NICOTINE ENHANCES THE EXPRESSION OF A CONDITIONED PLACE PREFERENCE IN ADULT MALE RATS

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Submitted to the Undergraduate Faculty of

Kenneth P. Dietrich School of Arts and Sciences in partial fulfillment

of the requirements for the degree of

Bachelor of Philosophy in Psychology

University of Pittsburgh

2012

UNIVERSITY OF PITTSBURGH

KENNETH P. DIETRICH SCHOOL OF ARTS AND SCIENCES

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As the number of cigarette-related deaths has risen over the years, researchers have tried to study and understand cigarettes, their components, and why they are so addictive despite widespread knowledge of their potentially fatal health consequences. Research in recent decades has unveiled that the strength of cigarette addiction could be due in part to the primary reinforcing effects of nicotine. Although these findings may give researchers and medical professionals some answers about this addiction, it does not provide the whole picture. Research conducted more recently has shown that the reinforcement-enhancing effect, a property of nicotine that increases an organismôs behavior in response to an environmental stimulus, could also play a large role in tobacco addiction. Though many studies have observed this phenomenon in an operant paradigm, the effect has yet to be observed using a different model. The present study hypothesized that this effect could be produced using a conditioned place preference (CPP) paradigm. Animals were conditioned to prefer one of two chambers using either a sucrose or cocaine reward. The day after a post-conditioning preference test, the effect of a one-time, acute injection of nicotine on these expressed CPPs was then observed. Results showed a significant effect of nicotine on the expression of both sucrose and cocaine CPPs with animals spending more time in the reward-associated chamber relative to the first post-conditioning preference test

and a third preference test preceded by a saline injection. Furthermore, there was no significant difference between the results of the saline and injection-free post-conditioning preference tests. These results show that the reinforcement-enhancing effect can be observed in a classical conditioning paradigm. They also show that in this non-operant paradigm, the reinforcement-enhancing effect can be produced by one acute injection of nicotine in animals with no previous exposure to the drug. These findings can provide more insight into the reinforcing effects caused by a first experience with nicotine.

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1.0 INTRODUCTION

With nearly 500,000 people dying and 8.6 million others suffering from tobacco smoke-related illnesses each year, tobacco smoking, particularly in the form of cigarettes, constitutes the leading cause of preventable death, disease, and debilitation in the United States. Despite an increase in knowledge of these negative health consequences over the past fifty years, nearly 50 million U.S. adults continue to smoke cigarettes (Centers for Disease Control and Prevention, 2011). In recent decades, studies investigating the causes of tobacco addiction have pinpointed nicotine as the main tobacco constituent that reinforces and maintains smoking behavior (Stolerman & Jarvis, 1995).

These studies have demonstrated that nicotine acts as a primary reinforcer increasing behaviors that result in direct nicotine delivery. However, they have also shown that this property of nicotine is not very strong (Caggiula et al., 2009). Though cigarette smoking is associated with a high risk of dependence and relapse, the weak primary reinforcing effects of nicotine in isolation neither compare to those of other abused drugs nor foretell the low quit rates in chronic cigarette smokers (Anthony, Warner, & Kessler, 1994; Caggiula et al., 2001; Goldberg, Spealman & Goldberg, 1981; Rose & Corrigall, 1997; Rose & Levin, 1991). These findings raise a very important question: if nicotine is such a weak primary reinforcer, why do smokers

continue to smoke? Further investigation of nicotine reinforcement has revealed the key role of smoking-related stimuli in understanding compulsive cigarette use (Caggiula et al., 2009; Chaudri et al., 2006; Conklin & Tiffany, 2001; Niaura et al., 1998; Palmatier et al., 2007a,b; Perkins, Epstein, Grobe, & Fonte, 1994; Rose, Behm, Westman, & Johnson, 2000; Sayette, Martin, Wertz, Shiffman, & Perrott, 2001).

Clinical studies researching the subjective effects of smoking-related cues have shown that nondrug stimuli associated with smoking (e.g. the sight and smell of lit cigarettes and the throat sensations triggered by smoke inhalation) can increase craving and desire to smoke in smokers (Conklin & Tiffany, 2001; Niaura et al., 1998; Perkins et al., 1994; Rose & Levin, 1991). After repeated pairings with smoking in a smokerôs environment, originally neutral cues can elicit similar effects (Geier, Mucha, & Pauli, 2000; Lazev, Herzog, & Brandon, 1999; Mucha, Pauli, & Angrilli, 1998). Recognizing the apparent need to consider stimuli and cues in nicotine reinforcement, preclinical researchers have begun to more closely examine the relationship between nicotine and the stimuli standardly used in the operant paradigm (e.g. light and tone stimuli). In one nicotine self-administration study with male rats, Caggiula et al. (2009) examined the effects of nicotine on responding for a moderately reinforcing visual stimulus (VS), defined as a 1-second presentation of the cue light located directly above the active lever followed by the offset of the white house light for one minute. Results exhibited minimal rates of responding in ratsô active lever-pressing for saline compared to the more moderate response levels in animals pressing the active lever for either nicotine or the VS alone. In addition, there were no significant differences in responding in animals receiving either nicotine or VS presentations alone, demonstrating the similar, mild primary (unconditioned) reinforcing

properties of both nicotine and the VS. Rats pressing the active lever for paired nicotine infusions and VS presentations, however, demonstrated more robust responding, active lever-pressing approximately five times more than rats receiving either nicotine or VS presentations alone.

