7-22-2016

The Effects of Nicotine on Conditioning, Extinction, and Reinstatement in Humans Using a Virtual Reality Conditioned Place Preference Paradigm

Alexandra N. Palmisano
University of Connecticut - Storrs, alexandra.palmisano@uconn.edu

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Alexandra N. Palmisano
B.S., St. Lawrence University, 2011

A Thesis
Submitted in Partial Fulfillment of the
Requirements for the Degree of
Masters of Science
At the University of Connecticut

2016

APPROVAL PAGE

Masters of Science Thesis

The Effects of Nicotine on
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Presented by
Alexandra N. Palmisano, B.S.

Major Advisor_____
Robert Astur, PhD
Associate Advisor_____
Etan Markus, PhD
Associate Advisor_____
John Salamone, PhD
Associate Advisor_____
Harriet de Wit, PhD

University of Connecticut
2016

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Acknowledgements

I would like to extend my sincerest appreciation and gratitude to my graduate advisor and primary investigator, Dr. Robert S. Astur, for his continued support, advice, knowledge, and patience during the entirety of my time at the University of Connecticut thus far, but particularly throughout the research and preparation of my Master of Science thesis. I would also like to thank my committee members, Dr. Harriet de Wit, Dr. John D. Salamone, and Dr. Etan J. Markus for all of their advice and guidance during this process. In addition, I could not have completed this work without the support of my fellow lab members, Soniya Assudani-Patel and Emily Errante, my fellow Behavioral Neuroscience graduate students: Samantha Yohn, PhD and Jennifer Corriveau, PhD, and the hard-working undergraduates, namely Courtney McQuade and Eleanor Hudd, who have graciously provided me with moral and technical support on this project. Last, but certainly not least, I would like to thank my parents, Laurie and Rick Palmisano, my brother, Nick Palmisano, and my boyfriend, Ross Murphy, for their continued love and support in all that I do.

Abstract

Despite an abundance of evidence illustrating the harmful effects of nicotine use, only a small percentage of users successfully quit (Messer et al., 2008). Moreover, current treatments for nicotine cessation produce only a slight increase in the likelihood of successfully quitting, which emphasizes the need for more effective strategies that facilitate smoking cessation (Hopkins et al., 2001). Several studies suggest that difficulty in controlling nicotine use behaviors results from nicotine's ability to enhance the motivating function of cues associated with obtaining rewards. These studies indicate that it is of value to understand the behavioral and neuropharmacological mechanisms by which nicotine enhances responding for conditioned rewards. Unfortunately, despite ample non-human studies, there is a paucity of literature examining nicotine's ability to enhance reward responding in humans. Thus, in order to better understand the reward mechanisms that underlie the risk for becoming dependent, the aim of the current study was to examine nicotine's effects on conditioning, extinction, and reinstatement in humans. Using a novel virtual reality translation of the hallmark conditioned place preference paradigm to investigate the aforementioned objectives, our main findings suggest that nicotine (1) increases the sensitivity of reward properties by enhancing the strength of food-reward conditioning, (2) delays the rate of extinction of conditioned preferences, and (3) increases the reinstatement of previous conditioning. These findings demonstrate the efficacy of utilizing the virtual conditioned place preference paradigm in understanding the behavioral mechanisms by which nicotine enhances responding for conditioned rewards, and provide insight into how nicotine can be particularly resistant to treatment. Importantly, these data provide key information for future work aimed at increasing the understanding of how conditioning paradigms can help treat and prevent substance dependences.

Chapter 1: General Introduction

Nicotine dependence has been shown to result in devastating health problems, including heart disease, lung disease, and cancer, as well as an increased susceptibility to a variety of infectious diseases (Benowitz, 2009). Despite the risks associated with its use, 17.8% of United States adults aged 18 years or older currently smoke cigarettes (CDC, 2014). Although 68.8% of U.S. tobacco users reported in 2010 that they wanted to quit completely (CDC, 2014), it is estimated that 80% of smokers who attempt to quit on their own relapse within the first month of abstinence, and approximately 3% remain abstinent at six months (CDC, 2000). This illustrates the severity of nicotine dependence and the chronic nature of the disorder. While most of the toxicity of nicotine use is related to the added components of nicotine-containing products (i.e. cigarettes, cigars, snuff, and chewing tobacco), the pharmacologic effects of nicotine are primarily responsible for the production and maintenance of the dependence (Benowitz, 2009). Thus, an understanding of how nicotine produces dependence and influences usage provides a necessary foundation for optimal nicotine use prevention and treatment therapies.

Motivational factors that contribute to nicotine use

Acute pharmacological effects of nicotine on motivational states, like the relief of anxiety or stress and the induction of euphoria, are likely responsible for initial experimental nicotine use. Like other psychomotor stimulants, nicotine dependence has been associated with the drug's ability to produce subjective sensations that can be described as a "rush," "elation," or "buzz" (Baker et al., 2004). Studies indicate that nicotine exerts anxiolytic and mood-enhancing effects (Leventhal, 2010), and beneficially influences neural processing of affective information (Kobiella et al, 2014). The expectation that nicotine will produce positive emotional

consequences has been shown to predict urges to smoke during the course of ongoing smoking (Zinser et al. 1992), and has been shown to inversely predict cessation success (Wetter et al., 1994). In addition to mood-enhancement, nicotine has also been shown to significantly improve cognitive performance in terms of attention and working memory function (Rezvani and Levin, 2002); however, Heishman et al. (1999) argues that enhanced attention and cognition are unlikely to play a major role in the decision to begin or to continue smoking.

However, while the positively reinforcing effects of mood enhancement by nicotine administration seem to play an important role in nicotine use behavior, an accumulating literature implicates a greater role for negative reinforcement in nicotine dependence. For example, although positive affect imagery elicits smoking urges, it is less effective than negative affect imagery (Tiffany & Drobes, 1990). Smokers reliably report that they smoke more when they are stressed, angry, anxious, or sad (Ikard et al., 1969), and hold the expectation that smoking will alleviate such negative mood states (Baker et al., 2004). Numerous studies have also found a positive relationship between stress and increased cravings for nicotine (Childs & de Wit, 2010), as well as increases in the rate and intensity of smoking intake (Payne, 1991). Subjective self-reports of stress reduction from nicotine use are not uncommon, where several studies have revealed that smoking attenuates subjective stress not only in anticipation of a stressor, but in the direct presence of a stressor as well (Kassel et al., 2003). These stressors have included aversive noises (Woodson et al., 1986), viewing stressful imagery (Gilbert et al., 1989), and engaging in a public speaking procedure (Juliano & Brandon, 2002). In addition to demonstrating that levels of negative affect strongly influence cessation outcome, negative affect is also a factor of nicotine withdrawal that significantly predicts relapse (Cinciripini et al., 2003).

Some withdrawal symptoms in humans have been shown to manifest for up to 10 weeks following nicotine cessation (Hughes, 1992); however, averaged across individuals, these symptoms are typically observed to increase sharply during the first week of cessation and decrease to baseline values within 4 weeks (Cummings et al., 1985). Nicotine cessation and withdrawal produce a number of undesirable effects. In humans, acute nicotine withdrawal is characterized by somatic symptoms, such as bradycardia, gastrointestinal discomfort, and increased appetite leading to weight gain (Heishman, 1994), as well as affective symptoms like negative changes in mood (Hall et al., 2015), dysphoria, irritability, anxiety, frustration, increased reactivity to environmental stimuli (Hughes et al., 1992), and difficulty concentrating (Gross, 1993; Sweet, 2010). Correlational and human self-report studies commonly suggest that relapse to nicotine use is often brought about by these effects (Patterson et al., 2010). As posited by Baker and colleagues (2004), smokers learn to detect physiological cues associated with the early stages of nicotine withdrawal, which in turn prompt the rise of negative affect. As negative affect intensifies, the incentive salience of drug-related cues increases, as does the probability that an individual will partake in drug-taking behavior. Therefore, use of nicotine negatively reinforces drug-taking behavior by attenuating the symptoms of negative affect, resulting in drug dependence.

Given that urges and cravings may be theorized as subjective, motivational states responsible for ongoing drug use and the inception of relapse, it is of value to attempt to discriminate the relevant motivational factors that induce nicotine cravings and drive drug-taking behavior. Carver and White's (1994) Behavioral Inhibition and Inactivation Scales (BIS/BAS), based on Gray's (1987) biopsychological theory of emotion, have become widely used measures of incentive processes, and can be used to better understand the motivational factors that underlie

nicotine use. The aversive motivational system, called the behavioral inhibition system (BIS), is hypothesized to control the experience of anxiety and to inhibit behavior that might produce negative or painful outcomes. The BIS, according to Gray, is sensitive to cues of punishment, non-reward, and novelty, and BIS functioning is responsible for negative affects like fear, anxiety, frustration, and sadness in response to these cues (Carver & White, 1994). Therefore, sensitivity to the BIS would reflect an individual's propensity toward anxiety when provided with the appropriate cues, and heightened avoidance of such anxiety-inducing experiences. On the other hand, the psychologically independent behavioral activation system (BAS) is believed to induce positive affects like elation and happiness in response to cues of reward, and thus motivates goal-driven behavior toward cues.

The BIS scale is consistently defined as unidimensional in that it is said to singularly regulate aversive motives in which the goal is to move away from something unpleasant (i.e. "I feel worried when I think I have done poorly at something"), while the BAS scale can be broken down into three distinct subscales: Drive, Reward Responsiveness, and Fun Seeking. In examining the items that comprise these scales, the Drive scale exclusively assesses behavioral responding (i.e. "If I see a chance to get something I want, I move on it right away"), Reward Responsiveness exclusively evaluates affective responding (i.e. "When I get something I want, I feel excited and energized"), and the Fun Seeking scale measures both affective and behavioral responding (i.e. "I crave excitement and new sensations" and "I will often do things for no other reason than that they might be fun;" Carver and White, 1994).

