CONCURRENT SELF-ADMINISTRATION OF
ALCOHOL AND NICOTINE IN AN OPERANT PARADIGM

by

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A thesis submitted in conformity with the requirements
for the degree of Master of Science
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Rationale and objectives: Alcohol and nicotine are the most commonly abused drugs and they are often taken together. To help address some of clinical issues regarding nicotine and alcohol co-dependence, a procedure in which rats self-administer nicotine intravenously and alcohol orally during the same operant session has been developed. 

Methods: Male Wistar rats were trained to self-administer alcohol (12%, w/v; 0.19 ml/delivery) or implanted with jugular catheters and trained to self-administer nicotine (30 µg/kg IV/infusion) by pressing a lever or were trained to self-administer both drugs, some with alcohol first, and others with nicotine first. Results: Animals readily co-administered alcohol and nicotine concurrently. Access to alcohol reduced nicotine self-administration significantly. Conclusions: These results show that rats will self-administer relevant amounts of intravenous nicotine and oral alcohol concurrently. They also provide further support for the important relationship between nicotine and alcohol.
To my father, who taught me to work hard and play hard.

To my mother, who always wants me to be the best of the best.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
<tr>
<td>1. GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1. Nicotine and Alcohol Studies in Humans</td>
<td>2</td>
</tr>
<tr>
<td>1.1.1. Laboratory studies in humans</td>
<td>2</td>
</tr>
<tr>
<td>1.1.1.1. Effect of alcohol on tobacco cigarette seeking</td>
<td>2</td>
</tr>
<tr>
<td>1.1.1.2. Effect of cigarette smoking on alcohol drinking</td>
<td>3</td>
</tr>
<tr>
<td>1.1.1.3. Summary for laboratory studies in humans</td>
<td>4</td>
</tr>
<tr>
<td>1.1.2. Genetic studies in humans</td>
<td>4</td>
</tr>
<tr>
<td>1.2. Experimental Studies in Animals</td>
<td>5</td>
</tr>
<tr>
<td>1.2.1. Behavioral studies</td>
<td>5</td>
</tr>
<tr>
<td>1.2.1.1. Effects of nicotine on alcohol consumption</td>
<td>5</td>
</tr>
<tr>
<td>1.2.1.2. Effects of alcohol on nicotine intake</td>
<td>7</td>
</tr>
<tr>
<td>1.2.1.3. Summary for behavioral studies</td>
<td>7</td>
</tr>
<tr>
<td>1.2.2. Genetic studies</td>
<td>8</td>
</tr>
<tr>
<td>1.2.3. Neuropharmacological studies on nicotinic acetylcholine receptors (nAChR)</td>
<td>8</td>
</tr>
<tr>
<td>1.2.3.1. Effects of nicotine on nicotinic acetylcholine receptors</td>
<td>8</td>
</tr>
<tr>
<td>1.2.3.2. Effects of alcohol on nicotinic acetylcholine receptors</td>
<td>10</td>
</tr>
<tr>
<td>1.2.3.3. Summary of neuropharmacological studies on nicotinic acetylcholine receptors</td>
<td>11</td>
</tr>
<tr>
<td>1.2.4. Neurochemical studies</td>
<td>12</td>
</tr>
<tr>
<td>1.2.4.1. Pedunculopontine mesencephalic tegmentum</td>
<td>13</td>
</tr>
</tbody>
</table>
1.2.4.1. Neuroanatomy of pedunculopontine mesencephalic tegmentum  
1.2.4.1.2. Role of pedunculopontine mesencephalic tegmentum in nicotine reinforcement

1.2.4.2. Mesolimbic dopamine system
1.2.4.2.1. Neuroanatomy of mesolimbic dopamine system
1.2.4.2.2. Role of mesolimbic dopamine system in nicotine reinforcement
1.2.4.2.3. Involvement of mesolimbic dopamine in alcohol reinforcement
1.2.4.2.4. Mesolimbic dopamine in the interaction between nicotine and alcohol

1.2.4.3. Summary for neurochemical studies

1.3. Animal Models

1.3.1. Nicotine self-administration

1.3.1.1. Route of nicotine self-administration
1.3.1.1.1. Oral administration
1.3.1.1.2. Intravenous self-administration
1.3.1.1.3. Summary for route of nicotine self-administration

1.3.1.2. Rate of intravenous nicotine infusion
1.3.1.3. Schedule of access
1.3.1.4. Session duration
1.3.1.5. Dose

1.3.2. Alcohol self-administration
1.3.2.1. Two-bottle choice paradigm
1.3.2.2. Techniques to increase oral alcohol intake
1.3.2.3. Session duration

1.4 Summary

2. PURPOSE OF INVESTIGATION
3. GENERAL MATERIAL AND METHODS

3.1. Animals

3.2. Apparatus
   3.2.1. Drinking cages
   3.2.2. Operant chamber

3.3. Surgery – Implantation of Intravenous Catheters
   3.3.1. Anaesthesia
   3.3.2. Implantation of Intravenous Catheters
      3.3.2.1. Construction of intravenous catheters
      3.3.2.2. Implantation

3.4. Experimental Procedures
   3.4.1. Two-bottle choice paradigm
   3.4.2. Sucrose self-administration
   3.4.3. Alcohol self-administration
   3.4.4. Nicotine self-administration
   3.4.5. Nicotine and alcohol co-administration

3.5. Drugs

3.6. Data analysis and presentation

4. CONCURRENT SELF-ADMINISTRATION OF INTRAVENOUS NICOTINE AND ORAL ALCOHOL IN RODENTS
   Experiment 1: Self-Administration of Alcohol prior to Nicotine

4.1. Introduction
4.2. Materials and Methods 55
  4.2.1. Animals 55
  4.2.2. Experimental design 55
  4.2.3. Statistics 57

4.3. Results 58

4.4. Discussion 64

5. CONCURRENT SELF-ADMINISTRATION OF INTRAVENOUS NICOTINE AND ORAL ALCOHOL IN RODENTS 69
  Experiment 2: Self-Administration of Nicotine prior to Alcohol

  5.1. Introduction 69

  5.2. Materials and Methods 72
    5.2.1. Animals 72
    5.2.2. Experimental design 72
    5.2.3. Statistics 75

  5.3. Results 74

  5.4. Discussion 76

6. GENERAL DISCUSSION 89

7. FUTURE WORK 102

  7.1. Future Investigations 102
    7.1.1. Determination of nicotine and alcohol dose-response 103
    7.1.2. Effect of session duration on concurrent self--administration of nicotine and alcohol 104
7.1.3. Effect of a block design on self-administration of nicotine and alcohol 106

7.2. Applications of Concurrent Self-administration Procedure 107
   7.2.1. Effect of pharmacological agents on co-administration of nicotine and alcohol 107
      7.2.1.1. Naltrexone 108
      7.2.1.2. Varenicline 109

8. APPENDIX 111
   Experiment 3: Extinction and Reinstatement in Rats with Concurrent Access to Alcohol and Nicotine

8.1. Introduction 111

8.2. Materials and Methods 112
   8.2.1. Animals 112
   8.2.2. Experimental design 112
   8.2.3. Statistics 114

8.3. Results 114

8.4. Discussion 119

REFERENCE 121
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
</tr>
<tr>
<td>ADH</td>
<td>alcohol dehydrogenase</td>
</tr>
<tr>
<td>CYP</td>
<td>cytochrome P450</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DHβE</td>
<td>dihydro-beta-erythroidine</td>
</tr>
<tr>
<td>FR</td>
<td>fix-ratio</td>
</tr>
<tr>
<td>g/kg</td>
<td>gram per kilogram of body weight</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
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<tr>
<td>IM</td>
<td>intramuscular</td>
</tr>
<tr>
<td>IP</td>
<td>intraperitoneal</td>
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<tr>
<td>IV</td>
<td>intravenous</td>
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<tr>
<td>LDT</td>
<td>laterodorsal tegmental</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>MLA</td>
<td>methyllycaconitine</td>
</tr>
<tr>
<td>NAc</td>
<td>nucleus accumben</td>
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<tr>
<td>nAChR</td>
<td>nicotinic acetylcholine receptor</td>
</tr>
<tr>
<td>NP</td>
<td>alcohol non-preferring</td>
</tr>
<tr>
<td>P</td>
<td>alcohol-preferring</td>
</tr>
<tr>
<td>PCP</td>
<td>phencyclidine</td>
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<td>PMT</td>
<td>pedunculopontine mesencephalic tegmentum</td>
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<tr>
<td>PPT</td>
<td>pedunculopontine tegmental</td>
</tr>
<tr>
<td>PR</td>
<td>progressive-ratio</td>
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<tr>
<td>SC</td>
<td>subcutaneous</td>
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<tr>
<td>SN</td>
<td>substantia nigra</td>
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<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
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<tr>
<td>w/v</td>
<td>weight per volume</td>
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<tr>
<td>α</td>
<td>alpha</td>
</tr>
<tr>
<td>α-BTX</td>
<td>alpha-bungarotoxin</td>
</tr>
<tr>
<td>α-CTXMII</td>
<td>alpha-conotoxin MII</td>
</tr>
<tr>
<td>β</td>
<td>beta</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 4.1.  \textit{Mean (± SEM) nicotine and alcohol intake in Experiment 1}  

Table 5.1. \textit{Mean (± SEM) nicotine and alcohol intake in Experiment 2}
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Schematic anatomy of a rat brain</td>
<td>14</td>
</tr>
<tr>
<td>1.2</td>
<td>Schematic representation of some ascending mesolimbic dopaminergic pathways</td>
<td>16</td>
</tr>
<tr>
<td>3.1</td>
<td>Schematic representation of the operant chamber interior</td>
<td>37</td>
</tr>
<tr>
<td>3.2</td>
<td>Catheter used for intravenous nicotine infusion in rodent self-administration research</td>
<td>39</td>
</tr>
<tr>
<td>4.1</td>
<td>Illustration of the nicotine and/or alcohol training regimens in Experiment 1</td>
<td>55</td>
</tr>
<tr>
<td>4.2</td>
<td>Nicotine or alcohol self-administration and co-administration of both drugs in Experiment 1</td>
<td>60</td>
</tr>
<tr>
<td>4.3</td>
<td>Training for co-administration of nicotine and alcohol in Experiment 1</td>
<td>61</td>
</tr>
<tr>
<td>5.1</td>
<td>Illustration of the nicotine and/or alcohol training regimens in Experiment 2</td>
<td>72</td>
</tr>
<tr>
<td>5.2</td>
<td>Comparison of nicotine or alcohol self-administration of both drugs in Experiment 2</td>
<td>78</td>
</tr>
<tr>
<td>5.3</td>
<td>Training for co-administration of nicotine and alcohol in Experiment 2</td>
<td>79</td>
</tr>
<tr>
<td>5.4</td>
<td>Pattern of self-administration in extension of the session duration in Experiment 2</td>
<td>81</td>
</tr>
<tr>
<td>5.5</td>
<td>Comparison of alcohol self-administration in nicotine experienced alcohol group with alcohol alone group in Experiment 2</td>
<td>82</td>
</tr>
<tr>
<td>5.6</td>
<td>Comparison of pattern of self-administration in nicotine experienced alcohol group with alcohol alone group in Experiment 2</td>
<td>83</td>
</tr>
<tr>
<td>8.1</td>
<td>Responding of animals trained to co-administer nicotine and alcohol subject to different extinction conditions</td>
<td>116</td>
</tr>
<tr>
<td>8.2</td>
<td>Reinstatement induced by nicotine or alcohol primes in rats trained to co-administer nicotine and alcohol subject to different extinction conditions</td>
<td>118</td>
</tr>
</tbody>
</table>
Chapter 1
GENERAL INTRODUCTION

Alcohol and nicotine, in the form of tobacco, are two of the most frequently used psychoactive substances in society and are often used together. Epidemiological studies have shown that 80 to 95 percent of alcoholics smoke (DiFranza and Guerrera, 1990; Patten et al., 1996; Miller and Gold, 1998) as compared to 20 to 30 percent of the general population who are smokers (Hughes, 1995). Smokers are 1.32 times more likely to consume alcohol regularly than non-smokers (Shiffman and Balabanis, 1995). In addition, heavier drinkers tend to be heavier smokers who smoke each cigarette more intensely than non-alcoholic smokers (Keenan et al., 1990). Alcoholics, on average, consume significantly more tobacco cigarettes than non-alcoholics. Seventy percent of alcoholics smoke more than one and half packs of tobacco cigarettes per day, but only 10 percent of non-alcoholic smokers smoke this much (Hughes, 1995). Several epidemiological studies have demonstrated that alcohol increases the amount and rate of cigarette smoking (for a review, see Istvan and Matarazzo, 1984). These studies all suggest that there is a positive correlation between alcohol consumption and tobacco cigarette use.

In this chapter, human studies in a controlled laboratory environment, and genetic studies in humans will be reviewed to further explore the positive relationship between alcohol drinking and cigarette smoking. Then, laboratory animal studies concerning the nicotine and alcohol interactions will be reviewed.
1.1 Nicotine and Alcohol Studies in Humans

Controlled laboratory studies in humans exploring the relationship between alcohol drinking and cigarette smoking have shown there is a pharmacological interaction between alcohol and nicotine. Similarly, genetic studies in humans have shown there is a strong genetic basis for the co-transmission of the proclivity to abuse alcohol and nicotine.

1.1.1 Laboratory studies in humans

1.1.1.1 Effect of alcohol on tobacco cigarette seeking

The majority of studies investigating the relationship of alcohol and nicotine co-abuse in humans have examined the effects of alcohol on measures of tobacco cigarette seeking and consumption. Acute administration of alcohol is known to increase the number of cigarettes smoked by alcoholics (Griffiths et al., 1976; Henningfield et al., 1984), and non-alcoholics (Mello et al., 1980; Mitchell et al., 1995). Studies using non-consumptive measures of craving have found that alcohol increases the urge to smoke in non-alcoholic cigarette smokers (Burton and Tiffany, 1997; Epstein et al., 2007) in a dose-dependent manner (King and Epstein, 2005). Consistent with this, alcohol consumption increases self-rated measures of smoking satisfaction, and potentiates the higher rated satisfaction of nicotinized (Glautier et al., 1996), compared to denicotinized cigarettes (Rose et al., 2004) for both men and women (King et al., 2008; King et al., 2009). However, the alcohol-induced smoking behavior was only observed in men as measured by choice of cigarette (nicotinized versus denicotinized cigarette), puff count,
puff volume, and puff duration (King et al., 2009). Together, these studies point out that alcohol consumption increases craving and smoking urges in both men and women. Such increases in smoking urges induced by exposure to alcohol, however, results only in an increase in cigarette or nicotine consumption in men but not in women. The mechanisms underlying such gender differences in nicotine consumption induced by alcohol remains to be explored.

1.1.1.2 Effect of cigarette smoking on alcohol drinking

The effects of cigarette smoking on alcohol drinking also have been examined. Acute cigarette smoking is known to increase alcohol related responding in the male non-alcoholic regular smoker (Perkins et al., 2000), and also in male occasional smokers (Barrett et al., 2006). In one study, male smokers who were asked to smoke regular or denicotinized cigarettes while performing a progressively more demanding task were rewarded with the opportunity to drink alcoholic beverages. Participants who smoked nicotine-containing cigarettes drank more, and worked harder for alcohol than those who smoked the denicotinized cigarettes (Barrett et al., 2006). Furthermore, the acute effects of alcohol, such as alcohol-induced euphoria and the feeling of relaxation, were reported to be enhanced by nicotine pretreatment in men (Kouri et al., 2004). Interestingly, the effects of nicotine on alcohol consumption may be sex dependent. Male subjects pretreated with nicotine consumed more alcohol than placebo, but similar treatment reduced alcohol consumption in women (Perkins et al., 1995; Perkins et al., 2000; Acheson et al., 2006). These studies suggest the potential of nicotine for increasing alcohol consumption, at least in men.
1.1.1.3 Summary for laboratory studies in humans

All these aforementioned studies in humans show that the increased consumption of either nicotine or alcohol can be induced by administration of the other drug in men. While there are potential sex differences in the effect of alcohol on tobacco cigarette seeking, as well as in the effect of cigarette smoking on alcohol drinking, nevertheless, the urge and satisfaction of taking either nicotine or alcohol are potentiated by the other drug.

1.1.2 Genetic studies in humans

A number of studies suggest that genetic predisposition to use nicotine and alcohol is co-inherited. Genetic factors are known to contribute to nicotine and alcohol use in humans (Tyndale, 2003). Results from twin and family studies have consistently reported substantial genetic influence on both nicotine addiction (Carmelli et al., 1992) and alcohol addiction (Hettema et al., 1999; Mérette et al., 1999; Enoch and Goldman, 2001); and these genetic factors may predispose human to co-abuse nicotine and alcohol (Swan et al., 1997; Bierut et al., 2004; Goldman et al., 2005). For example, researchers have reported that the best-fitting model for the lifetime co-occurrence of nicotine and alcohol addiction includes a substantial genetic correlation between these disorders (True et al., 1999).
1.2 Experimental Studies in Animals

Experimental studies with animals have shown a pharmacological basis for the co-use of nicotine and alcohol, and they have provided potential mechanisms underlying this co-abuse. In this section, the evidence from animal studies concerning the interaction between nicotine and alcohol in self-administration will be presented, and is followed by a review of potential neuropharmacological and neurochemical mechanisms that support such an interaction.

1.2.1 Behavioral studies

1.2.1.1 Effects of nicotine on alcohol consumption

Numerous studies on laboratory rodents have examined the effects of nicotine on alcohol consumption. The most common behavioral finding is that repeated or chronic administration of nicotine to rodents increases voluntary alcohol consumption. Rats implanted with subcutaneous nicotine pellets consumed much more alcohol than did rats which had placebo pellets implanted. However, the implantation of subcutaneous nicotine pellets did not modify the consumption of a flavored fluid, which was the caloric equivalent of the alcohol (Potthoff et al., 1983). This indicates that nicotine specifically results in increased alcohol self-administration. Similarly, chronic and repeated treatment with nicotine either by subcutaneous injections (Blomqvist et al., 1996; Smith et al., 1999; Lê et al., 2000; Söderpalm et al., 2000; Olausson et al., 2001; Lê et al., 2003) or by osmotic minipump (Clark et al., 2001) has significantly increased home-cage intake (Potthoff et al., 1983; Blomqvist et al., 1996; Smith et al., 1999; Olausson et al., 2001)
and operant self-administration of alcohol (Lê et al., 2000; Clark et al., 2001). Another group has, however, reported decreases in alcohol intake with repeated nicotine injections (Sharpe and Samson, 2002).

The disparity in results between most of these studies and the one conducted by Sharpe and Samson (2002) may be due to a number of reasons. Most of the studies which found that repeated administration of nicotine increased voluntary alcohol consumption in animals have used the Wistar strain of rats (Blomqvist et al., 1996; Smith et al., 1999; Lê et al., 2000; Olausson et al., 2001) rather than the Long-Evans strain of rats (Sharpe and Samson, 2002; Lê et al., 2003). Differences in behavioral responses to nicotine (Acri et al., 1991; Faraday et al., 1998) and alcohol (Gauvin et al., 1993) have been observed between different strains of rats. As well, different methods employed to initiate alcohol consumption as well as the selection of high and low alcohol consumption subjects might play a role in the observed effects of nicotine on alcohol consumption (Blomqvist et al., 1996; Smith et al., 1999; Lê et al., 2000; Olausson et al., 2001; Lê et al., 2003). In addition, the duration of the test sessions in most of the studies was longer than an hour (Potthoff et al., 1983; Blomqvist et al., 1996; Smith et al., 1999; Lê et al., 2000; Olausson et al., 2001; Lê et al., 2003) as compared to 20 minutes (Sharpe and Samson, 2002). Studies have shown that most of the increase in voluntary alcohol consumption induced by nicotine in a test session occurred later in the session rather than during the first 20 to 30 minutes (Lê et al., 2000).
1.2.1.2 Effects of alcohol on nicotine intake

Contrasted with the many studies examining the effects of nicotine on alcohol intake in laboratory animals, there are only few published studies with laboratory animals investigating the effects of alcohol on nicotine self-administration. One study, with twice-daily intraperitoneal (IP) injections of 0.905g/kg alcohol for 3 days, reported moderate increases in intravenous nicotine self-administration in laboratory rodents in a 24 hour access design (Hanson et al., 1979). However, another study showed that repeated treatment with daily IP injections of alcohol (0.25g/kg and 0.50g/kg) for 10 days did not have any effect on intravenous nicotine self-administration in daily 1 hour sessions (Wang, 2003). The difference in results between these two studies may be due to the different strains of rats (i.e. Sprague-Dauley versus Long-Evan) and session durations (unlimited access versus limited access). In addition, the difference in the dose of alcohol given (i.e. 0.905g/kg versus 0.50g/kg and 0.25g/kg) and the unit dose of nicotine for self-administration (i.e. 60μg versus 30μg) are other possible factors. Several behavioral studies with rodents have shown the level of response to nicotine and the total nicotine intake are dependent on the unit dose of nicotine for self-administration (Cox et al., 1984; Corrigall and Coen, 1989; Donny et al., 1995).

