ALCOHOL VS. SACCHARIN: A STUDY ON REWARD CHOICE IN ALCOHOL DEPENDENT AND NON-DEPENDENT ANIMALS

by

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ABSTRACT

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Maladaptive decision-making characterized by drug use despite the availability of many alternative rewards is a key component of substance use disorders. However, the role of changes in decision-making in the enhanced susceptibility to drug dependence has been little studied. The present thesis examined the choice between ethanol and a non-drug reward, saccharin, using the discrete-choice model in ethanol dependent and non-dependent animals. In a separate experiment, rats were trained to self-administer intravenous (i.v.) nicotine and oral saccharin. The two main findings of this thesis are: (1) rats choose oral ethanol and saccharin to a similar degree, in contrast to near-exclusive choice of oral saccharin over i.v. drugs, and (2) ethanol dependence shifts reward preference to exclusive ethanol choice. The increased choice of ethanol over saccharin produced by dependence may be explained by a dependence-induced increase in the positive reinforcing effects of ethanol and a decrease in its negative reinforcing effects.

To my father, who has taught me not to take life too seriously.

To my mother, who has shown me what it means to be a strong woman.

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
BAL	blood ethanol level
CPA	conditioned place aversion
CPP	conditioned place preference
DA	dopamine
EtOH	ethanol
FR	fixed-ratio
g/kg	grams per kilogram
hrs	hours
ITI	inter-trial interval
LAP	limited access paradigm
mg%	milligram per 100 millilitre
min	minutes
NAc	nucleus accumbens
P	ethanol-preferring
PC	place conditioning
PR	progressive-ratio
QEtOH	quinine-adulterated ethanol
SA	self-administration
Sacc	saccharin
sec	second
TO	timeout
VTA	ventral tegmental area
w/v	weight per volume
v/v	volume per volume

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Chapter 1
GENERAL INTRODUCTION

1.1. Alcohol and Drug Use Disorders

Substance use disorder can be described as a chronic relapsing disorder characterized by continued use of a drug despite its negative consequences (American Psychiatric Association, 2013). The behaviour of an individual abusing drugs is often impulsive and compulsive, reflecting altered decision-making processes (de Wit, 2009; World Health Organization, 1993). While dependence to a drug of abuse refers to the physiological processes that change in response to chronic drug exposure, the abuse of a substance relies on a complex interaction between the individual, the physiological and neurochemical effects of the abused substance, and society (National Alliance on Mental Illness, 2013).

There is considerable literature on abnormalities in decision-making in addicted individuals (American Psychiatric Association, 2013; Ahmed, Lenoir & Guillem, 2013; Guttman, Moeller & London, 2017). Drug-dependent individuals typically forgo other forms of reward in order to consume the drug in question. Substance use disorders may therefore be described as stemming from maladaptive decision-making, because natural environments usually provide access to a number of different types of rewards. The behavioural processes involved in these abnormalities in choice, and how they contribute to addiction and relapse are poorly understood.

1.2. Statement of Problem

A study conducted by the United Nations Office on Drugs and Crime (2011) reported that substance use disorders are a global problem that affect 210 million people worldwide. As will be reviewed below, alcohol and nicotine are the most commonly used drugs in our population. In addition, illicit drug use with opioids and psychotimulants pose an additional burden to our society.

Costs related to alcohol abuse in Canada equalled $14.6 billion in 2002 with the majority of services being dedicated to law enforcement, health care and loss of productivity (Rehm et al., 2006). In 2016, 19% of Canadians aged 12 years and older were classified as heavy drinkers (Statistics Canada, 2017). While a total of 79% of individuals reported alcohol use, 14% of these individuals were dependent on alcohol (Canadian Alcohol and Drug Use Monitoring Survey, 2012). In addition, alcohol-impaired driving was the leading cause of criminal death in 2008 and a recent survey reported that an estimated 1-5% of children in grade 1 are affected by fetal alcohol spectrum disorders (Perreault, 2013; May et al., 2018).

In relation to nicotine use, Statistics Canada (2014) reported that 18.1% of the population smokes cigarettes. In 2011, it was reported that smoking was the leading cause of premature death and accounted for a large portion of deaths caused by lung cancer. Notably, the health care costs related to smoking can account for 6-15% of a family's annual healthcare costs (Statistics Canada, 2012).

Finally, the abuse of illicit drugs continues to be problematic. In 2012, the Canadian Alcohol and Drug Use Monitoring Survey reported that, among users of opioid pain relievers and psychostimulants, 5.2% and 40% of them had problems with abusing each drug respectively. Harm (in physical health, friendships and social life, legal problems, employment opportunities, etc.) associated with illicit drug use in 2012 was reported to be 16.6% amongst drug users and this was more prevalent in youth (Canadian Alcohol and Drug Use Monitoring Survey). In 2002, the Canadian government spent approximately $8 billion to care for individuals dependent on illicit drugs, $4.7 billion of which was used for loss of productivity and premature death (Rehm et al., 2006).

Relapse is the major problem to overcome in the treatment of drug addiction. Forty to sixty percent of individuals treated for drug dependence return to using the drug in question one-year after discharge from an addictions treatment program (McLellan, Lewis, O'Brien & Kleber, 2000). Relapse is associated with re-exposure to the drug of

abuse, stressful life events, or exposure to environmental cues and contexts previously associated with drug taking (O'Brien, Childress, Mclellan, & Ehrman, 1992). Dependent alcohol users, for example, have been shown to be significantly more vulnerable to relapse during a period of abstinence after experiencing stressors (Brown, Vik, Patterson, Grant, & Schuckit, 1995). In addition, individuals dependent on alcohol have been shown to be both subjectively and physiologically more sensitive to alcohol-related cues (Sinha, Fox, Hong, Hansen, Tuit, & Kreek, 2011). The rate for relapse to alcohol and nicotine use can range from 80-95%, 12-months after treatment discharge (Hendershot, Witkiewitz, George, & Marlatt, 2011). There is, therefore, a critical need for more effective treatments for alcohol and drug use disorders.

A number of pharmacological and psychological treatments have been developed to reduce the high rates of relapse associated with alcohol use, perhaps the most abused drug. Subjective ratings of alcohol craving in abstaining addicts have been shown to decrease with administration of pharmacological interventions. These include (1) Acamprosate, and (2) Naltrexone, which act by reducing the rewarding effects felt by alcohol consumption, and (3) Disulfiram (i.e. Antabuse), which leads to aversive symptoms following alcohol intake (Heilig & Egli, 2006).

It is important to note that treatment adherence to these medications is typically low when given alone. In fact, clinical outcomes are best when both pharmacological and behavioural treatments are given concurrently (National Institute of Health, 2014). Behavioural interventions include: medical management by a healthcare professional, cognitive behavioural therapy, motivational enhancement therapy, and 12-step-facilitation. These methods combined with naltrexone have been shown to produce the greatest improvements on drinking measures (Anton et al., 2006). Nevertheless, relapse is still high even with the use of the most effective combined treatments.

Another approach that incorporates alternative reinforcers is known as contingency management. These programs provide alternative reward, in the form of money, vouchers, or tokens, in exchange for clean drug tests and treatment program adherence

(as reviewed in Stitzer & Petry, 2006). Contingency management programs have been shown to be one of the most effective methods of promoting abstinence for a substance of abuse by allowing addicts to take full advantage of clinical outcomes (Prendergast, Podus, Finney, Greenwell, & Roll, 2006). Substance use disorders are characterized by maladaptive decision-making in an environment with many alternative rewards available and this treatment program targets the core of this issue by using salient, alternative rewards in order to help improve a dependent individual's pattern of decision-making. While the use of contingency management programs as well as combined behavioural and pharmacological interventions have been shown to help alcohol dependent individuals abstain from drinking, there continues to be major issues with high rates of relapse.

While the use of clinical research is critical for understanding substance use disorders as a social, psychological, and multifaceted mental illness, the use of animal models of drug addiction is needed to help determine the neural underpinnings of substance use disorders. Acquiring a better understanding of the neural pathways, and neurotransmitters involved in compulsive drug use is necessary for the development of pharmacotherapies used to prevent drug relapse in humans. In the following sections I will review animal models of drug reward, reinforcement, relapse, dependence, and choice under dual-reward conditions. I will also discuss the neurophysiological properties of ethanol.

1.3. Animals Models of Drug Reward and Reinforcement

1.3.1. Conditioned place preference

The place conditioning (PC) paradigm is used to study the inherently rewarding properties of drugs of abuse. The basic characteristics of this paradigm involve learned associations between a particular environment and a drug treatment. Inversely, associations between the absence of drug (i.e. vehicle treatment) and a completely different environment are also made (Prus, James, & Rosecrans, 2009). The apparatus used for PC consists of a compartmentalized box made up of a neutral holding area that

separates sides A and B of the PC apparatus. In order to counterbalance treatment conditions, the side in which animals are given the drug treatment and vehicle treatment is randomized but made consistent throughout experimentation. Importantly, compartments A and B are very distinct from one another (e.g. black walls vs. white walls, tactile stimuli, etc.) so that, after multiple pairings, animals learn the environmental cues associated with each treatment condition through Pavlovian conditioning (Mucha, Van der Kooy, O'Shaughnessy, & Bucenieks, 1982). Following this, the rewarding or aversive properties of the drug in question can be tested by placing the animal in the neutral holding area and allowing it free access to compartments A and B, in a drug-free state. More time spent in the drug-paired compartment is indicative of conditioned place preference (CPP), whereas more time spent in the vehicle-paired compartment is representative of conditioned place aversion (CPA) (Prus et al., 2009). An overview of the development CPP or CPA to a variety of drug classes will be reviewed below.

1.3.1.1. Opiates and psychostimulants

CPP has been clearly demonstrated for various opiates. For example, morphine has been shown to produce CPP in rats and mice (Campbell, Wood & Spear, 2000; Vekovischeva et al., 2004). This has been shown within morphine doses ranging from 0.08-15 mg/kg (Mucha et al., 1982). A study by Mucha and Herz (1985) determined the effects of kappa vs. mu opioid receptor agonists on CPP. This study showed that animals will preferentially show CPP for mu opioid receptor agonists like morphine, fentanyl, and sufentanil. In contrast, kappa opioid receptor agonists were shown to either cause an absence of place conditioning or CPA (Mucha & Herz, 1985). In line with this, the mu opioid receptor agonist heroin has been shown to cause CPP with doses of 0.05-1 mg/kg (Hand, Stinus & Le Moal, 1989).

Similarly, the psychostimulants cocaine and amphetamine have been shown to produce CPP in rodents, however, they also produce CPA at higher doses (Vekovischeva et al. 2004; Spyraki, Fibiger & Phillips, 1982). In addition, CPP for 3,4-methylenedioxy-methamphetamine (MDMA) has also been demonstrated (Daza-Losada et al., 2007).

While the approach behaviour towards the drug-paired side has been demonstrated and replicated many times for illicit drugs of abuse, the induction of CPP by non-illicit drugs, such as nicotine and ethanol, has proven to be more difficult (Tzschentke, 2007).

1.3.1.2. Nicotine

Studies using PC for nicotine administration have found that CPP can only be induced within a narrow dosage range (0.2-0.6 mg/kg) whereas higher doses typically produce CPA (0.8-1.2 mg/kg) in adult male rats (Laviolette & Van der Kooy, 2003; Le Foll & Goldberg, 2005). Strain differences exist as well, one study found that adult Lewis rats exhibit CPP at a dose of 0.6 mg/kg but Fischer-344 rats do not. In contrast, Fischer-344 rats were unresponsive (i.e. did not change nicotine place-preference behaviour) to higher doses of nicotine (0.9 mg/ kg) whereas Lewis rats showed a clear decrease in CPP behaviour when the same dose was administered (Philibin et al., 2005). Different strains of adult mice also show differences in CPP behaviour. C5BL/ 6J mice exhibit significant CPP at a 0.3 mg/kg nicotine dose but DBA/ 2J mice do not (Grabus, Martin, Brown & Damaj, 2006). In addition to strain differences, the effect of nicotine CPP appears to be age-dependent. Adolescent Sprague-Dawley rats have been shown to acquire CPP at the 0.6 mg/kg nicotine dose but adult rats will not (Vastola, Douglas, Varlinskaya & Spear, 2002). In line with this, Shram and colleagues (2006) found that periadolescent animals will show pronounced CPP behaviour at the 0.8 mg/kg nicotine dose but adult animals will not exhibit any place preference or aversion. Similar findings were reported in a separate study by Belluzzi and colleagues (2004). Adolescent male mice also exhibit increased sensitivity to nicotine as shown by CPP acquisition at doses ranging from 0.05-0.5 mg/kg while adult mice only showed CPP at the 0.5 mg/kg nicotine dose (Kota, Martin, Robinson & Damaj, 2007). Taken together, nicotine-induced CPP appears to be more easily demonstrated in adolescent rats and mice while studies conducted on adult rodents have been shown to be less consistent across strains.

1.3.1.3. Ethanol

Differing results are seen for ethanol conditioning as well. One of the determining factors for these differences lies in the experimental contingencies related to when, in the PC procedure, ethanol is administered. For example, one study found that CPP was produced when ethanol was administered both before and after the PC procedure but produced CPA when administered midway through the procedure (Cunningham, Smith & McMullin, 2003). Another study found that administration of ethanol before PC sessions produced CPP whereas administration of ethanol after produced CPA (Font, Aragon & Miquel, 2006). Notably, pre-exposure to ethanol before conditioning sessions has been reported to favour CPP but it was found that magnitude of pre-exposure was negatively correlated with time spent in the drug-paired compartment (Nocjar, Middaugh & Tavernetti, 1999). The route of administration of ethanol has also been shown to cause differences in PC behaviour. Fidler and colleagues (2004) reported that intragastric administration (as opposed to intraperitoneal injections, which is more commonly used) of ethanol caused CPA in both naive and ethanol pre-exposed animals. Age- and strain-dependent differences have been shown to exist for ethanol CPP in mice. A study by Roger-Sánchez and colleagues (2012) showed that early male adolescent mice exhibit CPP with ethanol doses of 1.25 and 2.5 g/kg while late adolescent male rats do not. In addition, DBA/2J mice appear to be more sensitive to ethanol when compared to C57BL/6J mice. DBA/2J mice have been shown to acquire ethanol CPP while C57BL/6J mice have not (Cunningham, Niehus, Malott & Prather, 1992). Of important note is the fact that all of the aforementioned findings regarding CPP and/or CPA of ethanol were completed in mice. There exists a limited number of studies showing successful CPP or CPA after the administration of ethanol in rats (Asin, Wirtshafter & Tabakoff, 1985; Tzschentke, 2007). In fact, studies examining PC for ethanol in rats have commonly shown CPA but this has been shown to be subject to manipulation by a number of factors. For example, animals trained extensively on ethanol drinking prior to being introduced to the PC procedure acquired CPP but animals that were never introduced to ethanol prior to conditioning showed CPA (Gauvin & Holloway, 1992). Similarly, Bozarth (1990) found that animals with extensive conditioning days prior to PC testing

acquired CPP. Taken together, these studies suggest that prior exposure to ethanol in conjunction with the length of this period may be critical factors in determining ethanol-induced CPP vs. CPA in rats. In addition, it has been shown that rats genetically selected for ethanol preference will exhibit CPP and this effect becomes more robust with pre-exposure to ethanol prior to PC sessions and testing (Colombo et al., 1990; Ciccocioppo, Panocka, Froldi, Quitadamo, & Massi, 1999).

To summarize this section, CPP for illicit drugs, like opioids, psychostimulants and MDMA, has been established in the literature for many years. However, PC behaviour for drugs like nicotine and ethanol is not as clear-cut. Alongside the strain- and species-dependent differences highlighted above, studies examining PC for nicotine have showed that CPP is also age-dependent. For ethanol, PC studies in mice have shown greater levels of CPP, which may be dependent on the length of ethanol pre-exposure. PC studies in rats have typically shown CPA but this behaviour is subjected to manipulation caused by a history of pre-exposure to ethanol as well as strain- and species-specific differences. While it has been proven to be more challenging to show CPP or CPA for non-illicit drugs in rats, the PC paradigm is used as an index to determine the inherently rewarding properties of a number of drugs of abuse while applying a simple and fast methodology to do so (Tzschentke, 1998). However, the difficulties in establishing reliable CPP or CPA behaviour with specific drug dose ranges acts as a limitation. In addition, the absence of voluntary drug consumption restricts the PC paradigm to being a model of drug reward as opposed to a model of drug reinforcement (Bardo & Bevins, 2000).

1.3.2. Operant self-administration

In the context of substance use disorders, reinforcement can be described as the ability of the pleasurable effects of a drug of abuse to increase the frequency of drug-taking and drug-seeking. In an animal model, this is analogous to the likelihood of an operant response (e.g. lever press or nose-poke) to be increased by the contingent delivery of a drug of abuse that subsequently produces a feeling of pleasure in the organism (Sanchis-Segura & Spanagel, 2006). In contrast to the PC paradigm, operant self-administration

procedures allow for the animal to voluntarily self-administer the drug in question. Given this and the parallels between drug reinforcement in humans and animals, procedures involving operant self-administration are more translatable to the human condition. In fact, operant self-administration procedures have been shown to be one of the most valuable and reliable animal models in the pre-clinical research of drug addiction (Panlilio and Goldberg, 2007).

In models of operant self-administration, an animal is placed into an operant conditioning box that typically contains: an active lever, an inactive lever, a houselight, and a visual or auditory cue. The onset of a self-administration session is signified by the presentation of a white houselight in the operant conditioning box. Upon responding on the active lever, an animal receives a reinforcement. This is most commonly done via an intravenous infusion of drug (e.g. nicotine, heroin, cocaine, methamphetamine), or by the delivery of a drug solution (e.g. ethanol) into a drinking cup located near the lever, which is then orally consumed. The drug delivery is also usually accompanied by exposure to a visual or auditory cue, which tends to enhance the self-administration. After multiple pairings, the cues associated with reinforcement delivery have also been shown to hold conditioning properties in and of themselves (Schuster & Thompson, 1969). Responding on the inactive lever causes no programmable responses; the presence of an inactive lever is helpful in determining the presence of non-specific behaviour from the animal and ensures that behaviour is being driven by the drug of abuse or natural reward being delivered (Gardner, 2000). Importantly, self-administration behaviour is sensitive to the nature of the relationship between response and reinforcement (Panlilio & Goldberg, 2007). This relationship is referred to as the schedule of reinforcement and describes the required number of active lever responses needed to produce a reinforcement, how much time must pass before the next reinforcement becomes available, and which cues signify the availability of reinforcement (Panlilio and Goldberg, 2007). Animals are initially trained to self-administer a drug of abuse or natural reward with a continuous schedule of reinforcement in which one active lever response is required for one reinforcement delivery. The demand to receive one reinforcement is then increased based on the schedule (i.e. fixed or variable ratio) or the amount of time an animal must wait before

receiving a subsequent reinforcement (i.e. fixed or variable interval) (Schuster & Thompson, 1969). Of important note is the progressive ratio schedule of reinforcement, which is used to determine the reinforcing efficacy of the reward being administered. Here, the response requirement on the active lever increases with successive reinforcements throughout the operant conditioning session. The reinforcing efficacy of the reward in question is determined by the "final-ratio" achieved or the maximum number of responses an animal is willing to produce for the delivery of one reinforcement (i.e. the breakpoint) (Roberts, 1989). Operant self-administration has been used to study a number of drugs of abuse under many different types of schedules of reinforcement in both nonhuman primates and rodents. Studies related to illicit drugs of abuse like heroin, cocaine, and amphetamine will be touched upon. In addition, studies concerning nicotine and ethanol self-administration will be reviewed in more detail below.

Operant self-administration procedures have been used with nonhuman primates and rodents. To start, nonhuman primates have been shown to intravenously self-administer a number of illicit drugs like, cocaine, morphine, codeine, and methamphetamine under fixed-ratio and second-order schedules of reinforcement (Deneau, Yanagita & Seevers, 1969; Goldberg, 1973). Similarly, rodents have been shown to self-administer and "work" for intravenous administration of various drugs of abuse. As reviewed in Planeta (2013), rats have been shown to lever press or nose-poke for drugs like heroin, cocaine, and amphetamine. Given that nicotine and ethanol are of more relevance to the current thesis, a comprehensive review of studies concerning self-administration of these drugs is included below.

1.3.2.1. Nicotine self-administration

Nonhuman primates have been shown to intravenously self-administer nicotine under fixed-ratio (FR), progressive ratio (PR), and second-order (i.e. FR10 or fixed-interval 1) schedules of reinforcement (Spealman & Goldberg, 1981; Le Foll, Wertheim & Goldberg, 2007). The first published study examining intravenous nicotine self-administration in rats, by Corrigall and Coen (1989), reported that rats will respond for

nicotine at a FR-5 schedule of reinforcement. This same study revealed that an animal's nicotine intake is dependent on the size of the unit dose being infused into the bloodstream. More specifically, animals will increase their lever pressing for nicotine at unit doses between 0.01 and 0.03 mg/kg/infusion but will decrease intake at unit doses below or above this range; this results in an inverted-U shape dose-response curve (Corrigall & Coen, 1989). The 30 μg unit dose has generally been shown to be the optimal infusion dose resulting in the highest number of responses for intravenous nicotine and is, therefore, the "standard" dose for nicotine self-administration studies (Donny, Caggiula, Knopf & Brown, 1995; Shoaib & Stolerman, 1999). Animals have also been shown to exhibit high levels of motivation for nicotine infusions under a progressive ratio schedule of reinforcement. One study found that an animal will cumulatively press up to 185 times for one infusion of a 30 μg dose of nicotine (Donny et al., 1999). Another study found that the average breakpoint for the same dose of intravenous nicotine was 115 (Shram et al., 2008).

There are a number of strain- and sex-dependent differences in nicotine self-administration. For example, a study by Shoaib and colleagues (1997) found that Long-Evans and Sprague-Dawley rats will readily nose-poke for nicotine but Fischer-344 and Lewis inbred strains fail to do so. In addition, research by Caggiula and colleagues (2002) has shown that female rats are more sensitive to the rewarding effects of nicotine and this is compounded by the inclusion of a visual stimulus. To be more specific, while both males and females respond similarly for lower nicotine doses, female rats respond at a higher rate for 0.15 mg/kg/infusion of nicotine even with the absence of a nicotine-paired visual cue. Conversely, when visual cue presentation is initiated, both males and females increase their rates of responding but this was shown to be more salient in females at the 0.06 mg/kg/infusion dose (Caggiula et al., 2002). In line with this, another study found that females are quicker to respond for lower doses of nicotine and showed that they will work harder for nicotine infusions under a progressive ratio schedule of reinforcement across a wide range of doses (Donny et al., 2000). Taken together, these data suggest that intravenous nicotine self-administration is a reliable phenomenon and can be used to study its reinforcing effects, which can be modulated by several factors.

1.3.2.2. Ethanol self-administration

Nonhuman primates

When given unlimited access to ethanol, nonhuman primates have been shown to intravenously self-administer solutions of ethanol in high amounts (34 drinks/ day) (Deneau et al. 1969; Woods, Ikomi & Winger, 1971; Winger & Woods, 1973; Grant & Bennett, 2003). However, high levels of intoxication experienced in these animals often led to physical dependence, overdose, and death (Grant & Bennett, 2003). In addition, these patterns of behaviour were shown to be highly variable and included days of self-imposed abstinence and days of extreme intake. Importantly, nonhuman primates have also been shown to self-administer ethanol though the oral route in relatively lower amounts (2-16 drinks/ day) (Higley, Suomi & Linnoila, 1996; Grant and Johanson, 1988; Vivian et al., 2001).

Rats

Rodents have been shown to orally consume varying concentrations of an ethanol solution but, in contrast to nonhuman primates, they are less likely to consume pharmacologically relevant levels of the solution (Samson, Pfeffer & Tolliver, 1988). Procedures giving animals access to 2-bottle choice have reported that animals will drink ethanol over plain water at low concentrations (1-5%) (Samson et al., 1988), but consumption of higher concentrations is low, due to the bitter taste of ethanol. A number of procedures have been developed to enhance this low intake so that rats can be used in preclinical models of human ethanol misuse.

Sucrose fading procedure

The sucrose fading procedure is often used to train rats to drink higher concentrations of ethanol, in order to allow the animals to become accustomed to the taste of ethanol. The sucrose fading procedure involves having animals learn to self-administer sucrose

sweetened solutions of ethanol whereby the ethanol content is increased (and sucrose content decreased) over time. For example, animals will begin self-administration with a 20 % (w/v) sucrose solution until stable responding, then a 10% sucrose 5% (v/v) ethanol solution, a 5% sucrose 10% ethanol solution, and, finally a 10% ethanol solution (Samson et al., 1988). Using this procedure, animals have been shown to self-administer ethanol solutions of up to 40% (v/v) and to show overt signs of intoxication (Grant & Samson, 1985). While this procedure produces stable and reliable ethanol self-administration, it has been criticized on the basis of being confounded by the presence of sucrose, which is highly rewarding and activates similar brain pathways as drugs of abuse (Koob & Weiss, 1990; Lenoir, Serre, Cantin & Ahmed, 2007; Spangler et al. 2004). Therefore, due to their increased validity, operant procedures without the involvement of sucrose may be more desirable.

Prior exposure to ethanol

Intermittent access: Introduction to ethanol prior to the initiation of operant self-administration has also been shown to enhance the development of stable levels of ethanol responding. To start, animals provided with 24 hrs intermittent access to highly concentrated ethanol solutions have been shown to be successful in subsequently producing stable self-administration behaviour. Typically in this procedure, animals are exposed to intermittent access to 20% (v/v) ethanol via bottles in the home cage every other day and on this regimen, they show an escalation in ethanol drinking (Simms et al., 2008; Carnicella, Ron & Barak, 2014). This history of ethanol consumption was subsequently shown to facilitate the acquisition of operant responding for ethanol (Carnicella et al., 2014).

Limited access: The limited access procedure is another method shown to produce stable levels of ethanol self-administration. In order to allow animals to become accustomed to the taste of ethanol, they are exposed to increasing concentrations of ethanol, from 3% to 6% to 12% (w/v), daily for sessions lasting up to 1 hr, typically over about 3 weeks (Linseman, 1987; Le, Israel, Juzytsch, Quan & Harding, 2001). Using this method,

animals have been shown to lever press for a 12% (w/v) ethanol solution during subsequent self-administration sessions (Le & Shaham, 2002).

Intravenously administered ethanol

Rats have been shown to self-administer intravenously delivered ethanol (Smith & Davis, 1974; Gass & Olive, 2007). However, the maintenance of intake and adaptation to differing schedules of reinforcement has proven to be difficult to demonstrate (Grupp, 1981). In addition, a recent review by Le and Kalant (2017) puts into question the validity of intravenous ethanol self-administration in rats. More specifically, the low number of total reinforcements being received in an intravenous ethanol session has led to questioning the magnitude of reinforcement from ethanol with this specific route of administration. Taken together, procedures that train the animal to orally consume a sucrose-free ethanol solution are the preferred methods to acquire stable self-administration and ethanol intake levels.

Sex and strain differences

A recent review of the sex differences related to ethanol intake in rodents highlighted that a number of studies have found that female rodents will consume more ethanol than male rats under 2-bottle choice conditions (Becker & Koob, 2006). However, sex differences under operant self-administration parameters have proven to be less clear-cut. One study has shown that genetically selected ethanol-preferring female rats exhibit greater ethanol intake during the self-administration acquisition phase but will operantly respond in a similar amount to males after this period (Moor & Lynch, 2015). Another study found that females are more sensitive to the rewarding effects of ethanol relative to males when tested in a place conditioning procedure (Torres, Walker, Beas & O'Dell, 2014). However, a study by Priddy and colleagues (2017) did not find any sex differences in animals responding for 10% (w/v) ethanol. This study also elucidated potential strain differences, and found no difference between self-administration in Wistar and Long-Evans rats. Sprague-Dawley rats that acquired stable levels of self-administration,

however, were shown to have greater responding for ethanol than Lewis rats but Lewis rats consumed more ethanol under 2-bottle choice conditions (Wilson, Neill & Costall, 1997). Given the aforementioned results, it appears as though sex and strain differences in ethanol consumption differ depending on the type of procedure being used (i.e. self-administration vs. 2-bottle choice).

The literature concerning animal models of drug reward and reinforcement confirms that both nonhuman primates and rodents are capable of experiencing the inherently rewarding properties of drugs of abuse and are motivated to produce behaviour in order to feel these rewarding effects. The use of PC and operant self-administration procedures has been helpful in determining the behavioural and neural underpinnings of drug reward and reinforcement. Interestingly, animals have also been shown to exhibit relapse behaviour that is triggered by similar stressors and environmental factors as in humans, and this will be discussed in the next section.

1.4. Animal Models of Drug Relapse

1.4.1. Reinstatement procedure

As previously stated, re-exposure to the drug of abuse, psychosocial stressors, and environmental cues and contexts that are associated with drug taking have been shown to trigger relapse in abstaining individuals (O'Brien et al., 1992). The use of animal models of drug relapse, like the reinstatement procedure, has showed that these same three triggers can induce relapse-like behaviour in rodents (de Wit & Stewart, 1981; Meil & See, 1996; Shaham & Stewart, 1995). The reinstatement procedure involves animals experiencing multiple drug pairings (self-administration or experimenter-delivered) with a specific context or set of cues, followed by the absence of drug administration in the same environment (i.e. extinction). Subsequently, animals are exposed to any of the three "relapse triggers" mentioned above and are tested on drug-seeking behaviour. Reinstatement of drug-seeking behaviour can be used in the context of self-administration paradigms as well as the CPP paradigm.

1.4.1.1. Operant procedures

As previously mentioned, self-administration training occurs in operant conditioning boxes (i.e. drug-paired context) where drug delivery is presented with a specific set of cues (i.e. drug-paired cues). Upon stable levels of self-administration, animals are subjected to a period of extinction whereby responding on the drug-paired lever yields no drug reinforcement, typically over multiple daily trials. Following this period of experimenter-imposed abstinence with such extinction trials, animals are placed back into the drug-paired context. Similarly to extinction trials, appropriate responding on the drug-paired lever causes the presentation of drug-paired cues but the absence of drug reinforcement (de Wit & Stewart, 1981). Previous research has found that non-contingent priming injections of the self-administered drug, stress, and exposure to the drug-paired cues and context will cause reinstatement of lever-pressing behaviour (i.e. drug-seeking behaviour) (as reviewed in Shaham & Miczek, 2003).

