DEVELOPMENT OF A TRANSLATIONAL MODEL OF CO-USE OF ALCOHOL AND NICOTINE FOR TESTING POTENTIAL PHARMACOTHERAPIES

Sarah Elizabeth Maggio

University of Kentucky, sema253@uky.edu

Digital Object Identifier: https://doi.org/10.13023/etd.2019.395

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Recommended Citation

https://uknowledge.uky.edu/psychology_etds/167

This Doctoral Dissertation is brought to you for free and open access by the Psychology at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Psychology by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
**STUDENT AGREEMENT:**

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

**REVIEW, APPROVAL AND ACCEPTANCE**

The document mentioned above has been reviewed and accepted by the student’s advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student’s thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Sarah Elizabeth Maggio, Student

Dr. Michael Bardo, Major Professor

Dr. Mark Fillmore, Director of Graduate Studies
DEVELOPMENT OF A TRANSLATIONAL MODEL
OF CO-USE OF ALCOHOL AND NICOTINE
FOR TESTING POTENTIAL PHARMACOTHERAPIES

__________________________

DISSERTATION

__________________________

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Arts and Sciences
at the University of Kentucky

By
Sarah Elizabeth Maggio
Lexington, Kentucky

Director: Dr. Michael Bardo, Professor of Psychology
Lexington, Kentucky

2019

Copyright © Sarah Elizabeth Maggio 2019
ABSTRACT OF DISSERTATION

DEVELOPMENT OF A TRANSLATIONAL MODEL OF CO-USE OF ALCOHOL AND NICOTINE FOR TESTING POTENTIAL PHARMACOTHERAPIES

Co-users of alcohol and nicotine are the largest group of polysubstance users worldwide. Although pharmacotherapies are available for alcohol (EtOH) or tobacco use disorders individually, it may be possible to develop a single pharmacotherapy to treat heavy drinking tobacco smokers through capitalizing on the commonalities in their mechanisms of action. Towards this goal, several models of concurrent access to EtOH and nicotine were explored as potential preclinical models of co-use using female alcohol-preferring (P) rats. Additionally, potential pharmacotherapeutics for the treatment of EtOH and nicotine co-use disorder were tested using different variations of our model. Treatments tested included (1) varenicline, a nicotinic acetylcholine receptor (nAChR) partial agonist with high affinity for the α4β2* subtype; (2) r-bPiDI, a subtype-selective antagonist at α6β2* nAChRs; (3) (R)-modafinil, an atypical inhibitor of the dopamine transporter (DAT); and (4) naltrexone, a clinically available µ-opioid receptor antagonist used to treat alcohol use disorder (AUD).

Results from the current dissertation show success in developing a translational animal model in female P rats for co-use of EtOH and nicotine under which pharmacologically relevant levels of both EtOH consumption and nicotine intake are achieved. Additionally, our model was successfully used in testing potential pharmacotherapeutics for the treatment of EtOH and nicotine co-use disorder. Although none of the drugs tested were effective as a monotherapy, results from testing the known smoking cessation agent varenicline and the known AUD treatment naltrexone indicate that our model is effective for selectively measuring changes in EtOH and nicotine intake separately, which suggests the beneficial utility of this model for future treatment research.

Furthermore, by applying behavioral economic principles to our findings, we found that EtOH acts as an economic substitute for nicotine. Additionally, our behavioral economic analyses revealed that when the cost of nicotine is changed via response requirements vs dose per infusion, there are differences in the elasticity of demand for concurrently available EtOH and nicotine. Finally, the relatively flat consumption curve for EtOH following varenicline pretreatment suggests that pretreatment with varenicline
acts to disrupt the relationship between EtOH and nicotine such that EtOH no longer acts as an economic substitute for nicotine.

KEYWORDS: alcohol, nicotine, co-use disorder, behavioral economics, price elasticity of demand, p rats

Sarah Elizabeth Maggio

09/10/2019

Date
DEVELOPMENT OF A TRANSLATIONAL MODEL 
of co-use of alcohol and nicotine 
for testing potential pharmacotherapies

By
Sarah Elizabeth Maggio

Dr. Michael Bardo
Director of Dissertation

Dr. Mark Fillmore
Director of Graduate Studies

09/10/2019
Table of Contents

List of Tables ............................................................................................................. vi

List of Figures .......................................................................................................... vii

Chapter One: Introduction
  1. General Introduction ...................................................................................... 1
     1.1. Epidemiology of Alcohol and Nicotine Use .......................................... 1
     1.2. Co-Use of EtOH and Nicotine .............................................................. 2
     1.3. Pharmacokinetics of Ethanol .................................................................. 3
        1.3.1. Absorption ...................................................................................... 3
        1.3.2. Distribution .................................................................................... 4
        1.3.3. Metabolism and Excretion ............................................................... 4
     1.4. Pharmacological Effects of EtOH ............................................................ 6
     1.5. Psychological Effects of EtOH ................................................................. 6
     1.6. Pharmacodynamics of EtOH ................................................................... 7
        1.6.1. Dopamine ....................................................................................... 7
        1.6.2. Glutamate ....................................................................................... 7
        1.6.3. GABA ........................................................................................... 8
        1.6.4. Serotonin ...................................................................................... 8
        1.6.5. Endocannabinoids ......................................................................... 8
        1.6.6. Acetylcholine ............................................................................... 9
     1.7. Pharmacokinetics of Nicotine ................................................................ 11
        1.7.1. Absorption .................................................................................... 11
        1.7.2. Distribution .................................................................................... 12
        1.7.3. Metabolism and Excretion ............................................................... 12
     1.8. Pharmacodynamic and Psychological Effects of Nicotine ...................... 13
     1.9. Neuronal nAChRs ............................................................................... 14
     1.10. Common Substrates of EtOH and Nicotine ......................................... 16
        1.10.1. The Mesocorticolimbic DA System .............................................. 17
        1.10.2. The Cholinergic Pathway ............................................................. 18
     1.11. Current Therapeutic Treatments for Cessation of EtOH or Nicotine .... 19
        1.11.1. Disulfiram ................................................................................... 20
        1.11.2. Naltrexone .................................................................................. 21
        1.11.3. Acamprosate .............................................................................. 22
        1.11.4. Nicotine Replacement Therapy (NRT) ......................................... 22
        1.11.5. Bupropion .................................................................................. 23
        1.11.6. Varenicline ................................................................................ 24
     1.12. Translational Animal Models ................................................................. 25
     1.13. Behavioral Economics of Demand ........................................................ 27
     1.14. Overall Goals of Current Dissertation .................................................. 29

Chapter Two: Study 1
  2. Effects of the nicotinic agonist varenicline, nicotinic antagonist r-bPiDI, and

iii
DAT inhibitor R-modafinil on co-use of ethanol and nicotine in female P rats .................................................................30

2.1. Introduction ..................................................................................................................30
2.2. Methods .......................................................................................................................33
2.3. Procedures ..................................................................................................................35

2.3.1. Experiment 1: Drug Pretreatments During Co-use of EtOH and Nicotine (Phase 3) ...........................................................................35
2.3.2. Drug Pretreatments ..............................................................................................38
2.4. Experiment 2: Drug Pretreatments During Use of EtOH Only (Phase 1) ...........................................................................................................39

2.4.1. Data Analysis .........................................................................................................39
2.5. Results ........................................................................................................................40

2.5.1. Experiment 1: Pretreatments During Co-Use of EtOH and Nicotine .......................................................................................40
2.5.2. Experiment 2: Pretreatments During Use of EtOH Only .................................................43
2.6. Discussion ...................................................................................................................44

Chapter Three: Study 2

3. An improved model of ethanol and nicotine co-use in female P rats: Effects of naltrexone, varenicline, and the selective nicotinic α6β2* antagonist r-bPiDI. 65

3.1. Introduction ..................................................................................................................65
3.2. Methods .......................................................................................................................67

3.2.1. Procedures ..............................................................................................................68
3.2.2. Drug Pretreatments ..............................................................................................70
3.2.3. Data Analysis .........................................................................................................70

3.3. Results ........................................................................................................................71

3.3.1. Baseline EtOH and Nicotine Intake in Phase 1 (Experiment 1) and Phase 2 (Experiment 2) ........................................................................71
3.3.2. Effects of Naltrexone in Phase 1 (Experiment 1) and Phase 2 (Experiment 2) ..................................................................................72
3.3.3. Effects of Varenicline and r-bPiDI Pretreatments in Phase 2 (Experiment 2) ..................................................................................72
3.3.4. Within-session Nicotine Self-Administration .........................................................73

3.4. Discussion ...................................................................................................................74

Chapter Four: Study 3

4. A behavioral economic approach to a translational model of co-use of EtOH and nicotine using female P rats .........................................................................................86

4.1. Introduction ..................................................................................................................86
4.2. Methods .......................................................................................................................89

4.2.1. Procedures ..............................................................................................................90
4.2.2. Data Analysis .........................................................................................................92
4.3. Results ........................................................................................................................94

4.3.1. Demand Elasticity .................................................................................................94
4.4. Discussion ...................................................................................................................95
Chapter Five: Study 4

5. Effects of varenicline on demand elasticity for co-use of EtOH and nicotine:
   Comparing the effects of varying doses of nicotine to varying response requirements for nicotine using female P rats......................100
5.1. Introduction..........................................................................................100
5.2. Experiment 1: Varying nicotine cost by varying dose vs FR
   Requirement..........................................................................................103
   5.2.1. Method.......................................................................................103
   5.2.2. Procedures...............................................................................104
   5.2.3. Data Analysis...........................................................................106
5.3. Experiment 1 Results..........................................................................109
5.4. Experiment 1 Discussion..................................................................111
5.5. Experiment 2: Effect of varenicline on cross-price elasticity derived by varying nicotine cost with FR requirement...............112
5.6. Experiment 2 Results.......................................................................112
5.7. Experiment 2 Discussion..................................................................113
5.8. Study 4 General Discussion..............................................................113

Chapter Six: Conclusions...........................................................................119

APPENDICES ............................................................................................126

References...............................................................................................130

Curriculum Vita.........................................................................................154
LIST OF TABLES

Table 1, Unit Price Conversion for Nicotine..........................................................116
LIST OF FIGURES

Figure 1a, Chemical structure of varenicline .......................................................... 54
Figure 1b, Chemical structure of r-bPiDI ................................................................. 54
Figure 1c, Chemical structure of RMOD ................................................................. 54
Figure 2, Modified operant chamber apparatus .................................................... 55
Figure 3a, Results from Study 1, Experiment 1
   Acquisition across sessions for EtOH consumption in Phase 1 .................... 56
Figure 3b, Results from Study 1, Experiment 1 .................................................... 56
   Acquisition across sessions for EtOH consumption in Phase 3
Figure 3c, Results from Study 1, Experiment 1 .................................................... 56
   Acquisition across sessions for water consumption in Phase 1
Figure 3d, Results from Study 1, Experiment 1 .................................................... 56
   Acquisition across sessions for water consumption in Phase 3
Figure 3e, Results from Study 1, Experiment 1 .................................................... 56
   Number of active and inactive lever presses for nicotine in Phase 2
Figure 3f, Results from Study 1, Experiment 1 .................................................... 56
   Number of active vs. inactive lever presses for nicotine in Phase 3 ............ 56
Figure 4a, Results from Study 1, Experiment 1
   Average intake differences for EtOH consumption in Phase 1 vs Phase ... 57
Figure 4b, Results from Study 1, Experiment 1
   Average intake differences for water consumption in Phase 1 vs Phase ... 57
Figure 4c, Results from Study 1, Experiment 1
   Average intake differences for number of infusions of nicotine in Phase 2
   vs Phase 3 ........................................................................................................... 57
Figure 5, Correlation between EtOH consumption and nicotine infusions in Phase 3 ... 58
Figure 6, Blood EtOH concentrations in Phase 1 vs Phase 3 ............................ 59
Figure 7a, Results from Study 1, Experiment 1
   Effects of varenicline on EtOH consumption ............................................. 60
Figure 7b, Results from Study 1, Experiment 1
   Effects of varenicline on water consumption ............................................. 60
Figure 7c, Results from Study 1, Experiment 1
   Effects of varenicline on number of active lever presses for nicotine ....... 60
Figure 7d, Results from Study 1, Experiment 1
   Effects of varenicline on number of inactive lever presses ...................... 60
Figure 8a, Results from Study 1, Experiment 1
   Effects of r-bPiDI on EtOH consumption .................................................. 61
Figure 8b, Results from Study 1, Experiment 1
   Effects of r-bPiDI on water consumption .................................................. 61
Figure 8c, Results from Study 1, Experiment 1
   Effects of r-bPiDI on number of active lever presses for nicotine ............ 61
Figure 8d, Results from Study 1, Experiment 1
   Effects of r-bPiDI on number of inactive lever presses ......................... 61
Figure 9a, Results from Study 1, Experiment 1
   Effects of RMOD on EtOH consumption .................................................. 62
Figure 9b, Results from Study 1, Experiment 1  
Effects of RMOD on water consumption ...........................................62
Figure 9c, Results from Study 1, Experiment 1  
Effects of RMOD on number of active lever presses for nicotine ..........62
Figure 9d, Results from Study 1, Experiment 1  
Effects of RMOD on number of inactive lever presses .......................62
Figure 10a, Results from Study 1, Experiment 1: Within-session nicotine self-administration as active lever presses per 10-min interval  
Following pretreatment with naltrexone ....................................63
Figure 10b, Results from Study 1, Experiment 1: Within-session nicotine self-administration as active lever presses per 10-min interval  
Following pretreatment with r-bPiDI ...........................................63
Figure 10c, Results from Study 1, Experiment 1: Within-session nicotine self-administration as active lever presses per 10-min interval  
Following pretreatment with RMOD ...........................................63
Figure 11a, Results from Study 1, Experiment 2  
Effects of varenicline on EtOH consumption ...................................64
Figure 11b, Results from Study 1, Experiment 2  
Effects of varenicline on water consumption ...................................64
Figure 11c, Results from Study 1, Experiment 2  
Effects of r-bPiDI on EtOH consumption ......................................64
Figure 11d, Results from Study 1, Experiment 2  
Effects of r-bPiDI on water consumption .......................................64
Figure 11e, Results from Study 1, Experiment 2  
Effects of RMOD on EtOH consumption ......................................64
Figure 11f, Results from Study 1, Experiment 2  
Effect of RMOD on water consumption ........................................64
Figure 12a, Baseline intakes of EtOH and nicotine for Experiments 1 and 2  
EtOH consumption .......................................................................80
Figure 12b, Baseline intakes of EtOH and nicotine for Experiments 1 and 2  
Nicotine infusions .......................................................................80
Figure 13a, Results from Study 2, Experiment 1  
Effect of naltrexone on EtOH consumed ......................................81
Figure 13b, Results from Study 2, Experiment 1  
Effect of naltrexone on water consumed ......................................81
Figure 14a, Results from Study 2, Experiment 2  
Effect of naltrexone on EtOH consumed ......................................82
Figure 14b, Results from Study 2, Experiment 2  
Effect of naltrexone on water consumed ......................................82
Figure 14c, Results from Study 2, Experiment 2  
Effect of naltrexone on active lever presses for nicotine ..................82
Figure 14d, Results from Study 2, Experiment 2  
Effect of naltrexone on inactive lever presses ................................82
Figure 15a, Results from Study 2, Experiment 2
Effect of varenicline on EtOH consumed........................................83
Figure 15b, Results from Study 2, Experiment
Effect of varenicline on water consumed........................................83
Figure 15c, Results from Study 2, Experiment 2
Effect of varenicline on active lever presses for nicotine..............83
Figure 15d, Results from Study 2, Experiment 2
Effect of varenicline on inactive lever presses...........................83
Figure 16a, Results from Study 2, Experiment 2
Effect of r-bPiDI on EtOH consumed.............................................84
Figure 16b, Results from Study 2, Experiment
Effect of r-bPiDI on water consumed.............................................84
Figure 16c, Results from Study 2, Experiment 2
Effect of r-bPiDI on active lever presses for nicotine..................84
Figure 16d, Results from Study 2, Experiment 2
Effect of r-bPiDI on inactive lever presses.................................84
Figure 17a, Results from Study 2, Experiment 2: Within-session active lever presses for nicotine per 10-min interval
Pretreatment with naltrexone.......................................................85
Figure 17b, Results from Study 2, Experiment 2: Within-session active lever presses for nicotine per 10-min interval
Pretreatment with varenicline....................................................85
Figure 17c, Results from Study 2, Experiment 2: Within-session active lever presses for nicotine per 10-min interval
Pretreatment with r-bPiDI.........................................................85
Figure 18, EtOH consumption across changing response requirements for nicotine........98
Figure 19, Intake of EtOH and nicotine across changing unit price of nicotine........99
Figure 20, Elasticity of demand for EtOH and nicotine..........................117
Figure 21, Effect of varenicline on elasticity of demand for EtOH and nicotine........117
CHAPTER ONE

1. General Introduction

1.1. Epidemiology of Alcohol and Nicotine Use

In the United States, cigarette smoking is the leading cause of preventable death (World Health Organization, 2017), accounting for more than 480,000 deaths per year (Jamal et al., 2016; Warren, Alberg, Kraft, & Cummings, 2014). Following closely behind as the third leading cause of preventable death (K. Gonzales et al., 2014; Mokdad, Marks, Stroup, & Gerberding, 2004; Warren et al., 2014), excessive ethanol (EtOH) use leads to approximately 88,000 deaths per year in the United States (Stahre, Roeber, Kanny, Brewer, & Zhang, 2014). Results from the National Survey on Drug Use and Health (NSDUH) for 2017 show that, despite the alarming fatality rates associated with the use of EtOH and tobacco, slightly more than half (51.7%), or approximately 140.6 million, of Americans aged 12 or older reported being current EtOH drinkers and approximately 22.4%, or 61.1 million, of Americans aged 12 or older reported being current tobacco users (Harford, Yi, Chen, & Grant, 2018).

In addition to overwhelmingly high death tolls, the economic burden of EtOH and tobacco use in the United States is immense. It is estimated that the cost of excessive EtOH drinking in the United States reached $249 billion in 2010 (Sacks, Gonzales, Bouchery, Tomedi, & Brewer, 2015). This figure includes expenses resulting from losses in workplace productivity (72% of the total cost), health care (11% of total), law enforcement and other criminal justice (10%), and losses from motor vehicle crashes associated with excessive EtOH use (5%) (Sacks et al., 2015). Similarly, the cost of tobacco-related illnesses in the United States reached more than $300 billion, including
direct medical care and losses in workplace productivity (Xu, Bishop, Kennedy, Simpson, & Pechacek, 2015). Unfortunately, successful treatment rates for EtOH use disorder (AUD) and tobacco use disorder (TUD) are low (Van Skike et al., 2016).

1.2. Co-Use of EtOH and Nicotine

Related to the problems of AUD and TUD separately, the co-use of EtOH and nicotine together presents a unique problem. Estimates show that roughly 80% of alcoholics are also regular tobacco smokers, making co-use of EtOH and nicotine the most prevalent polysubstance use disorder (Falk, Yi, & Hiller-Sturmhofel, 2006). Estimates show that tobacco users are 10-14 times more likely to develop an AUD compared to non-smokers (DiFranza & Guerrera, 1990; Sherry A. McKee et al., 2013). Additionally, co-use poses a threat to successful cessation attempts of both substances, with the likelihood of a successful abstinence attempt being decreased in co-users when compared to users of either substance alone, and higher relapse rates following quit attempts in co-users (Chiappetta, Garcia-Rodriguez, Jin, Secades-Villa, & Blanco, 2014; Sherry A. McKee et al., 2013; Weinberger, Pilver, Hoff, Mazure, & McKee, 2013). Although pharmacological cessation treatments are available for AUD or TUD individually, the high rate of co-use, increased difficulty of cessation for co-use, and the dangers of combining multiple medications, highlight the critical need for development of a single therapeutic treatment for EtOH and nicotine co-use.

Despite high incidence of co-use, AUD and TUD have primarily been considered as separate substance use disorders (SUDs) (American Psychiatric Association, 2013), and medication development has focused on treating them individually. However, to the extent that there are commonalities in the mechanisms of action for EtOH and nicotine, it
may be possible to develop a single pharmacotherapeutic agent to treat cigarette smokers who are also heavy drinkers (Roche, Ray, Yardley, & King, 2016). The first step in developing a medication that capitalizes on the commonalities in the mechanisms of action for EtOH and nicotine is to understand the pharmacokinetics and pharmacodynamics of EtOH and nicotine and their interactions on these systems that are responsible for the unique characteristics of co-use of EtOH and nicotine that make it so difficult to treat (Littleton, Barron, Prendergast, & Nixon, 2007).

1.3. Pharmacokinetics of Ethanol

EtOH, (CH$_3$-CH$_2$-OH), is the psychoactive agent found in beverage alcohol. While there are several routes of administration that can be used, the most common route is oral administration (Pohorecky & Brick, 1988). EtOH is found in 12-14% concentrations in wine, usually about 5% in beers (but can reach up to ~10% in microbrews), and 40-50% in liquor (usually expressed as “alcohol proof”, which is twice the percent concentration) (Julien, Advokat, & Comaty, 2008). One alcoholic "drink" is defined as a can or bottle of beer, a glass of wine or a wine cooler, a shot of liquor, or a mixed drink with liquor in it, each of which contain 1/3 ounce of 100% EtOH (National Institute on Alcohol Abuse and Alcoholism (NIAAA), 2017).

1.3.1. Absorption

EtOH is both water and lipid soluble, can diffuse across all biological membranes, and thus it is rapidly absorbed throughout the entire gastrointestinal tract (Julien et al., 2008; Pohorecky & Brick, 1988). On an empty stomach, about 20% of EtOH is absorbed through the stomach, followed by the remaining 80% being absorbed in the intestines (due to the relatively larger surface area), with the only limiting factor being the time
taken to empty the stomach (Holt, 1981; Julien et al., 2008). Under normal drinking conditions, it is unlikely that more than 10% of EtOH would be absorbed by the stomach (Pohorecky & Brick, 1988). Following the last drink, it can take anywhere from 30-90 minutes for blood EtOH concentrations (BECs) to reach a maximal, but this is influenced by many factors, including gender, concentration of EtOH, type of EtOH, genetic factors, presence of food in the stomach, etc. (Holt, 1981; Julien et al., 2008; Pohorecky & Brick, 1988).

1.3.2. Distribution

Following absorption, EtOH is equally distributed throughout all bodily fluids and tissues in proportion to the relative water content of the tissue type (Cederbaum, 2012). Tissues with greater vascularization, such as the brain, lungs, kidneys, and liver, receive EtOH more rapidly (Pohorecky & Brick, 1988). EtOH freely and immediately permeates the blood-brain barrier (BBB) and the placenta (Julien et al., 2008).

1.3.3. Metabolism and Excretion

In humans, approximately 95% of EtOH is enzymatically metabolized by alcohol dehydrogenase (ADH), with about 85% metabolized in the liver and 15% in the stomach, while the remaining 5% remains unchanged and is excreted through sweat, urine, and exhaled air (Hiroshi & Yuko, 2002; Jörnvall & Höög, 1995; Julien et al., 2008). In rats, approximately 90% of EtOH is metabolized by hepatic ADH activity (Boleda, Julià, Moreno, & Parés, 1989). When ingested orally, EtOH passes through the ADH-containing cells lining the gastrointestinal tract and then through the liver (Levitt & Levitt, 1998). The drug metabolism here, known as first-pass metabolism, can markedly reduce the amount of EtOH that reaches the bloodstream (Hiroshi Matsumoto & Fukui,
The metabolism that occurs in the stomach can also be affected by the amount of food in the stomach, such that gastric emptying will occur more rapidly on an empty stomach, reducing the amount of time that EtOH is subjected to metabolism, resulting in higher BECs (Julien et al., 2008; von Wartburg, Bethune, & Vallee, 1964). During metabolism, ADH converts EtOH to acetaldehyde (the rate-limiting step of EtOH metabolism), with the help of the co-enzyme nicotinamide adenine dinucleotide. Then, acetaldehyde is converted to acetic acid by the enzyme aldehyde dehydrogenase in both rats (H Matsumoto, Fujimiya, & Fukui, 1994) and humans (Cederbaum, 2012). Finally, acetic acid is broken down into carbon dioxide and water to release energy in the form of calories (Cederbaum, 2012).