It has been suggested that the BAS is the basis for impulsivity, which in turn provides an inclination toward risky behaviors like drug-seeking and drug use (Dawe & Loxton, 2004). Zuckerman and Kuhlman (2000) argued that sensation seeking and impulsivity represent the

approach aspect of the reward/risk conflict. Another way to account for the observed association between impulsivity and risky behavior is in terms of disinhibition (Carlson et al., 2010), where risk-taking occurs due to a weak BIS system. Given that impulsivity is related to greater risk of substance use (Ham & Hope, 2003), Franken and Muris (2006) used the BIS/BAS questionnaire to examine the influence of impulsivity on drug and alcohol use in college students. Their results indicated that students' drug and alcohol use was positively correlated with BAS personality characteristics, and to a lesser extent, negatively correlated with BIS personality characteristics. The most substantial correlations were found between the BAS Fun Seeking and BAS Drive subscales and the number of illegal substances one had used, the quantity of alcohol use, and the frequency of binge drinking. Therefore, they conclude that impulsivity, as determined by BIS/BAS personality characteristics, significantly contributes to substance abuse.

Similar to the Behavioral Inhibition and Inactivation Scales, the Questionnaire on Smoking Urges (QSU) is a metric that can be used to evaluate the multi-dimensional nature of drug use, specifically in examining the motivational factors that drive urges and cravings. The QSU uses a two-factor item structure characterizing (1) a desire and intention to smoke with smoking anticipated as pleasurable (i.e. "I have an urge for a cigarette" and "I have no desire for a cigarette right now"), and (2) an anticipation of relief from negative affect and nicotine withdrawal with an urgent desire to smoke (i.e. "'All I want right now is a cigarette" and "My desire to smoke seems overpowering;" Tiffany & Drobes, 1991). The QSU has been shown to display high internal consistency across settings with smokers at differing stages of drug use, providing convenient and reliable assessment of desire to smoke (Cox et al., 2001); thus, in using this questionnaire, one may be able to discriminate the relevant motivational factors that induce nicotine cravings and drive drug-taking behavior. In an effort to correlate the relationship

between nicotine dependence and "background craving," defined as the craving that smokers experience irrespective of nicotine-associated cues (Ferguson & Shiffman, 2009), one study found that more dependent smokers reported higher background craving on both appetitive and distress relief factors of the QSU prior to any cue exposure (Dunbar et al., 2014).

As mentioned, impulsivity is an additional factor involved in the initiation of drug use. Impulsivity describes an individual's propensity to make rash behavioral decisions regardless of adverse consequences, or the loss of a delayed reward of greater magnitude (i.e. drug taking despite knowing the potential detrimental effects on health). Studies in laboratory animals can measure degrees of impulsivity by evaluating whether rodents preferentially lever press to receive smaller, immediately-available food rewards, or larger, delayed food rewards (Dierrgarde et al., 2008). Consistent with this measurement of delayed discounting, human dependence on nicotine has been found to be associated with high levels of impulsivity (Kolokotroni et al., 2011; Ryan et al., 2013) where nicotine users tend to opt for the smaller, more immediate reward, while rating the larger, delayed reward as having a relatively lower subjective value (MacKillop et al., 2011; Yi et al., 2010). The Monetary Choice Questionnaire (Kirby, 1996) is often used is human studies to measure individual differences in delayed discounting, which involves choosing between immediate, but smaller theoretical monetary rewards and delayed, but larger rewards. Recently, Konecky and Lawyer (2015) indicated that substance-abusing and substance-dependent adolescents evidenced significantly higher rates of delay discounting than did non-drug-abusing controls in that those who met criteria for substance abuse or dependence were more likely to choose a smaller-sooner reward over a larger-later reward than were non-abusing controls. Their findings were consistent with others linking higher rates of delay discounting with adolescent (Reynolds & Fields, 2012) and adult substance abuse (MacKillop et

al., 2011). Due to the significance of impulsivity on decisions of delayed discounting and increased rates of substance abuse, it is of relevance to assess the relationship between responses on the Kirby questionnaire and nicotine cravings and drug-taking behavior.

Reward learning in nicotine dependence

A multitude of diverse studies including self-administration, electrical self-stimulation, place preference, pharmacology, in vivo imaging, and cellular electrophysiology have suggested that the neurotransmitter, dopamine (DA), plays a critical role in both instrumental and classical conditioning(Dani, 2003). In classical conditioning, any change in behavior reflects an innate reaction to environmental events. Conversely, in instrumental conditioning, behavior is dependent on whether or not an animal gets a reward or punishment.

As mentioned, nicotine dependence in part stems from the expectation that nicotine use will produce positive emotional consequences, or relieve negative affect from stress and withdrawal. Therefore, an assertion that has guided much research is that the reinforcement mediated by psychostimulant drugs like nicotine is associated with enhanced DA release from the mesocorticolimbic system that originates in the ventral tegmental area (VTA) and innervates the amygdala, nucleus accumbens, prefrontal cortex, and striatum via dopaminergic projections(Di Chiara, 1988; Balfour, 2004). Nicotine has been shown to directly increase DA levels in the mesocorticolimbic system by interacting with nicotinic acetylcholine receptors (nAChRs) on dopaminergic neurons causing them to release more of the DA neurotransmitter (Koob & Valkow, 2010). These findings suggest that nicotine use taps into reward-based neuronal systems, reinforcing maladaptive behaviors. The primary reinforcing effects of nicotine use have been demonstrated by an increase in self-administration behavior in both human and

non-human studies to nicotine administration. For example, it has been shown that animals are significantly more likely to lever press when the press results in a nicotine infusion than when it results in placebo (LeFoll & Goldberg, 2009). Similarly, Henningfield and colleagues (1983) demonstrated that human cigarette smokers show increased patterns of responding for intravenous nicotine versus saline in a self-administration paradigm.

However, nicotine users do not simply self-administer nicotine, but they take the drug within the context of numerous external stimuli. These neutral stimuli can be either intrinsic or extrinsic to nicotine itself, and include things like the sight, smell, and taste of a cigarette, or the context where nicotine's actions take place. Nicotine-associated stimuli then promote nicotine-seeking behavior and use (DiChiara, 2000). The association between such cues and the resulting urge to use nicotine constitutes classical conditioning, in which dopamine is also implicated.

Classical/Pavlovian conditioning occurs when a neutral stimulus, for example, a drug-related cue, is repeatedly paired with a stimulus that evokes some type of innate response, termed an unconditioned stimulus (US). Self-administered drugs are considered effective unconditioned stimuli since they have been shown to possess powerful physiological and hedonic properties. Following consistent and repeated pairings of the neutral drug-related cue with the US, the cue may come to act as a conditioned stimulus (CS), triggering the same response as that produced by the US. The desire to use nicotine is partly maintained by such conditioning (Stewart et al., 1984). For example, smokers tend to have a cigarette after a meal or with friends who smoke. When these situations are repeated, they become cues for the urge to smoke. Demonstrating this phenomenon, several studies have shown that when smokers were exposed to smoking cues, like a lit cigarette resting in an ashtray, they reported increased cravings or “desire to smoke” (Wertz & Sayette, 2001; Sayette & Tiffany, 2013).

While the primary reinforcing effects of nicotine and the subsequent associative and classically-conditioned properties of nicotine-related stimuli are important in instigating nicotine cravings and drug use, recent studies suggest that non-associative effects of nicotine on reinforced behavior may also play an important role in that behavior changes in the absence of any apparent nicotine-associated stimulus or event (Caggiula et al., 2009). Studies utilizing animal models suggest that nicotine can promote the reinforcing properties of non-pharmacological rewards that are not directly associated with nicotine intake. In other words, nicotine may increase the incentive value of a non-nicotine stimulus by directly enhancing the reinforcing efficacy of other reinforcing stimuli in the environment.

In a study originally conducted by Donny et al. (2003), nicotine increased responding for a reinforcing, concurrently-available visual stimulus. Particularly, the increase in responding did not occur with non-contingent food delivery, and was regulated by nicotine delivery as demonstrated by an immediate reduction in responding when nicotine was replaced with saline (Chaudhri et al., 2007). Importantly, operant responding for the visual stimulus was sustained at high levels by nicotine that was neither temporally nor causally associated with behavior, suggesting that this effect was distinct from the actions of nicotine as a primary reinforcer (Rupprecht et al., 2015). Rodent studies by Guy and Fletcher (2013) also substantiate the hypothesis that nicotine increases appetitive responding for non-drug incentives by demonstrating that nicotine administered during conditioning promoted operant responding for the delivery of water. Furthermore, enhanced responding for water delivery induced by nicotine was blocked by mecamylamine and DHβ, nicotinic antagonists.

Findings of reinforcement enhancement by nicotine in non-humans have been replicated across a range of doses, routes of administration, schedules of reinforcement, and reinforcing

stimuli, including conditioned reinforcers (Chaudhri et al., 2006; Palmatier et al., 2006). However, literature regarding nicotine's ability to promote non-associative reinforcement in humans has received less attention. Of the few existing studies, observations are consistent with non-human literature. A study by Attwood et al. (2009) sought to determine the impact of nicotine on hedonic behaviors using ratings of facial attractiveness. Participants in withdrawal were randomized to smoke either a nicotine-containing or a denicotinized cigarette after which they completed ratings of attractiveness of male and female faces. Their data indicated that while nicotine treatment increased ratings of attractiveness, it did not significantly affect ratings of subjective mood, suggesting that the effects on attractiveness ratings were distinct and not reflective of a positivity bias in questionnaire responding for the nicotine condition.

Similarly, using a signal detection task designed to measure shift in responding toward a differentially (more) rewarded stimulus, Barr et al. (2008) concluded that transdermal nicotine increased response bias towards the more rewarded stimulus compared to placebo. Specifically, non-dependent and nicotine-naïve participants were asked to choose which of two stimuli (a short or long mouth) was displayed on a previously mouthless cartoon face. Correct identification of one stimulus was rewarded ("Correct!! You won 5 cents") three times more frequently compared with correct identification of the other stimulus. While participants were told that not all correct responses would receive a reward feedback, that lack of feedback did not indicate inaccuracy, and that they receive no feedback for errors, they were not informed about the differential reward schedule. Despite anticipated adverse effects like nausea, nicotine significantly increased response bias toward the more frequently rewarded condition at the expense of accuracy, and independent of effects on attention or overall vigilance (Barr et al.,

2008) supporting the hypothesis that nicotine increases the reinforcing salience of a non-nicotine stimulus.