This display of a synergistic, rather than additive, interaction between nicotine and the VS revealed a unique relationship between the two unconditioned reinforcers. As a primary reinforcer, nicotine promotes associative learning. In other words, stimuli repeatedly paired with nicotine become associated with and acquire the reinforcement power possessed by the drug. In this sense, the repeated pairings of nicotine infusions and VS presentations should have transformed the VS into a conditioned reinforcer giving it the added value of the reinforcing properties of the drug. Therefore, if the response rates for nicotine paired with the VS were a result of associative learning, animals should have pressed the active lever no more than twice as much as animals pressing the active lever for nicotine or the VS alone. The synergistic responding displayed by these animals seemed to suggest that a different, non-associative type of learning had occurred.

In an attempt to better understand the synergy between nicotine and the VS, Donny et al. (2003) conducted another nicotine self-administration study to determine whether or not a direct association between nicotine and the VS was necessary to engender robust responding for the VS. Results of the study revealed, as in the aforementioned experiment, that animals responding solely for nicotine or the VS displayed moderate levels of active lever responding. Animals responding for non-contingent nicotine (given continuously or in discrete infusions), however, displayed increased active-lever responding when the VS was available. More importantly, there

was no significant difference in responding for the VS when animals receiving non-contingent nicotine were compared with those receiving contingent nicotine. This observation indicated that the VS and nicotine did not need to be directly associated in order to see this potent effect. These findings led experimenters to conclude that not only does nicotine act as a response-contingent primary reinforcer, but it also alters responding for other stimuli through a non-associative process. This process is the reinforcement-enhancing effect of nicotine, the phenomenon whereby nicotine can non-contingently increase responding for mildly rewarding, nondrug stimuli (Donny et al., 2003). Within the last decade, studies have consistently produced results demonstrating the reinforcement-enhancing properties of nicotine including those in which rats received experimenter-administered, subcutaneous (SC) nicotine injections (Caggiula et al., 2009; Chaudri et al., 2006; Liu, Palmatier, Caggiula, Donny, & Sved, 2007; Palmatier et al., 2006; Palmatier et al., 2007a,b). Together, these experiments have demonstrated that SC injections as well as intravenous infusions of nicotine have the ability to produce reinforcement-enhancing effects in rats.

It is important to note that while the reinforcement-enhancing effect of nicotine has been studied at length in an operant conditioning paradigm, little research has been conducted on the phenomenon in other models. Conditioned Place Preference (CPP) is one of the oldest, most basic models utilized to study reward-related processes. In this paradigm, repeated pairings of an unconditioned stimulus with one of two neutral environments converts the paired context into a conditioned stimulus that elicits the same response and behavior as the unconditioned stimulus. As the reinforcement-enhancing effect can be applied to stimuli with conditioned reinforcement

properties, it appears the CPP model could constitute a useful context in which to study this non-associative process.

Though the interaction between nicotine and rewards in a CPP paradigm has been investigated, this relationship has not been examined using a non-associative design. For example, Thiel, Sanabria, and Neisenwander (2009) examined the interaction between nicotine and social rewards in adolescent rats within a CPP context. In their study, animals were trained to prefer their originally non-preferred chamber through repeated pairings of the chamber with both nicotine and social rewards. The results were a robust CPP relative to the lack of CPP in animals conditioned with nicotine or social rewards alone. However, the discrete pairings of nicotine, social rewards, and the chamber in which they co-occurred makes it difficult to rule out associative learning in the process. Furthermore, this design does not distinguish the study from others attempting to engender nicotine CPP, a phenomenon that focuses and relies on the primary reinforcing properties of nicotine. As a result, the Thiel et al. (2009) study is not a strong exemplar of the investigation of the reinforcement-enhancing effect of nicotine in a CPP context.

The main question driving the present study was: can the reinforcement-enhancing effect of nicotine be generalized and extrapolated to the classical CPP paradigm? This experiment sought to answer this question by 1) conditioning animals to prefer one distinct environment over another utilizing a non-nicotine reward, 2) testing animals to determine whether or not they have learned this association, and 3) giving these animals a one-time, acute nicotine injection to see if the drug could enhance this conditioned preference. To ensure that the process remained non-associative, nicotine was only administered prior to animals having access to the entire CPP apparatus. The first aim of this experiment was to determine if nicotine can enhance a sucrose-

induced CPP. In order to answer this question, it was first necessary to establish a reliable CPP paradigm. Once this task was completed using an unbiased design, it was hypothesized that a non-associative, SC injection of nicotine would enhance sucrose CPP. The procedures included three days of post-conditioning preference tests. On the first day, animals were assessed without injections to determine if they developed a CPP (preference test 1). The next day, animals were given a pre-assessment injection of SC nicotine to test the effects of nicotine on their established CPPs (preference test 2). On the third day, animals received a pre-assessment SC saline injection to compare the effects of saline against those of nicotine and no injection (preference test 3). It was predicted that nicotine would significantly enhance the expression of a sucrose CPP displayed during preference test 1 by increasing the amount of time animals spent in the sucrose-paired chamber. It was also hypothesized that in repeating the test with saline in place of nicotine, the enhancement effect would be eliminated showing that nicotine alone, and not the injection procedure, created the effect. For this reason, it was also expected that there would be no significant difference between the results of the saline and injection-free preference tests.