EtOH is metabolized according to Michaelis-Menten kinetics, by which a maximum rate of reaction is reached when the drug concentration fully saturates the enzyme (Fujimiya, Yamaoka, & Fukui, 1989; Hiroshi & Yuko, 2002). Also known as non-linear elimination kinetics, at extremely low concentrations, EtOH is cleared by first-order kinetics (elimination at a constant proportion per unit of time) and at high concentrations (100% ADH enzyme saturation) by zero-order kinetics (elimination of a constant amount per unit of time, regardless of the concentration of drug). This occurs because ADH becomes saturated at very low concentrations of EtOH (Cederbaum, 2012; Hiroshi & Yuko, 2002). While this type of elimination kinetics is not unique to EtOH, it is rarer than first-order kinetics, which is the elimination kinetics followed by most drugs.

On average, a person metabolizes EtOH at approximately 7 g/hr (~1 standard drink per hour), fairly consistently across different people (Cederbaum, 2012; Julien et al., 2008). Understanding of the elimination kinetics of EtOH allow for predictions of

1.4. Pharmacological Effects of EtOH

The primary pharmacological effect of EtOH is the graded, reversible depression of central nervous system (CNS) function, including behavioral and cognitive functioning. These effects are additive when combined with other sedative-hypnotic compounds. Increasing doses of EtOH cause progressive respiratory depression, which can lead to death at high BECs (Gilpin & Koob, 2008). EtOH also has anticonvulsant properties, although it is not used clinically for this purpose. Conversely, EtOH withdrawal produces hyperexcitability that can lead to seizures (Julien et al., 2008).

EtOH also affects circulation and the heart by acting to dilate blood vessels in the skin, resulting in a decrease in body temperature accompanied by a warm flush of the skin. Thus, drinking EtOH to keep warm can be especially dangerous in cold temperatures as it can increase the risk of hypothermia. Unfortunately, the cardioprotective effect of low doses of EtOH is counteracted by tobacco smoking. High doses of EtOH can also increase risk of stroke, while daily low doses can reduce the risk of stroke (Julien et al., 2008).

1.5. Psychological Effects of EtOH

As reviewed by Julien et al. (2006), at low doses, EtOH effects can be stimulant and depressive. At higher doses, effects of EtOH include increased sedation, decreased behavioral activity, and impaired perceptual speed. At doses of 0.05-0.09 average % BEC, users can experience increased sociability and talkativeness, as well as decreased inhibition, attention, judgement, and control. Effects of higher doses of EtOH also
include slowed information processing, loss of efficiency in performance (Julien at al. 2006), and increases in violent and destructive behavior (Wechsler, Davenport, Dowdall, Moeykens, & Castillo, 1994). Increasing the dose of EtOH ingested produces progressive impairments in memory, concentration, and insight, which are initially dulled and then lost. At a BEC of 0.25 average % or higher, EtOH produces the inability to transcribe memories, during which users will experience a “blackout”. Additionally, chronic use of EtOH can produce long-term impairments in cognitive functioning (Duka et al., 2004; Julien et al., 2008).

1.6. Pharmacodynamics of EtOH

1.6.1. Dopamine

At levels of EtOH intoxication, evidence shows moderate excitation of dopamine (DA) neurons in the ventral tegmental area (VTA) in brain slices, resulting in increased DA release in the nucleus accumbens (NAc) (Brodie, Pesold, & Appel, 1999; Brodie, Shefner, & Dunwiddie, 1990). In addition to the direct effects of EtOH on DA neurons, there are several other mechanisms by which EtOH can influence DA neurotransmission, such as EtOH-induced nicotinic receptor activity (Blomqvist, Söderpalm, & Engel, 1992; Söderpalm, Ericsson, Olausson, Blomqvist, & Engel, 2000). Although EtOH has been shown to directly influence the function of various ligand-gated ion-channels, the indirect actions of EtOH on mesocorticolimbic dopamine neurons remains to be elucidated.

1.6.2. Glutamate

EtOH inhibits glutamate neurotransmission (Nie, Madamba, & Siggins, 1994). The N-Methyl-D-Aspartic (NMDA) subtype of glutamate receptors are particularly sensitive to EtOH-induced inhibition (Nixon, Hughes, Amsel, & Leslie, 2004). EtOH
depresses NMDAR responsiveness to released glutamate to disrupt glutaminergic neurotransmission (Lovinger, White, & Weight, 1989; Roberto et al., 2004). Chronic EtOH use can lead to a compensatory upregulation of NMDAR, resulting in increases in excitatory receptors during withdrawal, which may lead to damage and loss of neurons (Heinz, Schäfer, Higley, Krystal, & Goldman, 2003; McGinnis, Morales, Alexander, & McCool, 2017; Nixon et al., 2004).

1.6.3. **GABA**

EtOH acts to increase chloride ion flow by activating GABA, which results in neuronal inhibition, producing muscle relaxation, sedation, and inhibition of cognitive and motor skills (Hunt, 1983; Mihic et al., 1997; Olsen & Liang, 2017). GABA-ergic activation by EtOH can also have downstream effects on DA neurons (Boehm, Piercy, Bergstrom, & Phillips, 2002; Melis, Camarini, Ungless, & Bonci, 2002; Theile, Morikawa, Gonzales, & Morrisett, 2008).

1.6.4. **Serotonin**

Intoxicating concentrations of EtOH have been shown to potentiate 5-HT action, with emphasis on EtOH’s action on 5-HT₃ receptors (Ding, Ingraham, Rodd, & McBride, 2015; Lovinger & White, 1991). The activation of 5-HT receptors by EtOH has been implicated in the reinforcing effects of EtOH (Liu, Thielen, Rodd, & McBride, 2006; W. J McBride et al., 2004). EtOH can influence DA neurotransmission through action on serotonin (5-HT) systems (Julien et al., 2008).

1.6.5. **Endocannabinoids**

Although endocannabinoids are not a direct target of EtOH, they can stimulate the formation of the endogenous neurotransmitter for cannabinoid receptors, anandamide,
which can eventually lead to the downregulation of receptors (Basavarajappa & Hungund, 1999a, 1999b; Julien et al., 2008). Studies have shown that CB1 receptors play a crucial role in voluntary EtOH consumption, preference, withdrawal, and relapse (Pava & Lovinger, 2014; Thanos, Dimitrakakis, Rice, Gifford, & Volkow, 2005; L. Wang, Liu, Harvey-White, Zimmer, & Kunos, 2003). Studies have also shown that CB1 receptors are relatively resistant to neurotoxic effects of binge EtOH exposure, suggesting the possibility of reducing EtOH-induced neurodegeneration through pharmacologic intervention targeting CB1 receptors (Liput, Pauly, Stinchcomb, & Nixon, 2017).

### 1.6.6. Acetylcholine

Although typically associated with nicotine action, nAChR subtypes may be both directly and indirectly affected by EtOH (Narahashi, Aistrup, Marszalec, & Nagata, 1999). This is not surprising, as both nAChRs and GABA<sub>A</sub> both belong to the “cys-loop” superfamily of ligand-gated ion channels (John A. Dani, 2015) and GABA<sub>A</sub> is commonly regarded as an important target site of EtOH (Narahashi et al., 1999). nAChRs are ligand-gated ion channels that bind endogenous ACh but also have a high affinity for nicotine. nAChRs are pentameric receptors made up of α, β, γ, δ, and ε subunits (Changeux, Galzi, Devillers-Thiéry, & Bertrand, 1992). Although nAChRs are found both centrally and peripherally, nAChRs in the CNS consist of two families of subtypes, α and β, with several members labeled in a numerical fashion (i.e. α2, α3, …α10, β2, β3, β4). Formations of different subtypes of nAChRs determine which ions can pass through the channel when it is open. Subtypes of nAChRs are made up of different combinations of subunits, forming either heteromeric (containing both α and β subunits) or homomeric
(containing only α subunits) receptors. The most common subtypes found in the brain are α4β2 and α7 (Neal L. Benowitz, Hukkanen, & Jacob, 2009).

nAChRs located in the mesocorticolimbic system contribute to the rewarding effects of both EtOH and nicotine through their activation of DA-ergic neurons in the VTA (Okamoto, Harnett, & Morikawa, 2006). As an important neurotransmitter system for co-use, the cholinergic system is a viable potential target for pharmacologic treatments for co-use of EtOH and nicotine (Van Skike et al., 2016; You, Vandegrift, & Brodie, 2018), which will be discussed in further detail in a later section.

Chronic EtOH consumption has been shown to alter the cholinergic system via hippocampal activity involving nAChRs (Nordberg, Larsson, Perdahl, & Winblad, 1982). Research has also shown that EtOH can interact with the cholinergic system by enhancing function of certain subtypes (i.e., α4β2, α4β4, α2β2, and α2β4), while inhibiting other certain subtypes (i.e. α7) (Cardoso et al., 1999; de Fiebre & de Fiebre, 2005; Yu et al., 1996). EtOH action on nAChRs in the mesocorticolimbic DA system has been shown to increase DA in nucleus accumbens (NAc) (Ericson, Blomqvist, Engel, & Soderpalm, 1998) and increase extracellular acetylcholine levels in VTA (Larsson, Edström, Svensson, SÖDerpalm, & Engel, 2005). Research has also shown that systemic injections of EtOH increase extracellular DA in NAc, a reaction that can be completely counteracted by systemic administration of the nicotinic antagonist mecamylamine at doses that have no effect on DA when given in the absence of EtOH (Blomqvist, 1996). This suggests that the increase in extracellular DA in NAc may be due, at least in part, to the effects of EtOH on nAChRs. Additionally, mecamylamine microinfusions in VTA, but not into NAc, counteract extracellular DA increases in NAc following systemic EtOH
injections (Blomqvist, Ericson, Engel, & Söderpalm, 1997), suggesting that nAChRs specifically in VTA are involved in EtOH-induced DA activation. These findings are also supported by behavioral data showing that P rats switch preference from EtOH to water following perfusions of mecamylamine in VTA (Weiss, Lorang, Bloom, & Koob, 1993).

While there is extensive evidence indicating that there is nAChR involvement in the effects of EtOH, specific mechanisms of action remain to be elucidated and are likely overlapping for both EtOH and nicotine. Importantly, these interactions between EtOH and nicotine at nAChRs are likely to contribute to the high rate of co-use of EtOH and nicotine, which is discussed later in more detail.

1.7. Pharmacokinetics of Nicotine

The alkaloid nicotine, chemical formula C_{10}H_{14}N_{2}, is the primary active ingredient in tobacco. One of over 4000 chemical components found in smoking and chewing tobacco, nicotine is considered to be primarily responsible for tobacco addiction and accounts for the acute pharmacological effects of smoking (Corrigall & Coen, 1989). Although there are several routes for nicotine administration, the most common route is inhalation via smoking processed tobacco in cigarettes, cigars, or pipe tobacco.

1.7.1. Absorption

When tobacco is burned, as in cigarette smoking, nicotine is distilled and inhaled (Gori, Benowitz, & Lynch, 1986). Nicotine is readily absorbed from every site in the body (Julien et al., 2008). When inhaled, nicotine is absorbed into the blood stream from the lungs, causing a rapid rise of nicotine levels in the blood. From the bloodstream, nicotine is directly transported into the brain within 10-20 seconds of inhalation, quickly producing behavioral reinforcement (N. L. Benowitz, 1990). One cigarette contains
approximately 10-20 mg of nicotine, only about 20% of which is inhaled and absorbed into the bloodstream, while the rest is metabolized by the hepatic enzyme CYP-2A6 (Julien et al., 2008).

1.7.2. Distribution

Distribution of nicotine occurs thoroughly and quickly throughout the body, with rapid penetration of the BBB, placenta, and in all bodily fluids. In bodily tissue, nicotine follows a steady-state volume of distribution (Neal L. Benowitz et al., 2009). Nicotine has a high affinity for binding to brain tissue, with increased binding in smokers when compared to non-smokers (Breese et al., 1997). This is due, at least in part, to a higher number of nicotinic cholinergic receptors in the brains of the smokers (Breese et al., 1997). Smokers can easily titrate the level of nicotine intake to achieve desired pharmacologic effects due to the fast rate at which nicotine reaches the brain, which contributes to the reinforcing value and abuse liability of nicotine (Neal L. Benowitz et al., 2009; Henningfield & Keenan, 1993). The elimination half-life of nicotine is about 2 hrs and smokers tend to continue smoking throughout the day to maintain nicotine blood levels of approximately 15 mg per liter (Henningfield & Keenan, 1993; Julien et al., 2008).

1.7.3. Metabolism and Excretion

Nicotine is metabolized primarily by the liver (Neal L. Benowitz et al., 2009), although local metabolism in brain also occurs (Crooks, Li, & Dwoskin, 1997). The primary metabolite of nicotine is cotinine, accounting for approximately 70-80% of nicotine metabolism (Neal L. Benowitz et al., 2009). First, nicotine is metabolized by CYP2A6 to produce nicotine-Δ1’ (5’)-iminium ion, which is then catalyzed by
cytoplasmic aldehyde oxidase, resulting in cotinine (Neal L. Benowitz et al., 2009). Other metabolites include nicotine N’-oxide, (S)-nicotine-N-β-glucuronide (N. L. Benowitz, Jacob, Fong, & Gupta, 1994), and nornicotine (Neurath, Orth, & Pein, 1991). Unlike EtOH, metabolism of nicotine follows first-order elimination kinetics, meaning that elimination occurs at a constant fraction of drug quantity present per unit of time (Neal L. Benowitz et al., 2009). Thus, elimination is proportional to the concentration of nicotine, i.e. the higher the concentration, the faster the rate of elimination. After metabolism by the liver, nicotine is excreted by the kidneys.

1.8. Pharmacodynamic and Psychological Effects of Nicotine

Smoking tobacco acts to increase heart rate, blood pressure, and cardiac contractility (Jolma, Samson, Klewer, Donnerstein, & Goldberg, 2002; Mishra et al., 2015). As explained in Julien et al. (2008), during early exposure instances, smoking can cause nausea and vomiting, but tolerance to these effects builds rapidly. Nicotine acts to stimulate the hypothalamus, producing a release in antidiuretic hormone to cause fluid retention. Afferent neuron activity is reduced, leading to a decrease in muscle tone. Nicotine also acts to decrease appetite. Additionally, nicotine increases psychomotor activity, cognitive functioning, sensorimotor performance, attention, and memory consolidation by increasing blood flow to the CNS structures that mediate arousal and reward (Julien et al., 2008).

Nicotine has also been shown to produce improvements in cognitive functioning, particularly in attention, learning, and memory performance (Levin, McClernon, & Rezvani, 2006; Levin & Simon, 1998; Logue & Gould, 2014; H. D. Mansvelder, K. I. van Aerde, J. J. Couey, & A. B. Brussaard, 2006). Most likely, this is a result of nicotinic
effects on multiple brain areas, such as hippocampus, amygdala, prefrontal cortex (PFC), thalamus, VTA, and substantia nigra (Levin et al., 2006; Huibert D. Mansvelder, Karlijn I. van Aerde, Jonathan J. Couey, & Arjen B. Brussaard, 2006). As previously stated, nAChRs are widespread in the central and peripheral nervous systems and can be located pre-synaptically on DA, acetylcholine (ACh), and glutamate neurons (Julien et al., 2008). Activation of nAChRs have been shown to increase DA release in the mesocorticolimbic system, including the VTA, NAc, and forebrain, which is presumed to be the action by which nicotine exhibits its potent behavior-reinforcing action (Okamoto et al., 2006).

1.9. Neuronal nAChRs

As previously discussed, nAChRs are ligand-gated ion channels found both centrally and peripherally (Neal L. Benowitz et al., 2009). Initially, nicotine acts as an agonist at all subtypes of nAChRs, but then quickly desensitizes nAChRs (A. L. Brody et al., 2006). Regular tobacco use produces prolonged exposure to low concentrations of nicotine, which results in significant receptor desensitization (John A Dani, Radcliffe, & Pidoplichko, 2000; Giniatullin, Nistri, & Yakel, 2005). Additionally, it is hypothesized that repeated exposure to nicotine binding and subsequent nAChR upregulation play an important role in nicotine dependence and addiction (Bardo, 1998; Sparks & Pauly, 1999; Wonnacott, 1990).

When an agonist is bound to nAChRs, the channel opens and allows the influx of sodium (Na⁺) and/or calcium (Ca²⁺) and the efflux of potassium (K⁺) cations, resulting in cell membrane depolarization. The increases in extracellular Ca²⁺ activates numerous cell-signaling pathways and causes the release of various neurotransmitters (Davis & de Fiebre, 2006; Hendrickson, Guildford, & Tapper, 2013).
As previously discussed, nAChRs are composed of 5 subunits with numerous compositions that make up a wide diversity of nAChR subtypes (Changeux et al., 1992). This wide variety of subtypes contributes to the many roles of nAChRs in the CNS, including learning, memory, attention, and processes involved in synaptic plasticity (Albuquerque et al., 1997; Aramakis & Metherate, 1998; Ge & Dani, 2005; Ji, Lape, & Dani, 2001). Dysfunction in nicotinic cholinergic mechanisms have also been implicated in schizophrenia, epilepsy, autism, Alzheimer’s disease (AD), and addiction (Court et al., 2001; John A Dani & Harris, 2005; Leonard et al., 2001; Raggenbass & Bertrand, 2002).

Although the nAChR upregulation seen in chronic smokers is thought to play an important role in nicotine dependence, especially upregulation involving the α4β2 and α7 subtypes, chronic exposure desensitizes α4β2 (Wonnacott, 1990). Thus, while there is an increase in receptors, these receptors are desensitized, requiring an increased amount of nicotine to produce the same response (Balfour, 1994; Marina R Picciotto, Addy, Mineur, & Brunzell, 2008). In regard to upregulation, a study by Brody et al. (2014) showed that patients with greater α4β2 upregulation were less successful in smoking cessation attempts and had higher rates of relapse compared to patients with less α4β2 upregulation (Arthur L Brody et al., 2014). This suggests that individual differences in treatment success may involve differential disruption of the processes involved in α4β2 upregulation.

Another component of nicotine use that contributes to its abuse liability is the extracellular DA release that occurs as a result of nicotine action on the dorsal striatum and NAc. This DA release is presumed to be primarily responsible for the reinforcing properties of nicotine (Corrigall, Franklin, Coen, & Clarke, 1992; Di Chiara et al., 2004).
Studies have shown that repeated exposure to nicotine can increase reward sensitivity through dopaminergic systems, as well as increase the reward sensitivity threshold (Kenny & Markou, 2006), which is likely to contribute to perpetuating the habit of smoking. Specifically, these results suggest that with repeated use, nicotine is likely enhancing the rewarding effects of other primary and secondary reinforcers, while decreasing the initial rewarding effects.

In summary, the complexities of nicotine and its ability to differentially effect dopamine release through desensitization and upregulation require special consideration with regard to nicotine interaction with the dopaminergic reward system and its subsequent effects on nicotine dependence. Additionally, EtOH also affects this system with unique interactions occurring when both substances are administered, further complicating examinations and understanding of EtOH and nicotine co-use and how they affect the cholinergic system. Thus, as previously mentioned, the interaction between EtOH and nicotine on nAChRs is important for understanding the co-use of both substances.

1.10. Common Substrates of EtOH and Nicotine

As previously discussed, there is a high rate of overlap in EtOH and nicotine use. Contributing factors are likely the many common molecular and cellular targets for EtOH and nicotine, but the interactions of EtOH and nicotine at these common substrates is still under investigation. Understanding these common substrates and the interactions of EtOH and nicotine at these substrates could help lead to the development of a single pharmacotherapeutic to treat the co-use of EtOH and nicotine. In this section, the interaction of EtOH and nicotine at these common substrates are reviewed.
1.10.1. The Mesocorticolimbic DA System

One major system which has been shown to play a critical role in the development of virtually all types of SUDs is the mesocorticolimbic DA system (Doyon, Thomas, Ostroumov, Dong, & Dani, 2013; Koob & Volkow, 2010). Within this system, activation of the VTA is the primary source of DA neuron release. Neurons from the VTA project to several forebrain structures, including the NAc, ventral pallidum, amygdala, and medial PFC (mPFC). One major hypothesis associated with this system is that drugs of abuse produce a prolonged release of DA that changes synaptic plasticity and shifts normal learning mechanisms to habit-learning (Wolf, 2002).

Virtually all drugs of abuse are associated with an increase of DA release in the NAc, including both EtOH and nicotine. Both EtOH and nicotine act directly and indirectly on the mesocorticolimbic DA system to increase DA transmission. Administration of either EtOH or nicotine increases the firing rate of DA neurons in the VTA (Avegno et al., 2016; Gessa, Muntoni, Collu, Vargiu, & Mereu, 1985; Schilstrom, Rawal, Mameli-Engvall, Nomikos, & Svensson, 2003). Specific concentrations of EtOH and nicotine together have also shown to produce a synergistic effect of DA firing of VTA neurons compared to either drug alone (Clark & Little, 2004). The increased firing of DA neurons produced by EtOH and nicotine is also associated with increased DA release in NAc, ventral pallidum, and mPFC (Bradberry, 2002; Gotti et al., 2010; Melendez, Rodd-Henricks, McBride, & Murphy, 2003; Nisell, Nomikos, & Svensson, 1994; Schier, Dilly, & Gonzales, 2013). Additionally, simultaneous administration of EtOH and nicotine produces an additive increase in DA release in NAc compared to
either drug alone (Tizabi, Bai, Copeland, & Taylor, 2007; Tizabi, Copeland, Louis, & Taylor, 2002; Tolu et al., 2017).

In summary, both EtOH and nicotine produce activation of the mesocorticolimbic DA system and when administered simultaneously, EtOH and nicotine produce additive increases in both DA firing and DA release in this system such that the increase is greater than the effects produced by either drug alone. These interactions seen in the mesocorticolimbic DA pathway suggest that understanding this pathway may be key in the development of a monotherapy to treat the co-use of EtOH and nicotine.

1.10.2. The Cholinergic Pathway

Within the mesocorticolimbic DA system, nAChRs have been shown to play an important role in drug addiction. Although nAChRs can be found throughout the CNS, they are robustly expressed in the VTA on DAergic and GABAergic neurons (Klink, de Kerchove d'Exaerde, Zoli, & Changeux, 2001; M. R. Picciotto et al., 1998; Tolu et al., 2013). Nicotine both activates and then quickly desensitizes nAChRs (A. L. Brody et al., 2006), a process that can be interrupted by EtOH (Marszalec, Aistrup, & Narahashi, 1999). Thus, EtOH not only affects normal signaling transmission of nAChRs, but also nicotine-induced signaling, an interaction that may contribute to the high rate of co-use for these substances.

Studies have also shown that long-term EtOH treatment can increase nicotinic binding in certain regions of the brain (Booker & Collins, 1997; Dohrman & Reiter, 2003; Tarren, Lester, Belmer, & Bartlett, 2017). Nicotine can also increase the number of nAChRs in the brain (Locker, Marks, Kamens, & Klein, 2016; Marks et al., 2011). Interestingly, EtOH and nicotine together can produce long lasting upregulation of
nAChR expression (Dohrman & Reiter, 2003; Ribeiro-Carvalho et al., 2009). Furthermore, these changes in nAChRs could be responsible for tolerance to both EtOH and nicotine. Studies have shown that EtOH treatment can produce tolerance to EtOH and cross-tolerance to nicotine. Similarly, nicotine treatment can produce tolerance to nicotine and cross-tolerance to EtOH (Burch, de Fiebre, Marks, & Collins, 1988; Collins, Burch, de Fiebre, & Marks, 1988; Funk, Marinelli, & Le, 2006). Research has also shown that EtOH enhances nicotine-induced excitatory activity in substantia nigra reticulata and ventral pallidum (Criswell et al., 1993). Additionally, it has been shown that chronic exposure to nicotine can reduce the neurotoxicity of EtOH withdrawal in hippocampus (Prendergast, Harris, Mayer, & Littleton, 2000), a region dense in nAChR expression. Taken together, this evidence suggests that the interaction of EtOH and nicotine at nAChRs may be critical for development of a pharmacotherapeutic treatment for the co-use of EtOH and nicotine.