1.2.1.3 Summary for behavioral studies

Taken together, these results provide evidence that nicotine can potentiate the rewarding effects of alcohol. However, the effect of alcohol on nicotine self-administration in laboratory animals remains unclear.
1.2.2 Genetic studies

Experiments with animals involving selectively bred rodents have demonstrated a genetic contribution to responses to nicotine and alcohol. For example, mice or rats bred for increased responsiveness to the sedative effects of alcohol demonstrate increased sensitivities to nicotine-induced hypothermia and hypoactivity (De Fiebre et al., 1987; De Fiebre et al., 1991). In other studies employing alcohol-preferring (P) and alcohol non-preferring (NP) rat lines, P rats were found to be more sensitive to nicotine than NP rats in a drug discrimination procedure (Gordon et al., 1993; McMillan et al., 1999). P rats also self-administered greater amounts of nicotine intravenously and sought nicotine much more robustly in reinstatement tests (Lê et al., 2006). Interestingly, P and NP rats do not differ in cocaine self-administration indicating that the underlying genetic differences are specific for nicotine and alcohol (Lê et al., 2006).

Genetic studies with experimental animals have shown that genetic factors are involved in the nicotine and alcohol dependence, and have further affirmed the predisposition to consume nicotine and alcohol is co-inherited.

1.2.3 Neuropharmacological studies on nicotinic acetylcholine receptors (nAChR)

1.2.3.1 Effects of nicotine on nicotinic acetylcholine receptors

Nicotine mediates its effects by directly binding to, and activating, nicotinic acetylcholine receptor (nAChR) (Benowitz, 1996; Dani and De Biasi, 2001). Each neuronal nAChR consists of 5 protein subunits, which traverse the cell membrane to form a complex around a central channel (Cooper et al., 1991; Lindstrom et al., 1996). Several
types of nAChR subunits are known (Gotti and Clementi, 2004). In the case of nAChR in
the brain, nAChR subunits are categorized into two families, alpha (α) and beta (β). Each
family has several members, which are labeled in numerical fashion. Of the nAChR
subtypes in the brain, the α-bungarotoxin (α-BTX) insensitive α4β2, and α-BTX sensitive
α7 nAChR subtypes are the two most prevalent ones (Davis and De Fiebre, 2006).
However, other nAChR subtypes such as α-Conotoxin MII (α-CTXMII) sensitive α3β2
and α6 have also been identified in the brain (Gotti and Clementi, 2004). Interestingly,
several nAChR subtypes, particularly the most prevalent ones, have been identified to be
harbored on mesolimbic dopamine (DA) neurons in the brain, especially in the ventral
tegmental area (VTA) (Larsson and Engel, 2004).

Besides activating the nAChRs, nicotine is also known to inactivate nAChRs via a
process called desensitization, which subsequently causes the upregulation of the receptor
number (Dani and De Biasi, 2001). This has been shown to occur in the brain (Marks et
al., 1985; Flores et al., 1992; Marks et al., 1992) as well as in cell culture systems (Peng
et al., 1994; Peng et al., 1997; Warpman et al., 1998). The suppression of nicotine-
induced locomotor activity elevation and nicotine intake in laboratory rodents after the
administration of mecamylamine, a non-competitive nicotinic antagonist, has further
supported the involvement of nAChRs in the rewarding effect of nicotine (Clarke and
Kumar, 1983b; Corrigall and Coen, 1989; Reavill and Stolerman, 1990).

There is a substantial interest in identifying the functional roles of the nAChR
subtypes in the rewarding effects of nicotine. The role of the most prevalent nAChR
subtype in the brain, α4β2 has been a foremost subject of research. An α4β2-specific
antagonist, dihydro-beta-erythroidine (DHβE) has attenuated nicotine self-administration
in laboratory rodents in a dose dependent manner (Corrigall et al., 1994; Watkins et al., 1999) and antagonized the nicotine-induced behavior (Stolerman et al., 1997). As well, varenicline, a partial α4β2 agonist, has attenuated nicotine self-administration (Rollema et al., 2007). Methyllycaconitine (MLA), a rather potent and selective antagonist of the α7 nAChR (Alkondon et al., 1992; Turek et al., 1995; Chavez-Noriega et al., 1997; Holladay et al., 1997) has been shown to antagonize nicotine self-administration (Markou and Paterson, 2001), nicotine-induced DA release in the nucleus accumben (NAc) (Larsson et al., 2002; Schilström et al., 2003), and nicotine-facilitated responses (Panagis et al., 2000). However, contradictory results have also been reported (De Fiebre et al., 1995; Brioni et al., 1996; Gordon et al., 1998; Grottick et al., 2000; Kempsill and Pratt, 2000). For example, microinjection of MLA into the VTA does not attenuate the level of nicotine self-administered by rodents or antagonize nicotine-induced hyperactivity (Grottick et al., 2000). Additionally, the α7-specific agonists have failed to stimulate locomotor activity, which was induced by nicotine (De Fiebre et al., 1995; Gordon et al., 1998). The functional roles of the α3β2 and α6 nAChR subtypes have also been investigated. Alpha-Conotoxin MII, a selective antagonist of the α3β2 (Cartier et al., 1996; Harvey et al., 1997) and α6 nAChR subtypes (Champtiaux et al., 2002) has been shown to inhibit nicotine-induced striatal DA release in slices and synaptosomes (Kulak et al., 1997).

**1.2.3.2 Effects of alcohol on nicotinic acetylcholine receptors**

Alcohol is known to increase agonist affinity of nAChRs (Forman et al., 1989), enhance the agonist-induced ion flux in nAChRs (Wu and Miller, 1994), and stabilize
nAChRs in the high affinity desensitized state (Wu et al., 1993). Alcohol also modulates nicotine-induced upregulation of nAChRs (Dohrman and Reiter, 2003), and increases the burst frequency of nAChR-containing neurons by augmenting the channel opening rate (Dilger et al., 1994; Liu et al., 1994). In vivo studies have shown that alcohol potentiates excitatory responses to nicotine (Criswell et al., 1993), as well as blocking the nicotine-induced inhibitory response in the brain (Yang et al., 1996).

Similarly, the functional roles of nAChR subtypes have been investigated for alcohol. Pretreatment with DHβE does not affect alcohol consumption in rodents (Lê et al., 2000), alcohol-induced locomotor activity (Larsson et al., 2002; Kamens and Phillips, 2008), DA overflow (Larsson et al., 2002; Ericson et al., 2003) or conditioned reinforcement to alcohol (Löf et al., 2007). However, another study has shown varenicline reduces alcohol consumption and seeking in rats (Steensland et al., 2007). As for identifying the functional role of α7 nAChR subtypes, MLA does not antagonize the alcohol-induced locomotor stimulatory effect nor the accumbal DA elevation (Larsson et al., 2002). Nicotinic acetylcholine receptor subtypes α3β2 and α6 have also been investigated. Alpha-Conotoxin MII has been shown to attenuate alcohol self-administration in laboratory rodents, and reduce alcohol-induced locomotor stimulatory and accumbal DA overflow (Larsson and Engel, 2004; Jerlhag et al., 2006; Löf et al., 2007).

1.2.3.3 Summary of neuropharmacological studies on nicotinic acetylcholine receptors

In summary, these reports suggest that the α4β2 nAChR subtype plays an important role in orchestrating rewarding profile of nicotine, but not of alcohol. The
reduction in alcohol consumption and seeking by varenicline (Steensland et al., 2007) may be due to its other pharmacological properties. Besides being a partial $\alpha_4\beta_2$ nAChR agonist, varenicline has a relatively high binding affinity at $\alpha_3$ nAChR subtype (Mihalak et al., 2006; Rollema et al., 2007; Steensland et al., 2007), which has been shown to play a significant role in alcohol self-administration (Larsson and Engel, 2004; Jerlhag et al., 2006; Löf et al., 2007). As for $\alpha_7$ nAChR subtype, its functional role in mediating the effect of nicotine is controversial while its involvement in mediating the effect of alcohol is absent. On the other hands, there are a number of studies showing the involvement of $\alpha_3\beta_2$ and/or $\alpha_6$ nAChR subtypes in mediating the effects of nicotine, and of alcohol (Larsson and Engel, 2004; Jerlhag et al., 2006; Löf et al., 2007), indicating that they may play a crucial role in modulating interaction between nicotine and alcohol. However, the functional roles of the $\alpha_3\beta_2$ and/or $\alpha_6$ nAChR subtypes remain to be further specified. Nevertheless, all these data provide strong support for the involvement of nAChRs in modulating interaction between nicotine and alcohol.

1.2.4 Neurochemical studies

A number of studies have demonstrated an important role for the pedunculopontine mesencephalic tegmentum (PMT) in the rewarding effects of nicotine. As well, many neurochemical studies investigating the mechanisms that underlie the rewarding effect of nicotine and alcohol have focused on a neural pathway in the brain known as the mesolimbic DA system. In this section, the neuroanatomy of these systems will be briefly reviewed. Then, the role of PMT in nicotine reinforcement, and the
involvement of mesolimbic DA in nicotine and in alcohol reinforcement will be examined.

1.2.4.1 Pedunculopontine mesencephalic tegmentum

1.2.4.1.1 Neuroanatomy of the pedunculopontine mesencephalic tegmentum

The pedunculopontine mesencephalic tegmentum is comprised of the pedunculopontine tegmental (PPT) nucleus and the laterodorsal tegmental (LDT) nucleus (Maskos, 2008), and it has been studied in relation to a variety of rewarded behaviors including the self-administration of drugs (Bechara and van der Kooy, 1989; Yeomans et al., 1993; Olmstead and Franklin, 1994; Olmstead and Franklin, 1997; Olmstead et al., 1998; Corrigall et al., 1999; Yeomans et al., 2000; Alderson et al., 2005; Lodge and Grace, 2006). The PPT nucleus extends from the posterior pole of the substantia nigra (SN) back to the lateral tip of the superior cerebellar peduncle (Martínez-Murillo et al., 1989). The LDT nucleus is dorsal and caudal to the neighbouring PPT nucleus, dorsally abutting the aqueduct, and at its caudal extreme positioned between the locus coerules and the fourth ventricle (Blaha et al., 1996), as schematically indicated in Figure 1.1. Both the PPT and the LDT contain cholinergic (Armstrong et al., 1983; Clarke and Kumar, 1983a; Sugimoto and Hattori, 1984; Beninato and Spencer, 1987; Beninato and Spencer, 1988; Gould et al., 1989; Bolam et al., 1991; Butcher et al., 1992) and non-cholinergic (Spann and Grofova, 1992; Honda and Semba, 1995; Steininger et al., 1997; Wang and Morales, 2009) neurons. The non-cholinergic neurons (e.g. glutamatergic neurons or γ-aminobutyratergic neurons) are interspersed within clusters of cholinergic
neurons in both the LDT and the PPT, and there is no segregation of different neuronal subtypes into clearly defined compartments (Clements and Grant, 1990). The PPT and the LDT send ascending, cholinergic as well as non-cholinergic projections to several regions, including the DA-producing cells of SN and the ventral tegmental area (VTA) (Sugimoto and Hattori, 1984; Hallanger et al., 1987; Hallanger and Wainer, 1988; Lee et al., 1988; Bevan and Bolam, 1995; Oakman et al., 1995). These ascending projections of the cholinergic population of PMT are topographically organized such that LDT and most caudal part of the PPT project primarily to the VTA, and the rest of the PPT innervates the SN (Oakman et al., 1995).

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**Figure 1.1. Schematic anatomy of a rat brain**

1: laterodorsal tegmentum (LDT)  
2: pedunculopontine tegmentum (PPT)  
3: substantia nigra (SN)  
4: ventral tegmental area (VTA)  
5: amygdala (amg)  
6: thalamus (TH)  
7: hippocampus (H)  
8: nucleus accumben (NAc)  
9: prefrontal cortex (PC)
1.2.4.1.2 Role of pedunculopontine mesencephalic tegmentum in nicotine reinforcement

The role of the cholinergic tegmentum of PMT in nicotine self-administration has mainly been assessed by a complete lesion of the PPT nucleus or LDT nucleus. Neurotoxin lesions of the cholinergic neurons in PPT reduce nicotine self-administration, as do intra-PPT microinfusions of the nicotinic antagonist DHβE (Lança et al., 2000a). A more sophisticated lesion paradigm shows the posterior PPT is responsible for the control of intravenous self-administration of nicotine in the laboratory rats (Alderson et al., 2006). All these reports postulate these cholinergic nuclei themselves are under the control of cholinergic mechanisms, and that their efferents to the midbrain DA system play a crucial role in nicotine reinforcement. Consistent with this postulation, Fos-positive nuclei have been observed in the cholinergic tegmentum after the administration of nicotine (Lança et al., 2000b). However, the same study also found Fos-positive nuclei almost exclusively in the non-cholinergic neurons (Lança et al., 2000b).

The LDT is also known to be critically involved in the nicotine self-administration. LDT-lesioned laboratory rats have been found to have significantly lower locomotor response to nicotine (Alderson et al., 2005). This is suggested to be due to a VTA dysfunction arising from the loss of a regulatory input from the LDT.

In summary, nicotine is known to activate the neurons of the PPT nucleus and LDT nucleus, possibly by targeting nicotinic receptors that may be located in non-cholinergic neurons. The activation of these non-cholinergic neurons may modulate the activity of cholinergic cells in the PMT, which in turn alters DA release the activity of DA neurons in the mesolimbic system.
1.2.4.2 Mesolimbic dopamine system

1.2.4.2.1 Neuroanatomy of mesolimbic dopamine system

The mesolimbic DA system is one of the dopaminergic pathways in the brain, as schematically indicated in Figure 1.2. This dopaminergic pathway originates from the VTA of the midbrain, and connects to the limbic system via the amygdala, the hippocampus, and the nucleus accumbens (NAc) (Dahlström and Fuxe, 1964; Koob et al., 1975; Shimada et al., 1976). Neurons in the VTA, which transmit signals to other brain cells by the release of a neurotransmitter called dopamine (DA), innervate various brain structures which modulate motivation, emotion, memory, and cognition (for a review, see Björklund and Dunnett, 2007). Of these brain structures with which VTA interacts, an area in the forebrain known as the nucleus accumbens (NAc) has been extensively studied with respect to its involvement in the reinforcing effect of drugs including nicotine and alcohol (Larsson and Engel, 2004).

*Figure 1.2. Schematic representation of some ascending mesolimbic dopaminergic pathways. The mesolimbic pathway is one of the dopaminergic pathways in the brain. The pathway begins from the ventral tegmental area (VTA) of the midbrain, and projects to the nucleus accumbens (NAc), the hippocampus (H), the amygdala (amg), and the prefrontal cortex (PC).*
1.2.4.2.2 Role of mesolimbic dopamine system in nicotine reinforcement

Neuroanatomical studies have shown that the mesolimbic dopaminergic neurons in rats’ brains possess nicotinic receptors both at the cell body and dendritic level in the VTA, and in their terminal fields in the NAc (Clarke and Pert, 1985; Deutch et al., 1987; Swanson et al., 1987; Wada et al., 1989). Electrophysiological studies have established that nicotine can activate mesolimbic neurons in VTA (Lichtensteiger et al., 1982; Grenhoff et al., 1986; Mereu et al., 1987; Calabresi et al., 1989). In addition, micro-infusions of the nicotinic antagonist, DHβE into the VTA, prior to the start of intravenous nicotine self-administration sessions, has resulted in a significant decrease in the number of nicotine infusions self-administered by laboratory rodents (Corrigall et al., 1994). As well, pretreatment with mecamylamine, a nicotinic antagonist that is both centrally and peripherally active, has attenuated intravenous self-administration of nicotine in animals (Goldberg et al., 1981; Corrigall and Coen, 1989).

Neurochemical studies have shown increased extracellular DA concentrations in the NAc after either systemic or intra-accumbens administration of nicotine (Imperato et al., 1986; Fung and Lau, 1988; Fung, 1989; Mifsud et al., 1989; Benwell and Balfour, 1992; Nisell et al., 1994). Consistent with these reports, the pretreatment with mecamylamine has prevented the stimulation of DA release induced by nicotine in the NAc, while the peripherally active nicotinic receptor antagonist hexamethonium has failed to influence the stimulation (Imperato et al., 1986). Furthermore, nicotine self-administration in laboratory rodents was attenuated by the pretreatment of selective DA receptor antagonists (Corrigall and Coen, 1991b), or by the DA-neurotoxin lesions at the NAc (Singer et al., 1982; Corrigall et al., 1992).
Taken together, these findings indicate that the reinforcing effects of nicotine arise from a central and direct action on the mesolimbic DA system via nicotinic receptors located in VTA and NAc. This indication is consistent throughout the observations from neuroanatomical, electrophysiological and neurochemical studies.

1.2.4.2.3 Involvement of mesolimbic dopamine in alcohol reinforcement

Similar to nicotine, electrophysiological and neurochemical studies have shown that alcohol dose-dependently increases the firing rate of ventral tegmental DA neurons (Gessa et al., 1985; Brodie et al., 1990; Bunney et al., 2001) and enhances the release of DA in the NAc (Gessa et al., 1985; Di Chiara and Imperato, 1988; Brodie et al., 1990; Weiss et al., 1993; Yim and Gonzales, 2000).

This modulation of DA release may subsequently underlie the reinforcing properties of alcohol. Laboratory rodents have been shown to self-administer alcohol directly into the posterior VTA (Gatto et al., 1994; Rodd-Henricks et al., 2000; Rodd et al., 2004). This intracerebral self-administration of alcohol can be extinguished by infusing quinpirole, a DA receptor selective agonist into the posterior VTA (Rodd et al., 2004). However, others have reported quinpirole to produce various effects on alcohol self-administration; microinjection of quinpirole at higher dose into VTA has no effect, or attenuates the oral self-administration of alcohol in laboratory rodents, but it increases oral self-administration of alcohol when micro-infused into the NAc core at low doses (Hodge et al., 1993; Hodge et al., 1997; Samson and Chappell, 2003). In addition, microinjection of the DA receptor selective antagonists (SCH23390, raclopride, or fluphenazine) into the NAc or VTA is known to reduce operant responding for oral
alcohol reinforcement (Rassnick et al., 1992; Hodge et al., 1997; Samson and Chappelle, 1999). Furthermore, the level of alcohol self-administration has been attenuated after laboratory rodents were systemically pretreated with either direct-acting DA receptor agonists (Pfeffer and Samson, 1988; Rassnick et al., 1993a; Cohen et al., 1999), DA receptor selective agonists (Weiss et al., 1990; Cohen et al., 1999), or DA receptor antagonists (Pfeffer and Samson, 1988; Files et al., 1998).

To further investigate the involvement of DA systems in alcohol reinforcement, many studies have utilized neurotoxins to produce the degeneration of dopaminergic neurons, and the commonly used neurotoxin in these studies has been 6-hydroxydopamine (6-OHDA). Selective lesioning of the NAc with 6-OHDA has produced inconsistent effects in alcohol drinking behavior. Micro-infusing 6-OHDA into NAc has resulted in no effect in both unselected heterogeneous rats (Myers and Quarfordt, 1991; Fahlke et al., 1994) and in alcohol-preferring rats (Ikemoto et al., 1997; Koistinen et al., 2001). However, an increase in alcohol drinking has also been observed after 6-OHDA lesions of the NAc (Quarfordt et al., 1991).

Unlike the aforementioned studies, which used two-bottle choice paradigm, studies investigating effects of 6-OHDA lesions on alcohol reinforcement under an operant condition have generated more consistent results. One study using an intravenous alcohol self-administration protocol has shown that the micro-injection of 6-OHDA into the NAc did not alter the acquisition or maintenance of the self-administration of alcohol in laboratory rodents (Lyness and Smith, 1992). A similar result was found in a subsequent study using oral alcohol self-administration. In this study it was shown that the lesioned group had a different pattern of responding, which involved a shift to shorter
interresponse intervals as compared to the control group (Rassnick et al., 1993b). These studies indicate that the destruction of dopaminergic afferents to the NAc had no overall influence on the maintenance of alcohol self-administration in rats. A possible interpretation of these findings is that the reinforcing effect of alcohol is a result of alcohol-induced alteration of mesolimbic DA transmission from its acting on VTA DA neurons and terminals in the NAc.

In summary, the involvement of mesolimbic DA in alcohol reinforcement is not clear as compared to involvement in nicotine reinforcement. However, a convergence of neuroanatomical, electrophysiological and neurochemical studies supports the idea that mesolimbic DA is important for alcohol reinforcement.

1.2.4.2.4 Mesolimbic dopamine in the interaction between nicotine and alcohol

Several other studies have identified the mesolimbic DA pathway as a major site for the interaction of nicotine and alcohol. Researchers have found that the level of alcohol voluntarily consumed in a two-bottle choice model (Blomqvist et al., 1996; Ericson et al., 1998) and self-administered in an operant condition (Lê et al., 2000) were attenuated when mecamylamine, a centrally active nicotinic receptor antagonist, was microinfused into the VTA (Ericson et al., 1998), or was systemically injected into laboratory rodents (Blomqvist et al., 1996; Lê et al., 2000). Mecamylamine is known to counteract alcohol-induced DA overflow in NAc when it is either microinfused into VTA (Tizabi et al., 2002; Ericson et al., 2003) or systemically injected (Blomqvist et al., 1993; Blomqvist et al., 1997). Consistent with these reports, an acute dose of IP alcohol with a central injection of nicotine into the VTA (Tizabi et al., 2002), as well as the systemic
administration of combined alcohol and nicotine (Tizabi et al., 2007) are known to result in an additive release of DA in the NAc, which can be blocked by pre-treatment with mecamylamine (Tizabi et al., 2002; Tizabi et al., 2007).