De Wit and Stewart (1981, 1983), for example, found that priming injections of cocaine and heroin will cause drug-seeking behaviour in animals with respective histories of cocaine and heroin self-administration. Similar results have been reported for ethanol-primed drug seeking (Le, Quan, Juzytch, Fletcher, Joharchi & Shaham, 1998).

Stress-induced reinstatement has been shown for drugs like heroin, cocaine, methamphetamine, ethanol, and nicotine (Mantsch, Baker, Funk, Lê & Shaham, 2015). Bäckström and Hyytiä (2004), found that following a period of extinction to an ethanol-paired lever and its associated cues, animals will show increased ethanol seeking when re-exposed to the ethanol-paired cues alone.

Similarly to re-exposure to discrete drug-cues, drug-seeking behaviour has also been observed when animals are re-exposed to an environmental context that has become associated with drug-intake using the renewal procedure (Crombag & Shaham, 2002). Briefly, the renewal procedure involves drug self-administration in one context and extinction of lever pressing in another context. Animals are subsequently tested for drug

seeking in the original drug-taking context (Bouton & Swartzentruber, 1991). Using this procedure, context-induced reinstatement has been found for cocaine, heroin, nicotine, and ethanol (Fuchs et al., 2005; Bossert et al., 2011; Diergaarde, De Vries, Raasø, Schoffelmeer & De Vries, 2008; Marchant, Hamlin & McNally, 2009).

1.4.1.2. Conditioned placed preference procedures

Relapse-like behaviour has not only been observed in the context of drug self-administration; animals have been shown to reinstate CPP after a period extinction followed by priming injections of the drug in question or exposure to stress. Here, extinction involves vehicle injection pairings with both compartments after CPP for the drug in question has been established (Mueller & Stewart, 2000). Using the CPP paradigm previously explained, reinstatement of CPP by drug priming to drugs like morphine, cocaine, methamphetamine, and nicotine has been shown (Wang, Luo, Zhang & Han, 2000; Sanchez & Sorg, 2001; Mueller & Stewart, 2000; Daza-Losada et al., 2007; Pascual, Pastor & Bernabeu, 2009). In addition, stress-induced reinstatement has been found for cocaine and morphine (Sanchez & Sorg, 2001; Wang et al., 2000).

The reinstatement procedure has been shown to have high face validity as a model of drug relapse (i.e. it measures what it is claiming to measure) because the factors that induce animals to seek a drug they have a history of consuming are the same factors that induce humans to relapse to a drug they are dependent on (i.e. re-exposure to the drug, the drug-paired context, and stress) (Katz & Higgins, 2003; Epstein, Preston, Stewart & Shaham, 2006). However, the presence of an extinction phase as well as an experimenter-imposed period of abstinence in the reinstatement procedure has received major criticism (Shaham, Shalev, Lu, de Wit & Stewart, 2003). This is because individuals dependent on drugs rarely experience extinction (unless it is part of a treatment program), and it is next to impossible for them to be completely removed from a "trigger-full" environment in which the drug is easily accessible and exposure to drug-associated cues is abundant. In addition, Epstein and colleagues (2006) have stated that the construct validity (i.e. its relation to theoretical concepts) of the reinstatement procedure has yet to be confirmed.

This results from difficulties in assessing feelings and behaviours related to craving in this animal model of drug relapse. This is an important consideration, because exposure to any of the aforementioned relapse triggers subjective feelings of craving in humans (O'Brien et al., 1992).

1.4.2. Ethanol deprivation effect

In modeling human ethanol use disorders, a widely used animal model of drug craving is the ethanol deprivation effect. In this model, animals trained to consume solutions of ethanol under 2-bottle free-choice access conditions have been shown to increase their ethanol drinking after a period of deprivation. This effect has been found in rats, mice, monkeys, and humans after both short- and long-term periods of deprivation (Rodd-Hendricks et al., 2000; Salimov, Salimova, Klodt & Mainsky, 1993; Kornet, Goosen & Van Ree, 1990; Burish, Maisto, Cooper & Sobell, 1981; Sinclair & Li, 1989; Sinclair, Walker & Jordan, 1973). It has also been shown in animals deprived from ethanol in the context of operant self-administration. That is, rodents with stable levels of ethanol self-administration have been shown to increase responding for ethanol after a period of deprivation (Sparta et al., 2008; Dayas, Martin-Fardon & Weiss, 2004). Reintroduction to ethanol after a period of deprivation leads to a "pronounced, although temporary, increase in voluntary ethanol intake" (Khisti, Wolstenholme, Shelton, & Miles, 2006). This effect is reliable and has been shown to have some predictive validity while being useful for testing "anti-craving" pharmacotherapies against excessive drinking and ethanol dependence (Koob, 2000). Interestingly, a deprivation effect has also been found for sweet, non-alcoholic, solutions. One study found that animals trained to self-administer a 25% glucose (i.e. sugar) solution show a robust increase in glucose consumption after 2 weeks of deprivation (Avena, Long & Hoebel, 2005). In addition, similar findings have been reported after the deprivation to a sweet-tasting, non-calorically relevant solution of saccharin (Sinclair & Li, 1989). In a study by Neznanova and colleagues (2002), animals were exposed to 4 weeks of unlimited access to water and 0.1% saccharin in their homecages. The amount of saccharin consumed post-deprivation was correlated with the number of deprivation days received. That is, there was little to no deprivation effect

reported 1 and 2 days after deprivation but the effect of saccharin deprivation became more evident as the number of deprivation days increased (Neznanova, Zvartau & Bespalov, 2002).

1.5. Methodological Considerations of Current Animal Models

The use of place conditioning, operant self-administration and reinstatement paradigms have been important to our current understanding of substance use disorders on a behavioural and neurological level. However, there are a number of methodological limitations that must be considered while keeping in mind that substance use disorders are characterized by maladaptive decision-making in an environment with many other rewards available (Ahmed, 2010). For the purpose of this thesis, one such limitation is the availability of one single reward, the drug in question, in current animal models of drug reinforcement.

The Rat Park experiment by Bruce Alexander and colleagues (1981) was one of the first studies exploring the effect of an alternate reinforcer, in the form of social interaction, on drug intake. In this study, animals that were housed in a colony were shown to consume significantly less of a morphine hydrochloride solution in comparison to animals housed in isolation. While this experiment focused on the effects of housing conditions on drug consumption its results instigated a large amount of thought regarding the aforementioned limitations of operant self-administration procedures. In line with this, the validity and translatability of single-reward operant self-administration procedures have been put under review (Ahmed, 2010).

The 5th edition of the Diagnostic and Statistical Manual of Mental Disorders refers to continued drug use despite the presence of and at the expense of other valuable activities as a defining feature of drug dependence (American Psychiatric Association, 2013). This is in direct contrast to continued drug use due to the absence or lack of these valuable activities (Ahmed et al., 2013). In fact, drug use as a result of the latter is considered to be an "expectable response to a situation or event"; a factor that meets the exclusion criteria

for a number of psychiatric disorders (Stein, Phillips, Bolton & Kendler, 2010). Given this, are the behaviours (i.e. pressing a lever to receive a reinforcement), observed in current animal models, driven by motivation to consume the available drug or is drug self-administration by animals merely a behaviour produced by the absence of other valued options (Ahmed, 2010)? In order to answer these questions, there has been a recent surge in the use of animal models, which provide the animal with the option to choose between a drug reward and a non-drug reward. These models have been used in nonhuman primates as well as rodents, and can provide the availability of both rewards in a restricted or unlimited manner.

1.6. Animal Models of Dual-Reward

1.6.1. Nonhuman primates

1.6.1.1. Concurrent access procedure

It is important to mention that procedures incorporating the aspect of choice were initially used in nonhuman primates in the 1980's. While the procedural method in these studies differs from those used in more recent publications concerning restricted choice in rodents (i.e. discrete-choice trials procedure), these studies provided useful information on the relative reinforcing properties of certain rewards as well as elucidated possible reward interactions (Carroll, Carmona & May, 1991). Carroll and colleagues (1991) explain that while human drug-taking behaviours occur in an environment with the availability of alternative and competing reinforcers, most research evaluating drug reinforcement occurs in a "well-controlled laboratory environment that is intentionally devoid of other reinforcers".

In order to determine the effect of alternative reward on drug self-administration in nonhuman primates, both rewards are made concurrently available in an unrestricted manner. In this procedure, nonhuman primates are given free access to both reward levers for 3 hrs and are required to respond under a fixed-ratio schedule of reinforcement.

Carroll and colleagues (1991) found that Rhesus monkeys will continue to self-administer phencyclidine in the presence of water but will decrease drug intake, by 30-40%, in the presence of sweet-tasting saccharin. Another study found that Rhesus monkeys will consistently self-administer more pentobarbital than water (DeNoble, Svikis & Meisch, 1982). The level of drug vs. non-drug reward intake has been shown to be influenced by differences in the schedule of reinforcement required for the delivery of each respective reward. For example, it was found that increasing the fixed-ratio (FR) requirement for phencyclidine will cause increased saccharin administration (Carroll et al., 1991; Campbell & Carroll, 2000). In addition, altering the concentration or amount of drug vs. non-drug reward delivered has also been shown to cause changes in choice behaviour. In a study by Nader and Woolverton (1991), increases in the amount of palatable food delivered caused a decrease in the frequency of cocaine choice. However, when the amount of food delivered was held constant and the cocaine concentration was altered, animals choice behaviour was reflected in a drug-dose related fashion (Nader & Woolverton, 1991; Gasior, Paronis & Bergman, 2004; Banks, Blough & Negus, 2013). Similar dose-dependent increases in drug choice were found in Rhesus monkeys given increasing doses of heroin (Negus, 2006).

1.6.1.2. Discrete-choice trials procedure

Another procedure that has been used to discern choice in nonhuman primates is the discrete-choice trials procedure. While its use in nonhuman primates is limited, it differs from the concurrent access procedure by restricting the number of choice trials available to the animal. One study showed that when Rhesus monkeys were given the choice between cocaine and candy they showed a clear preference for cocaine that remained even after multiple periods of extinction and re-testing (Foltin et al., 2015). In addition, pre-choice session exposure to candy alone did not cause any changes to cocaine preference. Even so, it was shown that decreasing the cocaine dose can cause the animals to exhibit an intermediate preference for both rewards (Foltin et al., 2015). Huskinson and colleagues (2015, 2016) found that Rhesus monkeys will choose cocaine over food (1) with the introduction of immediate cocaine in place of delayed food delivery and (2)

despite the presence of immediate food but delayed (0-120 sec) cocaine delivery. It was suggested that cocaine may be a more effective reinforcer than food in these subjects (Huskinson, Woolverton, Green, Myerson & Freeman, 2015). Overall, the results of these studies provide a distinction between choice behaviour in rodents vs. nonhuman primates.

1.6.2. Rodents

1.6.2.1. Concurrent access procedure

Due to the compelling evidence for the effects of alternative reinforcement on drug choice in nonhuman primates, the concurrent access procedure has also been studied in rodents. Similarly to the procedure used with nonhuman primates, rodents are given free access to both reward levers for sessions lasting 30 to 60 min and must respond on a fixed-ratio schedule of reinforcement. Panlilio and colleagues (2015) found that animals will respond on both levers when given concurrent access to nicotine and sucrose. It is important to note that pre-training history affected the level of reward consumption during concurrent access. For example, when animals received pre-training to both nicotine and sucrose, responses for saccharin were significantly higher but nicotine choice was greater when animals were pre-trained exclusively for nicotine responding (Panlilio, Hogarth & Shoaib, 2015). A study providing concurrent access to ethanol and food pellets, showed that responding on both levers can be made equal with the manipulation of schedules of reinforcement (e.g. FR-5 for ethanol reinforcement, FR-35 for food reinforcement) (Ginsburg & Lamb, 2013). A study by Lê and colleagues (2010) found that animals will readily self-administer both ethanol and nicotine when both are made available. Interestingly, ethanol was shown to cause significant decreases in nicotine intake in comparison to single-reward nicotine consumption while the availability of nicotine did not alter ethanol intake levels.

The aforementioned studies using the concurrent access procedure provide evidence that rodents will continue to self-administer a drug reward even in the presence of non-drug rewards or other drug rewards. In addition, providing the animal with access to more than

one reward renders this procedure more translatable and naturalistic to the human condition when compared to models in which one reinforcer is available (i.e. operant self-administration) because it allows for the determination of a preference of one reward over the other in the absence of a significant cost (i.e. both reinforcers can be freely self-administered) (Panlilio et al., 2015). Taken together, this model has improved face and predictive validity, and is considered to be a reliable method for determining the effectiveness of therapeutic agents in the context of substance use disorders (Panlilio et al., 2015).

1.6.2.2. Discrete-choice trials procedure

A major distinction between the discrete-choice trials procedure and the concurrent access procedure is that the former limits the number of possible reward choices by providing the animal with a maximum number of simultaneous reward presentations. In an effort to generate significant opportunity cost, the discrete-choice trials procedure requires animals to choose between a drug and nondrug reward, however, the selection of one precludes the availability of the other (Ahmed, 2010). Together, these factors render the discrete-choice trials procedure one of forced choice because the selection of one reward means one less opportunity to self-administer the other reward. Briefly, sessions of the discrete-choice trials procedure begin with sampling phases in which the animal is given the opportunity to sample each reward under single-lever conditions. The sampling phase is followed by choice trials in which both reward levers are made available. Selection of one reward causes the retraction of both reward levers for a 5-10 min inter-trial interval (ITI). Interest in this procedure, which has been primarily used in rats, has greatly increased since the early 2000s. It has been found that the majority of rats will robustly choose the non-drug reward over the drug reward. This has been found when animals are given the option to choose between a number of different drug rewards in conjunction with a number of different palatable nondrug rewards. These results (reviewed below) are quite surprising given what is known about the reinforcing effects of drugs of abuse relative to the nondrug rewards used in these studies. Palatable food pellets and sucrose pellets or solutions have typically been used as the nondrug reward

and have been shown to be rewarding due to their taste and post-ingestive properties. Interestingly, the selection of the nondrug reward over drug rewards has also been found with the use of saccharin, a non-biologically relevant substance, as the nondrug reward. Notably, similar results were found across all three nondrug rewards when paired with a number of different drug rewards suggesting that their effect, in the discrete-choice trials procedure, is equivalent.

Short-access self-administration training

A study by Lenoir and colleagues (2007) found that animals will consistently choose a non-biologically relevant sweet-tasting solution, saccharin, over intravenous infusion of cocaine. In addition, increasing the concentration of cocaine did not affect saccharin choice preference. In a similar experiment using the discrete-choice trials procedure, naive animals (i.e. with no history of drug administration) were shown to almost immediately prefer saccharin over intravenously administered heroin (Lenoir, Cantin, Serre, Vanhille & Ahmed, 2013). Increasing the dose of heroin, while causing a dose-dependent increase in sensitized locomotion, did not change animal's preference for saccharin. In a study by Huynh and colleagues (2015), the preference for nicotine and saccharin was determined. Here, animals were given pre-training with both nicotine and saccharin self-administration and initial choice sessions revealed an immediate and clear preference for saccharin over nicotine. Gradually decreasing the saccharin concentration did not cause changes to animals' choice behaviour, even when the saccharin solution was replaced by water. Animals with an increased number of pre-training sessions for nicotine self-administration continued to choose saccharin over nicotine, despite decreases in saccharin concentration and replacement with water. Finally, choice preference for saccharin was also impervious to the introduction of a higher nicotine dose (Huynh, Fam, Ahmed & Clemens, 2015). The discrete-choice trials procedure has also been used to determine a preference for methamphetamine or palatable food pellets (Caprioli et al., 2015). In line with the aforementioned studies, a robust and consistent preference for food pellets when both rewards were made available. In addition, a recent

publication showed that animals will choose solutions of saccharin over ethanol (Pelloux & Baunez, 2017).

Long-access self-administration training

It is important to mention that three of the above studies attempted to determine the effect of long-access drug pre-training on choice behaviour. These self-administration phases included longer daily self-administration sessions, usually lasting 6 to 9 hrs. Previous research using this method has showed that animals will show an escalation in drug taking, which has been suggested to be related to an addiction-like state in animals (Lenoir et al., 2007; Koob & Kreek, 2007). Animals in the study by Lenoir and colleagues (2007) were given 6 hrs daily access to cocaine training before being introduced to sessions of the discrete-choice trials procedure. Despite this additional training, animals still showed a robust preference for saccharin over cocaine. Notably, animals showed psychomotor sensitization to cocaine under these pre-training conditions, indicating an enhanced behavioural response to the drug over time (Kalivas & Stewart, 1991; Paulson, Camp & Robinson, 1991). Given this, it is surprising that animals chose saccharin over cocaine despite showing drug-sensitization and an escalation in cocaine intake, the latter of which is a hallmark of drug addiction (Lenoir et al., 2007). Interestingly, providing animals with a longer length of pre-training to heroin exposure did cause differences in choice behaviour. Animals given 6 hrs per day of heroin pre-training showed either indifferent preference between both rewards or a clear preference for heroin (Lenoir et al., 2013). Long-access to heroin has been shown to cause a robust escalation in drug intake over time and has been shown to cause neurological changes related to binge consumption of opiates and is, therefore, considered to be a model of dependence (Koob & Kreek, 2007). Given the differential choice behaviour reported in this study (i.e. heroin preference), it is possible that a key factor in an animal's decision-making is whether or not it is dependent on the drug being studied. Finally, animals given 6 or 9 hrs a day of methamphetamine self-administration pre-training continued to robustly prefer palatable food pellets when introduced to the discrete-choice trials procedure (Caprioli et al., 2015).

One major criticism of the discrete-choice trials procedure is that it imposes an "either-or" choice dilemma, which human drug users do not always have to face. In this paradigm, choosing one reward limits access to the other reward thus imposing a forced choice where selection of one reward means less opportunity for selection of the other. As explained by Panlilio and colleagues (2015), individuals' dependent on ethanol or nicotine, for example, will quantitatively allocate their behaviour between one reward and another (not between one or the other) in the real world. While there exists arguments against the translatability of the discrete-choice trials procedure to the human condition, the results of studies using this procedure provide valuable information on the relative rewarding value of drug and nondrug rewards. As such, it can be inferred that the rewarding efficacy of drugs of abuse may be low relative to the rewarding value of non-drug rewards (Ahmed, 2010). In fact, a study by Cantin and colleagues (2010) revealed that rats value cocaine approximately 10 times less than they do the alternative reward, saccharin. This was discovered by increasing the cost of saccharin (i.e. number of responses to receive saccharin reward delivery) relative to that of cocaine. In this study, animals showed a point of indifference between cocaine and saccharin only when the amount of work to obtain saccharin was approximately 10 times higher than that of cocaine. This point of indifference was shifted to a preference reversal, favouring cocaine, only when the relative cost of saccharin was increased to 16 times that of cocaine (Cantin, 2010). In addition to this, the absence of an experimenter-imposed period of abstinence (a key aspect in single-reward reinstatement procedures) is a novel feature that opens the door to potentially exploring relapse and therapeutic treatments in an animal model.

The replication of a consistent, robust, and almost immediate preference for the non-drug reward across a number of different studies has led the discrete-choice trials procedure to be considered a model of "voluntary abstinence" (Venniro, Caprioli & Shaham, 2016). That is, providing animals with the choice between a non-drug reward and a drug reward causes animals to, essentially, abstain from a drug of abuse that they previously self-administered when no other reward was available. This animal model has also been

described as being “analogous to the human condition of relapse to drug use after termination of long-term contingency management treatment” when used to study cue-induced drug seeking after a period of voluntary abstinence (Venniro et al., 2016; Caprioli et al., 2015).

As a secondary interpretation of the usefulness of the discrete-choice trials procedure; it is important to note, that a very small subset of animals (less than 10%) tested in the aforementioned studies showed a preference for the drug reward. Ahmed (2010) suggests that this small percentage of animals can be described as being dependent on the drug in question. Keeping in mind that only a fraction of people who use drugs of abuse actually transition to compulsive drug use (e.g. 6% of cocaine users become addicted within 2 years of drug use initiation); the minority of drug-preferring rats can be homologous to the minority of drug users that meet the criteria for having a substance use disorder (Anthony, Warner & Kessner, 1994; Ahmed et al., 2013). Therefore, it is suggested that this model be used to select for animals that have a greater predilection to become “addicted” to the drug in question while excluding animals that have been shown to be “resilient” to addiction, as shown by their preference for the non-drug reward (Ahmed, 2010). Doing so would allow for increased comparability between animal models of drug addiction and human substance use disorder as well as help determine drug-taking behaviour caused by addiction vs. a lack of choice (Ahmed, 2010). While the discrete-choice trials procedure may be extremely useful in determining choice of drug over alternative reinforcers, it has two major limitations. (1) The majority of previous research using this model has used intravenously administered drugs of abuse. Although humans do self-administer drugs by the intravenous route, it is relatively rare, and oral, intranasal or inhalational routes are much more common. For example, ethanol is the most commonly abused drug and is almost always taken orally. (2) The animals used in these previous studies on choice were not verified to be drug-dependent. As will be discussed, drug dependence is an important feature of addiction, and it results in strong and enduring changes, including increased intake and enhanced relapse risk.

1.7. Animal Models of Ethanol Dependence

As previously stated, dependence to ethanol can be defined as a chronic relapsing disorder characterized by the transition from recreational drinking to compulsive drinking despite negative consequences (American Psychiatric Association, 2013). In addition, those dependent to ethanol have been shown to have especially high relapse rates (relative to other drugs of abuse) despite efforts to treat their addiction psychologically and/or pharmacologically (Hendershot et al., 2011). Keeping this in mind, a major limitation of past preclinical research using dual-reward procedures is the use of nondependent animals. While helpful in comparing the relative reinforcing properties of drug vs. nondrug rewards, this limitation introduces a gap in the literature concerning choice in rodents because the choice behaviour exhibited in these studies is not one related to a state of drug dependence.

It has been suggested that in order for animals to be classified as drug-dependent they must exhibit: (1) escalation of drug use, (2) impaired control over behaviour, (3) resistance to extinction, (4) increased motivation for drugs, (5) drug preference over non-drug rewards, and (6) resistance to punishment (Vandershuren & Ahmed, 2013). In an effort to better link animal models to the human condition, a number of procedures have been developed to study ethanol dependence.

1.7.1. Liquid diet

The first of these models is the introduction of an ethanol-containing diet. In this model, animals are given free access to a liquid diet that includes all necessary nutritional requirements for growth (proteins, fats, carbohydrates, and fiber) with ethanol, and is presented without access to other food (Lieber, Jones, Mendelson & DeCarli, 1963). This model has been shown to induce changes in animals' behaviour as well as introduce health complications, which are common consequences of ethanol dependence in humans. Notably, animals on the ethanol-containing liquid diet show an escalation of ethanol consumption as well as liver lipid accumulation, tolerance to ethanol, physical

dependence and withdrawal, alcohol fetal syndrome, and a number of endocrine and metabolic disorders (Schulteis, Hyytiä, Heinrichs & Koob, 1996; Lieber & DeCarli, 1982). Despite the aforementioned commonalities to the human condition, this model does not have a high amount of experimental control because free access to the solution means that the animals determine how much of the liquid-diet they consume. In addition, a study by Rogers and colleagues (1979) found considerable variation in animal blood alcohol levels (BALs). More specifically, this method of dependence induction fails to produce consistently high BALs throughout experimentation because the dose of ethanol administered in the diet is dependent on the animal's nutritional state (Rogers, Wiener & Bloom, 1979).

1.7.2. Intragastric administration

A second method of inducing ethanol dependence is intragastric administration of ethanol. In this model, animals are gavaged with ethanol multiple times a day for 4-6 days. This experimenter-controlled deposition of ethanol directly into the stomach has been shown to produce stable and high BALs as well as overt signs of intoxication (e.g. sedation), withdrawal symptoms upon termination of ethanol administration, and an escalation of consumption in ethanol intake tests (Majchrowicz, 1975; Deutsch & Walton, 1977). In addition, this method of dependence induction has been described as mimicking the diurnal intake of ethanol in humans due to the cycles of high dose intake during the day, followed by abstinence overnight with the recurrent experience of early morning withdrawal symptoms. However, due to the invasive nature of the intubation procedure, a major limitation of this model is the uncertainty of whether or not the dependence exhibited in these animals can be sustained over extended periods of time (Braconi et al., 2010). In addition, the BALs of the animals in the studies published using this method, have been shown to be excessively high causing a number of health complications (e.g. excessive weight loss, seizures, etc.) and, sometimes, death (Majchrowicz, 1975).

1.7.3. Intermittent exposure to ethanol vapour

Due to the limitations of the ethanol-containing liquid diet and intragastric intubation methods described above, another model of dependence induction has been developed, ethanol vapour exposure (Goldstein & Pal, 1971; Rogers et al., 1979). Animals are exposed to ethanol vapour introduced via a continuous air stream in sealed chambers for a number of hours daily. This procedure has been shown to be most effective when exposure to ethanol vapour is intermittent as opposed to chronic. A study by O'Dell and colleagues (2004) found that animals exposed to ethanol vapour daily for 14 hrs "on" and 10 hrs "off", over four weeks, will exhibit more pronounced and prolonged increases in ethanol self-administration when compared to animals exposed to continuous (i.e. 24 hrs per day) ethanol vapour. It appears as though the repeated daily withdrawals experienced by intermittent vapour exposure facilitates the onset of ethanol intake escalation, which has been shown to subsequently endure after the cessation of withdrawal symptoms and after a period of protracted abstinence (Roberts, Heyser, Cole, Griffin & Koob, 2000; Griffin, Lopez, Yanke, Middaugh & Becker, 2009; Becker & Lopez, 2004). In addition, the BALs of animals exposed to intermittent ethanol vapour are stable and indicative of a state of physiological dependence (i.e. 150 to 200 mg%) (Valdez et al., 2002). Importantly, intermittent ethanol vapour exposure closely resembles the pattern of drinking in humans dependent to ethanol (i.e. drinking during day, and withdrawal at night) (O'Dell, Roberts, Smith & Koob, 2004). Another advantage of this model is that it has been shown to elicit compulsive drug-taking behaviour. Dependent animals previously exposed to intermittent ethanol vapour have been shown to continue to self-administer and consume an ethanol solution adulterated with bitter-tasting quinine (Vendruscolo et al., 2012). This behaviour has been described as being analogous to compulsive drug taking in humans because ethanol intake is persisting despite the negative consequences of consuming this, now, bitter tasting solution (Vendruscolo et al., 2012, Vendruscolo & Roberts, 2014). Dependent animals have also exhibited increased motivation for self-administering ethanol under a progressive ratio schedule of reinforcement (Vendruscolo et al., 2012). All in all, dependence induction via intermittent ethanol vapour exposure is more reliable than other methods in producing

stable BALs as well as causes measurable withdrawal symptoms, compulsive behaviour, and increased motivation for ethanol consumption. This method of ethanol dependence induction has been shown to produce differential activation and changes to neural substrates related to drug reinforcement and drug addiction (Heilig & Egli, 2006; Vendruscolo & Roberts, 2014).

1.8. Mechanisms of Ethanol Reward

The ventral tegmental area (VTA) and the substantia nigra house a large amount of dopaminergic cell bodies, which project to various areas of the brain. More specifically, the mesolimbic DA pathway comprises the VTA with afferent projections to the nucleus accumbens (NAc), hippocampus, ventral striatum, bed nucleus of the stria terminalis, amygdala, and other regions (Hui & Gang, 2014). This system modulates the physiological responses towards primary reinforcers such as food and sex. When activated, it produces feelings of euphoria and a state of well-being in both humans and rodents (Schultz, Dayan & Montauge, 1997). In addition, a number of psychoactive substances exert their effects by targeting this pathway (Kelley & Berridge, 2002; Wise, 1996). More specifically, low-to-moderate doses of ethanol have been shown to increase DA firing in the VTA, and to increase DA release in the NAc (Gessa, Muntoni, Collu, Vargiu & Mereu, 1985; Weiss, Lorang, Bloom & Koob, 1993). The following sections will include a review of the evidence supporting the role of DA in ethanol's rewarding effects.

1.8.1. Electrophysiology studies

Electrophysiology studies have provided information on the effect of ethanol on the cell firing of neurons in specific brain areas both in vitro and in vivo (Little, 1999). For example, an in vitro study by Brodie and colleagues (1990) showed that in isolated brain slices of the VTA, ethanol had direct action in stimulating cell firing. An in vivo study by Mereu and colleagues (1984) showed that intravenous injections of ethanol increases DA cell firing in the substantia nigra, which may underlie its locomotor stimulant effects at

low doses. Notably, neurons in the VTA are more sensitive to ethanol than those in the substantia nigra. To be more specific, relatively low doses of ethanol were shown to cause dopaminergic neuron firing in the VTA (Little, 1999). A separate study showed that this increased cell firing in the VTA was dependent on the dose of ethanol being intravenously administered (Gessa et al., 1985). Interestingly, withdrawal has been shown to cause ethanol-related changes in VTA firing. While acute ethanol intake was shown to increase DA neuron discharge in the VTA, a period of withdrawal reduced this discharge, which was only restored after re-administration of ethanol (Hui & Gang, 2014). The differential effects of ethanol intake and withdrawal in VTA cell firing suggest an important role for dopaminergic mesolimbic pathway in modulating the rewarding effects of ethanol.

1.8.2. Microdialysis studies

In order to determine extracellular levels of DA in specific brain areas of interest, like the NAc, the procedure of microdialysis has been utilized. Using this method, it has been shown that systemic injections of ethanol will cause dose-dependent increases in extracellular DA release in the NAc (Yim, Schallert, Randall & Gonzales, 1998). Voluntary ethanol drinking has also been shown to cause increases of accumbal DA in a dose-dependent manner (Weiss et al., 1993). Similar results were found for DA release in the amygdala after intraperitoneal injections of ethanol (Yoshimoto et al., 2000).

Microdialysis studies have also been conducted in rats that were genetically selected for high ethanol drinking. These studies provide convergence data that support the findings of studies related to the neurocircuitry of ethanol reward and addiction (Koob, 2006). More specifically, animals selectively bred for high ethanol drinking have been shown to have decreased basal levels of DA, which is suggestive of an innate deficiency in the mesolimbic DA system in animals with a genetic disposition towards high-ethanol drinking (Bustamante, Quintanilla, Tampler, Israel & Herrera-Marschitz, 2007). In a study by Smith and Weiss (1999), it was found that ethanol-preferring (P) rats given intraperitoneal injections of ethanol showed a marked increase in extracellular DA levels

in the NAc when compared to non-preferring rats. Finally, a tissue punching study discerning ex vivo DA levels found that P rats and their propensity to exhibit high ethanol-seeking behaviour was associated with lower content of DA in the NAc (McBride, Murphy & Lumeng, 1990). These studies provide evidence that animals, which are highly susceptible to ethanol dependence, have marked defects in the mesolimbic dopamine pathway (Hui & Gang, 2014).