1.11. Current Therapeutic Treatments for Cessation of EtOH or Nicotine

The next step in developing a novel treatment for co-use of EtOH and nicotine is exploring previous treatment for AUD or TUD alone and using this information to charter a logical direction for future medication development. Currently, three FDA-approved pharmacologic agents are available for EtOH abstinence and four agents are available for smoking cessation. These treatments target different aspects of dependence through various mechanisms of action. The three FDA-approved treatments for EtOH use disorders are disulfiram (Antabuse®), naltrexone (Revia®, Vivitrol®) and acamprosate (Campral®). Similarly, for smoking cessation, the FDA-approved agents are nicotine
replacement therapy (NRT, Nicorette®, Nicoderm®), varenicline (Chantix®), and bupropion (Zyban®).

Increased interest in varenicline continues due to findings in humans and animals that suggest it may also be useful in EtOH cessation (Bito-Onon, Simms, Chatterjee, Holgate, & Bartlett, 2011; Feduccia, Simms, Mill, Yi, & Bartlett, 2014; S. A. McKee et al., 2009; Sherry A. McKee et al., 2013; Wouda et al., 2011), but results also show that in co-users, smoking cessation with varenicline treatment is limited (S. A. McKee et al., 2009; Wouda et al., 2011). Importantly, all available cessation agents have limited long-term efficacy and are associated with high relapse rates (T. P. George & O'Malley, 2004; R. D. Hurt, 1999; R. D. Hurt et al., 2003; Wileyto et al., 2004), thus revealing a need for more efficacious alternative therapies.

1.11.1. Disulfiram

EtOH is converted to acetaldehyde in the body, which is then broken down by acetaldehyde dehydrogenase. Disulfiram (Antabuse®) acts to inhibit the enzyme acetaldehyde dehydrogenase, leading to a build-up of acetaldehyde in the body, which can cause unpleasant effects, similar to those experienced during a hangover (Franck & Jayaram-Lindström, 2013; Wright & Moore, 1990). These adverse effects occur upon EtOH ingestion, which can deter an individual from drinking. Individuals who are highly motivated to remain abstinent can benefit from this medication, but those who are motivated to drink EtOH can simply stop taking the medication to avoid adverse effects while continuing to drink. Thus, compliance for this medication is low. Clinical studies show treatment with disulfiram can reduce the number of drinking days and increase the number of days until relapse (Fuller et al., 1986; Krampe, Spies, & Ehrenreich, 2011).
Disulfiram has also been shown to be particularly effective in persons with inactive aldehyde dehydrogenase-2 (Yoshimura et al., 2014). A meta-analysis shows disulfiram is efficacious compared to other pharmacological treatments, including naltrexone and acamprosate, but only in open-label studies (Skinner, Lahmek, Pham, & Aubin, 2014). Evaluations of long-term efficacy of disulfiram show promise for those who undergo supervised outpatient treatment (Mutschler, Dirican, Gutzeit, & Grosshans, 2011). Overall, the efficacy of disulfiram for AUD treatment seems limited (Franck & Jayaram-Lindström, 2013; Skinner et al., 2014; Van Skike et al., 2016).

### 1.11.2. Naltrexone

Naltrexone (Revia®, Vivitrol®) and its active metabolite 6β-naltrexol act as non-selective competitive antagonists of opioid receptors, with the highest affinity for the μ-opioid receptor, followed by the κ- and δ-opioid receptors (Niciu & Arias, 2013). Naltrexone is available in both oral (Revia®) and extended release injectable (Vivitrol®) forms. Early studies showed some success of naltrexone in reducing craving, euphoria from drinking, and drinking (O'Brien, Volpicelli, & Volpicelli, 1996; Pettinati et al., 2006). However, a recent study found increased craving reports to EtOH cues and increased subjective reports of intoxication in naltrexone treated patients compared to those treated with placebo (Spagnolo et al., 2014). Additional studies of naltrexone show efficacy in reducing the number of drinks per drinking day, but no effects on the percent of heavy drinking days or the number of abstinence days (Garbutt, 2010; O'Malley et al., 2015; Pettinati et al., 2006). Overall, naltrexone treatment for EtOH drinking is effective in reducing heavy drinking, but not in maintaining abstinence from drinking due to
compliance issues resulting from significant anhedonia (Garbutt, 2010; Pettinati et al., 2006).

1.11.3. Acamprosate

The mechanism of action of acamprosate (Campral®) is presumed to be indirect modulation of NMDA receptors and enhancement of GABA_A receptor function (Berton, Francesconi, Madamba, Zieglsansberger, & Siggins, 1998; Daoust et al., 1992; Kalk & Lingford-Hughes, 2014). As previously discussed, EtOH withdrawal can lead to over-activation of glutamate receptors (Julien et al., 2008; Lovinger et al., 1989; Roberto et al., 2004), effects which are thought to be attenuated by the effects of acamprosate on NMDA and GABA_A receptors (Dahchour & De Witte, 2000; Kalk & Lingford-Hughes, 2014). A review of acamprosate treatment adjunct with psychosocial interventions for AUD found modest efficacy, with improvement outcomes similar to naltrexone treatment (Plosker, 2015).

1.11.4. Nicotine Replacement Therapy (NRT)

The first FDA-approved smoking cessation treatment was NRT, which is available in the form of gum, inhalers, lozenges, nasal spray, and patches (Corelli & Hudmon, 2002). NRTs are designed to expose individuals to low doses (5-20 mg per day) of nicotine throughout the day, with lower doses as treatment progresses. The goal of NRTs is to reduce craving for nicotine while also avoiding withdrawal symptoms (Stead et al., 2012). A review of the efficacy of all forms of NRTs indicates that the use of an NRT can increase the rate of quitting by approximately 50-70% during a 12-week treatment period when the medication was used consistently (Cahill, Stevens, & Lancaster, 2014; Stead et al., 2012; Zhang, Cohen, Bondy, & Selby, 2015). However,
among those who initially quit during NRT treatment, the relapse rate after 1 year is about 80% (Munoz et al., 2009; Secades-Villa, González-Roz, García-Pérez, & Becoña, 2017), indicating limited long-term efficacy of NRT treatment for smoking cessation.

1.11.5. Bupropion

The first non-nicotine approved smoking cessation therapy in the U.S. was bupropion, a norepinephrine-DA reuptake inhibitor (Dwoskin, Rauhut, King-Pospisil, & Bardo, 2006) and an nAChR antagonist with allosteric antagonist actions at α3β4, α4β2, α6β2, and α7 nAChRs (F. I. Carroll et al., 2014; Rauhut, Neugebauer, Dwoskin, & Bardo, 2003; Slemmer, Martin, & Damaj, 2000). Research has shown that bupropion is effective in the early stages of smoking cessation, presumably due to the attenuation of withdrawal symptoms (Ross & Williams, 2005; Wilkes, 2008). Bupropion has been shown to double quit rates when compared to placebo (Hall et al., 2002; Richard D Hurt et al., 1997; Simon, Duncan, Carmody, & Hudes, 2004). However, similar to NRT treatment, relapse rates after 1 year were approximately 80% (Richard D Hurt et al., 1997; Douglas E. Jorenby et al., 1999; Tønnesen et al., 2003).

In addition to bupropion treatment alone, combination treatment has also been explored. Research on bupropion administered in conjunction with NRT has shown increases in 1 year quit rates with the combination compared to either treatment alone (Douglas E. Jorenby et al., 1999; Mansourati, Borel, Munier, & Guevel-Jointret, 2005). However, more recent studies have shown that the combination of NRT with bupropion was no more effective in smoking cessation than either treatment alone (Stapleton et al., 2013). Thus, while bupropion is modestly effective as a smoking cessation treatment alone,
combination treatment with other cessation treatments may improve cessation rates, but further research is needed.

1.11.6. Varenicline

Varenicline (Chantix®), a clinically available partial nicotinic agonist with a high affinity for α4β2* nAChRs and with full agonism at α7, has been demonstrated to reduce nicotine self-administration in animals (O. George, Lloyd, Carroll, Damaj, & Koob, 2011; O’Connor, Parker, Rollema, & Mead, 2010; Rollema et al., 2007), reduce tobacco craving, withdrawal, and its reinforcing effects in humans (D. Gonzales et al., 2006; D. E. Jorenby, Hays, Rigotti, & et al., 2006; Sherry A. McKee et al., 2013), as well as increasing smoking abstinence rates (Ebbert, Croghan, Hurt, Schroeder, & Hays, 2016a; D. Gonzales et al., 2006; Nides et al., 2006). Currently, varenicline is regarded as the most effective smoking cessation agent available (Anthenelli et al., 2016; Oncken, Gonzales, Nides, & et al., 2006). Additionally, varenicline in combination with bupropion has shown greater efficacy in heavy smokers compared to either treatment alone (Ebbert et al., 2014; Rose & Behm, 2017; Vogeler, McClain, & Evoy, 2016).

Although varenicline is primarily regarded as an effective smoking cessation treatment, it has also been evaluated for AUD. Several studies have demonstrated that varenicline reduces EtOH consumption in humans (Falk, Castle, Ryan, Fertig, & Litten, 2015; Litten et al., 2013; Sherry A. McKee et al., 2013); however, conflicting evidence for the effects of varenicline on EtOH craving and consumption have been reported (de Bejczy et al., 2015; Schacht et al., 2014; Verplaetse et al., 2016). Several preclinical studies have demonstrated that pretreatment with varenicline reduces EtOH consumption in rodents when EtOH is available in the absence of nicotine (Froehlich et al., 2017; H.
M. Kamens, Andersen, & Picciotto, 2010; Sotomayor-Zarate et al., 2013; Steensland, Simms, Holgate, Richards, & Bartlett, 2007). Additionally, in a study by Froehlich et al. (2017), EtOH consumption in male alcohol-preferring (P) rats was reduced by daily repeated administration of varenicline. Furthermore, in preclinical examinations of co-use of EtOH and nicotine, there is conflicting evidence of the effectiveness of varenicline in reducing EtOH consumption (Funk, Lo, Coen, & Le, 2016; Randall, Jaramillo, Frisbee, & Besheer, 2015; Scuppa, Cippitelli, Toll, Ciccocioppo, & Ubaldi, 2015). Among the three preclinical studies that have examined the effects of varenicline on EtOH and nicotine co-use, only one study reported that varenicline significantly decreased both nicotine and EtOH self-administration during concurrent access (Cippitelli et al., 2015). In contrast, the two other studies showed that varenicline decreased nicotine self-administration, but not EtOH self-administration (Funk et al., 2016; Scuppa et al., 2015). These mixed results in both clinical and preclinical work indicate that more work is needed to determine the effectiveness of varenicline as a treatment for EtOH and nicotine co-use. Critical for the future of treatment discovery for co-use disorder is developing a translational animal model, as there are no currently accepted models of EtOH and nicotine co-use.

1.12. Translational Animal Models

Animal models have successfully been used to model and test treatments for both AUD and TUD separately (Van Skike et al., 2016), as well as for other SUDs (Koob, Kenneth Lloyd, & Mason, 2009), but relatively few studies have examined the voluntary co-use of EtOH and nicotine in the same animal (W. J. McBride, Rodd, Bell, Lumeng, & Li, 2014). It is imperative to develop a co-use model that produces pharmacologically
relevant levels of voluntary oral EtOH consumption and i.v. nicotine self-administration when both substances are available concurrently to more closely model human co-use behaviors.

Despite its high addiction liability in humans, nicotine has relatively weak intrinsic reinforcing effects in rats compared to other stimulants, such as cocaine, thus engendering relatively lower rates of drug-reinforced responding in traditional drug self-administration procedures (Peartree et al., 2012). However, reliable nicotine self-administration can be obtained by implementing an incrementing fixed-ratio (FR) schedule of reinforcement under limited-access conditions (Corrigall & Coen, 1989). This schedule produces stable and substantial behavioral output maintained on a nicotine-reinforced operant lever. Traditional models of drug self-administration utilize the i.v. route of administration, which is translationally relevant for nicotine as it not only allows for quantifiable doses of nicotine to be delivered, but also mimics the rapid onset of nicotine delivered through inhalation of cigarette smoke (Gardner, Dishion, & Posner, 2006).

Similarly, EtOH has weak intrinsic reinforcing properties in rodents. Thus, while many humans readily consume EtOH to intoxicating levels, this behavior is difficult to attain in rodents. Heterogeneous stock rats generally consume modest amounts of EtOH, producing only minimal BECs (Cicero & Smithloff, 1973). To ensure that rodents consume EtOH in quantities that are representative of binge drinking in humans, selective breeding techniques have been used (Richard L Bell et al., 2012).

Criteria for an animal model of AUD has been put forth based on the criteria for humans with AUD (Richard L Bell et al., 2012; Cicero & Smithloff, 1973; Lester &
Freed, 1973). The criteria are: 1) the animal should readily and voluntarily consume EtOH; 2) the amount of EtOH consumed should result in pharmacologically relevant BECs; 3) EtOH should be consumed for its post-ingestive pharmacological effects; 4) EtOH should be reinforcing; 5) chronic EtOH consumption should lead to the expression of tolerance; 6) chronic consumption of EtOH should lead to dependence (Richard L Bell et al., 2012; Lester & Freed, 1973); and 7) animals should display characteristics of relapse after extinction (W. J. McBride & Li, 1998). The only rat line from selective breeding techniques to have successfully fit all 7 of these criteria is the alcohol-preferring (P) rat, which has been developed and is currently maintained at Indiana University School of Medicine (Bell, Rodd, Lumeng, Murphy, & McBride, 2006). However, despite this strong scientific premise for using P rats, there is little published literature available on combined EtOH and nicotine self-administration using P rats (Van Skike et al., 2016). Important for the development of a co-use model is understanding the relationship between the concurrently available substances, which can be examined by applying economic principles of demand to drug-taking behaviors.

1.13. **Behavioral Economics of Demand**

In psychology, the field of behavioral economics has recently been gaining popularity as a means to examine drug-taking behaviors in the laboratory (Bickel, DeGrandpre, & Higgins, 1993; Hursh & Roma, 2016). In examination of the behavioral economics of drug self-administration, the two fundamental variables that control drug self-administration are response requirement (i.e. the amount of behavior allocation required to obtain a given dose of a drug reinforcer) and the dose of drug per administration (i.e. the dose of a drug that is obtained when a response requirement is
The relationship between reinforcer cost and reinforcer consumption can be plotted as a demand curve, providing a quantitative analysis of behavior.

In general, in a closed economy, as the price of a commodity increases, the consumption of that commodity decreases. The rate at which consumption changes shows the sensitivity to price for that commodity, which can be elastic (consumption decreases as price increases) or inelastic (consumption decreases at a slower rate than price increases) (Hursh & Roma, 2016). In behavioral economics of drug self-administration, drug consumption is a function of the unit price of a reinforcer. Manipulating the response requirement or reinforcer magnitude in drug self-administration produces the same functional effect of altering unit price. Thus, the amount of drug consumption can be changed via manipulation of response requirement or reinforcer magnitude (dose per infusion), creating a positively decelerating demand curve as unit price increases (Bickel et al., 1990).

In examining how SUDs are behaviorally expressed, economic principles have been applied to examinations of drug-taking behavior in both humans and animals, with the overall goals of finding the causes and treatments for various SUDs, including both AUD and TUD (Bickel, Jarmolowicz, Mueller, & Gatchalian, 2011). In humans, elasticity of demand for EtOH and nicotine have been examined using both operant drug self-administration paradigms (Nancy K Mello & Mendelson, 1965; Shahan, Bickel, Madden, & Badger, 1999) and purchase tasks (MacKillop et al., 2008; Murphy, MacKillop, Skidmore, & Pederson, 2009). Similarly, in animals, elasticity of demand for EtOH and nicotine can be examined using operant drug self-administration in
combination with changing the cost for a reinforcer (Heyman, 1996; Smith, Sved, Hatsukami, & Donny, 2014). Using methods of applying behavioral economic principles provides translational utility for drug-taking research by offering a quantitative approach to modeling behavior that is mathematically identical across species (Bentzley, Jhou, & Aston-Jones, 2014).


As a main goal of this dissertation, we have worked to develop a translational animal model of EtOH and nicotine co-use that produces pharmacologically relevant levels of both EtOH and nicotine intake. Using this model, we have also sought to test several potential pharmacotherapeutic treatments for co-use of EtOH and nicotine. Furthermore, we have applied behavioral economic principles to co-self-administration of EtOH and nicotine to describe the relationship between these drug reinforcers when they are concurrently available. The overall working hypothesis of this dissertation is that a single pharmacological treatment may be developed for the co-addiction to EtOH and nicotine using the novel co-use model developed in our laboratory.
CHAPTER TWO

2. Study 1: Effects of the nicotinic agonist varenicline, nicotinic antagonist r-bPiDI, and DAT inhibitor R-modafinil on co-use of ethanol and nicotine in female P rats.

2.1. Introduction

As previously discussed, animal models have been utilized successfully to model and test treatments for either EtOH or nicotine use disorders separately (Van Skike et al., 2016), as well as for other SUDs (Koob et al., 2009), but relatively few studies have examined voluntary co-use of EtOH and nicotine in the same animal (Bell et al., 2016; W. J. McBride et al., 2014). Critical for testing pharmacotherapeutics for EtOH and nicotine co-use disorder, is developing a translational animal model that produces pharmacologically relevant levels of concomitant voluntary oral EtOH consumption and i.v. nicotine self-administration.

Lê et al. (2010) developed a two-lever choice procedure for operant self-administration of EtOH and nicotine when both substances are available concurrently. Since that original report, variations of that procedure have been published, with each study showing that 2-lever choice will induce reliable EtOH and nicotine co-self-administration (Cippitelli et al., 2015; Funk et al., 2016; Scuppa et al., 2015). In this general procedure, two levers are available concurrently during a limited access (60 min) session; responding on one lever delivers EtOH (12% solution, 0.19 mL per delivery into a drinking receptacle) and responding on the other lever delivers nicotine (0.03
mg/kg/infusion i.v.). The response requirement on each lever is typically a fixed ratio (FR) 3.

While the original two-lever choice procedure (Lê et al., 2010) produces reliable co-administration of both EtOH and nicotine, there are some limitations to the model in terms of its utility for preclinical screening of candidate medications for potential efficacy in decreasing co-use. If a candidate medication decreases lever pressing for both EtOH and nicotine simultaneously, it is difficult to determine if the drug is specifically decreasing the reinforcing effects of both EtOH and nicotine concurrently or is simply producing a non-specific suppression of ongoing responding. To address this limitation, the current study employed a novel model in which a two-bottle choice (EtOH vs water) was combined with two-lever procedure (active vs inactive for nicotine). The intended advantage of this modified procedure was to determine if potential pharmacotherapies will specifically decrease both EtOH drinking and nicotine self-administration, while leaving both water intake and inactive lever pressing unchanged.

For this novel model, we used selectively-bred P rats, a translational genetic model of alcoholism (R. L. Bell et al., 2012; W. J. McBride et al., 2014) to ensure pharmacologically relevant levels of EtOH consumption (Barkley-Levenson & Crabbe, 2014; R. L. Bell et al., 2012). Importantly, P rats also voluntarily consume not only intoxicating amounts of EtOH which meet the proposed criteria for an animal model of AUD (R. L. Bell et al., 2012; Cicero & Smithloff, 1973; Lester & Freed, 1973), but also readily self-administer i.v. nicotine in amounts twice that of non-preferring, Wistar, and Long-Evans rats (Lê et al., 2006; Rezvani et al., 2010). Additionally, female P rats have been shown to voluntarily consume higher amounts of EtOH than male P rats (Bell et al.,
Thus, female P rats may be especially advantageous for screening potential medications for EtOH and nicotine co-use.

Using this novel model in female P rats, we evaluated three drugs: (1) varenicline; (2) 1,10-bis(3-methyl-5,6-dihydropyridin-1(2H)-yl)decane dihydrochloride (r-bPiDI); and (3) 2-[(R)-(diphenylmethyl)sulfinyl]acetamide ((R)-modafinil, RMOD; see Figure 1). As previously discussed, varenicline has been demonstrated to reduce nicotine self-administration in animals (O. George et al., 2011; O’Connor et al., 2010; Rollema et al., 2007), reduce tobacco craving, withdrawal, and its reinforcing effects in humans (D. Gonzales et al., 2006; D. E. Jorenby, Hays, Rigotti, & et al., 2006; Sherry A. McKee et al., 2013), as well as increasing smoking abstinence rates (Ebbert et al., 2016a; D. Gonzales et al., 2006; Nides et al., 2006). However, effects of varenicline on EtOH consumption in laboratory animals (Feduccia et al., 2014; Froehlich et al., 2017; Funk et al., 2016; Steensland et al., 2007) and in humans (de Bejczy et al., 2015; Plebani et al., 2013; Schacht et al., 2014; Verplaetse et al., 2016) have been mixed, indicating that more work is needed, especially regarding EtOH and nicotine co-use. r-bPiDI, a potent and selective α6β2* nAChR antagonist, has also been shown to decrease nicotine-evoked dopamine release and nicotine self-administration (Beckmann et al., 2015a). However, it is not known if r-bPiDI alters EtOH self-administration, tested either alone or when combined with nicotine. RMOD, an atypical inhibitor of the DA transporter (DAT) without abuse liability, was selected based on a report demonstrating that RMOD attenuates nicotine self-administration, nicotine-induced reinstatement, and cue-induced nicotine-seeking in P rats (X.-F. Wang et al., 2015). However, it is not known if RMOD also alters EtOH self-administration, tested either alone or combined with nicotine. For
each drug, pretreatments were given during either the co-use phase (Experiment 1) or the EtOH only phase (Experiment 2).

2.2. Methods

**Animals:** Selectively bred female P rats (n=25, selectively bred generations 79-83) were obtained from Indiana University School of Medicine (provided by NIAAA/NIH) and began training between PND 55 to 65. Rats were housed individually upon arrival in a temperature-controlled colony room under a 12:12hr light/dark cycle. All testing procedures occurred during the light phase (7:00 am – 7:00 pm), were in accordance with the NIH Guide for the Care and Use of Laboratory Animals (8th edition, 2011), and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky. Some of the rats (n=5) used in this study had a brief experimental history prior to the current experiment that involved exposure to saccharin- or quinine-flavored water and acute injections of a novel drug unrelated to the drugs tested here. In Experiment 1 (total n=17), animals were trained in both voluntary oral EtOH consumption and i.v. nicotine self-administration and were given drug pretreatments during the co-use phase. In Experiment 2 (n=8), animals were only trained in voluntary oral EtOH consumption and then were given drug pretreatments with varenicline, r-bPiDI, and RMOD, with each animal receiving treatment with each dose of each drug such that all doses of one drug were given in a random order before moving to the next drug treatment. The order of drug treatment given was randomized.

**Drugs:** EtOH was prepared in a concentration of 15% v/v 190 proof EtOH (Pharmco-AAPER, Shelbyville, KY) diluted in distilled water. Nicotine hydrogen tartrate (Sigma-Aldrich, San Diego, CA) was dissolved in a 0.9% NaCl (saline) solution, to
which NaOH was added to obtain a pH of 7.0 ± 0.05; nicotine dosage was based on freebase weight. Varenicline (6,7,8,9-tetrahydro-6,10-methano-6H pyrazino[2,3-h][3]benzazepine tartrate), a generous donation from the National Institute on Drug Abuse (NIDA) (Bethesda, MD), was dissolved in saline. r-bPiDI (1,10-\textit{bis}(3-methyl-5,6-dihydropyridin-1(2H)-yl)decane) was synthesized at the University of Arkansas for Medical Sciences (Little Rock, AK) and dissolved in saline. RMOD was synthesized at the National Institute on Drug Abuse-Intramural Research Program, Medicinal Chemistry Section (Baltimore, MD) and dissolved in sterile water containing 10% DMSO and 15% Tween-80. All test drug solutions were prepared immediately before each injection and administered i.p. 15 min prior to the start of the session, with doses based on formula weights. For surgery, rats were anesthetized via i.p. injections of 55/7.5/7.5 mg/kg ketamine (Henry Schein Animal Health, Dublin, OH)/xylazine (LLOYD Laboratories, Shenandoah, IA)/sterile water. Respective drug doses were determined from the literature (e.g., Beckmann et al. 2015; George et al. 2011; Wang et al. 2015).