Interestingly, systemic pretreatment with DHβE (Lê et al., 2000; Kuzmin et al., 2009), a competitive nicotinic receptor antagonist with the preference for the nAChR subtype α4β2, did not modify alcohol self-administration in laboratory rodents, and the microinjection of DHβE into VTA did not prevent the DA elevating effects of alcohol (Ericson et al., 2003). Furthermore, the nAChR subtype α7antagonist, MLA did not have an effect on alcohol-induced DA elevations in rodents (Larsson et al., 2002). Instead, the selective α3β2 and α6 nAChR antagonist α-CTXMII blocks the alcohol-induced effects on these parameters (Larsson et al., 2004; Jerlhag et al., 2006; Löf et al., 2007). In addition, the partial nAChR agonist, varenicline has reduced alcohol self-administration and seeking in rats (Steensland et al., 2007), which is possibly caused by its action at α3 nAChR subtype.

Altogether, these studies indicate that an interaction between nicotine and alcohol occurs in the mesolimbic DA system. However, the mesolimbic DA activating effects of alcohol may be due to an indirect rather than direct activation of ventral tegmental nAChRs of a subtype composition different from the α4β2.

1.2.4.3 Summary for neurochemical studies

All these data indicate that both nicotine and alcohol act in the mesolimbic DA pathway. The evidence points to the possibility that an interaction between nicotine and alcohol occurs within this system, which plays an important role in drug seeking. In
addition, neurochemical systems in the PMT, cholinergic and non-cholinergic also appear to play a major role in the interaction of nicotine with alcohol.
1.3 Animal Models

The interaction between nicotine and alcohol is observed at many biological levels ranging from the genetic to the behavioral. There is a vast volume of knowledge about this interaction gained through animal studies. It is, therefore, essential to understand the use of animal models as well as to examine the validity of these models for nicotine and alcohol addiction. In the following sections, methodological approaches that have been employed to induce nicotine and alcohol intake in experimental animals will be reviewed.

1.3.1 Nicotine self-administration

1.3.1.1 Route of nicotine self-administration

1.3.1.1.1 Oral administration

Studies have examined nicotine self-administration using both the oral and intravenous routes of administration. Oral self-administration is an easier and more convenient method to study nicotine dependence in animals due to the simplicity of the preparation. As well, animals which orally self-administer nicotine can be studied over a longer period of time without the worries of catheter patency. However, despite the success of studies where tree shrews voluntarily drank a nicotine solution (Opitz and Weischer, 1988) and non-deprived hamsters ingested commercial chewing tobacco (Ksir, 1983), most species are reluctant to consume nicotine, possibly due to its bitter taste. Rats and squirrel monkeys normally would not drink a nicotine solution and naïve rats do not
consume a nicotine solution that exceeds 5 µg/ml (Hutchinson and Emley, 1988; Flynn et al., 1989). Many attempts have been made to induce oral intake of nicotine. Various methods include schedule-induced polydipsia (Lang et al., 1977; Sanger, 1978; Latiff et al., 1980) or sweetening the nicotine solution (Smith and Roberts, 1995). Although these methods did induce higher nicotine solution drinking, their success was limited as no behavioral dependence on nicotine was induced. Non-deprived rodents in this paradigm prefer water to nicotine solution (Le Houezec et al., 1989). Rodents given access to sweetened nicotine solutions consistently consumed less than rodents given access to sucrose solutions without nicotine (Smith and Roberts, 1995). These studies suggested that the reinforcing effect of nicotine might be affected by relatively slow gastric absorption and the liver first-pass metabolism. Thus the bioavailability of nicotine in the circulatory system and the brain was not pharmacologically relevant, and the pharmacokinetic of nicotine was significantly different from nicotine obtained from cigarette smoking. Overall, oral nicotine self-administration fails to be a useful model for nicotine self-administration.

1.3.1.1.2 Intravenous self-administration

Nicotine has been intravenously self-administered by a broad range of species including rats, mice, dogs (Risner and Goldberg, 1983), baboons (Ator and Griffiths, 1983), and squirrel monkeys (Sannerud et al., 1994). One of the first reports of intravenous nicotine self-administration in rodents was published by Corrigall and Coen (1989). In this study, Long-Evans rats responded for nicotine at high rates in a stable and dose-dependent manner. The self-administration was acquired and maintained without
the use of concurrent reinforcement, nicotine pretreatment, or food deprivation schedules. As well, nicotine self-administration rates in Corrigall and Coen (1989) were attenuated by either saline substitution or the pretreatment of the central nicotinic blocker mecamylamine. Interestingly, years before the article by Corrigall and Coen (1989), DeNoble and Mele (2006) had actually conducted a similar study, and found similar results. However, their manuscripts were not published due to the censorship by a tobacco company (DeNoble and Mele, 2006). The essential features of the intravenous nicotine self-administration reported by Corrigall and Coen (1989) were replicated by Donny and his colleagues (Donny et al., 1995). These studies show that intravenous nicotine self-administration is pharmacologically and behaviorally more representative of human smoking because the rapid delivery of nicotine to the brain that occurs during smoking is mimicked by the intravenous infusion.

1.3.1.1.3 Summary for route of nicotine self-administration

Oral nicotine self-administration fails to simulate the pharmacological effects of nicotine in animals as the slow onset of effects after oral nicotine ingestion does not mimic the rapid delivery of nicotine to the brain following inhalation in humans. On the other hand, the intravenous delivery of nicotine is a better approach to use in self-administration studies in animals as nicotine is rapidly delivered to the brain when nicotine is injected intravenously. Therefore, the intravenous nicotine self-administration, in comparison with oral nicotine self-administration, is pharmacologically and behaviorally more representative of human smoking behavior.
1.3.1.2 Rate of intravenous nicotine infusion

The rate of nicotine infusion is important in intravenous nicotine self-administration paradigms as the rate of infusion should be calibrated to mimic the nicotine kinetics of cigarette smoking. Nicotine Bolus Hypothesis, a widely accepted nicotine theory of cigarette addiction, postulates that the rapid (5 to 10 seconds) transit time of nicotine from lung to brain, and the high arterial nicotine concentrations following each puff of smoke provide a reinforcing ‘hit’ of nicotine to the brain (Russell and Feyerabend, 1978). This hypothesis is consistent with many intravenous nicotine self-administration studies as most researchers who have established nicotine self-administration in animals utilized high (15 or 30 μg/kg) and fast (<1 to 4s) bolus infusions of nicotine (Hanson et al., 1979; Ator and Griffiths, 1983; Risner and Goldberg, 1983; Cox et al., 1984; Corrigall and Coen, 1989; Sannerud et al., 1994; Donny et al., 1995; Shoaib et al., 1997; DeNoble and Mele, 2006). Also, a few studies have reported that nicotine infusion durations longer than 3 seconds are poorly reinforcing (Valentine et al., 1997), and demonstrated a failure to obtain nicotine self-administration in adult rats have used slower (6 seconds) infusions (Belluzzi et al., 2005).

In contrast to the Nicotine Bolus Hypothesis, recent empirical evidence indicates that arterial concentrations of nicotine rise gradually and do not peak until 20 to 30 seconds after each cigarette puff (Rose et al., 1999), with brain nicotine concentrations peaking approximately 2 minutes after a puff (Rose et al., 2006). Interestingly, a recent study of intravenous nicotine self-administration has reported that rats prefer slow infusions of nicotine to fast infusions when given a simultaneous choice of the same unit dose. In addition, they self-administered slow (30 seconds) infusions of nicotine over a
range of unit doses, including a low-dose equivalent to one to two puffs of a cigarette (Sorge and Clarke, 2009). However, the number of nicotine reinforcements obtained in this study is quite low. The other study that has formally examined the effects of infusion rate on nicotine self-administration in animals has reported that faster infusions were self-administered more than slower infusions by rhesus monkeys (Wakasa et al., 1995).

Taken together, evidence that disproves the Nicotine Bolus Hypothesis is emerging from recent studies. However, many studies utilizing the conventional “fast/high” (e.g. 3-second infusions of 30 μg/kg) procedure for intravenous nicotine self-administration readily show nicotine self-administration behaviour, while those employing slow infusion procedures have produced conflicting results. The conventional “fast/high” method is thus preferred, although further research about the effects of infusion rate on the intravenous nicotine self-administration in animals is required.

1.3.1.3 Schedule of access

The schedule of access is an important factor in establishing a model of nicotine self-administration. Investigations of intravenous nicotine self-administration in rodents on continuous reinforcement schedules (in which each lever press results in the delivery of the reinforcer) show relatively low response rates for nicotine (Lang et al., 1977; Sanger, 1978; Hanson et al., 1979; Latiff et al., 1980). Higher rates of responding have occurred when intermittent schedules of access (in which the response is reinforced only part of the time) have been used in squirrel monkeys (Goldberg et al., 1981; Goldberg et al., 1983), and dogs (Risner and Goldberg, 1983). Intermittent schedules were also used in intravenous nicotine self-administration in rodents by Corrigall and Coen (1989). The
intermittent schedule is generally preferred in intravenous nicotine self-administration as a higher rate of responding to nicotine is observed.

1.3.1.4 Session duration

Intravenous nicotine self-administration has been conducted with continuous access (Cox et al., 1984; Valentine et al., 1997; O'Dell et al., 2007; O'Dell and Koob, 2007) and limited access methods (Corrigall and Coen, 1989; Donny et al., 1995; Shoaib et al., 1997). Several different results have been observed when the nicotine self-administration session was conducted with continuous access. In general, continuous access of intravenous nicotine self-administration results in dose-dependent increases in nicotine intake (Valentine et al., 1997; O'Dell et al., 2007), and induces physical signs of dependence induced by nicotine antagonist-precipitated withdrawal (O'Dell et al., 2007), which were not observed in limited access of nicotine self-administration. In addition, the nicotine deprivation effect, which has never been reported in studies with limited access to intravenous nicotine self-administration, was observed with intermittent (i.e. intravenous nicotine self-administration in 4-day cycles, each separated by three intervening days of abstinence) access to intravenous nicotine self-administration in rats (O'Dell and Koob, 2007).

1.3.1.5 Dose

Nicotine supports intravenous self-administration at several doses, particularly at 30 μg/kg/reinforcement. Per session, the unit dose of 30 μg/kg/reinforcement has the largest number of nicotine infusions and the greatest amount of total nicotine intake when
self-administered by a broad range of species such as dogs (Risner and Goldberg, 1983), baboons (Ator and Griffiths, 1983), and squirrel monkeys (Sannerud et al., 1994). Similar results have been also observed in various strains of rodents (Cox et al., 1984; Corrigall and Coen, 1989; Donny et al., 1995). For example, a study conducted by Corrigall and his colleagues (1989) using various unit doses (i.e. 3, 10, 30, and 60 μg/kg/infusion) in the limited-access paradigm reported that response to intravenous nicotine self-administration at 3 μg/kg dose was barely maintained, and that the number of responses at 60 μg/kg unit dose was only half of the number of responses at 30 μg/kg unit dose. Interestingly, the number of responses at 10 μg/kg unit dose was as high as the one at 30 μg/kg unit doses, which means the total intake at 10 μg/kg is two-thirds lower than the total intake at 30 μg/kg unit dose. Overall, 30 μg/kg/reinforcement is the ideal unit dose as all studies indicate this unit dose results in the largest number of nicotine infusions and the greatest amount of total nicotine intake.

1.3.2 Alcohol self-administration

1.3.2.1 Two-bottle choice paradigm

The oldest and most straightforward animal paradigm of alcohol self-administration has been to offer rodents a continuous choice between an alcohol solution and water or other drug-free fluids (Richter and Campbell, 1940; Richter, 1941). This model is often referred to as the “free-choice” or “preference” paradigm. In the most basic form of this paradigm, rodents are housed individually in a cage, and are offered alcohol along with unlimited access to food and water. However, this paradigm has been
severely criticized because the levels of alcohol consumption that most rodents achieved were lower than their average metabolic capacity for alcohol (Lester and Freed, 1973; Mello, 1973; Cicero, 1979; Kalant, 1983; Meisch, 1984). This consequently makes it difficult to determine whether physiologically relevant blood alcohol levels are achieved and pharmacological responses are triggered. As a result, various attempts have been made to increase oral alcohol self-administration by rodents.

1.3.2.2 Techniques to increase oral alcohol intake

The most common way to increase oral alcohol self-administration is to habituate rodents to the aversive taste of highly concentrated alcohol solutions.

In an experiment in which rodents had continuous access to both alcohol solutions and water, researchers observed that if the concentration of the alcohol was systematically increased over a number of days, rodents would subsequently consume higher doses and concentrations of alcohol than the control group which was not exposed to the regimen (Veale and Myers, 1969). Authors called this phenomenon an “acclimation effect”. In the same study (Veale and Myers, 1969), alcohol naïve rodents, that were forced to drink highly concentrated alcohol as the only source of fluid, showed a decrease in alcohol consumption when later offered a choice between alcohol solution and water. All these data illustrated the necessity for a gradual exposure to ascending concentrations and for the concurrent availability of water in order to bring about an increased preference for alcohol.

Another way to make rats habituate to the aversive taste of highly concentrated alcohol solutions is the “sucrose-fading” procedure, in which rats are first trained to make
a lever-pressing response for access to a concentrated, highly palatable solution of sucrose (Samson, 1986; Brown et al., 1998; Clark et al., 2001; Sharpe and Samson, 2002) or non-nutritive saccharin (Schulteis et al., 1996; Cohen et al., 1998; Cohen et al., 1999; Heyser et al., 1999). Over the course of several daily sessions, the sucrose concentration is gradually reduced with ethanol being increased. This eventually resulted in the rats drinking an ethanol-water solution without sucrose. With these procedures, the rats came to prefer the highly concentrated alcohol solution and consumed alcohol at a faster rate than the average metabolic elimination rate of alcohol for rats. However, this approach to alcohol self-administration leads to ambiguity about the ability of alcohol to serve as a reinforcer in itself and the conditions under which reinforcement of drug self-administration will occur.

These studies indicate that the most acceptable and the least controversial procedure to habituate rodents to self-administer alcohol orally is the one using the “acclimatization effect”.

1.3.2.3 Session duration

The most commonly used methods to assess alcohol consumption in rodents are the continuous free-choice drinking model (Stewart and Grupp, 1992), and the operant model in which rats are required to lever press for alcohol (Files et al., 1994; Files et al., 1997). In either method, the session duration for alcohol self-administration must be carefully defined, as rats drink alcohol in discrete bouts and drink mainly during the dark 12-hour period of their light/dark cycle (Aalto and Kiianmaa, 1984; Gill et al., 1986; Linseman, 1987). Thus, in a continuous access paradigm, the comparison of the daily
intake to the daily metabolic capacity may be misleading if the temporal distribution of
drinking within each 24-hour period is not considered. The limited access (i.e. 30 min or
1h) paradigm provides researchers with the benefit of controlling when and how much
alcohol the animals consume as well as with the convenience of determining a more
accurate blood alcohol level.

1.4 Summary

The review of literature has revealed the common comorbidity of nicotine and
alcoholism in humans. Nicotine and alcohol interact with each other at several biological
levels ranging from genetic to behavioral. Genetic studies have suggested the genetic
predisposition to alcoholism and nicotine dependence as being co-inherited. Similarly,
experimental studies with animals have further provided evidence for the interaction
between nicotine and alcohol in several ways: 1) neuropharmacological (i.e. receptor), 2)
behavioral, 3) neurochemical, and 4) genetic.
Chapter 2

PURPOSE OF INVESTIGATION

The review of literature indicates a high comorbidity in tobacco and alcohol abuse. Experimental studies in humans exploring this relationship have shown that alcohol potentiates nicotine intake, and nicotine stimulates the consumption of alcohol. As well, genetic studies in humans have established that the liability to use either nicotine or alcohol, or both together is co-inherited. Experimental studies with animals also have provided evidence for a biological basis for the co-use of nicotine and alcohol at several levels ranging from genetic, neuropharmacological, and neurochemical to the behavioral. Furthermore, alcohol self-administration in rats has been shown to be enhanced by pre-treatment with nicotine, and modulated by nicotinic receptor antagonists.

To date, with the exception of one study that examined the oral consumption of alcohol and nicotine solution in a two-bottle choice procedure (Marshall et al., 2003), preclinical research studying the relationship between nicotine and alcohol has typically relied on passive administration of nicotine or alcohol on self-administration of the other drug. Although these studies relying on the passive administration of drugs have provided the first important steps in understanding the relationship between nicotine and alcohol, their relevance is limited. In humans, nicotine and alcohol are voluntarily self-administered, and often taken together. Nicotine, alcohol, and other drugs of abuse are known to have different behavioral and biological effects depending on whether they are self-administered or administered passively (Wilson et al., 1994; Stefanski et al., 1999;
Donny et al., 2000). At present, there is no operant animal procedure examining voluntary and concurrent self-administration of alcohol and nicotine.

Since nicotine and alcohol are co-abused, there is a challenge in terms of treatment design. The few clinical studies that have addressed this question have produced mixed results (Kodl et al., 2006). There is evidence that discontinuation of smoking may increase the chances of relapse to alcohol use (Joseph et al., 2004a; Joseph et al., 2004b) while other studies have found that alcoholics who continue to smoke may be more likely to relapse to alcohol (Kalman et al., 2001; Joseph et al., 2004b). Others have reported that the inclusion of a smoking cessation program worsens the outcome of alcoholism treatment (Joseph et al., 1993; Grant et al., 2003), enhances the response to treatment for alcoholism (Miller et al., 1983; Hurt et al., 1994), or does not interfere with the success of alcoholism treatment (Bobo et al., 1987; Abrams et al., 1992).

The purpose of the present work is to develop an operant procedure in which animals would self-administer alcohol and nicotine voluntarily and together. Such a procedure would help to address some of these clinical issues. Operant self-administration is necessary in this procedure to demonstrate the voluntary intake of both drugs, and that the self-administration is maintained by the pharmacological effects of nicotine and alcohol, similar to the voluntary intake in humans. In order to develop and validate this model, two experiments were conducted. In the first experiment, the responding and intake of animals trained to self-administer nicotine or alcohol alone were compared with animals trained to co-administer both drugs. Then, in the second experiment, the effect of the order of the training (nicotine or alcohol first) on co-administration was determined.
Chapter 3

GENERAL MATERIAL AND METHODS

3.1 Animals

Male Wistar rats, weighing between 150-200 grams, were obtained from Charles River, Montreal, and allowed to acclimatize to the animal facility for 1 week prior to each experiment. The animals were individually housed and given free access to lab chow (Purina, Mississauga, ON, Canada) and tap water. The vivarium temperature was $21 \pm 1^\circ C$ and lights were on a 12-h reversed light/dark cycle (lights on from 7 p.m. to 7 a.m.). The experimental procedures followed the “Principles of Laboratory Animal Care” (NIH publication no. 85-23, 1996) and were approved by the local animal care committee of the Centre for Addiction and Mental Health.

3.2 Apparatus

3.2.1 Drinking cages

Wire cages (30.0 cm (L) x 20.0 cm (W) x 15.0 cm (H)) were used for the two-bottle choice paradigm, the initial part of training animals to self-administer alcohol. Each cage was equipped with two 25.00 ml Richter tubes. One of the Richter tubes contained either an alcohol or a sucrose solution, and the other one contained tap water.
3.2.2 Operant chamber

Self-administration of either nicotine or alcohol alone or co-administration of both drugs was conducted in 16 operant chambers housed in fan-ventilated and sound-attenuating enclosures, operated by a computer-controlled interface system (Med Associates, St. Albans, VT). The interior dimensions of the Plexiglas chambers were 30 x 21 x 21 cm. Each chamber was equipped with two retractable levers on one side panel located 2.5 cm above a removable grid floor (See Figure 3.1). In the case of self-administration of nicotine or alcohol alone, only one lever was extended. Appropriate responding activated an infusion pump (Razel Scientific, Stamford, CT, USA) on which were affixed syringes containing the nicotine or alcohol solutions. For alcohol, the infusion duration was 5 s delivering 0.19 ml of 12% (w/v) alcohol into a drinking receptacle. For nicotine, the infusion duration was 0.5 s to deliver 30 μg/kg nicotine/infusion via the intravenous catheter. The chambers were also equipped with a red house light located near the top of the chamber opposite the lever and a white cue light positioned above the lever. A modified 22-gauge cannula, which was attached to the intravenous catheter on a daily basis, was connected to a fluid swivel with Tygon tubing protected by a metal spring. The swivel was attached to the infusion pump syringe with Tygon tubing. During co-administration sessions, the chambers were equipped with two infusion pumps, one that delivered alcohol into the drinking receptacle and one that delivered intravenous nicotine. The two levers were extended during co-administration sessions. Appropriate responding on one of the levers resulted in intravenous nicotine delivery and, on the other, alcohol delivery into the receptacle. In all of the experiments, nicotine reinforcements were accompanied by a continuously illuminated white cue light,
while alcohol reinforcements were accompanied by a flashing white cue light (0.5 s on, 0.5 s off) during the timeout periods (described below). The orientation of the nicotine and alcohol-associated levers (right or left) was counterbalanced across animals in the co-administration groups.

Sucrose self-administration occurred in 16 similarly equipped operant chambers (Med Associates). Appropriate responding activated the white cue light (0.5 s on, 0.5 s off), and an infusion pump (Razel Scientific, Stamford, CT, USA) delivering 0.19 ml of 5% (w/v) sucrose solution into a drinking receptacle over 5 s.