Studies determining differences in extracellular DA levels in non-dependent vs. dependent animals are of special relevance. An early study by Weiss and colleagues (1996) showed that acute administration of ethanol in rats rendered dependent to ethanol, through a chronic ethanol-liquid diet, caused a marked increase of extracellular DA in the NAc. This effect was shown to be reduced during ethanol withdrawal and was restored upon ethanol intake (Weiss et al., 1996). In line with this, a marked decrease in striatal extracellular DA has been shown during periods of ethanol withdrawal in animals with a history of ethanol dependence through intragastric intubation (Rossetti, Melis, Carboni, Diana & Gessa, 1992). A similar study showed that decreased DA in the NAc was correlated to behavioural signs of withdrawal after cessation of chronic ethanol exposure through intragastric intubation (Diana, Pistis, Carboni, Gessa & Rossette, 1992). Animals exposed to an acute challenge of ethanol after 1 or 14 days after withdrawal from ethanol vapour exposure were shown to have a marked decrease in accumbal DA after 1 day of withdrawal but no differences after 14 days when compared to basal DA levels (Zapata, Gonzales & Shipennberg, 2006). In addition, voluntary ethanol intake has been shown to cause increases of DA release in the NAc. This increase is positively correlated with blood alcohol levels, which have been showed to restore dopamine deficits after a period of withdrawal (reviewed in Söderpalm & Ericson, 2013). These results suggest that ethanol dependence may render the brain more vulnerable to difficulties in recovering from ethanol exposure during periods of withdrawal (Hunt & Majchrowicz, 1974).

Importantly, a role for the dopaminergic pathway in the rewarding effects of ethanol has also been shown in imaging studies in humans. Notably, consumption of intoxicating doses of ethanol have been associated with dopamine release in the ventral striatum

(Boileau et al., 2003). This release has been correlated with subjective ratings of euphoria, stimulation, and drug wanting for ethanol (Yoder et al., 2007; Engel & Jerlhag, 2014).

The evidence supporting a strong association between the mesolimbic DA pathway and the reinforcing effects of ethanol is plentiful. In addition, it is important to consider the differences in neural activation and catecholamine content in the brains of dependent vs. nondependent animals, and ethanol-preferring vs. -nonpreferring animals. These differences confirm the necessity of using animals rendered dependent to ethanol in order to better determine their behavioural profiles and the associated brain correlates indicative of addiction.

Chapter 2
PURPOSE OF INVESTIGATION

While it is clear that substance use disorders are, at their core, multifaceted on a social, psychological, and neurological level, the high and increasing rates of drug abuse, relapse, and deaths by overdose signify the urgent need for pharmacotherapies and other treatments that will have higher levels of efficacy (Hendershot et al., 2011). In order to allow for the creation of these medications, animal models of substance use disorders must be used to determine the neural underpinnings related to maladaptive decision-making. Current animals models of drug addiction do not elucidate this problem because animals only have one reward to choose from, the drug of abuse being studied (Ahmed, 2010). Therefore, without the option to choose an alternative reward, maladaptive decision-making has not been studied in previous research using these models. The development and use of an animal model of drug addiction that can specifically study maladaptive decision-making in animal behaviour is necessary to determine: (1) the neural underpinnings that contribute to dysfunctional decision-making in drug addiction, and (2) potential targets for pharmacotherapies that can help decrease relapse rates.

As reviewed above, studies utilizing the discrete-choice trials procedure have found that most animals will robustly and consistently choose the non-drug reward over the drug reward when given the option to do so. This has been shown with intravenously administered drugs like nicotine, methamphetamine, heroin, and cocaine in conjunction with orally consumed nondrug rewards like palatable food pellets, and solutions of sucrose and saccharin (Huynh et al., 2015; Caprioli et al., 2015; Lenoir et al., 2013; Lenoir et al., 2007). The results from these studies present an important challenge because they demonstrate a preference for natural rewards over drugs of abuse, despite the fact that these drugs have been shown to be highly rewarding and highly addictive in a multitude of studies. In addition, these results provide information on the relative reinforcing value of drug vs. nondrug rewards experienced by each individual animal. That is, only animals that continue to show drug preference, despite this affecting their

access to the non-drug reward (i.e. negative consequences), are exhibiting maladaptive decision-making.

The work in the present thesis is designed to address the following questions:

1. Does such a high preference for natural rewards over drugs of abuse also occur with ethanol in the discrete-choice model? And what are the parameters that can influence this choice? The study of choice with ethanol is not only addressing the possible generalization of natural reward preferences to another drug, but also permits comparison of the reinforcing value of two substances that are consumed orally.
2. Most, if not all, of the previous studies using the discrete-choice trials procedure have been restricted to non-drug dependent rats. As dependence is an important feature of alcohol addiction or abuse, the second aim of this thesis is to investigate whether the reinforcing value of ethanol is enhanced over saccharin in ethanol-dependent animals.

In order to accomplish these goals, three experiments were conducted. The first experiment examined the choice between ethanol and saccharin in nondependent animals. Having found that animals showed a close to equivalent preference for ethanol and saccharin, we then examined the choice between nicotine and saccharin to help validate the experimental parameters used for the discrete-choice trials procedure in our lab. Given that animals showed a robust preference for saccharin over nicotine, thus confirming previous findings using the discrete-choice trials procedure, we conducted a final experiment that discerned the effects of ethanol dependence on reward preference. In addition, a number of experimental manipulations, which have been shown to effect drug taking in animals, were included in order to elucidate potential shifts in choice behaviour.

Chapter 3
GENERAL MATERIALS AND METHODS

3.1. Animals

Long Evans (Experiments 1 and 3) and Sprague-Dawley rats (Experiment 2) weighing 175-200 grams were obtained from Charles Rivers. Animals were double-housed for one week to allow for acclimatization to the animal facility. Rats were provided with ad libitum access to standard lab chow and tap water. After this period of habituation (Experiment 1 and 3) and catheterization surgeries (Experiment 2), animals were single-housed and fed approximately 23 grams of food per day. The vivarium temperature was set to 21°C ± 1°C and lights were maintained on a 12 hrs reverse light/dark cycle (lights on from 7 pm to 7 am). Experimental procedures were in compliance to the "Principles of Laboratory Animal Care" (8th Edition, 2011) and were approved by the Animal Care Committee at the Centre for Addiction and Mental Health.

3.2. Apparatus

3.2.1. Drinking cages

Animals (Experiments 1 and 3) were initially exposed to solutions of ethanol and saccharin during the limited access paradigm (LAP) at the beginning of the experiment. This occurred in wire cages (30 cm (L) x 20 cm (W) x 15 cm (H)), which was equipped with a 16 ml Richter tube containing the solution in question.

3.2.2. Operant chambers

Self-administration of ethanol and saccharin (Experiments 1 and 3) or nicotine and saccharin (Experiment 2) occurred in 16 operant conditioning chambers (30 x 21 x 21 cm). The chambers were enclosed within fan-ventilated and sound-attenuating enclosures, and were operated by a computer-interfaced system (Med Associates, St.

Albans, VT). Each chamber was equipped with two retractable levers located 2.5 cm above a grid floor and a single- (Experiment 2) or dual-receptacle (Experiments 1 and 3) for liquid dispension. Chambers contained a white houselight (located on the opposite side of the levers), and two cue lights located directly above each lever. Only one lever was inserted during single-reward self-administration sessions while both levers were extended during choice sessions. Appropriate responding on a lever activated an infusion pump located outside of the chamber. Responses for ethanol and saccharin (Experiments 1 and 3) activated a variable speed pump (PHM-100VS, Med Associates) and a single-speed pump (PHM-100; Med Associates) respectively. This allowed for the dispension of the liquid solution into its designated drinking receptacle. Similarly, responses for saccharin in Experiment 2 caused the dispension of solution into a receptacle by the activation of a single speed pump (PHM-100; Med Associates) while responses for nicotine activated a variable speed pump (PHM-100VS, Med Associates) and caused the intravenous infusion of the drug (0.03 mg/kg/infusion).

3.2.3. Ethanol Vapour Chambers

Dependence to ethanol was induced by chronic and intermittent exposure to ethanol vapour as previously described (O'Dell et al., 2004; Gilpin et al., 2008; Vendruscolo et al., 2012). Briefly, animals were exposed to 6 cycles of vapour for 14 hrs on and 10 hrs off daily for 5 consecutive days. Two ethanol vapour systems (La Jolla Ethanol Research Inc., San Diego, CA, USA) were utilized and each was equipped with 4 cages capable of housing 8 animals (a total of 16 animals were used). Each cage was fitted with a custom-built, sealed lid with a gasket, and air fittings to allow for the passage and removal of ethanol vapour, oxygen, and contaminated air (Gilpin et al., 2008). In addition, bedding-lined cages contained water bottles and food troughs, which animals had ad libitum access to.

Each system was attached to a 1 gallon container acting as an ethanol reservoir, which held 95% (v/v) ethanol. As a precaution against ethanol's flammable properties, the volume of liquid in each reservoir never exceeded more than 5 litres. Each reservoir was

attached to a "Q"-pump (Fluid Metering, Inc.) that controlled the speed at which ethanol was being dispensed into the vaporizing system. In addition, a spherical flask was placed in a hemispherical EM series electromantle heater (Electrothermal Engineering Ltd.). The 95% (v/v) ethanol would be dispensed into this flask while the heater caused the vaporization of the solution that would be passed through a series of tubes attached to each cage. In order to allow for the purification and circulation of cage air, each cage was additionally equipped with an air flow gauge (Series VF Visi-Float Flowmeter; Dwyer Instruments) that allowed for the control of ambient air influx. This was made possible by a series of air and vacuum tubes that pushed ambient air into the cages and pulled excess ethanol vapour out of the system.

Stoke rate of ethanol dispension into the heater as well as air flow rate of ambient air was experimenter-controlled and determined by behavioural and physiological monitoring throughout the duration of vapour exposure. A timer controlled the onset of vapour dispension for each system. This timer was programmed to turn on at 10 pm and off at 12 pm in order to allow for 14 hrs of ethanol vapour exposure daily. Animals were monitored 3-4 times daily for gait disturbances, mobility impairments, sedation, ataxia, hyperexcitability, tremors, and rigidity (Gilpin et al., 2008). In addition, animals were weighed daily after the ethanol system was turned off. Food and water were replaced on a daily basis and bedding was changed minimally every two days but more frequently if highly saturated. Ethanol levels were replenished as needed.

3.3. Catheter Implantation Surgery

3.3.1. Construction of intravenous catheters

In order to allow for the delivery of nicotine into the bloodstream by the infusion pump, a catheter was implanted into the right jugular vein of each rat. Prior to the surgical procedure required to do so, catheters were made in-house.

To start, a silicon tubing (Silastic® Medical-Grade Tubing, 0.3 mm internal-diameter (ID) x 0.64 mm outer-diameter (OD); Dow-Corning, Midland, MI, USA) was cut to 30 mm for insertion and catheterization of the jugular vein. In order to allow for the subcutaneous passage for the tubing from the vein to the catheterization port on the top of the animal's back a Silastic tubing was attached to 65 mm of durable and inelastic polyethylene tubing (PE10; 0.28 mm ID x 0.61 mm OD; BD-Canada, Mississauga, ON, Canada), which was subsequently attached to 170 mm of PE20 (0.38 mm ID x 1.09 mm OD; BD-Canada, Mississauga, ON, Canada).

Silastic tubing and PE10 were made to overlap with one another by inserting approximately 7 mm of PE10 into xylene-dilated Silastic tubing. A 6 mm piece of heatshrink (1.19 mm ID) was placed over this joint and heated in order to properly secure the Silastic-PE10 joint. Because of its elasticity and flexibility, the Silastic tubing end of this piece was to be inserted into the rat's vein.

The other end of this piece was to be made into the catheterization port that would subsequently be connected to the infusion pump for drug delivery. In order to do so, 170 mm of PE20 and the remaining PE10 of the piece described above were heat-welded into a bead joint to form one continuous line of tubing. Approximately 8 mm of a 16 mm heat shrink was fed through the open end of the PE20. Once heated, this part of the heat shrink was permanently attached to the PE20 while the other half was left as is, in order to allow for access to the catheter. This opening was then covered with a Silastic hub to protect the catheter from exposure to external particles. A nylon bolt was inserted and superglued (Loctite® Henkel, Canada Corp., Brampton, ON, CAN) overtop the PE20-heatshrink combined tubing. Two bends were then introduced to the catheter piece. This was accomplished by immersing the areas of interest in 90-100°C water. The first bend was a 180° turn in the PE10 just before the Silicon-PE10 joint; this allowed for the flow of the catheter during surgical insertion to be directed towards the heart. The second bend was a 90° bend that was made to the PE20 adjacent to the nylon bolt; this ensured that the PE20 lay flat when passed subcutaneously to the animal's back.

The final step of catheter construction consisted of affixing a biocompatible mesh (Marlex Mesh, BARD Cardio-surgery Division, Billercia, MA, USA), of approximately 30 mm in diameter, to the head of the nylon bolt with dental acrylic (Teets Cold Curing Dental Cement; A-M Systems Inc., Sequim, WA, USA). This allowed for the port of the catheter to be anchored to connective tissue during surgical recovery. All catheters were tested for integrity and patency through multiple pressure tests.

3.3.2. Surgical equipment

Prior to surgery, surgical drapes, gauze, swabs, and sutures (4-0 & 5-0 silk sutures, Surgical Specialties, Reading, PA, USA) were put in an autoclave for sterilization. Materials which were not suitable for autoclaving (e.g. catheters and delicate metallic surgical instruments) were soaked in 1.5% (w/v) benzalkonium chloride (Sigma-Aldrich, Oakville, ON, CAN), rinsed in saline, and placed on a sterile surface. Catheters were flushed with and subsequently soaked in sterile saline.

Surfaces of the surgical suite were also sanitized with the benzalkonium chloride solution. An absorbent sheet (Dri-Sorb® Underpads; Domtar Personal Care, Raleigh, NC, USA) was then placed on the sterilized surfaces upon which surgical drapes rested in preparation for surgery.

3.3.3. Anesthesia

Animals were sedated with a combination of 3-5% inhalation Isoflurane (99%USP; Halocarbon Products Corp., River Edge, NJ, USA) and oxygen. Gas ratios were adjusted through a vaporizer (53-T3ISO; Benson Medical Industries Inc., Markham, ON, CAN) and were delivered through a closed-air system. Upon sedation, animals were exposed to a steady flow of anaesthetic (4.5%) inside of a sealed induction chamber (internal dimensions of 23 cm x 10 cm x 10 cm). Following anaesthetic onset, animals were maintained at 3% Isoflurane through a nose cone. Animals were continuously monitored throughout the duration of surgery and gas ratios were adjusted accordingly.

3.3.4. Surgical preparation

Intravenous catheterization required two incisions: (1) a dorsal incision medial to the shoulder blade of the rat for the catheterization port, and (2) a ventral incision providing access to the right jugular vein. In order to prevent contamination of the surgical area, the fur on each animal's ventral neck and dorsal shoulder area was shaved with an electric razor. A nerve blocker, 1-2 mg/kg (0.125%) bupivacaine (Marcaine™), was injected subcutaneously at each of the two injection sites to provide local anesthesia. A Betadine surgical scrub (Purdue Frederick, Pickering, ON) was applied to both surgical sites followed by the application of 70% (v/v) ethanol and 10% USP povidone-iodine solution (Proviodine®; Teva Canada - OTC, Mirabel, QC, CAN) with sterile gauze. Animals were subsequently injected subcutaneously with: (1) 5 mg/kg ketoprofen (Anafen®), which acted as a local anti-inflammatory analgesic, (2) 0.1 ml Derapen (SQ/LA®), which acted as an antibiotic, and (3) 3 ml of 0.9% saline, which served to replace any fluids lost during surgery. Finally, an ophthalmic ointment (Lacri-Lube®; Allergan Inc., Markham, ON, CAN) was applied to the eyes of animals to prevent corneal drying.

3.3.5. Catheter implantation

Prior to incisions being made, level of anesthesia was verified through the absence of a pain withdrawal reflex. Animals were positioned ventrally on a sterile surgical drape and were incised with a 1 cm opening between the scapulae. Space was made for the eventual addition of the catheter port and mesh through blunt dissection. The dorsal incision was protected with sterile gauze before the animal was displaced to dorsal recumbency.

A 1 cm incision was made above the clavicle on the animal's right ventral side. Musculature was cleared through blunt dissection, and the jugular vein was exposed, striped of local fascia and isolated with suturing (5-0 surgical thread) placed underneath the vein for later retrieval. Vein moisture was maintained throughout with the addition of saline. A pair of forceps was used to form a subcutaneous pocket from the area of the

ventral incision, around the shoulder blade, to the top of the dorsal incision. To facilitate the passage of the catheter, a large-diameter polyethylene tube (PE380) was passed through this subcutaneous pocket and served as a trocar. The Silastic end of the catheter was fed through the trocar from the dorsal end in order to allow for easy access to the jugular vein. Upon proper positioning, the trocar was removed through the ventral end, leaving the catheter in place subcutaneously.

The 5-0 surgical threads previously used to isolate the jugular vein were used to manipulate the vein as a V-shaped incision was made through 2/3 of the vein. The silastic end of the catheter was passed into the vein through this incision until the heat-shrink connected to the PE10 tubing of the catheter made contact with the vein. This portion of the catheter was sutured to the outer surface of the vein at either end with 5-0 suture silk. A drop of cyanoacrylate (superglue) was placed on the underside of the heat-shrink to secure its placement to connective tissue. The superficial muscle layer was subsequently sutured with interrupted 5-0 suture silk before closure of the dermal layer with interrupted 4-0 silk sutures. The sutured incision was covered with sterile gauze before turning the animal over to allow for exposure of its dorsal side.

The excess PE20 tubing from the catheter was looped through the subcutaneous pocket, which was previously made, in such a way that allowed the mesh and catheter port to be laid flat and centered atop the incision between the scapula. With dermal tissue surrounding and securing the catheter port and mesh, interrupted 4-0 silk sutures were used to close the dorsal incision. Upon ensuring the absence of gaps or air pockets surrounding the base of the catheter port, a topical antibiotic powder (Cicatrin®; Glaxo Wellcome, Orange, CT, USA) was applied to prevent infection and facilitate recovery. Animals were then placed on a temperature-regulated heat pad (VL-20F; Fintronics Inc., Orange, CT, USA) to account for the hypothermic effects of anesthesia. Once animals were conscious and responsive, they were placed in a transfer cart, and subsequently placed back into their home cages. Animals were weighed and monitored daily throughout the recovery stage.

3.3.6. Maintenance and verification of catheter patency

A 1 mm Silastic hub, half of which was filled with cured liquid silicone adhesive (Dow-Corning) was used to cap the catheter port in order to prevent contamination. This hub remained on at all times, except for when animals were connected to the infusion pumps and receiving nicotine infusions. Catheter patency was maintained with intravenous injections of an anticoagulant, heparin sodium salt (50 units/ ml, Sigma-Aldrich, St. Louis, MO, USA), dissolved in 0.9% saline. The heparin solution was administered at a volume of 0.05 ml once daily, beginning two days post-surgery. A new solution was made every 3 days (stored at 2-8°C) and was filtered in order to maintain its sterility. Heparin was administered both before and after sessions of intravenous self-administration to ensure continuous catheter flow.

Upon completion of experimental protocol, catheter patency was behaviourally observed through intravenous infusions of 0.05 ml of a solution containing 20 mg/ ml xylazine (Rompum®, Bayer Inc., Toronto, ON, CAN) in heparin. Observable signs of ataxia (i.e. loss of muscle tone) were indicative of catheter patency. Animals showing the absence of a xylazine-heparin response were excluded from analyses due to compromised catheter patency.

3.4. Experimental Procedures

3.4.1. Limited access paradigm

Before exposure to either ethanol or saccharin in the operant conditioning boxes, animals were given 30 min sessions of limited access drinking to each reward respectively. This occurred in limited access paradigm (LAP) wire cages (as described above) in which animals had access to a 16 ml Richter tube.

Total volume consumed by each animal was calculated by taking the difference between the initial, pre-session solution volume and the post-session solution volume. Upon stable levels of LAP drinking, animals were subjected to sessions of operant self-administration.

3.4.2. Saccharin self-administration

Animals learned to self-administer a solution of 0.05% or 0.1% (w/v) saccharin in operant conditioning boxes described above. The saccharin solution was used to fill 20 ml (BD-Canada) syringes which were subsequently placed in single-speed syringe pumps (PHM-100; Med Associates). Syringes were connected to a 22-Gauge (22G) blunted needle tip which attached to Tygon™ tubing fluid lines (Saint-Gobain PPL Corp., Akron, OH, USA) to the drinking receptacles in the operant chambers. The initial schedule of reinforcement was set to fixed-ratio (FR) 1, which required animals to press the lever once in order to receive a reinforcement. Upon stable levels of saccharin reinforcement at FR-1 (4 days), the schedule of reinforcement was subsequently increased to FR-2 (2-4 days) and FR-3 (2-8 days).

Initiation of each session was indicated by the insertion of the saccharin lever and the illumination of the white house-light. Appropriate responding on the lever caused a 2 sec dispension of saccharin (0.19 ml) into the receptacle, the illumination of a stable, red cue light for 6 sec, and the retraction of the lever for a short timeout (TO; 6-30 sec) period. The volume of unconsumed saccharin was collected and measured at the end of each operant session and was converted into the equivalent number reinforcements in order to properly record the correct number of reinforcements consumed by each animal. As previously indicated, the position of the saccharin lever (left vs. right) was counterbalanced across rats.

3.4.3. Ethanol self-administration (Experiments 1 and 3)

Initial ethanol consumption training was completed as described in Section 2.4.1. Following this period, animals were trained to press a lever to self-administer 12% (w/v)

ethanol during 30 min operant conditioning sessions. As with saccharin self-administration, 20 ml syringes were filled with the ethanol solution and connected to a Tygon™ tubing, which was attached to the appropriate drinking receptacle. The pumps holding the syringes were variable-speed (PHM-100VS, Med Associates) in order to allow for proper control over the volume dispensed into the receptacle. The schedule of reinforcement began at a FR-1 and was increased to FR-2 (3-5 days) and FR-3 (6-9 days).

Sessions of ethanol self-administration began with the insertion of the ethanol lever and the illumination of the white-house light. Appropriate responding on the lever caused the dispension 0.19 ml of ethanol into its respective receptacle, the illumination of a flashing (0.5 sec on, 0.5 sec off), green cue light for 6 sec, and the retraction of the lever for a 6 sec TO period. The volume of unconsumed ethanol was collected and measured at the end of each operant session. This was converted into the equivalent number reinforcements in order to properly record the correct number of reinforcements consumed by each animal. The position of the ethanol lever was counterbalanced across animals.

3.4.4. Nicotine self-administration (Experiment 2)

Following a 7 day recovery period post-surgery, animals were subjected to sessions of intravenous nicotine self-administration. Operant boxes were configured similarly to those described above. Solutions of sterile nicotine (described below) were placed into 10 ml syringes before being attached to variable-speed pumps (PHM-100VS, Med Associates) and attached to a 22G needle holding Tygon™ tubing. This tubing was connected to the operant chamber by a freely-rotating fluid swivel (PHM-1151P, Med Associates) which in turn connected to a second set of Tygon™ tubing ending off with a 22G cannula that was used to connect the drug line to the animal's catheter port. The freely-rotating swivel allowed for animals to move freely while having their catheters attached to the drug line. It is important to note that the second set of Tygon™ tubing was enclosed in a metal spring that was screwed to the animals nylon bolt; this was to ensure proper protection and shielding of the drug line.

The infusion rate of nicotine delivery was determined daily, based on the animal's body weight. The variable-speed pumps allowed for the precise control of volume dispension under the control of the MED-PC interface. The animals' body weights were used to determined how to program each, individual pump in order to accommodate an infusion dose of 30 μg/kg/infusion (free base).

Before the session onset, animals were primed with a non-contingent infusion of nicotine in order to fill the empty space formed by the connection of the pump tygon to the catheter port. Doing so ensured that animals would be receiving nicotine directly into their bloodstream as of the first reinforcement obtained. Self-administration sessions were initiated by the insertion of the nicotine-paired lever and the illumination of a white houselight. The schedule of reinforcement used was as follows: FR-1 (4 days), FR-2 (2 days) and FR-3 (4 days) for the remainder of the study. Appropriate responding on the lever allowed for the infusion of a nicotine reinforcement, the presentation of a stable, green cue light for 6 sec, and the retraction of the lever for a 30 sec TO period. Nicotine self-administration sessions lasted 1 hr.

3.4.5. Discrete-choice trials procedure

Animals showing stable levels of drug reward (ethanol or nicotine) and non-drug reward self-administration (< 20% deviation from the mean) during single-reward sessions were trained on the discrete-choice trials procedure. The discrete-choice trials procedure consisted of 16 trials, which can be divided into two sequential phases: (1) the sampling phase, and (2) the discrete-choice trials phase.

The discrete-choice trials procedure commenced with a sampling phase, which gave animals the opportunity to sample each reward twice by providing them with access to only one lever at a time during the first 4 trials. For example, animals in Experiment 2 were exposed to each lever in a nicotine-saccharin-nicotine-saccharin order. Upon completion of the 4 sampling trials, animals were introduced to both levers at the same

time for the following 12 trials. It is important to note that animals only had 2 min to respond during each trial and that each trial was subsequently separated by a 2 min inter-trial interval (ITI). That is, if an animal did not respond within the allotted 2 min the levers were retracted and the 2 min ITI was initiated. However, if a response was made within the 2 min trial length, the reinforcement for the selected reward was delivered, its respective cue light would illuminate and both levers would retract for the 2 min ITI. Once the 2 min ITI was complete, both levers would be inserted into the conditioning box thus signifying the commencement of a new trial.

As with self-administration training, the cue associated with drug reward delivery was a flashing, green light whereas the saccharin reinforcement cue light was a stable, red light. An FR-3 schedule of reinforcement was maintained during this procedure; however, it is worth noting that animals needed to bar press on their lever of choice exclusively in order to receive a reinforcement. For example, if an animal pressed on the nicotine lever twice and then the saccharin lever once, the reinforcement requirement on the nicotine lever would be reset. The session length of the discrete-choice trials procedure varied depending on the speed at which animals responded during each 2 min length trial but the maximum length of any discrete-choice trials session was 1 hr.

3.4.6. Concurrent access procedure

Upon stable levels of choice behaviour during the discrete-choice trials procedure, animals were subjected to two sessions of concurrent access whereby they had unlimited access to both levers for 1 hr. During these sessions, both levers were made concurrently available without an ITI between reward presentations and without a maximum number of choice trials.

A concurrent access session began with the insertion of both reward levers and illumination of a white houselight. Appropriate and exclusive responding on the FR-3 schedule of reinforcement allowed for the delivery of the appropriate reward, the illumination of its respective cue light for 6 sec, and the retraction of both levers for a

short TO period. Following this 6-30 sec TO period both levers would be inserted into the box, allowing the animal to have access to both rewards in an unrestricted and continuous manner.

3.5. Drugs

Solutions of 0.1% (w/v) saccharin were prepared by dissolving saccharin sodium salt (Sigma-Aldrich) in tap water. 95% ethanol (Commercial Ethanols Incorporated, Tiverton, ON, Canada) was diluted to its desired concentration in tap water. Nicotine solutions were made fresh on a daily basis. Nicotine bitartrate (Sigma-Aldrich) was diluted in 0.9% saline to create a 0.3 mg/ml nicotine (free base) solution, thus creating a unit dose of 30 μg/kg/infusion. This was subsequently adjusted for a pH of 6.8 -7.2 and filtered into a sterile container before being put into the 10 ml pump syringes. All doses and concentrations mentioned for each reward have been shown to be commonly used in previous research; they have been shown to cause sufficient rates of self-administration (Shram et al., 2008; Le et al., 1998; Corrigall & Coen, 1989).

3.6. Statistical Analyses

All statistical analyses were performed using IBM SPSS version 24. Animals that showed low levels of operant behaviour (i.e. low number of self-administration reinforcements, and low number of completed choice trials) were excluded from all analyses. Unless otherwise specified, repeated-measures analysis of variance (ANOVA) was used to analyze dependent variables (i.e. reward reinforcements) that were continually being measured with a within-subjects factor of Day. Between-subjects analyses are unique to each experiment and will be discussed in their own sections. If Mauchly's test of sphericity returned a significant result, all statistics (the F values, p values and degrees of freedom) reported are taken from the Greenhouse-Geisser correction. The criterion for significance in any statistical result was set to a p value of < 0.05 and the Bonferroni post-hoc was utilized to further analyze significant effects.

Chapter 4
DUAL CHOICE BETWEEN ETHANOL AND SACCHARIN

4.1. Introduction

In the last 20 years, animal models of operant self-administration have been used to test a number of pharmacotherapies, some of which have been shown to decrease drug use and drug seeking. While helpful in gaining a better understanding of the neurochemical underpinnings related to the rewarding aspects of drug use, the technique of employing one single reward in these procedures acts as a limitation. These models have good face, constructive, and predictive validity for non-addictive drug use (i.e. recreational); however, their extension to drug dependence in humans is unknown (Ahmed, 2010). Part of this uncertainty can be attributed to the lack of choice for alternative, nondrug rewards in procedures using single-reward operant self-administration. In these procedures, the absence of other reward opportunities is in contrast to the potential multiple rewards available to human drug users (Ahmed, 2010). This discrepancy between animal models of self-administration and the realities of human drug use calls into question the translatability of data from pre-clinical research.

Given this, there has been a recent surge in the use of procedures, which give the animal the option to choose between a drug reward and nondrug reward. Previous studies using this model show that animals will choose a nondrug reward over intravenously administered drug rewards. More specifically, a study by Lenoir and colleagues (2007) found that almost all animals will choose saccharin or sucrose over intravenously administered cocaine. This pattern of choice behaviour remained even after extensive pre-training for cocaine administration and after increasing the dose of cocaine (Lenoir et al., 2007). Similar results have been found for the choice between drugs like methamphetamine, heroin, and nicotine in conjunction with nondrug rewards of palatable food pellets, and solutions of sucrose and saccharin (Caprioli et al., 2015; Lenoir et al., 2013; Huynh et al., 2015). In addition, a recent study showed that animals prefer saccharin over ethanol, both of which are orally consumed (Pelloux & Baunez, 2017).