\textbf{Apparatus:} All training and testing sessions were conducted in standard two-lever operant chambers (ENV-001; MED Associates, St. Albans, VT). Two response levers were located on the same wall of the operant chamber on either side of a recessed food tray. Located above each lever was a white cue light. Nicotine infusions were delivered by a syringe pump and food pellets were delivered by a pellet dispenser. A computer, linked to a MED Associates interface, recorded responses and controlled infusions during the experimental session. Each chamber was modified to allow access to two 100 ml Richter feeding tube glass bottles (Model 900010; Dyets, Inc., Bethlehem, PA) on the wall of the chamber opposite the levers. The design of the bottles allowed them to be
fixed securely to the outside of the chambers with lipped feeding tube holders (Model 901100; Dyets, Inc., Bethlehem, PA) such that only the drinking spout could be accessed by rats while inside the chambers (see Fig. 2).

2.3. Procedures

2.3.1. Experiment 1: Drug Pretreatments During Co-use of EtOH and Nicotine (Phase 3)

A timeline for training is shown in Appendix A.

EtOH Acclimation (pre-training): To allow for acclimation to the taste and smell of EtOH, rats were given one bottle of 20% EtOH as the sole source of liquid for 72 consecutive hours in the home cage (Simms, Bito-Onon, Chatterjee, & Bartlett, 2010). During this time, food was available ad libitum.

EtOH Access (Phase 1):

In Phase 1, rats began daily 60-min two-bottle choice sessions by being placed in the operant conditioning chambers with the levers retracted. For the duration of each session, rats were given free access to two bottles, one bottle of water and one bottle of 15% EtOH (v/v). Both bottles were presented on the same wall of the chamber, one on the left side and the other on the right, with the position of solutions alternating daily. During these sessions, rats could drink freely from both bottles. Access to water and EtOH were restricted to these daily 1-hr sessions.

EtOH and water consumption were measured by weighing each bottle immediately prior to and immediately after access sessions to determine differences in weight. Bottles were fixed to the side of the operant chamber to minimize spillage, but some spillage may have occurred. On the tenth day of EtOH access, BEC was determined
at a single time point (90 min after the start of the session) from plasma derived from tail blood. BEC was measured by a GM7 Analyser (Analox, London, UK). Animals were trained in this phase for at least 10 days until the average EtOH consumption stabilized, i.e. there were no significant differences in average consumption across 4 sessions (~14-20 days). Prior to the next phase, animals were placed back on free access to water in the home cage.

**Nicotine Access (Phase 2):** Animals were trained to lever press for i.v. injections of nicotine (0.03 mg/kg/infusion) using the general methods previously described (Bardo, Green, Crooks, & Dwoskin, 1999; Corrigall & Coen, 1989). At the beginning of Phase 2, rats were restricted to 8-12 g of food/day in the home cage until the completion of lever press training for food pellets. In the operant conditioning chambers, rats were initially trained to acquire lever pressing for palatable food pellets (45 mg Dustless Precision Pellets, Bio-Serv, Frenchtown, NJ). Responses on one lever (active lever, counterbalanced for position across rats) resulted in illumination of a cue light located directly above the lever and the delivery of one food pellet into the receptacle; responding on the other lever (inactive) had no programmed consequence. The cue light signaling the delivery of the food pellet remained illuminated for an additional 20-sec time-out (TO) period after pellet delivery; responding on either lever during this TO period had no programmed consequence. Response requirements for food pellet delivery increased under an incrementing FR schedule, beginning with FR1 (3 sessions), followed by FR3 (3 sessions), and then FR5, where it remained until responding for food stabilized (at least 5 consecutive sessions with no significant differences in responding between
sessions and at least two times more responding on the active lever vs inactive lever; ~14 days).

Prior to surgery, rats were given 24-hr ad libitum access to food and water in the home cage. Rats then underwent surgery under anesthesia to implant a chronic indwelling catheter into the jugular vein; catheters were flushed daily with heparinized saline to maintain patency. Following surgery, rats were given 5-7 days of recovery with ad libitum access to food and water in the home cage. Following the recovery period, rats were trained to self-administer nicotine (0.03 mg/kg/infusion i.v.) using a 2-lever procedure. During this time, rats were given 12-15 g of food/day in the home cage.

Similar to the food training procedure, during 60-min daily sessions in the operant chambers, responses on one lever (active lever, counterbalanced for position across rats) resulted in illumination of a cue light located directly above the lever and an infusion of 0.03 mg/kg nicotine (0.1 mL over 5.9 sec); responding on the other lever (inactive) had no programmed consequence. The cue light signaling the nicotine infusion remained illuminated for an additional 20-sec TO period after termination of the infusion; responding on either lever during this TO period had no programmed consequence. The FR-rate was increased incrementally and stabilized at FR5, such that the animals showed consistent FR5 responding across at least 3 consecutive sessions (~14 days), after which rats were moved to the concurrent access phase.

For one group of animals (n = 12), bottles were removed from the operant chambers throughout Phase 2; water (no EtOH) was available continuously in the home cage. For the remaining animals (n = 5), access to both water and EtOH bottles in the operant conditioning chambers continued throughout Phase 2. This procedural variation
had no effect on intake of EtOH, water, or nicotine when stable responding was reached in the final phase of the experiment (Phase 3), as described below. Stability in responding was defined as no significant difference in average EtOH consumption, water consumption, or nicotine intake across 5 sessions.

**Concurrent Access (Phase 3):** During Phase 3, the FR5 schedule for nicotine infusions remained as described in Phase 2, while access to 15% EtOH and water bottles (alternated daily between the left and right side of the chamber wall) was returned to the operant sessions (for the animals that underwent Phase 2 without bottle access (n = 12); see above). During Phase 3, water again was removed from the home cage as in Phase 1, and food provision (12-15 g per day) continued as in Phase 2. Each rat underwent at least 10 consecutive training sessions (with no significant differences in average daily EtOH consumption, water consumption, or nicotine intake across 5 sessions; ~10 days) in this phase prior to beginning drug pretreatment testing.

**2.3.2. Drug Pretreatments**

For animals in Experiment 1, drug pretreatments began after operant responding and drinking stabilized in Phase 3 (after at least 10 sessions and with no significant differences in average daily EtOH consumption, water consumption, or nicotine intake across 5 sessions; ~14-20 days). Each drug was prepared fresh prior to administration and was given 15 minutes prior to the start of the testing session. For varenicline, the test dose was 3 mg/kg; for r-bPiDI, the test doses were 10 or 20 mg/kg; and for RMOD, the test doses were 30, 56 or 100 mg/kg. Each drug dose was given in counterbalanced order, including the appropriate vehicle control. A minimum of 2 maintenance sessions (no pretreatment) separated each dose or drug pretreatment test session.
2.4. Experiment 2: Drug Pretreatments During Use of EtOH Only (Phase 1)

Animals in this experiment were trained under the same procedures described for EtOH acclimation (pre-training) and EtOH access (Phase 1) (see Appendix A). Drug pretreatments began on day 15 of Phase 1. After EtOH consumption stabilized (after at least 10 sessions and with no significant differences in average daily EtOH or water consumption across 5 consecutive sessions; 15 days), drug treatments began using the same doses and procedure as described in Experiment 1.

2.4.1. Data Analysis

For both experiments, consumption from the EtOH and water bottles were measured in g per kg body weight. For EtOH, the weight of liquid consumed during each session was converted to g of EtOH by multiplying the specific gravity of EtOH by the concentration of EtOH used (15% v/v/kg body weight/session). For Experiment 1, active and inactive lever presses for nicotine infusions were recorded by the automated system used to operate the operant chambers (ENV-001; MED Associates, St. Albans, VT). Consumption differences of EtOH and nicotine, averaged across the last 3 days of each experiment phase, were analyzed by two-tailed t-tests. A Pearson correlation analysis was used to analyze the association between EtOH intake and nicotine infusions.

Effects of varenicline on EtOH and water consumption and on lever presses for nicotine (active vs inactive) earned during concurrent access sessions, were analyzed by two-tailed paired t-test analyses (vehicle vs drug for each operant lever). Effects of r-bPiDI and RMOD on EtOH and water consumption (Experiments 1 and 2), and lever presses for nicotine (active vs inactive) earned during concurrent access sessions (Experiment 1), were analyzed by one-way, repeated-measures ANOVA. Post hoc
analyses using Dunnett’s test comparing each dose against the vehicle control (α = 0.05), were conducted when appropriate. All statistical analyses were conducted using Prism 5.0 (Graph Pad Software Inc., San Diego, CA). Within-session effects of varenicline, r-bPiDI, and RMOD on the number of nicotine infusions earned across 10-min intervals were analyzed by mixed model ANOVAs and post hoc analyses were conducted using subsequent Bonferroni posttests where appropriate (α = 0.05).

2.5. Results

2.5.1. Experiment 1: Pretreatments During Co-Use of EtOH and Nicotine

Acquisition across sessions for baseline levels of EtOH consumption, water consumption, active lever presses and inactive lever presses for nicotine across Phases 1-3 are shown in Figure 3. In Phase 1 (EtOH alone), both EtOH and water consumption gradually increased across the sessions (Figs 3A and 3C); for EtOH $F(13, 312) = 3.92, p < 0.05$ and for water $F(13, 312) = 4.19, p < 0.05$. However, both EtOH and water consumption eventually stabilized, as there were no significant differences in consumption across the last 4 sessions. In Phase 2 (nicotine alone), active lever pressing increased as the FR requirement increased and became stable across the last three FR5 sessions of this phase (Fig 3C); across all sessions $F(10, 131) = 51.72, p < 0.05$. In Phase 3 (EtOH + nicotine), EtOH consumption increased across sessions, $F(13, 237) = 2.10, p < 0.05$ (Figure 3B), while water consumption, active lever presses and inactive lever presses did not change significantly across sessions (Fig 3D and 3F).

A direct comparison of the average baseline levels of EtOH consumption, water consumption, active lever presses and inactive lever presses collapsed across the last 3 sessions within each of the Phases 1-3 are shown in Figure 4. Results from a two-tailed
paired t-test showed that there was a significant decrease in EtOH consumption between Phase 1 (EtOH alone) to Phase 3 (EtOH + nicotine); \( t(2) = 10.25, \ p < 0.05 \) (Fig 4A).

Similarly, there was a significant decrease in water consumption from Phase 1 to Phase 3; \( t(2) = 5.29, \ p < 0.05 \) (Fig 4B). In contrast, there was a significant increase in nicotine infusions from Phase 2 (nicotine alone) to Phase 3 (EtOH + nicotine); \( t(2) = 7.09, \ p < 0.05 \) (Fig 4C). Additionally, across individual rats, there was a significant negative correlation between EtOH consumption and nicotine infusions during Phase 3, \( r = -0.58, \ p < 0.05 \) (see Figure 5).

**Blood EtOH Concentrations.** Figure 6 shows BECs in Phases 1 and 3. Consistent with the decrease in EtOH consumption, there was a significant decrease in BEC in Phase 3 (EtOH + nicotine) versus Phase 1 (EtOH alone); \( t(23) = 4.63, \ p < 0.01 \).

**Effect of Varenicline Pretreatment.** Figure 7 shows EtOH consumption, water consumption, active lever presses for nicotine, and inactive lever presses after varenicline pretreatment in Phase 3 (n = 5). Analyses for Phase 3 revealed that varenicline had no significant effect on EtOH consumption (Fig 7A). While water consumption was increased slightly by varenicline, this effect was not statistically significant (Fig 7B). In contrast to liquid consumption, the number of active lever presses for nicotine was significantly reduced by varenicline (3 mg/kg) compared to vehicle; \( t(4) = 2.83, \ p < 0.05 \) (Fig 7C). However, varenicline did not significantly alter the number of inactive lever presses (Fig 7D).

**Effect r-bPiDI Pretreatment.** Figure 8 shows EtOH consumption, water consumption, active lever presses for nicotine, and inactive lever presses after r-bPiDI pretreatment in Phase 3 (n = 17). Analyses revealed there was no significant effect of r-
bPiDI on EtOH consumption or inactive lever presses at either dose (Figs 8A and 8D). However, there was a significant effect of r-bPiDI pretreatment on water consumption, $F(2,16) = 5.37, p < 0.05$ (Fig 8B). Subsequent Dunnett’s tests revealed that water consumption significantly increased at 20 mg/kg r-bPiDI compared to vehicle control. Analyses also revealed a significant effect of r-bPiDI treatment on active lever presses, $F(2,16) = 4.28, p < 0.05$ (Fig 8C). Subsequent Dunnett’s tests revealed that active lever presses for nicotine significantly decreased at 20 mg/kg r-bPiDI compared to vehicle control.

\textit{Effect of RMOD Pretreatment.} Figure 9 shows EtOH consumption, water consumption, active lever presses for nicotine, and inactive lever presses after RMOD pretreatment in Phase 3 ($n = 5$). Analyses revealed no significant effect of RMOD on either EtOH or water consumption (Figs 9A and 9B), and there was no significant effect of RMOD on inactive lever presses (Fig 9D). Analyses revealed a significant effect of RMOD on active lever presses, $F(3, 12) = 4.77, p < 0.05$ (Fig 9C), with a subsequent Dunnett’s test showing a significant decrease in active lever presses following 100 mg/kg RMOD compared to vehicle.

\textit{Within-session Nicotine SA.} Figure 10 shows the number of active lever presses for nicotine in 10-min intervals during the concurrent access phase (Phase 3). Analyses for varenicline reveal a significant main effect for dose, $F(1, 8) = 7.80, p < 0.05$, and a significant interaction, $F(5, 8) = 8.18, p < 0.05$. Subsequent Bonferroni posttests revealed a significant difference in responding between vehicle and 3.0 mg/kg varenicline during the first 10-min interval, $t(40) = 6.14, p < 0.05$ (Fig 10A). Analyses for r-bPiDI reveal a significant main effect for interval, $F(5, 48) = 28.93, p < 0.05$, with no significance
shown by the Bonferroni posttest (Fig 10B). Analyses for RMOD show a significant main effect for dose, $F(3, 35) = 4.75, p < 0.05$, a significant main effect for interval, $F(5, 35) = 3.18, p < 0.05$, and a significant interaction, $F(15, 35) = 1.72, p < 0.05$. Subsequent Bonferroni posttest revealed significant differences in responding during the first 10-min interval for 30, 56, and 100 mg/kg RMOD ($t(175) = 2.71, p < 0.05$; $t(175) = 2.94, p < 0.05$; $t(175) = 5.18, p < 0.05$, respectively) compared to vehicle. Additionally, analyses revealed a significant difference in responding for nicotine at 100 mg/kg RMOD during the 20-min interval compared to vehicle, $t(175) = 3.02, p < 0.05$ (Fig 10C). As there was no automated system to keep track of EtOH consumption rates, we are unable to provide this within-session data for EtOH.

2.5.2. Experiment 2: Pretreatments During Use of EtOH Only

Since EtOH use was low during the co-use phase (Experiment 1), this experiment tested the same drug pretreatments during Phase 1, when use of EtOH was relatively higher. Figure 11 shows EtOH consumption and water consumption for Experiment 2 (n=8) following drug pretreatments. Analyses revealed that varenicline (3 mg/kg) had no significant effect on EtOH consumption (Fig 11A). However, water consumption was significantly increased at this dose, $t(7) = 4.30, p < 0.05$ (Fig 11B). Analyses also revealed that r-bPiDI had no significant effect on EtOH consumption or water consumption (Figs 11C and 11D). For RMOD pretreatment, analyses revealed a significant effect of RMOD on EtOH consumption, $F(3, 21) = 14.97, p < 0.05$ (Fig 11E), with subsequent Dunnett’s test showing a significant decrease in EtOH consumption following 100 mg/kg RMOD compared to vehicle. There was no effect of any dose of RMOD on water consumption (Fig 11F)
2.6. Discussion

The current study used a novel model of EtOH and nicotine co-use in female P rats to assess the effects of varenicline, r-bPiDI, and RMOD on co-use behavior. In this study, the 2-bottle and 2-lever choice model allowed access to both EtOH and nicotine concurrently. However, in contrast to a previous co-use model (Lê et al., 2010), the current model also measured choice for a natural reward (water), as well as non-reinforced operant behavior (inactive lever pressing), thus allowing for assessment of nonspecific changes in ongoing behavior following pharmacotherapeutic pretreatments. With this new model, abuse-relevant levels of EtOH and nicotine intake were achieved when each substance was given alone. However, during the co-use phase (Phase 3), nicotine intake increased and EtOH intake decreased relative to the intake of each substance alone, consistent with results from previous co-use models (Funk et al., 2016; Scuppa et al., 2015).

When EtOH was given alone (Phase 1), rats voluntarily consumed ~1.6 g/kg/hr, which is comparable to binge drinking 5-6 standard alcoholic beverages for humans (Grant & Bennett, 2003; S. A. McKee, O'Malley, Shi, Mase, & Krishnan-Sarin, 2008; Udo, Harrison, Shi, Tetrault, & McKee, 2013). This amount of EtOH consumption produced a BEC of ~85 mg/dL paralleling our previous work (Bell et al., 2011), just above the definition of intoxication to impairment (National Institute on Alcohol Abuse and Alcoholism, 2017). Additionally, the P rats used in this study were selectively bred, generations 79-83. Results from previous research show that adult female P rats from generations 66-69 also consume approximately 1.6 g/kg/hr of EtOH (Bell et al. 2011), indicating consistent levels of EtOH drinking across a range of P rat generations. As one
note of caution, however, even though EtOH consumption and BECs were correlated, we cannot rule out that we overestimated the amount of EtOH consumed as a result of some spillage. In this regard, the model may be improved by using lickometers.

For nicotine, rats in the current study show rates of nicotine self-administration during both the nicotine access phase (~19 infusions of 0.03 mg/kg iv nicotine) and the concurrent access phase (~22 infusions of 0.03 mg/kg iv nicotine) that are comparable to those seen in a previous study by Lê et al. (2006), showing nicotine self-administration rates of ~20-25 infusions of 0.03 mg/kg iv nicotine in 44th generation selectively bred P rats. For nicotine, substantial intake is considered to be ~0.5 mg/kg/hr i.v. nicotine (16 infusions of 0.03 mg/kg nicotine), as this amount of intake is sufficient to produce pharmacologically relevant levels of nicotine and cotinine in plasma (Corrigall, 1992; Corrigall & Coen, 1989; Shoaib & Stolerman, 1992). The steady-state-peak plasma levels of nicotine seen in humans are on average 40 ng/mL (Hiroshi Yamazaki et al., 2010). In rats, it has been determined that 0.5 mg/kg/hr of i.v. nicotine results in ~65.4 ng/mL of plasma nicotine (Shoaib & Stolerman, 1992). Rats in the current study averaged ~0.65 mg/kg/hr of i.v. nicotine, enough to exceed pharmacologically relevant levels of plasma nicotine. Taken together, these results show that there is consistency in EtOH and nicotine intake across a wide range of selectively bred P rats, providing support for using P rats in the development of a translational model of EtOH and nicotine co-use.

One limitation to the co-use model described here relates to the relatively low EtOH consumption during the concurrent access phase (Phase 3). In contrast to the drinking levels during the EtOH access phase (Phase 1), when EtOH and nicotine were given concurrently (Phase 3), EtOH consumption was only ~0.5 g/kg/hr, which achieved
an average BEC of only ~25 mg/dL. This amount is comparable to humans drinking approximately 1-2 standard alcoholic beverages/hr (Grant & Bennett, 2003; S. A. McKee et al., 2008; Udo et al., 2013). Nonetheless, the decrease in EtOH intake in the presence of nicotine availability is consistent with previous concurrent access studies (Funk et al., 2016; Scuppa et al., 2015). Interestingly, however, the decrease in EtOH consumption in the presence of nicotine in the current study was greater than that observed previously (Funk et al. 2016; Scuppa et al. 2015). This may reflect a difference between P rats (current study) versus Marchigian Sardinian alcohol-preferring (msP) and Wistar rats (previous studies). Alternatively, in contrast to those previous studies that used a two-lever choice procedure (EtOH vs. nicotine), the current study included water, a natural reinforcer. Based on the notion of response competition among multiple drug and non-drug reinforcers (M. E. Carroll, Carmona, & May, 1991), the inclusion of both nicotine and water as alternative reinforcers may have been responsible for the greater suppression of EtOH consumption observed here. However, response competition is unlikely a complete explanation because EtOH consumption was decreased by lever pressing for nicotine, but not for food (self-administration pretraining phase).

The current results also show a significant increase in nicotine intake when EtOH is concurrently available. While this finding is consistent with clinical evidence that EtOH increases cigarette smoking (Henningfield, Chait, & Griffiths, 1984; S. A. McKee et al., 2009; N. K. Mello, Mendelson, Sellers, & Kuehnle, 1980), it conflicts with preclinical studies showing a decrease in nicotine self-administration when EtOH is available concurrently (Funk et al., 2016; Scuppa et al., 2015). In contrast to those previous preclinical studies, however, it is notable that the rate of nicotine self-
administration in the absence of EtOH in the current study was higher than the rates reported by Scuppa et al. (2015) and Funk et al. (2016). These differences across studies could reflect, at least in part, strain and/or sex differences because the current study used female P rats, whereas the study by Scuppa et al. (2015) used selective bred male msP rats and the study by Funk et al. (2016) used outbred male Wistar rats. Previous work has shown that P rats self-administer nicotine at higher rates than Wistar rats (Lê et al., 2006) and that females acquire nicotine self-administration and show more motivation for nicotine compared to males (Donny et al., 2000). Thus, the EtOH-induced increase in nicotine self-administration observed here may be unique to female P rats when compared to other breeds. Importantly, as previously stated, EtOH-induced increases in nicotine intake have been observed in humans (Henningfield et al., 1984; S. A. McKee et al., 2009; N. K. Mello et al., 1980), which suggests that female P rats display characteristics that more closely mimic the human condition, making them a better model for human behavior.

When tested during the co-use phase, varenicline specifically decreased nicotine-reinforced lever pressing, without altering inactive lever pressing, EtOH consumption or water consumption. Although several studies have demonstrated that varenicline reduces EtOH consumption in humans (Falk et al., 2015; Litten et al., 2013; Sherry A. McKee et al., 2013), conflicting evidence for the effects of varenicline on EtOH craving and consumption has been reported (de Bejczy et al., 2015; Schacht et al., 2014; Verplaetse et al., 2016). In conflict with the results of the current study, several preclinical studies have also demonstrated that pretreatment with varenicline reduces EtOH consumption in rodents (Froehlich et al., 2017; H. M. Kamens et al., 2010; Sotomayor-Zarate et al., 2013;
Steensland et al., 2007) when EtOH was available in the absence of nicotine. This may be explained by the different breeds used by Kamens et al. (2010), Sotomayor-Zarate et al. (2013), and Steensland et al. (2007). Additionally, in the study by Froehlich et al. (2017), EtOH consumption in male P rats (78th generation) was reduced by varenicline. However, these pretreatments occurred daily for 5 days, while the pretreatments in the current study were only given to females on one day. It is possible that the differences seen in the current study were due to the acute administration vs chronic administration of varenicline.

Furthermore, in preclinical examinations of co-use of EtOH and nicotine, there is conflicting evidence of the effectiveness of varenicline in reducing EtOH consumption in rodents (Funk et al., 2016; Randall et al., 2015; Scuppa et al., 2015). Among the three preclinical studies that have examined the effects of varenicline on EtOH and nicotine co-use, only one study reported that varenicline significantly decreased both nicotine and EtOH self-administration during concurrent access (Cippitelli et al., 2015). In contrast, the two other studies showed that varenicline decreased nicotine self-administration, but not EtOH self-administration (Funk et al., 2016; Scuppa et al., 2015), a finding that is corroborated by the current results. Thus, on balance, while it is possible that EtOH consumption levels were too low to detect significant effects of pretreatment, preclinical evidence to date does not support the utility of varenicline as a pharmacotherapeutic for heavy drinking tobacco smokers.