Figure 3.1. Schematic representation of the operant chamber interior. 
- a cue lights. b retractable levers. c receptacle. d metal spring. 
  e connector, where the Nylon bolt of the intravenous catheter was attached. 
  f 22-gauge needle for intravenous drug delivery.
3.3 Surgery – Implantation of Intravenous Catheters

3.3.1 Anaesthesia

Catheters were implanted into the jugular vein under surgical anesthesia induced by a ketamine/xylazine mixture (75 mg/kg ketamine/10 mg/kg xylazine; 2 ml/kg, IP). Incision sites were treated with a local anesthetic (0.1 ml Marcaine 0.125%, SC, Hospira Healthcare Corp., Montreal, QC, Canada). Buprenorphine (0.01 mg/kg, SC, Schering-Plough, Kirkland, QC, Canada) was administered as an analgesic and penicillin (30 000 U, IM, Rogar/STP, London, ON, Canada) was used as antibiotic treatment post the surgery.

3.3.2 Implantation of Intravenous Catheters

3.3.2.1 Construction of intravenous catheters

The catheter consists of three separate pieces of tubing, as illustrated in Figure 3.2: a 37 mm length of silicon rubber tubing (Silastic Medical-Grade Tubing, Dow-Corning, Midland, MI, USA), a 65 mm length of polyethylene tubing with an internal diameter of 0.28 mm (Intramedic, PE10, Beckton Dickinson, Sparks, MD, USA), and a 170 mm length of polyethylene tubing with a similar internal diameter, but a much thicker wall (PE20, Becton Dickinson). Silastic tubing is what is inserted into the vein, and the PE20 tubing, due to its strength, comprises part of the external end of the catheter. The PE10 tubing forms the junction between the PE20 and the silastic tubing.
The catheter was assembled in the following way. First, the Silastic-to-PE10 joint was made. To do this, the PE10 tubing was inserted into a Silastic tubing such that the two overlapped for approximately 7 mm. A 6 mm length of heat shrink tubing was placed over this overlap. Without puncturing the tubing, a piece of 32G wire (World Precision Instruments, Sarasota, FL, USA) was inserted through the lumen of the Silastic-PE10 assembly. Heat from a fine-tipped soldering iron was applied to the heat shrink tubing to secure the joint. In doing so, it was necessary to ensure that the heat shrink tubing did not extend beyond the area of overlap with the PE10 tubing as there was the risk that the lumen of the Silastic tubing might be occluded by the heat shrink tubing. Once the heat shrink tubing cooled and the joint was secure, the wire was removed from the Silastic-PE10 assembly.

The next step involved the construction of the joint between the Silastic-PE10 assembly and the PE20 tubing. To do this, both the PE10 assembly and the PE20 segment were fed onto a long piece of 30G wire (World Precision Instruments, Sarasota, FL, USA) in a manner such that the PE10 and PE20 met. With the wire serving as a mandrel...
(thereby preventing tubing occlusion), heat was applied to the ends of each PE tubing via a fine jet of hot air. Once the ends of the polyethylene tubing began to melt, the pieces of tubing were slid toward each other to form a small, smooth bead joint. Once the bead was cooled and the joint was secure, the 30G surgical wire was removed from the tubing.

To allow a reliable connection to be made to the drug delivery system, and to permit rapid connection and disconnection of the rat to the drug delivery line, the end of the catheter was constructed as follows. A nylon bolt (19.05 mm, 6-32) was prepared with an axially drilled hole sufficiently large to allow passage of the PE20 tubing. The end of the bolt opposite the head was countersunk with a larger hole, sufficient to accommodate PE20 tubing with a sleeve made of heat shrink tubing. The PE20 end of the catheter was threaded through the hole in the nylon bolt, starting at the head of the bolt. A piece of heat shrink tubing (1.19 mm), approximately 15 mm long, was passed over the PE20 tubing, to be used later. A 33-gage wire was then inserted into the end of the PE20 tubing, and the end of the tubing was heated in a jet of hot air to raise a smooth flanged end. The heat shrink tubing was then positioned over the end of the PE20 tubing such that it extended about 8 mm beyond the flange at the terminal end of the polyethylene.

A mandrel was prepared so that the terminal end of the catheter could be completed. This consisted of a 23-gauge blunt-cut hypodermic needle with a piece of 33-gauge wire extending beyond its tip. The mandrel was inserted into the PE20 tubing such that the end of the 23-gauge tubing rested against the flared polyethylene flange. The heat shrink cuff, detailed above, was then heated sufficiently to cause it to wrap snugly around the polyethylene tubing and needle to form a fluid-tight junction. The mandrel was removed when the tubing was cool. A small drop of cyanoacrylate adhesive was placed
on this last piece of heat shrink in the area over the PE20 tubing, and the heat shrink-tubing assembly was pushed gently into the countersunk hole in the bolt, so that only the flange and the heat shrink sleeve extended above it. The heat shrink provided a more resilient end at which connections can be made to the drug-delivery line, since polyolefin, the polymer it is made from, is harder than polyethylene and can be reshrunk to its previous size if it stretches during regular use.

After installation of the nylon bolt, two permanent bends were made in the polyethylene tubing components of the catheter. This was done by bending the tubing to the desired orientation, and quickly dipping the tubing into hot water (about 90 -100°C) and then into cold water. One bend, consisting of a 180° turn, was made in the PE10 tubing just past the heat shrink joint. The other was an approximate 90° bend made in the PE20 tubing just blow the nylon bolt. The 180° bend allows the catheter to be inserted into the jugular vein pointing toward the heart, while keeping the remainder of the catheter tubing pointing rostrally. The 90° bend ensures that the bolt can be positioned perpendicular to the animal’s back with the PE20 tubing lying flat subcutaneously.

Surgical mesh provides the means through which the bolt assembly was anchored to the animal’s skin when the catheter was implanted. A piece of this (Marlex Mesh, Bard Cardio-surgery Division, Billerica, MA, USA) was cut into an oval-shape with a long axis of about 30 mm. A small hole was made in the centre of the piece of mesh, and the mesh was pushed over the threaded part of the bolt until the head of the bolt rested against the mesh. The mesh was oriented in a way such that its long axis was perpendicular to the PE20 tubing, and fastened to the head of the bolt with a layer of dental acrylic liquid (Dentsply International, York, PA, USA). Once the dental acrylic
liquid hardened, it was burnished to ensure a smooth surface as sharp points on the dental acrylic could act as irritants. It is important to note that the dental acrylic was localized at the head of the bolt, leaving most of the mesh free to be encapsulated by connective tissue during healing.

To prevent undesired substances from entering the catheter, a removable closure for the external end of the catheter was made from a 15 mm long silastic tube partially filled with liquid silicon adhesive (Dow-Corning, Midland, MI, USA). Once the liquid silicon adhesive had cured, this closure cap was fitted over the end of the catheter.

The catheter was pressure-tested at various stages of construction to check for leakages, and was also tested at completion. To do so, a 1 cc syringe was filled with distilled water, equipped with a blunt-cut 22-gauge hypodermic needle, and connected to the heat shrink termination at the external end of the catheter. Fluid was then flushed to the end of the Silastic tubing. The end of the Silastic tubing was pinched off between the pads of the fingers, while pressure was applied to the catheter via the hypodermic syringe, and all joints were checked for leakages. Only catheters without leakage were used for implantation.

### 3.3.2.2 Implantation

To prevent contamination and infection of the surgical area, the hair on each rat’s right ventral neck and shoulder blade regions was shaved in the preparation area of the surgery room. Pressure from the blade to the neck area was well controlled to avoid stimulating the vagus nerve and/or damaging the trachea. Loose hair was removed by gentle brushing.
Each rat’s right ventral neck and shoulders were washed with Betadine scrub solution (Purdue Frederick, Pickering, ON), swabbed with 70% alcohol, and then painted with Betadine solution containing 10% povidone-iodine (Purdue Frederick, Pickering, ON). The rat was then placed on a sterile drape on the surgical platform and sterile gloves were donned.

Excess Betadine solution on the rat was removed with a sterile swab. The rat was draped, and a 1 cm incision was made through the skin to the subcutaneous space in the midline above the shoulder blades. The subcutaneous space was cleared to accommodate the loop of a catheter. The incision was covered by sterile gauze and the animal was repositioned so that the ventral neck region was exposed.

A 1 cm incision was made above the clavicle on the right side, and musculature was cleared using blunt dissection technique to expose the jugular vein. A section of the vein was isolated, stripped of fat and connective tissue and 2 ligatures (5-0 silk, Surgical Specialties, Reading, PA) were run underneath the vessel to facilitate later retrieval. The vein was kept moist with sterile saline at all times.

To tunnel the catheter subcutaneously, blunt dissection was used to pass the tips of a pair of forceps from the scapular incision to the ventral neck incision, traveling behind the animal’s right forelimb. To facilitate the passage of the catheter, a piece of large-diameter polyethylene tube (PE380) served as a trochar and was clamped with the forceps and drawn back through the subcutaneous channel. The catheter was then passed through this trochar in a dorsal-ventral direction. The trochar was then removed via the ventral incision, leaving the catheter in place subcutaneously.
Prior to jugular implantation of the catheter, a visual inspection was conducted to ensure that catheter length was appropriate. If necessary, the final length of the catheter was adjusted, so the tip of the catheter would lie just outside the heart. The vein was ligatured caudally by stretching the caudal ligature, filled with blood by massaging the vein gently, and tied off rostrally. With the vessel between the ligatures engorged, a small V-shaped cut was made in the vein between the two ligatures, and the silastic tip of the catheter was inserted into the vein until the shrink-tube was in contact with the vessel. The catheter was tested for patency, and the shrink-tube was sutured at either end to the outer surface of the vein with the 5-0 silk ligatures. As well, a drop of cyanoacrylate adhesive was placed on the underside of the heat shrink to secure it to underlying tissue. The PE-10 portion of the catheter was sutured to deep muscle. The muscle tissue was repositioned and sutured. The skin incision was closed using interrupted 3-0 silk sutures (Surgical Specialities, Reading, PA, USA), and dusted with neomycin antibiotic powder (Cicatrin; Glaxo Wellcome, Pickering, ON, Canada). As the animal was carefully turned over, the excess catheter tubing was looped into the subcutaneous pocket previously created at the scapular region. The Marlex Mesh base of the catheter was positioned under the skin, and the incision was closed with interrupted 3-0 silk sutures. Cicatrin powder was then applied to the site.

After surgical completion, each animal was allowed to recover in a recovery cage lined with bedding and a layer of paper towels. Heat lamps were used to carefully regulate the temperature of the recovery boxes, to allay the hypothermic effects of anaesthesia. Animals were moved regularly to avoid hypostasis and to monitor levels of
respiration. Once rats had fully recovered from anaesthesia, they were returned to their home cages.

In the days following surgery, animals were carefully monitored daily to assess recovery. Catheter patency was maintained by one 0.05 ml daily injection of sterile heparinized saline (50 units/ml; Sigma, St. Louis, MO, USA). If a catheter was suspected of being blocked, the animal was administered 0.1ml of sodium methohexitol (10 mg/ml; Monarch Pharmaceuticals, Bristol, TN, USA), an ultra short-acting barbiturate through the catheter. Patency was confirmed if the animal experienced ataxia and a few seconds of sedation. If the catheter was no longer venous, there would be little or no effect.

3.4 Experimental Procedures

3.4.1 Two-bottle choice paradigm

Rats were trained to consume alcohol or sucrose using a two-bottle choice paradigm as described previously by several researchers (Linseman, 1987; Lê et al., 2000). During this procedure, animals had free access to rat chow and water in their home cages. Animals were transported daily from the vivarium to a drinking room, where each was placed in a drinking cage with water and alcohol, or water and sucrose solution available in 25.00 ml modified Richter tubes. Animals were removed from the drinking cages 30 minutes later, and the amounts of water and alcohol or sucrose consumed during this period were recorded. Alcohol was provided in escalating concentrations: 3% (w/v) for the first 5 days, 6% (w/v) for the next 5 days, and 12% (w/v) for the last 10 days. Sucrose concentration was 5% (w/v).
3.4.2 Sucrose self-administration

Animals were trained to lever press for sucrose solution (5% w/v) in operant chambers during daily 1-hour sessions. Lever-pressing behavior was reinforced under an FR-1 reinforcement schedule. At the start of the sessions, the red house light was turned on and one of the retractable levers was extended. Appropriate lever-pressing resulted in a delivery of 0.19 ml of sucrose into the receptacle, which was followed by a 5-second timeout. Responses during the timeout period were recorded but were without consequence. During the timeout period, the white cue light associated with the lever flashed (0.5 second on, 0.5 second off). The volume of unconsumed sucrose solution in the receptacle was recorded, so the number of consumed sucrose reinforcements could be calculated. The position (left or right) of the extended retractable lever was counterbalanced across animals.

3.4.3 Alcohol self-administration

In experiments where alcohol training preceded nicotine training, initial alcohol training was accomplished using the two-bottle choice paradigm as described in section 3.4.1. Operant training followed this and animals were trained to lever press for alcohol (12% w/v) in the manner described below. In experiments where alcohol training followed nicotine training, the two-bottle choice procedure was not used and operant training was again conducted as detailed here.

Unless indicated otherwise, the duration of the operant self-administration sessions for alcohol alone, nicotine alone, and co-administration of both drugs was 1 hour per day. At the start of the sessions, the red house light was turned on and one of the
retractable levers was extended. Operant self-administration of alcohol was initiated at a fixed ratio 1 (FR-1) reinforcement schedule, in which a single reinforcement was delivered, contingent upon a single press on the active lever (with the exception of responses occurring within the timeout period). The FR-1 reinforcement schedule was carried out for approximately a week (i.e. 7-10 days). Subsequently, the response requirement was increased to an FR-2 for a shorter period of time (i.e. 3-5 days) and then to an FR-3 for the remainder of self-administration sessions. When the appropriate number of lever presses were made, 0.19 ml of alcohol solution would be delivered into the drinking receptacle. Each delivery of alcohol was accompanied by a timeout period, during which the white cue light associated with the lever flashed (0.5 seconds on, 0.5 seconds off) and the lever retracted. The duration of the timeout was 5 seconds at FR-1 in order to speed the training. The timeout was lengthened to 30 seconds at FR-2 and FR-3. Responses during the timeout period were recorded, but did not have any programmed consequence. At the end of each session in which alcohol was self-administered, the volume of unconsumed alcohol in the receptacle was recorded and used to calculate the numbers of reinforcements consumed. The position of the extended retractable lever was counterbalanced across animals.

3.4.4 Nicotine self-administration

In experiments where nicotine training preceded alcohol training, animals were first trained to self-administer sucrose as described in section 3.4.2.

Operant self-administration of nicotine began after full recovery from surgery. At the start of each session, the animal was placed in the operant chamber and hooked up to
the drug line. The drug line consisted of Tygon tubing which was connected to a single channel fluid swivel which was connected in turn to the infusion pump. The animal end of the drug line was fitted with a 22-gauge blunted needle, which plugged into the animal’s catheter. A protective steel spring was then lowered and threaded onto the nylon bolt to prevent the animal from chewing the drug line.

At the start of the sessions, the red house light was turned on, and one of the retractable levers was extended. The reinforcement schedule for nicotine self-administration was similar to one for alcohol self-administration; initiated at FR-1 reinforcement schedule for approximately a week (i.e. 7-10 days), increased to an FR-2 for relatively shorter period of time (i.e. 3-5 days), and to an FR-3 for the rest of self-administration sessions. Appropriate lever-pressing behavior would result in an intravenous infusion of nicotine (30 μg/kg per infusion). Each nicotine infusion was followed by a 30-second timeout, during which the white cue light associated with the lever illuminated steadily and the lever retracted. Responses during the timeout period were recorded but had no programmed consequence. The position of the extended retractable lever was counterbalanced across animals.

3.4.5 Nicotine and alcohol co-administration

Animals that had stable levels of alcohol and nicotine self-administration (< 20% deviation from the mean) during their training sessions were trained to self-administer both alcohol and nicotine concurrently in the operant chambers. At the beginning of the sessions, the red house light was turned on, and both retractable levers were extended. The lever-pressing behavior was reinforced under an FR-1 schedule for approximately a
week (7-10 days), and followed by an FR-2 schedule for 3-5 days, and then at FR-3 for the remaining self-administration sessions. Self-administration of either nicotine (30 μg/kg per infusion) or alcohol (0.19 ml of 12% (w/v) per reinforcement) was accompanied by a 30-second timeout, during which time both levers were retracted to avoid nonspecific response in the timeout period. During the period of timeout, the white cue light associated with the nicotine lever would illuminate steadily if nicotine has been infused; the white cue light associated with alcohol lever would flash (0.5 second on, 0.5 second off) if alcohol had been administered. At the end of each session, the volume of unconsumed alcohol in the receptacle was recorded and used to calculate the numbers of reinforcements consumed. The orientation of the nicotine and alcohol-associated levers (left or right) was counterbalanced across animals.

3.5 Drugs

Nicotine solutions (Sigma-Aldrich, Oakville, ON, Canada) were prepared daily using sterile saline, and pH was adjusted to 6.8 – 7.2. The unit dose for nicotine self-administration was 30 μg/kg/infusion, expressed as base (Corrigall and Coen, 1989; Shoaib and Stolerman, 1999; Lê et al., 2006). Alcohol (Commercial Alcohols Incorporated, Tiverton, ON, Canada) was diluted with tap water. The self-administration dose of alcohol was 0.19 ml of 12% (w/v) alcohol per reinforcement. These doses of nicotine and alcohol are commonly used in studies on self-administration (Corrigall and Coen, 1989; Lê et al., 2000; Caggiula et al., 2002; Heyser et al., 2003). Sucrose solutions were made from sucrose powder dissolved in tap water. The self-administration dose of sucrose was 0.19 ml of 5% (w/v) sucrose per reinforcement. Catheter patency was tested.
after each experimental phase using the rapid acting anesthetic, sodium methohexital (4 mg/kg, IV, 10 mg/ml).

3.6 Data analysis and presentation

Nicotine and alcohol reinforcements, g/kg/h alcohol intake and lever presses were analyzed with mixed analysis of variance (ANOVA), using the between factor of Group (alcohol alone, nicotine alone, co-administration) and within factor of Day. In the analysis of responding in 5-min intervals, the within factor of Interval was also utilized. Significant effects from ANOVA ($p$ values<0.05) were followed by post hoc tests using the Newman-Keuls procedure.

In the experiments involving the self-administration of alcohol, the volume of unconsumed alcohol left in the drinking receptacle was recorded and used to calculate the numbers of reinforcements consumed, and these values were used for presentation and analysis. Only a small minority of rats did not consume all of the delivered alcohol, and the overall mean volume of unconsumed alcohol measured in the experiments involving alcohol self-administration was less than 0.2 ml. Rats that consumed less than 0.4 g/kg/h alcohol during the alcohol training phases of the experiments were excluded from analysis.
Chapter 4

CONCURRENT SELF-ADMINISTRATION OF INTRAVENOUS NICOTINE AND ORAL ALCOHOL IN RODENTS

Experiment 1: Self-Administration of Alcohol prior to Nicotine

4.1 Introduction

As described previously, nicotine and alcohol are often used together. This phenomenon has been a subject of investigation for many years.

Behavioural pharmacological studies in humans have focused on how cigarette smoking or nicotine administration affects alcohol drinking, and vice versa. Consumption of alcohol has been shown to increase the urge to smoke either in the presence (Glautier et al., 1996; Burton and Tiffany, 1997) or absence of smoking cues (King and Epstein, 2005; Epstein et al., 2007), and to increase cigarette smoking behaviour as shown by a measure of the number of cigarettes smoked, number of puffs per cigarette, and the volume of puffs per cigarette (Griffiths et al., 1976; Mello et al., 1980; Henningfield et al., 1984; Mitchell et al., 1995; Perkins et al., 2000; Barrett et al., 2006). Conversely, nicotine administration, in the form of a transdermal patch, has been shown to increase desire (Kouri et al., 2004), and motivation to drink or actually increase alcohol consumption in human males (Acheson et al., 2006).

While the only two published experimental studies in animals investigating the effect of alcohol on nicotine self-administration have reported inconsistent results (Hanson et al., 1979; Wang, 2003), numerous studies have examined the effects of nicotine on alcohol consumption in laboratory rodents under various experimental
paradigms. Using the two-bottle choice paradigm, acute treatment with nicotine has been found to suppress alcohol consumption (Gauvin et al., 1993; Katner et al., 1997; Nadal et al., 1998). However, repeated or subchronic treatment with nicotine has been consistently found to increase alcohol consumption (Potthoff et al., 1983; Blomqvist et al., 1996; Smith et al., 1999; Lê et al., 2000; Söderpalm et al., 2000; Olausson et al., 2001). As well, Lê et al. (2000) have found that using the two-bottle choice paradigm, alcohol consumption after repeated nicotine treatment did not increase when the session duration was increased from 30 min to 60 min except when a higher dose of nicotine (800 μg/kg) was used. These studies indicate that under the two-bottle choice paradigm, the effect of nicotine on alcohol consumption is dependent on the nature of nicotine treatment (i.e. acute, repeated, or subchronic), and the dose of nicotine employed, but is independent on the duration of access to alcohol.

