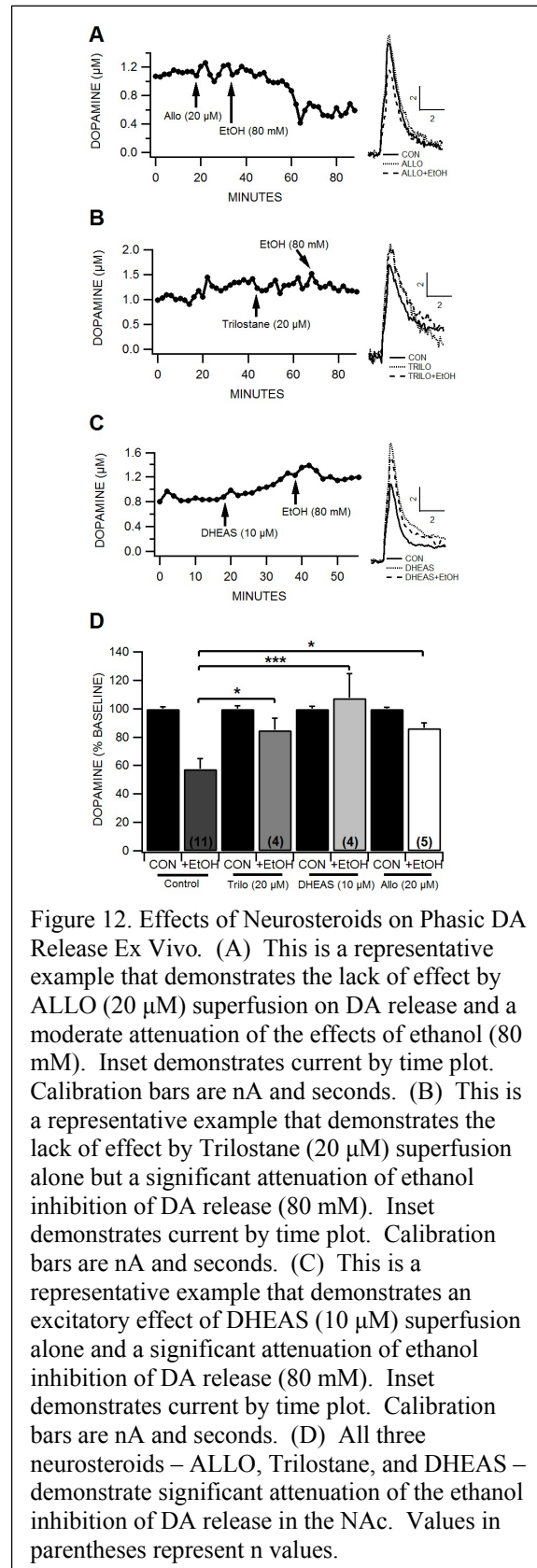
we utilized  $\delta$ -/- mice to evaluate their dose-response sensitivity to ethanol *ex vivo* at the same



concentration levels (20 mM – 160 mM) as WT mice. Compared to WT mice, the  $\delta^{-/-}$  mice demonstrated enhanced phasic DA release with exposure to all concentrations of ethanol (Fig. 11B). Two-way ANOVA revealed significance of strain ( $F_{1,59}=62.41$ ,  $p<0.0001$ ), concentration ( $F_{1,59}=8.09$ ,  $p=0.0006$ ), and strain by concentration ( $F_{1,59}=4.11$ ,  $p=0.0163$ ).

#### *Effects of Neurosteroids on Dopamine Release in the Nucleus Accumbens Ex Vivo*

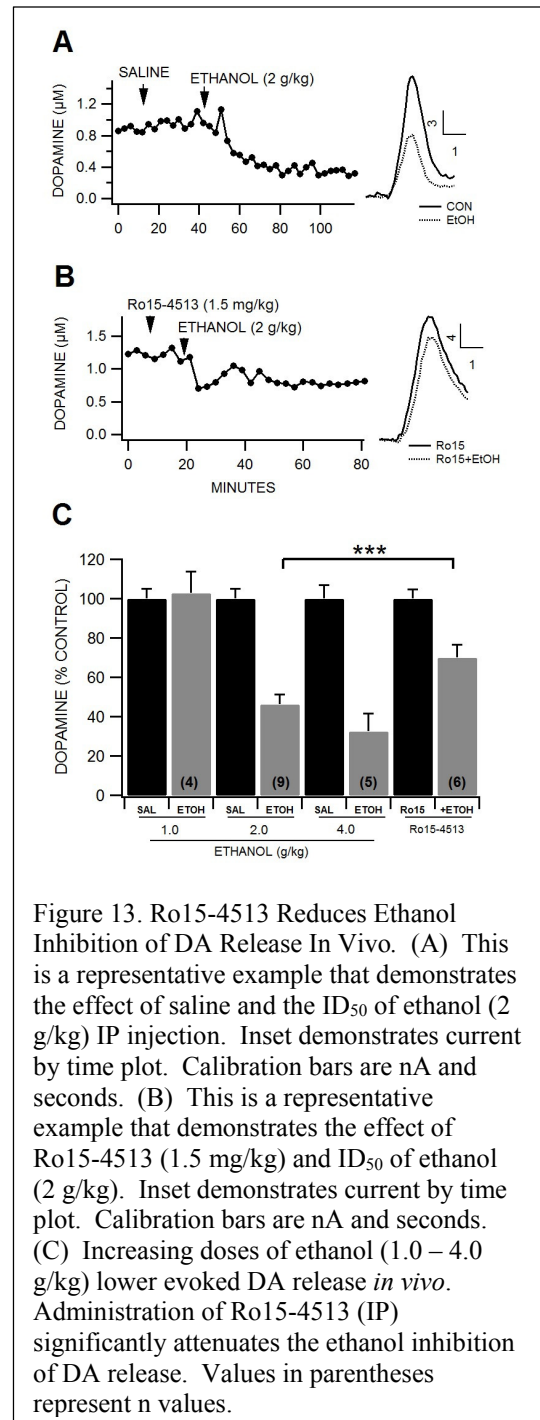
Superfusion of allopregnanolone (ALLO; 20  $\mu$ M), a potent GABA<sub>A</sub>R positive allosteric neurosteroid modulator, did not alter DA release on its own but slightly attenuated ethanol inhibition of DA release (Fig. 12A, D;  $F_{1,14} = 6.316$ ,  $p = 0.025$ ). Superfusion of (4 $\alpha$ ,5 $\alpha$ ,17 $\beta$ )-3,17-dihydroxy-4,5-epoxyandrost-2-ene-2-carbonitrile (Trilostane; 20  $\mu$ M), an inhibitor of 3 $\beta$ -hydroxysteroid dehydrogenase (effectively raising endogenous levels of the neurosteroid dehydroepiandrosterone-sulfate (DHEAS), produced no significant effects on DA release on its own, yet significantly



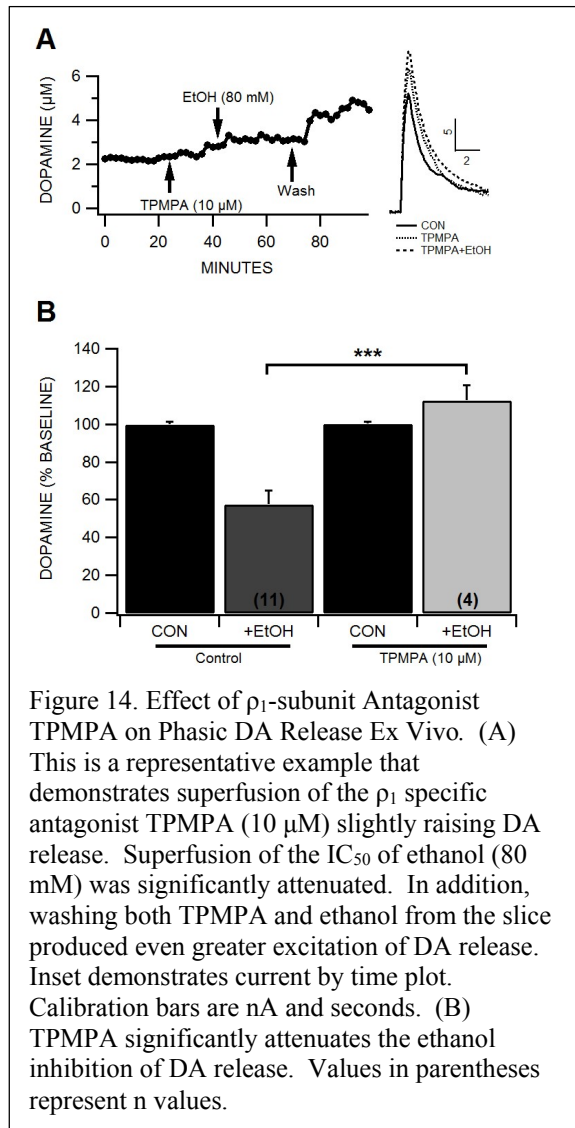
attenuated the effects of ethanol (Fig. 12B, D;  $F_{1,14}=6.073$ ,  $p=0.027$ ). Superfusion of the GABA<sub>A</sub>R negative allosteric modulator DHEAS (10  $\mu$ M) increased DA release and markedly attenuated the effects of ethanol on DA release (Fig. 12C, D;  $F_{1,13}=19.416$ ,  $p=0.001$ ).

#### *Effects of Ro15-4513 on Dopamine Release in the Nucleus Accumbens In Vivo*

To confirm the physiological relevancy of atypical GABA<sub>A</sub>R antagonists on ethanol inhibition of phasic DA release *ex vivo*, we evaluated the effects of the  $\delta$ -subunit selective GABA<sub>A</sub> antagonist, Ro15-4513 *in vivo*. We have previously demonstrated that ethanol reduces phasic DA release in vivo (Fig. 13A) with an IC<sub>50</sub> of 2.0 g/kg (Schilaty et al., 2014), similar to what others have shown. Administration of the Ro15-4513 (1.5 mg/kg, IP), did not affect DA release (Fig. 13B;  $F_{1,10}=0.509$ ,  $p=0.492$ ), but significantly attenuated ethanol inhibition of phasic DA release in the NAc in isoflurane-anesthetized mice compared to ethanol controls (Fig. 13C;  $F_{1,14}=12.223$ ,  $p=0.004$ ).



## Effects of GABA<sub>A</sub> $\rho$ -subunit Antagonist TPMPA on Dopamine Release in the Nucleus Accumbens



To confirm evidence of GABA<sub>A</sub>  $\rho_1$ -subunit pharmacology, we tested the  $\rho_1$ -subunit antagonist, 1,2,5,6-Tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA). Superfusion of TPMPA (10  $\mu$ M) produced a mild (but statistically insignificant) increase in evoked DA release on its own (Fig. 14A), but significantly attenuated ethanol inhibition of phasic DA release in the NAc (Fig. 14B;  $F_{1,13}=17.124$ ,  $p=0.001$ ).

## Immunohistochemistry of GABA<sub>A</sub> $\rho_1$ -subunit in the Nucleus Accumbens

To identify the presence of the GABA<sub>A</sub>  $\rho_1$ -subunits in the NAc, we observed GABRR1 labeled subunits using immunofluorescence. Images identified the presence of  $\rho_1$ -subunits (Fig. 15) in the NAc. Labeling of  $\rho_1$ -subunits was

observed in both GAD-GFP+ and non-GFP+ cell populations (with utilization of FITC filter) in CD-1 mice. Combined viewing clearly distinguished the  $\rho_1$ -containing neurons from the GABA containing neurons.

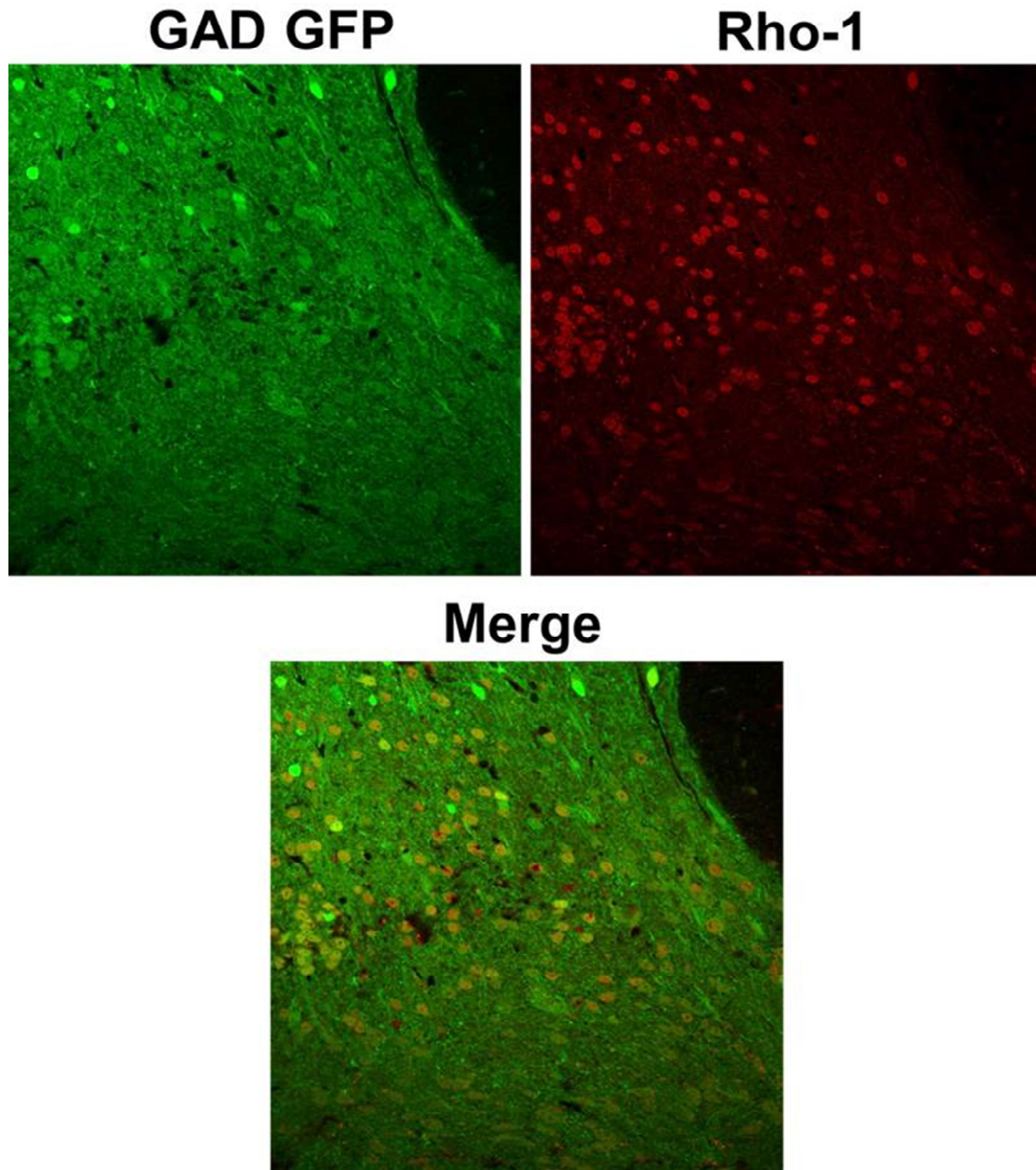


Figure 15. Expression of GABA<sub>A</sub>  $\rho_1$ -subunit in Nucleus Accumbens of CD-1 Mice. GABA neurons were revealed by green fluorescence from FITC filter (A) and GABA<sub>A</sub>  $\rho_1$  subunit immunoreactivity was revealed with red fluorescence in the NAc of CD-1 mice (B). This image shows that most GABA<sub>A</sub>  $\rho_1$ -subunits were in GABA neurons in the NAc (C). The bar indicates a scale of 100  $\mu$ m. AC = anterior commissure.