The second aim of the experiment was to test if nicotine can enhance a CPP using a drug unconditioned stimulus (UCS), intraperitoneal (IP) cocaine. Animals were split into two groups in a crossover design: one group followed the aforementioned preference test procedure (group 1) while the other group received a counterbalanced order of injections (group 2). It was hypothesized that SC nicotine would significantly enhance the amount of time animals spent in the cocaine-associated chamber. Furthermore, it was expected that a SC saline injection would not affect established cocaine CPP, producing the same results as injection-free preference test 1. Overall, it was predicted that a one-time, acute injection of SC nicotine -could enhance both non-

drug and drug-induced CPPs in - rats with no previous exposure to nicotine. It is necessary to emphasize that animals remained nicotine-naive before this one-time, acute injection. This lack of prior nicotine exposure eliminates the role of the sensitization effects of chronic nicotine exposure in any displayed reinforcement-enhancement. It also means that drug tolerance reversal could not have occurred, additionally removing this pharmacological effect as a possible cause of the studyôs outcomes.

2.0 METHOD

2.1 SUBJECTS

A total of 36 adult, male Sprague-Dawley rats (Harlan Farms, Indianapolis, IN) were utilized in this study. Weighing 220-225g and aged 60-65 days upon arrival, these rats were singly housed in suspended, wire mesh cages in a colony room temperature-controlled between 68 and 72ęF. All animals habituated to the home cages for at least 6 days (habituation time ranged from 6-15 days) during which they received *ad libitum* food. Access to water in the home cage remained unlimited throughout the length of the experiment. After postnatal day 75, animals were restricted to 20g of food per day. Animals following the cocaine CPP paradigm maintained this diet for the remainder of the study. In contrast, animals following the sucrose CPP paradigm were restricted further to 15g of food per day from the last day of home cage habituation until the end of the study. This measure was taken to encourage sucrose consumption during the conditioning phase of the study and prevent excessive weight gain from sucrose consumption over the course of the study. Animals were kept on a 12-hour reversed light/dark cycle with lights out from 7am to 7pm and all conditioning and testing taking place during the dark hours of the cycle. Animals were handled and weighed daily.

2.2 CONDITIONED PLACE PREFERENCE APPARATUS

Animals were conditioned and tested in three identical CPP apparatuses. Each apparatus had three chambers: Chambers A and B that were equal in size and dimension (27cm *l* x 21cm *w* x 20.5cm *h*) and a middle start chamber that divided the two larger chambers (12 cm *l* x 21cm *w* x 20.5cm *h*). Manual guillotine doors separated Chambers A and B from the start chamber. The start chamber had solid gray walls and metal grated flooring. Prior to the onset of the present study, the walls of Chambers A and B were solid white and solid black, respectively. With these color schemes, animals in various preliminary versions of this experiment displayed a strong initial bias for Chamber B (the black chamber) that proved difficult to overpower. Therefore, before the effects of nicotine on an established CPP could be tested, this general initial bias for Chamber A needed to be reduced. As a result, several changes were made to Chambers A and B. To balance out the darkness of each conditioning chamber, it was decided that both chambers should be both black and white. First, Chamber A had alternating horizontal black and white stripes while Chamber B had alternating vertical black and white stripes. After several unsuccessful CPP trials with these wall designs, it was deemed the chambers appeared too similar for animals to be able to distinguish between them. Therefore, Chamber A was modified further to contain alternating solid black and white walls while Chamber B maintained its alternating vertical black and white stripes. In the midst of all these changes, several alterations were also made to the floors of Chambers A and B. The design ultimately used for the present experiment, however, utilized the original floors designed for each chamber: grid flooring for Chamber A and metal bar flooring for Chamber B.

Once the design of the study was changed to incorporate the use of sucrose solution, Chambers A and B each had a small hole drilled in the back wall to allow for the insertion of standard water bottle spouts during conditioning sessions.

Stainless steel trays covered with a layer of bedding laid beneath the floors of each chamber during all phases of conditioning and testing. The top of each chamber was covered by a clear, Plexiglas lid with ventilation holes. Built-in yellow, opaque lights located in the center of each lid illuminated each individual chamber. Three knobs attached to the outer, right portion of the apparatus allowed for the control of the brightness of each chamber. Infra-red photo-beam sensors spaced 5.0 cm apart and located 3.5 cm above the floor of each chamber recorded how much time animals spent in each section of the apparatus over a given period of testing time. There were five photo-beam sensors in Chambers A and B and 2 sensors in the middle start chamber. Data were collected automatically. In order to filter out extra sound and light during testing, CPP apparatuses were encased in larger, sound-attenuating wooden cabinets.