Most of the studies using an operant procedure also have found repeated treatment with nicotine increases operant self-administration of alcohol (Lê et al., 2000; Clark et al., 2001; Lê et al., 2003) except for one study, which was conducted by Sharpe and Samson (2002) reporting the suppression of alcohol operant self-administration by repeated treatment of nicotine. However, the duration of access to alcohol in that study (Sharpe and Samson, 2002) was 30 min, and Lê et al. (2000) have found that nicotine produced a suppression, or had no effect on alcohol consumption when the duration of access to alcohol was 30 min, but produced an increase when the duration of access was 60 min. Consistent with this argument, Lê and his colleagues (2000) observed that most of the increase in alcohol self-administration induced by repeated treatment with nicotine occurred at a later time in the operant session rather than during the first 20 to 30 min. All
these reports indicate the effect of nicotine on operant alcohol self-administration is dependent on 3 factors: 1) nature of nicotine treatment, 2) duration of access to alcohol, and 3) dose of nicotine.

Although these experimental studies with laboratory animals demonstrate a relationship between nicotine and alcohol self-administration, their relevance is limited. In humans, both drugs are voluntarily self-administered. With the exception of one study that examined oral consumption of alcohol and nicotine solutions in a two-bottle choice procedure (Marshall et al., 2003), the animal studies to date have determined the effects of passive administration of nicotine or alcohol on the consumption of the other drug. Nicotine, alcohol, and other drugs of abuse are known to have different behavioural and biological effects depending on whether they are self-administered or administered passively (Wilson et al., 1994; Stefanski et al., 1999; Donny et al., 2000).

To help address these issues, an operant procedure in which rats have the opportunity to self-administer nicotine intravenously and alcohol orally during the same operant sessions has been developed. In this experiment, animals are trained to voluntarily self-administer alcohol first, and then are surgically implanted with catheters for the intravenous nicotine self-administration. Animals are trained to self-administer alcohol prior to nicotine because the attrition rate of implanted catheters increases as the experiment progresses, which would reduce the success rate of establishing this operant procedure of nicotine and alcohol co-administration. After animals have self-administered nicotine stably, animals are trained to self-administer both nicotine and alcohol concurrently. The responding and the intake in animals that voluntarily self-
administer nicotine or alcohol alone are compared with that of animals trained to co-administer both drugs.
4.2 Materials and Methods

4.2.1 Animals

Forty-four male Wistar rats weighing between 150-200 grams at the beginning of the experiment were used for this study. They were singly housed in the vivarium in the condition as described in section 3.1.

4.2.2 Experimental design

The training schedules of the 3 experimental groups during the operant phase are illustrated in Figure 4.1.

Animals receiving nicotine training self-administered it at a dose of 30 μg/kg/infusion at a final ratio of FR-3. For animals receiving training with alcohol, the final self-administered dose was 0.19 ml of 12% (w/v) alcohol/delivery at FR-3.

Figure 4.1. Illustration of the nicotine and/or alcohol training regimens in Experiment 1. Animals receiving nicotine training self-administered it at a dose of 30 μg/kg/infusion at a final ratio of FR-3. For animals receiving training with alcohol, the final self-administered dose was 0.19 ml of 12% (w/v) alcohol/delivery at FR-3.
All rats were first trained to consume alcohol in a two-bottle choice paradigm with limited access, in which alcohol was available for 30 min per day. Following this phase, rats were then trained to lever press for alcohol (12%, w/v) in the operant chambers during daily 1-h sessions as described in section 3.4.3. Lever presses were initially reinforced under an FR-1 ratio reinforcement schedule for 10 days. Subsequently, the response requirement was increased to an FR-2 for 5 days, and then to an FR-3 for 10 days.

Rats were then assigned into 3 groups according to their alcohol intake based on the last 3 days of operant alcohol self-administration phase, and were designated as the Nicotine alone group (n=16), the Co-administration group (n=16), and the Alcohol alone group (n=12). Rats from the Nicotine alone and the Co-administration groups were implanted with intravenous catheter for subsequent training for nicotine self-administration while rats from the Alcohol alone group were underwent sham surgery.

Alcohol alone

After recovering from surgery, rats were trained to self-administer alcohol (0.19 ml of 12%, w/v per reinforcement) in the operant chambers during daily 1-h sessions as described in section 3.4.3 for the remainder of the duration of the experiment.

Nicotine alone

After recovery, the rats were trained to self-administer nicotine (30 μg/kg per infusion) for 1 h/day for 24 days as described in section 3.4.4. For the first 10 days, active
lever responding was reinforced under a FR-1 schedule, the next 6 days at FR-2, and then under FR-3 for the remaining duration of the experiment.

Co-administration

After recovery, rats were trained to self-administer nicotine in the same condition as described above for the Nicotine alone group. Once rats had stable levels of nicotine self-administration at an FR-3 reinforcement schedule (24 days), the co-administration procedure as described in section 3.4.5 was introduced. During this co-administration training phase, both retractable levers were extended at the beginning of each session. Self-administration of either nicotine (30 μg/kg per infusion) or alcohol (0.19 ml of 12% (w/v) per reinforcement) was accompanied by a 30-second timeout, during which both levers were retracted, and the white cue light associated with the nicotine lever would illuminate steadily if nicotine has been infused; the white cue light associated with alcohol lever would flash (0.5 second on, 0.5 second off) if alcohol had been administered.

4.2.3 Statistics

Nicotine and alcohol reinforcements (intakes) from the self-administration phase were analyzed by mixed ANOVAs with repeated measures, using between factor of group and within factor of Days. The within factor of Interval was used in the analysis of responding in 5-min intervals. The Newman-Keuls procedure was utilized for post hoc testing where significant effects from ANOVA ($p$ values $< 0.05$) were found.
4.3 Results

Attrition

In this experiment, one rat from the Alcohol alone group was excluded due to low alcohol consumption. Five rats from the Nicotine alone group and four rats from the Co-administration group were excluded due to catheter blockage.

Table 4.1 shows the alcohol and nicotine intake of animals in Experiment 1. Each value is the averaged nicotine or alcohol intake for each experimental group during co-administration phase across 15 days.

Table 4.1 Mean (+ SEM) nicotine and alcohol intake in Experiment 1

<table>
<thead>
<tr>
<th>Drug intake/1h</th>
<th>Alcohol (g/kg)</th>
<th>Nicotine (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol alone</td>
<td>1.32 ± 0.03</td>
<td>—</td>
</tr>
<tr>
<td>Nicotine alone</td>
<td>—</td>
<td>0.54 ± 0.06</td>
</tr>
<tr>
<td>Co-administration</td>
<td>1.28 ± 0.09</td>
<td>0.40 ± 0.04</td>
</tr>
</tbody>
</table>

Figure 4.2 shows the responding of animals trained to self-administer nicotine or alcohol alone (a), or to co-administer nicotine and alcohol (b) during the last 15 self-administration sessions. A mixed ANOVA with the within factor of day (1 to 15) and between factor of group (nicotine alone, nicotine with alcohol access) done on numbers of nicotine reinforcements revealed a significant main effect of day ($F(14, 392)=3.377$, $p<0.05$) and group ($F(1, 28)=5.146$, $p<0.05$), as animals co-administering nicotine and
alcohol received significantly fewer nicotine reinforcements than those self-administering nicotine alone. The complementary analysis done on alcohol reinforcements showed a significant effect of day ($F(14, 350)=1.82, p<0.05$), but no effect of group or interaction, indicating that concurrent access to nicotine did not affect the numbers of alcohol reinforcements received and consumed.

Figure 4.3 (a) shows responding for alcohol and nicotine during the last three days of training for, respectively, self-administration of alcohol and nicotine alone and then when animals were given the opportunity to co-administer both drugs. The reintroduction of alcohol significantly reduced responding for nicotine, as shown by a repeated measures analysis done on the mean numbers of nicotine reinforcements of the 3 days prior to the reintroduction of alcohol and the 15 days after ($F(15, 542)=4.11, p<0.05$). Post hoc analysis revealed that this was due to significant reductions in responding for nicotine on each of the 15 days after alcohol was reintroduced (p’s <0.05). Analysis of alcohol reinforcements in the 15 days of co-administration revealed a significant effect of day, as there was a decrease in the numbers of reinforcements across the co-administration sessions ($F(14, 508)=7.27, p<0.05$). Post hoc analysis revealed that this was due to significant reductions in responding for nicotine on each of the 15 days after alcohol was reintroduced (p’s<0.05). Analysis of alcohol reinforcements in the 15 days of co-administration revealed a significant effect of day, as there was a decrease in the numbers of reinforcements across the co-administration sessions ($F(14, 508)=7.27, p<0.05$).
Figure 4.2. Nicotine or alcohol self-administration and co-administration of both drugs in Experiment 1.

a Mean (+sem) number of reinforcements earned by animals trained to self-administer nicotine (0.03 mg/kg/infusion, N=11) or alcohol (0.19 ml of 12%, w/v; N=11) alone at FR-3 in 1-h daily sessions.
b Mean nicotine and alcohol reinforcements in animals co-administering both drugs (N=12).
c Mean numbers of reinforcements of nicotine and alcohol obtained, averaged over fifteen days of access in animals trained to self-administer nicotine or alcohol alone in those trained to co-administer both drugs. In this experiment, eight rats were excluded due to catheter blockage and one due to low alcohol consumption. *p<0.05, significantly main effect of group.
Figure 4.3. Training for co-administration of nicotine and alcohol in Experiment 1. a Mean (± sem) numbers of reinforcements of nicotine and alcohol obtained prior (Day -3 to 3) and after (Day 4 to 18) the initiation of co-administration sessions. The numbers of alcohol reinforcements on the last three days of training with alcohol (0.19 ml of 12% w/v) alone at FR-3 are presented from days -3 to -1. On Days 1 to 3, the mean numbers of nicotine reinforcements (0.03mg/kg/infusion) earned on the last three days of training for nicotine self-administration at FR-3 are shown. B. Within-session analysis: Alcohol or Nicotine alone. C. Within-session analysis: Co-administration. *p<0.05, significantly different from preceding intervals in nicotine alone group.
In order to detail the relationship between nicotine and alcohol self-administration in animals co-administering the two drugs, Figure 4.3 shows the average number of nicotine and alcohol reinforcements in 5 min intervals earned by animals self-administering alcohol or nicotine alone (b), and by animals self-administering both concurrently (c) within single self or co-administration sessions.

Mixed ANOVAs with the within factor of interval (5-60 min) and between factor of group (single drug alone, co-administration) were done separately on nicotine and alcohol reinforcements. There was a significant effect of group (nicotine: $F(1, 65)=7.45; p<0.05$; alcohol: $F(1, 66)=24.69, p’s<0.05$), as overall, animals co-administering both drugs received fewer reinforcements than when they were self-administering either drug alone. There were also significant group x interval interactions in these analyses (nicotine: $F(11, 65)=1.98$; alcohol: $F(1, 66)=17.92, p’s<0.05$), which were followed by separate ANOVAs on alcohol and nicotine reinforcements for each of the different drug and co-administration conditions with the within factor of interval. The factor of interval was significant for both nicotine and alcohol alone (alcohol: $F(11, 33)=62.17, p<0.05$; nicotine: $F(11,32)=4.46, p<0.05$). Post hoc analyses showed that for alcohol alone, the first 5-, 10-, and 15-min intervals were significantly higher than all succeeding intervals, while for nicotine alone, responding was higher in the first 5 min of the session compared to intervals 10 to 50 min (b).

Overall, animals received fewer nicotine and alcohol reinforcements under conditions of co-administration (Figure 4.3c). Animals received the highest numbers of alcohol reinforcements during the first 10 min of the session; this was reflected in a significant effect of interval ($F(11, 33)=9.47, p<0.05$). Post hoc analysis showed that
numbers of alcohol reinforcements at 5 and 10 min were significantly higher than all succeeding intervals ($p$’s<0.05). The numbers of nicotine reinforcements earned per 5-min period did not vary significantly as a function of interval during co-administration.
4.4 Discussion

The most important result from this experiment is that rats self-administered both nicotine and alcohol in pharmacological relevant amounts, and in a stable manner, over a period of days when both drugs were concurrently available. When nicotine or alcohol was the only available drug, the amount of alcohol or nicotine self-administered by the animals in this experiment is comparable to other studies. The average number of alcohol reinforcements or amount of alcohol intake obtained by the animals that had access to alcohol only (i.e. the alcohol alone group) in this experiment is comparable to studies reported by others (Lê et al., 2000; Lê et al., 2003; Funk et al., 2005; Lê et al., 2005; Marinelli et al., 2007a; Marinelli et al., 2007b; Lê et al., 2009). Similarly, animals that had access to nicotine only (i.e. the nicotine alone group) in this experiment have self-administered in the range reported from the studies that employed a 1 h session and the same infusion dose (Corrigall and Coen, 1989; Corrigall and Coen, 1991a; Donny et al., 1995; Donny et al., 1999; Watkins et al., 1999; Wang, 2003; Paterson and Markou, 2004; Coen et al., 2009). When animals had concurrent accesses to both nicotine and alcohol (i.e. the co-administration group), the average amount of alcohol self-administered by the animals in this experiment was essentially similar to the amount of alcohol self-administered by the animals in the alcohol alone group. However, there was a slight but significant decrease in the number of nicotine reinforcements obtained by the co-administration group during the co-administration phase as compared to the nicotine alone group.

There was also a difference in the pattern of self-administration of nicotine and alcohol within self-administration sessions. Most of the alcohol reinforcements in the
alcohol alone group were self-administered during the first 15 min of the 1 h operant session, whereas the nicotine reinforcements in the nicotine alone group were self-administered in a steady manner across the session. These observations are in agreement with previous studies that used 1 h operant self-administration sessions (Corrigall and Coen, 1989; Donny et al., 1995; Lê et al., 2000; Williams and Broadbridge, 2009). As well, the pattern of nicotine or alcohol self-administration in 1 h operant sessions under the condition of co-administration was essentially the same as with animals self-administering nicotine or alcohol alone.

Interestingly, some of the results in the present experiment are inconsistent with previous work. In the present experiment, despite all the experimental groups having the conditions of access to drugs, there is a reduction in nicotine but not alcohol self-administration in the co-administration group relative to the nicotine or the alcohol alone group, respectively. However, the literature has shown that rats are willing to work harder for nicotine than for alcohol under a progressive ratio schedule of reinforcement (Donny et al., 1999; Rodd et al., 2003; Shram et al., 2008), suggesting nicotine has a higher reinforcing efficacy than alcohol.

Another interesting observation is an absence of increase in nicotine or alcohol self-administration in the co-administration group upon concurrent access to the other drug. Nicotine or alcohol has been shown to induce increased consumption of the other drug in humans (Griffiths et al., 1976; Mello et al., 1980; Henningfield et al., 1984; Mitchell et al., 1995; Perkins et al., 2000; Barrett et al., 2006; King et al., 2009), and passively administered nicotine has been shown to enhance alcohol consumption in
laboratory animals (Potthoff et al., 1983; Blomqvist et al., 1996; Smith et al., 1999; Lê et al., 2000; Söderpalm et al., 2000; Olausson et al., 2001; Lê et al., 2003).

A number of potential factors might account for the reduction in nicotine but not alcohol self-administration in the co-administration group. This concurrent self-administration of alcohol and nicotine may potentiate the depressant effects of nicotine (Lê et al., 2000; Brielmaier et al., 2007) and alcohol (Schechter and Lovano, 1982; White et al., 2002). Given the aforementioned difference in the pattern of self-administration between nicotine and alcohol in the co-administration group, there would be a greater impact on nicotine intake than alcohol. Another possibility is that the rats were trained longer with alcohol, and therefore their motivation to seek alcohol might be stronger than their motivation to seek nicotine.

An absence of an increase in alcohol self-administration in the co-administration group is also of interest, and a number of factors could explain this observation. Most of the animal studies that reported increased alcohol consumption after repeated or subchronic treatments with nicotine have utilized a “block design”; nicotine was passively administered at 15 – 30 min prior to the alcohol drinking session (Smith et al., 1999; Lê et al., 2000; Lê et al., 2003). As well, the reported increase in alcohol self-administration was only observed at 45 min after the passive administration of nicotine (Lê et al., 2000). In the present experiment, both nicotine and alcohol were concurrently available in the operant sessions. This concurrent self-administration design may be a limiting factor for detecting an increase in intake of alcohol within a relatively short duration of 1h operant sessions. In addition, there are possible additive effects of the depressant actions of nicotine (Lê et al., 2000; Brielmaier et al., 2007) and alcohol.
(Schechter and Lovano, 1982; White et al., 2002). Furthermore, the amount of nicotine self-administered by the co-administration group at the early portion of operant sessions may have been too little to induce increased alcohol self-administration, as increased alcohol self-administration is known to occur only after the exposure to the high dose (i.e. 80 μg/kg) of nicotine (Lê et al., 2003). Taken together, these results suggest that the limitations in design used in the present experiment may have contributed to the lack of increase in alcohol self-administration by nicotine in the co-administration group.

Similarly, the absence of increase in nicotine self-administration in the co-administration group might be accounted for the pattern of nicotine self-administration in combination with the time-dependent pharmacodynamic interaction between nicotine (Lê et al., 2000) and alcohol (Schechter and Lovano, 1982). The potentiation of the depressant effect from nicotine (Lê et al., 2000; Brielmaier et al., 2007) and alcohol (Schechter and Lovano, 1982; White et al., 2002) might have made a significant influence on nicotine self-administration, which is linearly dependent on time. Furthermore, studies using laboratory animals show no solid evidence on an increase in nicotine intake after pretreatment of alcohol (Hanson et al., 1979; Wang, 2003).

Interestingly, the experimental design utilized in most of laboratory studies in humans investigating on the effect of either alcohol or nicotine on tobacco cigarette seeking or alcohol drinking respectively is block design, like one used in the animal studies that showed nicotine-induced increases in alcohol intake. Most of the experimental studies in humans that reported increased urge and satisfaction of taking either nicotine or alcohol have the participants self-administered alcohol or nicotine respectively first, and then examined the levels of craving for the other drug at 15 – 20
min after the pre-treatment (Burton and Tiffany, 1997; Kouri et al., 2004; King and Epstein, 2005; Epstein et al., 2007; King et al., 2008). As well, many laboratory studies in humans, where actual consumption of alcohol or nicotine (both drugs) was involved, have been conducted in a similar way (Henningfield et al., 1984; Nil et al., 1984; Mintz et al., 1985; Perkins et al., 1995; King et al., 2009), and have produced results that were slightly different from studies that allow participants to consume either nicotine or alcohol during and immediately after the treatment (Mello et al., 1980). In that study, subjects could smoke during and immediately after consumption of the alcohol beverage, whereas in the experiment of Henningfield et al (1984), subjects could not begin to smoke until 20 min after consuming alcohol. It is possible that Henningfield et al (1984) did not observe an alcohol-related increase in smoking in non-alcoholics because of the difference in the time frame of drug administration.

An important limitation in the present experiment is the lack of an experimental group that self-administers nicotine without any exposure to alcohol. This experimental group would serve as a control to determine whether prior alcohol self-administration facilitates the acquisition of nicotine self-administration. However, this experimental group would be difficult to implement, due to the high risk of attrition due to catheter blockage.

In summary, an operant procedure, in which rodents have self-administered both nicotine and alcohol concurrently in significant amounts, and in a stable manner, over a period of days has been developed. This procedure can be utilized to study the relationship between nicotine and alcohol seeking. Further, this procedure may be helpful in determining ways to treat co-morbid nicotine and alcohol addiction.
Chapter 5
CONCURRENT SELF-ADMINISTRATION OF INTRAVENOUS NICOTINE AND ORAL ALCOHOL IN RODENTS

Experiment 2: Self-Administration of Nicotine prior to Alcohol

5.1 Introduction

Results from the previous experiment demonstrated that rats do self-administer alcohol and nicotine concurrently in pharmacologically relevant amounts when they are given access to both drugs. They maintained their alcohol intake in a similar amount as that obtained when alcohol was available, whereas their nicotine intake was slightly lower relative to intake by the nicotine alone group.

Initiating an operant self-administration of alcohol requires a long period of time, which generally ranges from 8 to 10 weeks, whether one employs the “sucrose fading” procedure (Samson, 1986; Brown et al., 1998; Clark et al., 2001; Sharpe and Samson, 2002), or the two-bottle choice paradigm with limited access to alcohol (Lê et al., 2000). On the other hand, stable operant self-administration of nicotine can generally be attained with 2 to 3 weeks of training. However, the patency of the intravenous (IV) cannula has always been one of the major methodological problems associated with IV drug self-administration. For studies that require longer length of time to be completed, problems arising as a result of cannula blockade are most common.

The previous experiment was the first attempt to study concurrent self-administration of oral alcohol and intravenous nicotine. Rats in the previous experiment were first trained to self-administer alcohol and were subsequently trained to self-
administer nicotine prior to concurrent access to both nicotine and alcohol. This design was utilized to minimize the length of the experiment and the potential problem regarding to the patency of the intravenous cannula. However, given that animals were first trained to self-administer alcohol, and that the training period of alcohol self-administration was much longer, it is possible that the co-administration of alcohol and nicotine has been confounded by the effect of order (i.e. alcohol first) as well as the differences in the duration of exposure to alcohol and nicotine. It could be argued that alcohol self-administration behavior was more robust than that of nicotine due to both the fact that animals were trained on alcohol first, as well as receiving a longer duration of exposure to alcohol. These factors might explain in part the observed reduction in nicotine but not alcohol self-administration during the co-administration phase.