### *Effects of Baicalein on Dopamine Release in the Nucleus Accumbens Ex Vivo*

To verify whether the isoflavone baicalein could serve as a potential nutraceutical agent for counteracting the effects of ethanol, we superfused baicalin hydrate (50  $\mu$ M) prior to the superfusion of ethanol (80 mM).

Baicalin hydrate appeared to significantly attenuate the inhibitory effects of ethanol on DA release in the NAc *ex vivo* (data not shown), however, we discovered that baicalin hydrate has a redox potential similar to DA and masked the effects seen by ethanol inhibition. Thus, we determined to use baicalein (as it does not have a redox potential similar to DA) dissolved in either DMSO or propylene glycol. With the *ex vivo* setup, concentrations of DMSO (0.1 – 10%) produced significant electrode

issues in which the DA signal would be lost. Thus, we switched to the solvent propylene glycol. Unfortunately, at reasonable concentration of propylene glycol (1 – 20%), we were not able to obtain complete solubility of baicalein for superfusion. Thus, with incomplete solubility of baicalein (50  $\mu$ M; and thus inaccurate parameters on concentration), baicalein significantly attenuated the ethanol inhibition of DA release (Fig. 17A,  $F_{1,16}=28.010$ ,  $p<0.001$ ).

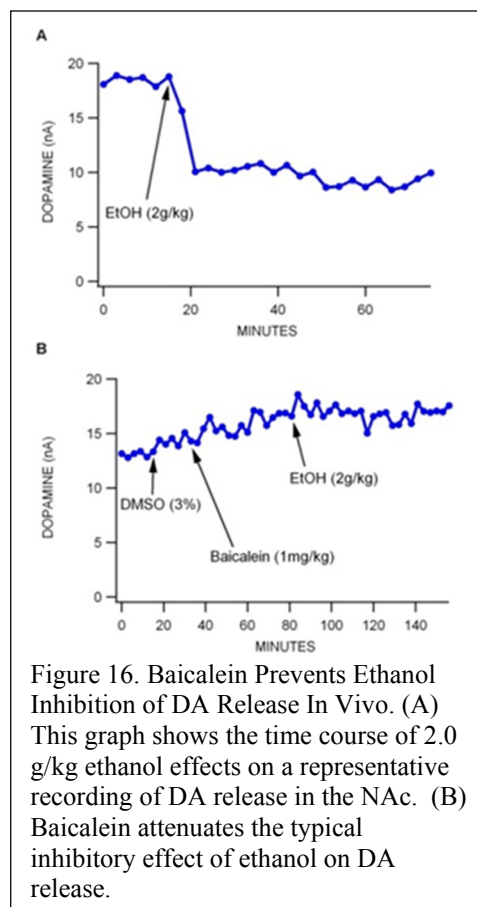
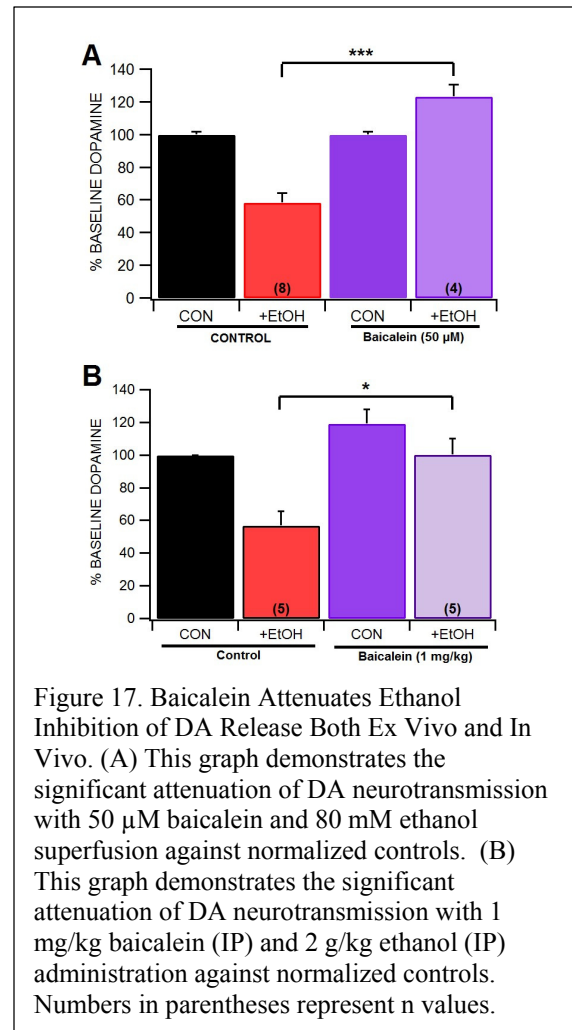


Figure 16. Baicalein Prevents Ethanol Inhibition of DA Release In Vivo. (A) This graph shows the time course of 2.0 g/kg ethanol effects on a representative recording of DA release in the NAc. (B) Baicalein attenuates the typical inhibitory effect of ethanol on DA release.

### *Effects of Baicalein on Dopamine Release in the Nucleus Accumbens In Vivo*

As *ex vivo* did not demonstrate accurate results based on the insufficiency of solubility of baicalein in both DMSO and propylene glycol and the redox potential of the baicalein metabolite baicalin hydrate, we dissolved baicalein (1 mg/kg) in DMSO (3 %) and administered it IP 30 minutes prior to ethanol (2.0 g/kg). Baicalein significantly attenuated the ethanol inhibition of DA release in the NAc *in vivo* (Fig. 16A, B; 17B;  $F_{1,8}=11.292$ ,  $p=0.01$ ).



## CHAPTER 4: Discussion

We demonstrated that evoked DA release is reduced by nicotine application in the NAc shell and core *ex vivo*. These inhibitory effects of nicotine were observed across all frequencies and were blocked by nAChR antagonists. Specifically, in the shell, nicotine's effects were blocked by the  $\alpha 4\beta 2^*$  selective antagonist DH $\beta$ E, but not by the  $\alpha 6$  nAChR antagonist  $\alpha$ -CtxMII. In contrast, nicotine modulation of DA signals in the core appears to be more through  $\alpha 6$  nAChRs, as  $\alpha$ -CtxMII, and the more selective  $\alpha 6$  antagonist  $\alpha$ -CtxMII [H9A; L15A], blocked nicotine's inhibition of evoked DA release. Similar to previous studies, we demonstrated that ethanol reduces stimulated DA release in the core in a frequency dependent manner, with greatest inhibition at higher stimulation frequencies. Ethanol's inhibitory effects were blocked by  $\alpha$ -CtxMII [H9A; L15A], but not MLA, suggesting that ethanol is acting through heteromeric  $\alpha 6^*$  nAChRs, and not  $\alpha 7$  homomeric receptors. We also demonstrated that the non-specific nAChR antagonist MEC also block's ethanol's inhibitory effects on evoked DA release under *in vivo* conditions.

### *Region Dependent Nicotine Modulation of Dopamine Release*

Nicotine decreased evoked DA release *ex vivo* in both the shell and the core of the NAc with an IC<sub>50</sub> of 0.1  $\mu$ M. Nicotine behaved similarly in the NAc shell and core except for at the highest concentration of 10  $\mu$ M, where the NAc core demonstrated significantly less inhibition of DA release (Fig. 1B, 2B), suggesting that the  $\alpha 6^*$  nAChRs in the NAc core desensitize to high concentrations of nicotine as compared to nAChRs in the NAc shell. More importantly, this significant variability in response to the higher concentrations of nicotine in the NAc core vs NAc shell demonstrates specific differences in nAChRs subunits in the NAc core and the NAc shell. Nicotine decreased evoked DA release in the core *in vivo*, but had no significant effect in



the shell. Although these results do not exactly correspond to our findings *ex vivo*, it is important to note that the *ex vivo* recordings are less influenced by intact circuit responses, but rather the behavior of stimulation at terminals of the neurons in the NAc. More importantly, the *in vivo* results demonstrate and further verify that there exists a significant variability in the nAChRs present in the NAc shell and core and provide compelling evidence that the *ex vivo* pharmacology demonstrated was valid.

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated cation (primarily  $\text{Na}^+$  or  $\text{Ca}^{2+}$ ) channels of a wide variety in the central nervous system (CNS). These channels can exist in either homomeric or heteromeric form, with the  $\alpha 7$  subunit composing the most common homomeric nAChR in the CNS, and pentameric mixture of alpha ( $\alpha 2$ -  $\alpha 10$ ) and beta ( $\beta 2$ - $\beta 4$ ) subunits, with  $\alpha 4\beta 2^*$ -nAChRs as the most common heteromeric CNS receptor (Taylor, Burman, et al., 2013). Although nAChRs are of a wide variety in the VTA (Wooltorton, Pidoplichko, Broide, & Dani, 2003), heteromeric  $\alpha 6^*$ -nAChRs are highly expressed in the mesolimbic DA system (Champtiaux et al., 2003; K. Yang et al., 2011; K. C. Yang, Jin, & Wu, 2009), and  $\alpha 6$  is the primary  $\alpha$  subunit that plays a prominent role in DA release (Quik, Perez, & Grady, 2011), and they are predominantly expressed in catecholaminergic systems (Brunzell, 2012).  $\alpha 6^*$ -nAChR subunits are functional in recombinant systems when paired with  $\beta$  subunits or hybrids of  $\alpha$  subunits. In fact, expression of the  $\alpha 6$  nAChR subunit is 16-fold higher than other subunit mRNAs in the VTA (K. Yang et al., 2009), and they have been implicated in DA transmission and nicotine dependence (Brunzell, 2012; Drenan et al., 2010; Drenan et al., 2008; Exley et al., 2008; Gotti et al., 2010; Jackson, McIntosh, Brunzell, Sanjakdar, & Damaj, 2009).

Nicotine inhibition of evoked DA release in the shell and the core of the NAc were blocked by nAChR antagonists *ex vivo*. However, the NAc core, but not the NAc shell, was

blocked by  $\alpha$ -conotoxins, suggesting that nicotine's effects are mediated by  $\alpha 6^*$ -nAChRs. Although it was previously determined that cholinergic interneurons excite presynaptic nAChRs on DA terminals (M. T. Brown et al., 2012b; I. W. Jones, Bolam, & Wonnacott, 2001; K. C. Yang et al., 2009; Zhang et al., 2009), we demonstrate here that acute nicotine administration causes a decrease in evoked DA release in the NAc, as reported previously by others (Perez, McIntosh, & Quirk, 2013; Zhang et al., 2009). Under the previous model, direct activation of the nAChR on the DA terminal would induce increased DA release, as a cationic influx into the presynaptic terminal would induce a higher magnitude of vesicular release. However, our results would suggest that there are interneurons regulating DA release in the NAc. It is likely that nAChRs are present on the terminals of these interneurons that then modulate the release of DA from DAergic neurons (Taylor, Steffensen, et al., 2013; K. Yang et al., 2011). Under this model, activation of the nAChRs on the interneurons would decrease the level of evoked DA release recorded by FSCV. Our experiments with the specific  $\alpha 6^*$ -nAChR antagonists  $\alpha$ -conotoxins in both the NAc core and NAc shell demonstrate a distinct difference in the nAChR distribution in these anatomical regions. Given that  $\alpha$ -CtxMII completely blocks the effects of nicotine in the NAc core and does not block the effects of nicotine in the NAc shell, yet DH $\beta$ E completely blocks the effects of nicotine in the NAc shell, these results indicate that the NAc core primarily operates via  $\alpha 6^*$ -nAChRs and the NAc shell primarily operates via  $\alpha 4\beta 2$  nAChRs. This verifies the results of those previously reported (Exley, McIntosh, Marks, Maskos, & Cragg, 2012).

#### *Ethanol Inhibits Dopamine Release Through nAChR Interactions*

Given that ethanol has been shown to decrease evoked DA release, as measured by FSCV, we sought to evaluate the role of ethanol on nAChRs in mediating evoked DA release in the NAc *in vivo* and *ex vivo*, as we are not aware of any previous studies that have systemically

focused on the involvement of ethanol inhibition via nAChRs at DA terminals. Local stimulation in the NAc slice preparation produces a robust increase in DA release with a single pulse, but evinces marked frequency modulation with multiple pulses, with optimal release at 20 Hz (10 pulses). Similar to nicotine, ethanol significantly decreased DA release at concentrations 20-160 mM and reduced phasic responses across all frequencies equally. Superfusion of the  $\alpha 7$ -subunit specific antagonist MLA had no effect on ethanol inhibition of evoked DA release, suggesting that ethanol is not acting in either the NAc core or NAc shell via  $\alpha 7^*$ -nAChRs. However, as  $\alpha$ -CtxMII and  $\alpha$ -CtxMII [H9A; L15A] significantly reduced ethanol inhibition of DA release in the NAc core, this suggests that ethanol is acting via  $\alpha 6^*$ -nAChRs in the NAc core. Thus, as  $\alpha$ -CtxMII significantly blocked both nicotine and ethanol in the NAc core, both nicotine and ethanol appear to be acting via the same  $\alpha 6^*$ -nAChRs. However, given that the block of  $\alpha$ -CtxMII was complete with nicotine and incomplete with ethanol, it is probable that ethanol is acting through more than one mechanism in the NAc, whether intracellular or through an extracellular receptor.

Ethanol reduced evoked DA release *in vivo* with an  $IC_{50}$  of 2.0 g/kg, a moderately intoxicating dose level. We determined this  $IC_{50}$  of ethanol *in vivo* through previous experimentation (data not shown). These studies provide the physiological relevancy of ethanol inhibition of evoked DA release at terminals in the NAc. As  $\alpha$ -CtxMII cannot be administered *in vivo*, due to its inability to cross the blood brain barrier, we utilized i.p. administration of MEC to block nAChRs. MEC blocked ethanol inhibition of evoked DA release, thus providing further evidence that the mechanism of ethanol inhibition of DA release occurs through a nAChR subunit. Comparing these physiologically-relevant results with our *ex vivo* experimentation, we conclude that MEC is antagonizing the  $\alpha 6^*$ -nAChRs in the NAc against the attenuation of

ethanol. Given that  $\alpha 6^*$ -nAChRs are located on GABA terminals and known to enhance GABA release (K. Yang et al., 2011), it is possible that ethanol inhibition of evoked DA release results from activation of  $\alpha 6^*$ -nAChRs on GABA terminals that inhibit evoked DA release. However, we acknowledge that the mechanism of nAChRs in the NAc may be much more complex due to the compelling evidence of nAChRs present on DA terminals (Exley et al., 2012; Zoli et al., 2002).

