

Effects of Nicotine Exposure and Anxiety on Motivation
for Gambling-like Cues

by

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Abstract

Introduction

More than half of disordered gamblers (60.4%) report comorbid tobacco dependence (compared to only 16.8% of the general population), suggesting a common mechanism for the pathology of these disorders. Furthermore, 41.3% of disordered gamblers report the diagnosis of an anxiety disorder (compared to only 10% of the general population), which is implicated as a risk factor for relapse, and is thought to promote the maintenance of maladaptive gambling and smoking behaviors. One of the most important characteristics common to all gambling games is the uncertainty associated with the probability of reward delivery and the magnitude of the reward. Evidence suggests that this uncertainty may powerfully enhance attraction to cues, such as the flashing lights and celebratory sounds of casino slot machines, and at times, may sensitize reward pathways in the brain. Nicotine is implicated in attention, particularly for cues. However, it is unknown how nicotine or anxiety might contribute to cue-attraction.

Methods

In the present study, we investigate the effects of nicotine (0.3 mg/kg, s.c.) on the desire for cues associated with uncertain rewards in male and female Long-Evans rats. Rats received injections of either nicotine or saline 15-minutes prior to each conditioning session. In an autoshaping task, rats learned to associate lever and tone cues (CS) with the delivery of sucrose pellet rewards (UCS) on either a certain or uncertain reward contingency. Under certain reward conditions, each CS presentation was immediately followed by the delivery of a single sucrose pellet (100% -1). In

contrast, under uncertain conditions, only half of all CS presentations were rewarded by a sucrose pellet, with the remaining half being unrewarded. When the CS presentation was followed by UCS delivery, the reward was either 1, 2 or 3 pellets (with equal probability) (50%-1-2-3), which resulted in the same total number of CS and UCS presentations as in the certain condition. Sign-tracking and goal-tracking behaviors were recorded as a measure of attraction to either the lever cue or the sucrose delivery dish. Subsequently, we tested the ability of gambling-like cues to serve as a conditioned reinforcer, and to promote motivation for the sucrose reward during a progressive ratio task. Finally, the Elevated Plus Maze was used to measure the effects of anxiety and its interaction with nicotine and uncertainty.

Results

Here, we demonstrate differences in cue attraction and motivation for certain versus uncertain rewards in the presence of nicotine. For example, during the autoshaping task, nicotine enhanced attraction to CS cues for certain conditions, but not for uncertain ones. Conversely, in the progressive ratio task, nicotine enhanced motivation to obtain the reward in uncertain conditions, but not for certain conditions. Females, in particular, appear to be vulnerable to the combined effects of nicotine and reward uncertainty on motivation to obtain rewards. While not significant, anxiety appears to play a role in moderating attraction-related behaviors, as rats in the uncertain condition exhibited higher levels of anxiety compared to rats in the certain condition.

Discussion

Understanding the effects of nicotine and uncertainty on cue-reactivity might provide insight into the comorbid relationship between pathological gambling and tobacco use. Here, nicotine increased attraction for cues associated with certain reward outcomes, and increased motivation to obtain rewards associated with uncertain outcomes. Since motivation to obtain rewards appears to be greater in females injected with nicotine, comorbidity rates may be sex specific rather than uniform across both sexes. Future studies should consider these sex-dependent effects when developing successful intervention programs for comorbid disordered gambling and tobacco behaviors. Additionally, the finding that uncertainty increases anxiety behavior suggests an interplay between anxiety and nicotine, which should be considered when treating patients with a comorbid anxiety diagnosis, GD diagnosis, and nicotine dependence. Since anxiety is a known contributor to the relapse and maintenance of maladaptive behaviors, it is crucial to understand its ability to promote attraction to uncertain rewards in the presence of nicotine.

INTRODUCTION

CHAPTER 1: GAMBLING DISORDER

The Continuum of Gambling Involvement

Gambling is a global health concern affecting nearly 1.8 billion individuals worldwide. In the United States, 85% of the adult population has gambled during some point in their lifetime (Shaffer and Hall, 2001; Cunningham-Williams et al., 2005; Kessler et al., 2008b). Furthermore, 63% to 82% of the population reports past-year participation in betting games (Welte et al., 2001; Welte et al., 2002; Kessler et al., 2008b). While gamblers comprise the majority of the population, individuals vary widely in the frequency and vigor to which they participate. For most, gambling is a pastime that occurs at sport events, in pubs, and various recreational milieus. However, for a subset of individuals (14%), gambling is a debilitating behavior that becomes a persistent and solitary activity, leading to social and economic ramifications (Slutske et al., 2010, 2011; Simpson and Balsam, 2016).

The intensity of gambling participation exists on a continuum ranging from recreational, to problem, to disordered play (Strong and Kahler, 2007). Of the individuals that gamble, some seldom participate, while others participate frequently, but without social or economic consequences. For others, gambling becomes a daily obsession that leads to enamored and incessant play (Blaszczynski and Nower, 2002). These individuals, referred to as problem gamblers, become infatuated with betting, typically lose track of time when gambling, experience financial loss, and become socially isolated (Lesieur and Blume, 1987; Lesieur and Rosenthal, 1991; Petry,

2005). In the most severe cases, problem gamblers develop a pathological form of behavior, termed gambling disorder (GD), which leads to severe hardship in nearly every aspect of life (extreme financial loss, employment difficulties, trouble with the law, etcetera) (Ladouceur et al., 1994; Steel and Blaszczynski, 1998; Griffiths and Parke, 2002; Ratelle et al., 2004). According to several epidemiological studies, approximately 12.5% of the general population meets the criteria for subclinical problem gambling, and 2.5% qualify for a gambling disorder diagnosis (GDD) (Shaffer et al., 1999; Shaffer and Hall, 2001; Cunningham-Williams et al., 2005; Kessler et al., 2008b; Slutske et al., 2011; Nower et al., 2015; Simpson and Balsam, 2016).

Gambling Disorder

Gambling disorder is defined by the *Diagnostic and Statistical Manual, Fifth Edition (DSM-V)* as a behavioral addiction characterized by persistent and recurrent gambling behavior that is problematic or impairs quality of life (Grant and Kim, 2006; American Psychiatric, 2013). In recent years, the DSM transitioned from the fourth to fifth edition, which reclassified gambling from a disorder of impulse control to one of addiction. This decision reflects the physiological similarities between the effects of gambling and highly addictive drugs, such as cocaine, alcohol, and nicotine (Blanchard et al., 2000; Breen and Zimmerman, 2002; Blaszczynski et al., 2008; Leeman and Potenza, 2012). Both addictive substances and disordered gambling behavior are characterized by a loss of control, excessive preoccupation with paraphernalia, persistence in the face of adverse consequences, tolerance, and withdrawal (Blaszczynski et al., 2008; Leeman and Potenza, 2012). For example,

disordered gamblers and drug users both escalate their involvement to achieve a previous degree of stimulation, have a diminished regard for the negative consequences associated with the maladaptive behavior, reduced inhibitory behaviors, and dysfunction involving similar brain regions (American Psychiatric, 2000; Holden, 2001; Wareham and Potenza, 2010; Leeman and Potenza, 2012). Such behaviors encourage repeated drug taking, and may also promote and sustain gambling behaviors (Tamminga and Nestler, 2006). In order to qualify for a GD diagnosis, individuals must meet four or more of the qualifying attributes over a period of twelve months (American Psychiatric, 2000, 2013). Such criteria relate to feelings of disinhibition, craving, obsession, and lack of self-control.

One major change from the DSM-IV to the DSM-V was the lowering of the diagnostic threshold, which decreased from the endorsement of five criteria to the endorsement of four (American Psychiatric, 2000, 2013). The lowered diagnostic threshold appears to better reflect the population of disordered gamblers, has led to a more accurate diagnosis of GD, and has reduced the rate of false negatives within the clinical setting (Stinchfield, 2003; Reilly and Smith, 2013). Furthermore, the DSM-V requires symptoms to be present over a period of 12-months, whereas the DSM-IV lacked this requirement (American Psychiatric, 2000, 2013). Because the DSM-V was released in 2013, much of the existing literature pertains to the DSM-IV criteria. With this in mind, studies conducted using the DSM-IV criteria for a diagnosis of pathological gambling (PG), and the DSM-V criteria for a diagnosis of gambling disorder, are concurrently cited in this review. The term “gambling disorder” will be used to refer to both PG and GD diagnoses.

Epidemiology of Gambling Disorder

Epidemiological studies have identified sub-populations that are susceptible to a gambling disorder diagnosis. Largely due to stereotypes and perceptions of men versus women, GD is typically considered to be a male-dominant disorder. This perception is a disservice to the large and growing population of women suffering with disordered gambling behavior. While males do in fact comprise the majority of the GD population, a study of clients from a counseling service revealed that 46% of treatment-seeking gamblers are female (Crisp et al., 2004), and several other studies support this claim (Crisp et al., 2004; Blanco et al., 2006; Sacco et al., 2011). Comparisons between the male and female populations of treatment-seeking gamblers reveal sex-dependent differences in betting behavior. Of the individuals that choose the gamble, females are far more likely than males to use electronic gambling machines (females, 91.1%; males, 61.4%), are older when seeking treatment (females, 39.6 years old; males, 36.1 years old), are more likely to be married (females, 42.8%; males, 30.2%), to have a family (females, 78.9%; males, 61.5%), and to have dependent children (females, 48.4%; males, 35.7%) (Crisp et al., 2004). On the contrary, men are more likely than women to have severe gambling debt (males, \$19,091; females, \$7,342) (Crisp et al., 2004). A study using a nationally representative sample of adults from the National Epidemiologic Survey on Alcohol and Related Conditions, revealed that women are less likely than men to endorse the diagnostic criterion for preoccupation with gambling games, but are more likely to gamble as a form of escapism (Sacco et al., 2011). Such trends suggest that men and women must be evaluated separately when considering disordered gambling, or with

the acknowledgement that the populations differ in their characteristics, pattern, and method of gambling behavior.

In addition to sex-differences, betting behavior appears to vary by race, ethnicity, and age. Research suggests that racial and ethnic minorities, such as Asians, African Americans, and Latinos, are more likely to bear the diagnosis of GD (Cunningham-Williams et al., 2000). However, these trends seem to disappear when controlling for other factors such as socioeconomic status, suggesting that lifestyle and access to resources may influence the development of a diagnosis (Cunningham-Williams et al., 2005). With regards to age, some studies suggest that middle-aged groups bear the greatest risk for disordered gambling, while others suggest that young adults bear a greater risk. According to a study conducted by the National Research Council, 4% to 8% of adolescents between the ages of 12 and 17 have a severe gambling problem, and another 10% to 15% are at risk for developing GD. These estimates exceed the prevalence rates of problem and pathological gambling in the U.S. adult population, which hover around 1% to 2%. Despite the lack of consensus, the youth appear to be an increasingly vulnerable population for problem and disordered gambling (Griffiths and Wood, 2000; Chambers and Potenza, 2003; Daníel Thor Ólason, 2006). In fact, an analysis of self-reported data collected between 1989 and 1999 revealed an increase in the prevalence of adolescent betting in North America. Between the years of 1984 and 1988, the median fraction of middle and high school students that gambled within the last 12 months was 45%, while the median fraction of students that gambled between the years of 1984 and 1999 was 66% (Jacobs, 2000; Daníel Thor Ólason, 2006). Such trends are largely

attributed to the rapid and widespread proliferation of technology, which has brought gambling from private venues to locally accessible devices and spaces, such as the internet. The accessibility of betting games has increased the availability and convenience of gambling, while decreasing the ability to enforce age-restrictions and legislation (Griffiths, 1999, 2003; Griffiths et al., 2006). In cyberspace, gambling is available to anyone with access to a computer, and can become an anonymous route to betting for underage individuals (Gupta and Derevensky, 1996; Griffiths and Wood, 2000; Daníel Thor Ólason, 2006).

Technologically Savvy Gambling Platforms Promote Disordered Play

Technology has modernized several industries, and the American Gambling Association has leveraged these advances to expand the popularity of betting games. New gambling platforms, such as online gambling, interactive television games, and telephone wagering have been developed (Griffiths and Wood, 2000; Messerlian et al., 2004). In addition, the attractiveness of older gambling platforms, such as electronic gambling machines and Video Lottery Terminals, has been increased. Presently, internet gambling appears to be the most lucrative betting method, and has developed into a multibillion dollar industry with more than 400 websites offering online gambling games (Griffiths, 1999, 2003; Messerlian et al., 2004).

A digitized gambling industry encourages the shift of gambling from casino, pub, and entertainment environments, to unspecified gambling venues, such as homes and workplaces (Griffiths, 1999). This presents an alarming health concern because betting in non-gambling environments is known to cause disinhibition, anonymity, and associability—all of which may accelerate the development and increase the

intensity of disordered behavior (Griffiths, 2003; Griffiths et al., 2006). Furthermore, a growing body of evidence suggests that increased exposure and access to gambling is related to an increase in the amount of money spent when betting, and heightened rates of problem gambling (Messerlian et al., 2004). One study of treatment-seeking gamblers at the University of Connecticut Health Centre revealed that 8.1% of patients gambled online, and the prevalence rates were significantly greater for younger people (Ladd and Petry, 2002; Messerlian et al., 2004).

General Comorbidity of Gambling Disorder

Numerous clinical reports suggest a co-occurrence between GD and drugs of abuse, such as alcohol, cocaine, marijuana, and tobacco (Smart and Ferris, 1996a; Petry, 2005; Kessler et al., 2008b; Kessler et al., 2008a; McGrath and Barrett, 2009). Up to 63% of pathological gamblers qualify for a substance abuse disorder, and conversely, approximately 9% to 16% of patients with a substance abuse disorder qualify for a gambling disorder diagnosis (Crockford and el-Guebaly, 1998). In addition, the first-degree relatives of disordered gamblers report higher rates of substance abuse compared to the general population (Crockford and el-Guebaly, 1998; Petry and Armentano, 1999). Interestingly, rate of drug use appears to be greater for individuals that report the use of technologically advanced methods of gambling, such as digitized slot machines.

As with drugs of abuse, studies report a significantly greater incidence of psychiatric disorders in the population of treatment seeking gamblers. Numerous studies show that problem gamblers often bear the diagnosis of affective agoraphobia, obsessive-compulsive disorder, antisocial personality disorder, depression, and

anxiety. Lifetime depression rates among disordered gamblers range between 70% to 76%, and 87% of disordered gamblers bear the diagnosis of a personality disorder—specifically, mood disorder (60%), anxiety disorder (40%), and antisocial personality disorder (33%) (Crockford and el-Guebaly, 1998; El-Guebaly et al., 2006). In all, the greater the severity of gambling behavior, the more likely an individual will meet the criteria for substance abuse and psychological disorders such as major depression, mania, antisocial personality disorder, alcohol abuse, and nicotine dependence (Crockford and el-Guebaly, 1998; Cunningham-Williams et al., 2000). Therefore, it is crucial to recognize the effects of comorbid substance and psychological diagnoses when studying and treating the disordered gambling population.