Finally, in an attempt to determine the generalizability of nicotine's ability to enhance reinforcement, one study assessed the effects of nicotine via smoking on enhancement of positive reinforcers like money or music, or negative reinforcers like the termination of an aversive noise (Perkins & Karelitz, 2013). Using a within subjects design, dependent and non-dependent smokers participated in several sessions involving an "Applepicker" computer task (Norman & Jongerius, 1985) in which participants were required to search for apples on virtual trees. Participants received visual feedback when an apple was found, signaling that a unit of reward selected for that trial had been earned. Each 15-minute trial per session differed in the type of reward that was available as a reinforcer. Upon earning a reinforcer, subjects could continue responding on the task to earn additional units of the reinforcer, or they were free to stop responding at any point and could simply wait quietly, or read provided magazines until the end of the 15-min task period. Virtually all participants stopped responding at some point before the end of the trial indicating that maximal responding for a reinforcer was reached. Prior to each session, participants who abstained from smoking overnight smoked either a nicotine-containing or denicotinized cigarette, or smoked nothing at all.

Similar to previous findings, the results of the study determined that reinforced responses were significantly greater following smoking of nicotine cigarettes, compared to smoking denicotinized cigarettes or no smoking. Furthermore, these reinforcement enhancing effects of nicotine via smoking did not differ between dependent and nondependent smokers (Perkins & Karelitz, 2013). Notably, nicotine only enhanced responding for music reinforcers, but not monetary reinforcers or the negatively reinforcing termination of aversive noise, suggesting that

additional human research is necessary in better understanding the specificity of nicotine's reinforcement enhancement.

The conditioned place preference paradigm

In non-human studies, the reward properties of drugs are frequently assessed using an experimental design called conditioned place preference (CPP), which measures the extent to which a rodent chooses to be in an environment that has been repeatedly paired with the appetitive effects of a stimulus. Drugs of abuse induce preference for the environment that has been repeatedly paired to its effects, resulting in what is termed a "conditioned place preference" (Carr et al., 1989). Generally, the task involves two contextually distinct compartments joined by a connecting tunnel. The two compartments may differ across modalities such as visual, auditory, tactile and olfactory cues. During the task, the animal is confined to one of the two compartments for a fixed amount of time, and is given a rewarding substance, like food or drug. Later, in a separate session, the animal is confined to the other compartment and receives a placebo for an equal amount of time. These pairings are repeated to strengthen the association between context and presence or absence of the reward. Following the pairing sessions, a test session is given in which the animal receives unrestricted access to both compartments without any reward or placebo. Typically, animals demonstrate a strong preference for the room in which the reward was previously paired despite the reward no longer being present (van der Kooy et al., 1987). Pavlovian conditioning is the most widely accepted explanation for the CPP since it is believed that the context paired with the reward becomes a conditioned stimulus that predicts the presence of the reward.

Advantages to CPP have been documented in a thorough review by Carr et al. (1989), indicating that the task is sensitive to low drug doses, can be obtained in a single drug-pairing (Bardo, 1999), does not require a surgical procedure, and measures the effects of both reward and aversion. Furthermore, the test measurements are made in the absence of the drug; therefore, drug effects like motor impairment or sedation do not confound the results. CPP can be elicited by a number of natural rewards such as food, water, copulatory opportunity, and opportunity for social interaction (Tzschentke, 1998), as well as by a variety of drugs (Mattson et al., 2003). Nicotine has been shown to produce CPP over a wide range of doses after both peripheral (Fudala et al., 1985; LeFoll & Goldberg, 2004) and central administration (Iwamoto, 1990). One study reported that effects of nicotine CPP persisted for 3 and 12 weeks without further conditioning (Forget, Hamon & Thiebot, 2005). Furthermore, mecamylamine, a nicotine antagonist, has been found to block the effects of nicotine (Fudala et al., 1985), and thus block nicotine-induced CPP.

Another advantage of CPP is that it is adaptable to a variety of laboratory animals (Hughes et al., 1995; Foltin & Evans, 1997), and recently has been translated to human studies (Childs & de Wit, 2009). Our lab has previously demonstrated that food-deprived undergraduates display a significant CPP for a virtual reality room previously-paired with a chocolate reward (Astur et al., 2014). To extend the standard CPP paradigm to humans, we created a virtual reality (VR) conditioned place preference task. The virtual environment consisted of two visually distinct virtual rooms connected by a neutral hallway. Using a two-day procedure, food-deprived undergraduates completed six, 6-minute conditioning sessions on the first day, navigating the environment with a joystick. One room was paired with real-life M&Ms for three sessions where participants were instructed to eat the M&Ms as they were dispensed, while the opposite room

was paired with no food for three sessions. The room that was paired with M&Ms and the orders of the pairing sessions were counterbalanced. Twenty-four hours following the conditioning sessions, participants returned for a test session where they were placed in the same virtual environment, but started in the neutral hallway. They had access to both rooms for the entire 6-minute session; however, no M&Ms were dispensed throughout the test session. Our results indicate that participants displayed a significant CPP as evidenced by amount of time spent in the previously-M&M paired room. Furthermore, using subjective self-report measures similar to those implemented by Childs and de Wit (2009), we observed that participants explicitly indicated that they preferred the food-paired room, and showed a trend for rating that room more favorably. Hence, both implicit and explicit measures indicated a CPP to chocolate in humans (Astur, 2014). Given the success of the CPP task in assessing basic reward mechanisms in non-humans, we were interested in examining whether this task could lend insight into the mechanisms underlying the known enhancement of reward-sensitivity by nicotine.

Chapter Two: Present Experiment

Introduction to the current study

The present study sought to examine nicotine's ability to increase the sensitivity of reward properties by enhancing responding for non-drug incentives in humans using a virtual CPP task. Not only is there a lack of information concerning nicotine's role in human reinforcement enhancement, but even fewer studies have been conducted utilizing the hallmark conditioned place preference paradigm to analyze the multi-faceted effects of nicotine on reward. Therefore, using a novel virtual reality translation of the CPP task, one goal of the present study is to investigate whether nicotine will enhance CPP for a chocolate food reward.

In addition to enhancing the reinforcing properties of non-nicotine stimuli, nicotine has also been shown to delay the rate of extinction for previous conditioning in that animals continue to respond for previously-rewarded stimuli that are no longer reinforced. One study demonstrated that nicotine infusions increased operant responding for alcohol, and that extinction of responding for alcohol was delayed by the infusion of nicotine (Clark et al., 2001). In examining the effects of nicotine administration on the extinction of conditioned fear memories, Elias et al. (2010) found that nicotine enhanced extinction when administered only prior to extinction sessions. However, when nicotine was administered before both the acquisition of the conditioned fear response and before extinction, the rate of extinction was delayed. These findings indicate that while nicotine may facilitate extinction, when it is administered at both training and extinction, nicotine strengthens contextual associations formed during training, and these enhanced contextual associations can interfere with extinction when extinction occurs in the same context as used for training (Elias et al., 2010) suggesting a somewhat complicated role for nicotine in the extinction process.

Extinction for CPP occurs when non-rewarded exposure to environmental contexts previously paired with rewarding stimuli reduces subsequent CPP. Brenhouse and Andersen (2008) found that rats formed reliable place preferences for cocaine, and that adolescent rats required significantly more extinction sessions than adults to extinguish cocaine CPP. However, to date, no studies have been conducted examining the effects of nicotine on extinction using place conditioning. Therefore, the present study aims to determine whether nicotine slows the rate of extinction in humans for a virtual room paired with a chocolate food reward.

Finally, nicotine has been shown to increase vulnerability to reward-primed reinstatement after extinction where non-contingent administration of the reward is administered in a neutral

context and reinstates behavior after periods of non-responding (de Wit & Stewart, 1981). In other words, animals are more sensitive to the recovery of former conditioning if they have previously received nicotine. In studying the effect of nicotine on alcohol self-administration and reinstatement after extinction, Le and colleagues (2003) found that nicotine significantly reinstated alcohol seeking after extinction of the alcohol-reinforced behavior, leading to intake levels that were more than twice of those of the saline-treated group. Furthermore, this effect was strongly enhanced by prior nicotine exposure. In the previously-mentioned study by Brenhouse & Andersen (2008), not only did cocaine delay adolescent rats' ability to extinguish learned place preferences for cocaine-paired environments, but it was found that adolescents were also more vulnerable to drug-primed reinstatement of CPP after extinction, displaying significant reinstatement for a lower conditioning dose of cocaine than adults, and spending more time than adults in a previously drug-paired environment. Again, however, there exists an absence of literature examining nicotine's effects on reinstatement using the CPP task. Therefore, the present study aims to determine whether nicotine will promote the reinstatement of an extinguished CPP.

Combined, these studies indicate that it is of value to understand the behavioral, emotional, and neuropharmacological mechanisms by which nicotine enhances responding for conditioned rewards. Accordingly, the aim of the present study was to (1) assess whether nicotine increases the sensitivity of reward properties by enhancing the strength of food-reward conditioning, (2) determine whether nicotine delays extinction when exposure to a virtual room previously-paired with food is no longer rewarded, and (3) determine whether nicotine increases the possibility of reinstatement of previous conditioning. We hypothesize that participants who receive nicotine prior to conditioning will demonstrate a stronger conditioned place preference in

our virtual paradigm in terms of the amount of time spent in and the subjective ratings of the previously-rewarding room. Additionally, we hypothesize that nicotine administration will significantly delay the rate of extinction of the CPP, as demonstrated by continued preference for the previously-rewarding room despite it no longer being paired with a reward. And finally, we hypothesize that nicotine will increase the strength of reinstatement of the CPP, such that participants will spend significantly more time in the food-paired room relative to the extinction sessions.

Materials and Method

Participants

Ninety-six University of Connecticut undergraduates (avg. age = 19.5 yrs; *SD* = 1.18; 25 females) were recruited from introductory psychology classes. Of these participants, Day 1 data from 30 participants was discarded, and Day 2 data from 24 participants was discarded due to ineligibility (i.e. ate before experiment, used nicotine before experiment as monitored by CO detector, felt nauseated during experiment, incorrect lozenge administration, did not enjoy chocolate). This resulted in usable Day 1 data from 72 participants (avg. age = 19.3 yrs; *SD* = 1.12; 16 females), and usable Day 2 data from 62 participants (avg. age = 19.3 yrs; *SD* = 1.19; 16 females; Day 1/Day 2: Nicotine/Nicotine, *n* = 20; Nicotine/Placebo, *n* = 38; Placebo/Nicotine, *n* = 22; Placebo/Placebo, *n* = 32). On average, 10.6 (*SD* = 8.9) nicotine-containing products were used weekly. Participants were required to abstain from eating and from using nicotine for six hours prior to the experiment. In order to participate, participants were required to have no pre-existing cardiac conditions, and female participants could not be pregnant. It was also required that participants were willing to eat chocolate for the purposes of this experiment. Participants

received class credit for their participation. Approval for this study was obtained from the University of Connecticut Institutional Review Board.