Interestingly,  $\alpha 6$  knock-out mice fail to self-administer nicotine. However, once the  $\alpha 6$  subunit is re-expressed via a lentiviral vector, the mice develop sustained acute self-administration behavior (Pons et al., 2008). The VTA has  $\alpha 6^*$ -nAChRs on presynaptic GABA terminals, but not on the somata of the DAergic neurons (Taylor, Steffensen, et al., 2013; K. C. Yang et al., 2009). Thus, nicotine can modulate both DA and GABA terminal neurotransmitter release (Mansvelder, Keath, & McGehee, 2002; Yin & French, 2000), although the majority of endogenous cholinergic inputs into the VTA appear to contact GABA rather than DA neurons (Fiorillo & Williams, 2000; Garzon, Vaughan, Uhl, Kuhar, & Pickel, 1999). More specifically, nAChRs play a crucial role in modulating GABA release onto DA neurons in the VTA (Taylor, Steffensen, et al., 2013; K. Yang et al., 2011). The majority of GABA neurons in the VTA express  $\alpha 4$  and  $\beta 2$  nAChR subunits, which can be blocked by the non-competitive antagonist MEC, or by the competitive antagonist dihydro- $\beta$ -erythroidine hydrobromide (DH $\beta$ E) (Mansvelder et al., 2002). It is the emerging view that the early acute effects of nicotine in the VTA predominantly affect GABA neurons, and that the nAChRs that have been associated with these cells desensitize rapidly, leading to a long-lasting excitation (disinhibition) of DA neurons through removal of the inhibitory influence of GABA (K. Yang et al., 2011). Recent optogenetic studies have demonstrated that selective activation of VTA GABA neurons drives conditioned

place aversion (Tan et al., 2012) and disrupts reward consumption (van Zessen et al., 2012), providing compelling evidence for the importance of GABA neurons in regulating DA transmission in the mesocorticolimbic reward pathway, and in particular interactions between ethanol and nicotine reward signaling. Thus, GABA inhibition is a critical regulator of DA neurotransmission in the mesocorticolimbic system and for the rewarding properties of both ethanol and nicotine.

### *Ethanol Modulation of DA Release*

Using voltammetry, we demonstrated that electrically-evoked, phasic DA release is inhibited in a dose-dependent manner by moderate to high ethanol in *ex vivo* and *in vivo* preparations, respectively. In consideration of the diverse effects of ethanol on mesolimbic DA activity as measured by both microdialysis and voltammetry, we sought to address the underlying mechanisms of ethanol's actions on evoked DA release at terminals in the NAc by examining ethanol's effects in the presence of various GABA<sub>A</sub>R agonist and antagonists. As the *ex vivo* preparation lacks the cell bodies of the projecting DA neurons and stimulation occurs locally, this setup allows us to specifically examine the phasic release of DA at terminals. Additionally, the *in vivo* preparation provides relevancy to the *ex vivo* preparation as afferent pathways remain operational.

The exact mechanism by which ethanol enhances GABA<sub>A</sub>R-mediated responses remains unclear. Ethanol potentiates GABA transmission in many brain areas (Siggins, Roberto, & Nie, 2005; Weiner & Valenzuela, 2006), and modulation of GABAergic synaptic transmission is still regarded as one of the main factors underlying alcohol withdrawal-related phenomena (Liang & Olsen, 2014). GABA<sub>A</sub>Rs undergo allosteric modulation by ethanol, anesthetics, benzodiazepines and neurosteroids, and have been implicated in the acute as well as the chronic effects of ethanol,

including tolerance, dependence and withdrawal [for a more complete review see (Enoch, 2008)]. We have reported in multiple studies that GABA neuron firing rate is inhibited by GABA<sub>A</sub> and GABA<sub>B</sub> receptor agonists (Steffensen et al., 2000; S. H. Stobbs et al., 2004) and by ethanol in rats *in vivo* with an IC<sub>50</sub> of 1.0 g/kg (Kimberly H. Ludlow et al., 2009; Scott C. Steffensen et al., 2009; S. H. Stobbs et al., 2004). These inhibitory effects on GABA neurons are believed to contribute to ethanol's excitatory effects on DA cell firing activity via disinhibition. This is one mechanism wherein ethanol might enhance spontaneous DA release. However, phasic DA release at terminals appears to be affected differentially. This may be due to GABA neurons present in the VTA that project to the NAc and modulate the release of DA in the NAc or GABA neurons in the NAc that influence phasic DA release through cholinergic interaction.

Since ethanol is known to influence neuronal excitability through direct inhibitory actions on GLU NMDA receptors (Wang et al., 2007), we first tested the effects of the NMDA antagonist APV on ethanol attenuated DA release. APV did not significantly affect ethanol inhibition of DA release. Perhaps this is due to GLU inputs to the NAc that are not operational on DA terminals, but on NAc MSNs or a cholinergic interneuron.

#### *GABA<sub>A</sub> Receptor Pharmacology in the Nucleus Accumbens*

Superfusion of the GABA<sub>A</sub>R agonist muscimol failed to alter DA release in the NAc at the typical dose levels used to block GABA<sub>A</sub>Rs. Not until extremely high doses of muscimol (350  $\mu$ M) were superfused did effectiveness similar to ethanol become manifest. Additionally, superfusion of the potent GABA<sub>A</sub> drugs CDX (30  $\mu$ M) and pentobarbital (100  $\mu$ M) had no effect on inhibiting DA release in the NAc and did not alter the effects of ethanol inhibition of DA release. The lack of effectiveness of muscimol, CDX, and pentobarbital led us to the possibility of either an atypical GABA<sub>A</sub>R or a metabotropic GABA<sub>B</sub>R responsible for the behavior of the

DA neurons to ethanol. In order to determine if these other classes of GABA receptors are involved in ethanol-mediated inhibition of DA release, we tested common GABA<sub>A</sub>R antagonists to determine whether they could block the effects of ethanol. Surprisingly, we did not observe any significant effect of either bicuculline or picrotoxin within the expected ranges of concentration effectiveness.

#### *GABA<sub>B</sub> Receptor Pharmacology in the Nucleus Accumbens*

In order to identify whether GABA<sub>B</sub>Rs were sufficient for inhibiting DA release in the NAc, we superfused the GABA<sub>B</sub> agonist baclofen at multiple concentrations (1  $\mu$ M – 2 mM). Baclofen did not demonstrate similar characteristics to ethanol inhibition of DA release at customary doses, but did demonstrate a significant attenuation of DA release at 100  $\mu$ M, a very high dose considering that baclofen inhibits VTA GABA neurons with an IC<sub>50</sub> of 1  $\mu$ M. CGP55845 (5-50  $\mu$ M) demonstrated a robust increase of DA release and a significant block of the effects of ethanol. As baclofen did not demonstrate a significant decrease of DA release, we superfused the GABA<sub>B</sub> agonist SKF97541 (considered ten times more potent than baclofen) and it had no effect on phasic DA release, suggesting that GABA<sub>B</sub> receptors were not involved in ethanol inhibition of DA release at terminals in the NAc.

Due to the lack of effect from stereotypical GABA<sub>A</sub>R agonists and antagonists and the lack of effect of GABA<sub>B</sub>R agonists, we examined the effects of GABA itself on DA release under multiple pulse stimulation conditions. We tested the effects of GABA on terminal DA release through a very broad concentration spectrum (100 nM – 10 mM). Interestingly, GABA only reduced DA release at 10  $\mu$ M and at extremely high concentrations (3 – 10 mM), suggesting that the GABA<sub>A</sub>Rs mediating ethanol inhibition of DA release might contain  $\rho$ -subunits, which are not very sensitive to GABA. These subunits are characterized by insensitivity to the

GABA<sub>A</sub>R antagonist bicuculline, resistance to the GABA<sub>B</sub>R agonist baclofen, and lack of response to the GABA<sub>A</sub>R modulators, benzodiazepines, barbiturates, and neurosteroids (R. M. Woodward, Polenzani, & Miledi, 1993), which is consistent with our findings. However, as  $\rho$ -subunits are typically homomeric GABA<sub>A</sub>p receptors, which is characteristic of those found in the retina, we did not consider this as a viable direction for our study. However, it has been previously reported that  $\alpha 4\beta 3\delta$  GABA<sub>A</sub>Rs have distinct pharmacology, are insensitive to modulation by benzodiazepine ligands, and neurosteroid effectiveness is greatly enhanced at  $\alpha 4\beta 3\delta$  (N. Brown, Kerby, Bonnert, Whiting, & Wafford, 2002). Thus, we determined to explore the effects of the  $\delta$ -subunit for GABA<sub>A</sub>R pharmacology in the NAc.

#### *Effectiveness of Ethanol on the $\delta$ -subunit of the GABA<sub>A</sub> Receptor*

With the previous effectiveness of Ro15-4513 on ethanol consuming WT mice (Melon & Boehm, 2011), we determined to superfuse this antagonist as it would selectively block GABA<sub>A</sub>R  $\delta$ -subunits. The competitive antagonist, Ro15-4513, effectively blocked ethanol inhibition of DA release both *ex vivo* and *in vivo*, with no effect on its own to the baseline signal, suggesting the involvement of  $\delta$ -subunit GABA<sub>A</sub>Rs. Although its direct effects on alcohol behavioral antagonism remain controversial, Ro15-4513's receptor interactions with ethanol include  $\delta$ -subunit containing GABA<sub>A</sub>Rs [for review see Wallner and Olsen (2008)]. To further elucidate the role of  $\delta$ -subunits in ethanol DA inhibition, we examined ethanol's effects on DA release in NAc slices of  $\delta$ -/- mice. Our results strongly demonstrate that  $\delta$ -/- mice have increased phasic DA release after ethanol superfusion compared to WT mice, further implicating GABA<sub>A</sub>  $\delta$ -subunits in ethanol's inhibitory effects on evoked DA transmission. With this solid evidence confirming  $\delta$ -subunit GABA<sub>A</sub>R involvement, we tested the  $\delta$ -subunit selective agonist THIP to demonstrate its effectiveness to agonize the receptor similar to ethanol. Surprisingly, THIP had



no effect on DA release at the expected dose ranges for  $\delta$  effects, even though THIP has higher efficacy at  $\alpha_4\beta_3\delta$  GABA<sub>A</sub>Rs than GABA (N. Brown et al., 2002) and dramatically increased THIP sensitivity (Meera, Wallner, & Otis, 2011).

*Effects of Neurosteroids in Attenuating the Effects of Ethanol at GABA<sub>A</sub> Receptors in the Nucleus Accumbens*

The  $\delta$ -subunit is responsible for neurosteroid sensitivity (Wohlfarth, Bianchi, & Macdonald, 2002) and alcohol sensitivity (Hancher, Dodson, Olsen, Otis, & Wallner, 2005). Extrasynaptic  $\delta$  subunit-containing GABA<sub>A</sub>Rs show distinct pharmacology, including low efficacy to GABA, and high sensitivity to positive modulation by neurosteroids, ethanol, and general anesthetics (Olsen & Sieghart, 2008). Thus, as ethanol and neurosteroids demonstrate similar selectivity on  $\delta$ -containing GABA<sub>A</sub>Rs, neurosteroids might provide further insight into the effects of ethanol on the GABA<sub>A</sub>R subtypes modulating the release of DA in the NAc.

Superfusion of the neurosteroid ALLO (20  $\mu$ M), a potent positive allosteric modulator of GABA<sub>A</sub>Rs, significantly blocked ethanol inhibition of DA release. Superfusion of Trilostane (20  $\mu$ M), an inhibitor of the enzyme 3 $\beta$ -hydroxysteroid dehydrogenase (an enzyme that raises endogenous release of DHEAS), also significantly attenuated the effects of ethanol inhibition of DA release. In addition, superfusion of DHEAS (10  $\mu$ M), a negative allosteric modulator of the GABA<sub>A</sub>R, had a slight excitatory effect on DA release and significantly attenuated the effects of ethanol inhibition of DA release. Thus, given the sensitivity of the GABA<sub>A</sub>  $\delta$ -subunits to neurosteroids, this data further demonstrated the likelihood of a  $\delta$ -subunit being expressed in the GABA<sub>A</sub>R present in the NAc.

### *Confirmation of an Atypical GABA<sub>A</sub> Receptor in the Nucleus Accumbens*

With pharmacological and molecular evidence in support of the presence of a  $\delta$ -subunit containing GABA<sub>A</sub>R modulating DA release with the effects of ethanol both *ex vivo* and *in vivo*, it was necessary to explore the lack of response of the GABA<sub>A</sub>R to THIP, a potent  $\delta$ -subunit GABA<sub>A</sub>R agonist. Previous published data indicated that THIP is weakest at  $\rho_1$ -subunits (Kusama et al., 1993). As mentioned previously, a search of the literature revealed that GABA<sub>A</sub> $\rho$  receptors are characterized by insensitivity to the GABA<sub>A</sub>R antagonist bicuculline, resistance to the GABA<sub>B</sub>R agonist baclofen, and lack of response to benzodiazepines, barbiturates, and neurosteroids (R. M. Woodward et al., 1993). Given that our  $\delta$ -subunit pharmacology did not give us the expected results with THIP, we determined to explore the possibility of an atypical GABA<sub>A</sub>R with a  $\rho$ -subunit. GABA<sub>A</sub> $\rho$  receptors are expressed in many brain regions, and it has recently been theorized that GABA<sub>A</sub>  $\rho$ -subunits would soon be discovered in the caudate with the possibility of, “a co-assembly of GABA  $\rho$  with GABA<sub>A</sub> subunits, which would mask the typical GABA $\rho$  pharmacology” (Martinez-Delgado et al., 2010). Also, due to high sequence conservation between  $\rho$ - and  $\beta$ -subunits, this “may account for some GABA receptors in the CNS that exhibit atypical characteristics” (Milligan et al., 2004). Our high correlation to these characteristics of  $\rho$ -subunits (except the neurosteroids) and recent publications indicating a possibility of  $\rho$ -subunits in the caudate, we felt confident pursuing GABA<sub>A</sub>  $\rho$ -subunit pharmacology. Referencing the Allen Brain Atlas which demonstrates *in situ* hybridization of RNA expression, we noted a significant distribution of GABRR1 ( $\rho_1$ -subunit) in the ventral striatum (Lein et al., 2007), indicating that somata in this region are expressing  $\rho_1$ -subunit mRNA.

Superfusion of the selective, competitive  $\rho_1$  antagonist TPMPA demonstrated a moderate increase of DA release on its own and a complete block of ethanol inhibition of DA release. In addition to this positive data on attenuating the effects of ethanol on DA release, we confirmed our results with immunohistochemistry, successfully identifying neurons in the NAc that selectively express  $\rho_1$ -subunits. Similar to the  $\delta$ -subunit pharmacology, we wanted to confirm our  $\rho_1$ -subunit findings pharmacologically with a specific  $\rho_1$  agonist; however, the pharmacology for  $\rho_1$ -subunits is not specific and precluded us from pursuing this direction.