One caveat to this experiment is that only one dose of varenicline (3.0 mg/kg) was tested. While Cippitelli et al. (2015) found that varenicline reduced EtOH consumption at 1.5 mg/kg, Scuppa et al. (2015) found that a higher dose of 3 mg/kg had no effect on
EtOH consumption and results from Funk et al. (2016) show that 3 mg/kg varenicline reduced both EtOH consumption and food intake. These results indicate that perhaps a lower dose of varenicline could be more effective for reducing EtOH consumption in co-use models, but more research is needed.

When tested in the co-use phase, like varenicline, r-bPiDI decreased nicotine SA, but not EtOH consumption. The decrease in nicotine self-administration was expected based on the findings of a previous report (Beckmann et al. 2015). These results, in combination with previous investigations of the neuropharmacology of r-bPiDI, suggest that α6β2* nAChRs play an important role in the maintenance of nicotine intake, but not EtOH intake. Additionally, the decrease in nicotine-evoked DA release produced by r-bPiDI is likely to contribute, at least in part, to the decrease in nicotine self-administration produced by r-bPiDI (Beckmann et al. 2015). While other less selective nAChR antagonists, such as mecamylamine, decrease nicotine self-administration, cue-induced reinstatement, and nicotine-seeking behavior in animals (DeNoble & Mele, 2006; Glick, Visker, & Maisonneuve, 1996) and in humans when combined with a transdermal nicotine patch (Jed E Rose, 2006; Rose, 2008; Jed E Rose, Frederique M Behm, Eric C Westman, Edward D Levin, Roy M Stein, & Gail V Ripka, 1994), they can also produce aversive peripheral side effects, which limits their success in clinical trials (Bevins & Caggiula, 2009; Shytle, Penny, Silver, Goldman, & Sanberg, 2002). The selectivity of r-bPiDI for central α6β2* nAChRs may eliminate problems with peripheral side effects seen with previously tested nAChR antagonists, but further research is needed.
The failure of r-bPiDI to decrease EtOH consumption in the current study is inconsistent with a study by Jirawoot Srisontiyakul, Hanna E. Kastman, Elena V. Krstew, Piyarat Govitrapong, and Andrew J. Lawrence (2016). Importantly, however, the study by Srisontiyakul et al. used the quaternary ammonium bPiDI (N,N-decane-1,10-diyl-bis-3-picolinium diiodide), whereas the current study used the neutral chemically reduced, tertiary amino derivative of bPiDI (i.e., r-bPiDI) which presumably allows for greater bioavailability and blood-brain barrier penetration. These findings suggest that while α6β2* nAChRs may be involved in both nicotine and EtOH maintenance, it is possible that there are important pharmacokinetic and/or pharmacodynamic differences between the actions of bPiDI and r-bPiDI affecting these behaviors. Unfortunately, beyond the current study, there are no other studies on the effects of these compounds on EtOH and nicotine co-use.

Further, when tested in the co-use phase, like varenicline and r-bPiDI, RMOD decreased nicotine self-administration, but not EtOH consumption. This finding is consistent with previous research showing that RMOD decreases nicotine self-administration using P rats (X.-F. Wang et al., 2015). These effects are likely due, at least in part, to RMOD binding to DAT and thereby preventing nicotine-induced dopamine release in Acb (X.-F. Wang et al., 2015), an action that also is seen with r-bPiDI (Beckmann et al., 2015b). Further examination of RMOD using electrophysiology has shown slowed dopamine neuron firing in a dopamine D2 receptor-dependent manner, which may also relate to its nicotine self-administration decreasing effects (Avelar, Cao, Newman, & Beckstead, 2017). Taken together, these results suggest that nicotine-induced dopamine release plays an important role in the maintenance of nicotine self-
administration and that blocking this dopamine release is an effective method for
reducing nicotine reinforcement. Clearly, pharmacotherapeutic agents that act to block
nicotine-induced dopamine release merit further exploration as potential smoking
cessation treatments.

To our knowledge, there have been no other investigations of the effects of
RMOD on EtOH intake. We hypothesized that the atypical DAT blocker RMOD would
decrease EtOH intake based on previous findings demonstrating that EtOH potentiates
DAT function and increases DAT expression in cell-based models (Mayfield, Maiya,
Keller, & Zahnisier, 2001; Methner & Mayfield, 2010; Riherd, Galindo, Krause, &
Mayfield, 2008). Additionally, work with rats selectively bred for high EtOH
consumption has revealed that chronic EtOH intake increases dopamine reuptake in Acb
of both P (Sahr, Thielen, Lumeng, Li, & McBride, 2004) and high alcohol-drinking
HAD1 rats (M. R. Carroll, Rodd, Murphy, & Simon, 2006). Consistent with our
hypothesis, RMOD decreased EtOH intake during Phase 1 when nicotine access was not
available, however, RMOD failed to decrease EtOH consumption when both EtOH and
nicotine were available concurrently. This suggests that this DAT modulator may not be a
viable pharmacotherapy to treat co-use of EtOH and nicotine, but it may be a viable
option for treatment EtOH use in individuals who are not smokers. Alternatively, results
from the triple monoamine uptake inhibitor, amitifadine, show robust decreases in both
nicotine self-administration (Levin et al., 2015) and EtOH self-administration or drinking
(O'Tousa et al., 2015; Warnock et al., 2012) when tested separately. It remains to be
determined if blockade of multiple monoamine transporters would be effective in an
EtOH and nicotine co-use model.
Given that concurrent access makes it difficult to disentangle the time-course that each reinforcer is self-administered, it may be that EtOH consumption was initiated before nicotine self-administration, which would be consistent with previous research and descriptions of EtOH “loading” at the beginning of EtOH self-administration sessions (Williams & Broadbridge, 2009). Subsequently, the rats may have focused on the more salient interoceptive cues of intravenous nicotine relative to EtOH consumption. Conversely, it may be that EtOH consumption and/or its cues enhanced nicotine self-administration. This latter hypothesis has some support from the literature, such that co-administration of EtOH and nicotine produces an additive effect on their reinforcing effects and associated dopamine release in nucleus accumbens (Acb) (Ericson, Lof, Stomberg, & Soderpalm, 2009; Sajja, Dwivedi, & Rahman, 2010; Sajja & Rahman, 2012; Tizabi et al., 2007).

Furthermore, results from within-session interval data in the current study show that following pretreatment with vehicle, lever pressing for nicotine is highest during the first 10-min interval and decreases to a steady level for the rest of the 1-hr session. Interestingly, lever pressing for nicotine was significantly decreased during the first 10-min interval following pretreatment with varenicline (3 mg/kg) and RMOD (30, 56, and 100 mg/kg), with suppression of responding for nicotine continuing into the 20-min interval for only the highest dose of RMOD. These results show that overall decreases in nicotine intake are primarily due to the decreases in responding during the first 10-min interval of the session.

In summary, toward the development of a preclinical model for screening potential pharmacotherapies for EtOH and nicotine co-use, the novel model used here
offers the advantage of including control for nonspecific suppression of behavior (i.e., water consumption and non-reinforced lever pressing). However, relatively low levels of EtOH consumption were obtained during the EtOH and nicotine co-use phase. With the drugs tested in this model, varenicline, r-bPiDI, and RMOD all reduced nicotine self-administration, but not EtOH consumption during the co-use phase, while producing no significant suppressant effect within the dose ranges tested. Interestingly, although RMOD did not decrease EtOH consumption during concurrent access, EtOH consumption was significantly decreased when EtOH was available alone. These results indicate that therapeutics which may be useful for smoking cessation via selective inhibition of α4β2 or α6β2* nAChRs, or DAT inhibition as afforded by the atypical inhibitor RMOD, may not be sufficient to treat EtOH and nicotine co-use. Further optimization of the current co-use model was beneficial for assessing novel medications that may be effective in treating tobacco smokers who are heavy drinkers.
Figure 1. Chemical structures of A) varenicline (6,7,8,9-tetrahydro-6,10-methano-6H pyrazino[2,3-h][3]benzazepine), B) r-bPiDI (1,10-bis(3-methyl-5,6-dihydropyridin-1(2H)-yl)decane), and C) RMOD, (R)-modafinil (2-[(R)- (diphenylmethyl)sulfinyl]acetamide)
Figure 2 Picture of the modified operant chamber apparatus used. Shows drinking tubes on the left, operant levers on the right, and the i.v. infusion line in the center.
**Figure 3** Results from Study 1, Experiment 1. Acquisition across sessions for A) EtOH consumption in Phase 1 (EtOH alone), B) EtOH consumption in Phase 3, C) water consumption in Phase 1 (EtOH alone), D) water consumption in Phase 3 (nicotine and EtOH), E) number of active and inactive lever presses for nicotine in Phase 2 and F) number of active vs. inactive lever presses for nicotine in Phase 3. Values represent mean±SEM.
**Figure 4** Results from Study 1, Experiment 1. Total intake across the access phases.

Graphs depict average intake differences for **A**) EtOH consumption in Phase 1 (EtOH alone) vs Phase 3 (EtOH and nicotine), **B**) water consumption in Phase 1 vs Phase 3, and **C**) number of infusions of nicotine in Phase 2 (nicotine alone) vs Phase 3. Values represent mean±SEM. *p < 0.05 vs Phase 1 or 2
Figure 5 Results from Study 1, Experiment 1. Scatter plot of individual rats showing a significant negative correlation between EtOH consumption and number of nicotine infusions during Phase 3 (EtOH and nicotine); $r = -0.58$, $p < 0.05$. 
Figure 6 Results from Study 1, Experiment 1. Blood EtOH concentration (BEC) in Phases 1 (EtOH alone) and 3 (EtOH and nicotine). Values represent mean±SEM BEC in mg/dL of tail blood. *p < 0.05 vs Phase 1
Figure 7 Results from Study 1, Experiment 1. Pretreatment with varenicline (0 and 3 mg/kg) in Phase 3 (EtOH and nicotine) (n = 5). Graphs depict the effects of varenicline on A) EtOH consumption, B) water consumption, C) number of active lever presses for nicotine, and D) number of inactive lever presses. Values represent mean±SEM. *p < 0.05 vs vehicle (0)
Figure 8 Results from Study 1, Experiment 1. Pretreatment with r-bPiDI (0, 10, and 20 mg/kg) in Phase 3 (EtOH and nicotine) (n = 17). Graphs depict the effects of r-bPiDI on A) EtOH consumption, B) water consumption, C) number of active lever presses for nicotine, and D) number of inactive lever presses. Values represent mean±SEM. *p < 0.05 vs vehicle (0)
Figure 9  Results from Study 1, Experiment 1. Pretreatment with RMOD (0, 30, and 56 mg/kg) in Phase 3 (EtOH and nicotine) (n = 5). Graphs depict the effects of RMOD on A) EtOH consumption, B) water consumption, C) number of active lever presses for nicotine, and D) number of inactive lever presses. Values represent mean±SEM. *p < 0.05 vs vehicle (0)
Figure 10 Results from Study 1, Experiment 1. Within-session nicotine self-administration as active lever presses per 10-min interval following pretreatments with
A) varenicline (0 and 3 mg/kg), B) r-bPiDI (0, 10, and 20 mg/kg), and C) RMOD (0, 30, 56, and 100 mg/kg). Values represent mean±SEM. *p < 0.05 vs vehicle (0)
Figure 11 Results from Study 1, Experiment 2. Pretreatment with varenicline (0 and 3 mg/kg), r-bPiDI (0, 10, and 20 mg/kg), and RMOD (0, 30, and 56 mg/kg) (n = 8). Graphs depict the effects of varenicline on A) EtOH consumption and B) water consumption, the effects of r-bPiDI on C) EtOH consumption and D) water consumption, and the effects of RMOD on E) EtOH consumption and F) water consumption. Values represent mean±SEM. *p < 0.05 vs vehicle (0)
3. **Study 2: An improved model of ethanol and nicotine co-use in female P rats:**

Effects of naltrexone, varenicline, and the selective nicotinic α6β2* antagonist r-bPiDI.

### 3.1. Introduction

Another recent study from our laboratory (Maggio, Saunders, Baxter, et al., 2018) used female P rats, a translational genetic model of AUD (R. L. Bell et al., 2012; W. J. McBride et al., 2014), to improve on our model of EtOH and nicotine co-use. That study used a two-bottle choice (EtOH vs water) procedure combined with a two-lever operant [active vs inactive for i.v. nicotine] procedure; nicotine was available on a FR5 operant schedule. Under those co-use conditions, we determined the effects of two potential pharmacotherapies that target nicotinic acetylcholine receptors (nAChRs), i.e., varenicline and r-bPiDI. Varenicline is a clinically available partial agonist with high affinity for α4β2* nAChRs that reduces nicotine self-administration in rats (O. George et al., 2011; Rollema et al., 2007) and increases smoking abstinence in humans (Ebbert, Croghan, Hurt, Schroeder, & Hays, 2016b; Nides et al., 2006). However, the effects of varenicline on EtOH consumption have been mixed in both laboratory animals (Hauser et al., 2017; Steensland et al., 2007) and humans (de Bejczy et al., 2015; Plebani et al., 2013; Schacht et al., 2014; Verplaetse et al., 2016). r-bPiDI is the reduced form of the potent and selective quaternary ammonium antagonist for α6β2* nAChRs, \(N,N'-\text{decane-1,10-diyl-bis-3-picolinium diiodide (bPiDI)}\), a compound that decreases both EtOH consumption (J. Srisontiyakul, H. E. Kastman, E. V. Krstew, P. Govitrapong, & A. J.
Lawrence, 2016) and nicotine self-administration (Wooters et al., 2011); it has physiochemical properties which confer greater brain penetration than bPiDI and also reduces nicotine self-administration (Beckmann et al., 2015a). However, one limitation of the study by Maggio et al. (2018) was that while the FR5 schedule maintained a high level of nicotine intake (>20 infusions of 0.03 mg/kg/infusion in 60 min), co-use of EtOH was relatively low (~0.5 g/kg in 60 min). Thus, the ability of varenicline and r-bPiDI to selectively decrease nicotine intake may have been due to the low consumption of EtOH in the co-use phase (i.e., floor effect) or the absence/limitations of neuroadaptations associated with chronic EtOH.

Thus, to mitigate this problem, during the co-use phase, the current study increased the “price” of nicotine by gradually increasing the FR requirement from an FR5 to FR30. We hypothesized that this change would increase EtOH consumption without markedly decreasing nicotine intake, thus allowing assessment of the effects of varenicline and r-bPiDI in our co-use model when intake of both substances is pharmacologically relevant. In addition to assessing the effects of varenicline and r-bPiDI, the current study also determined the effect of naltrexone on EtOH and nicotine co-use. Naltrexone is a clinically available mu-opioid receptor antagonist used to treat AUD (Heilig & Egli, 2006), but has also been examined as a treatment for TUD and co-use of EtOH and nicotine. Naltrexone has been demonstrated in preclinical studies to reduce EtOH intake in rats (Dhaher et al., 2012; Williams & Broadbridge, 2009) with a higher efficacy in rats exposed to both EtOH and nicotine (Lê, Funk, Lo, & Coen, 2014). Additionally, treatment with naltrexone reduces EtOH use in heavy drinking smokers, but not in non-smokers (Fridberg, Cao, Grant, & King, 2014; Fucito et al., 2012).
3.2. Methods

*Animals:* Female P rats (n=14, selectively bred generations 79-81) were obtained from Indiana University School of Medicine (provided by NIAAA/NIH) and began training between PND 50-60. Females were used because they voluntarily drink more EtOH compared to male P rats (Bell et al., 2011). Rats were housed individually in a temperature-controlled colony room under a 12:12 hr light/dark cycle. All testing procedures occurred during the light phase (7:00 am – 7:00 pm) and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals (8th edition, 2011), and were approved by the IACUC at the University of Kentucky.

*Drugs:* EtOH was prepared in a concentration of 15% v/v 190 proof EtOH (Pharmco-AAPER, Shelbyville, KY) and diluted in distilled water. Nicotine hydrogen tartrate (Sigma-Aldrich, San Diego, CA) was dissolved in a 0.9% NaCl (saline) solution, to which NaOH was added to obtain a pH of 7.0 ± 0.05; nicotine dosage was based on freebase weight. Naltrexone (5α)-17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-one) and varenicline (6,7,8,9-tetrahydro-6,10-methano-6H pyrazino[2,3-h][3]benzazepine tartrate), supplied by the National Institute on Drug Abuse (NIDA, Bethesda, MD), were dissolved in saline. r-bPiDI (1,10-*bis*(3-methyl-5,6-dihydropyridin-1(2H)-yl)decane) was synthesized at the University of Arkansas for Medical Sciences (Little Rock, AK) and dissolved in saline. All test drug solutions were prepared fresh daily and were administered s.c. 15 min prior to the start of the session, with doses based on formula weights. For surgery, rats were anesthetized via i.p. injections of 55/7.5/7.5 mg/kg ketamine (Henry Schein Animal Health, Dublin, OH)/xylazine (LLOYD Laboratories, Shenandoah, IA)/sterile water.
**Apparatus:** All training and testing sessions were conducted in standard two-lever operant conditioning chambers (ENV-001; MED Associates, St. Albans VT). Two response levers were located on the same wall of the chamber on either side of a recessed food tray. Located above each lever was a white cue light. Nicotine infusions were delivered by a syringe pump and food pellets were delivered by a pellet dispenser. A computer, linked to a MED Associates interface, recorded responses and controlled infusions during experimental sessions. Each chamber was modified to allow access to two 100 mL Richter feeding tube glass bottles (Model 900010; Dyets, Inc., Bethlehem PA) on the wall of the chamber opposite the levers. The design of the bottles allowed them to be fixed to the chambers with lipped feeding tube holders (Model 901100; Dyets, Inc., Bethlehem, PA) such that only the drinking spout could be accessed by rats while inside the chambers (see Figure 2).

### 3.2.1. Procedures

A timeline for training procedures is shown in Appendix B. Pre-training and EtOH access (Phase 1) was conducted using procedures similar to those described by Maggio et al. (2018). Briefly, during pre-training, to allow for acclimation to the taste and smell of EtOH, rats were given one bottle of 20% EtOH as the sole source of liquid for 72 consecutive hours in the home cage (Simms et al., 2010); food was available *ad libitum*. Following pre-training, rats were trained during daily 60-min sessions in which rats were given free-choice access to two bottles in the operant chamber; one bottle contained water and the other bottle contained 15% EtOH (v/v), counterbalanced for side daily. Animals were trained in this phase for at least 15 days until the average EtOH consumption stabilized, i.e., there were no significant differences in average consumption
across 5 consecutive sessions (*Mean* = 20 days). After stable EtOH drinking was achieved, one group of animals (n=6) was pretreated with naltrexone (Experiment 1); results evaluating varenicline and r-bPiDI were reported previously (Maggio, Saunders, Baxter, et al., 2018). A second group of animals (n=8) advanced to training for Experiment 2 (concurrent access, Phase 2) without drug pretreatment during Phase 1. In Experiment 2, naltrexone, varenicline and r-bPiDI were each tested separately using a within-subject design.

During the concurrent access phase (Phase 2; Experiment 2), animals were first trained to acquire lever pressing for palatable food pellets (45 mg Dustless Precision Pellets, Bio-Serv, Frenchtown NJ) using the general methods described previously (Maggio, Saunders, Baxter, et al., 2018), with some modifications. Rats were trained to lever press for food pellets using a standard 2-lever operant procedure (active vs inactive levers) with 2-bottle choice for EtOH (0% vs 15%) concurrently available during sessions. Rats then underwent surgery under anesthesia to implant a chronic indwelling catheter into the jugular vein, followed by 5-7 days of recovery with *ad libitum* access to food, water, and one bottle of 15% EtOH in the home cage.

Following the recovery period, rats were trained to self-administer nicotine (0.03 mg/kg/infusion, with a 20-sec time-out period following each infusion) using a 2-lever procedure, with both 15% EtOH and water access restricted to daily operant sessions. The FR requirement for nicotine was increased incrementally and maintained for 3 consecutive sessions before the FR value was increased. The FR progression was 1, 3, 5, 8, 12, 20 and 30. Each rat underwent at least 5 consecutive training sessions at FR30,
during which there were no significant differences in average EtOH, water, or nicotine intake across 5 sessions of the experiment \( (\text{Mean} = 5 \text{ days}) \).

3.2.2. **Drug Pretreatments**

After operant responding and EtOH drinking stabilized in Phase 1 (Experiment 1) or Phase 2 (Experiment 2), pretreatments were given 15 min prior to test sessions. For naltrexone, test doses were 0.15, 0.3, or 0.6 mg/kg; for varenicline, test doses were 1.5 or 3 mg/kg; and for r-bPiDI, test doses were 10, 20, or 40 mg/kg. Doses were selected based on previous literature (e.g., Beckmann et al., 2015; George et al., 2011; Williams and Broadbridge, 2009). Each animal in Experiment 2 received each drug and each dose in counterbalanced order, including the appropriate vehicle control. A minimum of 2 maintenance sessions (no pretreatment) separated each pretreatment test session.

3.2.3. **Data Analysis**

All statistical analyses were conducted using Prism 5.0 software (Graph Pad Software Inc., San Diego, CA, USA). Consumption from EtOH and water bottles was measured in g/kg body weight and the number of active lever presses for nicotine and inactive lever presses were recorded automatically. Consumption differences in EtOH and water across sessions were analyzed by one-way repeated measure ANOVA during Phase 1 and at each FR value during Phase 2. Active and inactive lever presses for Phase 2 were also evaluated by one-way repeated measures ANOVA. EtOH and nicotine consumption differences between experimental phases were analyzed by two-tailed t-tests. Effects of naltrexone, varenicline, and r-bPiDI on EtOH and water consumption, and on lever presses (active for nicotine vs inactive) earned during concurrent access sessions, were analyzed by one-way, repeated-measure ANOVA. A priori multiple
comparison analyses using Dunnett’s 2-tailed t-test comparing each dose to the vehicle control (α = 0.05) were conducted when appropriate.

3.3. Results

3.3.1. Baseline EtOH and Nicotine Intake in Phase 1 (Experiment 1) and Phase 2 (Experiment 2)

Figure 1 shows differences in EtOH consumption in Phase 1 in Experiment 1 on the final session of EtOH alone, at FR1, 3, and 5 for food (final session at each FR requirement) as well as EtOH and nicotine intake during Phase 2 in Experiment 2 under the FR5 and FR30 schedules (session 3 of each FR requirement) of nicotine self-administration. For Experiment 1, EtOH intake resulted in pharmacologically relevant levels (2 g/kg). There was a significant decrease in EtOH consumption when lever pressing for food was introduced to sessions, $F(3, 15) = 14.41, p < 0.05$, however, this only occurred at FR1 responding for food. EtOH consumption during FR3 and FR5 responding for food was not significantly different from EtOH consumption during the EtOH alone access. Additionally, EtOH consumption at FR1, 3, and 5 for food resulted in pharmacologically relevant levels ($M = 1.09$ g/kg, $M = 1.86$ g/kg, and $M = 2.66$ g/kg, respectively). Results revealed a significant decrease in EtOH consumption in Phase 2 (concurrent access) compared to Phase 1 (EtOH access only), $F(2, 17) = 30.07, p < 0.05$. Subsequent posttests revealed that EtOH consumption was significantly lower in Phase 2 compared to Phase 1 during both FR5 and FR30 response requirements for nicotine. However, for Experiment 2, analyses revealed a significant increase in EtOH consumption at FR30 for nicotine compared to FR5, $t(5) = 2.74, p < 0.05$ (Fig 1A). Additionally, EtOH consumption at FR30 for nicotine reached pharmacologically
relevant levels (~0.80 g/kg) in the 1 hr test session. Analyses for nicotine revealed no significant difference in nicotine intake at FR30 compared to FR5, with the amount infused being pharmacologically relevant (~0.33 mg/kg in the 1 hr session; Fig 12B). Increases in the FR requirement for nicotine had no significant effect on water consumption or inactive lever pressing (data not shown).