Apparatus

An IBM-compatible computer with a SVGA color monitor was used for testing. Participants seated at the computer navigated through the virtual environments by manipulating a joystick. A speaker connected to the computer was used to provide auditory feedback and a Med Associates Inc. ENV-203IR pellet dispenser was used to dispense M&Ms into a tray for the participant to consume.

Procedure

This was a two day study with each daily session lasting approximately one hour. On Day 1, food-deprived participants arrived in the morning between 8:30 and 10:30AM, and consent was obtained. All participants were required to blow into a CoVita Smokerlyzer carbon monoxide sensor, which was used to determine whether they had smoked within the last 6 hours. Anyone that had smoked within the last 6 hours (PPM <10; Perkins et al., 2012) was asked to reschedule their appointment. If the participant was female, she was asked to take a urinalysis pregnancy test that must be negative. Participants were then asked to complete a brief demographics questionnaire consisting of questions regarding age, sex, when the participant last ate, when the participant last used nicotine, and items like level of hunger on a 1-10 scale (1 being "not at all").

Following completion of the demographics questionnaire, participants were randomly selected to receive either a 4mg nicotine lozenge or a similar-tasting placebo. The 4mg lozenge

was chosen for nicotine treatment because studies investigating nicotine absorption from several nicotine replacement products have found that, of the smokeless products, the 4mg nicotine lozenge resulted in the greatest blood nicotine levels across a 1-hour timeline, with levels peaking 15-minutes after administration (McEwan et al., 2008). Participants were instructed to place the lozenge in their mouth, occasionally moving it from side to side to allow it to slowly dissolve over the course of 15 minutes. They were told to minimize swallowing, and not to chew or swallow the lozenge.

While the lozenge or placebo dissolved, participants completed several additional questionnaires administered in the order described. The Fagerstrom Test for Nicotine Dependence (Fagerstrom, 1989) is a standard instrument for assessing the intensity of physical dependence to nicotine. It contains six items that evaluate the quantity of cigarette consumption, the compulsion to use, and dependence. Given that cravings are one of the most prominent symptoms of nicotine dependence and are a significant predictor of nicotine relapse, we also use the Questionnaire on Smoking Urges (Tiffany & Drobes, 1991), a 10-item self-report measure. The Behavioral Inhibition and Activation Scale (BIS/BAS; Carver & White, 1994) is another questionnaire used that aims to assess the differences in the two motivational systems that likely contribute to cravings and relapse. Finally, the Kirby Monetary Choice Questionnaire (Kirby & Marakovic, 1996) is used to measure a person's level of impulsivity in terms of delay discounting– or whether or not an individual can delay reward choices.

After completing the surveys, and 15-minutes after administration of the lozenge or placebo to maximize absorption (McEwan et al., 2008), participants were guided through a brief tutorial on how to interact with the virtual environment using a joystick in a 90-second practice session where they were placed in a barren virtual reality (VR) room. To encourage exploration

in both the practice and experimental sessions, a downward facing arrow appeared periodically in random locations and participants were required to locate and collide with the arrow. Three to five M&Ms were dispensed during the practice session, and participants were instructed that throughout the experiment they are to eat the M&Ms as they were dispensed. Participants were allowed to ask questions at any time.

After completing the practice session, each participant completed six, three-minute experimental pairing sessions in a virtual environment. A short, 1-minute break followed each session. The environment consisted of two visually-distinct rooms connected by a neutral hallway (see Fig. 1A). In each of the six experimental sessions, the participants were confined to one of the two rooms and were to explore the environment using the joystick. One room was paired with real M&Ms for three sessions, while the opposing room was paired with no food for three sessions. The room paired with M&Ms and the orders of the pairing sessions were counterbalanced. One M&M was dispensed periodically into a cup next to the participant during the M&M sessions, and the participant was instructed to eat the M&Ms as they were dispensed. Specifically, an M&M was dispensed every 21s $\pm$ 5 s. Between 25-30 M&Ms total were dispensed over the course of the experiment, which is approximately half the amount in a regular 47.9g single size bag of M&Ms. After all six pairing sessions were completed, a 10-minute break was given before the test session (see Fig. 1B for a sample testing sequence).

For the test session, participants were placed in the same virtual environment and started in the neutral hallway. They had access to both rooms for the entire three-minute session via a door between the two rooms, but were locked out of the neutral hallway once they first entered a room. M&Ms were not dispensed during the test session. After the test, participants were given a survey. Questions asked which of the two rooms they preferred, how much they enjoyed each

room on a scale of 0-100 (0 being "not at all"), and how much they enjoy chocolate on a scale of 0-100 (0 being "not at all").

On Day 2, participants were again asked to complete the carbon monoxide test. Participants were then randomly selected to either receive a 4mg nicotine lozenge or the similar-tasting placebo. To test for extinction, the participant underwent three, 3-minute test sessions, as described on Day 1, in which they had unrestricted access to both VR rooms where no M&Ms were given. One-minute breaks were given between each session. After the test sessions, participants underwent a 60-second reinstatement session in which they received M&Ms in a neutral, novel VR room. After a 10-minute break, participants once more underwent a test session to test for possible reinstatement (see Fig. 1B for a sample testing sequence). After the test, participants were given a survey asking which of the two rooms they preferred, how much they enjoyed each room on a scale of 0-100 (0 being "not at all"), and how much they enjoy chocolate on a scale of 0-100 (0 being "not at all"). Finally, at several points throughout the experiment on both Day 1 and 2, participants were asked questions about their subjective mood on a visual analog scale (VAS). VAS questions inquired how buzzed the participant felt, how nauseated, how dizzy, how hungry, how anxious, and how much they craved nicotine and/or chocolate**.**

Results

In examining participants' levels of nicotine dependence, we found that most users in our sample demonstrated low levels of dependence with an average Fagerstrom score of 1 (*SD* = 1.43). While we did not collect information categorizing methods of consumption (i.e. e-cigarettes, cigarettes, snuff, etc.), participants did report using an average of 10.6 (*SD* = 8.9) nicotine-containing products weekly. Conditioned place preference scores were calculated as

difference scores by subtracting the amount of time spent in the non M&M-paired room from the amount of time spent in the M&M-paired room during the test session, such that any score greater than zero indicated a conditioned place preference for the M&M-paired room. Difference scores in ratings were also calculated this way.

In support of previous findings by our lab (Astur et al., 2014), placebo-treated participants demonstrated a significant CPP by spending significantly more time in the previously-paired M&M room on test day ($t(38) = 1.99$, $p = 0.04$; Figure 2A). Nicotine-treated participants, however, did not display a significant CPP in terms of time ($t(33) = 0.67$, $p = 0.51$; Figure 2A). Additionally, there were no significant differences in time between treatment groups ($F(1,72) = 0.25$, $p = 0.62$; Figure 2B). When examining subjective ratings as a measure of CPP, neither the nicotine group ($t(33) = 0.68$, $p = 0.50$) nor the placebo group ($t(38) = -0.41$, $p = 0.69$) demonstrated a significant CPP; in other words, there was no difference in ratings of how enjoyable the M&M-paired room was compared to the no M&M room after testing. Moreover, there were no significant ratings differences between treatment groups after testing ($F(1,72) = 0.64$, $p = 0.43$; Figure 2B).

In an attempt to determine whether individuals with greater nicotine dependence condition differently than those with lesser or no dependence, we specifically examined the 36 participants who scored greater than zero on the Fagerstrom questionnaire, a metric that assesses levels of nicotine dependence where a zero score indicates no dependence, a 1-5 score indicates low to moderate dependence, and anything greater than 5 indicates high dependence. For individuals with a Fagerstrom score greater than 0, the M&M-paired room was rated as significantly more enjoyable for the nicotine group compared to the placebo group ($F(1, 35) =$

4.72, $p = 0.04$; Figure 3). However, there were no significant differences between treatment groups in terms of time ($F(1, 35) = 0.25$, $p = 0.62$).

Given that urges and cravings have been shown to be responsible for ongoing drug use and the inception of relapse, we next attempted to discriminate whether an individual's subjective, motivational states affected CPP. In examining whether total BIS/BAS scores differentially affected CPP for the nicotine or placebo groups, we found no significant effects on time or ratings (Table 1).

Table 1.	**Nicotine**		**Placebo**	
	CPP Time	**CPP Ratings**	**CPP Time**	**CPP Ratings**
BIS	$F(1,33) = 0.21$, $p = 0.95$	$F(1,33) = 0.67$ $p = 0.78$	$F(1,38) = 0.65$, $p = 0.74$	$F(1,38) = 0.24$, $p = 0.98$
BAS	$F(1,33) = 0.40$, $p = 0.87$	$F(1,33) = 2.76$, $p = 0.45$	$F(1,38) = 0.45$, $p = 0.90$	$F(1,38) = 0.27$, $p = 0.98$

Specifically analyzing the individual BAS Subscales (Drive, Reward Responsiveness (RR), and Fun Seeking (FS)), we found that the nicotine group scored significantly higher on the BAS Drive subscale than did the placebo group ($F(1, 72) = 5.47$, $p = 0.02$). To examine this further, we performed median splits on all subscales to divide participants into groups of high and low responders. We found no significant time or ratings differences between treatment groups on any of the BAS subscales, regardless of high or low scores (Table 2). Furthermore, there were no significant differences between treatments in time or ratings regardless of whether participants scored in the upper or lower 50% on the BIS (Table 2).

Table 2.	**Time**		**Ratings**	
	High Responders (upper 50%)	**Low Responders (lower 50%)**	**High Responders (upper 50%)**	**Low Responders (lower 50%)**
BAS Drive	$F(1, 37) = 0.003$, $p = 0.96$	$F(1, 34) = 0.41$, $p = 0.53$	$F(1, 37) = 2.49$, $p = 0.12$	$F(1, 34) = 0.57$, $p = 0.45$
BAS RR	$F(1, 38) = 0.54$,	$F(1, 19) = 2.58$,	$F(1, 38) = 0.66$,	$F(1, 19) = 0.90$,

	$p = 0.47$	$p = 0.13$	$p = 0.42$	$p = 0.36$
BAS FS	$F(1, 28) = 0.13$, $p = 0.72$	$F(1, 43) = 0.18$, $p = 0.68$	$F(1, 28) = 0.84$, $p = 0.37$	$F(1, 43) = 0.002$, $p = 0.98$
BIS	$F(1, 35) = 0.001$, $p = 0.97$	$F(1, 36) = 0.14$, $p = 0.71$	$F(1, 35) = 0.89$, $p = 0.35$	$F(1, 36) = 0.004$, $p = 0.95$

Using a second measure to understand the association between underlying motivation to smoke and CPP, we assessed the relationship between CPP and scores on the Questionnaire on Smoking Urges and its two subscales: Urge to Smoke and Relief of Negative Affect. We found no significant correlations between time or ratings CPP and total QSU, Urge to Smoke, or Relief of Negative Affect scales (Table 3).