2.3 DRUGS AND SOLUTIONS

2.3.1 Nicotine

Nicotine solution utilized for these studies was made by dissolving (ï) nicotine hydrogen tartrate (Sigma, St. Louis, MO) in 0.9% saline solution. The pH of the solution was adjusted to 7.0 (± 0.2) using dilute NaOH. The dose of nicotine used was 0.4 mg/kg (free base concentration),

chosen based on the results of previous studies exploring the reinforcement-enhancing effects of subcutaneous nicotine injections (Caggiula et al., 2009; Palmatier et al., 2007b; Wing & Shoaib, 2010). Nicotine solution was sterilized before use by being pushed through a 0.22εm filter. Nicotine injections were SC, given at a volume of 1ml/kg.

2.3.2 Unconditioned Stimuli

The 25% sucrose solution used in this experiment was made by dissolving crystalline sucrose (Fisher Scientific, Waltham, MA) in water. Sucrose solution was chosen after trying numerous variations of the present experiment. In initial trials, sucrose pellets rather than solution were utilized as the UCS. Measurement of sucrose consumption proved difficult in these experiments as animals often flipped over their sucrose dishes spilling the pellets in bedding before they could even be eaten. Furthermore, animals did not appear to like the sucrose pellets as they did not display CPP in these trials. As a result, the next set of trials used sucrose solution instead of pellets. The switch to solution allowed for cleaner tracking of sucrose consumption as well as manipulation of concentrations to maximize sucrose CPP results. After tests with 1% and 10% solution produced lackluster results, it became clear sucrose concentration needed to be increased. A search through sucrose CPP literature produced a 1980s study that saw maximal CPP between 20 and 40% sucrose (White & Carr, 1985). To avoid encountering a ceiling effect once nicotine was introduced in post-conditioning testing, a 25% sucrose solution was selected and tested. This concentration proved effective and was subsequently used for the present study.

Cocaine hydrochloride (Sigma, St. Louis, MO) was dissolved in sterile saline to make a 10mg/kg dose solution. This particular concentration was selected based on the standard dose of cocaine used to induce cocaine CPP in previous studies (Harris & Aston-Jones, 2003). Cocaine injections were IP and given at a volume of 1ml/kg.

Sucrose served as the UCS for the first CPP paradigm while cocaine was the UCS for the second paradigm of the study. All animals remained nicotine-naïve until the post-conditioning preference test phase of the study during which they received a one-time, acute injection of nicotine prior to one of the three preference tests. Consequently, nicotine was not associated with either the UCS or the conditioning environments.

2.4 PROCEDURES

In the preliminary trials of this experiment, not only did animals show little to no CPP but they also generally spent more time in the gray start chamber than in either of the conditioning chambers during post-conditioning testing. While the changes made to the CPP apparatus and the increased salience of the sucrose UCS likely helped to temper this problem, some procedural changes still needed to be made in order to improve the construct validity of the study. In earlier versions of the study, initial assessment, conditioning, and post-conditioning assessment test began with a five-minute acclimation period in the middle start chamber. While this period likely reduced the novelty of the chamber, it may have also contributed to the exorbitant amount of time animals spent in the gray chamber during post-conditioning testing; it could have

encouraged them to remain in that safe, small dark space rather than explore the apparatus. As a result, this acclimation period was completely removed prior to the start of the present study.

Illumination settings likely also played a role in the amount of time animals spent in the gray chamber during testing. In all early trials of the study, the lights in all three chambers were set at the same level of brightness: 0.2 lux. To discourage animals from remaining in the confines of the gray chamber, the lux of the middle chamber lights were increased. All chamber light bulbs were tested to determine the maximum brightness of the bulbs. At one point, the brightest bulbs went up to 12.1 lux and were effective in keeping the animals from sitting in the middle chamber. However, some of these burned out after one assessment test and after repeated attempts with different bulbs, it became clear it was not possible to get all of the lights this bright. As a result, the middle chamber lights were brought down to the brightest setting of the least bright bulb: 8 ± 0.6 lux. This setting also functioned well in discouraging animals from spending too much time in the start chamber during testing and was therefore used for all phases of the present study. All other procedural changes made to arrive at the final method used in the present experiment are described in the procedures below.

2.4.1 Initial Preference Assessment

The day prior to the start of conditioning, all animals completed an initial preference assessment that also served as their habituation to the CPP apparatus. The manual guillotine doors remained open for the entirety of this 20-minute test. Testing time began when animals were placed directly into the middle start compartment. Each rat was allowed to explore the entire apparatus

for a full 20-minute period. The infra-red photo beams located at the bottom of each compartment recorded how much time animals spent in each compartment. The 9 animals with the most biased initial preferences (sucrose animals, n=5; cocaine animals, n=4) were excluded from the rest of the study. The less biased 27 animals (sucrose animals, n=14; cocaine animals, n=13) were randomly assigned into groups based on whether Chamber A or Chamber B would serve as their UCS chamber (the chamber paired with the UCS). Animals participating in the cocaine CPP paradigm were further randomly assigned to crossover injection groups for the post-conditioning preference test phase of the study.