These studies based on discrete choice have been useful in determining an animal's decision-making behaviour when given the option to administer two different types of reward. In addition, we have been able to learn important information regarding the relative reinforcing value of drug rewards vs. nondrug rewards in non-dependent animals. Notably, the discrete-choice trials procedure has yet to be studied in animals rendered dependent on any drug of abuse. Determining choice behaviour in dependent animals is of critical importance to our understanding of maladaptive decision-making and the transition to compulsive drug use; both of which are characteristic of substance abuse disorders. Given this, the main goal of my thesis was to determine choice behaviour in animals rendered dependent on ethanol. However, before determining the effects of dependence on this type of decision-making, an experimental framework for the choice between ethanol and saccharin had to be established in non-dependent animals. Therefore, as an important first step, this experiment was focused on determining initial choice behaviour between saccharin and ethanol alongside the effects of environmental and behavioural manipulations on decision-making in non-dependent animals.

4.2. Experimental Procedures

4.2.1. Animals

Forty Long-Evans rats weighing 175-200 grams at the beginning of the study were used for this experiment. As described in Section 3.1., they were initially pair housed in the Animal Care Facility before being single-housed and placed on a mild food restriction (≈23 grams of lab chow/ day) diet for the majority of the experiment.

4.2.2. Experimental design

Figure 4.1. provides details of the experimental timeline for the procedures described below. Briefly, animals were exposed to single-reward self-administration training sessions to ensure stable lever pressing for each reward respectively. Animals were then introduced to choice sessions. This was initially completed with the inclusion of a 0.05% (w/v) saccharin solution and 12% (w/v) ethanol solution. Following this phase, the saccharin concentration was increased to 0.1% and self-administration and choice baseline behaviour were re-established. Animals were subsequently subjected to environmental and behavioural manipulations in the form of the reward deprivation and increasing the inter-trial interval (ITI) during the discrete-choice trials procedure.

Limited access paradigm

Before exposure to ethanol in operant condition boxes, animals were subjected to the limited access paradigm (LAP), which exposed them to increasing concentrations of ethanol in wire cages for 30 min/ day. Animals had access to a solution of 3% (w/v) ethanol for 5 days, followed by 6% (w/v) ethanol for 7 days and, finally, 12% ethanol (w/v) for 13 days. To minimize environmental impacts (sound, lighting, etc.) on drinking behaviour, LAP sessions included the presence of a white noise generator in an isolated room with the lights turned off.

Ethanol volumes before and after the 30 min drinking period were recorded to allow for the determination of total volume consumed by each animal. This was completed in order to ensure animals were accustomed to the taste of ethanol before being required to respond for contingent delivery of a 12% (w/v) ethanol solution during the self-administration phase. After stable levels of ethanol drinking, animals were exposed to 5 days of 30 min sessions of LAP for a solution of 0.05% (w/v) saccharin.

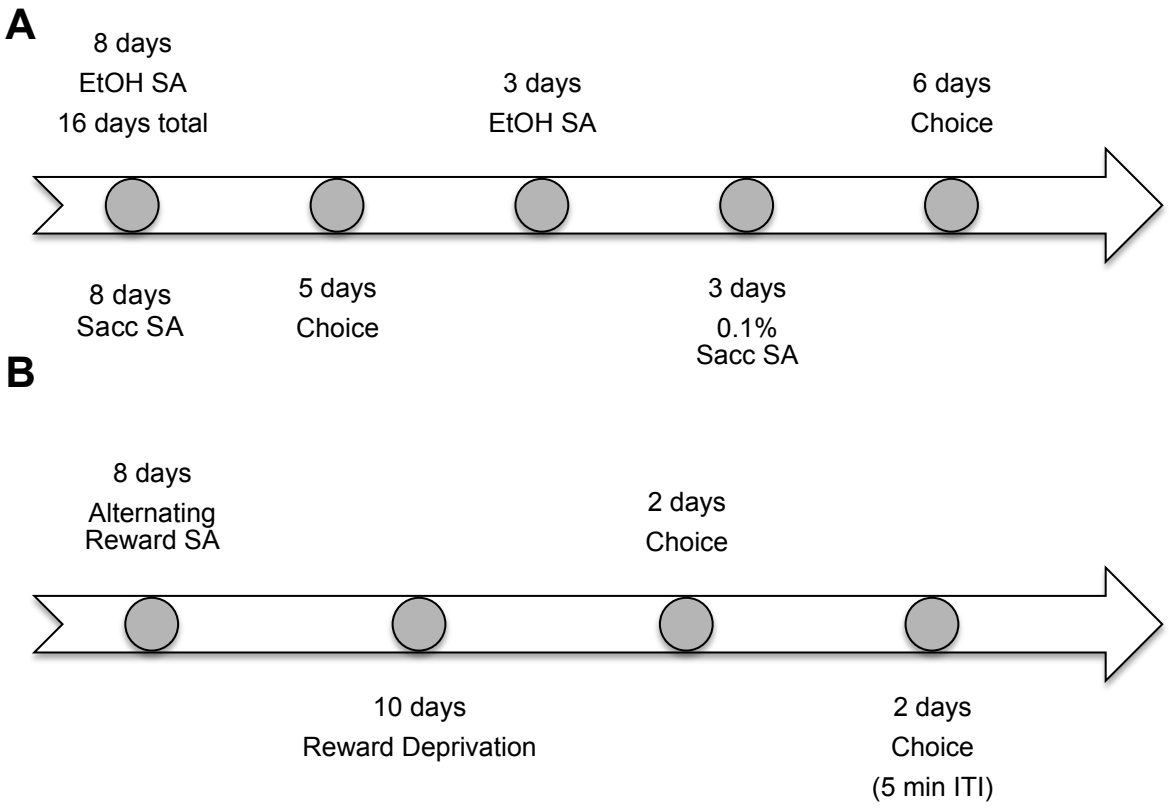

Figure 4.1. *Overview of experimental timeline for Experiment 1*. (A) Self-administration (SA) training for ethanol (EtOH) and saccharin (Sacc) on alternating days was followed by choice sessions. (B) Retraining of single-reward self-administration was followed by reward deprivation and choice testing. Finally, animals were tested with a 5 min inter-trial interval (n= 31) (initial n= 40).

Ethanol self-administration

After initial exposure to 12% ethanol during the LAP, animals began training to self-administer 12% ethanol in operant conditioning boxes. The schedule of reinforcement was increased from FR-1 (10 days), to FR-2 (5 days), and was maintained at FR-3 (6 days) for the remainder of the experiment.

Saccharin self-administration

Following ethanol administration training, animals learned to self-administer a 0.05% solution of saccharin. The schedule of reinforcement began at FR-1 (2 days), and was increased and maintained at FR-3 (2 days).

In order to ensure stable self-administration of both rewards, animals were subsequently subjected to alternating days of ethanol (8 days) and saccharin (8 days) self-administration for a total of 16 days. At this stage of the experiment, one-half of the animals ended on a saccharin self-administration day whereas the other half ended on an ethanol self-administration day; this was done to account for the order of reward session presentation and its potential effects on subsequent choice behaviour.

Discrete-choice trials procedure

0.05% Saccharin

Upon stable levels of 12% ethanol and 0.05% saccharin self-administration, animals were subjected to the discrete-choice trials procedure in which they were required to choose one reward over the other across 5 sessions. The saccharin concentration was subsequently increased in order to determine whether this manipulation would affect choice behaviour.

Animals were reintroduced to self-administration sessions for 12% ethanol (3 days) and 0.1% saccharin (3 days) on alternating days for a total of 6 days.

0.1% Saccharin

Following self-administration of the higher concentration of saccharin solution (0.1%) for 3 days, animals were given 6 days of sessions of the discrete-choice trials procedure with 12% ethanol and 0.1% saccharin.

Reward deprivation

Once rats showed stable baseline choice behaviour, they were given alternating days of ethanol and saccharin self-administration for a total of 6 days. This was followed by reward deprivation in their home cages for a total of 10 days before in the discrete-choice trials procedure (2 days).

Effect of ITI duration

Following the reward deprivation testing phase, the ITI was increased from 2 min to 5 min during 2 days of sessions with the discrete-choice trials procedure.

4.2.3. Statistics

Repeated-measures ANOVA with factors of Reinforcer (saccharin, ethanol) and Day or Concentration (0.05% or 0.1% (w/v) saccharin) were used to examine the role of reinforcer on self-administration and on choice during the discrete-choice trials procedure.

4.3. Results

Attrition

A total of 9 animals were excluded in this experiment. These exclusions were determined by: (1) low levels of ethanol and saccharin responding during single-reward training and (2) low number of total completed trials during sessions of the discrete-choice trials procedure.

Self-administration and choice behaviour with 0.05% saccharin and 12% ethanol

Rats acquired stable self-administration of both 0.05% saccharin and ethanol over the 8 days of training when rats received saccharin (mean ± sem= 25.56, ± 2.88) and ethanol (mean ± sem= 15.80 ± 1.95) self-administration sessions on alternate days (Fig. 4.2.). A repeated measures ANOVA with the factors of Reinforcer (saccharin, ethanol) and Training day (Day 1-Day 8) revealed a main effect of Reinforcer ($F(1, 25)= 13.711$, $p= 0.001$) because rats received significantly more deliveries of 0.05% (w/v) saccharin than 12% (w/v) ethanol.

A repeated measures ANOVA with the factors of Reinforcer (saccharin, ethanol) and Choice day (Day 1-Day 5) analyzing the choice of saccharin and ethanol across the 5 days of the discrete-choice trials procedure showed a Reinforcer x Day interaction, $F(4, 27)= 3.381$, $p= 0.021$. This was because, overall, choice of saccharin remained stable, while choice of ethanol tended to increase across choice sessions. There was also a main effect of Day ($F(4, 27)= 3.162$, $p= 0.033$) which was caused by this increased ethanol, but not saccharin choice across days. Pairwise comparisons revealed a significant difference in ethanol choice on Day 4 in comparison to Days 1 and 2 (Figure 4.3.). The ANOVA revealed no statistically significant differences in reward choice between 0.05% (w/v) saccharin and 12% (w/v) ethanol (Main effect of Reinforcer: $F(1, 30)= 0.038$, $p = 0.847$). A t-test conducted on the average number of saccharin choices and ethanol choices across the 5 days of discrete-choice trials testing revealed no statistically

significant differences between the two reinforcers, $t(30)= 0.947$, $p= 0.704$ (Figure 4.3. inset). Taken together, these data indicate that rats displayed equivalent preference for 0.05% saccharin and ethanol.

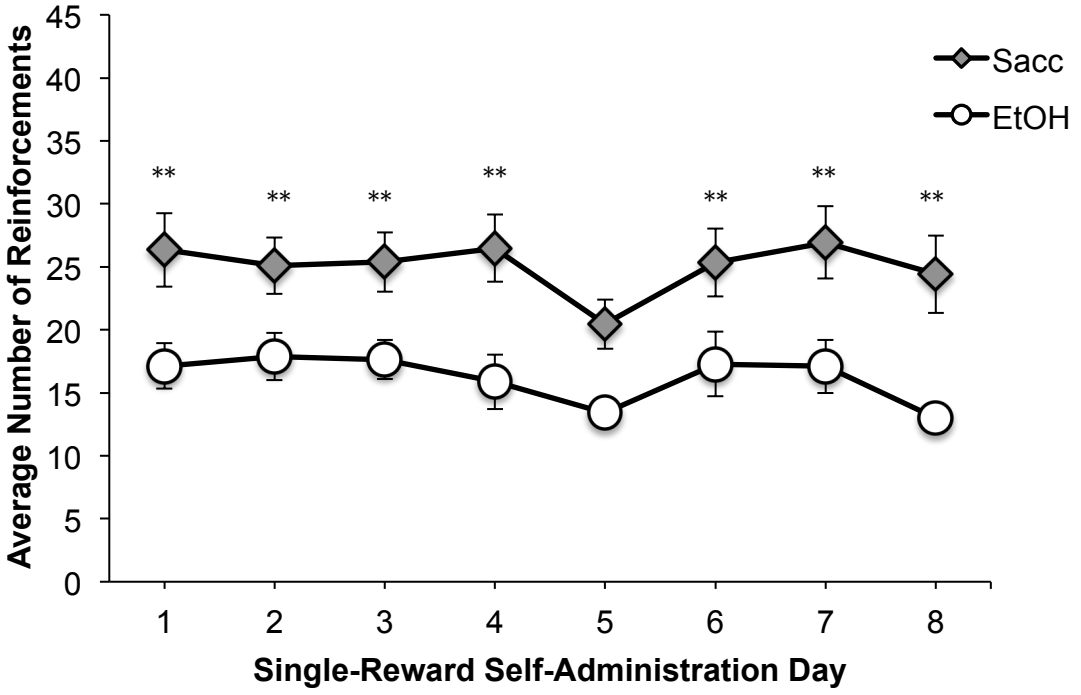

Figure 4.2. *Self-administration of ethanol or 0.05% saccharin*. Data are mean numbers (±SEM) of 12% (w/v) ethanol (EtOH; circles) and 0.05% (w/v) saccharin (Sacc; diamonds) reinforcements obtained during single-reward self-administration sessions (FR-3) (n= 31). $^{**}p < 0.01$ compared to ethanol reinforcement.

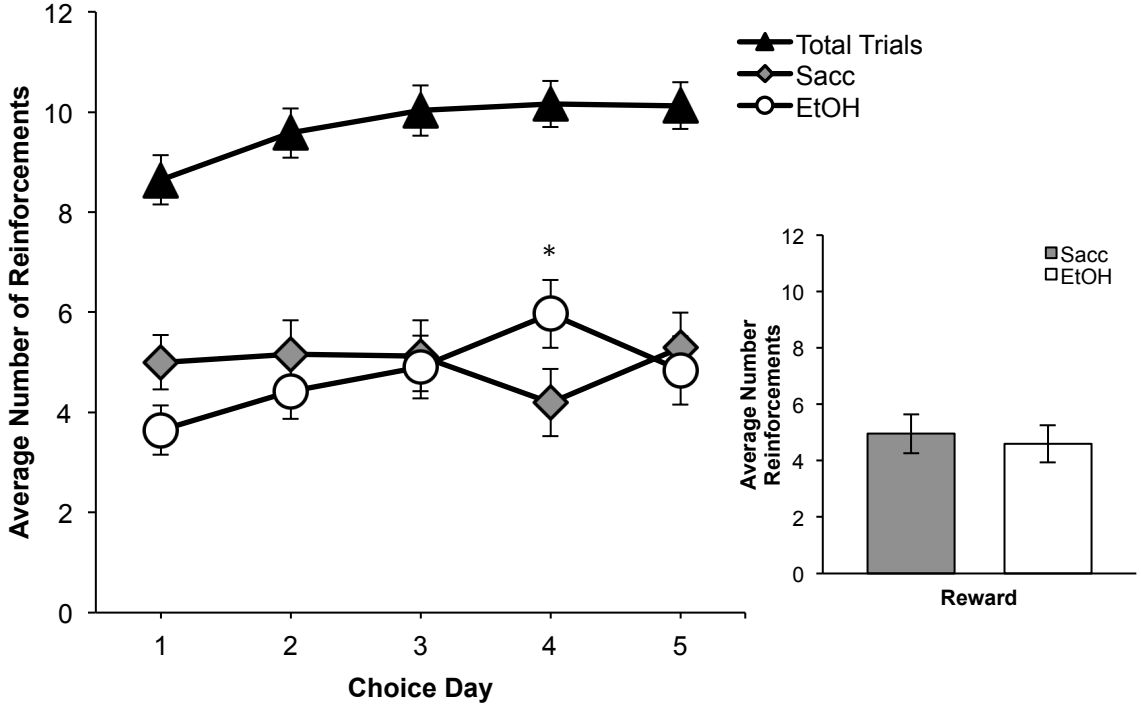

Figure 4.3. *Choice for ethanol and 0.05% saccharin with the discrete-choice trials procedure*. Data are mean numbers (±SEM) of 12% (w/v) ethanol (EtOH; circles) and 0.05% (w/v) saccharin (Sacc; diamonds) reinforcements obtained during 5 days of choice sessions (n= 31). Triangles represent mean total number of trials completed (i.e. ethanol + saccharin) (±SEM). * $p < 0.05$ compared to Day 1 and Day 2 for ethanol choice. Inset showing average saccharin and ethanol choice selection (±SEM) across 5 days.

Self-administration and choice behaviour with 0.1% saccharin and 12% ethanol

The concentration of saccharin was doubled in order to examine whether changing the reinforcer magnitude of the nondrug reward would shift reward choice. Repeated measures ANOVA on these data revealed a significant effect of Reinforcer ($F(1, 30)= 14.46$, $p= 0.001$) (Figure 4.4.), because rats self-administered more saccharin than ethanol during these sessions. In addition, analysis of saccharin self-administration at the 0.05% vs. 0.1% concentration revealed a main effect of Concentration ($F(1, 30)= 22.792$, $p= 0.000$), because animals self-administered more saccharin at the higher, 0.1% concentration (Figure 4.2 and 4.4.)

Repeated measures ANOVA on the saccharin (0.1%) and ethanol choice during the 6 daily choice sessions showed a near-significant main effect of Reinforcer, $F(1, 30)= 4.405$, $p= 0.053$. Pairwise comparisons revealed that saccharin choice was significantly greater than ethanol choice on Days 5 and 6 (Figure 4.5.). A paired t-test on the average of saccharin (0.1%) choice and the average ethanol choice across 6 days of choice sessions revealed statistically higher saccharin choice than ethanol choice, $t(30)= 1.909$, $p= 0.000$ (Figure 4.5. inset). To summarize, animals chose more 0.1% saccharin than ethanol during sessions of the discrete-choice trials procedure.

Separate repeated measures ANOVAs on saccharin choice and ethanol choice between low (0.05%) and high (0.1%) saccharin concentrations revealed a significant effect of saccharin concentration on saccharin choice, $F(1, 30)= 28.240$, $p= 0.000$ (Figure 4.6.). No statistically significant effects of saccharin concentration were found on ethanol choice, $F(1, 30)= 3.842$, $p= 0.059$. To summarize, increase in saccharin concentration from 0.05 to 0.1% did not affect ethanol choice but increased saccharin choice, which consequently increased the numbers of total trials completed.

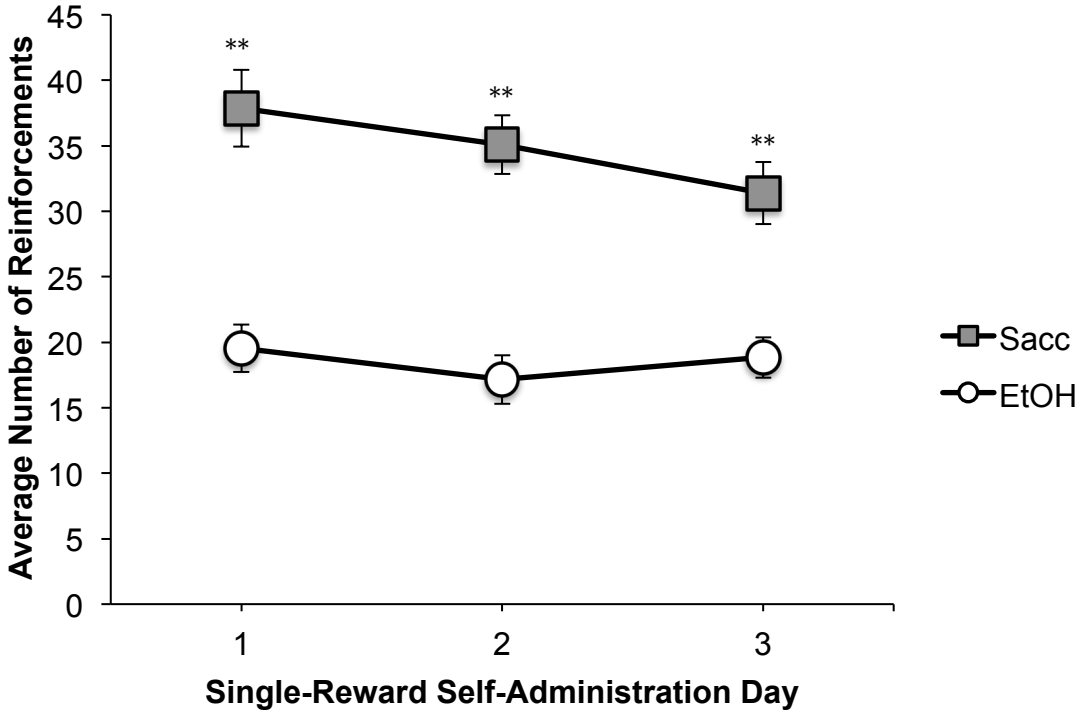

Figure 4.4. *Self-administration of ethanol or 0.1% saccharin*. Data are mean numbers (±SEM) of 12% (w/v) ethanol (EtOH; circles) and 0.1% (w/v) saccharin (Sacc; squares) reinforcements obtained during single-reward self-administration sessions (n= 31). ** $p < 0.01$ compared to ethanol reinforcement.

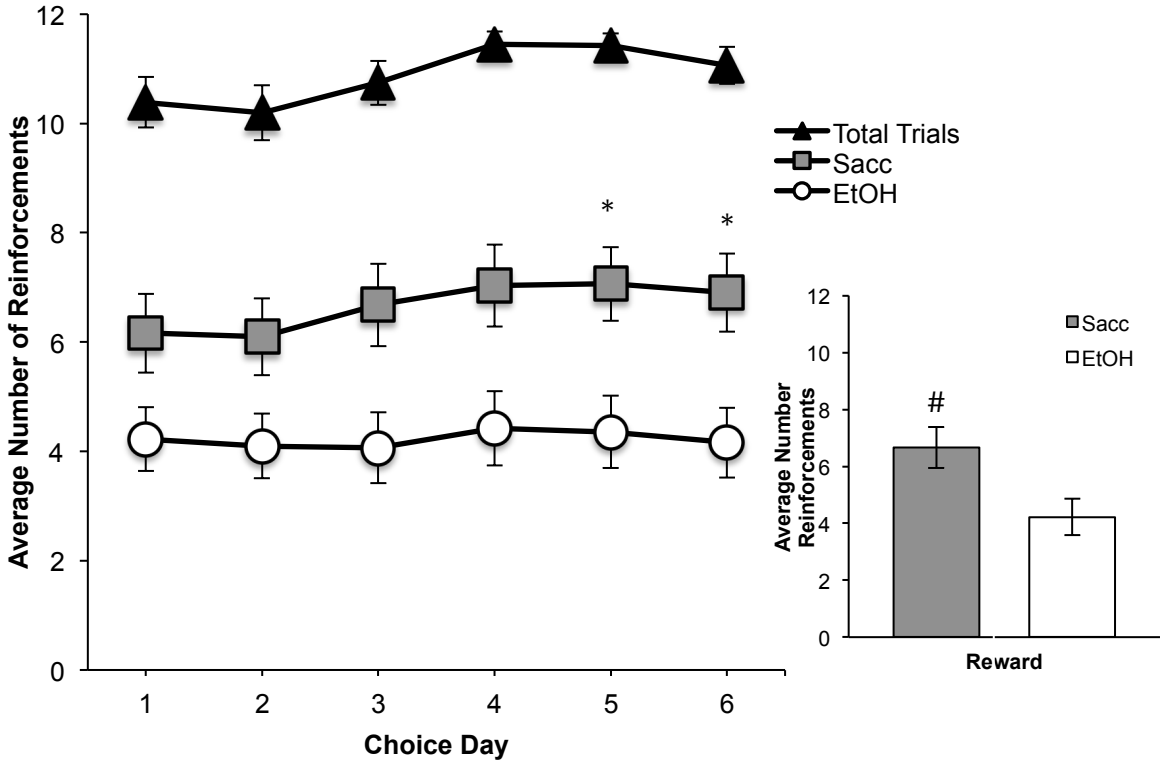

Figure 4.5. *Choice for ethanol and 0.1% saccharin with the discrete-choice trials procedure*. Data are mean numbers (±SEM) of 12% (w/v) ethanol (EtOH; circles) and 0.1% (w/v) saccharin (Sacc; squares) reinforcements obtained during 6 days of choice sessions. Triangles represent mean total number of trials completed (i.e. ethanol + saccharin) (±SEM). *$p < 0.05$ compared to ethanol reinforcement. Inset shows average saccharin and ethanol choice selection (±SEM) across 6 choice days. #$p < 0.01$ compared to ethanol choice.

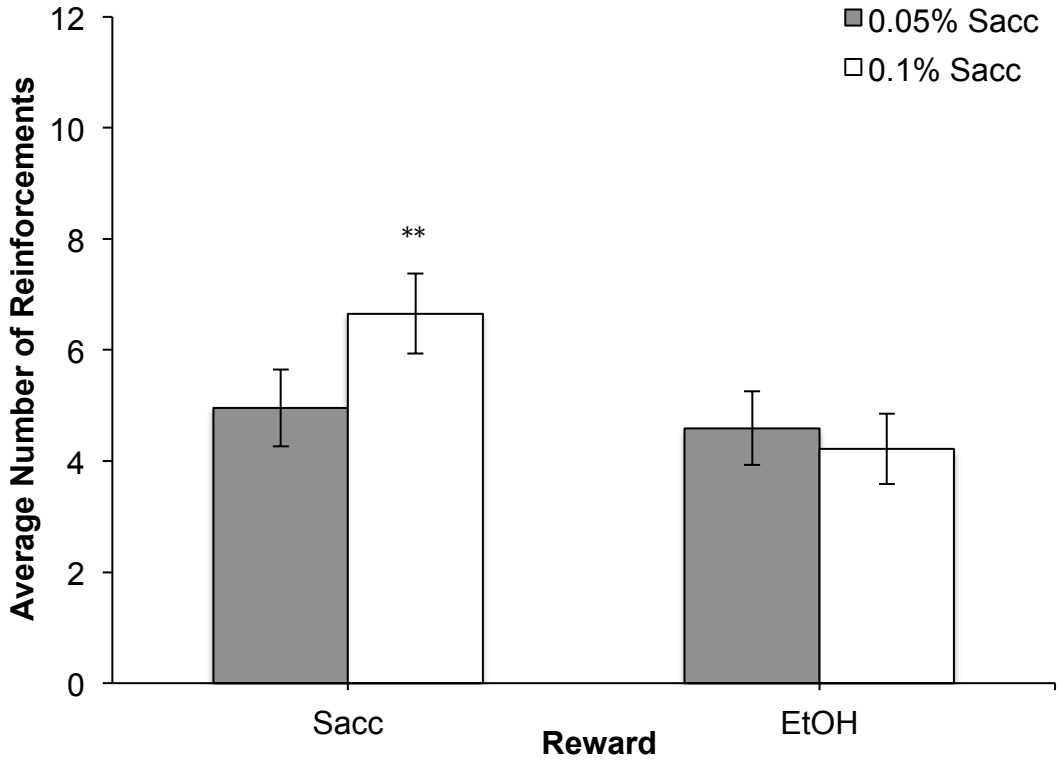

Figure 4.6. *Comparison of choice behaviour between 0.05% saccharin and 0.1% saccharin*. Data are mean numbers (±SEM) of 12% (w/v) ethanol (EtOH) and saccharin (Sacc) reinforcements obtained from choice sessions when 0.05% (grey bar) vs. 0.1% (w/v) saccharin (white bar) was available (n=31). $^{**}p < 0.01$ compared to 0.05% saccharin concentration.

Effect of reward deprivation on choice behaviour

After receiving alternating days of single-reward self-administration sessions to both rewards, animals were subjected to 10 days of deprivation followed by 2 days of testing with the discrete-choice trials procedure. An ANOVA comparing the average of the last 2 days of choice baseline vs. the 2 days of choice after 10 days of deprivation of both rewards revealed a significant Reinforcer by Day interaction ($F(2, 29)= 6.638$, $p= 0.000$), because saccharin choice increased as a function of Day, while ethanol choice decreased. There was a significant main effect of Reinforcer ($F(1, 30)= 14.857$, $p= 0.001$) indicating significantly greater choice for saccharin vs. ethanol. There was also a significant main effect of Day ($F(2, 29)= 3.498$, $p= 0.037$) because, when compared to baseline, saccharin choice increased on both days after reward deprivation whereas ethanol choice was significantly lower only on the second day of testing (Figure 4.7.).

Effect of a 5 min ITI on choice behaviour

Following the testing after reward deprivation, the effects of inter-trial interval (ITI) duration on choice of saccharin (0.1%) vs. ethanol was determined. ITI was increased from 2 min to 5 min for 2 days. Repeated measures ANOVA on saccharin and ethanol choice on the average of the last 2 days of choice with a 2 min ITI and the 2 days with a 5 min ITI revealed a significant Reinforcer by Day interaction ($F(2, 29)= 4.82$, $p= 0.025$) because ethanol choice was decreased on the first day with the 5 min ITI, while saccharin choice did not significantly change (Figure 4.8.). There was a main effect of Reinforcer because rats chose significantly more saccharin than ethanol across days ($F(1, 30)= 12.907$, $p= 0.001$). In addition, a main effect of Day was revealed ($F(2, 29)= 4.773$, $p= 0.012$) because, compared to choice with the 2 min ITI, ethanol choice was significantly lower with a 5 min ITI. Therefore, increasing the ITI from 2 to 5 min caused an increase in saccharin choice and decrease in ethanol choice. A paired t-test comparing choice averaged across both 5 min ITI testing days revealed significantly more saccharin choice than ethanol choice, $t(30)= 3.906$, $p=0.000$ (Figure 4.8. inset).