Comorbidity of Tobacco Dependence and Gambling Disorder

Cigarette smoking is highly prevalent among disordered gamblers, with rates of smoking ranging between 41% to 60% (Smart and Ferris, 1996b; Cunningham-Williams et al., 1998; McGrath and Barrett, 2009). While most of the consequences related to smoking are due to the inhalation of toxic vapors, it is well understood that attraction to cigarettes is maintained by the pharmacological effects of nicotine, the highly addictive ingredient found in nearly all tobacco products (Stolerman and Shoaib, 1991; Benowitz, 2009). Nicotine is a mild stimulant capable of crossing the blood brain barrier, and can do so within seven seconds of inhalation (Stolerman and Shoaib, 1991; Caggiula et al., 2001; Jiloha, 2010). Once inside the brain, it relaxes muscles, reduces anxiety, produces subjective states of euphoria, increases cognition, and produces a plethora of other pharmacological effects (Caggiula et al., 2001; Chiamulera, 2005). Compared to other drugs of abuse, nicotine dependence is one of

the most frequently reported addictions among disordered gamblers (60.4% comorbidity rate), ranking second to alcohol use disorder at 73% (McGrath and Barrett, 2009). Nicotine dependent individuals are seven times more likely to bear the diagnosis of GD compared to non-smokers. Within this trend, sex-differences emerge, with tobacco dependent women being fourteen times more likely to bear the diagnosis of GD than nonsmoking women, and tobacco dependent men being five times more likely to bear the diagnosis of GD compared to nonsmoking men (McGrath and Barrett, 2009).

Very few studies elucidate the syndromic relationship between maladaptive smoking and gambling behaviors. Some research suggests that smoking severity increases with gambling severity; however, other studies refute this claim. One study of treatment-seeking gamblers found current daily smokers to have greater difficulty with controlling gambling urges. These individuals reported a greater severity of gambling problems, gambled for longer periods of time, and spent more money gambling per month compared to nonsmokers (Petry and Oncken, 2002; McGrath and Barrett, 2009). A second study investigated nicotine comorbidity among a population of problem gamblers calling a helpline, and failed to find significant differences between smoking and non-smoking gamblers. However, greater social, financial, and behavioral problems were observed in the smoking gambling population (Potenza et al., 2004; McGrath and Barrett, 2009).

In addition to their syndromic comorbidity, nicotine and gambling co-occurs in the naturalistic setting. 80% of slot machine players report smoking while gambling, and some studies suggest that nicotine may influence gambling behavior

(McGrath and Barrett, 2009). Players appear to increase the vigor of gambling while smoking, and larger doses of nicotine are associated with more vigorous gambling involvement (Potenza et al., 2004; McGrath and Barrett, 2009).

Comorbidity of Anxiety and Gambling Disorder

The maladaptive relationship between anxiety and disordered gambling was first noted by Anderson and Brown in their novel model of gambling (Anderson and Brown, 1984; Brown, 1986; Blaszczynski and McConaghy, 1989; Blaszczynski and Nower, 2002). According to their theory, increased somatic and cortical arousal, combined with irregular schedules of reinforcement, produce disordered gambling behavior. Blaszczynski et al. (1986) refined their theory by suggesting that disordered gamblers are innately high in arousal and gamble in order to reduce anxiety or dysphoric mood (Blaszczynski et al., 1986). Due to the difficulty associated with studying anxiety and gambling behavior in a controlled setting, there is currently a lack of consensus regarding the relationship between the two factors. Recent studies suggests that anxiety and negative affect play a crucial role in the development and maintenance of gambling behavior (Coman et al., 1997). In particular, trait-anxiety appears to be related to gambling severity (Rodda et al., 2004); however, some studies do not find this link (Blaszczynski and McConaghy, 1989; Burtontont et al., 2000).

Within the disordered gambling population, it appears as if GD, anxiety, and nicotine dependence converge to exert a trifecta of effects. Recent studies suggest that negative affect and mood, such as anxiety and depression, is crucial for the maintenance of both gambling (Blaszczynski and McConaghy, 1989; Coman et al.,

1997; Ste - marie et al., 2002) and nicotine behavior (Coman et al., 1997; Audrain et al., 1998; Rodda et al., 2004). Although the relationship between all three of the disorders is not thoroughly researched, one study suggests that gambling scores predict tobacco scores, and anxiety is positively correlated with smoking status, gambling score, and tobacco dependence (Rodda et al., 2004). However, further research is needed to elucidate the relationship between all three factors. Since gambling behavior cannot be separated from players' affective states, and since it frequently occurs with nicotine use, it is important to investigate the combined roles of all three factors (please refer to the review article by Blaszczynski and Nower (2002) for additional information regarding the effects of anxiety on gambling behavior).

Gambling Disorder in the Clinic: Treatment Options

A few of the most common options for GD treatment include group psychotherapy, conjoint marital therapy, psychoanalysis, brief therapy, behavioral counseling, cognitive restructuring, hypnotherapy, and pharmacological and physiological treatments. Of these options, the cognitive and behavioral treatments, which include problem solving, social skills training, and relapse prevention, seem to provide the most promise (Petry and Armentano, 1999). However, there is no standard intervention for GD, and long-term rates of abstinence are alarmingly low. In fact, an analysis of various treatment outcomes revealed that 50% of individuals remain abstinent at 6 months post-gambling involvement, approximately 29% remain abstinent after one year, and only 15% are abstinent after two years (Walker, 1993; Stinchfield and Winters, 2001). Gamblers Anonymous, the most popular form of

intervention, has an alarmingly low abstinence rate, with only 8% of patients remaining abstinent after one year of treatment (Petry and Armentano, 1999). A study conducted using a sample of disordered gamblers revealed that relapse to gambling behavior is most likely to occur within three months of treatment. Furthermore, the main predictors of therapeutic failure appear to be inadequate money management, negative emotional states, drug abuse, craving, social pressure, and anxiety (Parke and Griffiths, 2006). In fact, an analysis of patients at the Pathological Gambling Centre of Rentereía in Spain found that the only significant difference between patients who dropped out and successfully completed GD treatment was the diagnosis of anxiety (Echeburúa et al., 2001).

At the moment, gambling disorder treatments fail to successfully address comorbidity, cultural influences, and socio-demographic differences within the gambling population. Studies of epidemiology and comorbidity demonstrate a large degree of variation within the disordered gambling population, and such factors are crucial to the maintenance of maladaptive gambling behavior. In order to successfully treat GD, interventions should be highly individualized, and should address comorbid diagnoses (for a more comprehensive review of the available treatment options for gambling disorder, please refer to work by Lesieur and Blume (1991)).

Concluding Remarks

Several studies report a relationship between high levels of anxiety and maladaptive behaviors such as nicotine use and disordered gambling. Interestingly, anxiety appears to interact with both nicotine and disordered gambling involvement, and it is possible that nicotine use and disordered gambling interact with each other.

For example, the syndromic and naturalistic comorbidity of cigarette smoking and gambling suggest that both disorders operate under similar mechanisms, and may possibly incite the involvement of one another. Therefore, maladaptive gambling behavior, nicotine use, and high levels of anxiety might promote one another and exert a trifecta of effects. However, there appears to be an overall lack of understanding regarding the relationship between gambling disorder, nicotine dependence, and anxiety. In order to better understand the mechanisms that contribute to disordered gambling behavior, future studies will need to address all of the factors that contribute to the development and maintenance of gambling behavior. Studying the trifecta of GD, nicotine abuse, and anxiety diagnoses is a crucial next step in understanding the underpinnings of GD and developing effective treatments.

CHAPTER 2: THE NEUROCOGNITIVE UNDERPINNINGS OF GAMBLING DISORDER

The Allure of Gambling Games

Monetary gain is often considered to be the main motivator of gambling behavior. However, several studies refute this claim. It is a well-known fact that gambling wins are typically small, and while big wins do exist, they are often very rare. Information regarding the structural characteristics of games, their hit frequency, and the odds of winning are generally unavailable to the public. However, one study received reports through the Freedom of Information and Protection of Privacy Act, and aimed to elucidate the probability of wins in *Lobstermaina*, a typical slot machine game (Harrigan and Dixon, 2009). In *Lobstermaina*, each time a payer wagers \$3.75, or 75 credits (5 credits on all 15 lines of the game), there are 649,847 possible

outcomes. The chance of winning a prize greater than or equal to \$150 is one in every 399 spins. Therefore, if a player were to bet every three seconds, they would receive a large win (\geq \$150) once every 20 minutes. However, with a bet of \$3.75 per spin, the player would likely become broke before hitting the jackpot (Harrigan and Dixon, 2009). For this reason, very few individuals describe betting as a source of income. Instead, gamblers report an infatuation with the game itself. They describe betting as a pleasant opportunity, and gamble to fulfill an urge or to gain satisfaction. Wins, no matter how big or small, contribute to the feeling of satisfaction by reinforcing behavior and encouraging players to engage for longer periods of time.

The observation that gamblers find pleasure in betting has raised interest in understanding the factors that motivate play and encourage prolonged gambling involvement. While this is a difficult question to address, studies suggest that individuals gamble because they are attracted to the features and design that are characteristic to games of chance. One key feature hypothesized to promote gambling behavior is the inability to predict when rewards will be delivered, or how large the rewards will be—a concept known as reward uncertainty.

Humans appear to be innately attracted to uncertain outcomes, which might have developed as an adaptive mechanism to ensure survival. During our years as foragers, the location and abundance of food was highly variable. If our ancestors were ambivalent or dismayed by this unpredictability, they would have been less motivated to seek sustenance, and our race might have extinguished centuries ago (Anselme, 2013). Instead, our ancestors were driven to seek food, even when it was unavailable (Evans, 1969; Forkman, 1993). Furthermore, environmental cues that

signaled a shortage of food supply or possible famine, such as the migration of birds, motivated them to seek food with more vigor (Evans, 1969; Newton, 1969). In the laboratory, this behavior has been modeled using the Mongolian Gerbil (Forkman, 1993). When placed in an environment containing an intermittent food source, gerbils are motivated to eat greater amounts of food than those placed in a stable environment that contains superabundant food. Such evidence suggests that attraction to uncertainty might serve as a source of motivation, possibly designed to ensure survival (Forkman, 1993).

It is hypothesized that the gambling industry, in their design of highly uncertain reward outcomes, might activate our innate attraction to persevere in the face of uncertainty (Anselme, 2013). Reward uncertainty appears to be a key component of all gambling games. However, modes of gambling that are highly unpredictable and require no degree of skill, such as electronic gambling machines, contain the greatest degree of this quality. Electronic gambling machines are specifically designed to be unpredictable, with the greatest degree of reward uncertainty—a complete toss-up between wins and losses. Providing the greatest degree of reward uncertainty captivates players by reinforcing their behavior with small, intermittent monetary gains. Furthermore, it encourages the player to bet while simultaneously ensuring the financial advantage of the house. Some studies suggest that large degrees of uncertainty are why electronic gambling games, such as slots, are highly attractive and the preferred mode of gambling for 70% of the disordered gambling population.

The Importance of Cues

In the aforementioned discussion about uncertainty and the foraging habits of our ancestors, environmental cues played an important role in heightening the vigor of motivated behaviors (Evans, 1969; Newton, 1969; Forkman, 1993; Anselme, 2013). Cues such as the migration of birds, signal a change in the environment that could threaten the availability of food. Such cues served as motivators that encouraged humans to seek and store food in preparation for famine (Evans, 1969; Newton, 1969). In this example, the environmental cues provided information about the availability of resources. This most likely occurred through a process of associative learning in which past famines were accompanied by changes in animal behavior (Anselme, 2013). Through repeated associations, cues gained meaning and provided valuable information in and of themselves.

Ivan Pavlov became the first to describe associative learning when he serendipitously discovered that canines would salivate in response to stimuli associated with food, even in the absence of the food reward. In his experiments that were initially designed to investigate the physiology of digestion, a light was illuminated immediately before dogs received access to food. During the first few pairings, dogs were ambivalent to the light, but salivated at the sight of food. However, after several pairings, Pavlov noticed that the canines began to salivate at the illumination of the light alone. Interestingly, the dogs began to salivate at the sight of Pavlov, even when he was not providing food. Any object or event that the dogs associated with food gained value and triggered the physiological salivary response (Pavlov, 1941). With this discovery, Pavlov outlined the theory of classical

conditioning. According to classical conditioning, the initial pairing of a neutral stimulus (NS), such as a light, with an unconditioned stimulus (UCS), such as food, will produce an unconditioned response (UCR), such as salivation. However, with repeated pairings, the neutral stimulus becomes a conditioned stimulus (CS), capable of producing a conditioned response (CR), which was salivation, in the absence of the unconditioned stimulus (Pavlov, 1941).

The shift in a response from a UCS to a CS has been observed across a wide range of organisms, including pigeons, raccoons, monkeys, and humans. In one experiment, raccoons were conditioned to pick up a coin (UCS) and drop it into a container in order to obtain food (CS) (Breland and Breland, 1961). While the raccoon was initially able to complete the task, it appeared to develop mild difficulty as trials progressed. Instead of dropping the coin into a box, the raccoon held, rubbed, and chewed the coin (CR). He would reach towards the box to place the coin inside, but would retract and firmly clutch the coin for several seconds. Finally, the raccoon would place the coin inside the box to obtain food. In this example, the coin became a CS that produced appetitive, CR behaviors. Interestingly, these behaviors occurred although they interfered with the ability of the raccoon to obtain immediate rewards (Breland and Breland, 1961).