In consideration of the  $\alpha$ -subunit present in the GABA<sub>A</sub>R expressed in the NAc, we examined our benzodiazepine and barbiturate data. The  $\alpha$ -subunits most sensitive to benzodiazepines are  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ , and  $\alpha_5$ ; the  $\alpha$ -subunits that are benzodiazepine-insensitive are the  $\alpha_4$  and  $\alpha_6$  subunits (Luscher, Baur, Goeldner, & Sigel, 2012). Thus, with our data demonstrating the insensitivity of this GABA<sub>A</sub>R to the benzodiazepine CDX, the likely  $\alpha$ -subunits would be either  $\alpha_4$  or  $\alpha_6$ . However, barbiturates are most sensitive at the  $\alpha_6$  subunit (Fisher & Fisher, 2010) and our superfusion of pentobarbital demonstrated a lack of sensitivity to this barbiturate. Thus, the most likely  $\alpha$ -subunit candidate for the GABA<sub>A</sub>R in the NAc would be the  $\alpha_4$ -subunit. Given the previous published data expressing the high sequence similarity of  $\rho$ - and  $\beta$ -subunits, we propose an atypical GABA<sub>A</sub>R in the NAc that modulates DA release consisting of two  $\alpha_4$ -subunits, two  $\rho_1$ -subunits, and a  $\delta$ -subunit ( $\alpha_4\rho_1\delta$ ). According to our understanding, this is the first proposed atypical GABA<sub>A</sub>R of this type in the nervous system. This proposal is strongly supported by pharmacological, molecular, and immunohistochemistry data, in addition to multiple recent publications hinting toward the possibility of an atypical GABA<sub>A</sub>R in the caudate. We have demonstrated that this atypical GABA<sub>A</sub>R is directly involved in ethanol's inhibitory effects on DA transmission in the NAc.

As we examined GABA<sub>B</sub>R agonists and antagonists as well as atypical GABA<sub>A</sub>R agonists and antagonists, a clear picture began to formulate regarding the atypical characteristics that are unique to GABA receptors in this region of the brain. With support from previously published research, we were able to identify a plausible hypothesis and re-direction regarding the  $\rho_1$ -subunit integrating in the GABA<sub>A</sub>R (Milligan et al., 2004) in place of the typical  $\beta$ -subunit (Martinez-Delgado et al., 2010). In addition to *in situ hybridization* data demonstrating  $\rho_1$ -subunits being present in the NAc (Lein et al., 2007), our own immunohistochemistry evidence clearly demonstrated the presence of the  $\rho_1$ -subunit, further confirming our pharmacology data of both  $\delta$ - and  $\rho_1$ -subunits regulating DA release in the NAc.

### *Baicalein*

Previous experimentation with baicalein has demonstrated neuroprotective (Mu, He, Yuan, Li, & Du) benefits as well as anti-inflammatory (Li, Wang, Pei, Liu, & Hong, 2005), antitumor (Chao, Su, & Liu, 2007), and antioxidant effects (Huang, Lee, Chien, & Chou, 2005) in neurons when exposed to various toxins and addictive drugs (Y. Liu et al.; F. Q. Li et al.; Wu et al.; Mu et al.; Cheng et al.). Given its promising characteristics and possible mechanisms of action, ethanol studies with baicalein were requisite.

We initially tested the effects of baicalein *ex vivo*, however we discovered significant solubility issues. When we initially dissolved baicalein in DMSO (0.1 – 3 %), we would lose our FSCV signal as a result of the DMSO. We then attempted to solubilize baicalein with the solvent propylene glycol. Unfortunately, we did not obtain complete solubility in reasonable ranges (1 – 20 %). With our preliminary attempts with baicalein (50  $\mu$ M) incompletely solubilized, we did see significance of attenuating ethanol's inhibition of DA release. However, in order to know the proper concentration of baicalein, we needed to solve the solubility issues.

We obtained a soluble metabolite of baicalein, baicalin hydrate, and attempted our experimentation. To our dismay, we soon realized that we could not properly record the results of DA release with baicalin hydrate as baicalin hydrate has a redox potential at the same voltage as DA. Thus, the oxidation of baicalin hydrate was masking the true effects caused by both baicalin and ethanol.

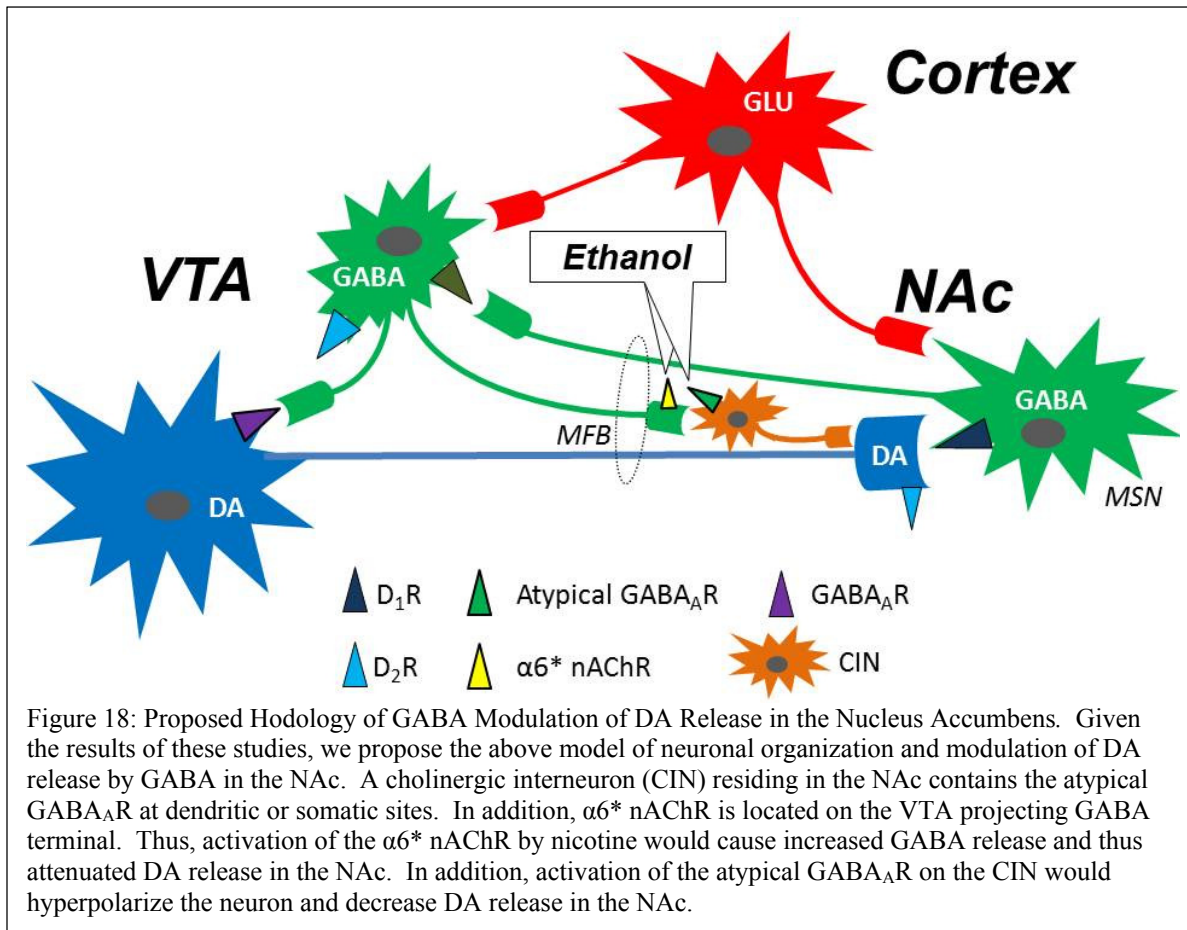
We determined to verify our preliminary findings *ex vivo* with baicalein administration *in vivo*. Dissolving baicalein in DMSO (3 %) and administering IP 30 minutes prior to ethanol (2 g/kg) and controlling for vehicle, we demonstrated that baicalein significantly attenuates the ethanol inhibition of DA release in the NAc. Thus, baicalein could be a possible nutraceutical intervention in alleviating the effects of acute ethanol in humans. We recognize that the acute exposure is much different from the chronic exposure (the addictive state). However, this data representing significance in the NAc under acute exposure allows us to further pursue baicalein effects *in vivo* in both the chronic non-dependent and withdrawal states of alcohol addiction.

## CHAPTER 5: Conclusions

These studies demonstrate an extensive look into the complex ethanol inhibition of DA release at terminals in the NAc. It has been stated that one that understands ethanol, understands the brain. The reason for this statement is due to the unrestrained nature of ethanol – it is capable of causing effects on multiple ionotropic receptor subtypes (i.e. acetylcholine, glutamate, and GABA receptors) in the nervous system and can affect systems both extra- and intracellularly as the molecule is small enough to diffuse through the cellular membrane. Thus, we recognize that even though this project has pharmacologically explored the extracellular effects of ethanol inhibition of DA release in the NAc, it is possible that DA release in the NAc can be affected by intracellular mechanisms as well.

Our study began with proposed experiments that hypothesized GABA inhibition of DA release in the NAc with the assumption that stereotypical GABA<sub>A</sub> agonists would produce similar effects to ethanol with its inhibition of DA release and that stereotypical GABA<sub>A</sub> antagonists would effectively block the inhibitory effects of ethanol of DA release. However, the results of these stereotypical GABA<sub>A</sub> agonists (including benzodiazepines and barbiturates) and antagonists quickly demonstrated to us that ethanol was acting via atypical GABA<sub>A</sub>R subunits or possibly through GABA<sub>B</sub>Rs. In addition, we recognized an important correlation of co-dependence between alcohol and nicotine and determined to explore the possibilities of both ethanol and nicotine acting through similar subunits in the NAc to modulate DA release. Thus, we began to explore these options further and it opened up a greater depth of understanding of the complexity of this modulatory system present in the NAc.

As we examined the effects of nicotine on DA release, we were surprised by the fact that nicotine caused an inhibition to the DA release similar to ethanol. As others have proposed



nAChRs on DA terminals due to cholinergic interneurons (Cachepe et al., 2012), activation of these nAChRs with nicotine would cause an increase of DA release due to the influx of  $\text{Na}^+$  into the DA terminal. Although the resultant inhibition could be due to desensitization of the nAChR, this would be unlikely at the nicotine concentrations that were being superfused. We soon discovered that both nicotine and ethanol act similarly in the NAc and are blocked in the core by superfusion of the  $\alpha 6^*$ -specific nAChR antagonist  $\alpha$ -CtxMII. Interestingly, whereas the effects of nicotine were completely blocked with  $\alpha$ -CtxMII in the NAc core, the effects of ethanol were only partially blocked with  $\alpha$ -CtxMII. This would indicate that ethanol is acting through activation at the nAChR as well as through other mechanisms, whether intra- or extracellularly.

These experiments verify that both nicotine and ethanol reduced phasic release of DA in the NAc, and that  $\alpha 6^*$ -nAChRs and an atypical GABA<sub>A</sub>R are involved in ethanol inhibition of evoked DA release in the NAc of C57/BL6 mice. Although these findings of an atypical GABA<sub>A</sub>R is confirmed only at this time in a mouse model and direct correlation to human subjects cannot be inferred, it is important to recognize that this finding when published will promote exploration into further GABA<sub>A</sub>R pharmacology in the NAc in both mouse and human models. In addition, the mouse model is widely used in neuroscience research as there are high correlations between the neural networks and constructs between human and mice. Additional confirmation of these findings will perpetuate scientific thought, research, and development and ultimately will lead to improved clinical therapeutics for those suffering with the disease of addiction, especially related to alcohol dependence / co-dependence.

#### *Future Directions*

Given the results of these studies, it would be logical to follow these acute ethanol exposure studies with those of chronic ethanol to verify how the ethanol inhibition of DA release is altered in the NAc and to verify whether GABA neurons are up-regulated in the NAc as we have seen in the VTA. As rodents fail to self-administer ethanol to the state of dependence, these chronic ethanol studies will be performed by placing rodents (C57/BL6 and  $\delta^{-/-}$ ) into vapor chambers that will supply the rodents with vaporized ethanol for 16 hours / day on a four day / three day on-off cycle. After the withdrawal period of three days, the rodents will be placed into individual chambers and will be given the election of consumption of 30 % ethanol or water (“Drink-in-the-Dark procedure”). The consumed volume of each liquid will be measured for analysis. After a dependent state of ethanol is developed by the rodents (usually after five weeks), the rodents will be sacrificed and FSCV analysis both *ex vivo* and *in vivo* will be



performed as described previously in our methods given our expected pharmacology characteristics. The pharmacological data can then be statistically analyzed against the acute data to verify induced changes to the atypical GABA<sub>A</sub>Rs modulating DA release in the NAc.

As there is a suspected interneuron expressing  $\rho_1$ -subunits in the NAc (Fig. 15), immunohistochemistry of acetylcholine neurons and GABRR1 can be utilized in a CD-1 GAD GFP mouse to verify whether the  $\rho_1$ -subunits are present on the suspected cholinergic interneuron (M. T. Brown et al., 2012a; Cachope et al., 2012) that has been shown to modulate DA release in the NAc or whether there are other interneurons involved in the synaptic hodology. This study will further elucidate the relationship of this interneuron to the GABA inhibition of DA release in the NAc.

For complete hodology confirmation, transmission electron microscopy (TEM) can be utilized with immunogold staining and silver enhancement to verify the presence of GABA terminals on DA terminals, or GABA terminals on cholinergic interneurons that then send a modulatory projection to DA terminals. This is a highly ambitious study and was undertaken for a few months by myself and a couple of other researchers. However, we acknowledge that this project will take a few years to successfully implement and will require additional expertise. We propose collaboration with one skilled in TEM and in TEM immunohistochemistry in order to obtain images that can confirm the presence of each of these hypothesized neural pathways.

Additional studies examining ethanol pharmacological activity on DA release in the NAc may provide novel mechanisms for testing ethanol pharmacotherapies, such as baicalein. The isoflavone baicalein demonstrated significant results in counteracting the acute effects of ethanol. As baicalein was only tested in the acute condition, baicalein must also be tested in both the

chronic non-dependent and withdrawal states. Promising results in this regard would propel baicalein forward as a candidate for alcohol addiction therapy.

Ultimately, this study provides unique insight into the complex regulation of DA release in the NAc via complex hodology and atypical receptor subtypes. As the dopaminergic systems of the nervous system are limited to confined regions in the brain and are important for behaviors such as motivation, reward, arousal, cognition, and movement, it would seem logical that the brain would regulate these dopaminergic systems in a different manner than other systems (i.e. glutamatergic and cholinergic) in order to maintain more precise and coordinated control. Disruption to homeostasis of these dopaminergic systems results in complicated disorders such as: Parkinson's disease, addiction, schizophrenia, and attention deficit hyperactivity disorder. Thus, as research continues in the field of neuroscience similar to the studies of this dissertation, we will become more aware of the fine-tuned capabilities of the nervous system to regulate DA release and will develop an understanding of how we can apply therapeutic applications to these disorders in a manner that does not disrupt other systems (i.e. side-effects).