2.4.2 Conditioning

Animals experienced eight conditioning sessions in the CPP. This number of conditioning sessions was selected based on the results of pilot versions of the present experiment determining that a minimum of four UCS and four control sessions was necessary for animals to learn to associate the UCS with the UCS chamber. Initially, these sessions were spread out over eight days partially separated by a weekend. However, in fine-tuning the design of the experiment and improving the flow of its preliminary trials, these eight days were eventually condensed to four consecutive days. Each day, animals underwent two conditioning sessions: one 20-minute session in the UCS chamber and one 20-minute session in the unpaired chamber (control chamber). Daily UCS and control conditioning sessions were separated by 3-4 hours. These sessions were also counterbalanced daily to prevent animals from associating one specific type of conditioning with a particular time of day. Both manual guillotine doors remained closed

during conditioning sessions so that animals only had access to the given conditioning chamber. Session time began when an animal was placed directly into the conditioning chamber.

2.4.3 Preference Tests

Animals experienced a total of three daily 20-minute post-conditioning preference tests. Preference test 1 was conducted in the same fashion as the initial preference assessment. Preference tests 2 and 3 followed the same procedure but were preceded by an acute SC injection five minutes prior to testing. Five minutes before preference test 2, each nicotine-naïve animal received a one-time, acute SC injection of 0.4 mg/kg nicotine. Five minutes before preference test 3, each animal received a 0.3 ml acute SC injection of 0.9% saline. This injection order was counterbalanced for half of the animals in the cocaine CPP paradigm. Each animal spent the 5-minute period between injection and preference testing in its home cage.

2.4.4 Paradigm-Specific Procedures

2.4.4.1 Paradigm 1: The reinforcement-enhancing effect of nicotine on sucrose CPP

In order to habituate the animals to the UCS they would receive during the conditioning phase of the study, animals (n=19) experienced 2 days of 2-hour home cage exposures to the sucrose solution. During this phase, each animal received its own bottle labeled with its ID number and filled with 25% sucrose solution. Bottles were positioned on the left side of the home cages so animals still had access their water bottles (located on the right side of the cage) during sucrose

exposure. Bottles were weighed before and after sucrose exposure in order to keep record of each animalôs sucrose consumption.

Following initial preference assessment, the 14 animals with the least biased initial preferences began the conditioning phase. In pilot versions of the present study, animals had access to a sucrose-filled or empty container for the entirety of each respective UCS and control conditioning session. Over time, however, this conditioning method proved problematic, particularly during post-conditioning testing. To test whether or not an animal has developed a CPP, the testing environment and conditions must remain the same as those of the conditioning environment but with two exceptions: 1) animals must have access to all chambers of the CPP apparatus and 2) the reward must be absent. Therefore, in this particular design, it was necessary for empty containers to be present in both the control and UCS chambers for the entire 20-minute test. As animals had previously learned to associate an empty container with the control chamber and discovered that they would not likely receive a reward within seconds of beginning the first post-conditioning test, this setup almost immediately extinguished any CPP the animals may have developed. For this reason, the conditioning design needed amendment to remedy this problem. The resultant design began each conditioning session with no reward or vehicle present. Ten minutes into each UCS conditioning session, the entire CPP apparatus was moved forward and the spout of the given animalôs sucrose bottle was inserted through the hole in the back wall of the UCS chamber. Ten minutes into each control conditioning session, these actions were repeated but with an empty bottle. Animals had access to these spouts for the full second half of each 20-minute conditioning session. Before and after UCS conditioning sessions, sucrose bottles were weighed in order to track and record individual sucrose consumption.

Like the conditioning session, each post-conditioning test began with no bottle spouts present in either chamber. Then, ten minutes into each session, the CPP apparatus was moved forward and back without the insertion of any bottle spouts. Animals were left undisturbed for the remainder of each test. With this setup, both the proper testing conditions and learned association between the sucrose reward and UCS chamber could be upheld. ..

2.4.4.2 Paradigm 2: The reinforcement-enhancing effect of nicotine on cocaine CPP

Animals (n=17) first completed an initial preference assessment. The 13 animals with the least biased initial preferences at the end of this test began conditioning the following day. Immediately prior to the start of UCS conditioning sessions, animals were given a 1ml/kg IP injection of 10mg/kg cocaine. Before control chamber conditioning, animals were injected with a 0.3ml IP injection of 0.9% saline. Cabinet doors were closed once animals were placed in the conditioning chamber, and they were left undisturbed for the entire 20-minute session. For post-conditioning preference testing, animals were split into two groups following a crossover design: group 1 followed the aforementioned order of injections (NIC/SAL, n=7) while group 2 had counterbalanced injections (SAL/NIC, n=6). . All animals received a 0.3ml IP injection of 0.9% saline immediately prior to all post-conditioning preference tests in order to mimic the injection procedureof the conditioning phase without providing the reward. Animals were left undisturbed in the CPP apparatuses for each, full 20-minute test.