3.3.2. Effects of Naltrexone in Phase 1 (Experiment 1) and Phase 2 (Experiment 2)

As shown in Figure 13, in Phase 1 (EtOH access), analyses revealed that naltrexone had no significant effect on EtOH or water consumption.

As shown in Figure 14, in Phase 2, there was a significant decrease in EtOH consumption following naltrexone pretreatment, $F(3, 18) = 1.57, p < 0.05$ (Fig 14A). Posttests revealed that EtOH consumption was decreased at all doses of naltrexone vs vehicle. However, analyses also showed a decrease in water consumption following naltrexone pretreatment, $F(3, 18) = 5.65, p < 0.05$ (Fig 14B), with posttests showing that water consumption was decreased only at the highest dose (0.6 mg/kg vs vehicle).

Naltrexone treatment had no effect on active lever presses for nicotine (Fig 14C), but significantly decreased inactive lever pressing, $F(3, 18) = 3.38, p < 0.05$ (Fig 14D). Posttests revealed that inactive lever pressing was decreased only at the lowest dose (0.15 mg/kg) vs vehicle.

3.3.3. Effects of Varenicline and r-bPiDI Pretreatments in Phase 2 (Experiment 2)

Figure 15 shows EtOH and water consumption, as well as active and inactive lever presses after varenicline pretreatment. Analyses revealed that varenicline had no
significant effect on EtOH or water consumption (Figs 15A and 15B). However, active and inactive lever responding was significantly decreased by varenicline treatment; $F(2, 14) = 2.89, p < 0.01$ and $F(2, 14) = 3.29, p < 0.05$, respectively (Figs 15C and 15D). Posttests showed that only the highest dose (3 mg/kg) decreased both active and inactive lever presses vs vehicle).

Figure 16 shows EtOH and water consumption, as well as active and inactive lever presses after r-bPiDI pretreatment. Analyses revealed that r-bPiDI had no significant effect on EtOH or water consumption (Figs 16A and 16B). In contrast, the number of active and inactive lever presses were significantly decreased by r-bPiDI treatment; $F(3, 21) = 3.34, p < 0.01$ and $F(3, 21) = 5.21, p < 0.01$, respectively (Figs 16C and 16D). Posttests revealed that the highest dose of r-bPiDI (40 mg/kg) significantly decreased both active and inactive lever presses, whereas 20 mg/kg r-bPiDI decreased inactive lever presses only.

3.3.4. **Within-session Nicotine Self-Administration**

Figure 17 shows the number of active lever presses for nicotine in 10-min intervals during Phase 2. Analysis of the naltrexone data revealed a significant main effect of time interval, $F(5, 20) = 4.83, p < 0.05$, but no significant differences among the doses at any interval (Bonferroni -hoc test $p > 0.05$ ) (Fig 17A). Analysis of the varenicline data revealed a significant main effect of dose, $F(2, 15) = 7.01, p < 0.05$, and a significant dose x interval interaction, $F(10, 75) = 2.82, p < 0.05$. Varenicline (3.0 mg/kg) decreased responding compared to vehicle during the first three 10-min intervals, Bonferroni $t(75) = 3.85, t(75) = 5.22$, and $t(75) = 3.11$, respectively, $p$’s < 0.05 (Fig 17B). Analysis of the r-bPiDI data revealed a significant main effect for interval, $F(5, 20) =$
28.93, \( p < 0.05 \) and for dose, \( F(3, 20) = 4.30, p < 0.05 \), with no significant dose x interval interaction. r-bPiDI (40 mg/kg) decreased responding compared to vehicle during the 20- and 30-min intervals, Bonferroni \( t(100) = 3.23 \) and \( t(100) = 3.01 \), respectively, \( p \)'s < 0.05 (Fig 17C).

Unfortunately, the operant chambers used herein did not provide a means to monitor cumulative EtOH consumption during the 10-min intervals across the session.

3.4. Discussion

The present findings revealed that our modified EtOH-nicotine co-use protocol resulted in pharmacologically relevant levels of concurrent EtOH intake and nicotine self-administration. When nicotine self-administration was on an FR30, EtOH consumption was \(~0.80\) g/kg/hr, an amount that is the human equivalent dose of approximately \(0.13\) g/kg (Nair & Jacob, 2016), which is approximately 2 standards drinks (National Institute on Alcohol Abuse and Alcoholism [NIAAA], 2004). Although increasing the schedule requirement to FR30 tended to decrease nicotine self-administration, the decrease was not statistically significant. At the FR30, rats earned \(~11\) infusions, with each infusion containing \(0.03\) mg/kg of nicotine, thus yielding a dose of \(~0.33\) mg/kg nicotine in each 60-min operant session. This level of responding exceeds the number of infusions (i.e., \(10\)) traditionally used as a criterion for demonstrating robust nicotine self-administration during a 60-min limited access session (Corrigall & Coen, 1989). Previous research has also shown that 10 infusions of \(0.03\) mg/kg i.v. nicotine produces nicotine plasma levels of \(~65\) ng/mL in male hooded Lister rats (Shoaib & Stolerman, 1999), well above peak plasma levels \((~15-40\) ng/mL\) found in human chronic smokers (Feyerabend, Ings, & Russel, 1985; H. Yamazaki et al., 2010). Thus,
when using the selectively bred alcohol-preferring P rat line, the protocol described herein results in pharmacologically relevant intake of both EtOH and nicotine when both are available concurrently. The results also support previous observations that the P rat line can serve as a genetic animal model of poly-drug abuse (Bell et al., 2016).

Naltrexone was the only drug given as a pretreatment during EtOH access (Phase 1), as we previously reported that there are no effects of varenicline or r-bPiDI on EtOH consumption when tested in this phase (Maggio et al., 2018). The current findings indicate that while naltrexone was ineffective in reducing EtOH consumption in Phase 1, it reduced EtOH consumption during Phase 2 (co-use). The fact that subthreshold doses of naltrexone (null finding in Phase 1) resulted in significant decreases in EtOH consumption during Phase 2 provides some pharmacological validity for the co-use model described above. The present finding that doses of naltrexone having no efficacy when EtOH is given alone can reduce EtOH intake when nicotine is available concurrently parallels similar findings in outbred Long Evans rats trained to self-administer both EtOH and nicotine in a 2-lever alternating choice test (Lê et al., 2014). The current results are also congruent with previous clinical research showing that treatment with naltrexone is more effective in heavy drinkers or alcoholics who are nicotine-dependent (Fucito et al., 2012; King, Cao, Vanier, & Wilcox, 2009). In contrast, other reports have shown that naltrexone can reduce EtOH consumption in rats without nicotine exposure, which may be due to differences in sex and rat line, including male Wistar (Lê et al., 1999; Steensland et al., 2007) and male Long-Evans Hooded rats (Steensland et al., 2007; Williams & Broadbridge, 2009); methodological differences may also play a role, including differences in dose (Henderson-Redmond & Czachowski,
Importantly, in each of these latter studies, EtOH delivery was contingent on operant lever-press responding, whereas in the current experiment EtOH was freely available. The finding that naltrexone had no effect on nicotine intake is consistent with previous preclinical studies (Corrigall & Coen, 1991; Lê et al., 2014). In contrast, clinical research suggests that alcohol use promotes the increased effectiveness of naltrexone in reducing smoking (King et al., 2009). However, these latter clinical results were obtained with repeated treatments over one month, which contrast with the acute pretreatments used in the current preclinical study. Thus, chronic treatment with naltrexone may yield positive results when applied to preclinical basic research.

During co-use (Phase 2), varenicline significantly reduced nicotine self-administration, but not EtOH consumption. However, in contrast to previous studies showing a selective effect of varenicline on active lever pressing for nicotine alone (Maggio et al., 2018), the present results indicated that varenicline also decreased inactive lever pressing. It is possible that the high FR requirement (FR30) in the current study may have enhanced the sensitivity of the rats to nonspecific suppressant effects of varenicline. Nevertheless, the varenicline-induced decrease in nicotine intake observed here is consistent with previous preclinical results (Funk et al., 2016; Maggio, Saunders, Baxter, et al., 2018; Scuppa et al., 2015). Although several preclinical studies have shown decreases in EtOH consumption following pretreatment with varenicline (Czachowski, Froehlich, & DeLory, 2018; Froehlich et al., 2017; Steensland et al., 2007), those studies only examined EtOH consumption in the absence of nicotine availability. Overall, preclinical evidence provides limited support for varenicline’s efficacy as a pharmacotherapeutic for co-users of EtOH and nicotine.
Also consistent with our previous findings (Maggio et al., 2018), when tested in the co-use phase, r-bPiDI decreased nicotine intake, but not EtOH consumption. However, similar to the effect of varenicline, there was also a nonspecific decrease in inactive lever pressing. In combination with previous investigations of the neuropharmacology of r-bPiDI (Beckmann et al., 2015b), these results suggest that α6β2* nAChRs play an important role in the maintenance of nicotine intake, but not necessarily EtOH intake. Furthermore, as r-bPiDI did not disrupt EtOH or water drinking in the current experiment and similar doses of r-bPiDI do not disrupt operant responding for food, as shown by previous research (Beckmann et al., 2015), it is unlikely that r-bPiDI disrupted motor function or caused general sedation. While previous research shows that less selective nAChR antagonists such as mecamylamine have the potential to reduce nicotine intake in animals (DeNoble & Mele, 2006; Glick et al., 1996) and in humans (J. E. Rose, 2006; J. E. Rose et al., 1994), the aversive peripheral side effects decrease their utility in clinical trials (Bevins & Caggiula, 2009; Shytle et al., 2002). Since r-bPiDI is selective for central α6β2* nAChRs, it is possible that peripheral side effects would be reduced compared to those seen with previously tested nAChR antagonists, indicating further research is needed.

One limitation of the current study is that we only used female P rats. Although we did not examine the estrus cycle for this experiment, day to day intakes of EtOH and nicotine show little variation across days, indicating that changes in estrus cycle were unlikely to be affecting intakes of either substance. Additionally, it is well-documented that there are sex differences in consumption of EtOH and related behaviors (Erol & Karpyak, 2015; Schulte, Ramo, & Brown, 2009), as well as in nicotine use (Torchalla,
Okoli, Malchy, & Johnson, 2011). Additionally, previous clinical research suggests that there are sex differences in the efficacy of varenicline, with efficacy being greater in females (S. A. McKee, Smith, Kaufman, Mazure, & Weinberger, 2016). However, previous research with mice shows that there are no sex differences in the effects of varenicline on EtOH drinking (Helen M. Kamens, Silva, Peck, & Miller, 2018). Clinical research with naltrexone has also indicated differences in effects for men and women, but overall efficacy appears similar for both sexes (Baros, Latham, & Anton, 2008). Nonetheless, given the evidence for sex differences in several studies, it is important to use both male and female subjects in future research on medication development for the treatment of EtOH and nicotine co-use.

When nicotine self-administration results were examined across time within the session, naltrexone showed no effect during any 10-min time interval. In contrast, the highest doses of varenicline (3 mg/kg) and r-bPiDI (40 mg/kg) decreased active lever responding for nicotine early in the session but not later in the session. Importantly, responding for nicotine in the absence of pretreatment was higher during the early portion of the session, an effect that is sometimes referred to “loading” under limited access conditions (Williams & Broadbridge, 2009). Thus, the high rate of responding observed early in the session appears to be more sensitive to disruption by these compounds compared to lower response rates later in the session. Alternatively, it could be that the lack of effect late in the session may reflect attenuation of efficacy due to pharmacokinetics. This latter interpretation is not likely, however, as the half-life of varenicline is about 4 hours in rats (Obach et al., 2006). Thus, taken together, these results indicate that therapeutics which may be useful for treating AUD via opioid
receptor antagonism and those that may be useful for smoking cessation via selective inhibition of α4β2* or α6β2* nAChRs, may not be sufficient to treat EtOH and nicotine co-use. Alternatively, a combination of these compounds, titrated for effective dose ranges, may yield synergistic or additive efficacy, which will require continued research.

The procedures used in our previous co-use study in female P rats (Maggio et al., 2018) revealed that, while robust nicotine self-administration was achieved, EtOH intake was relatively low, thus hampering our ability to assess drug pretreatment effects on EtOH intake. The current study modified the procedures by increasing the FR requirement for a nicotine infusion from an FR 5 to an FR 30. As modified, the present co-use procedures resulted in increased levels of EtOH intake with no significant diminution in nicotine intake. Since interactions between EtOH and nicotine have been postulated to arise from neural substrates common to both drugs (for review see Van Skike et al., 2016), we assessed the effects of opiate and nicotine receptor-selective drugs. Naltrexone significantly decreased EtOH intake when nicotine reinforcement was concurrently available, but not when EtOH was available alone. Varenicline and r-bPiDI both reduced nicotine self-administration, but not EtOH drinking, in a dose-dependent manner. Thus, under the current procedures, these results suggest that none of the drugs tested are effective as a monotherapy for co-use of EtOH and nicotine.
Figure 12 Baseline EtOH intake and nicotine self-administration in Phase 1 (EtOH alone) and Phase 2 (co-use) of Experiments 1 and 2. Panel A: EtOH consumed in Phase 1 (Experiment 1) alone, and under FR1, 3, and 5 for food pellets, and Phase 2 under either FR5 or FR30 schedule of nicotine self-administration (Experiment 2). Panel B: Nicotine infusions earned in Phase 2 under either FR5 or FR30 schedules of nicotine self-administration (Experiment 2). Values represent mean ± SEM. *p < 0.05 vs EtOH alone, #p < 0.05 vs FR5 for nicotine.
**Figure 13** Results from Study 2, Experiment 1. Results showing the effect of naltrexone on EtOH consumed (A) and water consumed (B) in Phase 1 (EtOH alone). Values represent mean±SEM. *p < 0.05 vs vehicle (0)
**Figure 14** Results from Study 2, Experiment 2. Results showing the effect of naltrexone on EtOH consumption (A), water consumption (B), number of active lever presses for nicotine (C), and number of inactive lever presses (D) in Phase 2 (co-use). Values represent mean±SEM. *p < 0.05 vs vehicle (0)
**Figure 15** Results from Study 2, Experiment 2. Results showing the effect of varenicline on EtOH consumption (A), water consumption (B), number of active lever presses for nicotine (C), and number of inactive lever presses (D) in Phase 2 (co-use). Values represent mean±SEM. *p < 0.05 vs vehicle (0)
Figure 16 Results from Study 2, Experiment 2. Results showing the effect of r-bPiDI on EtOH consumption (A), water consumption (B), number of active lever presses for nicotine (C), and number of inactive lever presses (D) in Phase 2 (co-use). Values represent mean±SEM. *p < 0.05 vs vehicle (0)
Figure 17 Results from Study 2, Experiment 2. Results showing within-session number of active lever presses for nicotine per 10-min interval following pretreatments with naltrexone (A), varenicline (B), and r-bPiDI (C). Values represent mean±SEM. Color of * matches dose. *p < 0.05 vs vehicle (0)
CHAPTER FOUR


4.1. Introduction

One critical finding of our previous research was the significant negative correlation between EtOH consumption and nicotine infusions during concurrent access, such that high EtOH drinking was associated with low nicotine self-administration and, conversely, high nicotine self-administration was associated with low EtOH consumption (Maggio, Saunders, Baxter, et al., 2018). Upon further examination of the characteristics of this model, we discovered that during co-use, increasing the response requirement for nicotine successfully increased EtOH consumption to pharmacologically relevant levels while also maintaining pharmacologically relevant levels of nicotine intake, up to an FR30 requirement for nicotine (Maggio, Saunders, Nixon, et al., 2018). Based on these findings, we hypothesized that EtOH and nicotine may act as economic substitutes for one another in this model. Thus, a behavioral economic approach to examine the relationship between EtOH and nicotine could help elucidate factors that contribute to the high rate of co-use of EtOH and nicotine.

As previously discussed, examinations of the behavioral economics of drug self-administration include two fundamental variables, response requirement (i.e. the amount of behavior allocation required to obtain a given dose of a drug reinforcer) and the dose of drug per administration (i.e. the dose of a drug that is obtained when a response requirement is met) (Bickel et al., 1990). When plotted as a demand curve, the
relationship between reinforcer cost and reinforcer consumption can be examined quantitatively.

In the laboratory, it is possible to measure demand elasticity for drugs by examining the demand curves of the changes in reinforcers earned (consumption) as a function of the response requirement to obtain each reinforcer (cost), thus capturing the sensitivity of drug consumption to changes in unit price (Hursh & Roma, 2016). In the case of co-use of EtOH and nicotine, consumption of nicotine can be defined as the number of infusions self-administered within a session. To plot these factors as a demand curve, we plot the log of consumption as a function of cost using the Exponential Model of Demand (or Own-Price Elasticity of Demand) equation (Hursh & Silberberg, 2008):

$$\log Q = \log Q_0 + k \left( e^{-\alpha(Q_0 \cdot C)} - 1 \right)$$

Plotting data from nicotine self-administration using this equation tells us how the consumption of nicotine changes as a function of increases in the cost of each reinforcer. Cost ($C$) is measured by number of responses per unit of reinforcer (one infusion). Log of consumption ($\log Q$) is a function of cost, with maximal consumption occurring at zero cost ($\log Q_0$). The rate constant ($\alpha$) determines the rate of decline in relative consumption (log consumption) with increases in cost ($C$). As a reflection of the range of the data, $k$ is used as a scaling constant. The rate of change in demand elasticity is determined by the rate constant, $\alpha$, which shows the sensitivity of consumption to changes in cost (Hursh & Silberberg, 2008).

In addition to the availability of nicotine, in our experiments, EtOH was also made concurrently available during sessions. Previous research has shown that concurrently available reinforcers can influence the demand elasticity of drugs (Marilyn...
E Carroll, Rodefer, & Rawleigh, 1995; Smethells, Harris, Burroughs, Hursh, & LeSage, 2018; Wade-Galuska, Galuska, & Winger, 2011). To determine the slope of the function relating the consumption of this second commodity (EtOH) at a fixed price to the changes in price of the alternative commodity (nicotine), we can use the cross-price elasticity of demand equation (Hursh and Roma 2016):

\[ Q_B = \log(Q_{alone}) + Ie^{-\beta \cdot C_A} \]

where \( Q_{alone} \) is the level of demand for the constant price commodity B at infinite cost \( C \) for commodity A (zero consumption of commodity A), \( I \) is the interaction constant, \( \beta \) is the sensitivity of commodity B consumption to the cost of commodity A, and \( C_A \) is the cost of commodity A (Hursh and Roma 2016). Using the co-use model described above, commodity A is nicotine because the price of nicotine is varied via response requirement manipulations, and commodity B is EtOH with constant cost because it is made freely available during sessions (Maggio, Saunders, Nixon, et al., 2018). Importantly, if this cross-price function has positive slope, then the second commodity is a substitute for the first; if the slope is negative, then the second is a complement of the first; and finally, if the slope is zero, they are considered independent.

The current experiment employed a model of co-use with concurrently available oral EtOH (0 vs. 15%, 2-bottle choice) and nicotine (0.03 mg/kg/infusion iv, active vs. inactive lever) and applied behavioral economic principles of demand using the exponential model of demand equation (own-price elasticity) for nicotine and the cross-price elasticity of demand equation for EtOH. Using these equations, the current study aimed to determine how the changing price of nicotine effects consumption of both nicotine and EtOH when both commodities are available concurrently. This behavioral
economic approach to examining the consumption of EtOH and nicotine allows further investigation into the relative reinforcing effects and demand factors that contribute to the relationship between EtOH- and nicotine-taking behaviors. Deriving these demand equations will allow us to further understand and make predictions about co-use behavior for better medication development research.

4.2. Methods

**Animals:** Female P rats (n=6, selectively bred generation 81) were obtained from Indiana University School of Medicine (provided by NIAAA/NIH) and began training between PND 50-60. Rats were housed individually upon arrival in a temperature-controlled colony room under a 12:12hr light/dark cycle. All testing procedures occurred during the light phase (7:00 am – 7:00 pm), were in accordance with the NIH Guide for the Care and Use of Laboratory Animals (8th edition, 2011), and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky. All animals were experimentally naïve at the start of the current experiment.

**Drugs:** EtOH was prepared in a concentration of 15% v/v 190 proof EtOH (Pharmco-AAPER, Shelbyville, KY) diluted in distilled water. Nicotine hydrogen tartrate (Sigma-Aldrich, San Diego, CA) was dissolved in a 0.9% NaCl (saline) solution, to which NaOH was added to obtain a pH of 7.0 ± 0.05; nicotine dosage was based on freebase weight. For surgery, rats were anesthetized via i.p. injections of 55/7.5/7.5 mg/kg ketamine (Henry Schein Animal Health, Dublin, OH)/xylazine (LLOYD Laboratories, Shenandoah, IA)/sterile water.

**Apparatus:** All training and testing sessions were conducted in standard two-lever operant chambers (ENV-001; MED Associates, St. Albans VT). Two response levers
were located on either side of a recessed food tray. Located above each lever was a white cue light. Nicotine infusions were delivered by a syringe pump and food pellets were delivered by a pellet dispenser. A computer, linked to a Med Associates interface, recorded responses and controlled infusions during the experimental session. Each chamber was modified to allow access to two 100 ml Richter feeding tube glass bottles (Model 900010; Dyets, Inc., Bethlehem PA) on the wall of the chamber opposite the levers. The design of the bottles allowed them to be fixed securely to the outside of the chambers with lipped feeding tube holders (Model 901100; Dyets, Inc., Bethlehem, PA) such that only the drinking spout could be accessed by rats while inside the chambers (see Figure 2).

4.2.1. Procedures

A timeline for training procedures is shown in Appendix C.

*EtOH Access (Phase 1)*: Pre-training and EtOH access (Phase 1) was conducted using procedures similar to those described by Maggio et al. (2018a & b). Briefly, during pre-training, to allow for acclimation to the taste and smell of EtOH, rats were given one bottle of 20% EtOH as the sole source of liquid for 72 consecutive hours in the home cage (Simms et al., 2010). During this time, food was available *ad libitum*. Following pre-training, rats were trained during daily 60-min two-bottle choice sessions during which rats were given free access to two bottles, one bottle of water and one bottle of 15% EtOH (v/v). EtOH and water consumption were measured by weighing each bottle immediately prior to and immediately after access sessions to determine differences in weight. Animals were trained in this phase for at least 15 days until the average EtOH consumption stabilized, i.e. there were no significant differences in average consumption
across 5 consecutive sessions. After stability in EtOH drinking was achieved, animals advanced to training for experiment 2 (Concurrent access, Phase 2).

**Concurrent access phase (Phase 2):** During this phase, animals were trained to acquire lever pressing for palatable food pellets (45 mg Dustless Precision Pellets, Bio-Serv, Frenchtown NJ) using the general methods previously described (Maggio, Saunders, Baxter, et al., 2018; Maggio, Saunders, Nixon, et al., 2018), with EtOH and water (2-bottle choice) available daily in the operant chambers during all training and testing sessions. Briefly, rats were trained to lever press for food pellets using a standard 2-lever operant procedure (active vs inactive levers). Response requirements for food pellet delivery increased under an incrementing FR schedule, beginning with FR1 (3 sessions), followed by FR3 (3 sessions), and then FR5, where it remained until responding for food stabilized (at least 5 consecutive sessions with no significant differences in responding between sessions and at least two times more responding on the active lever vs inactive lever; ~12 days). Rats then underwent surgery under anesthesia to implant a chronic indwelling catheter into the jugular vein; catheters were flushed daily with heparinized saline to maintain patency. Following surgery, rats were given 5-7 days of recovery with *ad libitum* access to food, water, and one bottle of 15% EtOH in the home cage.