## REFERENCES

- Allan, A. M., & Harris, R. A. (1986). Gamma-aminobutyric acid and alcohol actions: Neurochemical studies of long sleep and short sleep mice. *Life Sci.*, 39, 2005-2015.
- Amin, J., & Weiss, D. S. (1994). Homomeric rho 1 GABA channels: activation properties and domains. *Receptors Channels*, 2(3), 227-236.
- Anstrom, K. K., Cromwell, H. C., Markowski, T., & Woodward, D. J. (2003). Effect of baclofen on alcohol and sucrose self-administration in rats. *Alcohol Clin Exp Res*, 27(6), 900-908. doi: 10.1097/01.ALC.0000071744.78580.78
- Ariwodola, O. J., Crowder, T. L., Grant, K. A., Daunais, J. B., Friedman, D. P., & Weiner, J. L. (2003). Ethanol modulation of excitatory and inhibitory synaptic transmission in rat and monkey dentate granule neurons. *Alcohol Clin Exp Res*, 27(10), 1632-1639.
- Benke, D., Michel, C., & Mohler, H. (2002). Structure of GABAB receptors in rat retina. *J Recept Signal Transduct Res*, 22(1-4), 253-266. doi: 10.1081/RRS-120014600
- Berry, M. S., & Pentreath, V. W. (1980). The neurophysiology of alcohol *Psychopharmacology of Alcohol* (pp. 43-72).
- Besheer, J., Lepoutre, V., & Hodge, C. W. (2004). GABA(B) receptor agonists reduce operant ethanol self-administration and enhance ethanol sedation in C57BL/6J mice. *Psychopharmacology (Berl)*, 174(3), 358-366. doi: 10.1007/s00213-003-1769-3
- Bettler, B., Kaupmann, K., Mosbacher, J., & Gassmann, M. (2004). Molecular structure and physiological functions of GABA(B) receptors. *Physiol Rev*, 84(3), 835-867. doi: 10.1152/physrev.00036.2003
- Blackburn, J. R., Phillips, A. G., Jakubovic, A., & Fibiger, H. C. (1986). Increased dopamine metabolism in the nucleus accumbens and striatum following consumption of a nutritive meal but not a palatable non-nutritive saccharin solution. *Pharmacol Biochem Behav*, 25(5), 1095-1100.
- Blanchard, B. A., Steindorf, S., Wang, S., & Glick, S. D. (1993). Sex differences in ethanol-induced dopamine release in nucleus accumbens and in ethanol consumption in rats. *Alcoholism, Clinical and Experimental Research*, 17(5), 968-973.
- Blomqvist, O., Engel, J. A., Nissbrandt, H., & Söderpalm, B. (1993). The mesolimbic dopamine-activating properties of ethanol are antagonized by mecamylamine. *Eur J Pharmacol*, 249(2), 207-213. doi: 0014-2999(93)90434-J [pii]
- Blomqvist, O., Ericson, M., Engel, J. A., & Söderpalm, B. (1997). Accumbal dopamine overflow after ethanol: localization of the antagonizing effect of mecamylamine. *European Journal of Pharmacology*, 334(2-3), 149-156.

- Bloom, F. E., Siggins, G. R., Foote, S. L., Gruol, D., Aston-Jones, G., Rogers, J., . . . Staunton, D. (1984). Noradrenergic involvement in the cellular actions of ethanol. In E. Usdin (Ed.), *Catecholamines, Neurology and Neurobiology*. (Vol. 13, pp. 159-168). New York: Alan R. Liss, Inc.
- Bonci, A., & Malenka, R. C. (1999). Properties and plasticity of excitatory synapses on dopaminergic and GABAergic cells in the ventral tegmental area. *J Neurosci*, 19(10), 3723-3730.
- Bormann, J. (2000). The 'ABC' of GABA receptors. *Trends Pharmacol Sci*, 21(1), 16-19.
- Bozarth, M. A., & Wise, R. A. (1981). Intracranial self-administration of morphine into the ventral tegmental area. *Life Sci*, 28, 551-555.
- Brodie, M. S., & Appel, S. B. (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., Shefner, S. A., & Dunwiddie, T. V. (1990). Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. *Brain Res*, 508(1), 65-69.
- Brot, M. D., Akwa, Y., Purdy, R. H., Koob, G. F., & Britton, K. T. (1996). The anxiolytic-like effects of the neurosteroid allopregnanolone: interactions with GABAA receptors. Submitted.
- Brown, M. T., Tan, K. R., O'Connor, E. C., Nikonenko, I., Muller, D., & Luscher, C. (2012a). Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. *Nature*, 492(7429), 452-456. doi: 10.1038/nature11657
- Brown, M. T., Tan, K. R., O'Connor, E. C., Nikonenko, I., Muller, D., & Luscher, C. (2012b). Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. *Nature*. doi: 10.1038/nature11657
- Brown, N., Kerby, J., Bonnert, T. P., Whiting, P. J., & Wafford, K. A. (2002). Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta GABA(A) receptors. *Br J Pharmacol*, 136(7), 965-974. doi: 10.1038/sj.bjp.0704795
- Brunzell, D. H. (2012). Preclinical evidence that activation of mesolimbic alpha 6 subunit containing nicotinic acetylcholine receptors supports nicotine addiction phenotype. *Nicotine Tob Res*, 14(11), 1258-1269. doi: 10.1093/ntr/nts089
- Budygin, E. A., Phillips, P. E., Robinson, D. L., Kennedy, A. P., Gainetdinov, R. R., & Wightman, R. M. (2001). Effect of acute ethanol on striatal dopamine neurotransmission in ambulatory rats. *J Pharmacol Exp Ther*, 297(1), 27-34.
- Cachope, R., Mateo, Y., Mathur, B. N., Irving, J., Wang, H. L., Morales, M., . . . Cheer, J. F. (2012). Selective activation of cholinergic interneurons enhances accumbal phasic

- dopamine release: setting the tone for reward processing. *Cell Rep*, 2(1), 33-41. doi: S2211-1247(12)00136-2 [pii]. 10.1016/j.celrep.2012.05.011
- Calver, A. R., Medhurst, A. D., Robbins, M. J., Charles, K. J., Evans, M. L., Harrison, D. C., . . . Pangalos, M. N. (2000). The expression of GABA(B1) and GABA(B2) receptor subunits in the CNS differs from that in peripheral tissues. *Neuroscience*, 100(1), 155-170.
- Carr, D. B., & Sesack, S. R. (2000). GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. *Synapse*, 38(2), 114-123. doi: 10.1002/1098-2396(200011)38:2<114::AID-SYN2>3.0.CO;2-R [pii]. 10.1002/1098-2396(200011)38:2<114::AID-SYN2>3.0.CO;2-R
- Carter, C. R., Kozuska, J. L., & Dunn, S. M. (2010). Insights into the structure and pharmacology of GABA(A) receptors. *Future Med Chem*, 2(5), 859-875. doi: 10.4155/fmc.10.178
- Champtiaux, N., Gotti, C., Cordero-Erausquin, M., David, D. J., Przybylski, C., Lena, C., . . . Changeux, J. P. (2003). Subunit composition of functional nicotinic receptors in dopaminergic neurons investigated with knock-out mice. *23(21)*, 7820-7829.
- Chao, J. I., Su, W. C., & Liu, H. F. (2007). Baicalein induces cancer cell death and proliferation retardation by the inhibition of CDC2 kinase and survivin associated with opposite role of p38 mitogen-activated protein kinase and AKT. *Mol Cancer Ther*, 6(11), 3039-3048. doi: 6/11/3039 [pii]. 10.1158/1535-7163.MCT-07-0281
- Chebib, M., & Johnston, G. A. (1999). The 'ABC' of GABA receptors: a brief review. *Clin Exp Pharmacol Physiol*, 26(11), 937-940.
- Colombo, G., Agabio, R., Carai, M. A., Lobina, C., Pani, M., Reali, R., . . . Gessa, G. L. (2000). Ability of baclofen in reducing alcohol intake and withdrawal severity: I--Preclinical evidence. *Alcohol Clin Exp Res*, 24(1), 58-66.
- Cunningham, C. L., Howard, M. A., Gill, S. J., Rubinstein, M., Low, M. J., & Grandy, D. K. (2000). Ethanol-conditioned place preference is reduced in dopamine D2 receptor-deficient mice. *Pharmacol Biochem Behav*, 67(4), 693-699. doi: S0091-3057(00)00414-7 [pii]
- Danaei, G., Ding, E. L., Mozaffarian, D., Taylor, B., Rehm, J., Murray, C. J., & Ezzati, M. (2009). The preventable causes of death in the United States: comparative risk assessment of dietary, lifestyle, and metabolic risk factors. *PLoS Med*, 6(4), e1000058. doi: 10.1371/journal.pmed.1000058
- David, V., & Cazala, P. (1994). A comparative study of self-administration of morphine into the amygdala and the ventral tegmental area in mice. *Behav Brain Res*, 65(2), 205-211.

- Deitrich, R. A., Dunwiddie, T. V., Harris, R. A., & Erwin, V. G. (1989). Mechanism of action of ethanol: initial central nervous system actions. *Pharmacological Rev*, 41, 489-537.
- Devine, D. P., & Wise, R. A. (1994). Self-administration of morphine, DAMGO, and DPDPE into the ventral tegmental area of rats. *J. Neurosci.*, 14, 1978-1984.
- Diana, M., Pistis, M., Carboni, S., Gessa, G. L., & Rossetti, Z. L. (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.
- Diana, M., Pistis, M., Muntoni, A., & Gessa, G. (1996). Mesolimbic dopaminergic reduction outlasts ethanol withdrawal syndrome: evidence of protracted abstinence. *Neuroscience*, 71(2), 411-415.
- DiFranza, J. R., & Guerrera, M. P. (1990). Alcoholism and smoking. *J Stud Alcohol*, 51(2), 130-135.
- Drenan, R. M., Grady, S. R., Steele, A. D., McKinney, S., Patzlaff, N. E., McIntosh, J. M., . . . Lester, H. A. (2010). Cholinergic modulation of locomotion and striatal dopamine release is mediated by  $\alpha 6\alpha 4^*$  nicotinic acetylcholine receptors. *J Neurosci*, 30(29), 9877-9889. doi: 10.1523/JNEUROSCI.2056-10.2010
- Drenan, R. M., Grady, S. R., Whiteaker, P., McClure-Begley, T., McKinney, S., Miwa, J. M., . . . Lester, H. A. (2008). In vivo activation of midbrain dopamine neurons via sensitized, high-affinity  $\alpha 6$  nicotinic acetylcholine receptors. *Neuron*, 60(1), 123-136. doi: 10.1016/j.neuron.2008.09.009
- Einhorn, L. C., Johansen, P. A., & White, F. J. (1988). Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. *J Neurosci*, 8(1), 100-112.
- El-Ghundi, M., George, S. R., Drago, J., Fletcher, P. J., Fan, T., Nguyen, T., . . . O'Dowd, B. F. (1998). Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. *Eur J Pharmacol*, 353(2-3), 149-158. doi: S0014-2999(98)00414-2 [pii]
- Enoch, M.-A. (2008). The role of GABA(A) receptors in the development of alcoholism. *Pharmacology, Biochemistry, and Behavior*, 90(1), 95-104.
- Ericson, M., Blomqvist, O., Engel, J. A., & Söderpalm, B. (1998). Voluntary ethanol intake in the rat and the associated accumbal dopamine overflow are blocked by ventral tegmental mecamylamine. *European Journal of Pharmacology*, 358(3), 189-196.
- Exley, R., Clements, M. A., Hartung, H., McIntosh, J. M., & Cragg, S. J. (2008).  $\alpha 6$ -containing nicotinic acetylcholine receptors dominate the nicotine control of dopamine

- neurotransmission in nucleus accumbens. *Neuropsychopharmacology*, 33(9), 2158-2166. doi: 10.1038/sj.npp.1301617
- Exley, R., McIntosh, J. M., Marks, M. J., Maskos, U., & Cragg, S. J. (2012). Striatal alpha5 nicotinic receptor subunit regulates dopamine transmission in dorsal striatum. *J Neurosci*, 32(7), 2352-2356. doi: 10.1523/JNEUROSCI.4985-11.2012
- Fiorillo, C. D., & Williams, J. T. (2000). Cholinergic inhibition of ventral midbrain dopamine neurons. *J Neurosci*, 20(20), 7855-7860.
- Fisher, M. T., & Fisher, J. L. (2010). Activation of alpha6-containing GABAA receptors by pentobarbital occurs through a different mechanism than activation by GABA. *Neurosci Lett*, 471(3), 195-199. doi: 10.1016/j.neulet.2010.01.041
- Gallegos, R. A., Criado, J. R., Lee, R. S., Henriksen, S. J., & Steffensen, S. C. (1999). Adaptive responses of GABAergic neurons in the ventral tegmental area to chronic ethanol. *J. Pharmacol. Exp. Ther.*, 291, 1045-1053.
- Garzon, M., Vaughan, R. A., Uhl, G. R., Kuhar, M. J., & Pickel, V. M. (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. doi: 10.1002/(SICI)1096-9861(19990726)410:2<197::AID-CNE3>3.0.CO;2-D [pii]
- Gessa, G. L., Muntoni, F., Collu, M., Vargiu, L., & Mereu, G. (1985). Low doses of ethanol activate dopaminergic neurons of the ventral tegmental area. *Brain Res.*, 348, 201.
- Gilpin, N. W., & Koob, G. F. (2008). *Neurobiology of Alcohol Dependence*. *Alcohol Research and Health*, 31(3), 185-195.
- Gotti, C., Guiducci, S., Tedesco, V., Corbioli, S., Zanetti, L., Moretti, M., . . . Zoli, M. (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. doi: 10.1523/JNEUROSCI.5095-09.2010
- Gysling, K., & Wang, R. Y. (1983). Morphine-induced activation of A10 dopamine neurons in the rat. *Brain Research*, 277, 119-127.
- Hanchar, H. J., Dodson, P. D., Olsen, R. W., Otis, T. S., & Wallner, M. (2005). Alcohol-induced motor impairment caused by increased extrasynaptic GABA(A) receptor activity. *Nat Neurosci*, 8(3), 339-345. doi: 10.1038/nn1398
- Harris, A. R., & Allan, A. M. (1989). Alcohol intoxication: ion channels and genetics. *FASEB*, 3, 1689-1695.