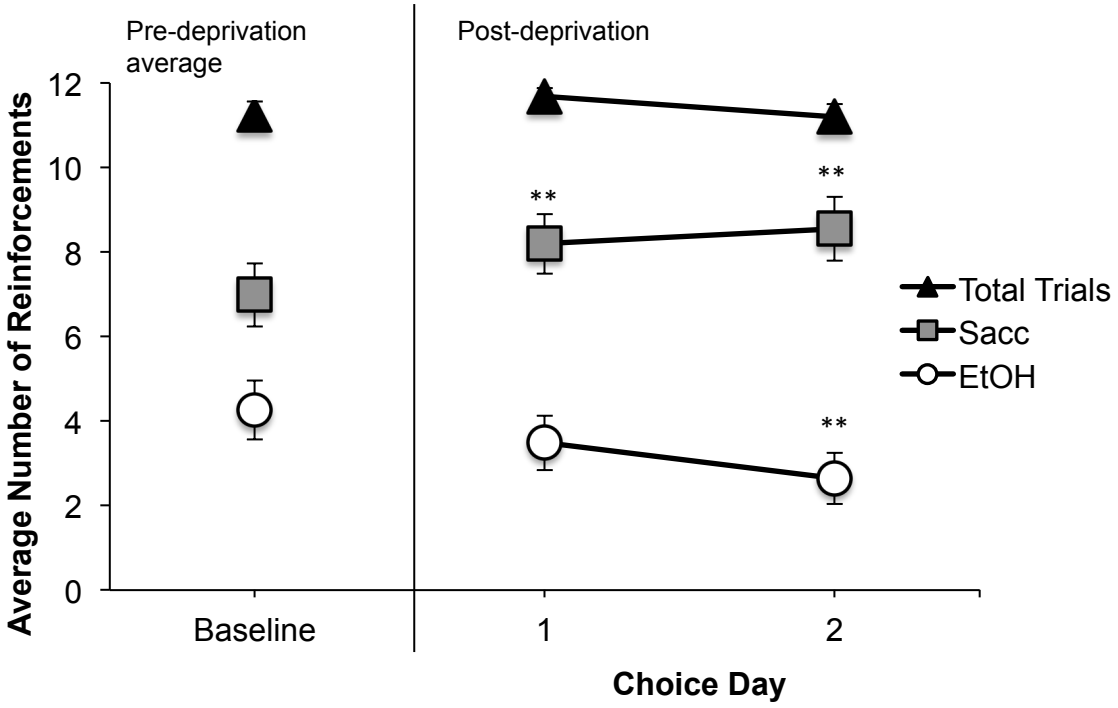

Figure 4.7. *Choice behaviour after deprivation to both rewards*. Data are mean numbers (±SEM) of 12% (w/v) ethanol (EtOH; circles) and 0.1% (w/v) saccharin (Sacc; squares) reinforcements obtained from the last two choice days of pre-deprivation baseline and 2 days of choice following 10 days of deprivation to both rewards (n=31). Triangles represent mean total number of trials complete (i.e. saccharin + ethanol) (±SEM). $^{**}p < 0.01$ compared to pre-deprivation baseline.

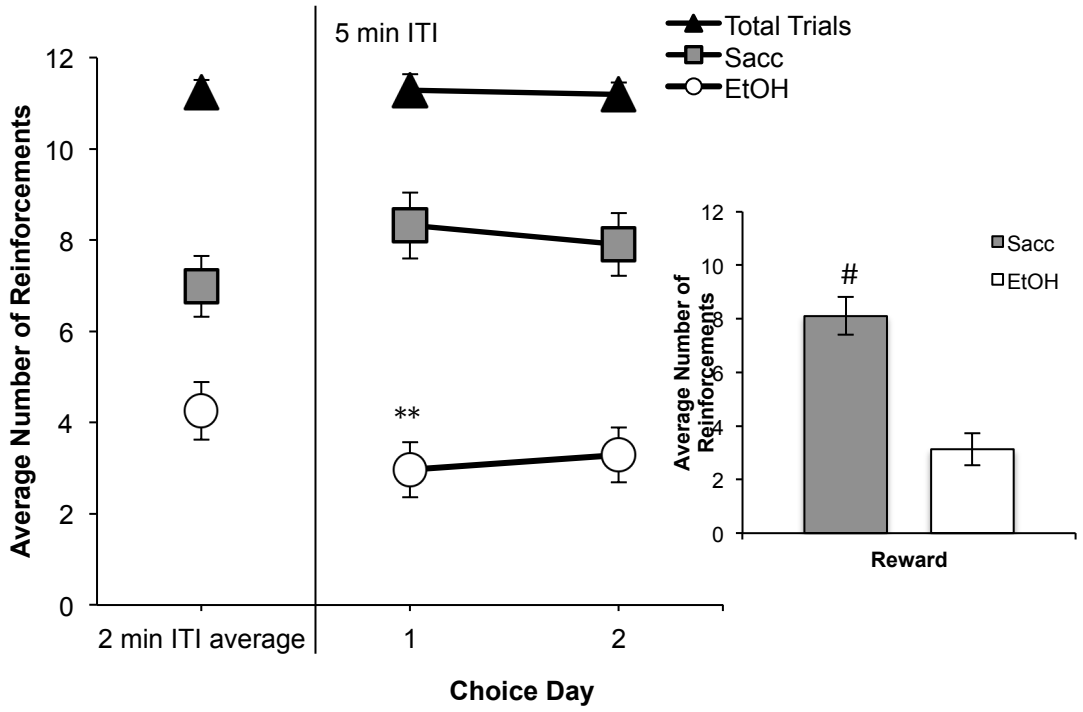

Figure 4.8. *Choice behaviour with the introduction of a 5 min inter-trial interval*. Data are mean numbers (±SEM) of 12% (w/v) ethanol (EtOH; circles) and 0.1% (w/v) saccharin (Sacc; squares) reinforcements obtained from the last 2 days of choice with a 2 min ITI and Day 1 and Day 2 with a 5 min ITI (n= 31). Triangles represent mean total number of trials complete (i.e. saccharin + ethanol) (±SEM). $^{**}p < 0.01$ compared to 2 min ITI average. Inset showing average saccharin and ethanol choice selection (±SEM) across 2 days with 5 min ITI. $\#p < 0.01$ compared to ethanol choice.

4.4. Discussion

Contrary to results from previous research using this paradigm, animals showed close to equivalent preference for saccharin and ethanol. There were no statistically significant differences in choice between 0.05% saccharin and 12% ethanol in the first phase of Experiment 1. Given previous research showing clear preference for the non-drug reward over the drug reward, these results were quite surprising.

When saccharin concentration was increased to 0.1% (w/v), self-administration was shown to be higher than at the 0.05% (w/v) concentration and remained much higher in comparison to levels of ethanol single-reward self-administration. When animals were given the option to choose between 0.1% saccharin and 12% ethanol, there was a slight and significant preference for saccharin over ethanol but rats continued to sample both rewards throughout the entirety of choice sessions. A comparison of choice behaviour in the presence of 0.05% saccharin vs. 0.1% saccharin revealed that ethanol choice behaviour did not change but the number of completed trials increased with the introduction of the higher saccharin concentration. In other words, changing the saccharin concentration increased saccharin choice, consequently, causing an increase in the number of completed trials while not affecting choice behaviour for ethanol. This increased number of completed trials could be due to animals learning the discrete-choice trials procedure after exposure to an increasing number of sessions. An alternate explanation could be that increasing the saccharin concentration increased its relative reinforcing value causing animals to show a slight preference for the sweet-tasting solution. Conversely, ethanol choice was maintained because its relative reinforcing value was not manipulated (i.e. remained at a concentration of 12%) or affected by changes in saccharin concentration.

Only one other study has used the discrete-choice trials procedure for ethanol and saccharin. This study found that animals showed a clear preference for saccharin over ethanol after multiple sessions of the discrete-choice trials procedure (Pelloux & Baunez, 2017). The preference for saccharin was more robust in the Pelloux & Baunez (2017)

study in comparison to the slight saccharin preference found in the present experiment. More specifically, animals in the Pelloux and Baunez (2017) study showed a 75% preference for saccharin where as animals in the present experiment had a 64% preference for saccharin. Notably, differences in experimental methodologies may help explain these discrepancies. To start, animals in the aforementioned study were given the choice between a 0.2% saccharin solution and a 20% (v/v) ethanol solution while the concentrations used in our study were notably lower. It is important to consider that rodents have been shown to prefer lower concentrations of an ethanol solution due to it's increased bitter taste at higher concentrations, such as 20% (v/v) (Spanagel & Hölter, 1999). In addition, Pelloux and Baunez (2017) discerned choice behaviour for a total of 20 sessions and only found a clear preference for saccharin starting on the fifth day of exposure to the discrete-choice trials procedure; a behaviour which stabilized with increasing choice sessions. In contrast, animals in our study showed stable choice behaviour by the fifth and sixth sessions of the discrete-choice trials procedure at the 0.05% and 0.1% saccharin concentration respectively. Therefore, the additional training with the discrete-choice trials procedure, paired with the use of a higher saccharin concentration and more bitter-tasting ethanol solution may help explain the more robust preference for saccharin in the Pelloux and Baunez (2017) study.

Interestingly, reward deprivation to both ethanol and saccharin caused a significant increase in saccharin choice and a significant decrease in ethanol choice. Reward deprivation can be described as a robust but temporary increase in voluntary intake of the rewarding solution being studied (Khisti et al., 2006). This phenomenon has been shown for both ethanol and saccharin (Khisti et al., 2006; Avena et al., 2005; Sinclair & Li, 1989; Neznanova, et al. 2002). Given the results from the present study, it appears as though the effect of saccharin deprivation is being exhibited behaviourally and could be masking any effects of ethanol deprivation. This suggests that the effect of saccharin deprivation is more salient than that of ethanol deprivation, which provides further evidence for saccharin's increased rewarding value relative to ethanol.

Finally, it was shown that increasing the ITI from 2 min to 5 min caused a trend towards increased saccharin choice and a significant decrease in ethanol choice. These results suggest that the time between reward availability is relevant to an animal's decision-making pattern. More specifically, this may be related to the choice setting (i.e. the structure of choice session) and how manipulating this can affect the drug's influence on reward preference (Vandaele, Cantin, Serre, Vouillac-Mendoza & Ahmed, 2016). Previous research using the discrete-choice trials procedure included long inter-trial intervals ranging from 5 to 10 min (Caprioli et al., 2015; Huynh et al., 2015; Lenoir et al., 2013; Lenoir et al., 2007). In these studies, choice was made almost exclusively for the nondrug reward. It is possible that the inclusion of a shorter ITI allows for circulating levels of the drug to be pharmacologically relevant at the time of the next choice trial. In other words, shorter ITIs may cause the drug to influence subsequent choice behaviour whereas longer ITIs eliminate this possibility (Vandaele et al., 2016). Results from our study and those discussed above provide supporting evidence for this interpretation.

A potential limitation of this experiment is the decision to use saccharin, as opposed to sucrose or food, as the nondrug reward. It is important to consider that, in addition to their palatability, sucrose and food contain caloric value and have been shown to be more rewarding than saccharin (Reilly, 1999). Therefore, the nutritional and post-ingestive properties of sucrose and food may favour choice selection for these rewards over the drug in question. In addition, given that the present experiment required animals to orally ingest a liquid solution of ethanol, the nondrug reward selected would need to have a similar mechanism of ingestion. For this reason, the use of food pellets as the nondrug reward was ruled out. Importantly, the administration of sucrose with ethanol has been shown to cause a decrease in blood alcohol levels (Roberts, Heyser & Koob, 1999). Therefore, given that saccharin has no effect on blood alcohol levels and is void of caloric properties, it was selected as the nondrug reward. With that said, it can be argued that the caloric value of ethanol may confound the results by skewing animals' preference towards ethanol intake. However, it could be argued that the volume of ethanol consumed in each single-reward or choice session would not be enough for caloric consumption to play a critical role in choice behaviour, but this is difficult to confirm. In addition, despite

ethanol holding caloric properties and saccharin being calorie-free, animals still chose and consumed more saccharin because of its sweet-taste.

Chapter 5
THE CHOICE BETWEEN INTRAVENOUSLY ADMINISTERED NICOTINE AND ORALLY CONSUMED SACCHARIN

5.1. Introduction

In Experiment 1, animals were shown to have an almost equal preference for saccharin over ethanol. Given previous research using the discrete-choice trials procedure, which showed a clear preference for the nondrug reward, these results are quite surprising. In addition, these results are unexpected when considering that ethanol is quite low in terms of reinforcing efficacy in comparison to intravenously administered drugs as well as orally consumed nondrug rewards. For example, animals trained on a progressive ratio schedule of reinforcement have been found to have a breakpoint of 115 for nicotine, 20 for saccharin, and 5 for ethanol (Shram et al., 2008; Vendruscolo et al., 2012). Taken together, we therefore expected to find a clear and robust preference for saccharin over ethanol. However, it is important to note that the parameters of the discrete-choice trials procedure used in our experiments are slightly different from those used in previous research. In order to ensure that results from Experiment 1 were not caused by these procedural differences, we decided to validate our specific experimental procedure and parameters. Since choice behaviour for oral saccharin is much higher than for intravenously administered drugs we conducted a second choice experiment with intravenous nicotine and saccharin, using the same parameters as in Experiment 1.

Differences between our experimental parameters and those used in previous choice studies include: (1) the concentration of the nondrug reward, (2) the nondrug reward selected, and (3) the length of the inter-trial interval. More specifically, the nondrug reward used in our experiment was a sweet-tasting non-biologically relevant saccharin solution with concentrations ranging from 0.05-0.1% (w/v). Previous research using saccharin as the nondrug reward have used initial saccharin concentrations of 0.2% (w/v) (Pelloux & Baunez, 2017; Lenoir et al., 2007) which is notably higher than the concentration range selected for our first experiment. Therefore, it is possible that the use

of a higher, more palatable saccharin concentration biased animal's preference towards the nondrug reward in these studies. In addition, sucrose and food have been commonly used in the discrete-choice trials procedure. However, in order to provide animals with the choice between two liquid solutions the use of food was ruled out. Given that saccharin, unlike sucrose, has no effect on blood alcohol levels and is void of caloric properties, it was selected as the nondrug reward for Experiment 1 and was maintained as such in the present experiment. Finally, previous research exploring the use of different ITI lengths suggests that choice behaviour may be, in part, determined by this experimental parameter. Notably, previous research determining choice for an intravenously administered drug and a nondrug reward has used longer ITIs ranging from 5 to 10 min (Caprioli et al., 2015; Huynh et al., 2015; Lenoir et al., 2013; Lenoir et al., 2007) while Experiment 1 of the present thesis utilized a shorter, 2 min ITI. Given that previous research has showed differential choice behaviour with the inclusion of a longer vs. shorter ITI, we wanted to determine if the use of varying ITI lengths would elicit different patterns of choice behaviour in animals choosing nicotine or saccharin.

5.2. Experimental Procedures

5.2.1. Animals

Experiment 2 used Sprague-Dawley rats, which are more commonly used in intravenous nicotine self-administration studies, because they are more robust after intravenous catherization.

Sixteen Sprague-Dawley rats weighing 175-200 grams at the beginning of the study were used. As described in Section 3.1., they were housed in pairs in the Animal Care Facility and placed on a mild food restriction (≈23 gram of lab chow/ day) diet for the majority of the experiment. Animals were treated daily with intravenous heparin during recovery from catheter surgery and after the daily behavioural testing throughout the rest of the experiment.

5.2.2. Experimental design

After 7 days of recovery from the intravenous catheterization surgery (Section 3.3), animals were subjected to self-administration training sessions for each reward on alternating days followed by baseline choice sessions. Animals were tested with sessions of increased inter-trial intervals (ITI) as well as sessions of concurrent, unlimited access to both rewards. Figure 5.1. represents a timeline of the experiments described below.

Home-cage drinking

Following recovery from the intravenous catheterization surgery, animals were exposed to a solution of 0.1% (w/v) saccharin in their home cages for 1 hr to allow for them to be accustomed to the taste of the sweet-tasting solution. Bottles were weighed before and after home-cage access in order to determine consumption.

Nicotine and saccharin self-administration

Nicotine (30 μg/kg/infusion) and saccharin (0.1% (w/v)) single-reward self-administration sessions occurred on alternating days for a total of 20 days. Animals had exposure to each reward at FR-1 for 4 days, FR-2 for 2 days, and FR-3 for 4 days. Animals were maintained on the FR-3 schedule of reinforcement for the remainder of the experiment. Operant conditions were as stated in Sections 3.4.2. and 3.4.4. for saccharin and nicotine self-administration respectively.

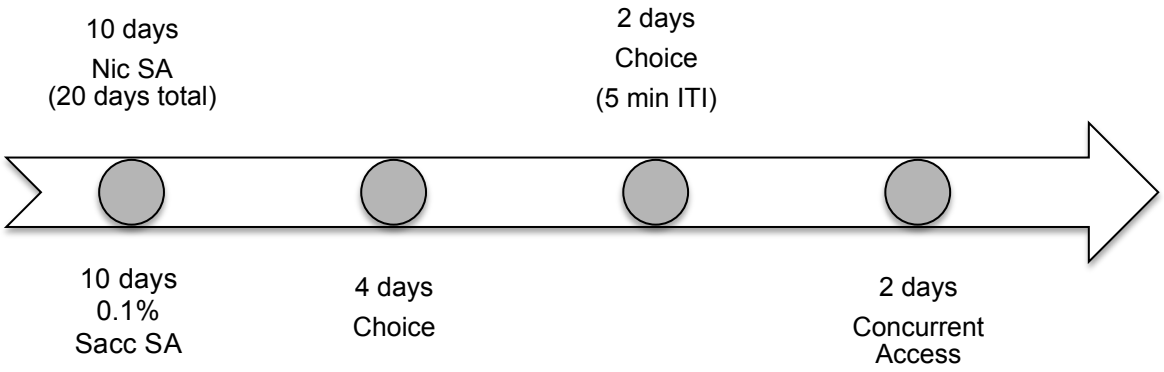

Figure 5.1. *Overview of experimental timeline for Experiment 2*. Animals were given alternating days of self-administration (SA) to nicotine (Nic) and saccharin (Sacc). This was followed by sessions of the discrete-choice trials procedure with varying inter-trial intervals (ITI). Finally, animals were given concurrent access to both rewards (n= 10) (initial n= 16).

Discrete-choice trials procedure

Upon stable self-administration of each reward, animals were subjected to 6 days of the discrete-choice trials procedure whereby they had the choice to administer intravenous infusions of nicotine vs. orally consumed 0.1% saccharin over 12 discrete-choice trials per session. The first 4 days of this phase consisted of choice sessions that included a 2 min ITI. This was followed by 2 days with an increased 5 min ITI.

Concurrent access

Following maintenance of baseline choice behaviour, animals were exposed to 2 days of the concurrent access procedure. As described in Section 3.4.6., animals had unlimited access to both reward levers over a 1 h period.

5.2.3. Statistics

As previously described, repeated-measures ANOVA were used to examine the role of reinforcer on self-administration and on choice during the discrete trials sessions. Paired t-tests were used to determine differences in the number of reinforcements between rewards averaged across days.

5.3. Results

Attrition

A total of 6 animals were excluded in this experiment. These exclusions were determined by: (1) low levels of nicotine and/or saccharin responding during single-reward training and (2) compromised catheter patency (see Section 3.3.6. for more details).

Self-administration with 0.1% saccharin and 30 µg/kg/infusion nicotine

Steady self-administration of both saccharin (mean ± sem)= 80.78, ± 12.07) and nicotine (mean ± SEM= 15.77, ± 3.10) was acquired by the end of single-reward training sessions at an FR-3 schedule of reinforcement (Figure 5.2.). A repeated measures ANOVA with the factors of Reinforcer (saccharin, nicotine) and Training Day (Day 1-10) revealed a main effect of Reinforcer ($F(9, 1)= 6.417$, $p= 0.003$), because there was an increase in saccharin self-administration across days vs. steady nicotine self-administration across days.

Discrete-choice trials procedure

Upon stable levels of self-administration to both saccharin and nicotine, animals were given 4 days of the discrete-choice trials procedure. A repeated measures ANOVA with factors of Reinforcer (saccharin, nicotine) and Choice day (Day 1-4) revealed a significant effect of Reinforcer ($F(1, 9)= 177.745$, $p= 0.000$) because animals robustly preferred saccharin over nicotine across choice testing with a 2 min ITI, (Figure 5.3.). A paired t-test conducted on the average number of reinforcements received confirmed these results, $t(9)= 14.61$, $p= 0.000$ (Figure 5.3. inset).

No statistically significant differences were found between the average of the last 2 days of choice with a 2 min ITI vs. 2 days with 5 min ITI, $F(1, 9)=1.964$, $p= 0.193$ (Figure 5.4.).

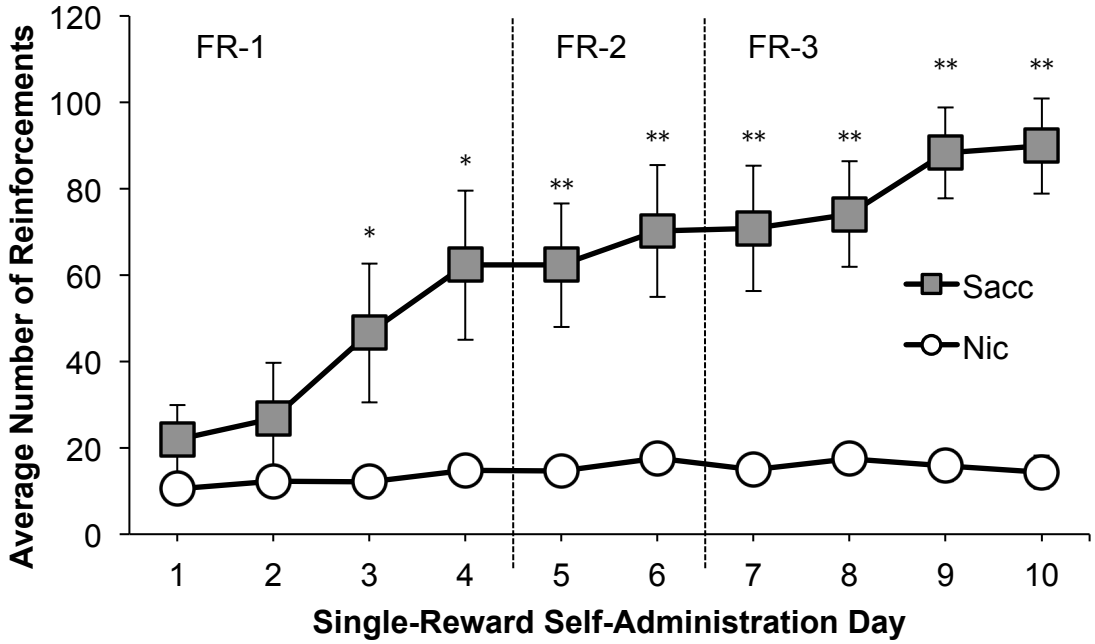

Figure 5.2. *Self-administration of nicotine or 0.1% saccharin*. Data are mean numbers (±SEM) of nicotine infusion 30 μg/kg (Nic; circles) and 0.1% (w/v) saccharin (Sacc; squares) reinforcements obtained during single-reward self-administration sessions (n= 10). *$p < 0.05$ and **$p < 0.01$ compared to nicotine reinforcement.

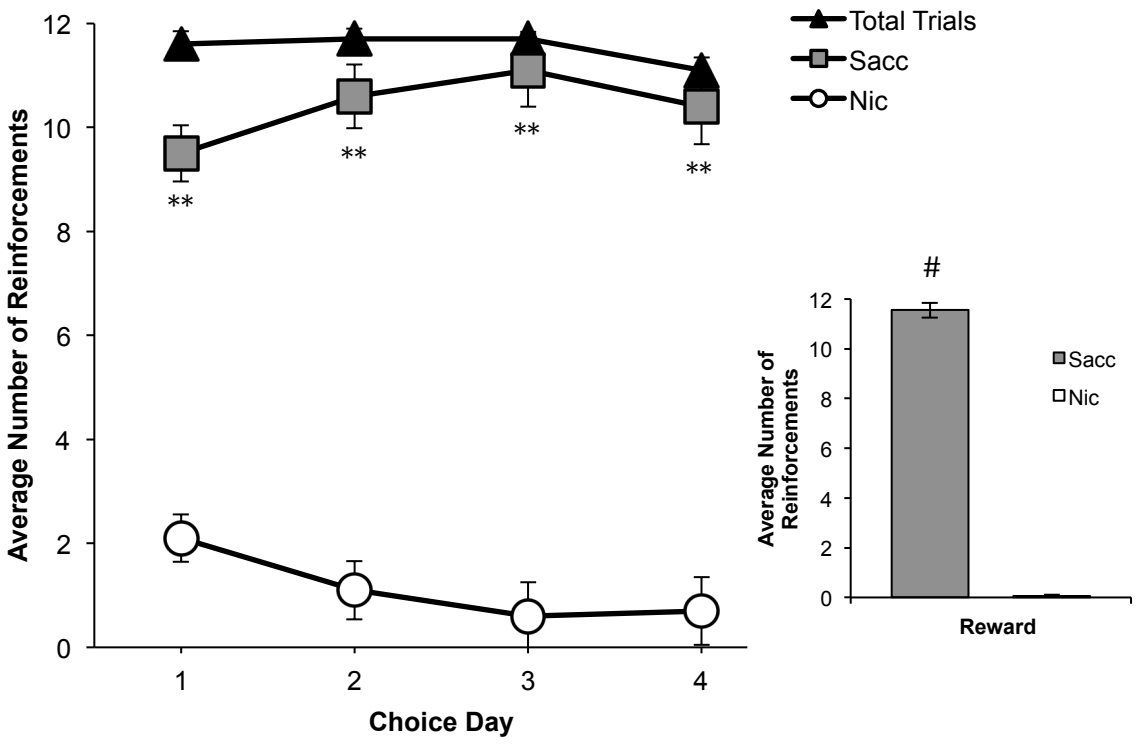

Figure 5.3. *Choice for nicotine and saccharin with the discrete-choice trials procedure (2 min inter-trial interval)*. Data are mean numbers (±SEM) of nicotine infusion 30 μg/kg (Nic; circles) and 0.1% (w/v) saccharin (Sacc; squares) reinforcements obtained during 4 days of choice sessions (n= 10). Triangles represent mean total number of trials complete (i.e. saccharin + nicotine) (±SEM). $^{**}p < 0.01$ compared to nicotine choice. Inset showing average saccharin and nicotine choice selection (±SEM) across 4 days. $\#p < 0.01$ compared to average nicotine choice.

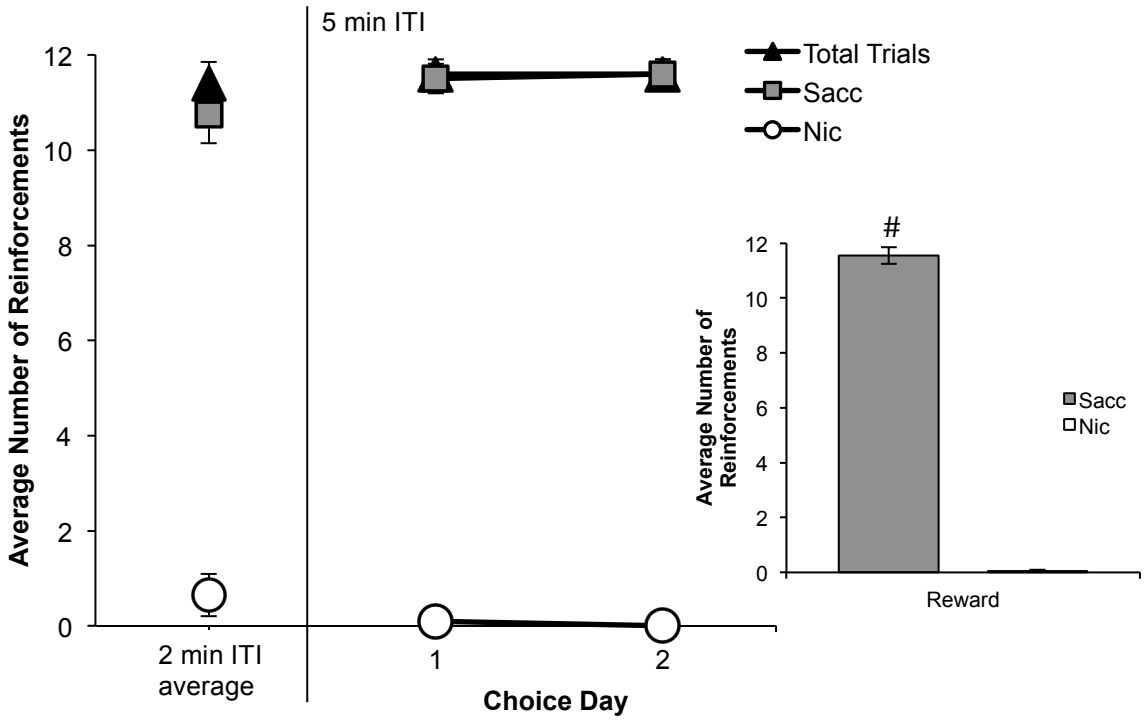

Figure 5.4. *Choice for nicotine and saccharin with the discrete-choice trials procedure (5 min inter-trial interval)*. Data are mean numbers (±SEM) of nicotine infusion 30 μg/kg (Nic; circles) and 0.1% (w/v) saccharin (Sacc; squares) reinforcements obtained from the last 2 days of choice with a 2 min ITI and Day 1 and Day 2 with a 5 min ITI (n= 10). Triangles represent mean total number of trials complete (i.e. saccharin + nicotine) (±SEM). Inset showing average saccharin and nicotine choice selection (±SEM) across 2 days with 5 min ITI. *#p* < 0.01 compared to average nicotine choice.

Concurrent access procedure

Animals were then given 2 days of choice under concurrent access parameters in which they had unlimited access to both rewards. A repeated measures ANOVA revealed a main effect of Reinforcer ($F(1, 9)= 37.821, p= 0.000$) revealing that saccharin was administered more than nicotine during both days of concurrent access. The Reinforcer by Condition interaction was not significant ($F(2, 8)= 3.707. p= 0.055$) indicating that providing concurrent access to both rewards did not change self-administration responding when compared to single-reward sessions (Figure 5.5.).