The duration of the daily operant self-administration session may be another factor that confounds the levels of co-administration of nicotine and alcohol as the sedative properties of nicotine (Brielmaier et al., 2007) and alcohol (White et al., 2002) are time-dependent (Schechter and Lovano, 1982; Lê et al., 2000). In the operant co-administration procedure described in the previous chapter, the duration of the daily operant self-administration session was limited to 1 h. It has been found that the total daily nicotine intake increases as the session duration is lengthened (Corrigall and Coen, 1989; Paterson and Markou, 2004; Kenny and Markou, 2006; O'Dell et al., 2007). The pattern of nicotine self-administration also changes when the duration of the session is extended (Corrigall and Coen, 1989; Donny et al., 1995; Paterson and Markou, 2004; O'Dell et al., 2007). In daily 1 h operant sessions, nicotine reinforcements are earned in a steady manner across the session (Corrigall and Coen, 1989; Donny et al., 1995). When
the duration of daily operant sessions is extended to 6 h, one-third of the total nicotine reinforcements are obtained in the 1st h of the session (Paterson and Markou, 2004). In the unlimited access to nicotine self-administration, the rate of nicotine intake is highest in the mid-hours of dark cycle (O'Dell et al., 2007). On the other hand, the duration of the access to alcohol has no marked effect on the pattern of alcohol self-administration in rats. Alcohol is usually consumed in episodes (Marcucella and Munro, 1987; Files et al., 1994; Hölter and Spanagel, 1999; Lê et al., 2000). However, the level of alcohol consumption in each episode increases as the duration of the access to alcohol is shortened (Marcucella and Munro, 1986; Marcucella and Munro, 1987; Files et al., 1994).

To explore these issues, and to address the potential confounds, a co-administration experiment, in which rats are been trained to self-administer nicotine prior to alcohol was conducted. In addition, the length of training for alcohol self-administration was shortened to determine whether a longer duration of alcohol exposure strengthens alcohol self-administration behavior. Then, the duration of the daily sessions was extended to determine the impact of session duration on co-administration. Additionally, to help determine whether prior experience with nicotine self-administration would facilitate subsequent alcohol self-administration, another experimental group, in which rats self-administered nicotine alone, and then alcohol alone for the rest of the experiment, was included.
5.2 Materials and Methods

5.2.1 Animals

Fifty-two male Wistar rats weighting between 150-200 grams at the beginning of the experiment were used for this study. They were singly housed in the vivarium in the condition as described in section 3.1.

5.2.2 Experimental design

The training schedules of the 4 experimental groups during the operant phase are illustrated in Figure 5.1.

![Figure 5.1. Illustration of the nicotine and/or alcohol training regimens in Experiment 2. Animals receiving nicotine training self-administered it at a dose of 30 μg/kg/infusion at a final ratio of FR-3. For animals receiving training with alcohol, the final self-administered dose was 0.19 ml of 12% (w/v) alcohol/delivery at FR-3.](image)
All rats were first trained to self-administer sucrose for 5 days in order to speed the learning of the operant response. Subsequently, they were assigned to one of four groups designated as the Nicotine alone group (n=12), the Co-administration group (n=16), the Nicotine experienced alcohol group (n=12), and the Alcohol alone group (n=12). Rats from the Nicotine alone, the Co-administration, and Nicotine experienced alcohol groups were implanted with intravenous catheters for nicotine self-administration training while rats from the alcohol alone group underwent intravenous sham surgery.

Alcohol alone

After recovering from sham surgery, rats were trained to self-administer 3% alcohol at FR-1, FR-2, and FR-3 for 8, 3, and 3 days, respectively; 6% alcohol at FR-3 for 5 days; and finally 12% alcohol at FR-3 for the rest of experiment. The operant conditions for alcohol self-administration are described in section 3.4.3.

Nicotine alone

After recovery from catheter surgery, rats were trained to self-administer nicotine (30μg/kg per infusion) in 1 h daily sessions for 22 days as described in section 3.4.4. For the first 8 days, active lever responding was reinforced under a FR-1 schedule, the next 3 days at FR-2, and then under FR-3 for the remainder duration of the experiment.

Co-administration

After recovery, rats were trained to self-administer nicotine (30μg/kg per infusion) at FR-1, FR-2, and FR-3 for 8, 3, and 11 days, respectively. Subsequently, they
were trained to self-administer alcohol alone beginning with 3% alcohol at FR-1 (8 days), FR-2 (3 days), and FR-3 (6 days), followed by 6% alcohol at FR-3 (5 days), and then 12% alcohol at FR-3 for the rest of the experiment. After rats showed stable responding for 12% alcohol at FR-3, they received sessions with concurrent access to alcohol and nicotine (both at FR-3). Animals were run under the co-administration conditions as described in section 3.4.5 for 2 weeks.

**Nicotine experienced alcohol**

After recovery, rats were trained to self-administer nicotine (30μg/kg per infusion) using the same parameters and schedule described for nicotine training in the co-administration group. Rats were then trained to self-administer alcohol alone beginning with 3% alcohol at FR-1 (8 days), FR-2 (3 days), and FR-3 (6 days), followed by 6% alcohol at FR-3 (5 days), and then 12% alcohol at FR-3 for the rest of the experiment.

**Extension of session duration**

Once rats in each experimental group self-administered their respective drug(s) in significant amounts, and in a stable manner, for 2 weeks, the duration of the daily self-administration sessions was extended from the original 1 h to 2 h for 9 days. With the exception of the change in duration, the conditions for self-administration session remained the same with respect to each experimental group as described in section 3.4.
5.2.3 Statistics

Nicotine and alcohol reinforcements (intakes) from the self-administration phase were analyzed by mixed analysis of ANOVAs with repeated measures, using the between factor of Group and within factor of Day. The within factor of Interval was used in the analysis of responding in 5-min intervals. The Newman-Keuls procedure was utilized for post hoc testing where significant effects from ANOVA ($p$ values < 0.05) were found.
5.3 Results

Attrition

Throughout this experiment, two rats from the nicotine alone group and four rats from the co-administration group were excluded due to catheter blockage.

Table 5.1 shows the alcohol and nicotine intake of animals in Experiment 2. Each value is the averaged nicotine or alcohol intake for each individual experimental group during co-administration phase across 9 days.

Table 5.1. Mean (+ SEM) nicotine and alcohol intake in Experiment 2

<table>
<thead>
<tr>
<th>Drug intake/1h</th>
<th>Alcohol (g/kg)</th>
<th>Nicotine (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol alone</td>
<td>0.91 ± 0.11</td>
<td>–</td>
</tr>
<tr>
<td>Nicotine alone</td>
<td>–</td>
<td>0.57 ± 0.11</td>
</tr>
<tr>
<td>Co-administration</td>
<td>0.70 ± 0.14</td>
<td>0.37 ± 0.06</td>
</tr>
<tr>
<td>Nicotine experienced alcohol</td>
<td>0.94 ± 0.08</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug intake/2h</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol alone</td>
<td>1.42 ± 0.28</td>
<td>–</td>
</tr>
<tr>
<td>Nicotine alone</td>
<td>–</td>
<td>1.00 ± 0.22</td>
</tr>
<tr>
<td>Co-administration</td>
<td>1.05 ± 0.20</td>
<td>0.59 ± 0.12</td>
</tr>
<tr>
<td>Nicotine experienced alcohol</td>
<td>1.33 ± 0.19</td>
<td>–</td>
</tr>
</tbody>
</table>
Figure 5.2 shows the responses of animals trained to self-administer nicotine or alcohol alone (a), or to self-administer nicotine, then alcohol, and subsequently given access to both (b) over the last 18 self-administration sessions. For 1 h self-administration sessions, the mixed ANOVA with the within factor of day (1 to 9) and between factor of group done on numbers of nicotine reinforcements revealed a significant main effect of group, as overall animals co-administering alcohol and nicotine self-administered less nicotine \( (F(1, 16)=13.33, p<0.001) \) than those self-administering it alone. For 2 h self-administration sessions, the mixed ANOVA with the within factor of day (10 to 18) and between factor of group done on numbers of nicotine reinforcements revealed a significant main effect of group as overall animals co-administering alcohol and nicotine self-administered less nicotine \( (F(1, 16)=4.88, p<0.05) \) than those self-administering it alone. There were no significant main effects of day or group or interaction in the mixed ANOVA done on alcohol reinforcements in animals trained to self-administer alcohol alone or to co-administer both drugs in either 1 h or 2 h self-administration sessions.

Figure 5.3 (a) shows the response to nicotine and alcohol during the last three days of training for, respectively, the self-administration of nicotine and alcohol alone and then when animals were given the opportunity to co-administer both drugs. The introduction of alcohol significantly reduced responding for nicotine, as shown by a repeated measures analysis including the average numbers of nicotine reinforcements for the 3 days prior to the introduction of administration of both drugs concurrently \( (F(16,118)=1.76, p<0.05) \).
Figure 5.2. *Comparison of nicotine or alcohol self-administration with co-administration of both drugs in Experiment 2.*

A. Mean (+sem) number of reinforcements earned by animals trained to self-administer nicotine (0.03 mg/kg/infusion) or alcohol (0.19 ml of 12% w/v) alone in their daily 1 h (day 1 to day 9) and 2 h (day 10 to day 18) operant self-administration sessions.

B. Mean nicotine and alcohol reinforcements in animals co-administering both drugs in 1-h (day 1 to day 9) and 2 h (day 10 to day 18) sessions.

C. Mean numbers of reinforcements of nicotine and alcohol obtained, averaged over nine days of 1 h or 2 h access in animals trained to self-administer nicotine or alcohol alone, and in those trained to co-administer both drugs. *p<0.05, significantly main effect of group.*
Figure 5.3. *Training for co-administration of nicotine and alcohol in Experiment 2.*

a Mean (± sem) numbers of reinforcements of nicotine and alcohol obtained prior to (Day -3 to 3) and after (Day 4 to 17) the initiation of co-administration sessions. The numbers of nicotine reinforcements (0.03mg/kg/infusion) on the last three days of training at FR-3 are presented from days -3 to -1. On Days 1 to 3, the mean numbers of alcohol (0.19 ml of 12% w/v) alone earned on the last three days of training at FR-3 are shown. The numbers of nicotine reinforcements obtained by animals in the alcohol alone and the nicotine alone group, are depicted in 5 min intervals. **p<0.05, significantly different from preceding intervals for nicotine. Generally,** *p<0.05, significantly different from preceding intervals for alcohol. #p<0.05, significantly different from preceding intervals for nicotine.*
Figure 5.3 (b) shows the average number of nicotine and alcohol reinforcements in 5 min intervals self-administered by the animals in the co-administration group when they were in the single drug self-administration training phases (self-administration of nicotine alone or alcohol alone). Animals in the co-administration group, when self-administering alcohol alone, responded at the highest rates in the first 10 min of the 1 h session, while the response was at a steady rate when animals self-administered nicotine alone. This observation was reflected in effects of the repeated factor of interval (alcohol: $F(11, 83)=7.60, p<0.001$; nicotine: $F(11,83)=1.12, p>0.05$).

Figure 5.3 (c) shows the average number of nicotine and alcohol reinforcements in 5 min intervals obtained by the animals in the co-administration group when they were co-administering both nicotine and alcohol in 1 h sessions. Animals, overall received fewer nicotine and alcohol reinforcements under conditions of co-administration. Animals, when co-administering both nicotine and alcohol, received the highest number of alcohol reinforcements during the first 5 min of the 1 h session ($F(11,83)=5.07$, $p<0.001$), and obtained the lowest nicotine reinforcements in the time period between the 15 min and 30 min marks ($F(11, 83)=2.69, p<0.05$).

Figure 5.4 details the relationship between nicotine and alcohol self-administration in the alcohol alone, the nicotine alone (a), and the co-administration (b) groups in 2 h sessions. Animals self-administering alcohol alone responded at the highest rates in the first 10 min of the 2 h session, while that of the nicotine alone was steady throughout the 2 h session. The separate analysis of alcohol or nicotine reinforcements showed significant effects of the repeated factor of interval (alcohol: $F(23, 239)=10.48$, $p<0.001$; nicotine: $F(23,167)=1.55, p>0.05$). When the co-administration of both nicotine
and alcohol was extended to 2 h, animals received the highest numbers of alcohol reinforcements during the first 5 min of the 2 h session; this was reflected in a significant effect of interval ($F(23,167)=2.13, p<0.05$). Meanwhile, the number of nicotine reinforcements was at the lowest level during the time period from the 20 min to the 40 min marks, as compared to the rest of the time intervals ($F(23, 167)=1.67, p<0.05$).

Figure 5.4. Pattern of self-administration in extension of the session duration in Experiment 2. 

a Mean numbers of nicotine and alcohol reinforcements obtained by animals in the alcohol alone and the nicotine alone group, depicted in 5 min intervals. 

b Mean numbers of reinforcements obtained by animals in the co-administration group in 5 min intervals. †$p<0.05$, significantly different from preceding intervals for alcohol. #$p<0.05$, significantly different from preceding intervals for nicotine.
Figure 5.5 compares the daily average numbers of alcohol reinforcements obtained by animals in the alcohol alone group and the nicotine experienced alcohol group during the last 18 self-administration sessions. A mixed ANOVA with the within factor of day and between factor of group (alcohol alone, alcohol with prior nicotine experience) revealed no significant main effects of day, group, or interaction in the 1 h ($F(1, 16)=1.85, p>0.05$) or 2 h sessions ($F(1, 16)=0.94, p>0.05$).

Figure 5.5. Comparison of alcohol self-administrations in nicotine experienced alcohol group with alcohol alone group in Experiment 2. a Mean (+sem) number of alcohol reinforcements obtained by animals with (i.e. nicotine experience alcohol group) or without (i.e. alcohol alone group) experience of nicotine self-administration in their daily 1 h or 2 h operant alcohol self-administration sessions. b Mean numbers of reinforcements of alcohol obtained, averaged over nine days.
Figure 5.6 shows the average number of alcohol reinforcements in 5 min intervals obtained by the alcohol alone group and nicotine experienced alcohol group in 1 h (a), or in 2 h (b) session. Animals in the nicotine experienced alcohol group responded at the highest rates in the first 10 minutes of the 1 h sessions \( (F(11,143)=64.22, p<0.001) \), and 2 h sessions \( (F(23,287)=14.36, p<0.001) \). The patterns of alcohol self-administration in the alcohol alone group and the nicotine experienced alcohol group were essentially similar in 1 h, or in 2 h self-administration sessions.

**Figure 5.6. Comparison of pattern of self-administration in nicotine experienced alcohol group with alcohol alone group in Experiment 2.**  
**a** Mean numbers of alcohol reinforcements obtained by animals (i.e. nicotine experience alcohol group) or without (i.e. alcohol alone group) prior experience of nicotine self-administration in a daily 1 h operant alcohol self-administration session, depicted in 5 min intervals.  
**b** Mean numbers of reinforcements obtained by nicotine experienced alcohol group and alcohol alone group in 5 min intervals in a daily 2 h operant alcohol self-administration session.  
\( \dagger p<0.05 \), significantly different from preceding intervals for alcohol.
5.4 Discussion

The results of the present experiment demonstrated that rats self-administered both nicotine and alcohol concurrently in pharmacologically relevant amounts when they were first trained to self-administer nicotine, and then alcohol prior to having access to both drugs. Similar to the previous experiment, alcohol self-administration did not change during the concurrent access phase relative to the group that received only alcohol at this time. However, nicotine intake was significantly reduced in the concurrent access group as compared to the nicotine alone group. Increasing the session duration to 2 h resulted in a significant increase in intake of both alcohol and nicotine in all experimental groups. The pattern of nicotine and alcohol intake in the concurrent access group was the same as in the other groups.

In this experiment, besides having rats trained to self-administer nicotine prior to alcohol, the length of the alcohol self-administration training was reduced as compared to the previous experiment. Surprisingly, with shorter length of alcohol self-administration training, a significant reduction was not observed in alcohol but nicotine intake during the concurrent access phase. In fact, in this study, while nicotine self-administration was still maintained, the reduction in nicotine intake during the concurrent access phase was more pronounced than that observed in the previous experiment. When the session duration was extended to 2 h, nicotine intake in the co-administration group was almost double of their intake during 1 h session (i.e. from 10 to 19 reinforcements). Nicotine intake, however, was still significantly lower than that of nicotine alone group subject to the 2 h session duration. Therefore, the relative reduction in nicotine intake in the co-
administration condition is independent of the order of the drug training as well as the
duration of the session.

While the results of this experiment have ruled out the potential impact of the
order effect in the training procedure as well as the potential differences in duration of
nicotine and alcohol exposure on subsequent intake of nicotine and alcohol during the co-
administration phase, it appears that rats still prefer alcohol more than nicotine during this
phase. A previous study by Manzaro et al (2002) has shown that rats prefer cocaine much
more than nicotine when given the choice of self-administering either drug intravenously.
This observation is not surprise given that the rewarding value of cocaine (Depoortere et
al., 1993; Li et al., 1994; French et al., 1995) is higher than nicotine (Shram et al., 2008;
Coen et al., 2009; Forget et al., 2009). However, another study, conducted by Lenoir et al
(2007), is of interest. They showed that rats prefer to self-administer sucrose or saccharin
orally over intravenous cocaine when cocaine and sucrose or saccharin were concurrently
accessible (Lenoir et al., 2007). This is somewhat puzzling given that the reinforcing
efficacy of cocaine, as measured on a progressive-ratio (PR) paradigm, is higher than that
of sucrose. Similarly, the reinforcing efficacy is much higher for nicotine relatively to
alcohol. In fact, the breakpoint or the final ratio of responses achieved for alcohol is quite
low, and for this reason, the PR procedure is not commonly employed in the alcohol
studies (Rodd et al., 2003; Pickering et al., 2007). Yet, when rats have the choice of
cocaine or sucrose or saccharin, or between alcohol and nicotine, they preferred sucrose
or saccharin over cocaine (Lenoir et al., 2007), and alcohol over nicotine. Taking the
results of these studies together with our present findings, one explanation may be
suggested. It is possible that the immediate oral stimulation induced by sucrose or alcohol
might overcome the heightened reward for the intravenous drugs. Oral sensation can stimulate the release of DA induced by sucrose sham feeding (Schneider, 1989; Smith, 2004) indicating that the effect can be independent of post-ingestive effects. Preference for sweet tasting solutions has been shown to be an important predictor of alcohol intake (Overstreet et al., 1993; Kampov-Polevoy et al., 1995; Kampov-Polevoy et al., 1999). Moreover, orally applied alcohol has also been shown to activate gustatory neural circuits that underlie sugar taste (Lemon et al., 2004).

Despite the changes that were made in the procedure used to initiate alcohol self-administration in this experiment, the amount of alcohol intake fell within the range of alcohol intake (0.7 – 1.5 g/kg/1h session) obtained by the alcohol alone group using extended training: 3-4 weeks on two-bottle choice drinking with limited access, followed by operant training (Lê et al., 2000; Lê et al., 2003; Funk et al., 2005; Lê et al., 2005; Marinelli et al., 2007a; Marinelli et al., 2007b; Lê et al., 2009).

Interestingly, without using the two-bottle choice drinking prior to the initiation of operant alcohol self-administration training, rats also self-administered high amounts of alcohol. It is possible that such acquisition of alcohol self-administration might be facilitated by prior nicotine exposure as well as by enhanced operant learning resulting from responding for nicotine. These, however, are unlikely explanations as the alcohol alone group, which has been trained without nicotine exposure, showed comparable responding for alcohol. It is also possible that animals readily acquired operant self-administration in this experiment due to the slow escalation of alcohol concentration such that the animals had the opportunity to habituate to the taste of alcohol solution. In fact, while the use of sweet tasting solutions such as sucrose or saccharin has been employed
in initiating alcohol self-administration in what has been known as the “sucrose fading technique”, it should be noted that the similarity between the “sucrose fading technique” and our procedure is that alcohol concentrations were slowly escalated over a period of time.

For both the nicotine alone and alcohol alone groups, drug intake significantly increased as the session duration was extended. With respect to nicotine self-administration, this phenomenon is consistent with previous studies from the literature (Paterson and Markou, 2004; O'Dell et al., 2007). However, it was surprising to observe a significantly higher alcohol intake in the alcohol alone group. In most instances, the majority of the alcohol reinforcements were self-administered in the early portion (i.e. first 10-15 min) of the session, and such a pattern of self-administration was also observed with the 2 h session duration. Time course analyses for the pattern of alcohol self-administration revealed that under both 1 and 2 h session durations, about 50% of the responding for alcohol occurred within the first 15-20 min. However, such responding did not occur subsequent to the first 15-20 min. One possible way to explain this is that the increase in alcohol self-administration with longer duration reflects the rats attempt to maintain their blood alcohol level.

While this experiment has addressed some of the issues related to the effects of concurrent access on self-administration of nicotine and alcohol, there are still unanswered question regarding session duration. The consumption of nicotine and/or alcohol in humans typically occurs over extended periods of time. It is possible that with the relatively short (1-2 h) session durations we employed, enduring changes in the physiological and behavioral effects of nicotine and alcohol are less likely to be observed.
In addition, differences in the pattern of nicotine self-administration in rats have been reported when the duration of the session was extended from 1 h (Corrigall and Coen, 1989; Donny et al., 1995; Paterson and Markou, 2004) to 6 h (Paterson and Markou, 2004) or 23 h (O'Dell et al., 2007). This offers support for the idea that longer sessions may be necessary in order to observe long-term changes.