2.5 DATA ANALYSIS

The main data of interest were collected during the initial preference assessment and three-day, post-conditioning preference test phases of this study. Two dependent variables were analyzed from the post-conditioning preference tests: drug pretreatment (the within-subjects variable) and order of injections (the between-subjects variable). Because the sucrose CPP paradigm contained only one group (i.e. all animals received the same order of injections), sucrose CPP data were only analyzed within subjects. Before analysis, data were organized by calculating difference scores (UCS chamber time ï control chamber time) for each animal during the initial preference assessment and each post-conditioning preference test. All differences scores were averaged and plotted on bar graphs. These individual difference scores were analyzed using repeated measures analyses of variance (ANOVAs). Difference scores from the initial preference assessment and preference test 1 of each CPP paradigm were compared using paired-samples *t*-tests. Paired samples *t*-tests were also used to directly compare the results of each post-conditioning preference test. ANOVA statistics were reported based on whether or not the data violated Mauchlyôs Test of Sphericity. For the data that passed this test, the Sphericity Assumed statistic was reported while the Greenhouse-Geisser statistic was reported for those that violated the assumption of sphericity. Data from each 20-minute initial preference assessment and post-conditioning preference test were analyzed and reported for both the sucrose and cocaine CPP paradigms. For the sucrose CPP paradigm, data from the first 10-minute half of each test were also analyzed and reported. As the animals learned to anticipate a sucrose reward during this

portion of conditioning, it was expected that its post-conditioning counterpart would be most representative of their conditioned behavior. For this reason, these data were analyzed.

One-tailed correlational analyses were also performed to determine if any linear relationships existed between the rewards, the CPPs they induced, and the extent to which nicotine enhanced these CPPs. For the sucrose CPP paradigm, two correlational analyses were performed. The first analysis examined the linear dependence of each animalôs CPP on its average sucrose consumption during conditioning. The amount of sucrose each animal consumed during conditioning sessions was averaged over four days and tested against its CPP, represented by its difference score calculated from preference test 1. The second analysis assessed the relationship between each animalôs CPP and how much that preference was enhanced by nicotine. Each animalôs CPP, represented as its difference score from preference test 1, was tested against its nicotine-manipulated, represented as its difference score from preference test 2. These analyses were performed for both the full 20-minute and first 10-minute portions of the post-conditioning preference tests. Results were reported as Pearson coefficients. For the cocaine CPP paradigm, only one correlational analysis was performed. Because each animal received the same amount of cocaine during conditioning, a linear relationship between the amount of cocaine received and the amount of CPP shown could not be analyzed. The linear dependence of nicotine-enhanced of CPP on the initial amount of CPP expressed could be analyzed, however. This analysis was performed in the same fashion as the corresponding analysis for the sucrose CPP paradigm. The test was performed using difference scores from the full 20-minute data only. The correlation was plotted in a scatter plot chart and the result of the analysis was reported as a Pearson coefficient.

3.0 RESULTS

Though data analyses were performed on the difference scores calculated from each pre- and post-conditioning preference test, it is vital to note the importance of the raw data. Without these data, the difference scores could not have been calculated. Furthermore, these raw numbers more accurately reflect the impact of the study design improvements. Prior to the many changes that lead to the final design of the present study, the average amount of time animals spent in the start chamber was consistently greater than the time they spent in either the UCS or control chamber. Also, from initial preference to preference test 1, the differences in the amount of time spent in the UCS versus the control chamber were not significant enough to suggest successful conditioning. The raw data from the present study tell a different story (Table 1).

In both CPP paradigms, animals spent less time in the start chamber than in the UCS and control chambers across all tests. During both initial preference assessments, animals spent nearly equal amounts of time in the UCS and control chambers on average. In relation to these initial assessments, animals showed an average increase in time spent in the UCS chamber and an average decrease in time spent in the control chamber in all post-conditioning preference tests. Together, these raw figures help provide a better view of the basic study results and reflect the increased construct validity of the study design as a whole.

Table 1: The average amount of time animals spent in each chamber during each 20-minute pre- and post-conditioning preference test (NIC = preference test preceded by nicotine injection; SAL = preference test preceded by a saline injection).

		Average Time (s) in Chamber (out of 1200 seconds)		
Paradigm	**Test**	**UCS Chamber**	**Start Chamber**	**Control Chamber**
Sucrose (n=14)	Initial Preference	431.33	298.45	485.27
	Preference Test 1	507.72	334.07	358.20
	NIC	713.00	242.15	244.85
	SAL	492.61	334.87	372.52
Cocaine (n=13)	Initial Preference	419.74	351.78	428.48
	Preference Test 1	570.20	311.48	318.32
	NIC	776.14	247.67	176.19
	SAL	577.17	317.00	305.82

3.1 PARADIGM 1: THE REINFORCEMENT-ENHANCING EFFECT OF NICOTINE ON SUCROSE CPP

Figure 1 displays the mean difference scores of animals from the first 10-minutes of the initial preference assessment and each sucrose CPP post-conditioning preference test. On average, there was a significant increase in the amount of time animals spent in their assigned UCS chambers between the initial preference assessment and post-conditioning preference test 1 [$t(13)=-3.03$, $p=0.01$]. These data show that sucrose CPP was successfully established. Across the post-conditioning preference tests, there was a significant effect of daily drug pretreatment ($F_{1.16, 15.03}=6.087$, $p<0.05$). Furthermore, the quadratic function of these data was significant ($F_{1, 13}=6.518$, $p<0.05$). Together, these statistics indicate that not only were there significant differences in the amount of time spent in the UCS chamber from test to test, but also, animals

spent the most time in the UCS chambers when nicotine was in their systems. Direct comparisons of each preference test confirmed a significant effect of nicotine on time spent in the UCS chamber relative to both saline [$t(13)=2.64$, $p<0.05$] and no injection [$t(13)=2.41$, $p<0.05$]. There was also no significant difference between the results of the injection-free and saline preference tests [$t(13)=0.77$, $p=0.46$].