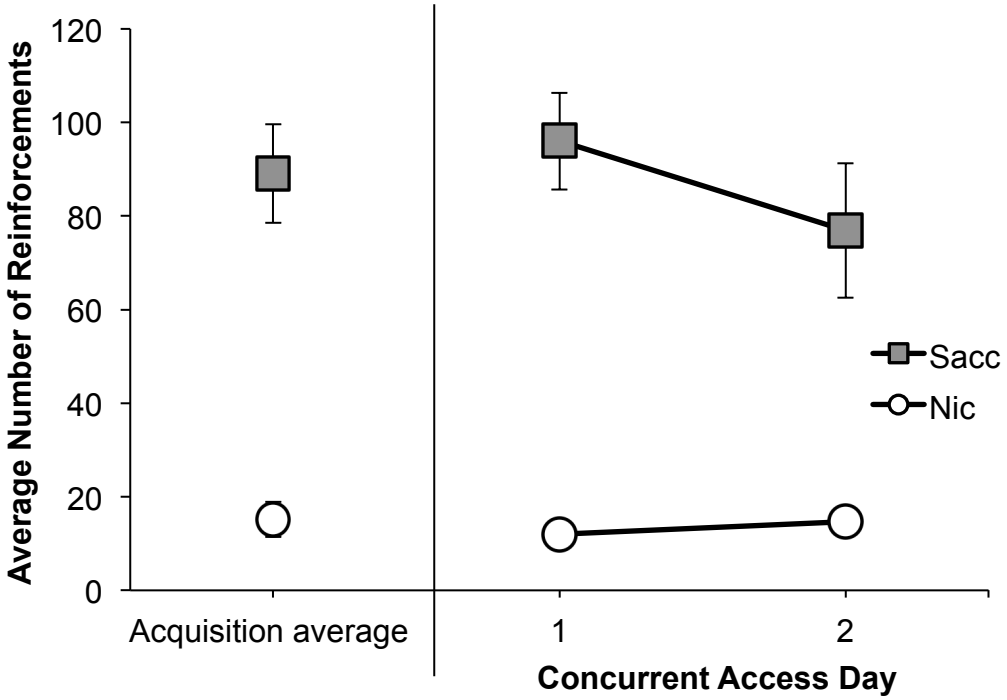

Figure 5.5. *Choice for nicotine and saccharin with the concurrent access procedure*. Data are mean numbers (±SEM) of nicotine infusions 30 μg/kg (Nic; circles) and 0.1% (w/v) saccharin (Sacc; squares) reinforcements obtained from the last 2 days of self-administration acquisition and Day 1 and Day 2 concurrent access sessions (n= 10).

5.4. Discussion

Animals self-administered more saccharin than nicotine during the single-reward self-administration training sessions. The number of nicotine reinforcements received remained stable throughout this experimental phase while saccharin administration increased as the fixed-ratio requirement increased.

A study conducted by Huynh and colleagues (2015) showed that animals prefer a solution of 0.2% (w/v) saccharin over intravenously administered nicotine. Similarly, animals in our study chose more saccharin than nicotine during testing with the discrete-choice trials procedure despite our use of a lower saccharin concentration (0.1% (w/v)). This suggests that even at lower concentrations, saccharin, a non-biologically relevant substance, has a greater reinforcing value relative to that of nicotine. While changing the ITI from 2 min to 5 min did not cause any statistically significant differences in saccharin choice, increasing the ITI to 5 min did result in an exclusive preference for saccharin. More specifically, under a 2 min ITI animals still chose nicotine 1-2 times throughout each choice session but the introduction of a 5 min ITI resulted in only saccharin choices and 0 nicotine choices. Similarly to Experiment 1, this suggests that the choice setting is of critical importance in an animal's decision-making behaviour. In addition, it appears that circulating levels of drug may modulate drug choice. That is, with no ITI (or a shorter ITI), which would likely result in significant levels of nicotine remaining upon initiation of the succeeding choice trial, animals continue to lever press for nicotine despite the presence of saccharin. However, if selection for the drug becomes costly (i.e. precludes saccharin selection) animals will omit drug intake in order to consume the sweet-tasting solution. Given the results of the present study, in conjunction with results from previous research, it appears that choice setting and whether or not this allows for the drug to influence subsequent choice behaviour are determining factors in an animal's overall reinforcer choice (Vandaele et al., 2016; Thomsen et al., 2013; Kerstetter et al., 2012).

Finally, animals self-administered nicotine and saccharin at equivalent levels to their acquisition training when given concurrent access to both reward levers. These results closely resemble those found by Panlilio and colleagues (2015). This study also showed that responding for sucrose remained higher relative to nicotine during sessions of concurrent access. These concurrent access results provide an important point of comparison to the choice behaviour determined during sessions of the discrete-choice trials procedure. More specifically, comparing choice elicited by both of these procedures provides information about what animals prefer when there is a significant choice cost vs. how animals behave when they have unrestricted access to both rewards. It is true that unlimited access to both rewards more closely mimics the human condition because humans are seldom ever forced to choose one reward over another. However, choosing one reward at the cost of access to the other reward elicits behaviour suggestive of a specific preference for the selected reward. With that said, the use of both procedures provides a more comprehensive understanding about animal's decision-making in different choice settings.

A potential limitation of the present study is that animals self-administered much more saccharin than nicotine during acquisition of single-reward responding. In addition, saccharin responding continued to increase while nicotine responding remained stable throughout. While saccharin single-reward self-administration was higher in comparison to ethanol in Experiment 1, the difference in single-reward responding is much greater in the present experiment. This may be explained by the differences in self-administration session length between experiments. Animals were given 30 min ethanol and saccharin self-administration sessions in Experiment 1 where as animals in Experiment 2 were given 60 min nicotine and saccharin single-reward sessions. In order to account for catheter patency in the present experiment, a shorter self-administration training phase was needed therefore longer daily self-administration sessions were provided. Given this, it is possible that longer daily access to saccharin contributed to the higher magnitude of saccharin self-administration in Experiment 2. However, the continued increase in responding for saccharin across self-administration days may act as a confound by introducing a bias to choose saccharin during the discrete-choice trials procedure. In

relation to this, it should be noted that the majority of studies introduced a cap (i.e. limit) to the number of reinforcements available in single-reward operant sessions (e.g. session ended after 15 infusions of cocaine were received) (Lenoir et al., 2007; Lenoir et al., 2013; Cantin et al, 2010; Cprioli et al., 2015; Huynh et al., 2015). Limiting the amount of drug received in these sessions was done "to prevent overdose" (Caprioli et al., 2015). Given that overdose to ethanol was not a factor to consider in Experiment 1, we decided to avoid using a cap on the number of reinforcements received during single-reward self-administration sessions. In order to remain consistent with the parameters used in Experiment 1, the use of a cap during self-administration sessions in Experiment 2 was ruled out. In addition, our results are comparable to those reported in another choice study that did not use a cap during single-reward sessions. In the study by Panlilio and colleagues (2015) animals self-administered significantly more sucrose than nicotine throughout self-administration training and choice behaviour during concurrent access was found to be comparable to responding during sessions of single-reward access. Our results replicate the self-administration behaviour as well as the concurrent access choice behaviour reported in this study. In addition, it appears as though the use of a cap during self-administration training does not affect animals' choice behaviour during the discrete-choice trails procedures because these studies consistently found nondrug reward preference, which we also replicated in the present experiment. Finally, it should be noted that, due to our awareness of this potential limitation, we tested animals on the discrete-choice trials procedure with the introduction of a lower, 0.05% (w/v) saccharin solution and found no differences in choice behaviour (data not presented). This finding suggests that the inclusion of a less rewarding saccharin solution would have yielded similar choice behaviour in our animals.

Chapter 6

THE EFFECT OF DEPENDENCE ON ETHANOL ON THE CHOICE BETWEEN ETHANOL AND SACCHARIN

6.1. Introduction

In the first experiment it was shown that animals choose roughly equivalent levels of saccharin and ethanol. These results were quite surprising when considering the findings from previous studies using the discrete-choice trials procedure showing a high level of choice of nondrug over drug rewards.

Given the differences in the experimental methodologies used in our laboratory compared to these earlier studies we conducted Experiment 2 to validate our changes to the discrete-choice trials procedure. The results from Experiment 2, showing a clear preference for saccharin over nicotine, replicate findings from previous studies using this procedure with intravenously administered drugs and orally consumed nondrug reward. These results suggest that an equal preference to ethanol vs. saccharin may be unique to this specific reward pairing.

Previous research utilizing the discrete-choice trials procedure was conducted in non-dependent animals. While this work in non-dependent animals is helpful in elucidating the relative reinforcing value of competing rewards, the extension of this procedure to dependent animals is vital to our understanding of the decision-making associated with the transition from recreational drug use to compulsive drug use. To this end, in Experiment 3, I examined choice behaviour in ethanol dependent and non-dependent rats.

Dependence on ethanol in humans is characterized by an escalation of ethanol consumption and the transition from recreational drinking to compulsive drinking (i.e. ethanol drinking despite negative consequences) (American Psychiatric Association, 2013). These characteristics defining ethanol dependence in humans are the same factors used to define drug dependence in animals (Vandershuren & Ahmed, 2013). While there are a number of ways to induce ethanol dependence in rodents, a commonly used method

is that of intermittent ethanol vapour exposure (Goldstein & Pal, 1971; Rogers et al., 1979). Over the last 3 decades, ethanol vapour exposure in conjunction with operant self-administration has demonstrated a number of behavioural features indicative of ethanol dependence. More specifically, animals exposed to ethanol vapour demonstrate an escalation in ethanol drinking as well as compulsive ethanol intake through the continued drinking of a quinine-adulterated ethanol solution. In addition, non-dependent vs. dependent animals respond differentially to a variety of existing pharmacological agents, including acamprosate, nalmefene, and naltrexone. More specifically, intermittent exposure to ethanol vapour has been shown to facilitate the onset of escalated ethanol drinking; a behaviour that remains even after a period of abstinence (Roberts et al., 2000). Inducing ethanol dependence also increases the motivational properties of ethanol increasing breakpoints under a progressive ratio schedule of reinforcement (Vendruscolo et al., 2012). Adulteration of ethanol with bitter-tasting quinine has been shown to cause significant decreases in ethanol self-administration in nondependent animals but affects self-administration less in dependent animals (Vendruscolo et al., 2012). This is a key effect of ethanol vapour exposure because this behaviour is analogous to the compulsive ethanol intake noted in humans (i.e. continued drug consumption despite negative consequences) (Vendruscolo et al., 2012; Vendruscolo & Roberts, 2014). In addition, studies determining the effects of pharmacotherapies on operant ethanol responding provide further evidence for differing behavioural and neurophysiological profiles in dependent vs. nondependent animals. For example, it was shown that, chronic treatment with mifepristone decreased escalation of alcohol self-administration in vapour-exposed animals but had no effect on responding for ethanol in non-dependent animals (Vendruscolo et al., 2012). Another study showed that nalmefene was more effective than naltrexone in decreasing ethanol self-administration in vapour-exposed animals compared to ethanol self-administration in air-exposed animals (Walker & Koob, 2008). In line with this, a separate study reported that naltrexone blocked escalated ethanol drinking after ethanol vapour exposure but not in controls (O'Dell et al., 2004; Morse & Koob, 2002). The effect of dependence induction on ethanol intake, dopamine release, and the efficacy of pharmacotherapies as explained here and in the introduction provide

substantial evidence for the need to conduct preclinical research in ethanol dependent animals.

It is important to note, however, that effects of dependence have only been examined with animal models in which a single reward is made available and, moreover, there has been limited attention paid to examining the effects of dependence on choice behaviour. Experiment 3 was designed to address this gap in the literature by inducing dependence in a group of animals and determining choice behaviour under discrete-choice and concurrent access conditions. In addition, we examined compulsive ethanol administration induced by dependence on choice behaviour by manipulating the taste of the ethanol solution through the addition of varying concentrations of the bitter tastant, quinine.

6.2. Experimental Procedures

6.2.1. Animals

Forty Long-Evans rats weighing 175-200 grams at the beginning of the study were used for this experiment. As described in Section 3.1., they were housed in pairs in the animal care facility and placed on a mild food restriction (≈23 grams of lab chow/ day) diet for the majority of the experiment.

6.2.2. Experimental design

The experimental timeline discussed in detail below is visually represented in Figure 6.1. Briefly, after acquiring stable drinking with the home cage intermittent access procedure and the limited access paradigm (LAP), animals were trained to self-administer ethanol and saccharin in separate sessions. This was followed by choice sessions, in which animals were forced to choose one reward over the other. Animals were then split into the control and dependent group, the latter of which was exposed to 5 cycles of ethanol vapour. Each cycle was followed by a series of behavioural tests in the form of ethanol self-administration, saccharin self-administration, discrete-choice trials procedure, concurrent access procedure, and/ or testing with quinine-adulterated ethanol.

Home-cage drinking

In order to allow animals in Experiment 3 to receive exposure to ethanol before both LAP and operant conditioning, bottles of 12% (w/v) ethanol were placed in each animal's home cage for 24 hrs every other day. This occurred for a total of 10 days, with animals having access to ethanol for 5 days. The weight of the bottles was measured before and after each 24 hrs access period in order to determine the total volume of ethanol consumed by each animal.

Limited access paradigm

After acquiring stable levels of ethanol drinking in the home cage, animals were exposed to 5 days of 30 min LAP access to 12% (w/v) ethanol followed by 2 days of 30 min access to 0.1% (w/v) saccharin. The lights in the LAP drinking room were turned off and a white noise generator was used to reduce environmental effects on drinking behaviour. In order to determine the total volume consumed by each animal, the solution volume was recorded before and after each LAP session.

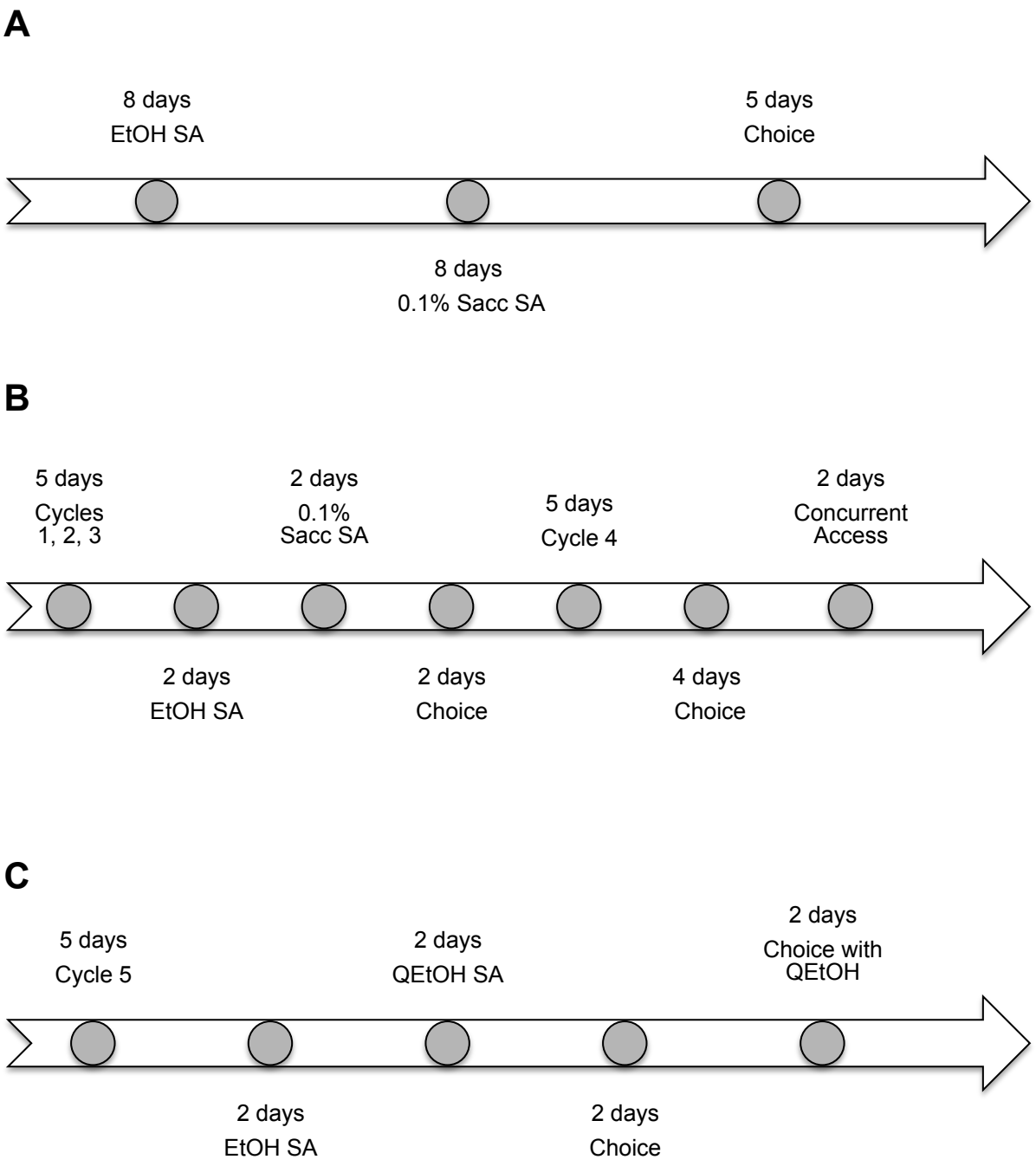

Figure 6.1. *Overview of experimental timeline for Experiment 3*. (A) Self-administration (SA) training for ethanol (EtOH) and saccharin (Sacc) on alternating days followed by choice sessions (n= 31) (initial n= 40). (B) Behavioural testing after cycles 1, 2 and 3 of ethanol vapour exposure and after cycle 4 (control group, n= 16; dependent group, n= 15). (C) Behavioural testing with quinine-adulterated ethanol (QEtOH) after cycle 5 (control group, n= 16; dependent group, n= 15).

Ethanol self-administration

Upon stable levels of 12% (w/v) ethanol drinking during the LAP, animals began training to self-administer 12% ethanol in operant conditioning boxes. The schedule of reinforcement was increased from FR-1 (7 days), to FR-2 (5 days), and was maintained at FR-3 (9 days) for the remainder of the experiment.

Saccharin self-administration

Following ethanol administration training, animals learned how to self-administer a 0.1% (w/v) solution of saccharin. The schedule of reinforcement began at FR-1 (2 days), and was increased and maintained at FR-3 (8 days).

Discrete-choice trials procedure

After acquiring stable levels of ethanol and saccharin self-administration at a FR-3 schedule of reinforcement, animals were subjected to 5 days of discrete trials choice sessions. During each session they had 12 opportunities to choose between both rewards. The parameters for the discrete-choice trials procedure are the same as described in Section 3.4.5.

Ethanol dependence induction

Upon exhibiting stable choice behaviour, animals were split into matched groups (control and dependent) on the basis of ethanol and saccharin self-administration and choice behaviour. As previously described (Section 3.2.3.), animals in the dependent group were placed into chambers and were exposed to ethanol vapour for 5 days (14 hrs ethanol vapour, 10 hrs clean air/day) while control animals remained in their home cages. Animals were exposed to a total of 6 cycles, however, due to experimental error (see below), only 5 cycles are considered in this thesis.

Behavioural testing during ethanol dependence induction

After 5 days of ethanol vapour exposure during Cycle 1, animals in both groups were subjected to 2 days of ethanol self-administration, 2 days of saccharin self-administration, and 2 days of choice sessions. Animals were tested in the same manner after Cycles 2 and 3. After the 5 days of ethanol vapour exposure during Cycle 4, animals in both groups were given 4 days of choice sessions followed by 2 days of concurrent access. In between Cycle 4 and Cycle 5, animals were exposed to an additional cycle of ethanol vapour and subsequently tested on ethanol self-administration and choice behaviour with quinine adulterated ethanol (QEtOH) but the quinine used during testing was found to have degraded and to have reduced potency. Given this, all data and analyses from this cycle are omitted. Upon receiving a new stock of quinine, animals were subjected to a final EtOH vapour exposure cycle (Cycle 5) and tested with two different QEtOH concentrations, 0.05 g/L and 0.1 g/L. Testing at each concentration occurred during sessions of QEtOH self-administration (2 days) and choice (2 days).

6.2.3. Statistics

A repeated measures ANOVA, with factors of Reinforcer and Day, was used to compare average number of reinforcements received per day of self-administration and choice sessions respectively prior to vapour exposure. Paired t-tests were used to determine differences in the number of reinforcements received between rewards averaged across days. Once alcohol vapour exposure was initiated, separate mixed ANOVAs on saccharin and ethanol deliveries were used with Cycle as the within-group factor, and Group as the between-subject factor. A mixed ANOVA was used on ethanol single-reward self-administration with Quinine concentration as the within-subjects factor and Group as the between-subjects factor. Quinine adulteration on choice behaviour was analysed by separate mixed ANOVAs on saccharin and ethanol choice with Quinine concentration as the within-subject factors and Group as the between-subjects factor.

6.3. Results

Attrition

Due to the fact that only 16 animals could be accommodated by the ethanol vapour chambers, 32 animals (16 ethanol dependent and 16 controls) were selected to continue in Experiment 3 upon completion of baseline choice sessions conducted prior to the initiation of ethanol vapour exposure. The criteria for exclusion were: (1) low levels of ethanol and saccharin responding during single-reward training and (2) low numbers of total completed trials during the discrete-choice trials sessions. Finally, one animal died during exposure to ethanol vapour, therefore a total of 31 animals were included in the analyses presented below.

6.3.2. Self-administration with 0.1% saccharin and 12% ethanol

Rats acquired stable self-administration of both saccharin and ethanol over the 8 days of training when rats received saccharin (mean ± sem= 28.99 ± 2.59) and ethanol (mean ± sem= 17.80 ± 0.93) self-administration sessions on alternate days at FR-3 (Figure 6.2.). A repeated measures ANOVA revealed a significant Reinforcer by Training day interaction ($F(7, 23)= 11.851$, $p= 0.000$) indicating that the pattern of saccharin self-administration differed across days in comparison to ethanol self-administration. More specifically, a main effect of Training day was found ($F(7, 23)= 9.062$, $p= 0.000$) because saccharin self-administration increased across days while ethanol self-administration remained stable. In addition, a main of effect of Reinforcer revealed that more saccharin was self-administered than ethanol during single-reward operant sessions, $F(1, 29)= 25.934$, $p= 0.000$.

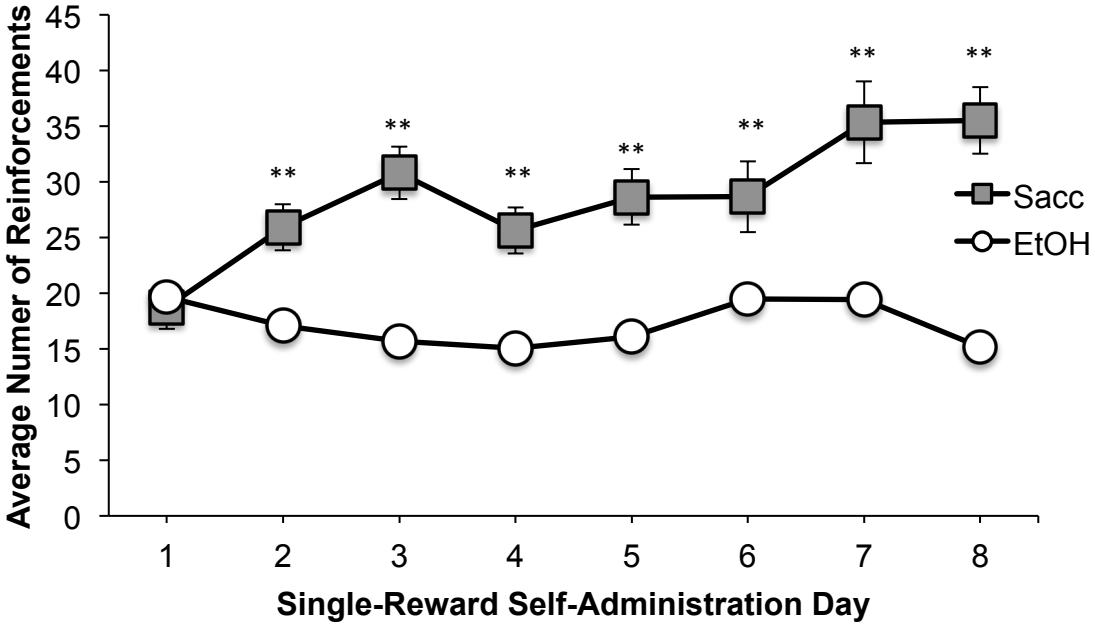

Figure 6.2. *Self-administration of ethanol or 0.1% saccharin*. Prior to dependence induction, data are mean numbers (±SEM) of 12% (w/v) ethanol (EtOH; circles) and 0.1% (w/v) saccharin (Sacc; squares) reinforcements obtained during 8 days of single-reward self-administration sessions (FR-3) (n= 31). $^{**}p < 0.01$ compared to ethanol reinforcement.

6.3.3. Discrete-choice trials procedure

Following acquisition of self-administration to both rewards, animals were subjected to 5 days of the discrete-choice trials procedure. A two-way repeated measures ANOVA with Reinforcer (saccharin, ethanol) and Choice days (Day 1-Day 5) revealed no significant differences between reward choice, $F(4, 27)= 0.894$, $p= 0.433$ (Figure 6.3.). In addition, a paired t-test conducted on the average number of reinforcements received across all 5 days of testing revealed no significant differences between reward administration, $t(30)= 0.186$, $p= 0.819$ (Figure 6.3. inset).

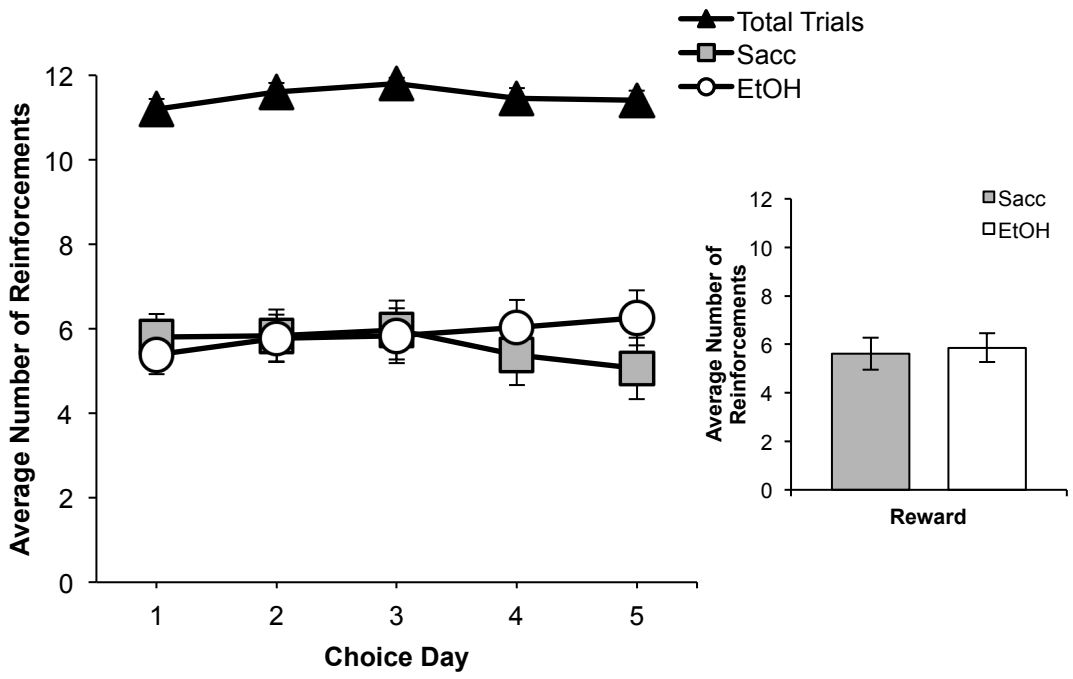

Figure 6.3. *Choice for ethanol and saccharin with the discrete-choice trials procedure*. Data are mean numbers (±SEM) of 12% (w/v) ethanol (EtOH; circles) and 0.1% (w/v) saccharin (Sacc; squares) reinforcements obtained during 5 days of choice sessions (n= 31). Triangles represent mean total number of trials complete (i.e. saccharin + ethanol) (±SEM). Inset showing average saccharin and ethanol choice selection (±SEM) across 5 choice days.

Behavioural testing during ethanol dependence induction

Single-reward self-administration after Cycle 1, 2, and 3

It is important to note that only the last day of testing at each cycle was used in the analyses of ethanol administration, saccharin administration, and choice. This was done because we noted that animals exposed to ethanol vapour were still showing overt signs of withdrawal and exhibited low levels of operant responding 24 hrs after removal from the vapour chambers.

Saccharin self-administration was tested on two days after Cycles 1, 2, and 3 respectively. A repeated measures ANOVA with Cycle (Cycle 1-Cycle 3) and Group (control, dependent) factors revealed no significant differences between the control and dependent group ($F(2, 28)= 1.236$, $p= 0.292$). In addition, within-group comparisons revealed no statistically significant differences in saccharin self-administration across cycles, $F(2, 28)= 2.177$, $p= 0.136$ (Figure 6.4a).

A repeated measures ANOVA on ethanol self-administration revealed a significant Cycle by Group interaction ($F(2, 28)= 8.022$, $p= 0.001$) indicating that ethanol self-administration was different between groups across each cycle (Figure 6.4b). More specifically, a main effect of Cycle was found ($F(2,28)= 5.763$, $p= 0.006$) because ethanol self-administration was higher in the dependent group, which showed significantly more ethanol consumption after Cycle 3.