Following the recovery period, rats were trained to self-administer nicotine (0.03 mg/kg/infusion) using a 2-lever procedure, during which time access to one bottle of 15% EtOH and one bottle of water remained during the 60-min daily operant sessions (both water and 15% EtOH access was restricted to daily sessions), using the procedure described in Maggio et al. (2018b). During this phase, the FR requirement for nicotine
was increased incrementally, starting at FR1, such that the animals trained at each FR value for 3 consecutive sessions before the FR value was increased per the following progression: 1, 3, 5, 8, 12, 20, 30, 45, 60, 85, 105, 135. Once rats reached an FR30 response requirement for nicotine, each rat underwent at least 5 consecutive training sessions at FR30 (with no significant differences in average EtOH consumption, water consumption, or nicotine intake across 5 sessions) in this phase. At the FR30 response requirement, rats were also given drug pretreatments with naltrexone, varenicline, and r-bPiDI. Results from drug pretreatments are presented in Maggio et al. (2018b). Following this experiment, rats were given 3 training sessions at FR30 for nicotine before continuing the incremental increase in FR requirement up to a terminal FR135, with 3 consecutive sessions at each FR value before the FR requirement was increased.

4.2.2. Data Analysis

Consumption from the EtOH and water bottles was measured in g per kg body weight. Active and inactive lever presses for nicotine infusions were recorded by the automated system used to operate the operant chambers (ENV-001; MED Associates, St. Albans VT). The values for EtOH consumption and nicotine intake were normalized to the same scale by representing the value for consumption/intake on the last day of each FR requirement for nicotine (FR1-FR135) as a percent of the maximum consumption/intake for individual subjects. Statistical analyses were conducted using both Prism 5.0 (Graph Pad Software Inc., San Diego, CA, USA) and R (R Foundation for Statistical Computing, Vienna, Austria). A two-way repeated measured ANOVA was run to compare the changes in consumption of nicotine and EtOH across changing price of nicotine.
Normalizing Intake Values for Nicotine and EtOH

Consumption of EtOH and water was measured in g/kg, while nicotine intake was measured in lever presses, infusions, and mg/kg. To compare intake of EtOH, water, and nicotine, values were transformed to the log of consumption/intake on the last day of each FR requirement for nicotine (FR1-FR135) as a percent of the maximum consumption/intake for individual subjects across all FR requirements. Equations for the normalized values can be found in Appendix D.

Own-Price Elasticity of Demand for Nicotine

Consumption of nicotine was defined as total mg/kg of nicotine self-administered within a session (dose of nicotine x number of infusions). Cost of nicotine was defined as the number of lever presses (FR value) to obtain one nicotine infusion. To plot nicotine intake as a function of the changing cost of nicotine using behavioral economic principles, we plotted the log of nicotine intake as a function of cost (FR value) using the Exponential Model of Demand equation (Hursh & Silberberg, 2008):

$$\log Q = \log Q_0 + k \left( e^{-\alpha \cdot (Q_0 \cdot C)} - 1 \right)$$

Cost (C) was measured by number of lever presses per unit of nicotine. Log of nicotine intake (log Q) was plotted as a function of the cost of nicotine, with maximal intake occurring at zero cost (log Q0). The rate constant (α) determined the rate of decline in relative nicotine intake (log consumption) with increases in cost (C). As a reflection of the range of the data, k was a scaling constant. The rate of change in demand elasticity was determined by the rate constant, α, which showed the sensitivity of nicotine intake to changes in FR value (Hursh & Silberberg, 2008).
**Cross-Price Demand Elasticity for EtOH**

To determine the slope of the function relating the consumption of a second commodity (EtOH) at a fixed price to the changes in FR requirement for nicotine (alternative commodity), we used the equation for cross-price elasticity of demand. The cross-price elasticity of demand for EtOH was determined using the equation (Hursh and Roma 2016):

\[ Q_B = \log(Q_{alone}) + l e^{-\beta \cdot C_A} \]

where \( Q_{alone} \) was the level of demand for EtOH (the constant price commodity B) at infinite cost (C) for nicotine (commodity A), \( l \) was the interaction constant, \( \beta \) was the sensitivity of EtOH consumption to the cost of nicotine, and \( C_A \) was the cost of nicotine (FR value). Using the co-use model described above, commodity A was nicotine because the price of nicotine was varied, and commodity B was EtOH with constant price. If this cross-price function has a positive slope (determined by the value of the interaction constant), then EtOH is considered a substitute for nicotine; if the slope is negative, then EtOH is considered a complement of nicotine; if the slope is zero, then EtOH and nicotine are considered independent (Hursh & Roma, 2016).

**4.3. Results**

**4.3.1. Demand Elasticity**

Results show that EtOH consumption significantly decreased when nicotine was made available concurrently, \( F(3, 15) = 9.89, p < 0.05 \). A Dunnett’s test revealed that consumption during EtOH alone (Phase 1) was significantly different from EtOH consumption when the response requirement for nicotine was FR1 or FR30: the FR30
result is important because it represents the response requirement at which varying doses of naltrexone, varenicline and r-bPiDI were tested (see Study 2). However, in the current study, when the FR requirement for nicotine was incremented up to FR135, there was no longer any difference in EtOH consumption compared to that observed in Phase 1 (Fig. 18).

Figure 19 plots the normalized intake of EtOH and nicotine as a function of the change in nicotine cost (FR requirement). Own-price analysis indicated that, with best-fit parameters $Q = 1.97$, and $k = 6.2$, increases in nicotine unit price significantly decreased nicotine intake ($\alpha = 0.00002$). When the changes in consumption for nicotine and EtOH were quantified via a cross-price elasticity measure, nonlinear mixed effects (NLME) modeling results, with best-fit parameter estimates of $Q = 1.84$ and $\beta = 0.04$, indicated that EtOH is an economic substitute for nicotine, $I = -0.79$ ($p < 0.05$). NLME modeling also showed that water consumption was independent from unit price changes for nicotine ($Q = 1.74; \beta = -0.007; I = 0.03$; data not shown). The point of indifference ($UP_{50}$), at which preference was equal for both EtOH and nicotine, occurred at $\approx 1000$.

4.4. Discussion

The aim of the current study was to employ a model of co-use with concurrently available EtOH and nicotine and to apply behavioral economic principles of demand to determine how the changing price of nicotine effects consumption of both substances when they are available concurrently. The economic interaction between EtOH and nicotine when both are made concurrently available was examined using two behavioral economic demand curves: 1) own price elasticity of demand for nicotine and 2) cross-price elasticity of demand for EtOH. For nicotine, as expected, as the FR requirement
increased, nicotine intake decreased. For EtOH, we assessed the change in consumption of the fixed-price alternative commodity (EtOH) with changes in price of the manipulated-price commodity (nicotine). As nicotine intake decreased, EtOH consumption increased, showing that EtOH acts as an economic substitute for nicotine in female P rats. This suggests that common neurobehavioral mechanisms may influence the relationship between EtOH and nicotine co-use.

One system which may play a role in the relationship between EtOH and nicotine commodities is the mesocorticolimbic DA system, commonly known as the reward pathway. In the current study, EtOH acted as an economic substitute for nicotine. Increases in EtOH consumption that occur when the unit price of nicotine is increased indicate that the rewarding value of EtOH increases when more behavior allocation is required to obtain nicotine reinforcement (the preferred reinforcer up to FR30). Of course, future work would be required to test the speculation that elevated EtOH consumption in response to increases in nicotine unit price is accompanied by altered activation of the DA reward system.

Analyses also revealed that preference during concurrent access changed as the unit price for nicotine changed. For FR1 to FR30, consumption of nicotine was preferred over EtOH. This preference switched from nicotine to EtOH when the cost for nicotine exceeded FR30, providing further evidence that EtOH is an economic substitute for nicotine. Interestingly, intake levels of both EtOH and nicotine were pharmacologically relevant at this indifference point (EtOH: $M = 0.754 \text{ g/kg}, SD = 0.13$; Nicotine: $M = 0.32 \text{ mg/kg}, SD = 0.17$). Thus, future research could benefit from the use of this co-use model.
in evaluating the effectiveness of pharmacotherapeutic treatments for co-use of EtOH and nicotine.

Alternative to response requirement manipulations, dose manipulations can also act as price changes in behavioral economic demand investigations of drug self-administration (Hursh & Roma, 2016). As logic would follow, a decrease in nicotine dose (with constant response requirement per unit) would be thought of as an increase in unit price, as the response requirement to achieve the same total amount of nicotine would increase with decreases in dose and vice versa. However, previous research has shown that rats will increase responding for higher doses of nicotine (Chaudhri et al., 2007; Donny et al., 1999). Thus, although both response requirement (FR) and dose variations can be used as a measurement of cost for the commodity nicotine, variations in these factors could have very different effects on the elasticity of demand for nicotine or EtOH, especially during concurrent access to both substances.
Figure 18 Results from Study 3. EtOH consumption (g/kg) across Phase 1 (EtOH access), and changing response requirements for nicotine (FR1, FR30, and FR135). * = p < 0.05 compared to Phase 1
Figure 19 Results from Study 3. The normalized intake of EtOH and nicotine as a function of the changing unit price of nicotine. Values are expressed as (log) % of maximum intake for individual subjects. Curves were derived from their respective equations.
CHAPTER FIVE

5. Study 4: Effects of varenicline on demand elasticity for co-use of EtOH and nicotine: Comparing the effects of varying doses of nicotine to varying response requirements for nicotine using female P rats.

5.1. Introduction

In our previous research (Maggio, Saunders, Baxter, et al., 2018; Maggio, Saunders, Nixon, et al., 2018), it was determined that EtOH acts as an economic substitute for nicotine when both are available concurrently in our co-use model in P rats. In that work, as the cost of nicotine increased, defined as an increase in the response requirement (FR) for nicotine, nicotine consumption decreased and EtOH consumption increased (Maggio, Saunders, Nixon, et al., 2018). These findings are inconsistent with human studies, which indicate that EtOH acts as an economic complement to nicotine (Bickel, DeGrandpre, & Higgins, 1995). While this inconsistency may be due to species differences (human vs. rat), the clinical data are limited because they are based on self-report. In addition, while the commodity used in the clinical experiments was tobacco cigarettes, the commodity used in the preclinical experiments was i.v. nicotine. To further investigate the economic relationship between EtOH and nicotine, this study examined the effects of a known smoking cessation agent, varenicline, on the relationship between concurrently available nicotine and EtOH across changing cost of nicotine. To further examine this relationship, an alternative approach to changing the cost of nicotine was explored as a possible model for examining this co-use relationship.
Although our previous model for co-use involved price changes for nicotine via FR requirement changes, there are other dimensions by which price can be changed. Previous research has shown that rats will increase progressive ratio responding for increasing doses of nicotine (Chaudhri et al., 2007; Donny et al., 1999). Additionally, previous research has shown that multiple factors contribute to nicotine self-administration, including the primary reinforcing effect of nicotine, as well as the ability of nicotine to non-contingently enhance the reinforcing effect of ongoing behavior (Chaudhri et al., 2006). In preclinical research, the availability of EtOH has previously been shown to affect consumption and demand for nicotine (Lê et al., 2014; Lê et al., 2010; Maggio, Saunders, Baxter, et al., 2018; Maggio, Saunders, Nixon, et al., 2018), although it is not clear if EtOH availability differently affects the nicotine demand curves that are derived when nicotine price is manipulated by either changes in FR requirement or nicotine dose. For these reasons, the current study examined the relationship between concurrently available nicotine and EtOH intake by changing the cost of nicotine by varying the dose of nicotine per infusion, while keeping the response requirement constant.

From a behavioral economic perspective, measuring drug intake at various doses or different response requirements is essentially examining drug intake at different prices. Thus, price can be defined conceptually as the amount of work or effort required to obtain a unit dose of a reinforcer. In the current experiment, dose and FR requirement manipulations are used as dimensions by which the price of nicotine is changed. For analysis across dimensions, changes in unit price are standardized as unit price, which is measured as the work requirement to obtain a unit of reinforcer. Thus, if the fixed ratio
(FR) to obtain one infusion of nicotine in a dose of 0.03 mg/kg/infusion is set at FR1, the unit price is 1/0.03, or 33.33. The unit price ratio can be increased by either increasing the FR requirement (numerator) or by decreasing the dose per infusion (denominator). For example, to increase the unit price from 33.33 (FR1 requirement for 0.03 mg/kg/infusion of nicotine) to a unit price of 100, the FR requirement can be increased to 3, yielding a unit price ratio of 3/0.03. Alternatively, the unit price can be increased by decreasing the dose to 0.01 mg/kg/infusion at an FR1 requirement for a unit price ratio of 1/0.01, or unit price of 100. In both cases, the unit price is equivalent, but the dimension by which the price is changed is different. By setting equivalent unit prices for both methods of price changes, the amount of work required to obtain a specific amount of reinforcer is also equivalent.

Hursh and Roma (2016) propose that changes in the price of a commodity primarily drives changes in demand elasticity, which are adequately described by the equations for exponential demand, regardless of the dimension by which the price of a commodity is changed. This is because in the exponential model of demand equation the size of the reinforcer is considered as a component of price, separate from the elasticity changes reflected in α. Additionally, differences in the size of the reinforcer that would change demand when the price of the commodity is zero (Q₀) are accounted for by standardizing the price in the exponent of the exponential (Q₀ * C). Thus, changing the cost of nicotine via varying the unit dose per infusion is expected to result in demand elasticity patterns similar to those seen by varying response requirement changes because the equation should automatically consider different types of price manipulations (Hursh & Roma, 2016). Assuming this is true, when the cost of nicotine is changed via dose
variation, it is hypothesized that increasing cost (decreasing unit dose) of nicotine will
decrease nicotine intake while increasing EtOH intake, as seen in our previous model of
increasing cost of nicotine via response requirement changes. Additionally, it is
hypothesized that EtOH will act as an economic substitute for nicotine when both are
available concurrently, similarly to our previous co-use model.

This study consisted of two separate experiments. In the first experiment, cross-
price elasticity of demand was modeled across changing nicotine unit doses to determine
if the model fit was similar to that observed across changing FR requirements. In the
second experiment, the effect of varenicline on cross-price elasticity was determined
using the approach in which nicotine cost was varied by changing the FR requirement. It
was hypothesized that varenicline pretreatment (3.0 mg/kg) would decrease nicotine
consumption across all dose changes. That is, for own-price elasticity for nicotine, it is
hypothesized that varenicline would increase the elasticity of demand for nicotine across
increasing cost of nicotine. In contrast, for cross-price elasticity for EtOH, it was
hypothesized that varenicline-induced decreases in nicotine consumption would increase
in EtOH consumption, thus shifting the indifference point leftward relative to control (no
varenicline).

5.2. Experiment 1: Varying nicotine cost by varying dose vs FR requirement.

5.2.1. Method

*Animals:* Female P rats (n=20, selectively bred generations 79-83) were obtained
from Indiana University School of Medicine (provided by NIAAA/NIH) and began
training between PND 50-60. Rats were housed individually upon arrival in a
temperature-controlled colony room under a 12:12hr light/dark cycle. All testing
procedures occurred during the light phase (7:00 am – 7:00 pm). All animals were experimentally naïve at the start of the current experiment.

**Drugs:** Preparation of EtOH and nicotine was similar to that used in Studies 1-3. Surgical preparations were also identical to those used in Studies 1-3.

**Apparatus:** All training and testing sessions was conducted in identical operant chambers used in Studies 1-3 (see Figure 2).

### 5.2.2. Procedures

**EtOH Access (Phase 1):** Pre-training and EtOH access (Phase 1) was conducted using procedures similar to those described by Maggio et al. (2018a & b). Briefly, during pre-training, to allow for acclimation to the taste and smell of EtOH, rats were given one bottle of 20% EtOH as the sole source of liquid for 72 consecutive hours in the home cage (Simms et al., 2010). During this time, food was available *ad libitum*. Following pre-training, rats were trained during daily 60-min two-bottle choice sessions during which rats were given free access to two bottles, one bottle of water and one bottle of 15% EtOH (v/v). EtOH and water consumption was measured by weighing each bottle immediately prior to and immediately after access sessions to determine differences in weight. Animals were trained in this phase for at least 10 days until the average EtOH consumption stabilized, i.e. there were no significant differences in average consumption across 5 consecutive sessions as determined by a one-way repeated measures ANOVA.

After stability in EtOH drinking was achieved, animals advance to training for concurrent access, Phase 2.

**Concurrent access phase (Phase 2):** During this phase, animals were trained to acquire lever pressing for palatable food pellets (45 mg Dustless Precision Pellets, Bio-
Serv, Frenchtown NJ) using the general methods previously described (Maggio, Saunders, Baxter, et al., 2018; Maggio, Saunders, Nixon, et al., 2018), with EtOH and water (2-bottle choice) available daily in the operant chambers during all training and testing sessions. Briefly, rats were trained to lever press for food pellets using a standard 2-lever operant procedure (active vs inactive levers). Response requirements for food pellet delivery will increase under an incrementing FR schedule, beginning with FR1 (3 sessions), followed by FR3 (3 sessions), and then FR5, where it will remain until responding for food stabilized (at least 5 consecutive sessions with no significant differences in responding between sessions and at least two times more responding on the active lever vs inactive lever; ~12 days). Rats then underwent surgery under anesthesia to implant a chronic indwelling catheter into the jugular vein; catheters were flushed daily with heparinized saline to maintain patency. Following surgery, rats were given 5-7 days of recovery with ad libitum access to food, water, and one bottle of 15% EtOH in the home cage.

Following the recovery period, rats were trained to self-administer nicotine using a 2-lever procedure, during which time access to one bottle of 15% EtOH and one bottle of water remained during the 60-min daily operant sessions (both water and 15% EtOH access were restricted to daily sessions), using the procedure described in Maggio et al. (2018b). Each rat underwent at least 5 consecutive training sessions at FR1 (with no significant differences in average EtOH consumption, water consumption, or nicotine intake across 5 sessions) in this phase. Following stabilization of responding at FR1, unit price of nicotine was varied such that each rat underwent 3 consecutive sessions of each unit price. To compare elasticity of demand for unit price changes for nicotine, rats then
were tested in one of two different groups. For group 1, unit price for nicotine was changed via dose changes as follows: 0.03, 0.01, 0.006, 0.004, 0.003, 0.002, 0.001, 0.0007, 0.0005, 0.0004, 0.0003, and 0.0002 mg/kg in a randomized order. Doses were determined by matching the unit price for nicotine to the increasing FR requirements previously used with 0.03 mg/kg/infusion of nicotine (see Table 1). For group 2, unit price for nicotine was changed via FR requirement changes for nicotine infusions as follows: FR 1, 3, 5, 8, 12, 20, 30, 45, 60, 80, 105, and 135 in an increasing order (see Table 1 for unit price matching relative to dose changes).

5.2.3. Data Analysis

Consumption from EtOH and water bottles were measured in g per kg body weight. Active and inactive lever presses for nicotine infusions were recorded by the automated system used to control the operant chambers (ENV-001; MED Associates, St. Albans VT). The values for EtOH consumption and nicotine intake were normalized to the same scale by representing the value for consumption/intake on the last day of each unit price of nicotine as a percent of the maximum consumption/intake for individual subjects. Statistical analyses were conducted using both Prism 5.0 (Graph Pad Software Inc., San Diego, CA, USA) and R (R Foundation for Statistical Computing, Vienna, Austria). Consumption differences of EtOH and nicotine, averaged across the last 3 days of each experimental phase, were analyzed by two-tailed t-tests.

Normalizing Intake Values for Nicotine and EtOH

To compare intake of EtOH, water, and nicotine, values were transformed to the log of consumption/intake on the last day of each unit price of nicotine as a percent of the...
maximum consumption/intake for individual subjects across all doses. Equations for the normalized values can be found in Appendix D.

*Own-Price Elasticity of Demand for Nicotine*

Consumption of nicotine was defined as total mg/kg of nicotine self-administered within a session (dose of nicotine x number of infusions). Cost of nicotine was defined as the number of infusions required to achieve the equivalent of one unit dose of 0.03 mg/kg. To plot nicotine intake as a function of the changing unit price of nicotine using behavioral economic principles, the log of nicotine intake as a function of cost was plotted using the Exponential Model of Demand equation (Hursh & Silberberg, 2008):

$$\log Q = \log Q_0 + k (e^{-\alpha(Q_0 \cdot C)} - 1)$$

Where C equals the number of infusions required for one-unit dose of nicotine (0.03 mg/kg), $Q_0$ represents nicotine intake at zero price, $\alpha$ is the rate of decline in relative nicotine intake (log consumption) with increases in cost (C), and $k$ acts as a global scaling constant. The rate of change in demand elasticity were determined by the rate constant, $\alpha$, which shows the sensitivity of nicotine intake to changes in dose per infusion (Hursh & Silberberg, 2008).

*Cross-Price Demand Elasticity for EtOH*

To determine the slope of the function relating the consumption of a second commodity (EtOH) at a fixed price to the changes in dose of nicotine (alternative commodity), the equation for cross-price elasticity of demand was used. The cross-price elasticity of demand for EtOH was determined using the equation (Hursh and Roma 2016):

$$Q_B = \log(Q_{alone}) + 1e^{-\beta \cdot C_A}$$
where $Q_{alone}$ is the level of demand for EtOH (the constant price commodity B) at infinite cost ($C$) for nicotine (commodity A), $I$ is the interaction constant, $\beta$ is the sensitivity of EtOH consumption to the price of nicotine, and $C_A$ was the cost of nicotine (Hursh & Roma, 2016).

*Nonlinear Mixed-Effects Modeling*

Nicotine and EtOH intake were measured for each rat at each unit price for nicotine. This generated 12 values of nicotine intake and 12 values of EtOH intake for each rat for dose change and FR change groups in Experiment 1. For Experiment 2, nicotine and EtOH intake were measured for each rat at each unit price for nicotine in control conditions (no treatment) and following varenicline pretreatment, generating 24 values of nicotine intake (12 in control conditions and 12 following varenicline pretreatment) and 24 values of EtOH intake (12 in control conditions and 12 following varenicline pretreatment) for each rat. Nicotine and EtOH intakes were analyzed as functions of unit price for nicotine. For nicotine, exponential demand functions (Hursh and Silberberg, 2008) were fit to nicotine intake at each unit price for each individual rat. For EtOH, cross-price demand functions (Hursh and Roma, 2016) were fit to EtOH intake at each unit price of nicotine for each individual rat. The demand functions were fit to the data via nonlinear mixed effects modeling (NLME) (Beckmann & Chow, 2015; Pinheiro, Bates, DebRoy, & Sarkar, 2006; Young, Clark, Goffus, & Hoane, 2009) using the NLME tool in the R statistical software package (Pinheiro et al., 2006), with $Q_0$ and $\alpha$ as free parameters and $k$ as a global constant for nicotine equations and with $Q_{alone}$, $\beta$, and $I$ as free parameters for EtOH equations. For Experiments 1 and 2, the NLME models defined unit price as a fixed, continuous, within-subject factor and subject as a random
factor for both nicotine and EtOH models. For Experiment 1 only, the NLME models defined type of price change (dose or FR change) as a fixed, nominal, between-subject factor. For Experiment 2 only, the NLME models defined treatment (control or varenicline) as a fixed, nominal, within-subject factor.

NLME analysis provides advantages over traditional ANOVA, by significantly increasing power, reducing Type I error rates, and allowing analyses that are closer to specific underlying relationships in the data by using defined functions (Beckmann & Chow, 2015; Young et al., 2009). Similar to linear mixed effects modeling (Giuliano, Robbins, Wille, Bullmore, & Everitt, 2013), NLME is a hierarchical, multi-level modeling technique that employs maximum likelihood estimation (Myung, 2003) to determine parameter estimates of a predefined non-linear function, in this case the own- and cross-price demand equations (Hursh & Roma, 2016; Hursh & Silberberg, 2008). This type of modeling allows for fitting data over different experimental conditions, as well as incorporating model fits from each individual, providing metrics of goodness of fit and determining statistical significance of parameter estimates across levels of experimental conditions (Beckmann & Chow, 2015; Young et al., 2009).