Incentive Sensitization Theory of Addiction

Nicotine, gambling, and other abused substances are rewarding for many individuals. However, only a subset of users will develop the compulsive pattern of drug-seeking and drug-taking that is characteristic to addiction. The theory of incentive sensitization describes the behavioral changes that are caused by rewards,

and explains how their repeated exposure might alter brain chemistry in susceptible individuals, ultimately leading to addiction (Robinson and Berridge, 1993, 2000, 2008b, a). According to the theory, repeated exposure to potentially addictive substances, under particular circumstances, causes persistent excitability of the brain circuits associated with the attribution of incentive salience. Incentive salience is a cognitive process that assigns a subconscious desire, called ‘wanting’ to rewards and the stimuli that are associated with them (Robinson and Berridge, 1993, 2008b, a; Berridge et al., 2009). The hyperexcitability of these brain circuits are thought to cause a hypersensitive (“sensitized”) system, which assigns a pathological degree of incentive salience to rewards and their cues. Interestingly, the process of ‘wanting’ is a physiological response that manifests as a subconscious attraction and desire, and will occur despite the fact that the user might not subjectively enjoy the reward itself (Robinson and Berridge, 2004; Robinson and Berridge, 2008b; Berridge et al., 2009). Repeated drug use appears to sensitize the motivational process of incentive salience, but does not affect the systems that mediate liking for a reward (Robinson and Berridge, 1993). This dissociation between the motivation to obtain rewards and feelings of subjective liking is an important characteristic of addiction, and a crucial concept of the incentive sensitization theory of addiction (Robinson and Berridge, 1993, 2008b, a).

The brain circuit thought to assign incentive salience to rewards is known as the mesolimbic dopamine reward system. Such a system is comprised of dopaminergic fibers that project from the medial forebrain bundle (comprised of fibers in the brainstem and ventral tegmental area) to limbic and cortical structures

(such as the nucleus accumbens) (Robinson and Berridge, 1993; Di Chiara, 1998). The mesolimbic system is implicated as a common neural substrate contributing to the self-administration of a wide range of addictive substances including nicotine, caffeine, barbiturates, alcohol, benzodiazepines, cannabis, and phencyclidine (Wise and Bozarth, 1987; Benwell et al., 1988; Di Chiara and Imperato, 1988). In support of the incentive sensitization theory, a vast body of evidence, in both rodents and humans, suggests that the mesolimbic system is preferentially active during exposure to rewards (Di Chiara and Imperato, 1988; Corrigall et al., 1994; Berridge and Robinson, 1998; Balfour et al., 2000). Sensitization caused by repeated drug use has been observed with many drugs of abuse, including nicotine (Benwell et al., 1988; Cadoni and Di Chiara, 2000; Laviolette and Van Der Kooy, 2004; Govind et al., 2009). For example, repeated exposure to acute nicotine administration has been shown to increase dopamine release within the core of the nucleus accumbens during a microdialysis experiment (Cadoni and Di Chiara, 2000). Therefore, repeated nicotine administration sensitizes the reward system and makes it hyper-responsive to the effects of subsequent administration. Furthermore, it is thought that reward uncertainty, like drugs of abuse, might sensitize animals to rewards and reward-related cues (Anselme et al., 2013; Robinson et al., 2014). When experienced together, nicotine and reward uncertainty might exert a dual-effect on the mesolimbic reward system, known as cross-sensitization. Cross-sensitization occurs when the enhanced motivational effect of one reward reinforces a second reward, causing an increase in the attribution of incentive salience for the second reward (Robinson and Berridge, 1993, 2000). The effects of cross-sensitization are seen in studies that

simultaneously administer two potent drugs. For example, the administration of caffeine and amphetamine increases locomotor activity in rats previously exposed to nicotine (Celik et al., 2006). Locomotor activity is a known behavioral measure for sensitization. Considering that nicotine and reward uncertainty separately excite the mesolimbic reward system, it is possible that the sensitization caused by nicotine might cause enhanced incentive salience to uncertainty, and vice versa, when both rewards are experienced by the same individual.

Cues in the Gambling Setting

The gambling setting is filled with cues such as flashing lights and celebratory jingles (Griffiths, 1993; Parke and Griffiths, 2006; Noseworthy and Finlay, 2009). While these cues are initially neutral stimuli, repeated gambling activity and pairing of cues to rewards might transform them into conditioned stimuli (Flagel et al., 2009). Through the process of classical conditioning, gambling cues in and of themselves are capable of producing urges to play, and this has been consistently portrayed in the literature (Symes and Nicki, 1997; Potenza et al., 2003; Kushner et al., 2008; Wulfert et al., 2009). In one study, individuals that played rounds of blackjack in a gambling-like environment reported greater gambling urges than individuals that played in a neutral context (Kushner et al., 2008). Similarly, an fMRI study that required disordered gambling men and healthy controls to view a video of a neutral or gambling scenario found increased attraction to cues in the gambling setting. GD men that viewed the gambling scenario showed increased activation in the right cuneus and right middle occipital gyrus, regions previously shown to be involved in emotional and motivational responses (Potenza et al., 2003). Interestingly, the effects

of cue-induced craving extend past the physical casino environment and to the virtual gambling environment. One study analyzed reports of craving in virtual casino environments and found gambling cues to increase subjective reports of craving in recreational gamblers (Park et al., 2015). In all, research suggests that cues can become instigators of gambling behavior. Therefore, games with a large number of cues or a high prevalence of flashing lights and sounds, such as electronic gambling machines, are risky, and might be particularly capable of eliciting motivated play.

In addition to driving behavior, cues provide an added element of uncertainty to the gambling setting (Anselme and Robinson, 2013). Reward uncertainty is heightened by the fact that cues do not reliably predict reward outcome (Anselme and Robinson, 2013; Robinson et al., 2014; Anselme, 2015). For example, in games of chance, such as slot machines, celebratory sounds may be present in win outcomes where the player receives a reward greater than their wager, as well as in loss outcomes when the player receives a reward less than their wager (Griffiths, 1993; Dixon et al., 2010). Since cues are salient and capture players' attention, they may distort perception of events, causing individuals to associate cues with wins despite their potential loss status (Dixon et al., 2010; Spewyn et al., 2010). In doing so, cues can become powerful motivations that leverage the effects of uncertainty to promote gambling behavior (Anselme and Robinson, 2013).

The Role of Cues in Nicotine Reinforcement

In the case of cigarette smoking, users do not just self-administer nicotine—the environment is an important aspect of the drug-taking process, and they do so within a context of various drug-related stimuli (Robinson and Berridge, 1993;

Caggiula et al., 2002). Objects such as ashtrays, lighters, and other smokers frequently accompany nicotine use (Caggiula et al., 2002). Through repeated cigarette smoking, nicotine is thought to increase the attraction for stimuli associated with drug taking (Rose and Levin, 1991; Berridge and Robinson, 1998; Berridge, 2003; Berridge and Robinson, 2003; Flagel et al., 2009; Versaggi et al., 2016).

Evidence of reactivity to nicotine-associated cues is used to explain the maintenance and relapse of cigarette smoking (Juliano and Brandon, 1998). Due to their motivational effects, nicotine cues are known to produce a wide range of physiological reactions in smokers (Barr et al., 1983; Perkins et al., 1994; Droungas et al., 1995; Juliano and Brandon, 1998; Dols et al., 2000; Wertz and Sayette, 2001; Chiamulera, 2005). A few of these subjective and autonomic responses include increased craving, heart rate, and skin conductance (Carter et al., 2006). For example, Perkins et al. (1994) demonstrated that exposure to a lit cigarette increased craving for nicotine in cigarette smokers. Following overnight abstinence, smokers performed a task to receive a puff of cigarette smoke while in the presence of a lit cigarette on an ashtray, which served as an environmental cue. The lit cigarette reinforced the value of smoking, and increased engagement with the task in order to receive a puff of nicotine (Perkins et al., 1994). Similarly, the ability of cigarette cues to evoke a psychological response was measured in a study assessing self-reports of cigarette craving. Nicotine-deprived and non-deprived participants were shown a series of positive, negative, neutral, and cigarette photos, as they provided self-reports of nicotine craving (Carter et al., 2006). Both deprived and non-deprived participants showed significantly enhanced craving in response to nicotine images. However, non-

deprived participants showed a slightly greater response compared to those that were deprived. Therefore, smoking associated stimuli mediate urges to smoke and cravings for cigarettes (Niaura et al., 1988; Carter and Tiffany, 1999)

In addition to increased craving, numerous experiments suggest that nicotine related cues shift focus and command attention (Mogg and Bradley, 2002; Bradley et al., 2003). In one study, smokers' gaze to neutral and nicotine associated cues was assessed as a measure of visual orienting and salience. Smokers maintained a longer gaze for images associated with smoking compared to non-smoker controls. Furthermore, smokers were fixated on nicotine related images for longer periods of time compared to neutral images, and fixations were positively correlated with increased urges to smoke (Mogg et al., 2003). These observations have been replicated in several studies, which all demonstrate the attention-grabbing effects of nicotine stimuli. Such data provides evidence for a bias in attention towards smoking related cues in cigarette smokers. This bias is thought to increase motivational valence of stimuli during administration, thus promoting the salience of cues (Mogg and Bradley, 2002; Bradley et al., 2003; Mogg et al., 2003).

Interestingly, cue attraction appears to differ across demographic populations. For example, laboratory studies have demonstrated sex-dependent differences in attraction for nicotine cues (Field and Duka, 2004). In one experiment, male and female subjects were exposed to smoking paraphernalia and neutral cues while their subjective and physiological responses were recorded (Field and Duka, 2004). Smoking paraphernalia increased levels of skin conductance in all participants, irrespective of sex. However, for females exclusively, smoking paraphernalia

increased craving for nicotine, and increased salivary response. In males, cigarette cues decreased salivation (Field and Duka, 2004).

The gambling population is thought to be particularly susceptible to the risks associated with cue-induced attraction to nicotine (Petry and Oncken, 2002; McGrath and Barrett, 2009). While rates of smoking across the general population have substantially decreased over the years, the population of gamblers appears to be a faction with relatively little changes in the prevalence of cigarette smoking (Petry and Oncken, 2002; Rodda et al., 2004). This is possibly due to the fact that the casino environment has many smoking cues such as cigarettes themselves, cigarette smoke, pictures of lit cigarettes, and ashtrays. Considering the large co-occurrence of smoking and gambling behavior, the casino environment appears to be an intense cue for smoking, and similarly, smoking appears to be an extreme cue for gambling (Petry and Oncken, 2002). Therefore, the nicotine cues and gambling cues are likely associated with similar environments, and it is possible that cues can drive craving for both behaviors (Petry and Oncken, 2002).

There is evidence to suggest that nicotine may enhance the rewarding and reinforcing properties of gambling games; however, such studies are inconclusive. In one experiment, daily smokers demonstrated significantly reduced responsiveness to a financially incentivized card-sorting task after a period of overnight nicotine-abstinence (Dawkins et al., 2006). Similarly, smokers who are given nicotine lozenges before a gambling task exhibit greater pleasure and enhanced responsiveness compared to smokers given placebo (Dawkins et al., 2006). In another experiment, non-smokers who were given nicotine patches experienced increased responding

towards positive cues and monetary reward compared to those given placebo (Barr et al., 2008). On the contrary, one study found that non-dependent smokers given acute doses of nicotine, administered nasally or via cigarette smoking, did not exhibit an increase in the number of responses in a task reinforced by money, music, and the termination of an aversive noise (Perkins and Karelitz, 2013). These findings suggest that nicotine may enhance the reinforcement value of stimuli that are not associated with smoking, and in the gambling setting, these stimuli would be gambling cues. Since nicotine and gambling occur at both the syndromic and event level, it is important to understand the mechanisms that contribute to their relationship, and possible enhanced reinforcing properties. Given the lack of consensus regarding the effects of nicotine on reward, it is important for future research to address this topic (Please refer to the review by McGrath and Barrett (2009) for a complete discussion of the comorbidity between gambling disorder and nicotine use).

Concluding Remarks

Several studies report a high comorbidity between gambling disorder and nicotine use at the syndromic and event level (Petry and Oncken, 2002; McGrath and Barrett, 2009). In the gambling setting, betting cues and nicotine cues may work synergistically to drive motivation to smoke and gamble, and the cross-sensitization of reward pathways caused by both factors might accelerate and worsen the pathology of gambling disorder. Both reward uncertainty and nicotine exposure appear to enhance attraction for cues (Barr et al., 1983; Perkins et al., 1994; Droungas et al., 1995; Juliano and Brandon, 1998; Dols et al., 2000; Wertz and Sayette, 2001; Chiamulera, 2005; Anselme and Robinson, 2013; Anselme et al., 2013; Robinson et

al., 2014; Anselme, 2015). However, no study to date has investigated the combined effects of these stimuli and their associated cues on attraction or motivated behaviors. It is important for research to investigate the combined effects of uncertainty and nicotine on motivation for gambling-like cues.

CHAPTER 3: THE VALUE OF RODENT MODELS IN ELUCIDATING THE MECHANISMS OF GAMBLING DISORDER

Modeling Attraction for Cues

Using humans to study the effects of reward uncertainty, nicotine use, and cue attraction, presents an ethical dilemma. For one, it is problematic to ask players to gamble in the laboratory setting. Secondly, asking problem and disordered gamblers to engage in maladaptive behaviors might worsen their symptoms (Peters et al., 2010). For this reason, animal models were developed as an ethical way to manipulate gambling-like settings and to measure the effect of these manipulations on maladaptive behavior (Madden et al., 2007; Weatherly and Derenne, 2007; Peters et al., 2010).

A method commonly used to study attraction to cues, called autoshaping, is a Pavlovian conditioning technique that pairs a reinforcer with an unconditioned stimulus in order to capture the incentive salience attributed to the CS (Flagel et al., 2007). In the classic paradigm, a CS lever is paired with a UCS palatable sucrose reward, and rodent behavior during cue presentation is measured. Although no action is required to obtain the reward, rats typically perform one of three behaviors in the presence of the CS. Rats will either approach, vigorously sniff, nibble, or press the CS

lever, known as sign-tracking, perform a similar interaction with a goal dish where the sucrose pellet is delivered, known as goal-tracking, or will exhibit a mixture of both sign-tracking and goal-tracking, known as intermediate behavior (Flagel et al., 2009; Robinson et al., 2014). Sign-tracking is conventionally operationalized as a measure of incentive salience for the predictive cue (Flagel et al., 2009; Anselme et al., 2013; Robinson et al., 2014).

A study conducted by Anselme et al. (2013) investigated the effects of uncertain reward contingencies on attraction to cues associated with reward uncertainty. Results demonstrated the greatest sign-tracking for conditions high in reward uncertainty paired with variable reward magnitude, where one, two, or three sucrose pellets was delivered during half of the trials, than in certain conditions where one pellet was consistently delivered during each trial. In addition, conditions high in reward uncertainty paired with uncertain reward magnitude led to greater cue attraction than in conditions high in reward uncertainty paired with certain reward magnitude, where two pellets were delivered during half of the trials (Flagel et al., 2009; Robinson et al., 2014). Together these results suggest increased incentive salience for conditions associated with uncertainty in reward delivery paired with variable reward magnitude.