Table 3.	**Time**	**Ratings**
Total QSU	$r(73) = 0.19$, $p = 0.87$	$r(73) = 0.07$, $p = 0.56$
Urge to Smoke	$r(56) = 0.12$, $p = 0.39$	$r(56) = 0.01$, $p = 0.92$
Relief of Negative Affect	$r(56) = 0.07$, $p = 0.61$	$r(56) = 0.07$, $p = 0.61$

However, performing a median split on the QSU subscales, we found that nicotine-treated participants who scored in the upper 50% on the Urge to Smoke subscale, demonstrating a strong desire and intention to smoke with smoking perceived as rewarding, rated the M&M-paired room as significantly more enjoyable compared to placebo-treated participants ($F(1,36) = 3.19$, $p = 0.04$). There was no significant difference in CPP between treatments in terms of time ($F(1, 36) = 0.27$, $p = 0.34$) using the Urge to Smoke subscale. Furthermore, regardless of whether individuals scored in the upper or lower 50% on the Relief of Negative Affect scale, there were no significant differences in treatments in terms of time or ratings (Table 4).

Table 4.	**Time**		**Ratings**	
	High Responders (upper 50%)	Lower Responders (lower 50%)	High Responders (upper 50%)	Lower Responders (lower 50%)
Relief of Negative Affect	$F(1, 30) = 0.21$, $p = 0.65$	$F(1, 34) = 0.00$, $p = 0.99$	$F(1, 30) = 0.16$, $p = 0.69$	$F(1, 34) = 0.08$, $p = 0.78$

Recognizing that an additional factor involved in the initiation of drug use is impulsivity, further Day 1 analyses found a no significant correlations between the Kirby Monetary Choice Questionnaire (Kirby) and treatment differences in ratings ($r(73) = -0.30$, $p = 0.79$), or time ($r(73) = 0.20$, $p = 0.10$). However, performing a median split on scores of the Kirby revealed a significant difference between treatment groups in terms of time spent in the M&M-paired room for participants who scored in the upper 50% on the Kirby Monetary Choice Questionnaire ($F(1,33) = 3.17$, $p = 0.02$). In other words, participants in the nicotine-treated condition who score as more impulsive on the Kirby questionnaire were more likely to show a CPP.

We next aimed to characterize the effects of nicotine on extinction and reinstatement. When examining extinction, it is worthwhile to analyze those who acquired a CPP on Day 1 since those who did not display a CPP may not have acquired the necessary learned associations needed to readily show extinction. Looking at Day 2 drug effects in nicotine-dependent participants who demonstrated a CPP on Day 1 (Fagerstrom > 0; CPP Difference Score > 0), there was no significant time CPP for the nicotine group or placebo group in extinction sessions 1 or 2 (Table 5). Furthermore, no significant differences in time were seen between Day 2 treatments for the first ($F(1, 18) = 0.02$, $p = 0.90$) or second ($F(1, 18) = 1.80$, $p = 0.20$) extinction sessions. However, we did find that those who received placebo on Day 2 showed a CPP during the third extinction session by spending significantly more time in the M&M-paired room than Day 2 nicotine-treated participants ($F(1, 18) = 5.01$, $p = 0.04$). What is more, individuals who received nicotine on Day 1 spent significantly more time in the M&M-paired room during the third extinction session than placebo-treated participants ($F(1, 18) = 13.7$, $p = 0.002$; Figure 4). Therefore, nicotine administration on Day 1 and placebo administration on Day 2 appear to be

the most influential in determining whether the participant will demonstrate a conditioned place preference during the third extinction session (Figure 6).

Table 5.	**Nicotine**	**Placebo**
Extinction Session 1 (E1)	$t(16) = 0.62$, $p = 0.56$	$t(17) = 0.57$, $p = 0.58$
Extinction Session 2 (E2)	$t(16) = -1.56$, $p = 0.14$	$t(17) = 0.42$, $p = 0.68$

Finally, while there were no significant differences between Day 1 treatments in terms of time during the reinstatement session (F (1, 18) = 3.39, $p = 0.83$), participants who received nicotine on Day 2 reinstated by a significantly greater change between the amount of time spent in the M&M-paired room during the last extinction session and the reinstatement session compared to placebo-treated participants ($F(1, 18) = 5.87$, $p = 0.03$; Figure 8).

Chapter Three: General Discussion

The present experiments were undertaken to characterize the effects of nicotine on conditioned responses in humans using a virtual CPP paradigm. More specifically, these studies were conducted to (1) determine whether nicotine increases the sensitivity of reward properties by enhancing the strength of food-reward conditioning, (2) assess whether nicotine delays extinction when exposure to a virtual room previously-paired with food is no longer rewarded, and (3) determine whether nicotine increases the possibility of reinstatement of previous conditioning after extinction.

Overall, the present results demonstrated that nicotine does seem to enhance conditioning for a food reward during the virtual CPP task as evidenced by participants who are dependent on nicotine rating the M&M-paired room as significantly more enjoyable when they receive nicotine on Day 1. Nicotine also seems to make individuals more resistant to extinction since those who received nicotine on Day 1 revealed an increased preference for the M&M room in the

last extinction session. Lastly, nicotine on Day 2 seems to promote reinstatement of the conditioned behavior following a small amount of M&Ms given in a neutral context after extinction.

Contributions of the current work to the literature

Day 1 of the present study demonstrated that by employing a novel virtual CPP task, nicotine enhances the sensitivity of non-pharmacological rewards in humans that are not directly associated with nicotine intake. For nicotine-treated participants who demonstrate some level of nicotine dependence, the M&M-paired room was rated as significantly more enjoyable compared to placebo-treated participants. This finding supports those of previous studies illustrating nicotine's ability to increase appetitive responding for non-drug incentives (Donny et al., 2003; Chaudhri et al., 2007; Raiff & Dallery, 2008; Atwood et al., 2009; Guy & Fletcher; 2013; Perkins & Karelitz, 2013). Understanding the relationship between nicotine dependence and conditioning is important in that dependence indices have been shown to predict cue-induced cravings and subsequent relapse. Sayette et al. (2003) reported that craving responses to cues are greater in dependent versus non-dependent smokers. Following this research, Erblich (2004) found that responses on a five-item self-reported craving scale to an in vivo cue (holding an unlit cigarette), but not to an imagined cue (thinking about smoking in certain situations), were associated with self-reported shorter duration of abstinence during a prior quit attempt. Furthermore, it has been shown that measures of nicotine dependence predict differential responses to smoking cessation treatments (Zelman et al., 1992). Therefore, differentiating dependence severity and understand the role of dependence in conditioning is of practical importance.

Interestingly, while we were able to demonstrate a CPP in placebo-treated participants in terms of the amount of time spent in the M&M-paired room, we were not able to produce a reliable CPP in nicotine-treated participants. CPP procedures designed to assess drug preferences in non-humans rely on certain implicit assumptions regarding the positive effects of drugs. Particularly, it is assumed that drugs produce similar subjective states in non-humans as they do in humans. Thus, when an animal spends more time in the previously reward-paired compartment of the place preference chamber, it is inferred that the environment has acquired rewarding properties through association with pleasurable drug effects. By examining subjective ratings of a reward-associated environment, it is assumed that there would be a logical relationship between measures of room preference and the incentive effects of the reward, such that more positive (elation and positive mood) and less negative (anxiety and dysphoria) subjective effects would predict liking of the rewarding room. Given that nicotine-treated subjects revealed significantly higher subjective ratings of the food-paired room, we infer that nicotine enhanced reward-sensitivity of the M&M room. Similar results of an explicit place preference, in terms of subjective ratings, in the absence of an implicit place preference, in terms of time, have been documented by others when examining drug effects on humans (Childs & de Wit, 2009; Childs & de Wit, 2011). Therefore, nicotine's influence on subjective responses to food-rewards contribute to its ability to establish place conditioning, but the relationship between quantitative ratings of liking of a room and whether this sufficiently corresponds to the behavioral measure obtained in animals in terms of time spent in one or the other remains to be investigated.

As mentioned, cue-induced cravings, or the urge or desire to smoke in response to the presentation of stimuli associated with smoking, has been implicated in the maintenance and

persistence of nicotine use (Perkins, 2009). Theoretically, it is believed that greater magnitudes of cue-induced craving predict increased risk of relapse when faced with smoking-associated stimuli in the natural environment given the strong likelihood that such stimuli become associated with smoking and acquire incentive motivational properties. Given that urges and cravings can be theorized as subjective emotional states responsible for continued drug use and the inception of relapse (Carver & White, 1994), we aimed to discriminate the effects of drug using behavior resulting from changes in subjective motivational states on CPP using both the BIS/BAS and the QSU. While we saw no effects of the BAS Reward Responsiveness or Fun Seeking subscales on CPP or differences between treatments, we did note that nicotine-treated participants scored significantly higher on the BAS Drive subscale than did placebo-treated participants. The BAS is said to motivate goal-driven behavior toward positively-reinforcing cues. The Drive subscale assesses behavioral responding, while the Reward Responsiveness scale evaluates affective responding, and the Fun Seeking scale measures both affective and behavioral responding. Therefore, given our results, it is possible that nicotine users with low levels of nicotine dependence have greater levels of behavioral responding for positively-reinforcing stimuli, which might increase their likelihood of rating the reward-paired room more favorably; however, it is important in future studies to investigate whether people with higher dependence would score differently on other BIS/BAS measures.

Using the QSU, we also found that nicotine-treated participants who scored in the upper 50% on the Urge to Smoke subscale of the Questionnaire on Smoking Urges rated the M&M-paired room as significantly more enjoyable compared to placebo-treated participants. Therefore, nicotine reward enhancement was evident in nicotine-treated participants who indicated that their

desire and intention to smoke was reward-seeking based, as opposed to smoking to relieve negative affect and nicotine withdrawal.