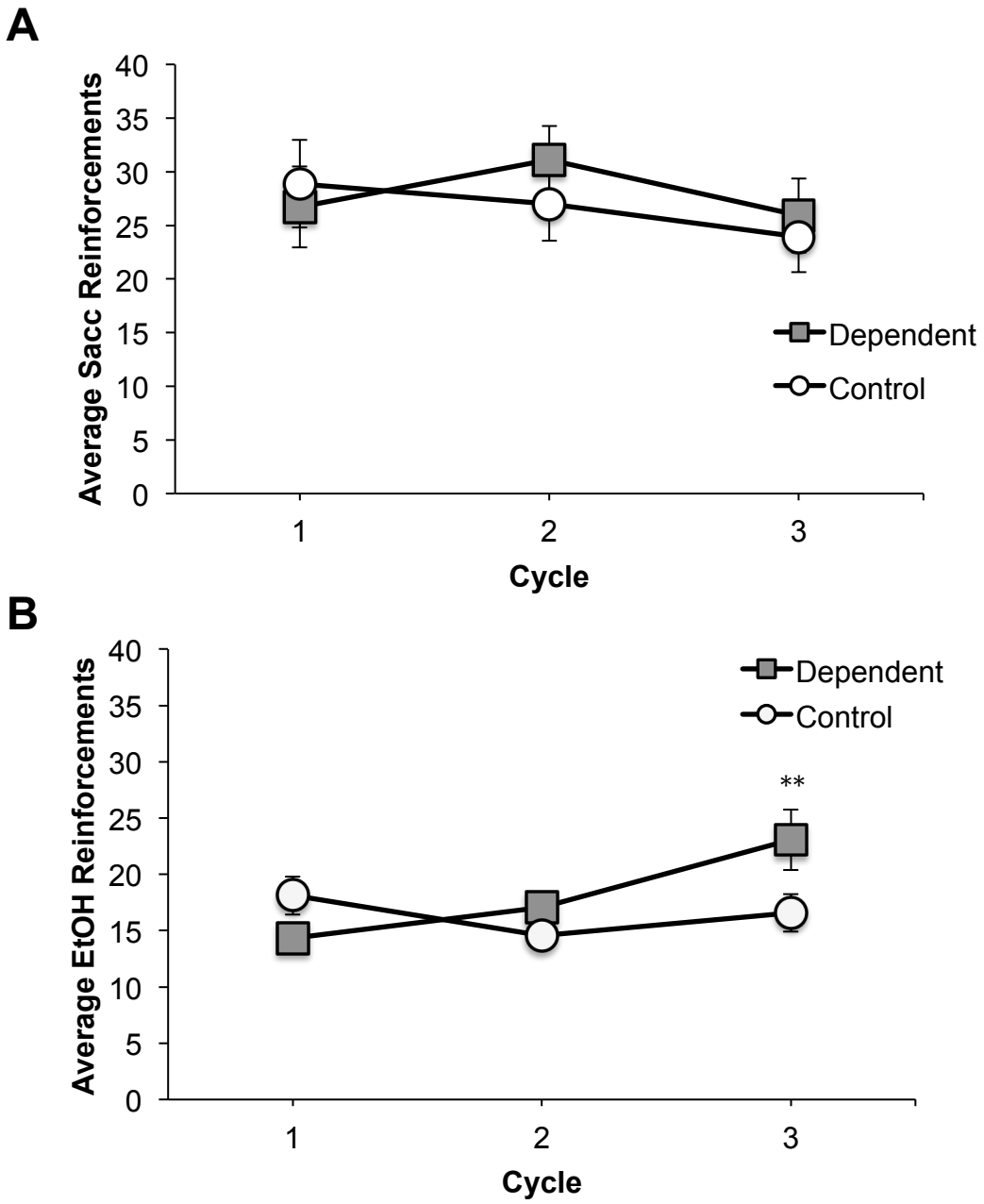

Figure 6.4. *Self-administration of saccharin or ethanol after Cycles 1-3 of ethanol vapour exposure*. Data are mean numbers (±SEM) of (A) 0.1% (w/v) saccharin (Sacc) and (B) 12% (w/v) ethanol (EtOH) reinforcements obtained after 3 cycles of ethanol vapour exposure for the dependent (grey; n= 15) and control (white; n= 16) group. $^{**}p < 0.01$ compared to control.

Discrete-choice trials procedure after Cycles 1, 2, 3, and 4

Following single-reward self-administration testing, animals were given 2 sessions of the discrete-choice trials procedure after Cycles 1, 2, and 3. Animals were given 4 sessions of the discrete-choice trials procedure after Cycle 4. A repeated measures ANOVA, with Group (dependent, control) and Cycle (Cycle 1-Cycle 4) as factors, was conducted for each reward separately.

A significant Group by Cycle interaction was found for saccharin choice ($F(3, 25)= 4.240$, $p= 0.019$), because animals in the dependent group decreased saccharin responding across cycles while the control group remained stable (Figure 6.5a). In addition, a main effect of Cycle ($F(3, 25)= 8.633$, $p= 0.001$) indicated that the dependent group selected saccharin significantly less than the control group during Cycles 3 and 4.

A significant Group by Cycle interaction was found for ethanol choice ($F(3, 27)= 5.841$, $p= 0.004$), because animals in the dependent group increased ethanol choice across cycles but the control group did not (Figure 6.5b). A main effect of Cycle was revealed ($F(3, 27)= 10.914$, $p= 0.000$), because animals in the dependent group chose more ethanol during Cycles 3 and 4 when compared to ethanol choice in the control group.

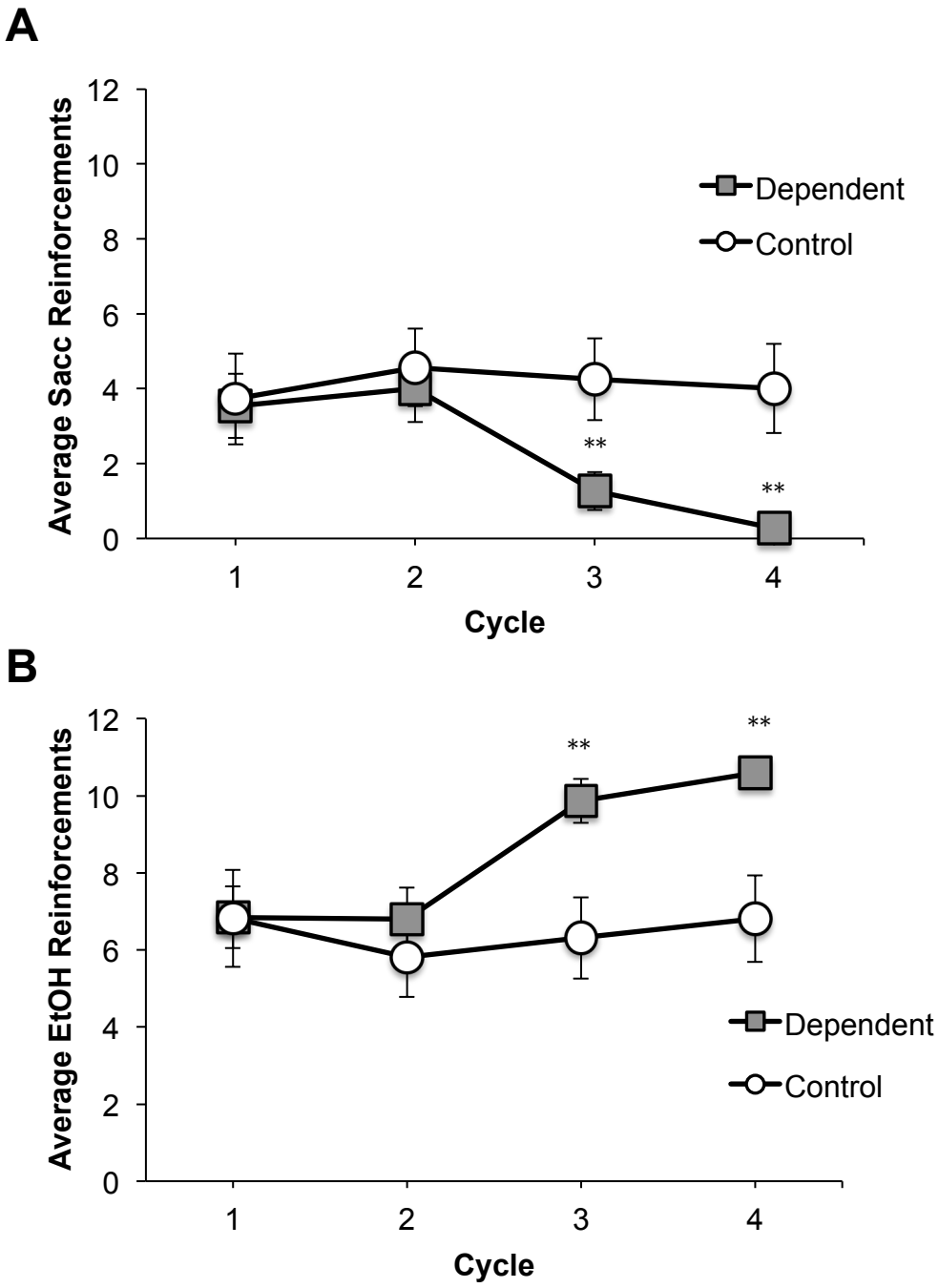

Figure 6.5. *Choice for ethanol or saccharin with the discrete-choice trials procedure in the control and dependent group*. Data are mean numbers (±SEM) of (A) 0.1% (w/v) saccharin and (B) 12% (w/v) ethanol reinforcement obtained during choice sessions after 4 cycles of ethanol vapour exposure for the dependent group (squares; n= 15) and the control group (circles; n= 16). $^{**}p < 0.01$ compared to control group.

Concurrent access after Cycle 4

Animals received 2 days of concurrent access after Cycle 4. A repeated measures ANOVA, with Group (dependent, control) and Condition (acquisition average, concurrent access) as factors, was used for the saccharin and ethanol responding respectively.

Analyses on saccharin responding revealed a main effect of Day ($F(2, 28)= 10.541$, $p= 0.000$), because the dependent group showed a significant decrease in saccharin self-administration on Day 2 of concurrent access (Figure 6.6a). The control group showed no statistically significant differences in saccharin administration.

Ethanol responding during the 2 days of concurrent access was not statistically different from ethanol self-administration acquisition training ($F(2, 28)= 0.321$, $p= 0.727$) or between the control and dependent groups, $F(2, 28)= 1.056$, $p= 0.353$ (Figure 6.6b).

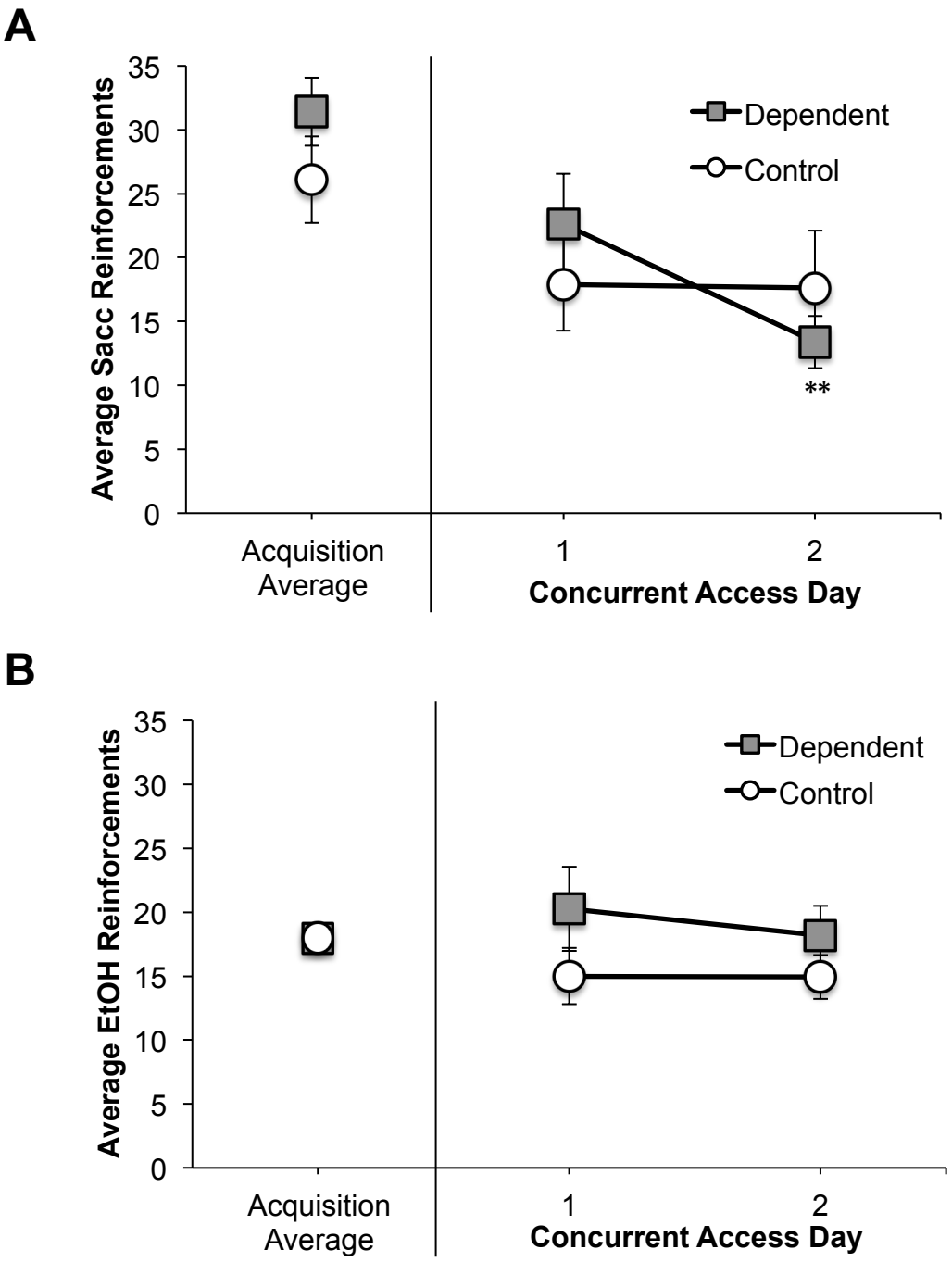

Figure 6.6. *Choice for ethanol or saccharin with the concurrent access procedure in the control and dependent group*. Data are mean numbers (±SEM) of (A) 0.1% (w/v) saccharin and (B) 12% (w/v) ethanol reinforcements obtained during two days of concurrent access after Cycle 4 of ethanol vapour exposure for the dependent group (squares; n= 15) and the control group (circles; n= 16). $^{**}p < 0.01$ compared to acquisition average.

Quinine adulteration after Cycle 5

After Cycle 5, animals were given 2 sessions of single-reward self-administration with quinine-adulterated ethanol at two concentrations, 0.05 and 0.1 g/L. A mixed ANOVA with the between factor of Group (control, dependent) and within factor of Quinine concentration (0.00, 0.05, 0.1 g/L) indicated a significant effect of Quinine concentration ($F(2, 28)= 24.76$, $p= 0.000$) because the addition of quinine to the ethanol solution caused a decrease in ethanol self-administration in both dependent and non-dependent groups (Figure 6.7.). A main effect of Group ($F(1, 29)= 469.05$, $p= 0.006$) indicated that dependent animals self-administered more quinine-adulterated ethanol than the control group at the 0.05 g/L concentration than the control group.

Animals were given 2 days of discrete-choice trials before the introduction of quinine-adulterated ethanol, which was tested at the 0.05 and 0.1 g/L quinine concentrations. Separate mixed ANOVAs, with the between factor of Group and within factor of Quinine concentration were conducted on saccharin choice and ethanol choice. Analysis of saccharin choice revealed a main effect of Group such that the control group made more saccharin choices than the dependent group ($F(2, 25)= 5.049$, $p= 0.033$) (Figure 6.8a). Post hoc tests showed that the control group's choice of saccharin was higher than the dependent group at the 0.05 g/L concentration. Analysis of ethanol choice revealed a main effect of Group ($F(2, 25)= 8.798$, $p= 0.006$) because the dependent group received more ethanol deliveries than the control group (Figure 6.8b). There was also a main effect of Quinine concentration ($F(2, 25)= 5.134$, $p= 0.009$) however post hoc analyses revealed no significant effect of quinine on ethanol choice for the control and dependent group.

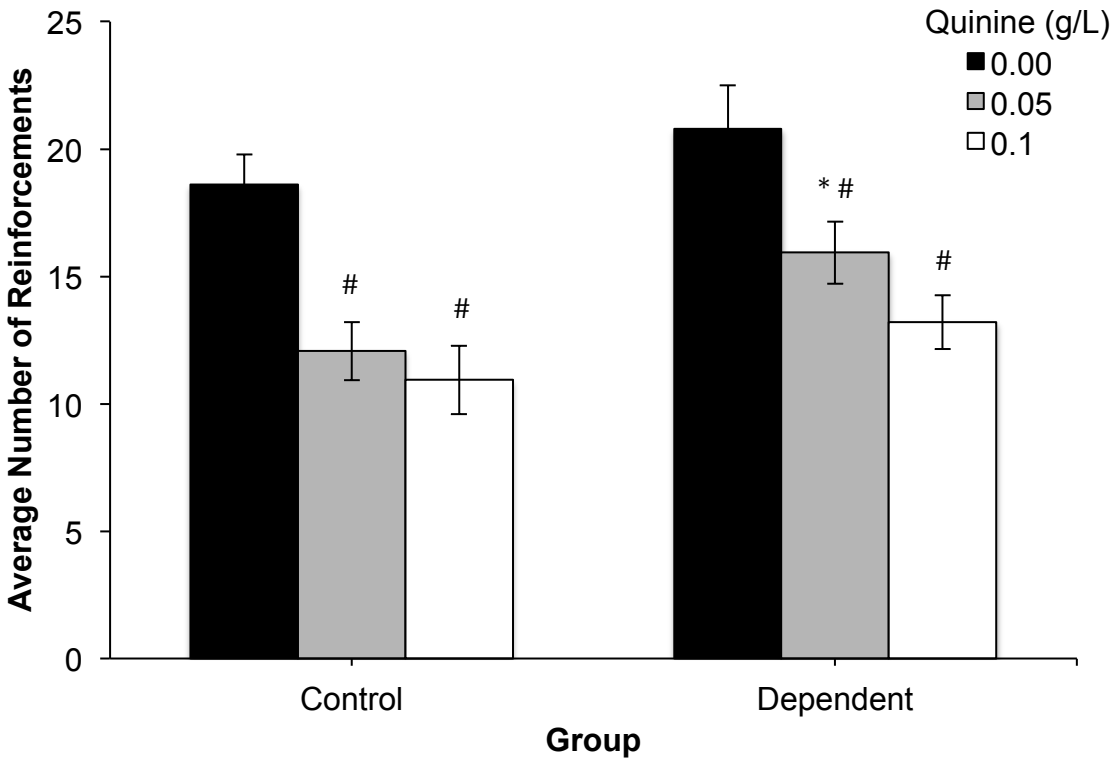

Figure 6.7. *Self-administration for quinine-adulterated ethanol for the control and dependent group*. Data are mean numbers (±SEM) of 12% (w/v) ethanol reinforcement obtained during single-reward sessions with quinine-adulterated ethanol at concentrations of 0.00 (black), 0.05 (grey), and 0.1 (white) g/L for the control group (n= 16) and dependent group (n= 15). #$p < 0.001$ compared to 0.00 g/L quinine concentration. *$p < 0.05$ compared to control group.

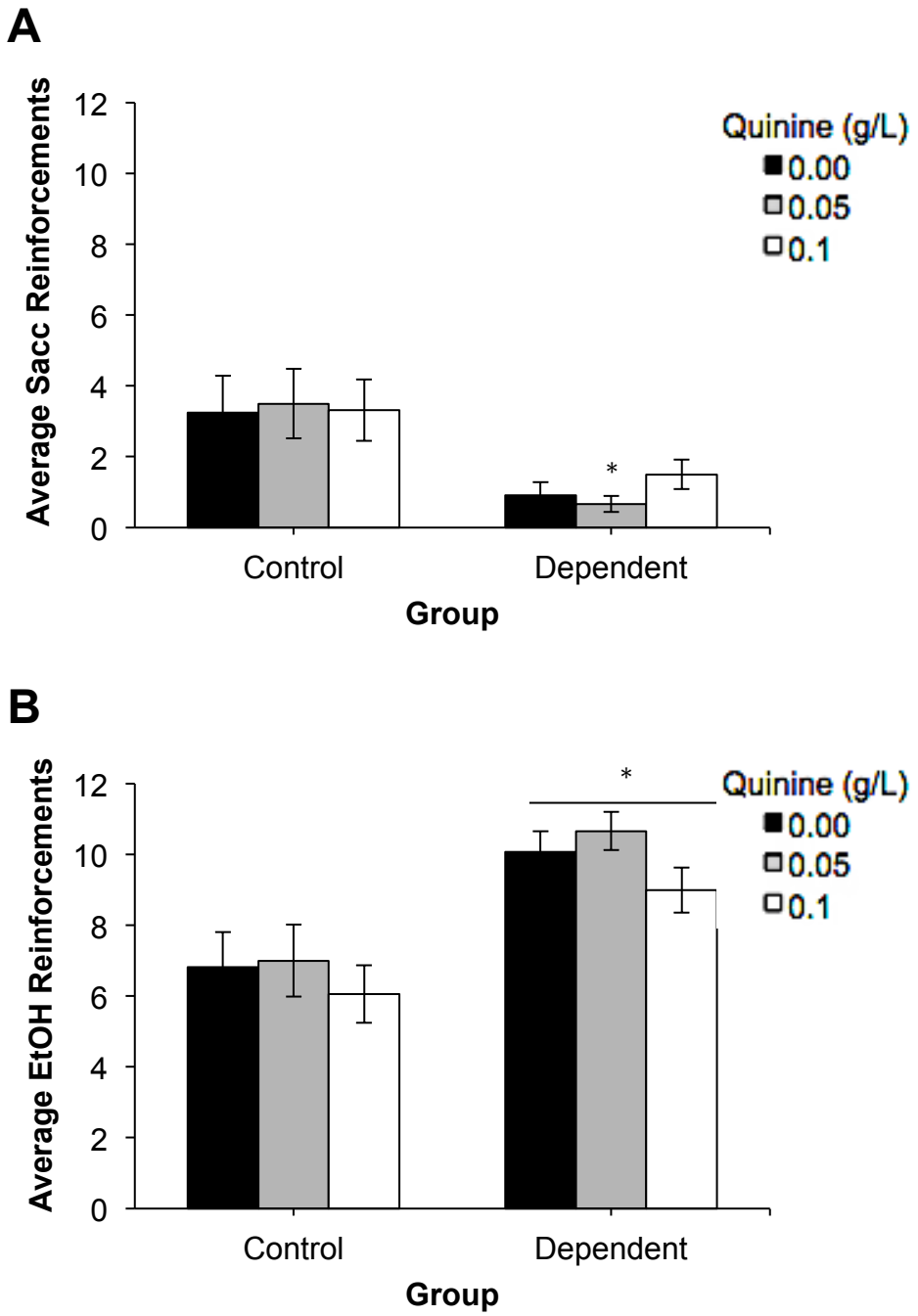

Figure 6.8. *Choice for saccharin or quinine-adulterated ethanol for the control and dependent group*. Data are mean numbers (±SEM) of (A) 0.1% (w/v) saccharin (Sacc) and (B) 12% (w/v) ethanol (EtOH) reinforcements obtained during choice sessions with quinine adulterated ethanol at concentrations of 0.00 (black), 0.05 (grey), and 0.1 (white) g/L for the control group (n= 15) and the dependent group (n= 16). *$p < 0.05$ compared to control group.

Blood alcohol levels during ethanol dependence induction

Serum derived from saphenous vein blood extractions taken from the vapour exposed animals during Cycles 2, 3, 4, and 5 were analyzed enzymatically for alcohol concentration (Analox AM-1). The blood alcohol levels of these samples were within or over the range known to induce a state of physiological dependence (150-200 mg%, Valdez et al., 2002). Specifically, the average (±SEM) blood alcohol levels obtained during Cycles 2, 3, 4, and 5 respectively were as follows: 165.74 (±15.97), 338.3 (±21.68), 187.00 (±23.76), 248.77 (±35.05).

6.4. Discussion

Similar to our findings in Experiment 1, prior to dependence induction, animals self-administered more saccharin than ethanol during single-reward operant training sessions. In addition, when given the choice between saccharin and ethanol in this experiment, animals showed similar preference for both rewards. These results closely mimic those found in Experiment 1 but it should be noted that the magnitude of preference differs slightly between these experiments. The preference for ethanol and saccharin was equal in the present study while animals in Experiment 1 showed a significant preference for saccharin. This could be due to different self-administration regimens before the introduction of choice sessions in these experiments. In Experiment 1 animals were given prior exposure to ethanol with the limited access paradigm and then trained to operantly self-administer ethanol. In contrast, animals in the current experiment were first given intermittent homecage access before being introduced to the limited access paradigm and subsequent self-administration training. In addition, animals in Experiment 1 had prior experience with 0.05% saccharin before being introduced to the higher concentration. These two differences may help explain the slight discrepancy in choice behaviour shown in Experiment 1 and the current experiment.

Exposure to ethanol vapour caused a gradual increase in ethanol self-administration, with dependent animals exhibiting significantly more ethanol consumption after the third cycle

of vapour exposure. This pattern resembles the escalation of ethanol consumption reported by a number of different research groups investigating the effects of dependence induced by ethanol vapour exposure (Roberts, Heyser, Cole, Griffin & Koob, 2000; Griffen, Lopez, Yanke, Middaugh & Becker, 2009; Becker & Lopez, 2004). While dependent animals do show a nonsignificant decrease in ethanol consumption after Cycle 1, this may be explained by the low levels of intoxication experienced by animals during this first cycle. Blood alcohol levels (BALs) were not collected during Cycle 1, however BALs from Cycle 2 were found to be on the lower end of the BAL range found in the present study (165-338 mg%), and at the lower end of the range reported to induce physical dependence (Valdez et al. 2002). Therefore, it is possible that animals were not as heavily intoxicated during Cycle 1, which may explain the lower levels of ethanol administration observed during testing. Finally, ethanol vapour exposure did not cause any significant changes in saccharin self-administration.

Animals were given sessions of the discrete-choice trials procedure after each cycle of ethanol vapour exposure. The most important finding is that dependent animals showed a gradual increase in ethanol choice and a gradual decrease in saccharin choice starting after the first cycle of ethanol vapour and this effect increased as a function of the number of vapour cycles the animals were exposed to. In other words, as the number of vapour cycles increased, the choice for ethanol became more robust to the point of exclusive ethanol choice after the fourth cycle. Although control animals maintained relatively constant levels of choice behaviour throughout testing, a trend towards increased ethanol choice was being exhibited by the third and fourth cycle of testing. A possible explanation of this is that these rats received multiple deprivation phases inherent to the experimental regimen. In order for both groups of animals to be treated as equally as possible, the control group was removed from the operant setting and remained in the home cage for the entire duration that dependent animals were exposed to ethanol vapour. This means that control animals received 5 deprivation phases in which they were not allowed to consume ethanol or saccharin. Given that the ethanol and saccharin deprivation effect have both been established in the literature and that this effect becomes more pronounced with repeated deprivation episodes, it is possible that we are seeing a

similarly mediated increase in our control animals (Khisti et al., 2006; Neznanova et al., 2002).

The addition of quinine to the ethanol solution during single-reward sessions and choice sessions was implemented in order to compare compulsive-like drinking behaviour in the control animals to those rendered dependent on ethanol. Animals were first exposed to differing concentrations of quinine in ethanol during single-reward sessions. Addition of quinine to the ethanol solution caused a significant decrease in ethanol responding across both the control and dependent groups. However, the magnitude of suppression of ethanol self-administration was more pronounced in the control group, which was exhibited by similar decreases in ethanol administration at both quinine concentrations. The dependent group, however, showed a more concentration-dependent decrease and consumed significantly more ethanol than the control group at the 0.05 g/L quinine concentration. Nevertheless, there was no interaction of group with quinine concentration. The inability to replicate a common phenomenon in ethanol dependence studies can act as a potential limitation of the present study. It is important to note, however, that previous studies using quinine adulteration to determine the presence of compulsive drinking behaviour have used a wider range of quinine concentrations. Vendruscolo and colleagues (2012), for example, found a decrease in ethanol administration at the lower quinine concentrations of 0.01 g/L and 0.025 g/L while no differences in QEtOH administration were shown between the dependent and control group at the 0.05 g/L concentration. Taking this into consideration, it is possible that compulsive-like behaviour simply could not be determined in the present study due to our selection of a higher range of quinine concentrations. Given this, the potential presence of compulsive drinking behaviour in the dependent animals should not be completely ruled out. It is also important to consider the finding that dependent animals continued to select ethanol much more than saccharin despite the addition of quinine during choice sessions. While quinine adulteration did not change choice behaviour in the control group, we showed that dependent animals will continue to administer bitter-tasting ethanol not only in favour of saccharin but with the complete preclusion of saccharin in subsequent choice-trials (i.e. despite negative consequences). In addition, the presence of saccharin

in the discrete-choice trials procedure may interact with the taste of quinine causing it to be less bitter. This possibility may explain why quinine-adulteration of the ethanol solution had minimal effects on choice behaviour.

This is one of the first studies investigating the effects of drug dependence on choice behaviour. We showed that animals rendered dependent on ethanol exhibit exclusive ethanol choice after multiple ethanol vapour exposures. Previously, one study determined the effects of long-access heroin pre-training on choice behaviour (Lenoir et al., 2013). Long-access to heroin (6-9 hrs) has been shown to cause a robust escalation in drug intake over time and has been shown to cause neurological changes related to binge consumption of opiates and is, therefore, considered to be a model of dependence (Koob & Kreek, 2007). Lenoir and colleagues (2013) reported two different choice profiles in animals given long-access to heroin: (1) 40% of animals showed an equal preference for heroin and saccharin, and (2) 60% of animals eventually showed a preference for heroin**.** These results support our findings on the effects of dependence on choice behaviour because, like in our study, long-access to heroin caused a shift toward drug choice. We showed, however that the effects of ethanol dependence on choice behaviour are much more robust. To be more specific, ethanol dependence caused ethanol preference in 100% of our animals while 87% (i.e. 13 out of 15) of these animals chose ethanol exclusively by the end of the fourth vapour cycle.

This shift to complete ethanol choice caused by exposure to ethanol vapour opens the door to further exploration of the effect of pharmacotherapies on ethanol preference. As explained by Banks and colleagues (2015), the purpose of treating drug addiction is "not only to decrease drug-maintained behaviours but also to promote a reallocation of behaviour towards alternative, nondrug reinforcers". In other words, an ideal pharmacotherapy for the treatment of drug dependence would be one that decreases drug self-administration while increasing the consumption of a nondrug reward (i.e. preference reversal). Keeping this in mind, investigation of the effect of pharmacotherapies on choice behaviour using dual-reward models in dependent animals would increase the

validity of pre-clinical research in the evaluation of candidate medications (Banks, Hutsell, Schwienteck & Negus, 2015).