Modeling the Reinforcing Power of Cues

Cues such as celebratory jingles and flashing lights are used to enhance the attractiveness of gambling games, and several studies hypothesize that these cues might entice players by increasing confidence, modulating arousal, and appealing to their sense of enjoyment (Griffiths, 1993; Griffiths and Dunbar, 1997; Spenwyn et al.,

2010). In addition to being attractive, it is possible that cues become ‘wanted’ in and of themselves, even in the absence of reward. Conditioned reinforcement tasks are used to measure ‘wanting’ for cues associated with reward (Berridge et al., 2009). In these experiments, a cue that is made a conditioned stimulus through Pavlovian conditioning becomes contingent on a novel behavior in the absence of the primary reinforcer (UCS) (Kelleher and Gollub, 1962; Williams, 1994). The animal is required to perform a novel behavior in order to gain access to solely the CS cues in the absence of reward, and the animals that acquire this novel behavior are said to show conditioned reinforcement behaviors (Williams, 1994).

Burrhus F. Skinner was one of the first scientists to demonstrate conditioned reinforcement (Kelleher and Gollub, 1962). In his experiment, rats were trained on a Pavlovian task to approach a food dish at the sound of the pellet dispenser, which predicted the delivery of food. After rats acquired this behavior, the pellet dispenser and food were removed from the contingency and replaced with a lever. Skinner found that rats learn to press the lever for access to the sound of the pellet dispenser, in the absence of food (Kelleher and Gollub, 1962). His results suggested that cues alone become valuable, wanted, and capable of producing a new, motivated behavior. In addition to lever pressing, rats will acquire a nose poke behavior in order to gain access to cues. Therefore, the motivational property of cues associated with nicotine and reward uncertainty can be determined using a conditioned reinforcement task. Past studies have shown that nicotine increases responding during conditioned reinforcement tasks (Olausson et al., 2004). However, to our knowledge, no study has investigated the effects of combined nicotine administration and reward uncertainty

on the conditioned reinforcer property of associated cues (refer to Williams (1994) for a review of conditioned reinforcement and classical conditioning).

Progressive Ratio as a Measure of Reward Strength

Progressive ratio is a well-known paradigm used to measure the strength of a reinforcer (Hodos, 1961; Richardson and Roberts, 1996). The technique requires rats to emit an increasing number of responses in order to obtain a single reinforcement, which systematically multiplies after each trial. Progressive ratio schedules of drug delivery are typically used to evaluate the strength of the drug reward because they require animals to exert effort, or work, to obtain the reinforcer. Reward strength is measured as the breaking point of the rodents behavior, which is the number of trials completed before the animal fails to complete the ratio necessary to obtain reinforcement (Hodos, 1961; Richardson and Roberts, 1996). Several studies have evaluated the rewarding properties of nicotine using such tasks (Donny et al., 1999; LeSage et al., 2006; Palmatier et al., 2012). One study found that nicotine administration increases rats willingness to work for a sucrose reward (Palmatier et al., 2012). However, no study to date has investigated the will to work for rewards paired with the combination of nicotine and reward uncertainty.

Measuring Anxiety in Rats

Rodent levels of anxiety are typically measured using the Elevated Plus Maze (EPM), a known assay for the investigation of rodent anxiety behaviors (Pellow et al., 1985; Rodgers and Dalvi, 1997). The Elevated Plus Maze consists of four narrow planks arranged in an intersecting fashion to form a plus (+) shape symbol. Two opposing arms are enclosed with tall dark walls, and two opposing arms are well lit

and exposed, without walls. At the start of the experiment, rats are placed in the center of the maze and behavior is recorded for a period of five minutes. Behavior is typically recorded on an overhead video camera device, and videos are used for later analysis.

The Elevated Plus Maze uses rodents' affinity for dark enclosed spaces to quantify levels of anxiety. Analysis of the session consists of recording the amount of time the rats spend in the open versus the closed arms over a period of five minutes. Rats that spend more time in the closed arms are said to have high levels of anxiety. Conversely, rats that spend a relatively greater amount of time in the open arms of the maze are considered to have low levels of anxiety.

Open arm versus closed arm exploration has been correlated to biological indicators of anxiety in rats (Süer et al., 1998; Rodgers et al., 1999). Pain latencies, skin conductance levels, and plasma corticosterone titres are shown to be significantly elevated in rats exposed to the EPM (Süer et al., 1998; Rodgers et al., 1999). Furthermore, plasma corticosterone titres are shown to be significantly elevated with increased exposure to the open arms (Rodgers et al., 1999). Since corticosterone is a glucocorticoid released by the adrenal cortex in times of stress, the Elevated Plus Maze is a validated behavioral measure of anxiety in rats.

The Present Study

Here, we sought to determine how nicotine exposure and reward uncertainty might contribute to the attribution of incentive salience and motivation for reward cues, which are associated with the development of disordered gambling behavior.

Specifically, we used a rodent model to study the effects of acute nicotine injections and uncertain reward outcomes (probability and magnitude) on attraction to gambling-like cues in autoshaping, conditioned reinforcement, and progressive ratio tasks. A second goal of this study was to determine the effects of reward uncertainty on anxiety behaviors, and the effects of anxiety behavior on the attribution of incentive salience to uncertain reward cues. There is substantial evidence for the role of anxiety as a risk factor for GD (Blaszczynski et al., 2008). We use the Elevated Plus Maze, a standard behavioral measure of rodent anxiety (Rodgers and Dalvi, 1997), to compare anxiety levels before and after conducting autoshaping and operant tasks. Evidence suggests that female EPM activity is unreliable due to fluctuations in hormones caused by the estrous cycle (Marcondes et al., 2001). However, we study EPM behavior in both male and female animals in attempt to have a sample that is representative of the population. In addition to studying the combined effects of nicotine exposure and reward uncertainty on levels of anxiety, we analyzed sex-dependent differences within our results.

Anxiety was measured at two time points during our study: before conditioning (pre-conditioning) and after conditioning (post-conditioning). Pre-conditioning levels were used to assign rats to one of four experimental conditions so that average anxiety levels were matched across all groups: (1) saline exposure and reward certainty, (2) nicotine exposure and reward certainty, (3) saline exposure and reward uncertainty, (4) nicotine exposure and reward uncertainty. After the experiment, pre- and post-conditioning data was compared to determine the effects of gambling-like conditions on rodent anxiety behavior.

In our autoshaping task, male and female rats were trained to associate the presentation of a lever, light, and tone (CS) with the delivery of sucrose pellets (UCS). Attraction to the CS cues was evaluated across sessions, and quantified as lever presses (sign-tracking) and magazine entries (goal-tracking). A conditioned reinforcement task was used to measure the operant responding for cues by determining if the animal will acquire a novel behavior, such as nose-poking, to obtain the CS cues. Following conditioned reinforcement, animals were trained on a progressive ratio task, which measured reward strength by requiring the animal to perform an increasing number of operant responses for each successive sucrose pellet reward.

Based on the literature, we hypothesized that nicotine exposure and reward uncertainty would enhance attraction to CS cues, as observed by an increase in sign-tracking and decrease in goal-tracking during the autoshaping task. Secondly, we hypothesized that the CS cues would become reinforcing, and that rats would acquire a novel nose poke behavior in order to access the lever and tone during conditioned reinforcement. In addition, we predicted that nicotine exposure and reward uncertainty would increase motivation for the sucrose pellet during the progressive ratio task. Lastly, we expected our Pavlovian and operant tasks to increase anxiety in rats assigned to uncertain autoshaping conditions, and to decrease anxiety in rats assigned to the certain autoshaping conditions. Furthermore, we expected rats injected with nicotine to exhibit heightened post-conditioning anxiety levels.

MATERIALS AND METHODS

SUBJECTS AND HOUSING CONDITIONS

Subjects were eighty adult (10-18 weeks old) Sprague-Dawley rats (female, n = 40; male, n = 40) bred in-house and weaned at postnatal day 21. Prior to the start of the experiment, rats were housed by sex in groups of 2-3 animals per cage with *ad libitum* access to water and Purina Chow. Cages were stored in climate controlled rooms, and housing rooms were regulated to a 12:12 hour reverse light:dark cycle, with white lights off at 8:30 am and white lights on at 8:30 pm. Approximately 6-8 days prior to the start of the experiment, animals were handled and habituated to the experimenter for a period of 2-3 days. After habituation, approximately 3-5 days prior to the start of the experiment, rats were food-restricted to 8-14 grams of Purina Chow per subject (female, 8-12 grams; male, 12-14 grams), or approximately 85-90% of initial body weight, and all rats received *ad libitum* access to water for the duration of the experiment. After each day of testing, rats were returned to their home cage and were given 8-14 grams of chow. All procedures were approved by the Institutional Animal Care and Use Committee at Wesleyan University.

APPARATUS

Elevated Plus Maze

The Elevated Plus Maze is a known behavioral measure used to index levels of anxiety in rodents. The apparatus contained two platforms that intersected to form a plus-shaped symbol (+) with two sets of perpendicular, alternate arms (fig. 1). Two alternate arms (96.4 x 15.3 cm) were partially enclosed by dark, non-transparent walls

(96.4 x 15.3 x 41.1 cm), and two alternate arms (96.4 x 15.3 cm) were exposed, without walls. The center of the maze, located at the intersection of the four arms, was open, without walls. The apparatus was exposed on top and elevated (96.8 cm) above the floor. An infrared video camera (Advidia™) was placed above the apparatus and was used for the visualization and manual recording of behavior under red light conditions. Experimentation was conducted in a designated red-light room.

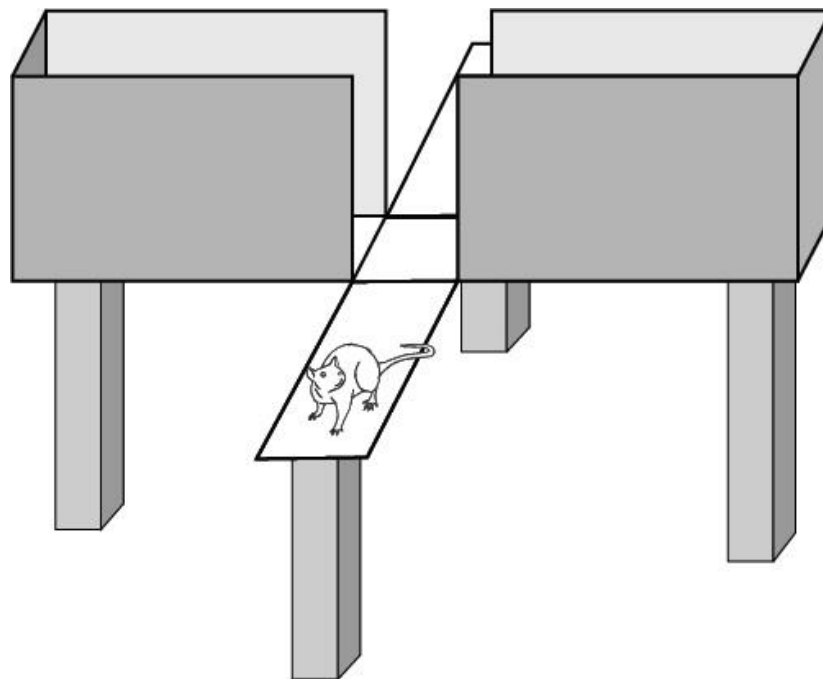


Figure 1: Elevated Plus Maze. Schematic of the Elevated Plus Maze apparatus, which consisted of two closed and two open arms. Both arms were elevated above the floor and the closed arms were partially enclosed by non-transparent walls. The open arms were exposed, without walls. The Elevated Plus Maze leverages rats natural fear of open space, and attraction to small dark spaces, in order to measure levels of anxiety from behavioral observation. During a 5-minute session, interaction with the open and closed arm was assessed, and rats that spent a greater amount of time in the open arms were said to have low levels of anxiety. Conversely, rats that rarely traveled to the open arms of the maze were said to have high levels of anxiety. Here, the rat is shown on the open arm of the maze, which indicates lower levels of anxiety.

Autoshaping Chamber

Autoshaping was conducted in Med Associates Inc. Modular Test Chambers (25.8 x 32.2 x 33.2 cm) (fig. 2). The door and far wall of the chamber were made from clear polycarbonate. The front and back walls were made from removable aluminum inserts arranged as three adjacent columns. The front wall contained two metal levers and one metal magazine dish, which were elevated from the base of the apparatus (levers, 6 cm; magazine, 3 cm). The magazine was located in the center column of the front wall, and the levers were located in the two metal columns parallel to either side of the magazine dish. One lever, termed the inactive lever, measured baseline lever attraction (fig. 2). The inactive lever, which was used to assess baseline lever attraction, was available for the duration of the session, and never retracted. The second lever, termed the active lever, served as a conditioned stimulus (CS), which predicted the delivery of sucrose pellets. During each trial, the active lever extended from the wall and into the chamber for 8 seconds prior to the delivery of the pellet reward. The position of the active and inactive lever was counterbalanced so that half of the subjects received an active lever located proximal to the test chamber door, while the other half of the subjects received an active lever located proximal to the far wall of the chamber. The active lever was accompanied by an auditory tone and light cue. Each autoshaping sessions contained 36 trials. The intertrial-interval was variable, averaging to 45 seconds. Subjects completed autoshaping sessions once per day for a total of 10 days. Sessions for all conditioning tasks lasted for 30 minutes. Lever presses and trials were automatically recorded by the MedPC program. All chambers used in our study contained a stainless-steel grid floor with a waste pan. An infrared video recorder was placed above the testing

chambers, and was used for the visualization and recording of behavior. Chambers were placed in sound-attenuating boxes in a designated red-light testing room.