Interestingly, using our QSU and BIS/BAS measures, we saw no influence of regulating aversive motives on the acquisition of CPP. Scores on the BIS did not affect CPP regardless of the degree of scoring on this scale, nor were there significant differences between treatment groups in response to this measure. Additionally, responses on the Relief of Negative Affect subscale of the QSU had no significant effect on CPP in terms of time or ratings, and did not differ by treatment. Negative affect is typically described as a factor of nicotine withdrawal that significantly predicts relapse with the expectation that nicotine use will attenuate negative mood symptoms. Both the Relief of Negative Affect subscale and the BIS are thought to control the experience of anxiety and to inhibit behavior that might produce negative or painful outcomes. Therefore, sensitivity to these scales would reflect an individual's propensity toward anxiety when provided with the appropriate cues, and heightened avoidance of such anxiety-inducing experiences. Pang et al. (2014) investigated the effects of negative affect and anxiety symptoms as moderators of nicotine dependence severity, and found a positive relationship between increasing levels of negative affect and anxiety symptoms and associations between smoking expectancies and nicotine dependence severity. Given that we saw no relation of negative reinforcement and CPP, it is possible that (1) the required 6-hour window of nicotine abstinence prior to participating in our study was not long enough to manifest negative mood states strong enough to elicit negative reinforcement, or (2) negative reinforcement plays a greater role for individuals with higher levels of nicotine dependence that were not represented by our sample.

As mentioned, it has been suggested that since BAS responding reflects motivated goal-driven behavior toward positively-reinforcing cues, it may represent the biological underpinnings

of impulsivity (Carver & White, 1994). Franken and Muris (2006) used the BIS/BAS questionnaire to examine the influence of impulsivity on drug and alcohol use in college students. Their results indicated that students' drug and alcohol use was positively correlated with BAS personality characteristics with the most substantial correlations found between the BAS Fun Seeking and BAS Drive subscales. Given that nicotine-treated participants scored significantly higher on the BAS Drive subscale than did placebo-treated participants in our study, our findings also demonstrate a relationship between substance use and impulsivity.

Another measure often used to assess levels of impulsivity, the Kirby questionnaire requires an individual to choose between immediate, but smaller theoretical monetary rewards and delayed, but larger rewards. High levels of impulsivity also seem to contribute to nicotine-enhanced CPP. A significant difference between treatment groups was found for participants who scored in the upper 50% on the Kirby Monetary Choice Questionnaire, where nicotine-treated participants who scored highly on the Kirby questionnaire spent more time in the previously-paired M&M room. Diergaarde and colleagues (2008) showed that impulsive choices were associated with enhanced motivation to initiate and maintain nicotine self-administration. Furthermore, impulsivity predicted a weakened ability to inhibit nicotine seeking during abstinence, and an enhanced vulnerability to relapse upon re-exposure to nicotine cues. Therefore, our findings complement those previously-published suggesting that nicotine-mediated learning is influenced by high levels of impulsivity, and that interventions aimed to improve impulse control might aid in the reduction of susceptibility to nicotine dependence and/or lead to successful smoking cessation. Additionally, due to its sensitivity in detecting appetitive responding for those who score highly on the Kirby and BAS-Drive questionnaires,

utilizing the virtual CPP task seems advantageous in detecting underlying impulsivity traits which may predict susceptibility to cue-induced cravings and higher rates of relapse.

While significant enhancement by nicotine on the acquisition of CPP in nicotine-dependent users was noted, we did observe a lack of significant CPP in non-dependent nicotine-treated participants. As described previously, numerous studies emphasize the inherently rewarding nature of nicotine as evidenced by increased self-administration behavior (Henningfield et al., 1983; LeFoll & Goldberg, 2009) and by nicotine's ability to produce CPP (Fudala et al., 1985; Iwamoto, 1990; LeFoll & Goldberg, 2004). However, CPP results using nicotine have been equivocal. It has been suggested that nicotine only produces CPP within a relatively narrow dose range with the exact range determined by the influence of other experimental parameters. Le Foll & Goldberg (2005) tested nicotine across a range of doses in rodents and found CPP at 0.1, 0.4, 1 and 1.4 mg/kg sc, but not at 0.01, 0.04 and 2 mg/kg sc. In the latter group of rats, 2 mg/kg sc nicotine produced a small but significant conditioned place aversion (CPA) where animals displayed a preference for the non-nicotine-associated environment. Numerous studies have reproduced investigations of dose-sensitive nicotine CPP with discrepant results across studies where nicotine has been shown to produce no effect, a CPP, or a CPA (Agatsuma et al., 2006; Spina et al., 2006; Laviolette & van der Kooy, 2003) alluding to the complexity of nicotine's effects on conditioning, and suggesting that nicotine can produce both rewarding and aversive effects. Therefore, it may be the case that non-dependent users are more sensitive to the aversive effects of nicotine treatment, thus accounting for the lack of a significant CPP in non-dependent, nicotine-treated participants.

The two main objectives for Day 2 of the present study aimed to determine the relationship between nicotine-treatment on extinction of CPP and reinstatement of the

extinguished behavior. It was hypothesized that if nicotine increased the reinforcing value of non-drug rewards, then this should result in a preference for the reward-paired context that is more resistant to extinction. However, we found no significant differences in time nor ratings between Day 2 treatments for the first or second extinction sessions when examining nicotine-dependent individuals who demonstrated a conditioned place preference on Day 1 (Fagerstrom > 0; CPP Difference Score >0). Interestingly, we did observe a CPP by the third extinction session such that individuals who received nicotine on Day 1 and placebo on Day 2 spent significantly more time in the M&M-paired room than placebo-treated participants.

Existing literature illustrates a complex relationship between nicotine and extinction. While some studies demonstrate that nicotine delays extinction of conditioned responses in that animals continue to respond for previously-rewarded stimuli that are no longer enforced (Clark et al., 2001), other studies found that the effects of nicotine on extinction can vary, and are dependent upon experiment parameters (Elias et al., 2010; Brenhouse & Andersen, 2008). As mentioned previously, Elias and colleagues (2010) demonstrated that the effects of nicotine on the extinction of fear conditioning were contingent upon when nicotine was administered and on the context during extinction. Nicotine facilitated extinction when administered only at extinction; however nicotine delayed extinction when administered at both training and extinction. Furthermore, this delay of extinction was dependent on the consistency of contextual information between training and extinction. Nicotine administered at both training and extinction delayed extinction only if the context was the same at both stages, but if the context changed between training and extinction, no effect of nicotine was seen. Furthermore, Raiff and Dallery (2008) showed that nicotine increased responding maintained by conditioned reinforcers, but did not increase resistance to extinction on any of the response types studied.

While one explanation for our results is that we are in fact seeing delayed extinction during the third extinction session as an effect of Day 1 nicotine and Day 2 placebo-treatment, an alternate explanation is that CPP elicited during the third extinction session, following the extinction of CPP seen in extinction sessions one and two, actually reflect spontaneous recovery. Spontaneous recovery is the return of a conditioned response elicited by a conditioned stimulus when time passes following extinction (Brooks & Bouton, 1993). In a study by Cohen and colleagues (2005), rats were allowed to self-administer nicotine in a specific environment, the behavior was extinguished in the same environment, animals were withheld from that environment for a certain time, and then behavior was recorded when animals were returned to the drug-taking environment. The results from the study showed that the nicotine-associated environmental cue effectively elicited recovery of nicotine-seeking responding after 25 days of abstinence from the drug and environment in rats. A study by Troisi (2003) found no evidence for spontaneous recovery two weeks following extinction, but partial recovery four weeks following the final extinction phase, while Shaham et al. (1997) found spontaneous recovery of the previously extinguished behavior when rats that had been housed for 21 days without being exposed to the drug-taking environment were then re-exposed to that environment. Literature concerning the timeline of spontaneous recovery following extinction is largely limited to non-human studies; however, these results suggest that spontaneous recovery is time-dependent (Bouton, 1988) and that it may take several weeks following extinction before the return of a conditioned response. Therefore, while we do see the return of conditioned behavior following extinction, the theory of spontaneous recovery is not fully supported in that each of our three extinction sessions is only 3-minutes long. This suggests that our participants have extinguished a CPP and recovered the conditioned behavior in an unreasonably short timeframe. Some have

coined this phenomenon “reacquisition” of a conditioned response post-extinction, which has been described as occurring faster than the initial acquisition. Reacquisition may indicate that the original learning was not destroyed but rather was “saved,” through the process of extinction (Bouton, 2004). Given these observations, it is important in future work to dissociate the effects of spontaneous recovery, reacquisition, and nicotine-induced resistance to extinction.

Finally, in examining the effect of nicotine on reinstatement we found that participants who received nicotine on Day 2 reinstated by a significantly greater change between the amount of time spent in the M&M-paired room during the third extinction session and the reinstatement session compared to placebo-treated participants. Reinstatement refers to the recovery of conditioned behavior produced by exposure to the unconditioned stimulus alone following extinction. In the traditional reinstatement model, the effect of non-contingent re-exposure to drugs on the reinstatement of drug-seeking is examined after training for self-administration of drugs and subsequent extinction of the drug-reinforced behavior (Stewart & de Wit, 1987). Using this procedure, investigators have shown that priming injections of nicotine reinstate extinguished nicotine-taking behavior (Chiamulera et al. 1996; Shaham et al., 1997). In an effort to expand upon this literature, we found that nicotine seems to promote the reinstatement of an extinguished CPP for a food reward after priming by several M&M’s in a novel, neutral virtual room. While our findings deviate somewhat from the traditional reinstatement model in that reward-priming after nicotine-treatment increased the magnitude of CPP after the third extinction session without inducing a significant CPP, we do feel confident that the results suggest that nicotine promotes reinstatement for conditioned behavior.

Future Directions

The current study has provided us with novel and informative data in understanding the role of nicotine in enhancing conditioned place preferences in humans using a virtual task. Furthermore, these data provide a foundation for future studies aimed at more thoroughly characterizing the reward mechanisms that underlie risks for maintaining nicotine use, as well as risks for relapse following cessation.