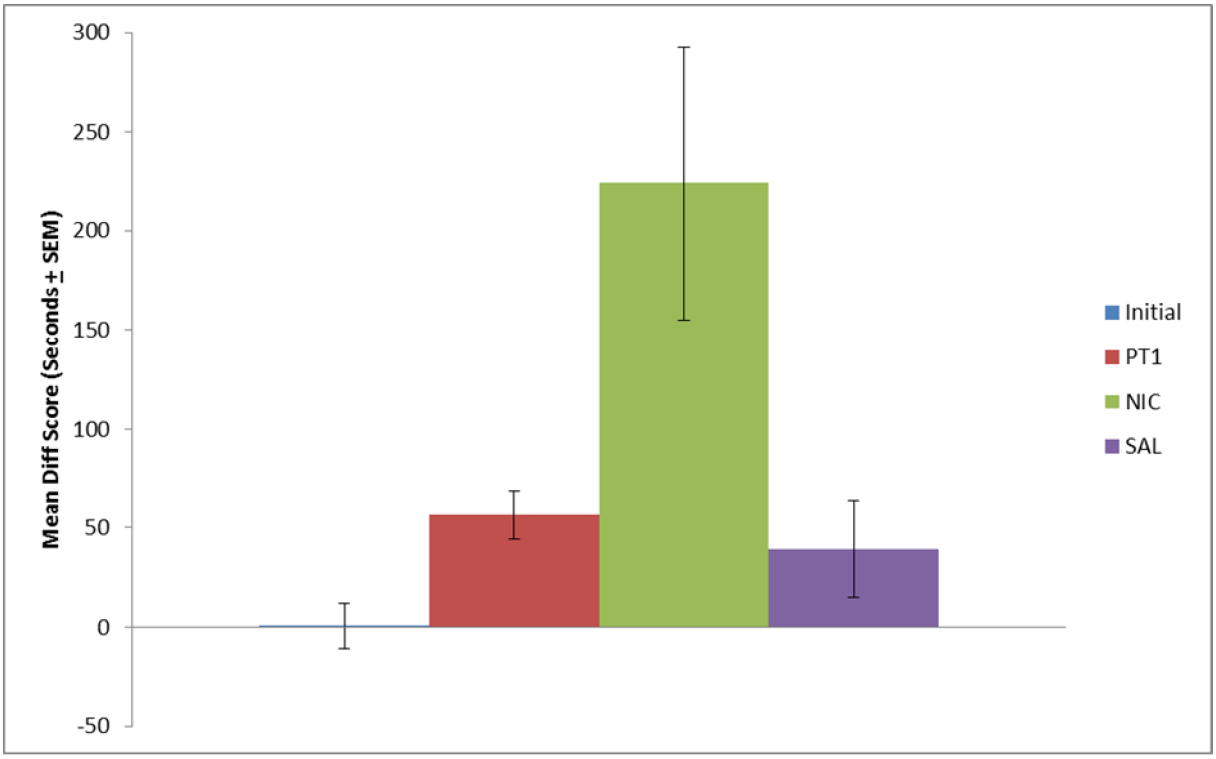

Figure 1. The effect of nicotine (0.4mg/kg SC; -5min) on the first 10 minutes of an established sucrose CPP. Data are represented as the mean difference score (UCS chamber time ï control chamber time; ±SEM) of animals during the first ten minutes of the initial preference assessment and each post-conditioning preference test (n=14).

Analyses across each full, 20 ï minute test yielded similar statistical results (Figure 2). The establishment of sucrose CPP was significant [t(13)=-2.99, p=0.01], and both daily drug pretreatments and the quadratic function of the data showed significant effects ($F_{1.21, 15.75}$=12.85, p<0.005; $F_{1,13}$=14.45, p<0.005). Nicotine also had a significant effect on the amount of time spent in the UCS chamber relative to both saline [t(13)=4.23, p=0.001] and no injection [t(12)=5.25, p<0.001]. The difference between the results of preference tests 1 and 3 was statistically insignificant [t(13)=0.80, p=0.44].