- Heimer, L., Zahm, D. S., Churchill, L., Kalivas, P. W., & Wohltman, C. (1991). Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience*.
- Hendrickson, L. M., Guildford, M. J., & Tapper, A. R. (2013). Neuronal nicotinic acetylcholine receptors: common molecular substrates of nicotine and alcohol dependence. *Front Psychiatry*, 4, 29. doi: 10.3389/fpsy.2013.00029
- Hoffman, P., Rabe, C. S., Moses, F., & Tabakoff, B. (1989). N-methyl-D-aspartate receptors and ethanol: inhibition of calcium flux and cyclic GMP production. *J Neurochem*, 52, 1937-1940.
- Hoffman, P. L., & Tabakoff, B. (1993). Ethanol, sedative hypnotics and glutamate receptor function in brain and cultured cells. *Alcohol Alcohol Suppl*, 2, 345-351.
- Huang, W. H., Lee, A. R., Chien, P. Y., & Chou, T. C. (2005). Synthesis of baicalein derivatives as potential anti-aggregatory and anti-inflammatory agents. *Journal of Pharmacy and Pharmacology*, 57(2), 219-225. doi: Doi 10.1211/0022357055371
- Hyman, S. E., & Malenka, R. C. (2001). Addiction and the brain: the neurobiology of compulsion and its persistence. *Nat Rev Neurosci*, 2(10), 695-703. doi: 10.1038/3509456035094560 [pii]
- Hyman, S. E., Malenka, R. C., & Nestler, E. J. (2006). Neural Mechanisms of Addiction: The Role of Reward-Related Learning and Memory. *Annu Rev Neurosci*. doi: 10.1146/annurev.neuro.29.051605.113009
- Ikemoto, S. (2007). Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev*, 56(1), 27-78. doi: S0165-0173(07)00075-6 [pii]10.1016/j.brainresrev.2007.05.004
- Ikemoto, S., McBride, W. J., Murphy, J. M., Lumeng, L., & Li, T. K. (1997). 6-OHDA-lesions of the nucleus accumbens disrupt the acquisition but not the maintenance of ethanol consumption in the alcohol-preferring P line of rats. *Alcohol Clin Exp Res*, 21(6), 1042-1046. doi: 00000374-199709000-00016 [pii]
- Jackson, K. J., McIntosh, J. M., Brunzell, D. H., Sanjakdar, S. S., & Damaj, M. I. (2009). The role of alpha6-containing nicotinic acetylcholine receptors in nicotine reward and withdrawal. *J Pharmacol Exp Ther*, 331(2), 547-554. doi: 10.1124/jpet.109.155457
- Jang, E. Y., Ryu, Y. H., Lee, B. H., Chang, S. C., Yeo, M. J., Kim, S. H., . . . Kim, H. Y. (2014). Involvement of reactive oxygen species in cocaine-taking behaviors in rats. *Addict Biol*. doi: 10.1111/adb.12159
- Jones, I. W., Bolam, J. P., & Wonnacott, S. (2001). Presynaptic localisation of the nicotinic acetylcholine receptor beta2 subunit immunoreactivity in rat nigrostriatal dopaminergic neurones. *J Comp Neurol*, 439(2), 235-247.



- Jones, S. R., Mathews, T. A., & Budygin, E. A. (2006). Effect of moderate ethanol dose on dopamine uptake in rat nucleus accumbens in vivo. *Synapse (New York, N.Y.)*, 60(3), 251-255.
- Kalivas, P. W., Sorg, B. A., & Hooks, M. S. (1993). The pharmacology and neural circuitry of sensitization to psychostimulants. *Behav Pharmacol*, 4(4), 315-334.
- Kasparov, S., Davies, K. A., Patel, U. A., Boscan, P., Garret, M., & Paton, J. F. (2001). GABA(A) receptor epsilon-subunit may confer benzodiazepine insensitivity to the caudal aspect of the nucleus tractus solitarii of the rat. *J Physiol*, 536(Pt 3), 785-796.
- Kauer, J. A., & Malenka, R. C. (2007). Synaptic plasticity and addiction. *Nat Rev Neurosci*, 8(11), 844-858. doi: nrn2234 [pii]10.1038/nrn2234
- Koob, G. F. (1992). Dopamine, addiction and reward. *Semin. Neurosci.*, 4, 139-148.
- Koob, G. F., Rassnick, S., Heinrichs, S., & Weiss, F. (1994). Alcohol, the reward system and dependence. In B. Jansson, H. Jörnvall, U. Rydberg, L. Terenius & B. L. Vallee (Eds.), *Towards a Molecular Basis of Alcohol Use and Abuse* (pp. 103-114). Basel, Switzerland: Birkhäuser.
- Kumar, S., Fleming, R. L., & Morrow, A. L. (2004). Ethanol regulation of gamma-aminobutyric acid A receptors: genomic and nongenomic mechanisms. *Pharmacol Ther*, 101(3), 211-226.
- Kusama, T., Spivak, C. E., Whiting, P., Dawson, V. L., Schaeffer, J. C., & Uhl, G. R. (1993). Pharmacology of GABA rho 1 and GABA alpha/beta receptors expressed in *Xenopus* oocytes and COS cells. *Br J Pharmacol*, 109(1), 200-206.
- Lambert, J. J., Peters, J. A., & Cottrell, G. A. (1987). Actions of synthetic and endogenous steroids on the GABAA receptor. *Trends Pharmacol Sci*, 8, 224-227.
- Lein, E. S., Hawrylycz, M. J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., . . . Jones, A. R. (2007). Genome-wide atlas of gene expression in the adult mouse brain. *Nature*, 445(7124), 168-176. doi: 10.1038/nature05453
- Li, F. Q., Wang, T., Pei, Z., Liu, B., & Hong, J. S. (2005). Inhibition of microglial activation by the herbal flavonoid baicalein attenuates inflammation-mediated degeneration of dopaminergic neurons. *J Neural Transm*, 112(3), 331-347. doi: 10.1007/s00702-004-0213-0
- Liang, J., & Olsen, R. W. (2014). Alcohol use disorders and current pharmacological therapies: the role of GABA(A) receptors. *Acta Pharmacol Sin*, 35(8), 981-993. doi: 10.1038/aps.2014.50

- Liljenquist, S., & Engel, J. (1982). Effects of GABAergic agonists and antagonists on various ethanol-induced behavioral changes. *Psychopharmacology*, 78, 71-75.
- Lima-Landman, M. T. R., & Albuquerque, E. X. (1989). Ethanol potentiates and blocks NMDA-activated single channel currents in rat hippocampal pyramidal cells. *FEBS Lett*, 247, 61-67.
- Lovinger, D. M., White, G., & Weight, F. F. (1989). Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science*, 243, 1721-1724.
- Lovinger, D. M., White, G., & Weight, F. F. (1990). Ethanol inhibition of neuronal glutamate receptor function. *Ann Med*, 22(4), 247-252.
- Lovinger, D. M., White, G., & Weight, F. F. (1990). NMDA receptor-mediated synaptic excitation selectively inhibited by ethanol in hippocampal slice from adult rat. *J. Neurosci.*, 10, 1372-1379.
- Ludlow, K. H., Bradley, K. D., Allison, D. W., Taylor, S. R., Yorgason, J. T., Hansen, D. M., . . . Steffensen, S. C. (2009). Acute and chronic ethanol modulate dopamine D2-subtype receptor responses in ventral tegmental area GABA neurons. *Alcoholism, Clinical and Experimental Research*, 33(5), 804-811.
- Ludlow, K. H., Bradley, K. D., Allison, D. W., Taylor, S. R., Yorgason, J. T., Hansen, D. M., . . . Steffensen, S. C. (2009). Acute and chronic ethanol modulate dopamine D2-subtype receptor responses in ventral tegmental area GABA neurons. *Alcohol Clin Exp Res*, 33(5), 804-811. doi: 10.1111/j.1530-0277.2009.00899.x
- Luscher, B. P., Baur, R., Goeldner, M., & Sigel, E. (2012). Influence of GABA(A) receptor alpha subunit isoforms on the benzodiazepine binding site. *PLoS One*, 7(7), e42101. doi: 10.1371/journal.pone.0042101
- Mansvelder, H. D., Keath, J. R., & McGehee, D. S. (2002). Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. *Neuron*, 33(6), 905-919.
- Margolis, E. B., Lock, H., Hjelmstad, G. O., & Fields, H. L. (2006). The ventral tegmental area revisited: is there an electrophysiological marker for dopaminergic neurons? *J Physiol*, 577(Pt 3), 907-924.
- Margolis, E. B., Toy, B., Himmels, P., Morales, M., & Fields, H. L. (2012). Identification of rat ventral tegmental area GABAergic neurons. *PLoS One*, 7(7), e42365. doi: 10.1371/journal.pone.0042365
- Martinez-Delgado, G., Estrada-Mondragon, A., Miledi, R., & Martinez-Torres, A. (2010). An Update on GABA rho Receptors. *Current Neuropharmacology*, 8(4), 422-433. doi: 10.2174/157015910793358141

- McIntosh, J. M., Azam, L., Staheli, S., Dowell, C., Lindstrom, J. M., Kuryatov, A., . . . Whiteaker, P. (2004). Analogs of alpha-conotoxin MII are selective for alpha6-containing nicotinic acetylcholine receptors. *Mol Pharmacol*, 65(4), 944-952. doi: 10.1124/mol.65.4.944
- McKinzie, D. L., Rodd-Henricks, Z. A., Dagon, C. T., Murphy, J. M., & McBride, W. J. (1999). Cocaine is self-administered into the shell region of the nucleus accumbens in Wistar rats. 877, 788-791.
- Meera, P., Wallner, M., & Otis, T. S. (2011). Molecular basis for the high THIP/gaboxadol sensitivity of extrasynaptic GABA(A) receptors. *J Neurophysiol*, 106(4), 2057-2064. doi: 10.1152/jn.00450.2011
- Melis, M., Diana, M., Enrico, P., Marinelli, M., & Brodie, M. S. (2009). Ethanol and acetaldehyde action on central dopamine systems: mechanisms, modulation, and relationship to stress. *Alcohol*, 43(7), 531-539. doi: S0741-8329(09)00106-2 [pii] 10.1016/j.alcohol.2009.05.004
- Melon, L. C., & Boehm, S. L., 2nd. (2011). GABAA receptors in the posterior, but not anterior, ventral tegmental area mediate Ro15-4513-induced attenuation of binge-like ethanol consumption in C57BL/6J female mice. *Behav Brain Res*, 220(1), 230-237. doi: 10.1016/j.bbr.2011.02.014
- Mereu, G., K-W., Y., Gessa, G., Naes, L., & Westfall, T. (1987). Preferential stimulation of ventral tegmental area dopaminergic neurons by nicotine. *Eur. J. Pharmacol.*, 141, 395-399.
- Meyerhoff, D. J., Tizabi, Y., Staley, J. K., Durazzo, T. C., Glass, J. M., & Nixon, S. J. (2006). Smoking comorbidity in alcoholism: neurobiological and neurocognitive consequences. *Alcohol Clin Exp Res*, 30(2), 253-264. doi: 10.1111/j.1530-0277.2006.00034.x
- Mihalek, R. M., Bowers, B. J., Wehner, J. M., Kralic, J. E., VanDoren, M. J., Morrow, A. L., & Homanics, G. E. (2001). GABA(A)-receptor delta subunit knockout mice have multiple defects in behavioral responses to ethanol. *Alcohol Clin Exp Res*, 25(12), 1708-1718.
- Mihic, S. J., & Harris, R. A. (1996). Alcohol actions at the GABAA receptor/chloride channel complex. In R. A. Deitrich & V. G. Erwin (Eds.), *Pharmacological Effects of Ethanol on the Nervous System* (pp. 51-72). Boca Raton: CRC Press.
- Miller, N. S., & Gold, M. S. (1998). Comorbid cigarette and alcohol addiction: epidemiology and treatment. *J Addict Dis*, 17(1), 55-66. doi: 10.1300/J069v17n01\_06
- Milligan, C. J., Buckley, N. J., Garret, M., Deuchars, J., & Deuchars, S. A. (2004). Evidence for inhibition mediated by coassembly of GABA(A) and GABA(C) receptor subunits in native central neurons. *Journal of Neuroscience*, 24(33), 7241-7250. doi: 10.1523/Jneurosci.1979-04.2004

- Moore, E. M., & Boehm, S. L., 2nd. (2009). Site-specific microinjection of baclofen into the anterior ventral tegmental area reduces binge-like ethanol intake in male C57BL/6J mice. *Behav Neurosci*, 123(3), 555-563. doi: 10.1037/a0015345
- Mu, X., He, G. R., Yuan, X., Li, X. X., & Du, G. H. Baicalein protects the brain against neuron impairments induced by MPTP in C57BL/6 mice. *Pharmacol Biochem Behav*, 98(2), 286-291. doi: S0091-3057(11)00025-6 [pii]10.1016/j.pbb.2011.01.011
- Nauta, W. J., Smith, G. P., Faull, R. L., & Domesick, V. B. (1978). Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. *Neuroscience*, 3(4-5), 385-401.
- Nie, Z., Yuan, X., Madamba, S. G., & Siggins, G. R. (1993). Ethanol decreases glutamatergic synaptic transmission in rat nucleus accumbens in vitro: Naloxone reversal. *J Pharmacol Exp Ther*, 266(3), 1705-1712.
- Nugent, F. S., & Kauer, J. A. (2008). LTP of GABAergic synapses in the ventral tegmental area and beyond. *J Physiol*, 586(6), 1487-1493. doi: jphysiol.2007.148098 [pii]10.1113/jphysiol.2007.148098
- Olsen, R. W., & Sieghart, W. (2008). International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev*, 60(3), 243-260. doi: 10.1124/pr.108.00505
- Overton, P. G., Richards, C. D., Berry, M. S., & Clark, D. (1999). Long-term potentiation at excitatory amino acid synapses on midbrain dopamine neurons. *Neuroreport*, 10(2), 221-226.
- Peng, C., Kimbrell, M. R., Tian, C., Pack, T. F., Crooks, P. A., Fifer, E. K., & Papke, R. L. (2013). Multiple modes of alpha7 nAChR noncompetitive antagonism of control agonist-evoked and allosterically enhanced currents. *Mol Pharmacol*, 84(3), 459-475. doi: 10.1124/mol.113.086462
- Perez, X. A., McIntosh, J. M., & Quirk, M. (2013). Long-term nicotine treatment down-regulates alpha6beta2\* nicotinic receptor expression and function in nucleus accumbens. *J Neurochem*. doi: 10.1111/jnc.12442
- Phillips, T. J., Brown, K. J., Burkhart-Kasch, S., Wenger, C. D., Kelly, M. A., Rubinstein, M., . . . Low, M. J. (1998). Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D2 receptors. *Nat Neurosci*, 1(7), 610-615. doi: 10.1038/2843
- Pierce, R. C., & Kumaresan, V. (2006). The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neurosci Biobehav Rev*, 30(2), 215-238.