Given that our lab has previously demonstrated much stronger CPPs in terms of time and ratings in undergraduate participants using 6-minute conditioning sessions (Astur et al., 2014), future studies will return to the 6-minute paradigm from the current 3-minute paradigm, in hopes of examining nicotine enhancing effects on CPP in terms of both time and ratings with a more powerful pairing paradigm. Additionally, after establishing a relationship between nicotine dependence and CPP, it is of interest to examine the effects of dependence severity on our behavioral measures. A majority of participants in our sample reported low levels of nicotine dependence (Fagerstrom score average = 1); therefore, future work should investigate whether individuals with increased dependence condition more strongly or have greater resistance to extinction, particularly because dependence indices have been shown to predict cue-induced cravings and subsequent relapse.

Expecting to find an effect of negatively-reinforcing motivational states on our behavioral measures, particularly given previous literature which agrees that a major motivational basis of drug dependence is the reduction or avoidance of aversive internal states (Baker et al., 2004), it was surprising to find that negative reinforcement did not affect the acquisition of CPP using the BIS or the QSU-Relief of Negative Affect scales, especially for those who received nicotine treatment. While lengthening the required 6-hour window of

nicotine abstinence prior to participating in our study may be necessary to manifest negative mood states strong enough to elicit negative reinforcement, it would also be interesting to examine the effects of nicotine on a Pavlovian aversive task, like the conditioned place aversion (CPA) task. Traditionally, the CPA paradigm involves repeatedly pairing an aversive unconditioned stimulus (i.e. an electrical shock) with a particular neutral context (conditioned stimulus, CS). These repeated pairings eventually result in a learned association between the CS and the aversive US, such that participants express a fearful conditioned response (CR) to the originally neutral conditioned stimulus. This unpleasant emotional reaction would then serve to negatively reinforce a response that terminates the CS, and thus terminates the conditioned response. In theory, when given unrestricted access to two contexts, one previously-paired with the US and the other neutral, participants should demonstrate a strong aversion for the room in which the shock was previously paired despite the shock no longer being present. Furthermore, nicotine administration prior to acquisition of the conditioned response should enhance a CPA.

Given the complexity of our Day 2 results, future research should further explore the effects of nicotine on extinction, but also attempt to better discriminate these results from spontaneous recovery and reacquisition. Currently we employ three 3-minute extinction sessions to assess whether nicotine delays extinction. Going forward, it may be best to use one longer extinction session and examine behavior throughout that session. Examining nicotine's effects on extinction after 1, 3, or 7 day delays might also be insightful in investigating whether longer acquisition-extinction intervals are more or less prone to spontaneous recovery.

Conclusions

Overall, the current body of work has provided a solid foundation for future work describing the effects of nicotine on conditioning, extinction, and reinstatement. Collectively, our results demonstrate that nicotine (1) enhances reward sensitivity of non-nicotine rewards, (2) slows extinction, and (3) promotes reinstatement. Additionally, our study demonstrates the efficacy of utilizing the virtual conditioned place preference paradigm in understanding the behavioral mechanisms by which nicotine enhances responding for conditioned rewards. Importantly, the current findings of our study, as well as those we have proposed, will allow for better understanding and interpretation of future studies with regard to mechanisms of nicotine dependence, and provide insight into how nicotine can be particularly resistant to treatment.

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Figures

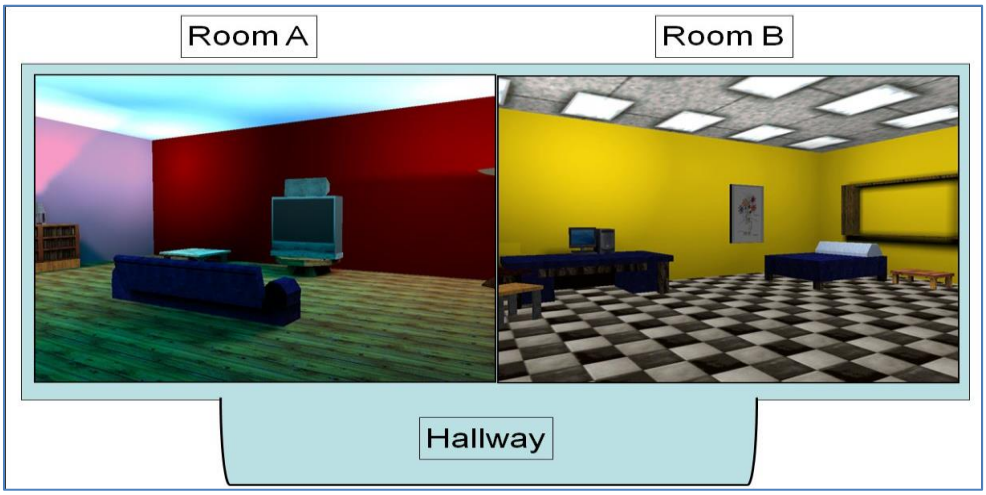

Figure 1A. Both rooms were identical in shape and size, but contained different items, colors and patterns.

Day 1	**Practice Session**	**Conditioning Sessions**						**Test Session**
	Neutral Room	1	2	3	4	5	6	Test
	Food	Food	No Food	No Food	Food	No Food	Food	No Food

Day 2	**Extinction Sessions**			**Reinstatement Session**	**Test Session**
	Test	Test	Test	Neutral Room	Test
	No Food	No Food	No Food	Food	No Food

Figure 1B. A sample testing order for one participant on Day 1 and Day 2. Across participants, testing order and M&M/Room pairings was counterbalanced.

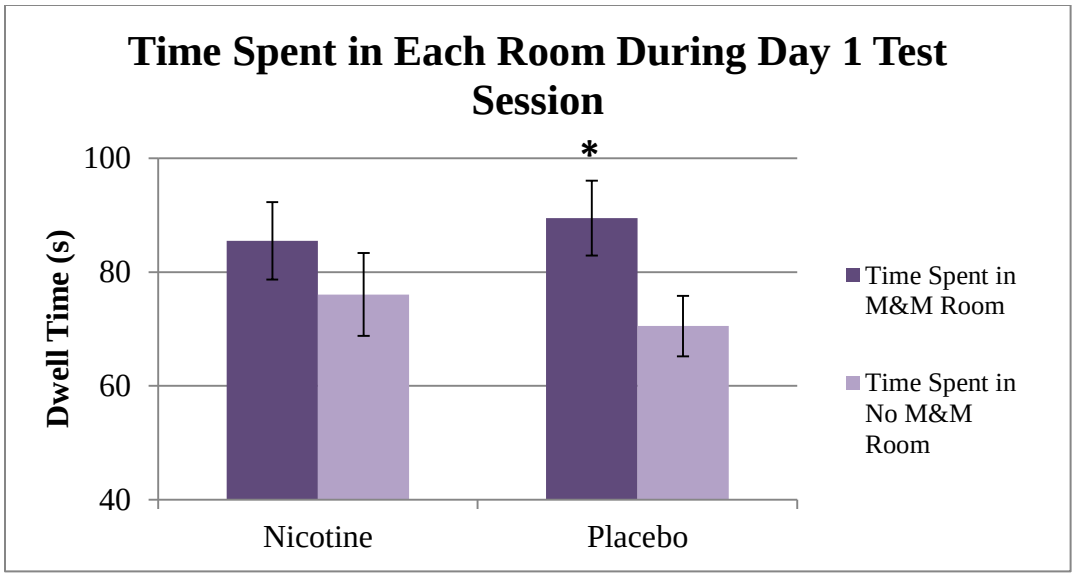

Figure 2A. Day 1 placebo group spends significantly more time in the previously-paired M&M room during the test session compared to the No M&M Room ($t(38) = 1.99$, $p < 0.05$). No significant difference in time for nicotine-treated participants ($t(33) = 0.67$, $p = 0.51$).

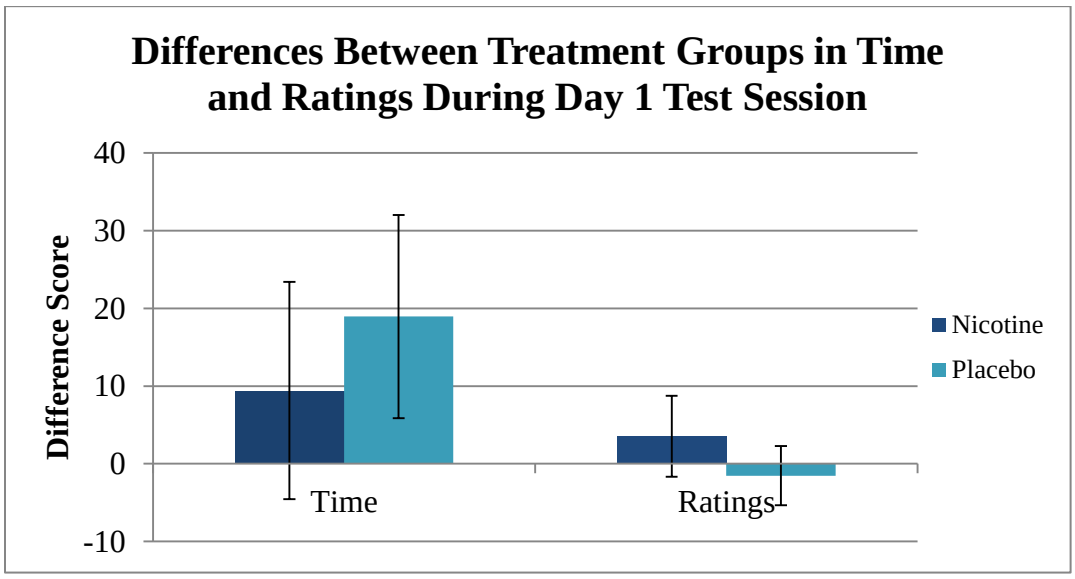

Figure 2B. No significant differences between treatment groups in the amount of time spent in M&M room on test day ($F(1,72) = 0.25$, $p = 0.62$), nor in ratings of the M&M-paired room on test day ($F(1,72) = 0.64$, $p = 0.43$).