5.3. Experiment 1 Results

For comparison, unit price for nicotine was matched for dose changes and response requirement changes (See Table 1). Figure 20 plots the normalized intake of EtOH and nicotine as a function of the change in nicotine unit price (dose changes in blue and FR changes in red). For nicotine, when the changes in consumption were quantified via own-price analysis using nonlinear mixed effects (NLME) modeling, results indicated that, with best-fit parameters $Q = 1.94$ and $k = 1.93$, increases in nicotine unit price
significantly decreased nicotine intake ($\alpha = 0.0005$) when nicotine price was varied via dose changes. When nicotine price was changed via FR manipulations, own-price analysis indicated that, with best fit parameters $Q = 1.96$ and $k = 1.93$, increases in nicotine unit price significantly decreased nicotine intake ($\alpha = 0.0001$). The intake of nicotine at zero price ($Q_0$), sensitivity to price changes, and global scaling constants were all significantly different from zero ($p < 0.05$ for all). Nicotine intake at zero price ($Q_0$) was not significantly different for dose changes compared to FR changes ($p = 0.73$). However, there was a significant difference between dose changes vs FR changes in sensitivity to price change ($\alpha$), $p < 0.05$, such that there was a greater sensitivity in nicotine intake to price changes when the price of nicotine was changed using dose manipulations.

For EtOH, when the changes in consumption were quantified via cross-price analysis using NLME modeling, results indicated that, with best-fit parameter estimates of $Q = 1.77$ and $\beta = 0.002$, EtOH served as an economic substitute for nicotine, $I = -0.39$, when the price of nicotine is varied via dose changes. When price of nicotine was varied via FR manipulations, with best-fit parameter estimates of $Q = 1.84$ and $\beta = 0.001$, indicated that EtOH is an economic substitute for nicotine, $I = -0.79$. Intake of EtOH at infinite price for nicotine ($Q_{alone}$), sensitivity of EtOH intake to price changes in nicotine ($\beta$), and interaction constants ($I$) were significantly different from zero ($p < 0.05$ for all). However, consumption of EtOH at infinite price for nicotine and sensitivity of EtOH to price changes in nicotine were not significantly different for dose change vs FR change ($p = 0.47$ and $p = 0.23$, respectively). Interestingly, the interaction constants for dose changes vs FR changes were significantly different, such that FR changes produced a
greater substitution relationship compared to dose changes ($p < 0.05$). For dose manipulations, UP50 was between unit prices 166.67 and 266.67 for nicotine. For FR manipulations, UP50 was between unit prices 666.67 and 1000 for nicotine.

### 5.4. Experiment 1 Discussion

In congruence with findings from our previous research, the present findings revealed that, under concurrent access conditions, as the unit price of nicotine increases, nicotine intake decreases. Additionally, as nicotine intake decreases, EtOH consumption increases. Results from the current experiment also reveal that these changes in demand for nicotine and EtOH occur when the unit price for nicotine is changed via response requirement changes, as well as dose changes.

A significantly higher value of $\alpha$ is observed in the elasticity of demand equation derived from the dose change data compared to the FR change data, indicating that nicotine intake is more sensitive to unit price changes when the price of nicotine is changed via dose manipulations (Hursh & Roma, 2016). Furthermore, although there is evidence of EtOH acting as an economic substitute for nicotine for both models explored in the current study, the value of the interaction constant ($I$) for dose changes ($I = -0.39$) is closer to zero compared to that for FR changes ($I = -0.78$), indicating that there is a weaker substitution relationship (Hursh & Roma, 2016) using dose changes to change the unit price for nicotine, a finding that was significant. However, there is no difference between dose change and FR change models when comparing demand level or sensitivity of EtOH intake to the changing price of nicotine. Similarly, there is no difference between nicotine intake at zero price when price is changed via dose changes vs FR changes.
5.5. **Experiment 2: Effect of varenicline on cross-price elasticity derived by varying nicotine cost with FR requirement.**

The animals from group 2 of Experiment 1 were used in Experiment 2. Procedures are described above, excluding the pretreatment day testing. On test days, rats were given drug pretreatment with varenicline (3 mg/kg s.c.) 15 minutes prior to the start of concurrent access sessions. Testing days occurred on the 4th consecutive day of each FR requirement for nicotine and the FR requirement was increased on the day following a pretreatment test day. Preparation of varenicline was identical to that described in Studies 1-3.

5.6. **Experiment 2 Results**

For nicotine, own-price analysis indicated that, under control conditions (no varenicline pretreatment) with best fit parameter $Q = 1.91$ and following treatment with varenicline with best fit parameter $Q = 1.60$ (shared $k = 1.60$), there was a significant ($p < 0.05$) decrease in nicotine intake as the price of nicotine was increased ($\alpha = 0.0002$ and $\alpha = 0.0003$, respectively). There was a significant difference in sensitivity to price ($\alpha$) between control conditions compared to varenicline pretreatment conditions ($p < 0.05$), such that price sensitivity was increased following varenicline pretreatment. There was also a significant decrease in nicotine intake at zero price ($Q_0, p < 0.05$) following pretreatment with varenicline.

Results from NLME modeling of cross-price elasticity of demand for EtOH show a significant difference in EtOH intake at infinite price for nicotine ($Q_{\text{alone}}$) in both control conditions and following EtOH treatment ($p < 0.05$). However, this was the only parameter that differed from zero, and there were no significant differences between
control conditions and varenicline pretreatment conditions for any of the parameters ($p > 0.05$ for all).

5.7. Experiment 2 Discussion

As predicted, there was a significant decrease in nicotine intake across increasing FR requirements, as well as following varenicline pretreatment, at all unit prices for nicotine. Varenicline also increased the sensitivity of nicotine intake to unit price changes for nicotine.

Additionally, EtOH intake was significantly decreased by increases in price for nicotine overall. However, unexpectedly, there were no significant differences in EtOH intake at infinite price for nicotine, sensitivity of EtOH to nicotine price changes, or interaction constants for EtOH demand following varenicline pretreatment.

5.8. Study 4 General Discussion

The goal of the current study was to examine the relationship between nicotine and EtOH consumption across changing unit price for nicotine, as well as to determine how this relationship is altered by varenicline pretreatment. In Experiment 1, results showed that the relationship between intake of concurrently available nicotine and EtOH is differentially affected by different dimensions of the changing cost (dose vs FR requirement), even when price equivalence is set, contrary to previous theories of economic demand (Hursh & Roma, 2016). Specifically, there is a greater sensitivity of nicotine intake to change with price when using a dose manipulation rather than an FR manipulation. Results also show a stronger substitution relationship between EtOH and nicotine when the price of nicotine is changed via an FR manipulation.
Hursh and Roma (2016) propose that when commodities differ in size, the true cost must be changed in order to maintain baseline demand. Thus, price equivalence must be set such that the unit price of a commodity provides the same amount of a reinforcer, regardless of the size of the reinforcer. In Experiment 1 of the current study, the size of the reinforcer nicotine was changed via changing the dose per infusion. To establish price equivalence with the model that changes nicotine price via FR requirement changes, we set the unit price of nicotine equivalent across each price change used for both models. Stated another way, when the dose of nicotine was 0.03 mg/kg/infusion at an FR requirement of 1 lever press, the unit price for nicotine was 33.33. Increasing the dose of nicotine per infusion from 0.03 to 0.01 changed the unit price of nicotine to 100, the same unit price when the FR requirement is changed from FR1 to FR3 when the dose remains at 0.03 mg/kg/infusion (see Table 1). Price equivalence was established in this way across all doses and FR requirement values for nicotine in the current study. However, the predictions of the model imply that by establishing price equivalence, the curves for the elasticity of demand for nicotine should follow the invariance assumption, and thus be equivalent. In our models, however, changing the price for nicotine via FR changes and dose changes produced curves that were significantly different in sensitivity to price (α) and scaling constant (k), violating the invariance assumption put forth by the demand equations used. Thus, although there were equivalent unit price values across conditions, the dimension by which price was changed affected both the shape and size of the curves.

Results from Experiment 2 of this study show a significant decrease in nicotine intake across increasing FR requirements, as well as following varenicline pretreatment, at all unit prices for nicotine. Unexpectedly, however, results also showed there were no
significant differences in EtOH intake between control conditions and following varenicline pretreatment. These results show the ability of varenicline to affect the nicotine intake while leaving EtOH intake relatively unchanged when these substances are made concurrently available.

In the absence of varenicline pretreatment, increasing the price of nicotine decreases nicotine intake and increases EtOH consumption. However, the relatively flat consumption curve for EtOH produced under similar conditions suggests that, following varenicline pretreatment, EtOH no longer acts as an economic substitute for nicotine, results that are supported by no significant differences in the interaction constants when compared to zero. One possible explanation for this is that the effects of varenicline as a partial agonist on nAChRs, a mechanism that is believed to reduce smoking by reducing cravings for and withdrawal symptoms from nicotine (D. Gonzales et al., 2006; D. E. Jorenby, Hays, Rigotti, Azoulay, et al., 2006; S. A. McKee et al., 2009), may also having concomitantly reduced craving for EtOH. These results suggest that treatment for co-use of EtOH and nicotine will likely benefit from a focus on disrupting the economic substitution relationship between these two substances via reduction in craving and withdrawal for both substances. Some caution is needed, however, because varenicline was only tested when the economic relationship between EtOH and nicotine was assessed with an FR manipulation, not a dose manipulation. Moreover, since varenicline was given over repeated sessions, we cannot rule out the possibility that tolerance or sensitization effects may have contributed to the overall economic relations observed between EtOH and nicotine observed here.
**Table 1.** Unit Price Conversions for Nicotine.

<table>
<thead>
<tr>
<th>FR Manipulations</th>
<th>Dose Manipulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR</td>
<td>Dose</td>
</tr>
<tr>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>0.03</td>
</tr>
<tr>
<td>8</td>
<td>0.03</td>
</tr>
<tr>
<td>12</td>
<td>0.03</td>
</tr>
<tr>
<td>20</td>
<td>0.03</td>
</tr>
<tr>
<td>30</td>
<td>0.03</td>
</tr>
<tr>
<td>45</td>
<td>0.03</td>
</tr>
<tr>
<td>60</td>
<td>0.03</td>
</tr>
<tr>
<td>80</td>
<td>0.03</td>
</tr>
<tr>
<td>105</td>
<td>0.03</td>
</tr>
<tr>
<td>135</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Figure 20 Results from Study 4, Experiment 1. The normalized intake of EtOH and nicotine as a function of the changing unit price of nicotine. Values are expressed as (log) % of maximum intake for individual subjects. Curves were derived from own-price (nicotine) and cross-price (EtOH) elasticity of demand equations.
Figure 21 Results from Study 4, Experiment 2. The normalized intake of EtOH and nicotine as a function of the changing unit price of nicotine during baseline conditions and following pretreatment with 3 mg/kg varenicline. Values are expressed as (log) % of maximum intake for individual subjects. Curves were derived from own-price (nicotine) and cross-price (EtOH) elasticity of demand equations.
CHAPTER SIX

6. Conclusions

The current dissertation aimed to develop a translational animal model of pharmacologically relevant intake of EtOH and nicotine when both substances were made available concurrently for use in testing potential pharmacotherapeutic treatments for co-use of EtOH and nicotine. This dissertation also sought to describe the economic relationship between concurrently available EtOH and nicotine by applying behavioral economic principles to derive elasticity of demand equations from co-use data collected. Using these equations, the current dissertation aimed to elucidate factors that contribute to the high rate of co-use of EtOH and nicotine to aid in the ongoing research directed towards discovery of a single pharmacological treatment for EtOH and nicotine co-use disorder.

The first study used a novel 2-bottle and 2-lever choice model of EtOH and nicotine co-use in female P rats to assess the effects of varenicline, r-bPiDI, and RMOD on co-use behavior. Results from this experiment showed that pharmacologically relevant levels of both EtOH and nicotine intake were achieved when each substance was given alone. However, only pharmacologically relevant levels of nicotine intake were maintained when both EtOH and nicotine were available concurrently. One possible explanation for these results is the differences in the pharmacokinetics of EtOH and nicotine. The onset of action for i.v. nicotine is approximately 20-30 seconds (Jed E Rose, Frederique M Behm, Eric C Westman, Edward D Levin, Roy M Stein, James D Lane, et al., 1994), whereas oral EtOH can take 30-90 minutes to reach peak effects.
(Pohorecky & Brick, 1988). The faster pharmacologic effects of nicotine may contribute to the observed preference for nicotine of EtOH during these restricted access sessions.

Results for the drugs tested in this model, varenicline, r-bPiDI, and RMOD, revealed that all reduced nicotine self-administration, but not EtOH consumption during co-use. These results indicate that pharmacotherapeutics which may be successful smoking cessation aids via selective inhibition of α4β2 or α6β2* nAChRs, or DAT inhibition as afforded by the atypical inhibitor RMOD, may not be sufficient in treating co-use of EtOH and nicotine (Maggio, Saunders, Baxter, et al., 2018). However, the inability of these drugs to reduce EtOH intake may be due, at least in part, to the low levels of EtOH intake observed in concurrent access phases.

The second study of this dissertation sought to explore modifications to our original procedure with the goal of finding a model that produces pharmacologically relevant levels of intake for both EtOH and nicotine. Findings from the second study of this dissertation revealed that modifying our EtOH-nicotine co-use protocol by increasing the FR requirement for nicotine to FR30 was successful in achieving pharmacologically relevant levels of EtOH and nicotine intake when both substances were made concurrently available. These co-use procedures were also used to assess the effects of opiate and nicotine receptor-selective drugs. Naltrexone significantly decreased EtOH intake only when nicotine was concurrently available. Varenicline and r-bPiDI both dose-dependently reduced nicotine self-administration, but not EtOH drinking. While these results suggest that none of the drugs tested are effective as a monotherapy for co-use of EtOH and nicotine, results from the modified version of our co-use model demonstrated that this procedure was effective as a translational animal model for achieving
pharmacologically relevant levels of EtOH and nicotine intake during concurrent access sessions.

The aim of the third study was to apply behavioral economic principles of demand to determine how the changing price of nicotine affects consumption of concurrently available EtOH and nicotine. Using the co-use model developed in our laboratory, co-use of concurrently available EtOH and nicotine was examined using two behavioral economic demand curves: 1) own price elasticity of demand for nicotine and 2) cross-price elasticity of demand for EtOH. As the price of nicotine increased, nicotine intake decreased. Across increasing price for nicotine, EtOH acted as an economic substitute for nicotine such that decreases in nicotine intake were accompanied by increases in EtOH consumption. Preference switched from nicotine to EtOH when the cost for nicotine exceeded FR30, at which intake levels of both EtOH and nicotine were pharmacologically relevant, highlighting the value of this model for use in the future of testing treatments for EtOH and nicotine co-use disorder.

In the final study of the current dissertation, the main goal was to test the effects of varenicline pretreatment on elasticity of demand for concurrently available EtOH and nicotine across changing price for nicotine. However, although FR requirement changes are traditionally used for price changes in animal studies, research has shown that the dimension of the reinforcer critically impacts the demand curve derived from behavior to obtain reinforcers (Hursh & Roma, 2016). Specifically, the sensitivity of nicotine intake to changes in price was significantly greater when price was changed via a dose manipulation compared to an FR manipulation. Additionally, the substitution relationship between EtOH and nicotine was stronger when the price of nicotine was changed via an
FR manipulation compared to a dose manipulation. These results violate the invariance assumption that suggests setting price equivalence between our two conditions would produce equivalent curves, as the curves produced for dose change and FR change were significantly different in both sensitivity to price change and scaling constant.

In Experiment 2 of this study, varenicline pretreatments were given prior to sessions for each FR value used in this study. As expected, nicotine intake decreased as FR requirement increased. Following varenicline pretreatment, nicotine intake decreased at all unit prices for nicotine. Results also showed there were no significant differences in EtOH intake between control conditions and following varenicline pretreatment. Thus, showing varenicline pretreatment affects nicotine intake, but not EtOH intake, across increasing price for nicotine. Interestingly, decreases in nicotine intake did not result in increases in EtOH intake across increasing price for nicotine, despite previous results that show in the absence of varenicline pretreatment, increasing the price of nicotine decreases nicotine intake and increases EtOH consumption. Thus, in contrast to the economic substitution relationship that is seen when the unit price of nicotine is increased in the absence of varenicline pretreatment, it appears that EtOH demand is acting independently of varenicline-induced decreases in nicotine across increasing unit price for nicotine. This suggests that the ability of a pharmacotherapeutic to reduce craving and withdrawal for nicotine may also be useful in reducing EtOH co-use by reducing craving to substitute for nicotine intake decreases via increases in EtOH consumption.

One limitation of the current dissertation is that co-use was only studied in female rats. As noted by Frezza et al. (1990), there are important sex differences in the pharmacokinetics of EtOH. Research has shown that women have decreased activity in
ADH, and therefore less gastric metabolism of EtOH, compared to men. Additionally, men have a greater ratio of muscle to fat, which can dilute EtOH more in men due to the larger vascular compartment resulting from greater muscle ratio. Finally, women also have higher body fat than men. Since fat contains little EtOH, the concentration of EtOH in the blood is higher in women, drink for drink, even after adjusting for weight. Taken together, at similar levels of EtOH consumption, these differences can increase BEC by approximately 7% in women compared to men, even after adjusting for body weight (Frezza et al., 1990). Previous research in rats has revealed sex differences in nicotine-taking behaviors between males and females. Research shows that female rats tend to self-administer more nicotine than males (Flores, Uribe, Swalve, & O'Dell, 2017) and have a higher motivation for nicotine self-administration (Donny et al., 2000). In humans, research indicates that there may also be sex differences in the reactivity to smoking cues (Dumais et al., 2017), self-reported reinforcing effects (Perkins et al., 2006), and general use of nicotine (Torchalla et al., 2011). For these reasons, future research will benefit from examining co-use of EtOH and nicotine in both male and female subjects.

Another limitation of the current studies is that the effects of varenicline were only examined in the model that used an FR manipulation as the dimension for changing the price for nicotine. Results showed that the dimension by which the price of nicotine is changed impacted the pattern of EtOH and nicotine intakes across price changes and that the relationship between EtOH and nicotine intake was affected by pretreatment with varenicline. Additionally, using an FR manipulation to change the price for nicotine was limited by the necessity to change nicotine price in an increasing fashion across sessions. In the between-session FR change model, varenicline pretreatments were given at each
FR increase for nicotine. Since each increase in nicotine price was accompanied by an increase in the number of varenicline pretreatments each subject underwent, this introduced the possibility that tolerance to repeated varenicline pretreatments prior to each FR increment might have induced tolerance. That is, using the dose change model would have eliminated this problem, as it allows for randomization of price changes as opposed to only increasing nicotine price. For these reasons, the effects of varenicline pretreatment using the dose change model should be examined in future research.

Overall, the current dissertation was successful in developing a translational animal model in female P rats for co-use of EtOH and nicotine under which pharmacologically relevant levels of both EtOH consumption and nicotine intake are achieved. This model was successfully used in testing potential pharmacotherapeutics for the treatment of EtOH and nicotine co-use disorder. Although none of the drugs tested were effective as a monotherapy, results from testing the known smoking cessation agent varenicline and the known AUD treatment naltrexone indicate that our model is effective for selectively measuring changes in EtOH and nicotine intake separately, which suggests the beneficial utility of this model for future treatment research. Additionally, by applying behavioral economic principles to our findings, we were able to determine that there are differences in the elasticity of demand for concurrently available EtOH and nicotine when the cost of nicotine is changed via response requirements vs dose per infusion, findings that will require further investigation in order to determine how the dimension of drug reinforcers impacts demand. Finally, with regard to the effect of varenicline on cross-price elasticity of demand, the relatively flat consumption curve for EtOH suggests that, following varenicline pretreatment, EtOH no longer acts as an
economic substitute for nicotine. These findings suggest that varenicline may act to disrupt the economic substitution relationship between these two substances via reduction in craving and withdrawal for both substances.
APPENDICES

Appendix A: Timeline for Pilot 1 Experiment

Phase 1
15% EtOH

Phase 2
Food
Surgery/Recovery
Nicotine

Phase 3
15% EtOH
+ Nicotine

FR1 FR3 FR5 FR1 FR3 FR5

Days
Appendix B: Timeline for Pilot 2 Experiment
Appendix C: Timeline for Pilot 3 Experiment
Appendix D: Equations for the Normalized Intake of Nicotine and EtOH

For nicotine:

\[
\left(\frac{\text{Nicotine intake in mg/kg}}{\text{Individual's maximum nicotine intake in mg/kg}}\right) \times 100
\]

= Nicotine intake as a % of maximum nicotine intake

For EtOH:

\[
\left(\frac{\text{EtOH intake in g/kg}}{\text{Individual's maximum EtOH intake in g/kg}}\right) \times 100
\]

= EtOH intake as a % of maximum EtOH intake

For water:

\[
\left(\frac{\text{water intake in g/kg}}{\text{Individual's maximum water intake in g/kg}}\right) \times 100
\]

= water intake as a % of maximum water intake
References


Basavarajappa, B. S., & Hungund, B. L. (1999a). Chronic ethanol increases the cannabinoid receptor agonist anandamide and its precursor N-


Boeker, T. K., & Collins, A. C. (1997). Long-term ethanol treatment elicits changes in nicotinic receptor binding in only a few brain regions. *Alcohol (Fayetteville, N.Y.), 14*(2), 131-140. doi:https://doi.org/10.1016/S0741-8329(96)00116-4


doi: http://dx.doi.org/10.15585/mmwr.mm6544a2


Levin, E. D., McClernon, F. J., & Rezvani, A. H. (2006). Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and
doi:10.1007/s00213-005-0164-7

doi:10.1007/s002130050667

doi:10.1016/j.ejphar.2015.06.041


Effects of naltrexone, varenicline, and the selective nicotinic α6β2* antagonist r-bPiDI. *Drug Alcohol Depend, 193,* 154-161.


Oncken, C., Gonzales, D., Nides, M., & et al. (2006). Efficacy and safety of the novel selective nicotinic acetylcholine receptor partial agonist, varenicline, for smoking cessation. *Archives of internal medicine, 166*(15), 1571-1577. doi:10.1001/archinte.166.15.1571


Sotomayor-Zarate, R., Gysling, K., Busto, U. E., Cassels, B. K., Tampier, L., & Quintanilla, M. E. (2013). Varenicline and cytisine: two nicotinic acetylcholine


dependent decline in mice. *Proceedings of the National Academy of Sciences, 100*(3), 1393-1398.


Sarah Elizabeth Maggio
Baltimore, MD

Education

B.A. Degree in Psychology 2012
*University of North Carolina – Wilmington, Wilmington, NC.*
Concentration in Psychopharmacology and Animal Behavior;
Graduated with University Honors, cum laude with 3.57 G.P.A.

M.A. Degree in Psychology 2014
*University of North Carolina – Wilmington, Wilmington, NC.*
Concentration in Behavioral Psychopharmacology;
Graduated with 3.89 G.P.A.

PhD. In Psychology 2019
*University of Kentucky, Lexington, KY.*
Concentration in Behavioral Neuroscience and Psychopharmacology;
Current 3.9 G.P.A.

Career History

**Research Assistant, Bardo Lab,**
*Department of Psychology, University of Kentucky* Fall 2014-Summer 2019

**Teaching Assistant, Advanced Behavioral Neuroscience,**
*Department of Psychology, University of Kentucky* Fall 2017 & Fall 2018

**Teaching Assistant, Learning and Cognition,**
*Department of Psychology, University of Kentucky* Spring 2018

**Teaching Assistant, Introduction to Psychology,**
*Department of Psychology, University of Kentucky* 2015

**Teaching Assistant, Cognitive Psychology and Experimental Psychology,**
*UNCW Department of Psychology* 2012-2014

**Psychology Research Graduate Assistant,**
*UNCW Department of Psychology* 2012-2013

**Graduate Assistant,**
*UNCW Center for the Support of Undergraduate Research and Fellowships* 2014

Honors and Awards

**T32 Pre-doctoral Research Fellowship** Fall 2015-Summer 2017
*Training in Drug Abuse Related Research – CRAN Supplement*
College of Pharmacy, Department of Pharmaceutical Sciences
*University of Kentucky, Lexington, KY*

**NIDA Director’s Travel Award** Summer 2017
*College on Problems of Drug Dependence*

**Lipman Research Fund for the Prevention of Drug and Alcohol Abuse** 2018
*UK Department of Psychology*
Publications