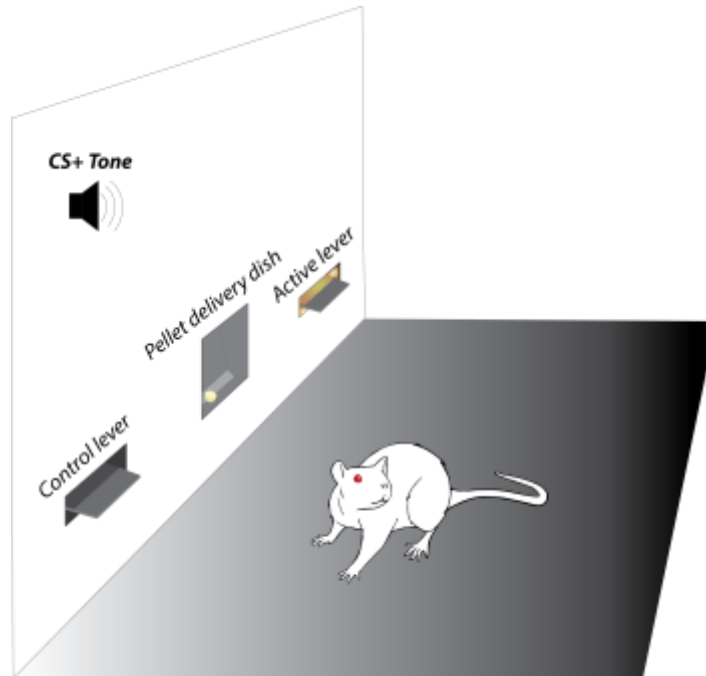


Figure 2: Autosshaping Chamber. The autosshaping chamber consisted of two retractable levers, a tone and light cue, and a magazine dish. The inactive (control) lever was available for the duration of the 30-minute autosshaping experiment. The active lever and cues became available for 8 seconds during each trial, and were reinforced with sucrose delivery under specific conditions. Sucrose pellets were delivered to the magazine dish, which was equidistant from the active and inactive levers (illustration created by Ellen Lesser).

Conditioned Reinforcement Chamber

After autosshaping, the Med Associates Inc. Modular Test Chambers were reconstructed so that the levers and magazine dish were removed from the front wall of the apparatus, and were replaced with metal plates (fig. 3). The back wall was reconfigured to contain one metal lever and two nose pokes, which were elevated from the base of the chamber (lever, 6 cm; nose pokes, 4.5 cm). The lever was located in the center column of the back wall, and the nose pokes were located in the two metal columns parallel to either side of the lever. Both nose pokes were illuminated

with a muted yellow light. One nose poke, termed the inactive poke, served as a control that measured baseline attraction to the nose poke. The second nose poke, termed the active poke, served as a reinforcer that provided access to the lever and cues, which were present for a duration of 3 seconds (fig. 3). The position of the active and inactive nose poke was counterbalanced so that half of the subjects received an active poke that was proximal to the test chamber door, while the other half of the subjects received a poke located proximal to the far wall of the chamber.

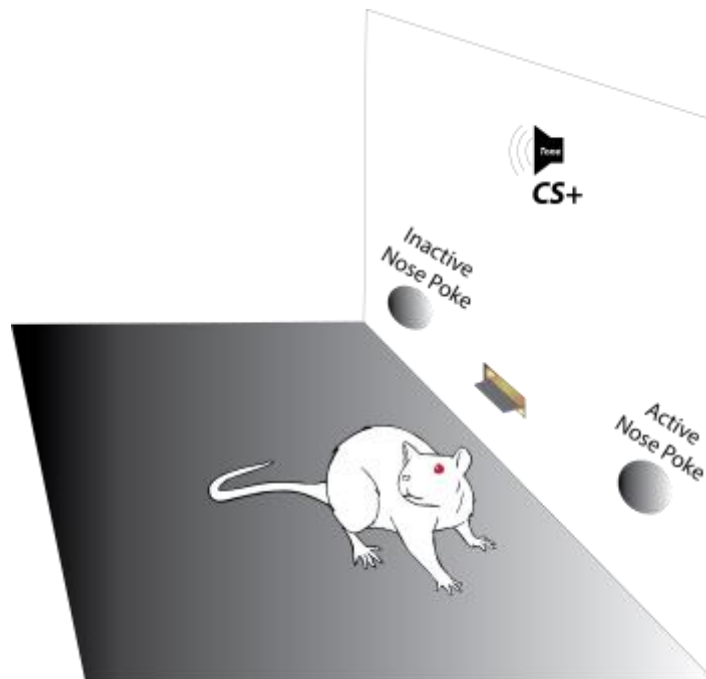


Figure 3: Conditioned Reinforcement Chamber. The conditioned reinforcement chamber consisted of two nose poke holes and the tone, light, and lever cues associated with the autoshaping procedure. Both nose pokes were illuminated for the duration of the experiment. The active nose poke provided access to the tone, light, and lever cues for a duration of 3 seconds. The inactive hole served as a control that measured baseline attraction to the nose pokes (illustration created by Ellen Lesser).

Progressive Ratio Chamber

After conditioned reinforcement, the chambers were reconfigured to match the autoshaping setup. The front wall contained two metal levers and one metal magazine dish. The magazine dish was located in the center of the wall, with one lever on each

side. The active lever used during the autoshaping task remained active during progressive ratio. Similarly, the inactive lever used during autoshaping remained inactive during the progressive ratio task.

PROCEDURE

Experimental Timeline



Figure 4: Experimental Timeline. An overview of the progression of tasks and procedures conducted throughout the experiment.

Elevated Plus Maze

Rats were placed in the center of the maze, with the head and tail facing the open arms. Exploratory behavior was assessed for a period of five minutes. After each trial, the apparatus was cleaned with 90% Fisherbrand™ Versa-Clean™ Multi-Purpose Cleaner. Rats were returned to their home cage and given 8-14 grams of Purina Chow. Videos were manually assessed for the length of time spent in the open, closed, and center regions of the maze. Longer open arm exploration times were associated with lower levels of anxiety.

Autoshaping

The autoshaping procedure consisted of one day of sucrose exposure, one day of magazine training, and ten days of pavlovian conditioned approach (fig. 4). ***Sucrose Exposure.*** Two days prior to the first autoshaping session, 30-50 sucrose pellets were placed in the home cage, to reduce neophobia and habituate the rats to sucrose

consumption. **Magazine Training.** One day prior to autoshaping, rats received a magazine training session. During the 30-minute training session, rats were habituated to the environment of the testing chamber and collected 30 sucrose pellets from the magazine dish. Pellets were delivered on a variable intertrial-interval, averaging to 45 seconds. **Autoshaping Sessions.** Rats received one autoshaping session per day for a period of 10 days. Each session consisted of 36 trials, and lasted for approximately 30-35 minutes. Rats were trained to associate a metal lever (CS) with the delivery of a sucrose pellet (UCS). During each trial, an 8 second auditory tone was delivered while an illuminated lever extended into the chamber. After 8 seconds, the tone silenced, the lever retracted, and sucrose pellets fell from an overhead dispenser into the metal magazine dish. Although the delivery of reward was independent of behavior, rats develop two distinct conditioned responses (CR): sign-tracking and goal tracking. Sign-tracking was observed as a compulsive responding towards the lever cue, quantified as presses on the CS lever. Goal-tracking was observed as the preoccupation with the magazine dish, quantified as head dives into the magazine dish. Pellets were dispensed according to two reward contingencies: 100%-1 and 50%-1-2-3. In the 100%-1 reward contingency, each CS presentation, during every trial, resulted in the delivery of 1 sucrose pellet to the magazine dish. In the 50%-1-2-3 reward contingency, half of the CS presentations (18 trials) resulted in the delivery of 0 sucrose pellets, while the other half of the CS presentations (18 trials) resulted in the delivery of 1, 2, or 3 sucrose pellets. The 50%-1-2-3 reward contingency created uncertainty in the probability and magnitude of reward delivery. However, despite the reward contingency, all rats received 36 pellets

by the end of each autoshaping session, and were therefore equally exposed to the CS cues and rewards. After autoshaping, rats were removed from the testing chamber, and the apparatus was cleaned with 90% Versa-Clean™.

Conditioned Reinforcement

After autoshaping, rats completed a one-day conditioned reinforcement task, which evaluated the incentive value of the CS lever. Conditioned reinforcement required rats to acquire a novel nose poke behavior in order to obtain the previously incentivized lever. Nose pokes were illuminated and contained an infrared detector. The auditory and tone cues became active when rats inserted their nose into the active poke, and the lever extended from the wall and into the chamber for a period of 3 seconds. The number of active pokes, inactive pokes, lever presentations, and lever presses were recorded for the duration of the session. The conditioned reinforcement task lasted for 30 minutes. After the session, rats were removed from the testing chamber, and the apparatus was cleaned with 90% Versa-Clean™. Rats were returned to their home cage and were given 8-14 grams of Purina Chow.

Progressive Ratio

The progressive ratio procedure consisted of one day of fixed ratio 1 training, one day of random ratio 2 testing, and one day of progressive ratio testing (fig. 4).

Fixed Ratio 1. The fixed ratio (FR) reward contingency required rats to execute one lever press for the delivery of one sucrose pellet. During the one-day task, rats freely pressed a metal lever for sucrose pellets until the end of the 30-minute session.

Random Ratio 2. Following the FR1 task, rats completed a two-day random ratio reward contingency (RR) task. The RR2 program required rats to press a lever an

unspecified number of times to obtain a single sucrose pellet. While the number of presses required for the delivery of a pellet was random, an average of two lever presses was required to obtain a single reward. Rats freely pressed the metal lever for sucrose pellets until the end of the 30-minute training session. ***Progressive Ratio Session.*** After RR2, rats completed a one-day progressive ratio task. Progressive ratio assessed rats willingness to expend effort in order to obtain a sucrose reward. The number of presses required to obtain a single sucrose pellet increased on an exponential progressive ratio schedule (1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95 ...) determined by the equation (progressive ratio = $[5e^{(\text{reward number} \times 2)}]-5$). The highest number of lever presses completed to obtain a single sucrose pellet, known as the breaking point, signified the value of the reinforcer.

Drugs

Saline. All rats, irrespective of group assignment, received a saline injection 15 minutes prior to the start of the magazine training session. Rats assigned to the saline autoshaping groups (saline injection, 100%-1 reward contingency; saline injection, 50%-1-2-3 reward contingency) received one subcutaneous saline injection prior to each autoshaping, conditioned reinforcement, FR1, RR2, and PR session. Each injection was given 15 minutes prior to the start of testing. After each injection, rats were placed in the home cage. ***Nicotine.*** (-)-Nicotine hydrogen tartrate salt obtained from Glentham Life Sciences was administered at a dose of 0.3 mg/kg, and a pH of 7. Rats assigned to the nicotine groups (nicotine injection, 100%-1 reward contingency; nicotine injection, 50%-1-2-3 reward contingency) received one

subcutaneous nicotine injection 15 minutes prior to each of the aforementioned training sessions, and were returned to the home cage until the start of testing.

GROUPS

Autoshaping Groups

Baseline anxiety data, measured using the Elevated Plus Maze, was used to assign animals to one of four experimental conditions: (1) saline injection, 100%-1 autoshaping reward contingency; (2) saline injection, 50%-1-2-3 autoshaping reward contingency; (3) nicotine injection, 100%-1 autoshaping reward contingency; (4) nicotine injection, 50%-1-2-3 autoshaping reward contingency. Rats were grouped according to litter, sex, and baseline anxiety levels so that anxiety was matched across all groups, and within each sex.

STATISTICAL ANALYSIS

Data was analyzed using IBM SPSS Statistics Software, and graphs were created using GraphPad Prism. Analysis of Variance (ANOVA) was used to analyze day and group effects of repeated measure data. One-way ANOVA, repeated measures ANOVA, unpaired T-test, and paired T-test was used to analyze data. Due to fluctuations in the behavior of animals across all conditions on day 10 of autoshaping, only days 1-9 of the autoshaping procedure were used to analyze behavior.

RESULTS

PRE-CONDITIONING ANXIETY LEVELS

Average pre-conditioning anxiety levels were similar across all conditions.

Prior to the start of autoshaping, levels of anxiety were measured using the Elevated Plus Maze. The duration of time spent in both open arms of the maze was operationalized as a measure of anxiety, and rats that spent a greater amount of time in the open arms were said to be less anxious than rats that spent less amounts of time in them. Open arm time was used to assign rats to one of four conditions so that the pre-conditioning anxiety levels were matched across all groups: saline certain (100%-1): 60.90 ± 10.57 s, $n = 20$, saline uncertain (50%-1-2-3): 65.75 ± 10.18 s, $n = 20$, nicotine certain (100%-1): 61.15 ± 7.50 s, $n = 20$, and nicotine uncertain (50%-1-2-3): 64.20 ± 9.74 s, $n = 20$. Animals were assigned to groups so that an equal number of males and females were in each of the four experimental conditions (males: $n = 10$ per group; females $n = 10$ per group). We confirmed that there was no significant difference in the baseline anxiety levels across all conditions ($F_{(3,76)} = 0.061$, $p = 0.980$, $n = 20$) (fig. 5).

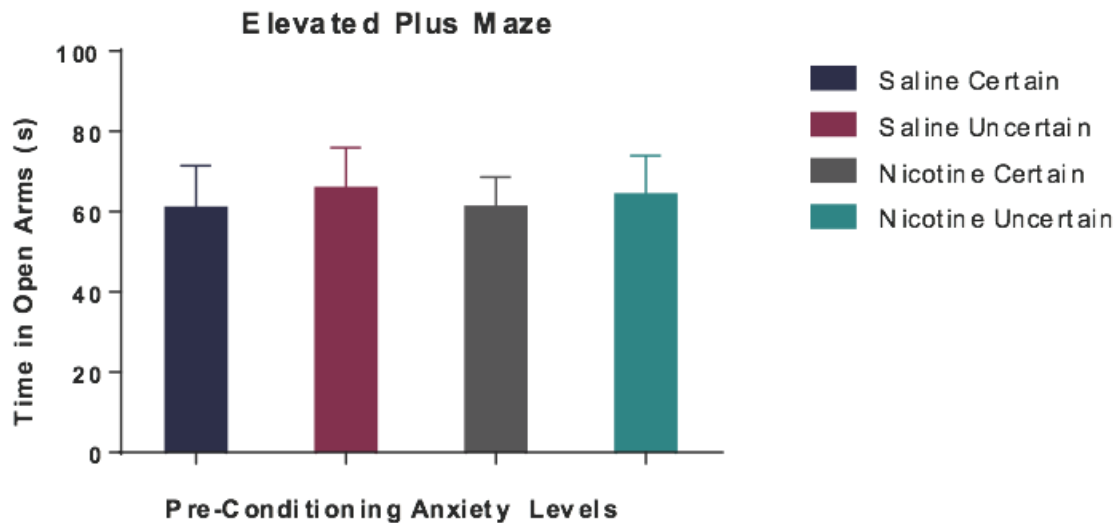


Figure 5: Average pre-conditioning anxiety levels were equal across all conditions. The Elevated Plus Maze was used as a behavioral measure for levels of anxiety in rats. Time spent in the open arms of the maze was operationalized as a measure of anxiety, and greater open arm time was associated with decreased levels of anxiety. Rats were assigned to experimental conditions so that average pre-conditioning anxiety levels were similar across all conditions ($F_{(3,76)} = 0.061$, $p = 0.980$, $n = 20$).