- Pons, S., Fattore, L., Cossu, G., Tolu, S., Porcu, E., McIntosh, J. M., . . . Fratta, W. (2008). Crucial role of alpha4 and alpha6 nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. *J Neurosci*, 28(47), 12318-12327. doi: 10.1523/JNEUROSCI.3918-08.2008
- Quirk, M., Perez, X. A., & Grady, S. R. (2011). Role of alpha6 nicotinic receptors in CNS dopaminergic function: relevance to addiction and neurological disorders. *Biochem Pharmacol*, 82(8), 873-882. doi: 10.1016/j.bcp.2011.06.001
- Rahman, S., & McBride, W. J. (2000). Feedback control of mesolimbic somatodendritic dopamine release in rat brain. *J Neurochem*, 74(2), 684-692.
- Risinger, F. O., Freeman, P. A., Greengard, P., & Fienberg, A. A. (2001). Motivational effects of ethanol in DARPP-32 knock-out mice. *J Neurosci*, 21(1), 340-348. doi: 10.1523/JNEUROSCI.2111-01.2001 [pii]
- Risinger, F. O., Freeman, P. A., Rubinstein, M., Low, M. J., & Grandy, D. K. (2000). Lack of operant ethanol self-administration in dopamine D2 receptor knockout mice. *Psychopharmacology (Berl)*, 152(3), 343-350.
- Roberto, M., Madamba, S. G., Moore, S. D., Tallent, M. K., & Siggins, G. R. (2003). Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. *Proc Natl Acad Sci U S A*, 100(4), 2053-2058.
- Roberto, M., Schweitzer, P., Madamba, S. G., Stouffer, D. G., Parsons, L. H., & Siggins, G. R. (2004). Acute and chronic ethanol alter glutamatergic transmission in rat central amygdala: an in vitro and in vivo analysis. *J Neurosci*, 24(7), 1594-1603.
- Robinson, D. L., Howard, E. C., McConnell, S., Gonzales, R. A., & Wightman, R. M. (2009). Disparity between tonic and phasic ethanol-induced dopamine increases in the nucleus accumbens of rats. *Alcohol Clin Exp Res*, 33(7), 1187-1196. doi: 10.1111/j.1530-0277.2009.00942.x [pii]
- Robinson, D. L., Howard, E. C., McConnell, S., Gonzales, R. A., & Wightman, R. M. (2009). Disparity between tonic and phasic ethanol-induced dopamine increases in the nucleus accumbens of rats. *Alcoholism, Clinical and Experimental Research*, 33(7), 1187-1196.
- Robinson, D. L., Volz, T. J., Schenk, J. O., & Wightman, R. M. (2005). Acute ethanol decreases dopamine transporter velocity in rat striatum: in vivo and in vitro electrochemical measurements. *Alcohol Clin Exp Res*, 29(5), 746-755.
- Schilaty, N. D., Hedges, D. M., Jang, E. Y., Folsom, R. J., Yorgason, J. T., McIntosh, J. M., & Steffensen, S. C. (2014). Acute ethanol inhibits dopamine release in the nucleus accumbens via alpha6 nicotinic acetylcholine receptors. *J Pharmacol Exp Ther*. doi: 10.1124/jpet.113.211490

- Schwenk, J., Metz, M., Zolles, G., Turecek, R., Fritzius, T., Bildl, W., . . . Bettler, B. (2010). Native GABA(B) receptors are heteromultimers with a family of auxiliary subunits. *Nature*, 465(7295), 231-235. doi: 10.1038/nature08964
- Shefner, S. A. (1990). *Electrophysiological effects of ethanol on brain neurons* (Vol. 2). Boca Raton, FL: CRC press.
- Siggins, G. R., Bloom, F. E., French, E. D., Madamba, S. G., Mancillas, J., Pittman, Q. J., & Rogers, J. (1987). Electrophysiology of ethanol on central neurons. *Ann Ny Acad Sci*, 492, 350-366.
- Siggins, G. R., Roberto, M., & Nie, Z. (2005). The tipsy terminal: presynaptic effects of ethanol. *Pharmacol Ther*, 107(1), 80-98. doi: S0163-7258(05)00026-4 [pii]10.1016/j.pharmthera.2005.01.006
- Simmonds, M. A. (1991). Modulation of the GABA-A receptor by steroids. *Seminars in the Neurosciences*, 3(231), 231-239.
- Soderpalm, B., & Ericson, M. (2012). Neurocircuitry Involved in the Development of Alcohol Addiction: The Dopamine System and its Access Points. *Curr Top Behav Neurosci*. doi: 10.1007/7854\_2011\_170
- Soderpalm, B., Ericson, M., Olausson, P., Blomqvist, O., & Engel, J. A. (2000). Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. *Behav Brain Res*, 113(1-2), 85-96. doi: S0166432800002035 [pii]
- Soderpalm, B., Lof, E., & Ericson, M. (2009). Mechanistic studies of ethanol's interaction with the mesolimbic dopamine reward system. *Pharmacopsychiatry*, 42 Suppl 1, S87-94. doi: 10.1055/s-0029-1220690
- Steffensen, S. C., Bradley, K. D., Hansen, D. M., Wilcox, J. D., Wilcox, R. S., Allison, D. W., . . . Edwards, J. G. (2010). The role of connexin-36 gap junctions in alcohol intoxication and consumption. *Synapse*, 65(8), 695-707. doi: 10.1002/syn.20885
- Steffensen, S. C., Nie, Z., Criado, J. R., & Siggins, G. R. (2000). Ethanol inhibition of N-methyl-D-aspartate responses involves presynaptic gamma-aminobutyric acid(B) receptors. *J Pharmacol Exp Ther*, 294(2), 637-647.
- Steffensen, S. C., Svingos, A. L., Pickel, V. M., & Henriksen, S. J. (1998). Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. *J Neurosci*, 18(19), 8003-8015.
- Steffensen, S. C., Taylor, S. R., Horton, M. L., Barber, E. N., Lyle, L. T., Stobbs, S. H., & Allison, D. W. (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., Walton, C. H., Hansen, D. M., Yorgason, J. T., Gallegos, R. A., & Criado, J. R. (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. doi: S0091-3057(08)00354-7 [pii]10.1016/j.pbb.2008.10.012
- Steffensen, S. C., Walton, C. H., Hansen, D. M., Yorgason, J. T., Gallegos, R. A., & Criado, J. R. (2009). Contingent and non-contingent effects of low-dose ethanol on GABA neuron activity in the ventral tegmental area. *Pharmacology, Biochemistry, and Behavior*, 92(1), 68-75.
- Stobbs, S. H., Ohran, A. J., Lassen, M. B., Allison, D. W., Brown, J. E., & Steffensen, S. C. (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. doi: 10.1124/jpet.104.071860 [pii]
- Stobbs, S. H., Ohran, A. J., Lassen, M. B., Allison, D. W., Brown, J. E., & Steffensen, S. C. (2004). Ethanol suppression of ventral tegmental area GABA neuron electrical transmission involves NMDA receptors. *J Pharmacol Exp Ther*, 311(1), 282-289.
- Stromberg, M. F. (2004). The effect of baclofen alone and in combination with naltrexone on ethanol consumption in the rat. *Pharmacol Biochem Behav*, 78(4), 743-750. doi: 10.1016/j.pbb.2004.05.006
- Suzdak, P. D., Glowa, J. R., Crawley, J. N., Schwartz, R. D., Skolnick, P., & Paul, S. M. (1986). A benzodiazepine that antagonizes alcohol actions selectively. *Science*, 234, 1243-1247.
- Tan, K. R., Yvon, C., Turiault, M., Mirzabekov, J. J., Doehner, J., Labouebe, G., . . . Luscher, C. (2012). GABA neurons of the VTA drive conditioned place aversion. *Neuron*, 73(6), 1173-1183. doi: 10.1016/j.neuron.2012.02.015
- Taylor, D. H., Burman, P. N., Hansen, D. M., Wilcox, J. D., Larsen, B. R., Blanchard, J. K., . . . Steffensen, S. C. (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).
- Taylor, D. H., Steffensen, S. C., & Wu, J. (2013). Nicotinic Acetylcholine Receptors in the Ventral Tegmental Area are Important Targets for Nicotine and Ethanol Co-dependence. *Biochem Pharmacol*, 1(1), In Press.
- Ticku, M. K., Lowrimor, P., & Lehoullier, P. (1986). Ethanol enhances GABA-induced Cl<sup>-</sup> influx in primary spinal cord cultured neurons. *Br. Res. Bull.*, 17, 123-126.
- Tripathi, A., Prensa, L., Cebrian, C., & Mengual, E. (2010). Axonal branching patterns of nucleus accumbens neurons in the rat. *J Comp Neurol*, 518(22), 4649-4673. doi: 10.1002/cne.22484

- Usuda, I., Tanaka, K., & Chiba, T. (1998). Efferent projections of the nucleus accumbens in the rat with special reference to subdivision of the nucleus: biotinylated dextran amine study. *Brain Res*, 797(1), 73-93. doi: S0006-8993(98)00359-X [pii]
- Van Bockstaele, E. J., & Pickel, V. M. (1995). GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Br. Res.*, 682, 215-221.
- van Zessen, R., Phillips, J. L., Budygin, E. A., & Stuber, G. D. (2012). Activation of VTA GABA neurons disrupts reward consumption. *Neuron*, 73(6), 1184-1194. doi: 10.1016/j.neuron.2012.02.016
- Walker, B. M., & Koob, G. F. (2007). The gamma-aminobutyric acid-B receptor agonist baclofen attenuates responding for ethanol in ethanol-dependent rats. *Alcohol Clin Exp Res*, 31(1), 11-18. doi: 10.1111/j.1530-0277.2006.00259.x
- Wallner, M., & Olsen, R. W. (2008). Physiology and pharmacology of alcohol: the imidazobenzodiazepine alcohol antagonist site on subtypes of GABAA receptors as an opportunity for drug development? *Br J Pharmacol*, 154(2), 288-298. doi: bjp200832 [pii]10.1038/bjp.2008.32
- Wang, J., Carnicella, S., Phamluong, K., Jeanblanc, J., Ronesi, J. A., Chaudhri, N., . . . Ron, D. (2007). Ethanol induces long-term facilitation of NR2B-NMDA receptor activity in the dorsal striatum: implications for alcohol drinking behavior. *J Neurosci*, 27(13), 3593-3602. doi: 10.1523/JNEUROSCI.4749-06.2007
- Weiner, J. L., & Valenzuela, C. F. (2006). Ethanol modulation of GABAergic transmission: the view from the slice. *Pharmacol Ther*, 111(3), 533-554.
- Weiss, F., Lorang, M. T., Bloom, F. E., & Koob, G. F. (1993). Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. *The Journal of Pharmacology and Experimental Therapeutics*, 267(1), 250-258.
- Welzl, H., Kuhn, G., & Huston, J. P. (1989). Self-administration of small amounts of morphine through glass micropipettes into the ventral tegmental area of the rat. *Neuropharmacology*, 28(10), 1017-1023.
- White, G., Lovinger, D., & Weight, F. (1990). Ethanol inhibits NMDA-activated current but does not alter GABA-activated current in an isolated adult mammalian neuron. *Brain Res.*, 507, 332-336.
- Wieland, S., Belluzzi, J. D., Stein, L., & Lan, N. C. (1995). Comparative behavioral characterization of the neuroactive steroids of 3a-OH,Sa-pregnan-20-one and 3a-OH,Sb-pregnan-20-one in rodents. *Psychopharmacology*, 118, 65-71.



- Wise, R. A. (1996). Addictive drugs and brain stimulation reward. *Annu. Rev. Neurosci.*, 19, 319-340.
- Wise, R. A., & Bozarth, M. A. (1987). A psychostimulant theory of addiction. *Psychol. Rev.*, 94(1-24).
- Wohlfarth, K. M., Bianchi, M. T., & Macdonald, R. L. (2002). Enhanced neurosteroid potentiation of ternary GABA(A) receptors containing the delta subunit. *J Neurosci*, 22(5), 1541-1549.
- Woodward, J. J., & Gonzales, R. A. (1990). Ethanol inhibition of N-methyl-D-aspartate-stimulated endogenous dopamine release from rat striatal slices: reversal by glycine. *Journal of Neurochemistry*, 54(2), 712-715.
- Woodward, J. J., Machu, T., & Leslie, S. W. (1990). Chronic ethanol treatment alters omega-conotoxin and Bay K 8644 sensitive calcium channels in rat striatal synaptosomes. *Alcohol (Fayetteville, N.Y.)*, 7(4), 279-284.
- Woodward, R. M., Polenzani, L., & Miledi, R. (1993). Characterization of bicuculline/baclofen-insensitive (rho-like) gamma-aminobutyric acid receptors expressed in *Xenopus* oocytes. II. Pharmacology of gamma-aminobutyric acidA and gamma-aminobutyric acidB receptor agonists and antagonists. *Mol Pharmacol*, 43(4), 609-625.
- Wooltorton, J. R., Pidoplichko, V. I., Broide, R. S., & Dani, J. A. (2003). Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. *J Neurosci*, 23(8), 3176-3185. doi: 23/8/3176 [pii]
- Wu, P. H., Shen, Y. C., Wang, Y. H., Chi, C. W., & Yen, J. C. (2006). Baicalein attenuates methamphetamine-induced loss of dopamine transporter in mouse striatum. *Toxicology*, 226(2-3), 238-245. doi: S0300-483X(06)00404-5 [pii]10.1016/j.tox.2006.06.015
- Xia, Y., Driscoll, J. R., Wilbrecht, L., Margolis, E. B., Fields, H. L., & Hjelmstad, G. O. (2011). Nucleus accumbens medium spiny neurons target non-dopaminergic neurons in the ventral tegmental area. *J Neurosci*, 31(21), 7811-7816. doi: 31/21/7811 [pii]10.1523/JNEUROSCI.1504-11.2011
- Yamaguchi, T., Sheen, W., & Morales, M. (2007). Glutamatergic neurons are present in the rat ventral tegmental area. *Eur J Neurosci*, 25(1), 106-118. doi: EJN5263 [pii]10.1111/j.1460-9568.2006.05263.x
- Yang, K., Buhlman, L., Khan, G. M., Nichols, R. A., Jin, G., McIntosh, J. M., . . . Wu, J. (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. doi: 10.1523/JNEUROSCI.3003-10.2011

- Yang, K., Hu, J., Lucero, L., Liu, Q., Zheng, C., Zhen, X., . . . Wu, J. (2009). Distinctive nicotinic acetylcholine receptor functional phenotypes of rat ventral tegmental area dopaminergic neurons. *J Physiol*, 587(Pt 2), 345-361. doi: 10.1113/jphysiol.2008.162743
- Yang, K. C., Jin, G. Z., & Wu, J. (2009). Mysterious alpha6-containing nAChRs: function, pharmacology, and pathophysiology. *Acta Pharmacol Sin*, 30(6), 740-751. doi: aps200963 [pii]10.1038/aps.2009.63
- Yin, R., & French, E. D. (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. doi: S0361-9230(00)00237-9 [pii]
- Yorgason, J. T., Ferris, M. J., Steffensen, S. C., & Jones, S. R. (2013). Frequency-Dependent Effects of Ethanol on Dopamine Release in the Nucleus Accumbens. *Alcohol Clin Exp Res*. doi: 10.1111/acer.12287
- Zhang, T., Zhang, L., Liang, Y., Siapas, A. G., Zhou, F. M., & Dani, J. A. (2009). Dopamine signaling differences in the nucleus accumbens and dorsal striatum exploited by nicotine. *J Neurosci*, 29(13), 4035-4043. doi: 10.1523/JNEUROSCI.0261-09.2009
- Zimatkin, S. M., Pronko, S. P., Vasiliou, V., Gonzalez, F. J., & Deitrich, R. A. (2006). Enzymatic mechanisms of ethanol oxidation in the brain. *Alcohol Clin Exp Res*, 30(9), 1500-1505. doi: ACER181 [pii]10.1111/j.1530-0277.2006.00181.x
- Zoli, M., Moretti, M., Zanardi, A., McIntosh, J. M., Clementi, F., & Gotti, C. (2002). Identification of the nicotinic receptor subtypes expressed on dopaminergic terminals in the rat striatum. *J Neurosci*, 22(20), 8785-8789.