In summary, the concurrent self-administration of both nicotine and alcohol in pharmacologically relevant amounts was clearly established in this study. Such co-administration was observed to be stable, and was observed over many days. The amounts of nicotine and alcohol self-administered under conditions of concurrent access were independent of the order of drug training. The self-administration of nicotine and alcohol was independent of the length of the training for each drug. As to the effect of session duration, the patterns of intake of nicotine and alcohol did not change significantly from 1 to 2 h.
Chapter 6

GENERAL DISCUSSION

The high prevalence of tobacco abuse in individuals with alcohol dependence poses a number of important issues regarding the treatment of their addictions. First, it is not known whether the treatments for nicotine and alcohol co-dependence should be given simultaneously or sequentially (Sussman, 2002; Hurt and Patten, 2003). The sequential treatment has a notable low success rate (Hughes, 1993; Sussman, 2002; Hughes and Kalman, 2006), but the simultaneous treatment could be detrimental to alcohol treatment outcomes (Kalman, 1998; Bowman and Walsh, 2003; Joseph et al., 2004b). Second, it is not known whether the cessation of alcohol intake would result in an increase in nicotine intake or vice versa. It is unclear whether the consumption of nicotine or alcohol would enhance relapse to the other drug, after a period of abstinence. Some studies show that discontinuation of smoking may increase the chances of relapse to alcohol use (Joseph et al., 2004a; Joseph et al., 2004b) while another study reports that alcoholics who continue to smoke may be more likely to relapse to alcohol (Kalman et al., 2001). Another question is whether smokers respond to the drugs used for treating alcoholism and vice versa. Despite the importance of these questions, few preclinical studies have addressed them. One of the main reasons for this is the lack of appropriate animal models of comorbid nicotine and alcohol abuse.

To address this issue, an operant nicotine and alcohol co-administration procedure, as presented in this thesis, was developed. In this procedure, rats were trained to self-administer nicotine or alcohol first, and then were trained to self-administer the
other drug. Then, they were trained to self-administer both nicotine and alcohol concurrently. The level of self-administration for either nicotine or alcohol during the co-administration phase was in the range reported from previous literature, in which nicotine (Corrigall and Coen, 1989; Corrigall and Coen, 1991a; Donny et al., 1995; Donny et al., 1999; Watkins et al., 1999; Paterson and Markou, 2004) or alcohol (Lê et al., 2000; Lê et al., 2003; Funk et al., 2005; Lê et al., 2005; Marinelli et al., 2007a; Marinelli et al., 2007b) was available alone. Furthermore, such levels of self-administration of nicotine and alcohol were maintained over many days. These results demonstrate the feasibility of using this animal model of concurrent oral alcohol and IV nicotine self-administration to model comorbid nicotine and alcohol addiction in humans.

While the interaction between nicotine and alcohol with respect to consumption or intake has been described in the literature (please refer to section 1.2.1), most of these studies have focused on examining the effect of passive administration of one drug on intake of the other (Blomqvist et al., 1996; Smith et al., 1999; Lê et al., 2000; Söderpalm et al., 2000; Clark et al., 2001; Olausson et al., 2001; Sharpe and Samson, 2002; Lê et al., 2003), or using a procedure in which no effort was required to obtain alcohol or nicotine (i.e. drinking from bottles) (Marshall et al., 2003). These experiments may yield important information about biological mechanisms of physical dependence, but have limited relevance to address processes underlying drug addiction (Fainsinger et al., 2006; Kalant, 2008; Kalant, 2010).

An ad hoc committee set up by the Royal Society of Canada to review addiction to tobacco has concluded that “the only elements common to all definitions of drug addiction are a strongly established pattern of repeated self-administration of a drug in
doses that produce reinforcing psychoactive effects reliably, and great difficulty in achieving voluntary long-term cessation of such use, even when the user is strongly motivated to stop.” The importance of studying self-administration versus passive drug exposure in relation to drug dependence has been elegantly articulated in a recent review by Kalant (2010). Comparing the consequences of repeated exposure to various drugs of abuse for different reasons such as use of opioids for analgesia, Kalant (2010), reveals that it is the self-administration of a drug, not simply the exposure to a drug that underlies addiction and dependence. With regards to preclinical studies, operant self-administration of alcohol, nicotine, or other drugs of abuse, in which the animals have to perform operant responses in order to obtain drug is considered a gold standard in the study of the reinforcing effects of drugs. The work conducted in this thesis, which attempted to deal with the issue of co-dependence on nicotine and alcohol has also utilized voluntary operant self-administration of nicotine and alcohol. Such a concurrent self-administration design is a much more relevant procedure to study co-dependence of nicotine and alcohol as compared to previous studies from literature utilizing passive administration.

The concurrent self-administration procedure, as depicted in this thesis, is essentially a two-lever self-administration preference paradigm, which offers self-administering rodents a choice between two concurrently available drugs. The concurrent availability of alternative non-drug reinforcers or other reinforcing drugs has been used extensively in laboratory studies with humans (Stitzer and Bigelow, 1984; Vuchinich et al., 1987; Zacny et al., 1992; Comer et al., 1998), non-human primate (Carroll, 1985; Nader and Woolverton, 1992a; Macenski et al., 1993), and recently with rodents (Manzardo et al., 2001; Manzardo et al., 2002; Lenoir et al., 2007; Ping and Kruzich,
This paradigm of concurrent access to different reinforcers was designed under the premise that drug dependent individuals give higher priority to their respective drug use than to other behaviours that once had greater value (Jaffe, 1990; Schuster, 1990). Under this premise, identifying factors that render drug taking the preferred choice relative to the larger number of alternative activities available to an individual would be an important functional approach to study drug dependence (Vuchinich and Tucker, 1988). More importantly, it would be necessary to understand how qualitatively different reinforcers interact with one another where they are concurrently available if drug dependence is viewed as a choice.

A number of human studies have shown that availability of an alternate reward such as money reduces self-administration of alcohol (Vuchinich et al., 1987; Zacny et al., 1992), cigarettes (Stitzer and Bigelow, 1984) or heroin (Comer et al., 1998). Extensive studies with non-human primates have also demonstrated that concurrent access to non-drug reinforcers such as food or sweetened solution (i.e. saccharin solution) can decrease cocaine (Nader and Woolverton, 1992b), phencyclidine (PCP) (Carroll, 1985), or pentobarbital (Macenski et al., 1993) self-administration. Concurrent access has also been used to evaluate the choice between different infusion doses of the same drug (Iglauer and Woods, 1974) or between two different drugs (Johanson and Schuster, 1975; Woolverton and Johanson, 1984).

The two-lever self-administration preference paradigm has been used in a few studies with rodents (Manzardo et al., 2001; Manzardo et al., 2002; Lenoir et al., 2007; Ping and Kruzich, 2008). Using this paradigm, the availability of a non-drug reinforcer such as sucrose pellets (Ping and Kruzich, 2008), sucrose or saccharin solutions (Lenoir
et al., 2007) has been shown to reduce methamphetamine intake (Ping and Kruzich, 2008) or preference for cocaine (Lenoir et al., 2007), respectively. In general, it can be said that the availability of an alternate reinforcer whether drug or non-drug has been shown to reduce the intake of the other reinforcer.

The present study, however, is somewhat different from those described above. While evaluating drug choice is one aspect of the present work, the issues in our studies are primarily dealing with whether animals would maintain self-administration of alcohol and nicotine when they are available concurrently over a sustained period of time, and whether they would enhance the intake one another as observed in humans. It should be noted that the majority of studies employing concurrent access have focused mainly on interaction between drug and non-drug (i.e. food, saccharin or sucrose) (Nader and Woolverton, 1992a; Comer et al., 1994; Paronis et al., 2002; Negus, 2003; Gasior et al., 2004; Lenoir et al., 2007; Weiss et al., 2007; Ping and Kruzich, 2008). There are few studies that have examined choice between two drug reinforcers (Carroll, 1987; Manzardo et al., 2001; Manzardo et al., 2002). Others, however, dealt with drugs of the same class such as cocaine versus procaine (Johanson and Aigner, 1981) or cocaine versus methylphenidate (Johanson and Schuster, 1975).

Having addressed the importance of self-administration in addiction, and rationalized the two-lever self-administration preference paradigm as the choice of the experimental paradigm to study nicotine and alcohol co-dependence, the following section will discuss issues arising from the current work. One of the issues arising from utilizing the two-lever self-administration preference paradigm with intravenous nicotine and oral alcohol as concurrent reinforcers is the reduction in nicotine self-administration.
This observation is at issue because it is not consistent with the observations made in earlier studies which demonstrated that repeated daily pretreatment with nicotine can increase alcohol self-administration (Lê et al., 2000; Clark et al., 2001; Lê et al., 2003).

It should be noted that in various studies with concurrent access to two different reinforcers such as food or sweetened liquid (i.e. sucrose or saccharin solution) versus drug (e.g. cocaine, methamphetamine, or PCP), a reduction of drug intake has always been observed (Nader and Woolverton, 1992a; Campbell and Carroll, 2000; Lenoir et al., 2007; Ping and Kruzich, 2008). Using oral operant self-administration in which monkeys have the concurrent choice to self-administer alcohol or PCP, Carroll (1987) found that concurrent delivery of alcohol decreases PCP intake, whereas PCP, at high concentrations reduce alcohol intake. These changes in drug intake due to access to alternate reinforcers (drug or non-drug) have been suggested due to changes in the reinforcing efficacy of the drug (Rodefer and Carroll, 1997).

The more important issue with the reduction in nicotine self-administration in the concurrent access to alcohol and nicotine is that the reduction occurred in nicotine but not alcohol intake. This is particularly interesting given what is known about the reinforcing efficacy of nicotine and alcohol. The progressive ratio (PR) technique in which the subject is required to emit a systematically increasing number of responses to receive successive reinforcers has been used as a method to assess the reinforcing efficacy of the drug (Hodos, 1961). The last completed ratio, where maximal responding occurs is termed the “breakpoint”, with higher breakpoints associated with higher reinforcing efficacy of a given drug. Using this technique, nicotine has been shown to have a much higher reinforcing efficacy than alcohol in the rat, with the average breakpoint for alcohol
being about 10-15 (Rodd et al., 2003) whereas for nicotine it is about 80-120 (Donny et al., 1999; Shram et al., 2008). It is, therefore, rather surprising that a reduction in nicotine but not alcohol intake was observed in the present study under concurrent access conditions. As concurrent access has been suggested as a method to assessing the relative reinforcing efficacy of different reinforcers (Johanson and Schuster, 1975), the present results suggest that alcohol has higher reinforcing efficacy than nicotine.

The above interpretation, however, has to be regarded with caution. In the present work, the concurrent access or self-administration session was restricted to one hour. Under this condition, the pattern of alcohol and nicotine self-administration is quite different as pointed out earlier. When alcohol or nicotine is the only reinforcer available, animals self-administer most of the alcohol reinforcements within the first 10-15 min of the session, whereas responding for nicotine is generally spaced out evenly over the entire 1 h session. The loading of alcohol during the early period of access is probably related to both oral sensory as well as the pharmacokinetic aspects of alcohol. Unlike IV delivery of nicotine where the animals would experience the effect of the drug quite rapidly upon infusion, alcohol is delivered orally, and it is slowly absorbed; each delivery is not sufficient to produce a pharmacological effect. These differences in temporal responding for alcohol and nicotine might help to explain the reduction in nicotine intake during concurrent access. The early responding for alcohol would potentially impact on the intake of nicotine particularly in later session, as the animals may be satiated with alcohol, this may cause motor suppression and influence responding for nicotine.

The difference in the temporal pattern of ethanol and nicotine self-administration within the same session might also explain the absence of increase in alcohol intake
induced by nicotine. In the study that demonstrated the stimulation of alcohol consumption or self-administration (Lê et al., 2003), nicotine was given at a high dose (0.8 mg/kg, SC) 15-30 min prior to the alcohol self-administration session. It is quite possible that, in concurrent access, most of the alcohol is consumed before sufficient nicotine is self-administered to produce pharmacologically relevant nicotine levels in the blood or brain.

The interaction between nicotine and alcohol at the neuropharmacological level might be another possible factor involved in the decrease in nicotine intake. Alcohol has been known to modulate the activity of nicotine at nicotinic acetylcholine receptors (nAChRs) by directly modulating it’s the affinity they have for agonists (Forman et al., 1989), degree of agonist-induced upregulation (Dohrman and Reiter, 2003), and channel opening rate (Dilger et al., 1994; Liu et al., 1994). Alcohol has also been known to modulate nicotine-induced stimulation of DA release in the mesolimbic system (Tizabi et al., 2002; Tizabi et al., 2007). Given that animals under concurrent access self-administered most of the alcohol in the first 10-15 min of the session, it may be speculated that the reinforcing efficacy of nicotine is modulated by the alcohol consumed earlier in the session, thereby leading to a decrease in nicotine self-administration (Hanson et al., 1979; Wang, 2003).

Another issue arising from utilizing the two-lever self-administration preference paradigm to study nicotine and alcohol co-dependence is the absence of an increase in either alcohol or nicotine self-administration with concurrent access. This issue might be related to the pharmacodynamic interaction between nicotine and alcohol. As mentioned earlier, although the intake of nicotine was reduced, the intake of alcohol and nicotine
under concurrent access was substantial even with this short duration of access. While not systematically measured, it was noticed that rats that concurrently self-administered alcohol and nicotine displayed a high degree of intoxication compared to those self-administering either alcohol or nicotine alone. In designing this study, we utilized experimental conditions (i.e. training procedures, drug doses, infusion rate, and etc.) that produced a high intake of alcohol and nicotine. For example, our procedure used to initiate and maintain operant alcohol self-administration that generally produces an average intake of 0.8 to 1.5 g/kg per hour (Lê et al., 2000; Lê et al., 2003; Funk et al., 2005; Lê et al., 2005; Marinelli et al., 2007a; Marinelli et al., 2007b; Lê et al., 2009), an intake that is higher than those that employed other procedures to induced alcohol self-administration in operant condition (i.e. average about 0.5 to 1.0 g/kg) (Samson, 1986; Brown et al., 1998; Sharpe and Samson, 2002). Similarly, the infusion dose for nicotine employed in the present work was the optimal one for producing high intake of nicotine (Corrigall and Coen, 1989; Donny et al., 1995; DeNoble and Mele, 2006). It should be noted that the infusion dose-response curve for nicotine is rather flat (Corrigall and Coen, 1989) and that at 10 μg/kg per infusion, the number of reinforcements obtained by rats was comparable to those induced by 30 μg/kg/infusion, although obviously with much lower intake. It is therefore likely that the experimental conditions employed in our studies were not optimized to observe enhancements in alcohol and nicotine intake under concurrent access conditions. Perhaps employing a lower ethanol concentration and a lower infusion dose of nicotine might increase the likelihood of observing an increase in alcohol or nicotine intake. An alternative approach is to maintain a constant infusion dose of alcohol with varying infusion dose of nicotine or vice versa.
Another factor making it difficult to detect enhanced intake of either nicotine or alcohol is the limited access sessions employed in the present work. Human consumption of both nicotine and alcohol typically occurs over extended periods of time. The 1 h access sessions might result in nicotine and alcohol being self-administered competitively, instead of synergistically. As well, the relative shortness of 1 h access sessions might have minimized the development of enduring physiological and behavioural changes. An alternate approach is to explore nicotine and alcohol consumption in concurrent access conditions in which alcohol or nicotine is available continuously over period of 23 to 24 h per day. In the continuous access condition, while animals consume more alcohol (Files et al., 1994) or nicotine (O'Dell et al., 2007), the intake per hour even during the peak intake time would never exceed the amount they would self-administer during the limited access condition. This would permit one to assess the topography of alcohol and nicotine intake over the day but more importantly to assess the relationship between alcohol drinking and nicotine intake.

The degree of alcohol or nicotine dependence is another possible factor involved in the absence of increases in alcohol or nicotine intake. Human studies have suggested that a history of alcohol intake plays a significant role in increased smoking (Henningfield et al., 1983; Henningfield et al., 1984; Mello et al., 1987). For example, Henningfield et al. (1983, 1984) have shown that pretreatment with alcohol significantly increased smoking in alcoholics but did not influence smoking in a consistent manner in social drinkers. Similarly, Mello et al. (1987) have also shown that heavy smoking and heavy drinking was associated with greater increases in smoking during drinking than moderate and occasionally smoking or occasional alcohol drinkers. In the present study, while our
animals consumed alcohol and/or nicotine daily, the amount of intake during their daily 1 h sessions might not be sufficient to induce dependence on either alcohol or nicotine. This is an issue that may be addressed by giving them unrestricted access to alcohol and/or nicotine daily over long periods of time.

The interaction between nicotine and alcohol at the pharmacokinetic level might also be a factor involved in the absence of increases in alcohol or nicotine intake. At the pharmacokinetic level, metabolic cross-tolerance occurs when drug-metabolizing enzymes are induced by one drug, leading to increased metabolism and decreased plasma levels of another drug. In rats, nicotine is metabolized to cotinine primarily by an enzyme known as cytochrome P450 (CYP) 2B1/2 (Nakayama et al., 1993), whereas the ethanol is metabolized to its primary metabolite, acetaldehyde by alcohol dehydrogenase (ADH) (Goldstein, 1983). However, some studies have suggested that an enzyme known as CYP2E1 metabolizes about 20% of ethanol at low blood alcohol concentrations (BAC, 10mM) (Lieber, 1994; Matsumoto et al., 1996), and 60% at high BAC (40-70 mM) (Koob et al., 1975) following the CYP2E1 induction by ethanol itself (Lieber, 1994). Interestingly, subchronic ethanol administration has been shown to induce CYP2B1, which results in enhanced nicotine metabolism (Schoedel et al., 2001). Similarly, hepatic CYP2E1 enzyme is induced by nicotine, and may in turn be metabolized by it (Howard et al., 2001). Activities of these CYP enzymes have been examined in several brain regions in rats. Cell- and region-specific induction of rat brain CYP2B1 (protein and mRNA) has been observed following subchronic nicotine administration (Miksys et al., 2000), but not subchronic ethanol administration (Schoedel et al., 2001). Increased CYP2E1 expression has, however, been observed in several brain regions following subchronic ethanol
administration (Howard et al., 2000; Schoedel and Tyndale, 2003). These data indicate that a metabolic cross-tolerance occurs between nicotine and ethanol, suggesting that pharmacokinetic profiles of nicotine and alcohol may change when both drugs are concurrently administered. The alteration in the pharmacokinetic profiles may complicate the pharmacodynamic effects, which are dependent on drug concentrations (Hukkanen et al., 2005; Vengeliene et al., 2008).

Conclusions

The most important finding in this thesis is that rats will self-administer significant amount of nicotine intravenously and alcohol orally when the two drugs are concurrently available in a two-lever self-administration preference paradigm. The current findings lay the groundwork for the development of this procedure into an animal model of nicotine and alcohol co-abuse.

The present work also demonstrates that alcohol and nicotine are self-administered by rats at high levels when available concurrently. While there are certain limitations in the use of the concurrent schedule for nicotine and alcohol under a limited access conditions (1 h session as discussed above), it offers several advantages for investigation of the co-use of nicotine and alcohol. First, as the levels of intake of nicotine and alcohol are in the range that produces pharmacological effects, the procedure provides a tool for investigating the effects of neurobiological manipulations on the intake of nicotine and/or alcohol. Second, in the limited access condition, since the time of nicotine and alcohol intake is known, it is easier to examine the effects of pharmacological agents or biological manipulations on concurrent self-administration.
An important future study is to determine the effects of pharmacological agents which alter alcohol consumption or nicotine intake on the intake of these drugs under concurrent access conditions.
Chapter 7

FUTURE WORK

The co-administration procedure developed from the present series of studies is intended to be used on research on the co-dependence of nicotine and alcohol. It is a better approximation of the co-abuse of nicotine and alcohol in humans. As described above, rodents do self-administer pharmacologically relevant amounts of both nicotine and alcohol concurrently. This procedure will be developed into a more detailed animal model of nicotine and alcohol co-dependence. It may have applications such as in determining better treatments for nicotine and alcohol co-dependence. The co-administration procedure, as presented here, however, has a number of shortcomings. In this chapter, further studies will be described that would help remedy this. Finally, the possible applications of this co-administration procedure will be elaborated upon.

7.1 Future Investigations

As mentioned in the General Discussion, there are two main limitations of the co-administration procedure. First, there is the absence of an increase in nicotine or alcohol self-administration in the co-administration group. Second, there is a reduction in nicotine but not alcohol self-administration when both drugs are available concurrently. These issues, which are inconsistent with observations from previous studies with humans and rodents, can be attributed to three major factors: 1) unit doses of nicotine and alcohol, 2) duration of the session, 3) experimental design. In this section, experiments
will be described to investigate the effect of each of these factors on the relationship between nicotine and alcohol self-administrations.