AUTOSHAPING

Nicotine enhances cue-directed behavior in rats assigned to the certain autoshaping condition, but not the uncertain condition.

In order to assess attraction for CS cues, rats underwent nine days of an autoshaping procedure that paired a CS lever and tone with the delivery of sucrose pellets, which were administered on either a certain (100%-1 pellet) or uncertain (50%-1-2-3 pellets) reward contingency. Each day prior to the start of the autoshaping task, rats were given an acute injection of either nicotine or saline based on weight. Day 9 autoshaping data was used to classify animals as “sign-trackers” (STs), “goal trackers” (GTs), or “intermediates” (INTs). STs were defined as animals that performed $\geq 70\%$ lever presses (LPs), GTs completed $\leq 30\%$ LPs, and INTs completed between 31% and 69% LPs. Of the animals included in this experiment,

92.5% of the population were STs (n = 74), 1.25% were INTs (n = 1), and 6.25% were GTs (n = 5), and the distribution of phenotypes was relatively equal across all four conditions: saline certain: STs (n = 19), GT (n = 0), INTs (n = 1); saline uncertain: STs (n = 19), GT (n = 1), INTs (n = 0), nicotine certain: STs (n = 17,) GT (n = 1), INTs (n = 0); nicotine uncertain: STs (n = 17), GT (n = 2), INTs (n = 0).

Results were analyzed using the measure of total cue-directed behavior, defined as the sum of lever presses and magazine entries on each day. Overall, animals demonstrated acquisition of the CS-UCS pairing as demonstrated by an increase in cue-directed behaviors across all days (LPs + MEs (n = 80), $F_{(1,29)} = 864.443$, $p = 0.000$). Further analyses revealed an injection-dependent enhancement in cue attraction for rats assigned to the certain autoshaping condition. Animals injected with nicotine and assigned to the certain autoshaping condition exhibited increased levels of cue-directed behavior compared to those injected with saline. As seen in figure 6, nicotine enhanced overall responding (days 1-9) to the lever and tone and magazine cues for animals assigned to the certain autoshaping condition, and this effect was evident for both the rate of behavioral acquisition (days 1-4) and behavioral expression (days 5-9) (Control vs. Nicotine: Overall, $F_{(1,38)} = 12.478$, $p = 0.001$; Acquisition, $F_{(1,38)} = 10.061$, $p = 0.003$; Expression, $F_{(1,38)} = 10.176$, $p = 0.003$, n = 20). Surprisingly, no effects of nicotine were observed for animals assigned to the uncertain autoshaping condition (Control vs. Nicotine, $F_{(1,38)} = 1.965$, $p = 0.169$, n = 20). Furthermore, no effects of uncertainty were observed on the quantity or rate of cue-directed behaviors in control animals (Certain vs. Uncertain, $F_{(1,38)} = 0.049$, $p =$

0.827, $n = 20$) or animals injected with nicotine (Certain vs. Uncertain, $F_{(1,38)} = 1.609$, $p = 0.212$, $n = 20$).

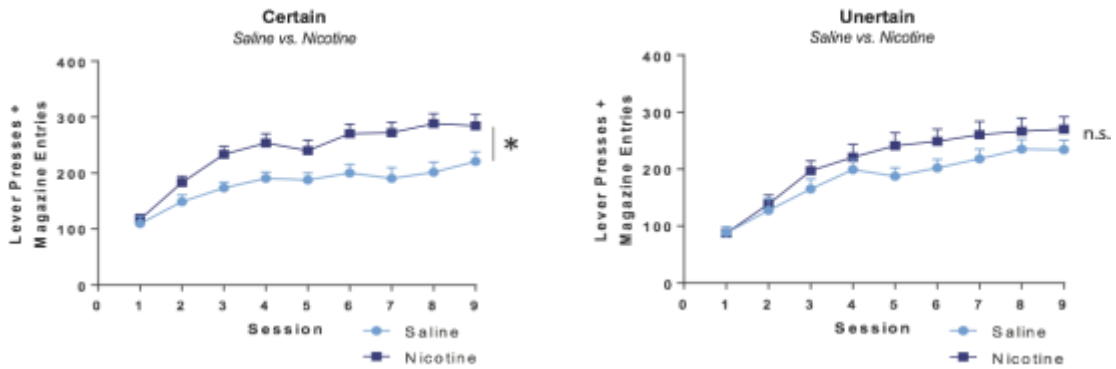


Figure 6: Nicotine and Reward Certainty Enhances Cue-Directed Behavior. Rats assigned to the certain autoshaping condition and injected with nicotine express more cue-directed behaviors than rats injected with saline ($p = 0.001$). This effect was present for both the acquisition ($p = 0.003$) and expression ($p = 0.003$) of behavior. No significant difference in the quantity of cue-directed behaviors for animals assigned to the uncertain autoshaping condition and injected with nicotine or saline were observed ($p = 0.169$).

Results were analyzed to identify sex-dependent trends in cue-directed behaviors (fig. 7). No differences in cue behavior between male and female rats were observed. Both males and females injected with nicotine and assigned to the certain autoshaping condition exhibited a heightened incentive motivation for cues (Males: Control vs. Nicotine, $F_{(1, 18)} = 6.202$, $p = 0.023$, $n = 10$; Females: Control vs. Nicotine, $F_{(1, 18)} = 8.456$, $p = 0.009$, $n = 10$). Interestingly, neither males nor females injected with nicotine and assigned to the uncertain autoshaping condition exhibited enhanced cue-directed behavior (Males: Control vs. Nicotine, $F_{(1, 18)} = 0.041$, $p = 0.842$, $n = 10$; Females: Control vs. Nicotine, $F_{(1, 18)} = 3.489$, $p = 0.078$, $n = 10$).

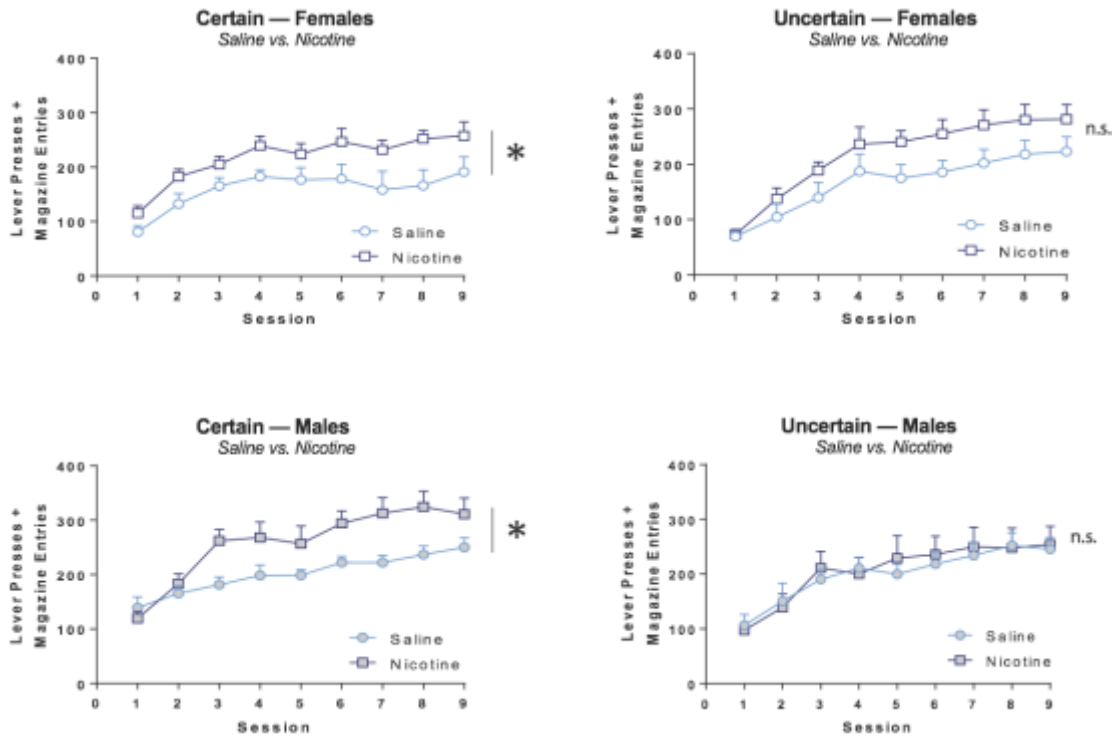


Figure 7: Nicotine and Reward Certainty Enhances Cue-directed Behaviors for Males and Females. Male and female rats assigned to the certain autoshaping condition and injected with nicotine express more cue-directed behaviors than animals injected with saline (Males, $p = 0.023$; Females, $p = 0.009$). No differences in the expression of behavior caused by nicotine for males or females assigned to the uncertain autoshaping condition were observed (Males, $p = 0.842$; Females, $p = 0.078$).

CONDITIONED REINFORCEMENT

Cues associated with reward become motivational magnets across all conditions.

Conditioned reinforcement was used to assess rats attraction to the lever and tone cues, which predicted the delivery of sucrose pellets during the autoshaping task. In conditioned reinforcement experiments, animals must acquire a novel behavior in order to gain access to a CS. Here, animals performed nose pokes (NPs) into an active hole in order to gain brief access to the CS lever and tone cues. As seen in figure 8,

all animals acquired the nose poke behavior and performed significantly greater active NPs compared to inactive NPs (Saline certain, $t_{(7.547)} = 19$, $p = 0.000$, $n = 20$; Saline uncertain, $t_{(7.646)} = 19$, $p = 0.000$, $n = 20$; Nicotine certain, $t_{(9.76)} = 19$, $p = 0.000$, $n = 20$; Nicotine uncertain, $t_{(9.176)} = 19$, $p = 0.000$, $n = 20$). Such evidence suggests that regardless of the condition, the lever and tone cues become attractive, and are conditioned reinforcers capable of producing behavior.

Analyses were conducted to determine sex-dependent differences in conditioned reinforcement behavior. Males in all experimental groups were attracted to the CS and performed a greater number of active NPs compared to inactive NPs (Males: Saline Certain, $t_{(7.357)} = 9$, $p = 0.000$, $n = 10$; Saline Uncertain, $t_{(6.993)} = 9$, $p = 0.000$, $n = 10$; Nicotine Certain, $t_{(6.034)} = 9$, $p = 0.000$, $n = 10$; Nicotine Uncertain, $t_{(5.360)} = 9$, $p = 0.000$, $n = 10$). Similarly, the lever and tone became conditioned reinforcers for females in all conditions (Females: Saline Certain, $t_{(5.881)} = 9$, $p = 0.000$, $n = 10$; Saline Uncertain, $t_{(4.230)} = 9$, $p = 0.002$, $n = 10$; Nicotine certain, $t_{(7.449)} = 9$, $p = 0.000$, $n = 10$; Nicotine uncertain, $t_{(3.482)} = 9$, $p = 0.007$, $n = 10$). Interestingly, this effect was present across all groups, despite the fact that the lever and tone cues have a diminished predictive value in uncertain reward conditions.

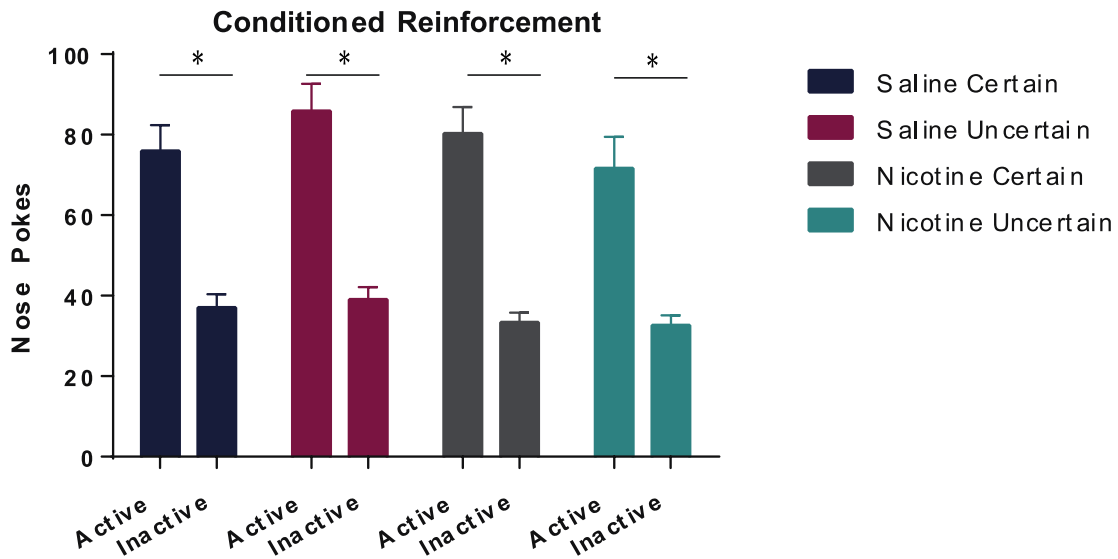


Figure 8: Cues become motivational magnets across all conditions. Rats in all conditions were willing to acquire a new behavior to gain access to cues, as seen by the significant increase in active compared to inactive nose pokes ($p = 0.000$). This effect was present in both males ($p = 0.000$) and females ($p \leq 0.007$).

PROGRESSIVE RATIO

Nicotine administration and uncertainty increases motivation to obtain rewards.

Results from the autoshaping task suggests that the combination of nicotine and reward certainty directs behavior towards CS cues. However, it is currently unclear as to whether nicotine or certain versus uncertain reward outcomes enhance attraction to cues, increase motivation for rewards, or both. The progressive ratio task was used to assess motivation to obtain the sucrose pellet reward in the absence of the lever and tone cues. Rats were required to work for a single sugar pellet on an exponential ratio of responding, and reward strength was measured as the last ratio completed by the animal, known as the breaking point. As seen in figure 9, rats treated with nicotine and assigned to the uncertain autoshaping condition work significantly harder to obtain the sucrose reward (Certain vs. Uncertain, $t_{(38)} = 2.484$,

$p = 0.018$, $n = 20$). However, no difference in breaking point was observed for animals treated with saline (Certain vs. Uncertain, $t_{(38)} = 0.061$, $p = 0.952$, $n = 20$). Therefore, the combination of nicotine and reward uncertainty appears to enhance the incentive-value of the reward itself.