## CURRICULUM VITAE

Nathan Dan Schilaty

### EDUCATION

- 2011 – 2014      Brigham Young University – Provo, UT  
PhD, Neuroscience – December 2014  
Laboratory of Scott C. Steffensen  
Addiction Hodology and Therapies for Addiction Recovery
- 2004 – 2006      Parker University – Dallas, TX  
DC (Doctorate of Chiropractic), Magna cum Laude  
BS, Health and Wellness, Magna cum Laude  
BS, Anatomy, Magna cum Laude
- 1999 – 2003      Salt Lake Community College – Taylorsville, UT  
AS, Chemistry, Summa cum Laude

### CAREER

- 2011 – Present      Research Assistant  
*Addiction Recovery Laboratory* – Ex Vivo / In Vivo /  
Neurobiochemical research of the nucleus accumbens and  
neuromelanin  
*Neuromechanics Laboratory* – Research of the wrist biomechanics,  
carpal tunnel syndrome, coordinative variability, and repetitive  
strain injuries  
*Memory Laboratory* – Research of recollection and familiarity  
with hippocampal structures
- 2011 – 2014      Teaching Assistant  
*Neuroanatomy Laboratory* – Assisted instruction of trifocal  
camera microscopes, neuroanatomy histology, behavior, and  
electrophysiology  
*Neurobiology* – Assisted instruction of an entry-level course of  
neuroscience for majors and non-majors, held bi-weekly review  
sessions, grading, teaching practicum at University level  
*Neuromechanics* – Assisted instruction of graduate-level course for  
mechanical engineers, exercise science, and neuroscience majors.  
Emphasis of instruction was on neuroscience of mechanics, spinal  
cord, and central nervous system.
- 2011 – Present      Circadian Wellness, Inc.  
President and Wellness Coach  
Health education website that correlates blog articles, webinars,  
personalized wellness coaching, and online school courses
- 2009 – 2011      Everest College Online

	Online Instructor, Medical Department
	Human Pathology
	Human Anatomy & Physiology
	Medical Terminology
	Medical Law & Ethics
2009 – Present	Missouri College Online
	Online Instructor, Medical Department
	Anatomy & Physiology
	Basic Anatomy & Physiology
2007 – 2011	Centra Chiropractic, Inc.
	President and Chiropractic Physician
	Acquired skills of business management, marketing, record keeping, accounting, patient recruitment, and clinical expertise
2007 – 2011	Front Range Community College
	Adjunct Professor, Natural Applied and Environmental Sciences
	Basic Human Anatomy & Physiology
	Human Anatomy & Physiology I
	Human Anatomy & Physiology II
	Human Anatomy & Physiology Laboratory
	Human Cadaver Dissection
2006	Princeton Review – Dallas, Texas
	Bilingual High School Teacher
	PSAT Preparation

## INTERNSHIPS

2005 – 2006	Clinic Intern – Dallas, TX
	Parker College of Chiropractic
2006	Clinical Assistant – Dallas, TX
	Parker College of Chiropractic
	Honorary program for high academic aptitude and clinical performance

## LICENSES

2011 - Present	Utah Chiropractic Physician
	License granted based upon satisfactory qualifications by the Utah Department of Professional Licensing
2007 – 2011	Colorado Chiropractic Physician
	License granted based upon satisfactory qualifications by the Colorado Board of Chiropractic Examiners

## CERTIFICATIONS

July 2007	Applied Kinesiology, 120-Hour Certified
-----------	---

	Neuromuscular biofeedback for diagnosis of neurovascular, neurolymphatic, acupuncture meridians, cranial faults, proprioceptive rehabilitation, neurology, and endocrinology
Sept. 2006	Physiotherapy National Board of Chiropractic Examiners

## FUNDING

2012	BYU Graduate Fellowship Award Brigham Young University – Graduate Studies \$15,000 fellowship award for superior graduate research
------	--

## AWARDS

2013 / 2014	Research Society on Alcoholism Graduate Travel Award
2013	1st Place BYU Grad Talk Double elimination process for finalist Presentations peer-voted for quality of research and presentation
2012	BYU Elwood Christensen Scholarship Brigham Young University – College of Life Sciences
2012 / 2013	Research Presentation Award Travel award to present original research of student
2011 - 2014	Research Assistant / Tuition Award Brigham Young University – Neuroscience
2006	Outstanding Dallas Clinic Intern Parker College of Chiropractic Honor for high clinical knowledge and performance

## PUBLICATIONS

### *Refereed Journal Articles:*

- 1) Jang EY, Ryu YH, Lee BH, Chang SC, Yeo MJ, Kim SH, Folsom RJ, Schilaty ND, Kim KJ, Yang CH, Steffensen SC, Kim HY. Involvement of reactive oxygen species in cocaine-taking behaviors in rats. *Addiction Biology* (2014) Epub ahead of print
- 2) Wu Y, Zhao Z, Yang Y, Yang X, Jang EY, Schilaty ND, Hedges DM, Kim SC, Cho IJ, Zhao R. Effects of the aqueous extract of Schizandra chinensis fruit on ethanol withdrawal-induced anxiety in rats. *Chin Med J* (2014) 127:1935-1940
- 3) Schilaty ND, Hedges DM, Jang EY, Folsom RJ, Yorgason JT, McIntosh JM, Steffensen SC. Acute ethanol inhibits dopamine release in the nucleus accumbens via  $\alpha 6$  nicotinic acetylcholine receptors. *J Pharmacol Exp Ther* (2014) 349(3):559-67
- 4) Shin SI, Andersen DJ, Hansen DM, Yorgason JT, Schilaty ND, Busath DD, Steffensen SC. Connexin-36 Knock-Out Mice Have Increased Threshold for Kindled Seizures: Role of GABA Inhibition. *Biochem & Pharmacol* (2013) S1: 006

*Submitted and Under Review or Revision:*

- 1) Steffensen SC, Schilaty ND, Hedges DM, Jang EY. Addiction Theory: A Review. *Journal of Clinical Psychological Science*.
- 2) Hedges DM, Schilaty ND, Perez A, Watt RJ, Steffensen SC. Parametric analysis of physiologically-relevant dopamine melanization. *Journal of Biological Chemistry*.
- 3) Schilaty ND, Hedges DM, Jang EY, Ekins TG, Solomon JP, Hansen MD, Yorgason JT, Wallner M, Jones SR, Steffensen, SC. Ethanol Inhibition of Evoked Dopamine Release in the Striatum Involves Atypical GABA<sub>A</sub> Receptors. *Addiction Biology*.

*Published Abstracts / Conference Presentations:*

- 1) Woodward TJ, Shin SI, Mabey JK, Nelson AC, Schilaty ND, Taylor DH, Wu J, McIntosh M, Steffensen SC. 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
- 2) Schilaty ND, Hedges DM, Okelberry TD, Perez AW, Steffensen, SC. Neurosteroids block the inhibitory effects of ethanol through GABA(A) receptors on dopamine terminals in the nucleus accumbens. Soc. Neurosci Absts 39 (2014) 53.02
- 3) Hedges DM, Schilaty ND, Perez AW, Watt RK, Steffensen SC. Physiologically relevant oxy-radical formation of neuromelanin by photostimulation: Effects of iron and calcium. Soc. Neurosci Absts 39 (2014) 144.05
- 4) Chang S, Jang E, Ryu Y-H, Lee B, Folsom RJ, Schilaty ND, Kim K, Yang C, Steffensen SC, Kim H. Involvement of reactive oxygen species in cocaine taking-behaviors in rats. Soc. Neurosci Absts 39 (2014) 616.01
- 5) Steffensen SC, Hedges DM, Schilaty ND, Jang EY, Yorgason JT, Bellinger FP. Acute methamphetamine induces reactive oxygen species in dopamine terminals of the nucleus accumbens. Soc. Neurosci Absts 39 (2014) 809.22
- 6) Shin SI, Mabey JK, Nelson AC, Woodward TJ, Schilaty ND, Taylor DH, Wu J, McIntosh JM, Steffensen SC. 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)
- 7) Folsom RJ, Jang EY, Schilaty ND, Linzey J, Friend L, Edwards J, Burnett S, Burton G, Steffensen SC. Rapid adaptation of central and peripheral D2 dopamine receptor responses following acute ethanol. *Alcoholism: Clin. Exp. Res.* (2014) 38(S1) 8A(30)
- 8) Schilaty ND, Folsom RJ, Hanson S, Jang EY, Lee JY, Yang CH, Steffensen SC. Ethanol inhibits dopamine release at GABA terminals in the nucleus accumbens via alpha-6 nicotinic acetylcholine receptors. *Alcoholism: Clin. Exp. Res.* (2014) 38(S1) 9A(31)
- 9) Jang EY, Schilaty ND, Folsom RJ, Hedges DM, Shin SI, Mabey JK, Yang CH, Kim HY, Steffensen SC. Role of reactive oxygen species in ethanol effects on dopamine release. *Alcoholism: Clin. Exp. Res.* (2014) 38(S1) 83A(329)
- 10) Mabey JK, Shin SI, White DN, Nielson CA, Schilaty ND, Ting-A-Kee R, Vargas-Perez H, van der Kooy D, Steffensen SC. Functional switch in GABA<sub>A</sub> receptors on VTA GABA neurons by chronic ethanol. *Alcoholism: Clin. Exp. Res.* (2013) 37(2) 238A(908)

- 11) Shin SI, Mabey JK, White DN, Nielson CA, Schilaty ND, Taylor DH, Wu J, McIntosh JM, Steffensen SC. 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)
- 12) Jang EY, Schilaty ND, Hanson S, Folsom RJ, Steffensen SC. Rapid adaptation of D2 dopamine receptor following acute ethanol. *Alcoholism: Clin. Exp. Res.* (2013) 37(2) 239A(915)
- 13) Yorgason JT, Schilaty ND, Hedges DM, Jang EY, Jones SR, Steffensen SC. Ethanol inhibits dopamine release at terminals in the nucleus accumbens via GABA<sub>B</sub> receptors. *Alcoholism: Clin. Exp. Res.* (2013) 37(2) 240A(916)
- 14) Schilaty ND, Folsom RJ, Hanson S, Jang EY, Lee JY, Yang CH, Steffensen SC. Baicalein reduces ethanol inhibition of mesolimbic dopamine transmission and ethanol self-administration. *Alcoholism: Clin. Exp. Res.* (2013) 37(2) 20A(038)
- 15) Schilaty ND, Yorgason JT, Hedges DM, Jang EY, Folsom RJ, Nelson A, Vera A, Hanson S, Wallner M, Jones SR, Steffensen SC. Ethanol inhibits dopamine release at terminals in the nucleus accumbens via GABA receptors. *Soc. Neurosci Absts* 38 (2013) 349.10
- 16) Mabey JK, Shin SI, White DN, Nielson CA, Schilaty ND, Ting-a-Kee R, Vargas-Perez H, Van der Kooy D, Steffensen SC. Functional switch in GABA<sub>A</sub> receptors on VTA GABA neurons by chronic ethanol. *Soc. Neurosci Absts* 38 (2013) 349.12
- 17) Hedges DM, Schilaty ND, Badal W, Morrison CE, Watt RK, Steffensen SC. Physiologically relevant oxy-radical formation of neuromelanin by photostimulation: Effects of iron and calcium. *Soc. Neurosci Absts* 38 (2013) 432.23
- 18) Steffensen SC, Schilaty ND, Jones SR, Yorgason JT. Role for GABA<sub>A</sub> receptors in ethanol inhibition of evoked dopamine release in the nucleus accumbens. *Proceedings of the 10th International Catecholamine Symposium* 10 (2012) 92.

*Dissertation:*

Schilaty, ND (2014, Dec). Alcohol Modulation of Dopamine Release. A dissertation submitted to the faculty of Brigham Young University, College of Life Sciences. Provo, UT.

*Editorial and Ad Hoc Journal Review Activities (Journal / Number of Articles Reviewed):*

- 1) Addiction Biology (1)
- 2) Neuroscience (1)

PRESENTATIONS

*Invited Seminar Presentations:*

- 1) Schilaty, ND, Steffensen, SC (2014, Aug). Ethanol modulation of dopamine release in the striatum involves extrasynaptic GABA<sub>A</sub>Rs on dopamine terminals. 15th International Monitoring Molecules in Neuroscience, Los Angeles, CA.

- 2) Schilaty ND, Steffensen, SC (2013, July). Ethanol inhibits dopamine release at terminals in the nucleus accumbens via GABA receptors. LDS Life Science Research Symposium, Salt Lake City, UT.

*Invited Guest Lecture Presentations:*

- 1) Schilaty ND (2014, Nov). ExSc 501 – Sports Medicine Pathology & Pharmacology. Addiction & Opioids. Brigham Young University, Provo, UT.
- 2) Schilaty ND (2014, July). StAc 191 – Beginning Weight Training. Spinal Care and Core Wellness. Brigham Young University, Provo, UT.
- 3) Schilaty ND (2014, Apr). ExSc 501 – Sports Medicine Pathology & Pharmacology. Addiction & Opioids. Brigham Young University, Provo, UT.
- 4) Schilaty ND (2014, Feb). Biol 100 – Principles of Biology. Principles of Addiction. Brigham Young University, Provo, UT.

*Institutional Seminar Presentations:*

- 1) Schilaty ND (2014, Oct). Ethanol modulation of dopamine release in the striatum involves atypical extrasynaptic GABA<sub>A</sub>Rs on dopamine terminals. Department of Physiology, Pharmacology, and Neuroscience. University of South Carolina School of Medicine, Columbia, SC.
- 2) Schilaty ND (2014, Oct). Ethanol modulation of dopamine release in the striatum involves atypical extrasynaptic GABA<sub>A</sub>Rs on dopamine terminals. Post-doctoral Recruitment Event. Ohio State University, Columbus, OH.
- 3) Schilaty ND (2014, Sept). Ethanol and METH: Role of atypical extrasynaptic GABA<sub>A</sub>Rs and reactive oxygen species on dopamine release in the striatum. Department of Physiology & Pharmacology. Wake Forest University, Winston-Salem, NC.
- 4) Schilaty ND (2014, Feb). Addiction: Re-wired. BYU Graduate Studies – 3-Minute Thesis Competition. Brigham Young University, Provo, UT.
- 5) Schilaty ND (2013, Oct). Ethanol inhibits dopamine release at terminals in the nucleus accumbens via  $\alpha 6^*$ -nAChRs. Physiology and Developmental Biology / Neuroscience Seminar Series. Brigham Young University, Provo, UT.
- 6) Schilaty ND (2013, June). Addiction and the Brain. Graduate Studies – Grad Talk Competition. Brigham Young University, Provo, UT.
- 7) Schilaty ND (2012, Nov). Baicalein attenuates the inhibitory effect of ethanol on dopamine terminals. Physiology and Developmental Biology / Neuroscience Seminar Series. Brigham Young University, Provo, UT.

MEMBERSHIPS

2013 – Present	Society for Neuroscience
2013 – Present	Research Society on Alcoholism
2011 – Present	American Association for the Advancement of Science
2007 – 2008	International College of Applied Kinesiology
2004 – 2006	American Chiropractic Association



## PROFESSIONAL DEVELOPMENT

### LabVIEW Programming

Ability to generate user-friendly software to interface research hardware and work more efficient and effective

## FOREIGN LANGUAGE

Spanish                      Fluent in written and verbal communication