Chapter 7:
GENERAL DISCUSSION

The current thesis investigated animal decision-making when given the choice between a drug reward and a nondrug reward. In Experiment 1, we established choice behaviour for ethanol and saccharin in non-dependent animals with previous self-administration training to both rewards respectively. This experiment demonstrated that rats have a close to equivalent preference for solutions of ethanol and saccharin. In addition, the introduction of a number of experimental manipulations caused a shift towards saccharin preference. More specifically, reward deprivation to both rewards caused an increase in saccharin choice. Increasing the inter-trial interval (ITI) from 2 min to 5 min also caused a shift in reward preference towards saccharin. In order to validate the parameters of the methodologies used in our experiments, animals were given the option to choose between intravenously administered nicotine and orally consumed saccharin in Experiment 2. Here, animals displayed a robust preference for saccharin, a result that replicated findings from previous studies using the discrete-choice trials procedure with intravenously administered drugs (Lenoir et al., 2007; Lenoir et al., 2013; Caprioli et al., 2015; Huynh et al., 2015). In addition, introducing sessions of concurrent access yielded similar results to those reported by Panlilio and colleagues (2015). More specifically, animals self-administered both rewards in comparable levels to those administered during single-reward sessions. Finally, in Experiment 3 we examined the effects of ethanol dependence on choice behaviour. In addition to finding an escalation in single-reward ethanol self-administration, animals' reward preference was shown to favour ethanol choice to the point of exclusivity after multiple cycles of ethanol vapour exposure. Concurrent access to both rewards caused similar patterns of responding to those shown in single-reward self-administration sessions for non-dependent animals while dependent animals showed a decrease in saccharin responding but maintained ethanol responding. Finally, quinine-adulteration of the ethanol solution caused decreases in single-reward ethanol administration but had no effect on choice in both dependent and non-dependent animals.

7.1. Choice between a drug and nondrug reward in non-dependent animals

In Experiment 1, animals that were trained to self-administer solutions of ethanol and saccharin were shown to have an equal preference for both rewards when given the option to choose one over the other in the discrete-choice trials procedure. These results are quite surprising given: (1) the relative administration of both rewards during single-reward sessions, (2) what we know about the reinforcing properties of ethanol, and (3) the results from previous studies using the discrete-choice trials procedure. To be more specific, animals in our study were shown to self-administer much more saccharin than ethanol during the single-reward self-administration period. In addition, the relative reinforcing value of ethanol is quite low in comparison to other drugs and nondrug rewards as shown by previous research making use of the progressive ratio schedule of reinforcement (Shram et al., 2008; Vendruscolo et al., 2012). Finally, all previous research using this procedure has shown that animals will choose the nondrug reward over the drug reward; a pattern of behaviour, which remains despite a number of environmental manipulations. These results have been shown for intravenously administered drugs of abuse like cocaine, heroin, methamphetamine, and nicotine in conjunction with nondrug rewards like sucrose, food, and saccharin (Lenoir et al., 2007; Lenoir et al., 2013; Caprioli et al., 2015; Huynh et al., 2015). These results were replicated in Experiment 2 of the present study where we found that animals will choose saccharin over nicotine under our experimental parameters. Similar patterns of choice behaviour were also found in a recent study by Pelloux and Baunez (2017), which found that animals will preferentially administer oral solutions of saccharin over ethanol after multiple choice sessions. This study showed that animals had an equal preference for either reward up until the fifth choice session in which a preference for saccharin was demonstrated and maintained for the remainder of the 20 choice sessions. It should be noted that this study used a higher 0.2% (w/v) saccharin concentration, which may help explain the higher magnitude of saccharin preference found in this study. Even with the consideration of these methodological differences, our results from Experiment 1 closely mimic the results reported by Pelloux & Baunez (2017). Overall, these results suggest that saccharin is higher in reinforcing value when compared to ethanol. Further evidence

for this comes from our results regarding reward deprivation. We included a reward deprivation manipulation because this has been shown to cause an increase in ethanol administration after a period of abstinence (Khisti et al., 2006). This effect has also been shown after deprivation from a sweet-tasting solution (Avena et al., 2005). Testing with discrete-choice trials procedure after 10 days of deprivation to both rewards yielded a significant increase in saccharin choice and a decrease in ethanol choice showcasing a more salient preference for the nondrug reward.

In order to further determine choice behaviour for ethanol and saccharin, and intravenous nicotine and saccharin a number of experimental manipulations were introduced.

7.1.1. Role of saccharin concentration on choice

The concentration of saccharin was increased from 0.05% to 0.1% (w/v) while the ethanol concentration was maintained at 12% (w/v). Animals were shown to have an equal preference for both rewards at the 0.05% saccharin concentration but the preference shifted towards saccharin choice with the increased 0.1% concentration. While this slight increase in saccharin preference is statistically significant, animals chose both rewards throughout choice sessions. This finding is unique to results in previous studies that showed a near-exclusive preference for the nondrug reward. It is important to note that increasing the saccharin concentration had no effect on ethanol choice. In other words, the introduction of a low vs. high saccharin concentration caused a slight increase in saccharin choice, no change in ethanol choice, and a slight increase in the total number of trials completed. Overall these results suggest that the relative reinforcing value of saccharin is moderately higher than ethanol, especially at higher concentrations, which is in agreement with the finding of Pelloux & Baunez (2017). This is in contrast to previous studies with intravenously administered drugs that found a clearer preference for saccharin (Lenoir et al., 2007, Huynh et al., 2015). These differences in the magnitude of nondrug reward preference suggest that the relative reinforcing value of two orally consumed drugs (i.e. ethanol and saccharin) is more comparable than that of an intravenously administered drug and an orally consumed nondrug reward.

7.1.2. Role of inter-trial interval on choice

In addition to changing the saccharin concentration, we determined the effects of changing the length of the inter-trial interval (ITI) on choice behaviour. In Experiment 1, we showed that increasing the ITI from 2 min to 5 min caused a shift in preference towards saccharin. Conversely, in Experiment 2, animals showed a robust preference for saccharin at the 2 min ITI and increasing the ITI to 5 min caused this preference to be completely exclusive. Taken together, it appears as though shorter ITIs favour drug choice, and this may be related to the drug setting, or the influence of the pharmacological effects of the drug, on subsequent choice behaviour. The majority of previous studies using the discrete-choice trials procedure used an ITI ranging from 5 to 10 min. One study explained the selection of a longer ITI in an effort to decrease the effect of drug satiety on choice behaviour (Caprioli et al., 2015). Drug satiety is very similar to food satiation in that they represent a threshold in which additional drug (or food) becomes aversive to the point of ceasing self-administration behaviour (Wise, 1987). Notably, this effect could negatively impact choice behaviour by causing an overt bias towards the nondrug reward. In addition, other studies have used an ITI of 10 min because this is the amount of time required for the locomotor effects of the drug (e.g. cocaine or heroin) to dissipate (Lenoir et al., 2007, 2013; Cantin et al., 2010; Vandaele et al., 2016). This too is an important consideration because locomotor sensitization occurs after multiple exposures to drugs like cocaine, amphetamine, and heroin, and causes a hyperesponsiveness to the effects of the drug in question, which may confound subsequent choice behaviour (Paulson et al., 1991). Given that neither drug satiety nor drug-sensitized locomotion are characteristic of ethanol consumption, we decided to select a shorter 2 min ITI in our experiments. Given the differences caused by changing the ITI in our study, a review of previous literature determining the effects of drug setting is necessary.

A recent review by Ahmed (2017) explains the need for longer ITIs in order to avoid the possible interactions between the drug and nondrug reward. For example, cocaine has been shown to have anorexigenic effects on saccharin administration (i.e. suppresses the

value of saccharin intake) while heroin has opposing, orexigenic effects (i.e. enhances to the value and intake of saccharin) (Ahmed, 2017). The use of a higher ITI prevents the drug of abuse from having a pharmacological influence on subsequent choice behaviour by inhibiting these potential drug vs. nondrug reward interactions. In contrast, the use of a shorter ITI or no ITI at all allows for pharmacologically relevant levels of the drug in question to be present in the brain at the introduction of subsequent choice trials. This could act as a potential confound because it could allow for drugs like cocaine and heroin to have anorexigenic and orexigenic effects on saccharin's relative value. In fact, a study exploring this showed that, with a 10 min ITI, animals will choose saccharin over both cocaine and heroin (Vandaele et al., 2016). However, under continuous choice conditions with no ITI, animals initially preferred saccharin then sampled both heroin and saccharin throughout the choice session. When given concurrent access to cocaine and saccharin, animals showed an initial preference for saccharin before switching to a near exclusive preference for cocaine. This same pattern of cocaine choice was observed with a 1 min ITI (Vandaele et al., 2016). Due to our selection of a shorter ITI, the possible interaction between the pharmacological effects of the drug and nondrug reward consumption is relevant to the current thesis. Nicotine, for example, has been shown to have anorexigenic effects on sweet substances and has been shown to cause conditioned-taste aversion when paired with a novel, sweet reward (Grunberg, Bowen, Maycock & Nespor, 1985; Pescatore, Glowa & Riley, 2005). However, this effect is improbable in the present study given that animals were shown to have a robust preference for saccharin at both the 2 min and 5 min ITI. In addition, concurrent access (with no ITI) to nicotine and saccharin did not cause any decreases in saccharin administration despite continued responding for nicotine on both days of testing. Similarly, ethanol has been shown to cause conditioned-taste aversion for saccharin (Roma, Flint, Higley & Riley, 2006). However, this interaction does not seem to affect the results presented in Experiment 1 where increased saccharin choice was found at both ITIs and concurrent access to saccharin and ethanol caused no change in saccharin self-administration in non-dependent animals. To conclude this section, the use of the discrete-choice trials procedure in the exploration of animals' decision-making should include procedures with varying ITI lengths in order to gain a full understanding of choice under different drug settings as well as to elucidate potential

interactions of the two reinforcers that may act as a confound.

7.1.3. Role of reward type on choice

Another important consideration is the selection of saccharin as the nondrug reward as opposed to food or sucrose, which have both been commonly used in previous research with a dual-reward model of drug addiction. While all three substances are orally ingested, liquid solutions of sucrose or saccharin are most comparable to ethanol solutions; for this reason, the use of food pellets as the nondrug reward was ruled out. Saccharin and sucrose are very similar in their sweet taste but they differ in their caloric value. Sucrose contains calories and is therefore consumed for nutritional purposes in addition to its rewarding properties. While ethanol does contain caloric properties and solutions of ethanol and sucrose can be made equicaloric, sucrose has also been shown to decrease blood alcohol levels when administered in conjunction with ethanol (Roberts et al., 1999). Given that the primary drug used in the present thesis was ethanol, this property of sucrose could act as a potential confound, especially in Experiment 3 where animals consumed ethanol and were rendered dependent to it. A major goal in our selection of the type of nondrug reward was that its magnitude of reinforcement be similar to that of ethanol. That is, we wanted to ensure that animals' decision-making would not be strongly biased towards the nondrug reward. Given that sucrose has been shown to be more rewarding than saccharin, we selected the latter as the nondrug reward and started with a low concentration (Reilly, 1999). It is important to note that an almost equal preference for both rewards was found despite the fact that saccharin was self-administered at higher rates during single-reward sessions. In addition, the 2 min ITI and the availability of only 12 choice trials meant that the selection of one reward over the other came at a significant cost. That is, the selection of one reward meant one less opportunity to administer the other reward. Interestingly, animals continued to sample ethanol even if this decision meant that access to saccharin would be precluded. It can be argued that our findings may be explained by ethanol's caloric properties and saccharin's lack of caloric properties. However, animals were fed 23-25 grams of food daily, which allowed them to grow and gain a healthy amount of weight throughout experimentation.

In addition, the relative amount of saccharin administered in single-reward sessions suggests that the absence of caloric properties in this liquid solution did not inhibit animals from consuming it at higher rates than ethanol. In order to assess this potential limitation, we tested the effects of food satiation on choice behaviour between ethanol and 0.1% saccharin (results not presented). After 7 days of ad libitum food access, animals were tested on two days of choice behaviour and it was shown that food satiation in nondependent animals did not cause any change to reward preference. Taken together, the caloric confound from ethanol on choice is unlikely.

Role of route of self-administration on choice behaviour

Given the finding that choice behaviour for ethanol vs. saccharin and nicotine vs. saccharin was notably different in Experiments 1 and 2, a potential explanation for this difference will be explored in the current section. To start, a major difference between the self-administration of ethanol vs. other drugs of abuse in animal models of drug addiction is the route of administration. A characteristic that is unique to the oral ingestion of liquid solutions is the activation of taste receptors upon entering the mouth. The activation of taste receptors is immediate and the taste of a solution has been shown to become associated with its pharmacological effects after multiple pairings, which may modulate its rewarding properties (Bassareo, Cuca, Frau & Di Chiara, 2017). In contrast, the effect of intravenously administered drugs depends on the speed at which it reaches the brain. In support of this, a number of studies show that the speed of drug infusion may influence drug taking in animals. For example, one study found that a longer rate of infusion (e.g. 100 sec in comparison to 5-50 sec) results in lower numbers of cocaine and amphetamine reinforcements received under a FR-1 schedule of reinforcement (Crombag, Ferrario & Robinson, 2008). In addition, it has been shown that faster infusion rates of nicotine and cocaine can potentiate psychomotor sensitization, which has been linked to an increased susceptibility to drug reward (Samaha, Yau, Yang & Robinson, 2005; Samaha, Mallet, Ferguson, Gonon & Robinson, 2004). It should be noted that one study showed a significant time lag, of approximately 6 sec, between the moment of drug infusion and the perception of behavioural change caused by that infusion in animals self-

administering cocaine (Lenoir et al., 2007). Taken together, it appears as though the speed at which a drug reaches the brain is an important factor in when its reinforcing effects are felt, which may play a role in the transition to compulsive drug intake (Samaha & Robinson, 2005).

While ethanol takes a longer time to reach the brain and exert its rewarding effects (due to its passage through the gastrointestinal tract), it is possible that the taste of ethanol can in and of itself become a conditioned stimulus. If this is the case, after repeated pairings, the activation of taste receptors may in turn activate brain reward centers before pharmacologically relevant levels of ethanol are in the brain. In addition, this activation from taste would be faster than that of intravenously administered drugs of abuse, which need to pass through the bloodstream before reaching the brain. The differential activation of dopamine in the nucleus accumbens caused by ethanol, sucrose, and nicotine administration may provide evidence for the involvement of taste receptors in promoting drug addiction. A study by Bassareo and colleagues (2017) determined extracellular levels of dopamine in the nucleus accumbens (NAc) shell and core through microdialysis during sucrose and ethanol operant sessions in animals with previous self-administration training for either reward. An increase in dopamine levels in the NAc shell was observed only at the start of the sucrose self-administration session. In contrast, animals self-administering ethanol were shown to have increased dopamine levels in both the NAc shell and core throughout the entire operant session (Bassareo et al., 2017). Interestingly, another study found that non-contingent intraoral infusions of ethanol caused differential dopamine responsiveness in ethanol naïve vs. pre-exposed animals. Here, an intraoral infusion of ethanol in naïve animals was shown to cause a biphasic release of dopamine in the NAc shell (Bassareo, DeLuca, Aresu, Aste & Di Chiara, 2003). The early rise in dopamine was attributed to the taste of ethanol while the later rise was related to physiologically relevant levels of ethanol present in the dialysate. However, the animals with pre-exposure to ethanol were shown to have a monophasic pattern of dopamine release that was directly correlated to the levels of ethanol present in the dialysate sample (i.e. physiological level of ethanol in the brain). Importantly, this study showed that the taste of ethanol alone can elicit DA changes in the NAc. Finally, a

study determining the effects of intravenous nicotine self-administration on extracellular dopamine levels in the NAc found that self-administration was associated with a preferential increase of dopamine in the NAc shell, (Lecca et al., 2006).

These results suggest that the dissociation of choice behaviour between nicotine and saccharin vs. ethanol and saccharin may be rooted in the activation of taste receptors by ethanol and saccharin. The activation of taste receptors upon consumption of a sweet-tasting solution and ethanol have both been shown to cause an initial increase of dopamine in the NAc after multiple exposures to reward ingestion (Bassareo et al., 2017). Due to ethanol's rewarding properties, this initial increase is maintained for as long as there are pharmacologically relevant levels of ethanol present in the NAc (Bassareo et al., 2017). In addition, the taste of ethanol can undergo habituation as a primary unconditioned stimulus and be converted into a conditioned stimulus after multiple exposures. Therefore, even though ethanol is low on the reinforcement value ladder, the activation of taste receptors in conjunction to its long-lasting effects on dopamine may increase its overall reinforcing value; especially when compared with a sweet-tasting solution that only produces short-term activation of the brain's reward centers (Vendruscolo et al., 2012; Bassareo et al., 2017). Conversely, the clear preference for saccharin over nicotine may be related to the rate at which the brain is activated by each of the reinforcers, and not so much the duration of this activation. As previously stated, the speed of brain activation caused by rewarding stimuli has been shown to be positively associated with their reinforcing properties (Samaha & Robinson, 2005). Keeping in mind that the activation of taste receptors by an orally consumed solution is much more immediate than the activation caused by an intravenously administered drug, it is possible that the oral ingestion of saccharin is more rewarding relative to slower acting intravenously administered drugs. In addition, the introduction of an inter-trial interval may inhibit the ability of cumulative nicotine infusions to produce longer-lasting reinforcing effects. Taken together, the activation of taste receptors unique to the ingestion of rewarding solutions may provide a possible explanation for the differences in choice between ethanol and saccharin, and intravenous nicotine and saccharin in the present thesis.

7.2. Effect of ethanol dependence in choice between ethanol and saccharin

An important consideration of previous research using dual-reward procedures to determine choice behaviour for a drug vs. nondrug reward is that these studies were conducted in non-dependent animals. The use of non-dependent animals has been valuable in the study of choice behaviour and the relative reinforcing value of competing rewards. However, the use of drug dependent animals in discrete choice studies is needed for a better understanding of maladaptive decision-making that is associated with dependence in humans.

To resolve this issue, we used the technique of intermittent chronic exposure to ethanol vapour to induce dependence, and examined its effects on choice between ethanol and saccharin using the discrete-choice trials procedure. Intermittent vapour exposure is an accepted model of dependence induction and causes an escalation in ethanol drinking as well as operant responding for ethanol, and upon cessation of vapour exposure, physical withdrawal symptoms are observed (O'Dell et al., 2004). In addition, ethanol vapour exposure has been shown to cause compulsive ethanol drinking as demonstrated by continued self-administration of ethanol despite the addition of bitter-tasting quinine. The quinine-adulteration test is analogous to compulsive drug taking in human drug addiction because dependent animals are continuing to self-administer ethanol despite the negative consequences associated with doing so (i.e. consuming something bitter) (Vendruscolo et al., 2012). Another advantage of intermittent ethanol vapour exposure is that the transition to physical dependence can be tightly controlled by observation of signs of intoxication and by adjusting the concentration of ethanol vapour that the rats are exposed to, in order to maintain blood alcohol levels in the range known to induce dependence.

Given the validity of the ethanol vapour exposure method as well as the importance of elucidating choice behaviour in dependent animals, our third experiment attempted to clarify these gaps in the literature. Upon stable levels of reward preference, which yielded equivalent choice for solutions of ethanol and saccharin, some animals were exposed to 5

cycles of ethanol vapour exposure while the rest remained in their home cages without such exposure. Ethanol and saccharin self-administration, as well as choice under discrete-choice trials and concurrent access parameters across vapour cycles did not differ in comparison to baseline measures in the control group. In contrast, animals exposed to ethanol vapour were shown to exhibit robust changes in behaviour as a function of the number of vapour cycles. Animals showed significant increases in ethanol self-administration by the third cycle which is consistent with previous studies that employed ethanol vapour exposure to induce dependence, but did not show change in saccharin self-administration.

The key finding in the present study was that dependent animals were shown to have a gradual increase in ethanol choice and a gradual decrease in saccharin choice across vapour exposure cycles. Interestingly, choice for ethanol was exclusive by the fourth cycle. These results show, for the first time, the effects of dependence on choice behaviour with the use of the discrete-choice trials procedure. More specifically it was shown that drug consumption in dependent animals occurs in favour of the consumption of a nondrug reward that was previously self-administered at high levels in single-reward sessions. In addition, when exposed to sessions of concurrent access, dependent animals were shown to exhibit similar ethanol intake levels as single-reward self-administration sessions while showing a robust decrease in saccharin intake by the second day of testing. These results show that the effects of dependence on choice behaviour can be exhibited under conditions of unlimited reward access in addition to conditions of discrete-choice where the selection for one reward precludes access to the other.

Finally, we included a quinine adulteration manipulation in which quinine was added to the ethanol solution, giving it a bitter-taste. This was included in an effort to determine compulsive drug administration in our dependent animals, as shown in previous studies using this method (Vendruscolo et al., 2012; Vendruscolo & Roberts, 2014). Here, animals were exposed to quinine adulterated ethanol solutions in both single-reward sessions as well as sessions of the discrete-choice trials procedure. During single-reward sessions, overall ethanol consumption still remained higher in the dependent group but

exposure to quinine-adulterated ethanol caused similar decreases in the dependent and nondependent group. When introduced in the choice setting, quinine-adulteration was expected to cause a decrease in ethanol choice, having a much greater effect on the control group. Our results showed that quinine-adulteration at both the low and high concentrations had no effect on choice behaviour in both groups of animals. Given that this is the first study exploring the effects of quinine-adulteration on choice behaviour, the reasons for a clear decrease in ethanol responding in single-reward sessions vs. no effect on choice behaviour in sessions of the discrete-choice trials procedure are not known. However, it can be speculated that presence of saccharin during choice sessions circumvented the bitter taste of the quinine-adulterated ethanol solution, which consequently impeded the ability to discern compulsive ethanol administration in the context of choice.

Determination of ethanol dependence

Importantly, while the effects of dependence were clearly exhibited behaviourally, we were also able to observe blood alcohol levels and physiological symptoms of withdrawal indicative of physical dependence in rodents. More specifically, animals in our study were found to have blood alcohol levels exceeding 150 %mg throughout cycles 2, 3, 4, and 5. Visual inspection of animals 6 hrs and 24 hrs after removal from the vapour chambers yielded observations of mobility impairments, tail rigidity and decreased food intake all of which are associated with a state of ethanol withdrawal (Valdez et al., 2002). Taken together, these findings confirm that the animals in Experiment 3 were, in fact, physically dependent on ethanol. All in all, this is the first study to show that dependence, confirmed by blood alcohol measures and observations of withdrawal symptoms, has the ability to cause a robust and exclusive change in choice behaviour towards preference for the drug reward. While previous studies using the discrete-choice trials procedure have implemented a model of dependence commonly used for intravenously administered drugs, the magnitude of dependence exhibited by these animals is unknown due to the lack overt measurements of factors indicating physical dependence (i.e. withdrawal symptoms).

Previous work approaching dependence

It is important to note that previous studies using the discrete-choice trials procedure have used long-access procedures to induce dependence but their validity as models of dependence remains to be confirmed in these studies. One study showed that animals provided with short-access to heroin pre-training (for 1 hr per day) exhibited a clear preference for saccharin. In contrast, animals provided with long-access to heroin pre-training (for 6 hrs per day) showed an overall equal preference for both rewards, with some animals showing a clear heroin preference (Lenoir et al., 2013), results which are closely mimicked by our study. Animals in this study showed an escalation in heroin intake during single-reward sessions, showcased by 13 times more heroin consumed over 6 hrs than the short-access group did over 1 hr. Another study by Lenoir and colleagues (2007) examined the effects of long-access to cocaine on choice behaviour. This study showed that animals with a long history of cocaine administration (6 hrs per day, for three weeks) will robustly choose sucrose over cocaine despite exhibiting a clear escalation in cocaine intake during single-reward sessions. Finally, the investigation of short-term extended daily drug access vs. long-term limited daily drug access has been determined for the choice between methamphetamine and palatable food pellets (Caprioli et al., 2015). This study found that neither method of methamphetamine self-administration has an effect on the preference for food pellets. The common trend in the aforementioned studies is that methods of long-access drug sessions do not have an effect on choice behaviour with cocaine or methamphetamine as the drug reward but do cause marked changes in choice behaviour with heroin and ethanol (the latter which is showcased in our study). While the mechanisms for these differences are unknown, chronic exposure to stimulants (i.e. cocaine and methamphetamine) vs. depressants (i.e. ethanol and heroin) may be causing differential effects on choice behaviour. For example, overt withdrawal symptoms have been associated with the cessation of heroin and ethanol exposure where as physical withdrawal symptoms for cocaine and methamphetamine have been shown to be less obvious (Wise & Bozarth, 1987). Keeping this in mind, it is possible that this dissociation in withdrawal behaviour between drug classes could contribute to the effect of dependence on choice behaviour.

General implications and additional considerations

An important characteristic of human drug dependence is continued drug use in the place of alternative rewards (American Psychiatric Association, 2013). Therefore, a major advantage of choice procedures is that they have the potential to model human substance abuse more closely than models that only offer one reward. Previous research using choice procedures in rodents have mainly done so in non-dependent animals. In order to account for this gap in the literature, the main purpose of this thesis was to establish choice behaviour for ethanol and saccharin in nondependent and dependent animals.

To gain a better understanding of animal decision-making when given the option to choose between a drug and non-drug reward, three different manipulations shown to greatly effect ethanol administration, were examined. First, reward deprivation was shown to cause an increase in saccharin choice while having minimal effects on ethanol choice. This effect has been previously shown to increase both ethanol and saccharin intake after a period of deprivation to both rewards respectively (Khisti et al., 2006; Avena et al., 2005). Secondly, we showed that, in addition to replicating an escalation of ethanol consumption in single-reward sessions, dependent animals also exhibit a gradual and eventual exclusive preference for ethanol. Finally, we examined choice behaviour with the introduction of bitter-tasting quinine to the ethanol solution and found that it had no effect on choice behaviour. Taken together, it appears as though ethanol dependence was the only manipulation successful in causing a robust and overt change in animals' choice behaviour. These results suggest that choice behaviour, elicited by the discrete-choice trials procedure, is quite resistant to change. The reasons for this "choice resilience" are unknown but it can be speculated that dependence induction was the only manipulation to produce significant changes in choice behaviour because it is the only manipulation that truly altered an animals' physiological state. In addition, this dependence was directly demonstrated in animal's choice behaviour. Taken together, the unique ability of dependence induction to alter choice behaviour renders the use choice procedures in dependent animals a key component in discerning maladaptive decision-making in an animal model of drug addiction. In addition, these results suggest that the

discrete-choice trials procedure is able to detect dependent animals' increased motivation for consuming the drug over alternative reinforcers, an important factor in human drug dependence.

7.3. Relevance of findings to drug addiction treatments

The use of the dual-reward paradigm, especially in dependent animals, may provide a useful way to screen pharmacotherapies for addiction. A key factor in successful treatments for drug relapse is that they not only decrease drug-intake and drug seeking but that they also promote behaviour towards nondrug rewards (Banks et al., 2015). Contingency management programs build upon this idea by providing alternative reward, in the form of money, vouchers, or tokens, in exchange for clean drug tests and treatment program adherence (as reviewed in Stitzer & Petry, 2006). These programs have been shown to be one of the most effective methods of promoting abstinence for a substance of abuse by allowing addicts to take full advantage of clinical outcomes (Prendergast, Podus, Finney, Greenwell, & Roll, 2006). As previously stated, drug use disorders are characterized by maladaptive decision-making in an environment with many alternative rewards available. Contingency management programs target the core of this issue by using salient, alternative rewards in order to help improve a dependent individual's pattern of decision-making. Keeping this in mind, the discrete-choice trials procedure appears to mimic the same features of contingency management programs. It can, therefore, be used as a powerful model to determine which behavioural, environmental, and neurophysiological factors are contributing to maladaptive decision-making in drug addiciton. Importantly, the present thesis found that ethanol dependence causes animals to exhibit an exclusive ethanol preference, despite the preclusion of a previously self-administered nondrug reward, saccharin. The replication of this finding could allow for the evaluation and testing of potential pharmacotherapies (e.g. naltrexone and acomprosate) by determining if a shift from dependence-induced ethanol preference can be switched to saccharin preference following the administration of these therapies.

Chapter 8:
FUTURE DIRECTIONS

The effect of dependence on the choice between ethanol and saccharin introduces an exciting avenue to explore aspects of substance use disorders with the use of a valid and more translatable preclinical animal model of drug addiction. Future research should be focused on extending the examination of dependence on choice to other drugs of abuse, like nicotine, cocaine, methamphetamine, and heroin. This can be accomplished with the use of methods that have been shown to cause overt withdrawal symptoms indicative of physical drug dependence. Dependence to a number of intravenously administered drugs has been made possible through the implantation of osmotic minipumps, which allows for the continuous, subcutaneous infusion of drugs like nicotine, cocaine, and heroin (Vann et al., 2006; Joyner, King, Lee & Ellinwood, 1993; Shaham, Rajabi & Stewart, 1996).

In order to determine the differences discovered in the present thesis between choice with oral rewards vs. intravenous rewards, a direct comparison using the administration of the same drug and nondrug rewards but with different routes of drug administration is needed. A potential method for accomplishing this would be to determine choice behaviour in animals given the option between oral solutions of ethanol and saccharin vs. intravenously administered ethanol and orally consumed saccharin. A similar experiment could be conducted with nicotine because animals have been shown to self-administer oral solutions of nicotine (Glick, Visker & Maisonneuve, 1996). While the acquisition of intravenous ethanol self-administration and orally consumed nicotine have both proven to be difficult (Le & Kalant, 2017; Flynn, Webster & Ksir, 1989), these experiments would also allow us to gain a better understanding of the involvement of taste receptors in the reinforcing efficacy of certain rewards.

The implication of dopamine in the rewarding effects of a number of drugs of abuse has been established in the literature for quite some time. Given the prominent role of dopamine and the mesolimbic system in drug addiction, the involvement of this reward

circuitry in an animal's reward preference should be elucidated. This could be accomplished by determining the effects of a dopaminergic modulator (e.g. agonist or antagonist) on choice behaviour in both dependent and nondependent animals. In addition, the involvement of certain brain areas potentially playing a role in an animal's pattern of decision-making should be explored as well. A particular area of interest would be the prefrontal cortex because it has been shown to be heavily involved in executive functions such as inhibition, decision-making, and the temporal integration of voluntary behaviour (Dalley, Cardinal & Robins, 2004). The level of involvement from the prefrontal cortex in choice behaviour exhibited by dependent vs. nondependent animals could potentially aid in having a better understanding of the neuroanatomy involved in maladaptive decision-making.

Finally, the use of the dual-reward paradigm in dependent animals opens the door to further exploration of the effect of pharmacotherapies on not only ethanol intake but ethanol preference. The use of pharmacotherapies like acamprosate and naltrexone in animals that are showing a clear preference for ethanol will allow for the determination of these medications' effects on the pattern of decision-making that is associated with the transition to compulsive ethanol consumption in dependent rodents. The ability of these pharmacotherapies to cause a change in choice behaviour has yet to be determined.

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