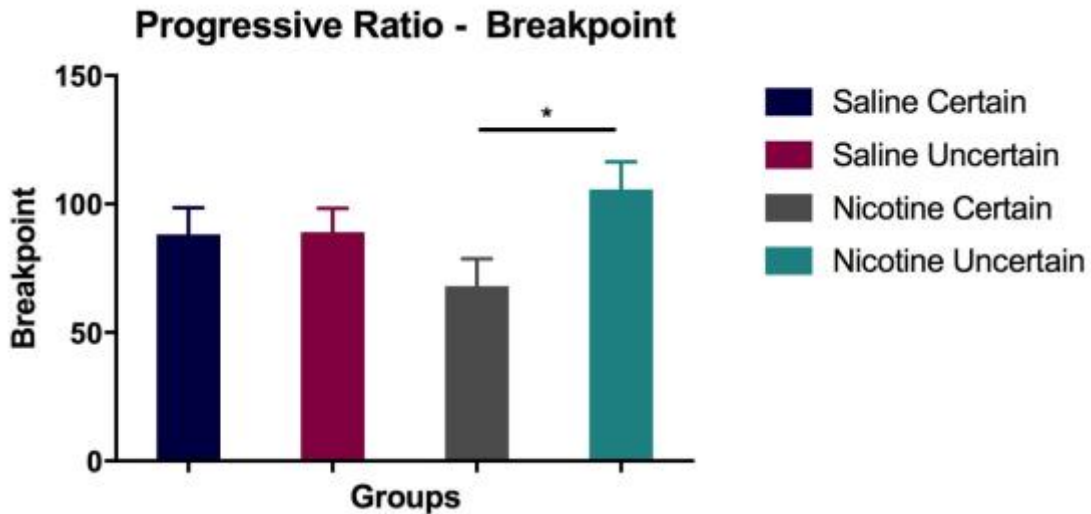


Figure 9: Nicotine and reward uncertainty increases motivation for rewards. Of the rats injected with nicotine, rats assigned to the uncertain reward condition are willing to work significantly harder to obtain the reward ($p = 0.018$). No difference in the willingness to work for sucrose was observed between certain and uncertain conditions for rats injected with saline ($p = 0.952$).

Analyses by sex suggest that females injected with nicotine might be more sensitive to the effects of uncertainty, and tend to work harder for the reward. As seen in figure 10, females treated with nicotine and assigned to the uncertain autoshaping condition appear to have a greater breaking point compared to those assigned to the certain condition (Certain vs. Uncertain, $t_{(18)} = 2.092$, $p = 0.051$). However, this effect was trending and not significant. Breaking point trends were not observed for females treated with saline (Certain vs. Uncertain, $t_{(18)} = 0.534$, $p = 0.600$, $n = 10$), or males treated with nicotine (Certain vs. Uncertain, $t_{(18)} = 1.691$, $p = 0.108$, $n = 10$). Therefore, females treated with nicotine might be particularly responsive to uncertain

reward outcomes, and might assign greater incentive-value to rewards in this condition.

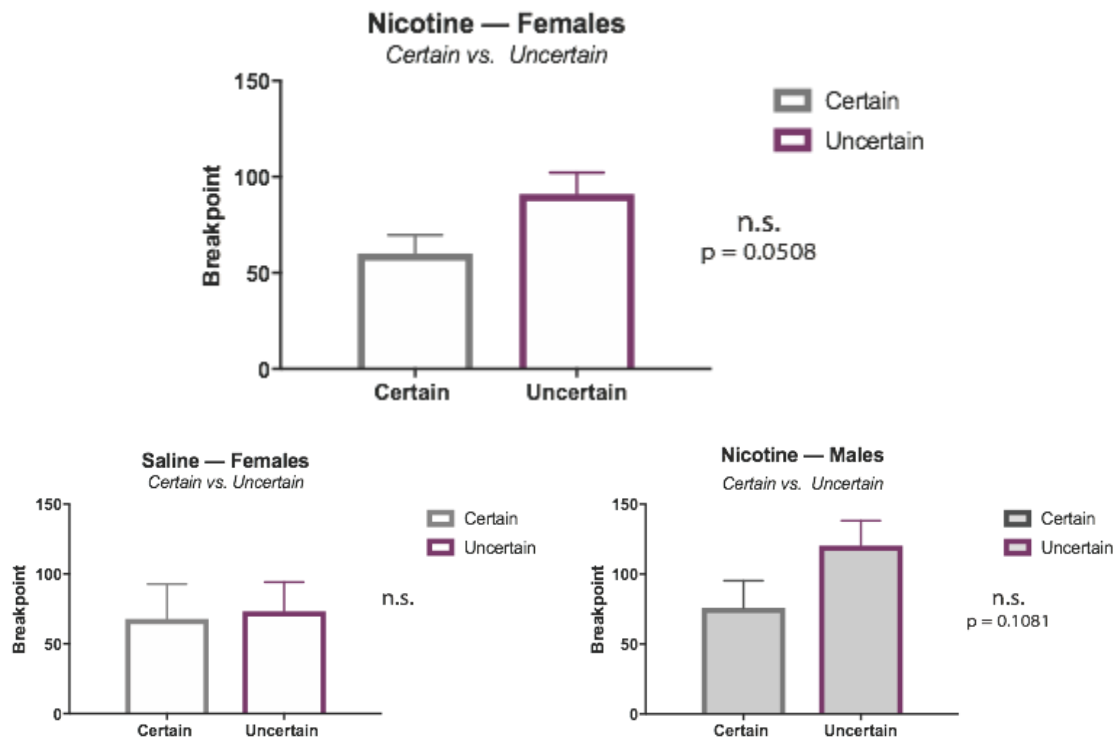


Figure 10: Females might be particularly sensitive to the motivational effects of nicotine and reward uncertainty. Females injected with nicotine and assigned to the uncertain autoshaping condition to work harder for sucrose than those assigned to the certain condition; however, this trend was not significant ($p = 0.0508$). No differences in breaking point were observed for males injected with nicotine ($p = 0.1081$). No difference between motivation to work for sucrose was observed between animals treated with saline and assigned to the certain and uncertain conditions ($p = 0.600$).

POST-CONDITIONING ANXIETY LEVELS

Neither nicotine administration nor chronic exposure to uncertainty affected levels of anxiety in rats.

Following the autoshaping, conditioned reinforcement, and progressive ratio tasks, levels of anxiety were re-measured using the Elevated Plus Maze apparatus. To create a sense of novelty, the apparatus was relocated to the opposite side of the testing room, and the arms of the maze were rotated 45° about the origin. Behavioral

and statistical analyses were identical to those conducted during pre-conditioning anxiety testing. As seen in figure 11, no significant difference between pre- and post-conditioning anxiety levels were observed across any of the conditions or within either sex. Although not significant, rats assigned to the uncertain autoshaping condition might have increased post-conditioning anxiety levels (Certain vs. Uncertain, $t_{(38)} = 0.211$, $p = 0.834$). Similarly, rats assigned to the certain autoshaping condition might have decreased levels of anxiety post-conditioning (Certain vs. Uncertain, $t_{(38)} = 0.398$, $p = 0.693$). However, these comments are based on averages of behavior, and not significant. Future research is needed to determine whether chronic exposure to uncertain reward outcomes increases levels of anxiety in rats.

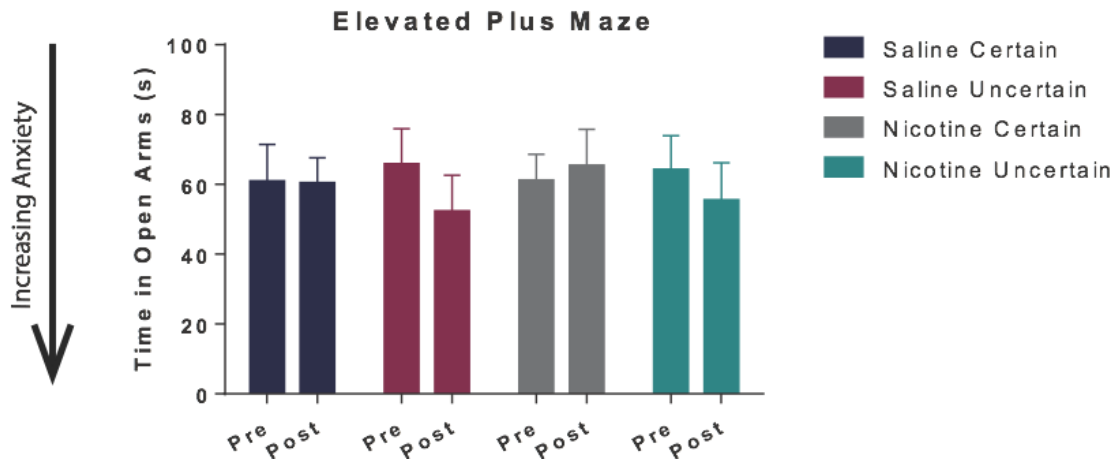


Figure 11: Post-conditioning anxiety levels were equal across all conditions. Rats in all conditions have similar post-conditioning levels of anxiety ($p < 0.05$). No significant difference between pre- and post-conditioning anxiety levels was observed ($p < 0.05$).

DISCUSSION

General Discussion

The effects of nicotine exposure and reward uncertainty are well-reported in the literature (Baker et al., 2004; Attwood et al., 2009; Anselme and Robinson, 2013; Anselme et al., 2013; Robinson et al., 2014; Anselme, 2015). However, these studies investigate the effects of either nicotine exposure or reward uncertainty on motivation for gambling-like cues or rewards, and no study to date has investigated the combined effects of these factors. For example, research conducted by Anselme and colleagues explored the effects of reward uncertainty on attraction to cues, but did not investigate how nicotine might alter the attribution of incentive salience in uncertain reward conditions (Anselme et al., 2013; Robinson et al., 2014; Anselme, 2015). In addition, studies that investigate the motivational value of nicotine typically examine the primary reinforcing properties of the drug by measuring vigor of self-administration. They hardly explore the ability of nicotine to impact motivation for a secondary reinforcer, and fail to consider its effect on motivation for cues associated with uncertain rewards. Here, we contribute to the current body of research by investigating the combined effects of nicotine and reward uncertainty on incentive motivation for a secondary reinforcer. We investigate the effects of acute nicotine administration on attraction to cues, the reinforcing value of cues, and motivation to obtain sucrose rewards in a pavlovian autoshaping procedure, conditioned reinforcement experiment, and progressive ratio task.

The unpredictability associated with reward probability and magnitude is an important characteristic for the allure of gambling games (Anselme and Robinson, 2013; Anselme et al., 2013; Robinson et al., 2014; Anselme, 2015). With this in

mind, we used reward uncertainty as a model for the improbability and ambiguity that is characteristic to games of chance. We sought to measure motivation for cues that are associated with a gambling-like setting, and employed an autoshaping procedure that consisted of two conditions—one that paired CS cues with the delivery of certain rewards, and a second that paired CS cues with the delivery of uncertain rewards. To observe the effects of nicotine, we injected rats with either a dose of nicotine or saline prior to each training session.

Nicotine Enhances Incentive Salience for Cues Associated with Certain Rewards.

The studies conducted by Anselme and colleagues show reward uncertainty to increase incentive motivation during a pavlovian autoshaping task (Anselme et al., 2013; Robinson et al., 2014). In the experiment conducted by Anselme et al. (2013), rats assigned to an uncertain reward condition exhibit increased sign-tracking behavior compared to rats that were assigned to a certain reward condition. Furthermore, the study conducted by Robinson et al. (2014) found exposure to high levels of uncertainty, compared to low levels of uncertainty, to increase incentive salience for predictive cues. Uncertainty is thought to excite the mesolimbic dopamine system, which intensifies the motivational value attributed to cues (Anselme et al., 2013; Robinson et al., 2014; Anselme, 2015). Given this information, we expected an enhancement in incentive motivation for cues that are associated with uncertain reward outcomes. From our population of control animals, we hypothesized that rats assigned to the uncertain autoshaping condition would express a greater amount of cue-directed behavior compared to rats assigned to the certain condition.

Interestingly, we did not observe an effect of uncertainty in our experiment. This finding is surprising, as our task closely mimicked that of Robinson et al. (2014), who previously reported an increase in incentive motivation for CS cues that predicted uncertain rewards during an autoshaping task. Like Robinson et al. (2014), we utilized a certain and uncertain autoshaping condition with a CS light, tone, and lever. Also, we conducted our experiment using Sprague-Dawley rats between 10 and 18 weeks of age, which matched the genotype and closely matched the age of subjects used in their experiment.

One possible explanation for our inability to replicate the effect of uncertainty is that our subjects are too phenotypically homogenous to observe substantial differences in behavior. The large majority (92.5%) of our animals were sign-trackers. Only one goal-tracker was assigned to the certain reward contingency, and three goal-trackers were assigned to the uncertain reward contingency. However, in the experiment conducted by Robinson et al. (2014), a substantial number of goal-trackers (> 30%) were assigned to the certain autoshaping condition. It is hypothesized that the behaviors of sign-trackers, as opposed to goal-trackers, more reliably capture the attribution of incentive salience to cues (Flagel et al., 2009). Therefore, it is possible that the phenotypic variability in the certain autoshaping condition of the study conducted by Robinson et al. (2014) attenuated the overall behaviors observed in the group; thus, accentuating the difference between the performance of rats in the certain and uncertain autoshaping conditions.

Our inability to replicate previous findings can also be explained by the ceiling effect. As suggested by our large population of sign-trackers, the rats used in

our colony are bred to express the sign-tracking phenotype. Therefore, it is possible that our rats express the greatest degree of sign-tracking behavior possible, and for this reason, it is impossible to observe differences in cue-directed behaviors between the certain and uncertain groups. In the present study, the average amount of lever presses for each autoshaping session exceeded 200 presses. Therefore, rats perform approximately five lever presses during each trial, during the eight second period when the lever is available. While we did not find an effect of reward uncertainty, we also did not observe an effect of reward certainty. Therefore, animals exposed to certainty and uncertainty produced equal levels of cue attraction. Such evidence is further support for the ceiling effect. In the future, we aim to extend the availability of the CS lever from eight seconds to twelve seconds. Doing so might allow us to better index cue-attraction, and possibly observe differences in motivation for cues between the certain and uncertain groups. Furthermore, we aim to add phenotypic variability to our population by purchasing goal-trackers from an outside breeder and incorporating them into our colony.