7.1.1 Determination of nicotine and alcohol dose-response

In the studies in this thesis, only single unit doses of intravenous nicotine (30\(\mu\)g/kg/infusion) and oral alcohol (0.19ml of 12% (w/v) per reinforcement) for self-administration were used. A reduction in nicotine self-administration was consistently observed when alcohol became concurrently available. It is important to determine how different unit doses of alcohol and nicotine influence this relationship. In addition, the apparent absence of an increase in nicotine or alcohol self-administration in the co-administration group could be attributed to a ceiling effect created by these unit doses. Rodents generally have maximum responses to these unit doses of intravenous nicotine and oral alcohol when each drug is self-administered alone (Corrigall and Coen, 1989; Donny et al., 1995; Lê et al., 2000; Lê et al., 2003).

In an experiment designed to investigate this, three groups of rodents (i.e. alcohol alone, nicotine alone, and co-administration groups) will be employed in a mixed design with the between-subjects factors of self-administration condition, and within-subject factor of nicotine dose or alcohol concentration. The nicotine doses will be 10, 15, 30 \(\mu\)g/kg per intravenous infusion, and these nicotine doses are chosen because they result in a similar number of responses during self-administration (Corrigall and Coen, 1989; Donny et al., 1995). The alcohol concentrations will be 3, 6, 12% (w/v), and they are utilized because stable and high responding for these doses have been shown in a previous experiment (Lê et al., 2000). The effects of varying the infusion dose of nicotine
(10, 15, 30 μg/kg per intravenous infusion) on self-administration will be examined in the nicotine alone and co-administration groups. During this time, the alcohol alone groups will continue to self-administer alcohol at the concentration available during training (0.19ml of 12%, w/v per delivery). Following the examination of nicotine dose-response, alcohol dose-response (3, 6, 12%, w/v) will be examined in the alcohol alone, and the co-administration groups. During the alcohol dose-response test, the nicotine alone group will self-administer nicotine at 30 μg/kg per infusion. The order of presentation of the drug doses in each nicotine or alcohol dose-response test will be counterbalanced. Each test will be separated by 2 to 3 days, during which rodents will self-administer 30 μg/kg nicotine infusion and/or 12% (w/v) alcohol.

7.1.2 Effect of session duration on concurrent self-administration of nicotine and alcohol

Differences in the patterns of self-administration of nicotine and alcohol were observed in the present experiments. Response rates for nicotine were consistent within the self-administration sessions. The relatively short duration of the self-administration sessions may therefore have possibly contributed to the reduced in nicotine self-administration in the co-administration group. It would be of interest to employ self-administration sessions of longer duration. This may produce enduring physiological and behavioural changes not seen with shorter periods. Therefore, in this proposed experiment, session duration will be taken as an independent variable. An added benefit of this is that human consumption of both nicotine and alcohol typically occurs over
extended periods of time, and the model would therefore better approximate the human condition.

Session duration will be varied systematically in rodents trained to self-administer nicotine and alcohol. They will be trained to self-administer nicotine and/or alcohol, and will be separated into 3 groups of 12 subjects each (i.e. nicotine alone, alcohol alone, and co-administration groups). Rats will undergo the same training procedures as described above except that the session duration will increase every two weeks. The session duration will be increased in this order: 1 h, 6 h, 12 h, to 24 h, chosen based on the previous studies from the literature, which have shown a difference in the pattern of self-administration between these session durations for either nicotine or alcohol (Marcucella and Munro, 1986; Marcucella and Munro, 1987; Corrigall and Coen, 1989; Files et al., 1994; Paterson and Markou, 2004; O'Dell et al., 2007). Nicotine and alcohol intake is expected to increase with longer durations of access to the two drugs. The effects of session duration on the relative intake of, or pattern of intake of nicotine and alcohol are difficult to predict as the pattern of nicotine self-administration changes when the duration of the session is extended (Corrigall and Coen, 1989; Donny et al., 1995; Paterson and Markou, 2004; O'Dell et al., 2007). In daily 1 h operant sessions, nicotine reinforcements are obtained in a steady manner across the session (Corrigall and Coen, 1989; Donny et al., 1995). When the duration of daily operant sessions is extended to 6 h, one-third of the total nicotine reinforcements are obtained in the 1st h of the session (Paterson and Markou, 2004). In the unlimited access to nicotine self-administration, the rate of nicotine intake is highest in the mid-hours of dark cycle (O'Dell et al., 2007).
7.1.3 Effect of a block design on self-administration of nicotine and alcohol

In the present series of studies described in this thesis, both nicotine and alcohol were concurrently available to self-administering rats. However, most of the animal studies that have reported increased alcohol consumption after repeated or subchronic treatments with nicotine have utilized a block design. It is possible that concurrent self-administration of nicotine and alcohol may potentiate the depressant effect from nicotine (Lê et al., 2000; Brielmaier et al., 2007) or alcohol (Schechter and Lovano, 1982; White et al., 2002), and thus results in an absence of increase in nicotine or alcohol self-administration under conditions of concurrent access. Similarly, most of laboratory studies in humans investigating on the effect of either alcohol or nicotine on tobacco cigarette seeking or alcohol drinking have also utilized block design, and most of them have reported increased urge and satisfaction of taking either nicotine or alcohol. It is, therefore, important to determine whether using a block design will increase the subsequent self-administration of nicotine or alcohol, respectively.

Rats will be trained to self-administer nicotine and/or alcohol, and will be separated into 4 groups of 12 subjects each. There will be one nicotine alone, one alcohol alone, and two co-administration groups. One of the co-administration groups will self-administer nicotine in the first half, and alcohol in the second half of the operant sessions. The other co-administration group will self-administer in reverse order; alcohol in the first half, and then nicotine in the second half. The single drug groups will self-administer their respective drug only (i.e. nicotine or alcohol alone) for the entire duration of the operant sessions. Previous behavioural studies using laboratory rodents have shown an increase in alcohol intake after nicotine pretreatment, but not an increase in nicotine
intake after alcohol pretreatment. Although predictions about the self-administration with a block design are difficult, since previous studies used non-contingent administration, and different routes of administration, alcohol self-administration is expected to increase in the co-administration group that self-administers nicotine in the first half of the operant session. However, it is very difficult to speculate whether the nicotine self-administration will increase after alcohol self-administration.

7.2 Applications of the Concurrent Self-administration Procedure

7.2.1 Effect of pharmacological agent on co-administration of nicotine and alcohol

Studies from the literature report that most common combination of drugs used by humans is alcohol and nicotine. This co-dependence presents serious social and medical problems, more so than with either drug alone. Previous animal studies have also demonstrated significant interactive effects between nicotine and alcohol. However, the effect of clinically used drugs for treating alcohol- or nicotine- dependence has not been evaluated on this interaction. In this proposed experiment, the effects of such clinically used drugs on co-administration of nicotine and alcohol will be examined in rodents. This extension may aid in the determination of more effective means to treat co-morbid nicotine and alcohol addiction.
7.2.1.1 Naltrexone

Naltrexone, an opiate antagonist, is clinically used to treat alcohol dependence. Studies investigating the effect of naltrexone on smoking in humans have produced conflicting results. Some studies reported reduction in the urge to smoke (King and Meyer, 2000; Epstein and King, 2004) while others presenting null results (O'Malley et al., 2006; Rohsenow et al., 2007). In this proposed study, the effect of naltrexone will be examined on rodents self-administering alcohol alone, nicotine alone, and nicotine and alcohol concurrently.

Rats will be assigned to one of 3 groups: the alcohol alone group, the nicotine alone group, and the co-administration group. These three groups will be employed in a mixed design, with between-subject factors of the Self-administration condition, and within factors of self-administration day and Naltrexone dose (0, 0.3, 1.0, 3.0 mg/kg, SC). Each test is separated by 2 to 3 days, during which rodents will self-administer nicotine and/or alcohol in the same way as during training.

As studies with rodents have shown naltrexone-induced reductions in alcohol self-administration alone (Lê et al., 1999; Williams and Broadbridge, 2009), but not nicotine (Corrigall and Coen, 1991a), a decrease in self-administration in the alcohol alone group but not the nicotine alone group is hypothesized. However, it is very difficult to predict the effect of naltrexone on rodents co-administering both nicotine and alcohol. Possibly, a reduction in alcohol consumption, but no change in nicotine intake in the co-administration group will be observed. It is also possible that an increase in nicotine self-administration will be observed if there is a compensation effect for the reduced alcohol self-administration by naltrexone.
7.2.1.2 Varenicline

Varenicline, an α4β2 nAChR partial agonist, is clinically used as a smoking cessation aid. To date, there is only one experimental study in humans investigating the effect of varenicline on alcohol consumption, showing that 7 days of varenicline pretreatment (2mg/day) significantly reduced alcohol self-administration in non-alcohol-dependent heavy drinkers (McKee et al., 2009). There is only one study using laboratory animals, that demonstrated varenicline to attenuate operant alcohol self-administration without affecting locomotor function (Steensland et al., 2007). In this proposed study, the effect of varenicline will be examined on rats self-administering alcohol alone, nicotine alone, and those co-administering nicotine and alcohol concurrently.

Rats in this experiment will be assigned into 3 groups: the alcohol alone group, the nicotine alone group, and the co-administration group. These three groups will be employed in a mixed design, with between-subject factors of the Self-administration condition, and within factors of Self-administration day and Varenicline dose (0, 1.0, 2.0, 3.0 mg/kg, subcutaneous injection). Each test is separated by 2 to 3 days, during which rodents will self-administer nicotine and/or alcohol at the concentration available during training.

As studies using experimental animals has shown a reduction in alcohol self-administration alone (Steensland et al., 2007), and nicotine self-administration alone ((Rollema et al., 2007) with pretreatment of varenicline, self-administration is expected to decrease in alcohol alone and nicotine alone groups. However, it is very difficult to predict whether a reduction in alcohol or nicotine self-administration will occur in
rodents co-administering both nicotine and alcohol as there are significant interactive
effects between nicotine and alcohol.
Chapter 8

APPENDIX

Experiment 3: Extinction and Reinstatement in Rats with Concurrent Access to Alcohol and Nicotine

8.1 Introduction

The fact that nicotine and alcohol are commonly co-abused by humans presents a challenge in terms of treatment design. At issue is whether nicotine or alcohol co-dependence should be treated simultaneously or individually. The few clinical studies on this issue have produced mixed results. Discontinuation of smoking may increase the chances of relapse to alcohol use (Kozlowski et al., 1989). Another study found that alcoholics who continue to smoke may be more likely to relapse to alcohol (Stuyt, 1997). Others have reported that smoking cessation programs do not interfere with the success of alcohol treatment (De Soto et al., 1989) or that unaided smoking cessation might actually enhance the response to treatment for alcoholism (Karam-Hage et al., 2005). The reasons for these discrepant results are not known.

Despite the fact that treatment design is a major clinical issue in the co-dependence of nicotine and alcohol, pre-clinical research has paid little attention to this issue. This state of affairs is likely due to the lack of an animal procedure that allows animals to self-administer both nicotine and alcohol concurrently. This experiment, therefore, utilizes the co-administration procedure described in this thesis to help address the question of whether alcoholism or nicotine addiction or both should be treated when they occur comorbidly in humans. This experiment determines how the continued
availability of alcohol and nicotine affects extinction of responding for the other drug, and compares these conditions to one where responding for both drugs was extinguished.

8.2 Materials and Methods

8.2.1 Animals

Thirty-four male Wistar rats weighing between 150-200 grams at the beginning of the experiment were used for this study. They were singly housed in the vivarium in the condition as described in section 3.1.

8.2.2 Experimental design

All rats were first trained to self-administer alcohol (0.19 ml of 12%, w/v alcohol), received IV surgery and recovery, and were then trained to self-administer nicotine alone (30 μg/kg per infusion) as described section 4.2.2. After animals showed stable responding for nicotine at FR-3, they received sessions with concurrent access to alcohol and nicotine. After delivery of each alcohol or nicotine infusion, a 30-s timeout period occurred; during the timeout, both levers retracted. Alcohol reinforcements were paired with a flashing white cue light and nicotine with a continuously illuminated light for the duration of the timeout period. Animals were run under these co-administration conditions in daily sessions for 3 weeks prior to the start of extinction.
Extinction of nicotine and/or alcohol self-administration

Animals were assigned to one of three groups matched on responding for alcohol and nicotine. In the first group, alcohol self-administration was extinguished while nicotine self-administration was maintained. In the second, nicotine self-administration was extinguished while alcohol self-administration was maintained. In the third group, both alcohol and nicotine self-administration were extinguished. The conditions during this phase were the same as during co-administration, except that drug infusions were withheld in the groups where nicotine and/or alcohol responding was extinguished. The drug-associated cue lights were also present during these extinction sessions. Animals were run under these extinction conditions in daily sessions for 15 days.

Reinstatement induced by priming with nicotine or alcohol

After the 15 days of extinction, the effects of priming injections of nicotine or alcohol on reinstatement of responding for the extinguished drug and on self-administration of the remaining drug were examined.

Nicotine (150 μg/kg, SC) was injected 15 min prior to the test sessions in rats in the group in which both nicotine and alcohol was extinguished and in the group with nicotine extinguished that continued to have alcohol available. A single non-contingent infusion of alcohol (0.19 ml; 12%, w/v) was delivered into the drinking receptacle at the beginning of the test sessions in the group in which responding for both drugs was extinguished and the group where only alcohol was extinguished, with nicotine available.

Nicotine or alcohol was given in a counterbalanced manner with two to three extinction days between each test. Prior to initiating the priming tests, rats received three
daily SC injections of saline in order to habituate them to the injection procedure. Before testing the effects of each drug on self-administration and/or reinstatement of extinguished responding, a baseline test after a saline vehicle injection was determined. The cues previously associated with nicotine or alcohol self-administration were present during extinction and the reinstatement tests.

8.2.3 Statistics

Nicotine or alcohol infusions and active lever presses were analyzed separately with mixed two-way ANOVAs with the between factor of extinction condition and within factor of day. Significant effects from ANOVA ($p$ values<0.05) were followed by post hoc tests using the Newman-Keuls procedure. Throughout this experiment, 10 rats were excluded from analysis due to catheter blockage and 4 due to failure to meet the extinction criterion.

8.3 Results

*Extinction of nicotine and/or alcohol self-administration*

Extinction of responding for both drugs resulted in comparable and significant decreases in numbers of lever presses on the nicotine and alcohol associated levers (Figure 8.1a), as shown by separate ANOVAs with the within factor of day (baseline, extinction days 1-15) done on numbers of responses made on the levers previously associated with nicotine and alcohol (nicotine lever: $F(15, 191)=11.61$; alcohol lever: $F(15, 191)=16.14$, $p$’s<0.05).
In the group in which responding for nicotine was extinguished but alcohol access was maintained, numbers of lever responses for nicotine decreased significantly (Figure 8.1b). An ANOVA with the within factor of day (baseline, extinction days 1-15) and between factor of group (nicotine extinguished with alcohol available, both extinguished) revealed a significant effect of day \((F(1, 367)=24.43, p<0.05)\), but not of group or an interaction, suggesting that animals receiving extinction of both drugs extinguished at a rate similar to those receiving extinction of only nicotine.

In the group in which responding for alcohol was extinguished but nicotine access was maintained, numbers of alcohol lever responses decreased significantly (Figure 8.1c). The rate of extinction of responding for alcohol was significantly slower in these animals compared to those receiving simultaneous extinction of both drugs. An ANOVA done with the within factor of day (baseline, extinction days 1-15) and between factor of group (alcohol extinguished with nicotine available, both extinguished) revealed a significant effect of day \((F(15, 366)=13.64, p<0.05)\) and a significant day x group interaction \((F(15, 366)=2.359, p<0.05)\). Post hoc analyses revealed significantly decreased responding on the alcohol associated lever on extinction days 2 to 15 in the group receiving extinction of both drugs but only on extinction days 5, 7-9, and 12-15 in the group with only alcohol extinguished.
Figure 8.1. Responding of animals trained to co-administer nicotine and alcohol subject to different extinction conditions. 

a Mean (± SEM) responses on the nicotine and alcohol-associated levers in animals subject to extinction of responding for nicotine and alcohol (N=12).  

b Extinction of responding for nicotine with continued access to alcohol (N=11).  

c Extinction of responding for alcohol with continued access to nicotine (N=11). +, significantly different from the mean responses on the alcohol lever during the last 3 days of co-administration. *, significantly different from the mean responses on the nicotine lever during the last 3 days of co-administration.
Reinstatement induced by priming with nicotine or alcohol

In animals where responding for both nicotine and alcohol was extinguished, nicotine induced a significant reinstatement of responding on the levers previously associated with each drug, compared to the saline baseline (Figure 8.2a; nicotine lever: $F(1, 21)=29.35, p<0.05$, alcohol lever: $F(1, 21)=22.42, p<0.05$). Priming injections of alcohol in these animals induced a significant reinstatement of responding on both the nicotine and alcohol-associated levers (Figure 8.2b; nicotine lever: $F(1, 19)=5.87, p<0.05$; alcohol lever: $F(1, 19)=15.11, p<0.05$).

In animals whose responding for nicotine was extinguished but received continued access to alcohol, injections of nicotine significantly reinstated responding for nicotine ($F(1, 21)=26.72, p<0.05$; Figure 8.2c). Alcohol self-administration was not affected by priming injections of nicotine.

Priming injections of alcohol significantly reinstated responding on the lever previously associated with alcohol in alcohol-extinguished animals that received continued access to nicotine ($F(1, 17)=7.57, p<0.05$; Figure 8.2d). Nicotine self-administration was unaffected by the alcohol prime.
Figure 8.2. Reinstatement induced by nicotine or alcohol primes in rats trained to co-administer nicotine and alcohol subject to different extinction conditions. 

- **A:** Nicotine and alcohol extinguished: Nicotine prime
- **B:** Nicotine and alcohol extinguished: Alcohol prime
- **C:** Nicotine extinguished, alcohol available: Nicotine prime
- **D:** Alcohol extinguished, nicotine available: Alcohol prime

Nicotine (0.15 mg/kg, s.c.) was administered 15 min before the test. The alcohol prime comprised a non-contingent presentation of 0.19 ml of alcohol (12%, w/v) into the drinking receptacle at the onset of the test session.
8.4 Discussion

This experiment was done to help address the question of whether alcoholism or nicotine addiction or both should be treated when they occur comorbidly in humans. The effect of the continued availability of alcohol and nicotine on the extinction of responding for the other drug were determined, and these conditions have been compared to one where responding for both was extinguished.

A progressive decline in responding was found in animals that were trained to co-administer nicotine and alcohol when the delivery of both drugs was withheld. Like wise, animals showed a decline in responding for nicotine when it was withheld, when alcohol was still available. Interestingly, when alcohol was withheld with nicotine still available, the extinction of responding for alcohol was delayed. One interpretation of this is that continued self-administration of nicotine prolongs the motivation to seek alcohol. This is consistent with work showing that nicotine users have lower recovery rates when being treated for alcoholism (Stuyt, 1997).

A potential explanation for the reduction in the rate of extinction of responding for alcohol in animals with continued access to nicotine deals with the interaction of nicotine with cues. Nicotine self-administration has been shown to be greatly potentiated when response-contingent cues are present (Caggiula et al., 2002), and moreover, non-contingent injections of nicotine can increase responding for visual cues alone (Donny et al., 2003). In this study, the response-contingent visual and auditory cues were present during self-administration, extinction, and reinstatement. In this light, it may be that the self-administered nicotine in this group potentiated responding for the cue previously associated with alcohol during the extinction sessions. Given the importance of response-
contingent cues in nicotine self-administration, it would be of interest in future studies to specifically examine their role in co-administration and in extinction, and reinstatement after concurrent access.

Relapse or reinstatement in response to priming with the abused drug is another hallmark of addiction. Little is known about the effects of priming doses of nicotine or alcohol when their abuse is comorbid. We therefore determined the effects of nicotine or alcohol primes on responding in animals trained to co-administer whose lever pressing for nicotine, alcohol, or both was extinguished. In co-administering animals whose responding for both alcohol and nicotine were extinguished, priming injections of nicotine or presentation of alcohol reinstated responding on both the nicotine- and alcohol-associated levers. Continued access to nicotine or alcohol, while extinguishing responding for the other drug, did not make animals more or less susceptible to reinstatement by alcohol or nicotine primes, respectively. These results are consistent with findings in experimental animals that nicotine can reinstate extinguished responding for alcohol or nicotine and that alcohol priming induces reinstatement of responding for alcohol (Lê et al., 1998; Lê et al., 1999; Lê et al., 2003). They are also the first to show that alcohol can induce a modest reinstatement of responding for nicotine.
REFERENCES


Koob GF, Balcom GJ and Meyerhoff JL (1975) Dopamine and norepinephrine levels in the nucleus accumbens, olfactory tubercle and corpus striatum following lesions in the ventral tegmental area. *Brain Research* **94**:45-55.


Marcucella H and Munro I (1986) Patterns of ethanol and water consumption as a function of restricted ethanol access and feeding condition. Psychopharmacology 89:145-149.


Matsumoto H, Matsubayashi K and Fukui Y (1996) Evidence that cytochrome P-4502E1 contributes to ethanol elimination at low doses: effects of diallyl sulfide and 4-methyl pyrazole on ethanol elimination in the perfused rat liver. Alcoholism: Clinical and Experimental Research 20:12A-16A.


Mihalak KB, Carroll FI and Luetje CW (2006) Varenicline is a partial agonist at alpha4beta2 and a full agonist at alpha7 neuronal nicotinic receptors. Molecular Pharmacology 70:801-805.


Nisell M, Nomikos GG and Svensson TH (1994) Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. Synapse 16:36-44.