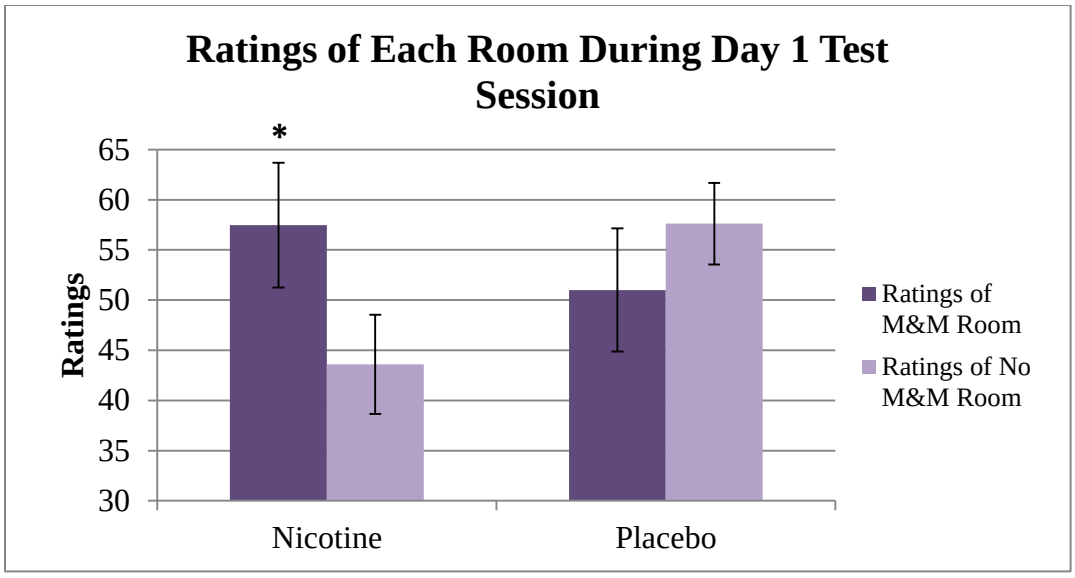

Figure 3. Day 1 nicotine group rates previously-paired M&M room more favorably than does placebo group when Fagerstrom score greater than zero ($F(1, 35) = 4.72$, $p < 0.05$).

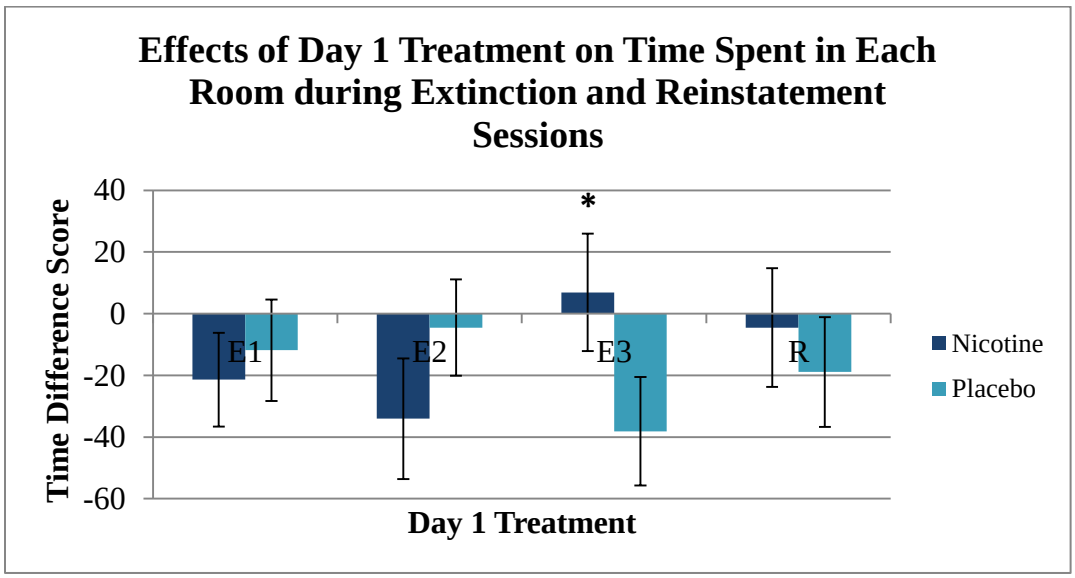

Figure 4. Participants who receive nicotine on Day 1 spend more time in previously-paired M&M room than placebo group during third extinction session ($F(1, 18) = 13.7$, $p < 0.05$).

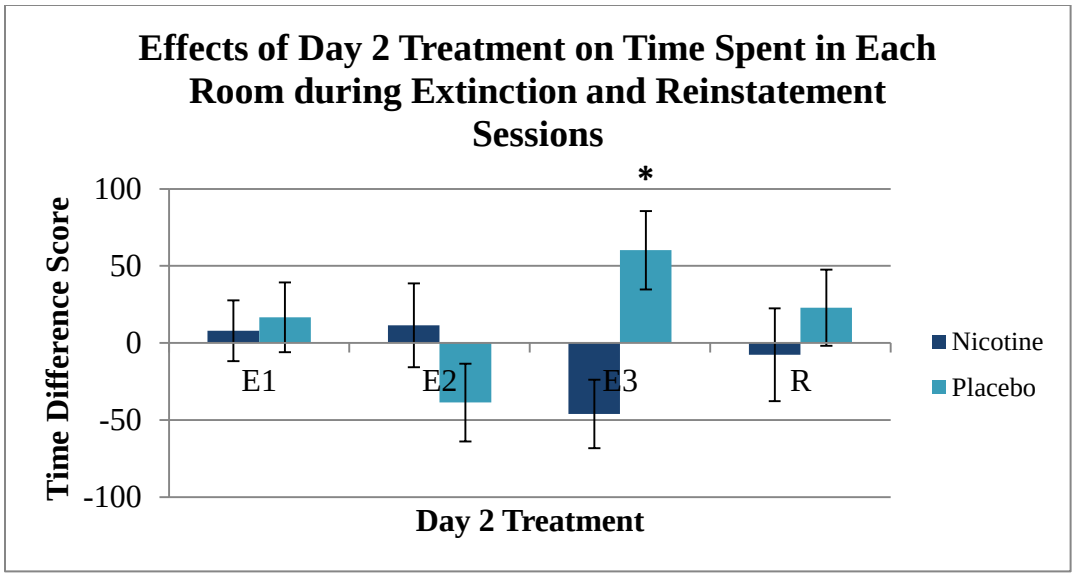

Figure 5. Participants who receive placebo on Day 2 spend more time in previously-paired M&M room than nicotine group during third extinction session ($F(1, 18) = 5.01$, $p < 0.05$).

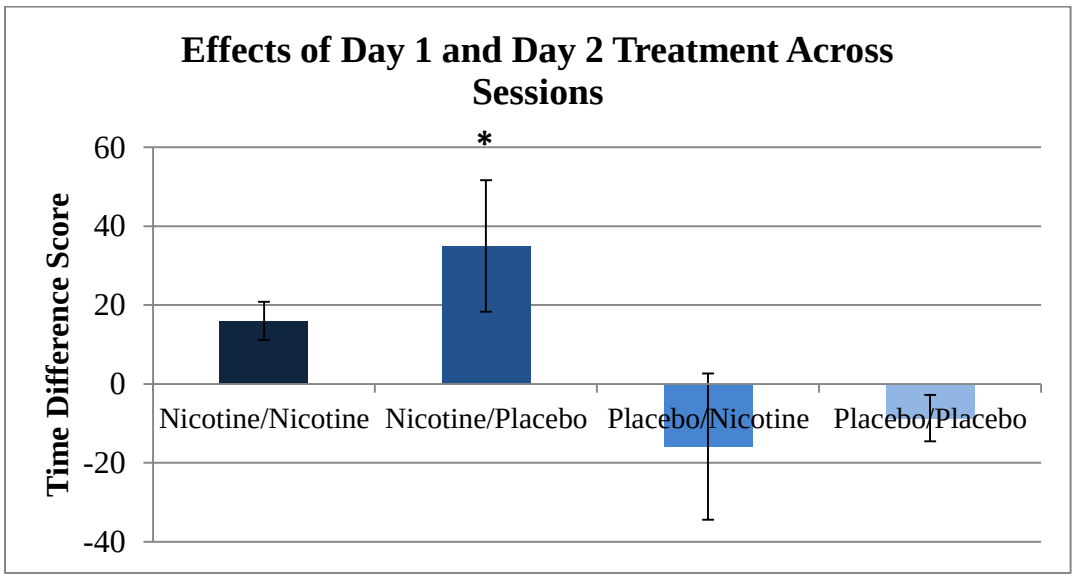

Figure 6. Effects of Day 1 and Day 2 treatments where nicotine on Day 1 and placebo on Day 2 are most likely to result in a conditioned place preference during extinction.

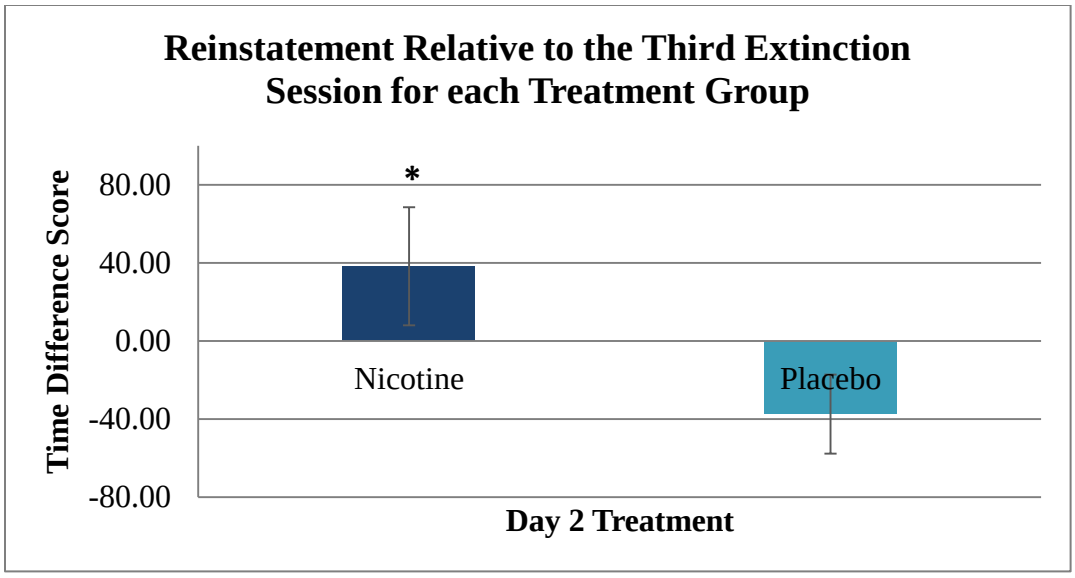

Figure 7. Day 2 nicotine-treated participants reinstate by a significantly greater change between the amount of time spent in the M&M-paired room during the third extinction session and the reinstatement session ($F(1, 18) = 5.87$, $p < 0.05$).