The main goal of this study was to investigate the effects of nicotine on motivation for CS cues that are associated with reward. To our knowledge, no study to date has investigated the combined effects of nicotine and reward uncertainty on attraction to cues or motivation for rewards. We chose to investigate the effects of nicotine because it is known to increase attraction to environmental stimuli such as ashtrays, cigarette smoke, or any other objects associated with the drug-taking context (Rose and Levin, 1991; Berridge and Robinson, 1998; Berridge, 2003; Berridge and Robinson, 2003; Flagel et al., 2009; Versaggi et al., 2016). In turn, these cues become

triggers that elicit craving and desire to smoke, even when cigarettes are not available (Caggiula et al., 2001; Franklin et al., 2007). Interestingly, nicotine does not only increase the attractiveness of cues associated with the drug-taking context, but also increases attraction to unrelated stimuli. For example, nicotine has been shown increases ratings of attractiveness for faces in humans (Attwood et al., 2009), and has been shown to increase attraction for a food reward in rats (Palmatier et al., 2007). In addition, nicotine is known to have an attention-grabbing effect, and increases attention and subjective sates of liking for cues (Caggiula et al., 2001; Chiamulera, 2005). Given this information, we hypothesized that nicotine would enhance the attractiveness of the CS lever, tone, and light cues during our autoshaping experiment.

In our study, nicotine increased cue-directed behavior for animals assigned to the certain autoshaping condition, but failed to increase responding for rats assigned to the uncertain autoshaping condition. Animals that were injected with nicotine and exposed to certain reward outcomes exhibited a greater amount of cue-directed behavior compared to animals that were injected with saline. This effect was not only evident when analyzing the full nine days of the autoshaping task, but was also apparent during the early days of the procedure (days 1-4), when behavior is typically acquired. Therefore, rats that are injected with nicotine and assigned to the certain autoshaping condition acquire cue-directed behaviors more rapidly than rats injected with saline, and they express significantly more motivation for cues early in their exposure to nicotine and reward certainty. The effects of nicotine and reward

certainty were also apparent after controlling for sex, as nicotine increased attraction to cues for both males and females in our population.

The results of our study can be taken to suggest that nicotine enhances attraction to cues associated with small certain rewards as opposed to large uncertain ones. Interestingly, our findings are supported by a study that compared the impulsivity of regular smokers and non-smokers that were given a choice between rewards that were small and immediate, or large and delayed (Mitchell, 1999). Nicotine increased the attractiveness for small immediate rewards, rather than large rewards with a delayed pay-out. Daily smokers selected small immediate monetary rewards significantly more than large rewards, and did so significantly more than their nonsmoking counterparts (Mitchell, 1999). Such evidence suggests that nicotine may enhance motivation in contexts that provide immediate gratification.

In our autoshaping task, the certain reward contingency is designed to deliver one sucrose pellet during each trial. However, the uncertain reward contingency is designed to deliver one, two, or three sucrose pellets during half of the trials, and zero sucrose pellets during the other half of the trials. Therefore, in the uncertain condition, nine trials might occur before one is reinforced. It is possible that the certain autoshaping condition simulates the immediacy of rewards that is appealing to smokers. On the contrary, the uncertain autoshaping condition might simulate the effect of delayed gratification, which is ill-preferred when combined with the effects of nicotine, at least in human smokers.

The effect of nicotine and reward certainty can explain the disordered gambling behavior that is observed in the casino setting. In a typical game of slots, such as *Lobstermaina*, the chances of winning a reward greater than or equal to \$150 is one in every 399 spins, providing that the player wagers \$3.75 and bets on 15 lines (Harrigan and Dixon, 2009). However, the total payback percentage of slot machines ranges between 85% and 97%, and the majority of the payouts are small wins that are of a lesser value than the wager (Harrigan, 2007). Therefore, games of chance are inconsistent and uncertain in their probability and magnitude of large rewards, but relatively certain in their delivery of small rewards.

Although players lose money when they are awarded less than they wager, slot machines celebrate small wins as if they were jackpot earnings (Griffiths, 1993). In fact, cues such as flashing lights and celebratory sounds are paired with small wins, despite their overall loss status. The celebration of small rewards is an intentional design that perpetrates cognitive bias (Dixon et al., 2010; Dixon et al., 2014). Small wins create the illusion of winning, and celebratory sounds increase the players' arousal, help them to disregard previous losses, and induce a pleasant affective state that encourages prolonged play. The delivery of small rewards promotes gambling while ensuring the financial advantage of the house (Griffiths, 1993; Griffiths and Parke, 2005; Parke and Griffiths, 2006). It is possible that nicotine increases attraction to small certain rewards in the naturalistic setting. This would increase incentive motivation for cues that are associated with small rewards, and would further entrench the cognitive biases that are exploited by the gambling industry. Therefore, individuals that are at-risk for developing problem gambling might be

further harmed by the effects of nicotine, which appear to enhance the incentive motivation for cues that are associated with the small, consistent rewards that promote cognitive biases. In all, incentive motivation for cues that are associated with nicotine and reward certainty might enhance the preoccupation and engrossment that is experienced by disordered gamblers.

Another explanation for the heightened incentive salience for cues associated with nicotine is that their combined effects produce an adaptive level of stress in rats assigned to the certain autoshaping condition, but a maladaptive level of stress in rats assigned to the uncertain autoshaping condition. Moderate levels of stress are known to exert a beneficial effect on motivation (Broadhurst, 1957), and have been shown to increase the release of dopamine in the nucleus accumbens (Oswald et al., 2005; Oswald et al., 2007). However, extreme levels of stress are known to decrease motivation and cause anhedonia (Willner et al., 1992; Konkle et al., 2003; Kleen et al., 2006; Henningsen et al., 2009). Past studies demonstrate nicotine to increase cortisol levels in humans (Kirschbaum et al., 1992; Steptoe and Ussher, 2006; Badrick et al., 2007), and similar results are observed in studies that use rats (Balfour et al., 1975; Benwell and Balfour, 1982; Cam and Bassett, 1983). One study found heightened plasma corticosterone levels for rats that were initially unstressed and given nicotine (Balfour et al., 1975). Similarly, reward uncertainty is known to impact the dopaminergic system, and might do so through a stress mechanism (Anselme et al., 2013). In all, it is possible that the administration of nicotine alone produces a beneficial level of stress that enhances the attribution of incentive salience to cues. However, the combination of nicotine administration and reward uncertainty might

elevate stress to levels that are above the threshold for its beneficial effects on incentive motivation. In the future, studies that measure corticosterone levels of rats injected with nicotine and exposed to certain versus uncertain reward outcomes are needed to validate this hypothesis.

Following our autoshaping experiment, we used a conditioned reinforcement task to measure the reinforcing property of CS cues. Cues gained motivational value through repeated associations with rewards during the autoshaping experiment, and were transformed into an effective conditioned reinforcer. Animals in all conditions acquired a novel behavior to gain access to cues during the conditioned reinforcement task, and no difference in the reinforcing value of cues between any of the conditions was observed. Therefore, cues that were associated with the certain autoshaping condition were able to reinforce behavior. Similarly, cues that were associated with the uncertain autoshaping condition became a conditioned reinforcer. In all, cues that reliably predicted rewards had the same reinforcing power as cues that did not reliably predict rewards. Therefore, consistency between cues and the availability of reward is not necessary for the attribution of incentive salience for cues. Hence, in games of chance, celebratory lights and sounds can become motivational magnets, even though they do not reliably predict jackpot winnings.

Nicotine enhances motivation for uncertain rewards.

To our knowledge, no study to date has investigated the effects of nicotine on motivation for a secondary reinforcer, such as food. Similarly, no study has investigated the combined effects of nicotine and reward uncertainty on the motivation to obtain rewards during a progressive ratio task. Past studies suggest that

the strength of a reinforcer is linked to the breaking point achieved during a progressive ratio task (Hodos, 1961; Richardson and Roberts, 1996). Therefore, the greater the value of the breaking point, the greater the value of the reinforcer. Given the motivational effects of reward uncertainty, we expected rewards associated with nicotine and uncertainty to have the greatest value.

In our autoshaping task, rats exposed to nicotine and reward uncertainty achieved a significantly higher breaking point compared to animals in all other conditions. Hence, rats assigned to the nicotine uncertain autoshaping condition were willing to work significantly harder to obtain the sucrose reward. Our results support our hypothesis that nicotine exposure and reward uncertainty increases motivation to obtain sucrose pellets. In all, rats that are exposed to nicotine attribute the greatest incentive value to rewards associated with the uncertain condition.

Interestingly, there appears to be a dichotomy between the effects of nicotine and reward probability on the attraction to cues versus the motivation to obtain rewards. The results from our study suggest that nicotine enhances the reinforcing property of rewards that are unpredictable in their magnitude and time of delivery. However, nicotine appears to enhance incentive salience for cues when they consistently predict the delivered of rewards. Therefore, nicotine appears to exert a different effect on motivation for the predictor of reward than it does on motivation for the reward itself. The main effect of nicotine might be its ability to heighten attraction to cues that reliably predict rewards, but to increase motivation for rewards that are unpredictable.

Neither nicotine administration nor chronic exposure to uncertainty affected levels of anxiety in rats.

The second goal of our study was to investigate the interaction between nicotine, incentive salience for gambling-like cues, and anxiety. Anxiety is a known risk factor for relapse to maladaptive behaviors, and is implicated in the maintenance of both nicotine use and gambling involvement. In our study, pre-conditioning and post-conditioning levels of anxiety were compared to determine how exposure to nicotine in a gambling-like setting might affect anxiety.

We found post-conditioning levels of anxiety across all conditions to be relatively similar to their baseline levels. Interestingly, further analysis of our data revealed a slight increase in the average levels of anxiety for rats assigned to the uncertain autoshaping condition, albeit the increase in anxiety was not significant. However, just as humans exhibit a wide range of anxiety behavior, we observed a wide range of anxiety levels across the population of animals used in our study. The variability of anxiety behavior in our population was quite large and could have hindered our ability to observe an effect. It appears as if a subset of animals exhibited high levels of anxiety, while an opposing subset exhibited low levels of anxiety. Furthermore, a subset of animals that initially expressed high levels of anxiety became substantially less anxious, and a subset of animals that initially expressed low levels of anxiety became more anxious after testing. Therefore, it appears as if our population can be further categorized by the degree of anxiety behavior, and by how levels of anxiety change across our autoshaping, conditioned reinforcement, and progressive ratio procedures. Considering high levels of trait-anxiety are related to gambling severity in humans, it is possible that our high-anxious subjects might have

a response to the conditioning tasks that is different from animals that are less anxious. Furthermore, nicotine is known to increase levels of anxiety. Therefore, it is likely that anxiety would impact behavior in the face of nicotine and reward uncertainty, and vice versa. Further analyses that investigate the autoshaping, progressive ratio, and conditioned reinforcement results by severity of anxiety is needed to determine the relationship between anxiety, nicotine administration, and uncertain reward outcomes.

In the future, we will conduct a more detailed analysis to uncover the effects of anxiety on motivation for cues, and the effects of conditioning on the expression of anxiety. To address this aim, we plan to account for variability in anxiety behavior by analyzing our data by the degree of anxiety behavior. Rats that exhibit high levels of anxiety will be analyzed as one group, and rats that exhibit lower levels of anxiety will be analyzed as a second group. We hypothesize that this analysis will help control for the variability that possibly dampens the effect of anxiety in our current analyses. In addition, we plan to use a second measure of anxiety, such as the open field test, to improve our measurement of anxiety behavior. We will also employ a glucocorticoid testing procedure to measure blood cortisol levels after conditioning.

CONCLUSION

In conclusion, we sought to elucidate the effects of nicotine exposure on incentive motivation for cues. To address this aim, we injected rats with nicotine or saline and utilized an autoshaping task that paired cues with the delivery of certain or uncertain rewards. Prior research suggests that cues associated with uncertain

outcomes gain incentive salience through the activation of the mesolimbic reward system (Anselme et al., 2013; Robinson et al., 2014). Furthermore, nicotine appears to increase attraction to cues, and is also known to sensitize the reward system, making it hyper-responsive (Celik et al., 2006). Therefore, both nicotine and uncertainty appear to sensitize the pathways that release dopamine and assign incentive salience to cues and their rewards. Interestingly, trait-anxiety appears to be related to gambling severity, and is a known risk factor for relapse to both maladaptive smoking and gambling behaviors. Thus, uncertainty in gambling, nicotine, and anxiety might interact and produce a trifecta of effects that promote disordered gambling behavior.

Contrary to expectations, the results of our study suggest that nicotine may drive the attribution of incentive salience to cues that are associated with certain rewards. However, nicotine may enhance motivation to obtain rewards that are associated with uncertainty. Therefore, cues that reliably predict rewards gain enhanced incentive salience, but rewards that are inconsistent in their delivery incite a willingness to work harder to obtain them. No effects of anxiety were observed in this study. However, further investigation is needed to assess how anxiety might affect cue-directed behaviors in the presence of nicotine and reward uncertainty, and vice versa.

Based on the results of the present study, individuals that smoke cigarettes and gamble might experience elevated craving and interest in games that frequently reward small monetary prizes. For example, slot machines award wins that are of lesser value than the wager 85% to 97% of the time. Cues such as flashing lights and

sounds celebrate these wins despite their overall loss status. Therefore, the pay-out of small rewards is quite consistent, and they are typically paired with cues. Nicotine may enhance the attraction to the cues associated with small, certain rewards, and may enhance feelings of pleasure or excitement that players experience while gambling. While small rewards are frequently delivered in games of chance, large rewards are infrequent, and their delivery and magnitude is unpredictable. Results from our study suggest that nicotine might enhance a player's willingness to work for large, uncertain rewards. Therefore, the effects of nicotine on disordered gambling behavior might be binary. On one hand, nicotine might increase attraction to cues associated with small, certain rewards. Thus, creating cognitive biases that promote prolonged play. On the other hand, nicotine might increase motivation to obtain the large, infrequent rewards. The effects of nicotine on motivation for cues and the willingness to work for rewards might converge to promote disordered gambling behavior.

In the future, studies that replicate this design should conduct their research using a population of rats with greater phenotypic diversity. In addition, the autoshaping procedure should be modified to extend the availability of CS cues. Perhaps a greater range of behaviors, and a longer period to perform them, would provide a better context for the measurement of incentive salience. In addition, future studies should use the Elevated Plus Maze behavioral measure of anxiety in conjunction with the open field test. Two anxiety measures might increase the ability to accurately measure levels of anxiety. Furthermore, cortisol samples should be collected from blood serum to provide a biological measure of anxiety in rats.

In all, this study contributes to the literature by suggesting that nicotine enhances attraction to cues and motivation for rewards in the gambling setting. Furthermore, we propose avenues for future research and suggest that the interaction between gambling, nicotine, and anxiety should be considered when investigating the behavioral dysfunction that is characteristic to gambling disorder.

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