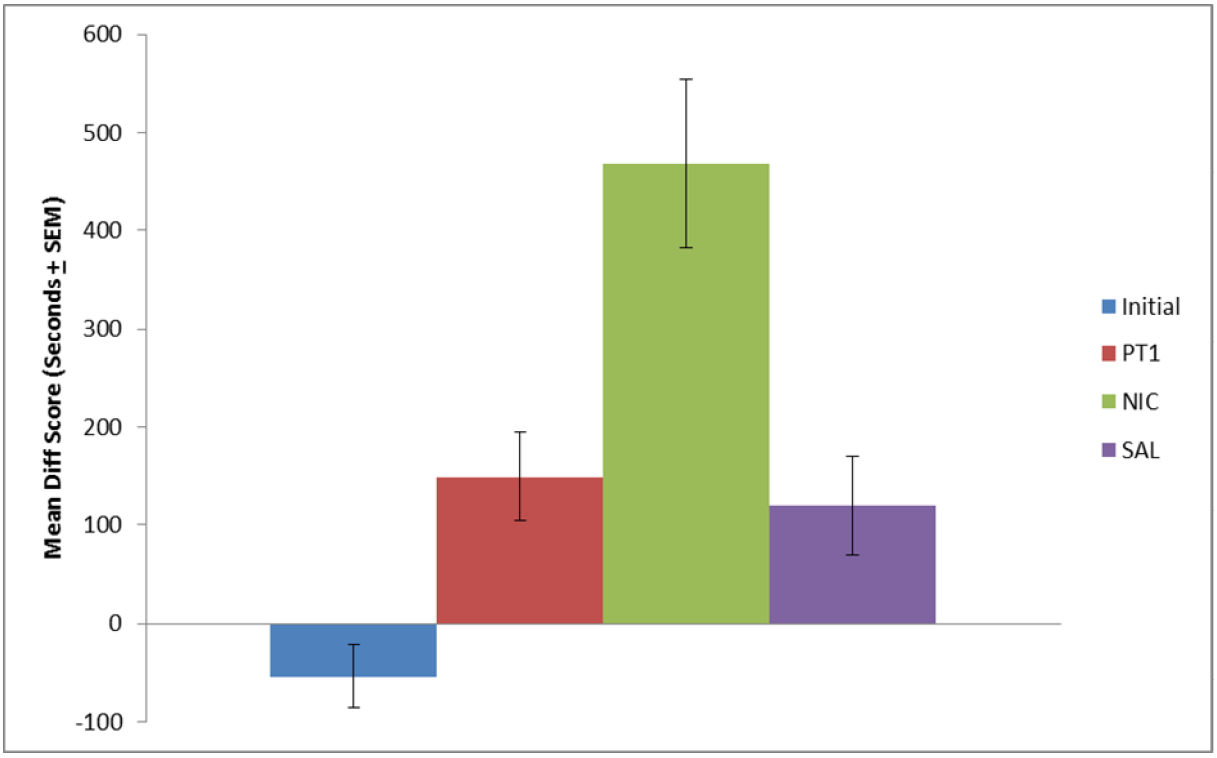

Figure 2. The effect of nicotine (0.4mg/kg SC; -5min) on established sucrose CPP. Data are represented as the mean difference score (UCS chamber time ï control chamber time; ±SEM) of animals during the full 20-minute length of the initial preference assessment and each post-conditioning preference test (n=14).

Correlational analyses performed on these data yielded insignificant results. The test of average sucrose consumption during conditioning against the first 10 minutes of sucrose CPP revealed a negative but insignificant linear relationship (R=-0.02, p=0.48). The test of sucrose consumption against the full length of sucrose CPP produced similar results (R=-0.23, p=0.22). These numbers suggest a weak, negative relationship between the degree to which animals liked sucrose and the strength of their preference for the UCS chamber during preference test 1. When the first half of sucrose CPP was tested against the first half of the preference test with nicotine pretreatment, a positive but insignificant linear relationship surfaced (R=0.04, p=0.50). The analysis performed between the full length of both tests unveiled similar results (R=0.02, p=0.47). These figures allude to a positive yet loose relationship between the strength of sucrose CPP and the extent to which nicotine enhanced this CPP.

3.2 PARADIGM 2: THE REINFORCEMENT-ENHANCING EFFECT OF NICOTINE ON COCAINE CPP

Figure 3a represents the average difference scores of cocaine animals across all four 20-minute preference tests regardless of crossover group or injection order. All animals showed a significant increase in the amount of time spent in the UCS chamber from initial preference assessment to post-conditioning preference test 1, indicating the achievement of a cocaine CPP [t(12)=-4.36, p=0.001]. Treating injection order as the between-subjects variable (Figures 3b and

3c), there was a significant effect of drug pretreatment ($F_{2, 22}$=22.99, p<0.001) indicating significant changes in total time spent in the UCS chamber from test to test. There was no significant effect of injection order demonstrating that animals in each group displayed similar behavior regardless of the order of injections ($F_{1, 11}$=0.60, p=0.46). Direct comparisons of the results of each preference test showed a significant effect of nicotine on the amount of time spent in the UCS chamber relative to saline [t(12)=5.25, p<0.001] and no injection [t(12)=-5.23, p<0.001]. There was no significant difference between the results of the injection-free and saline preference tests [t(12)=-0.593, p=0.56].

Figure 3a.

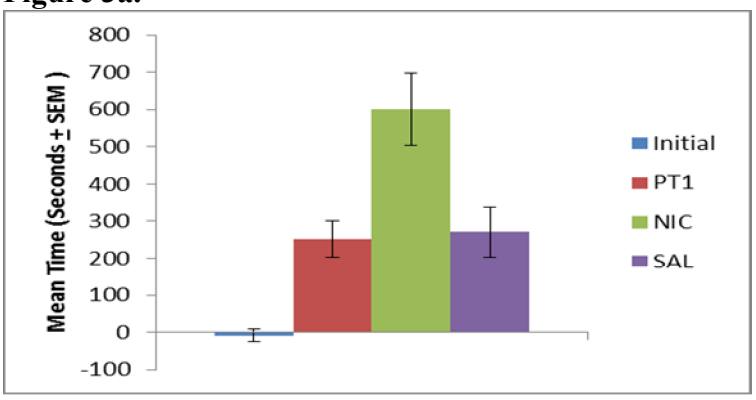

Figure 3b.

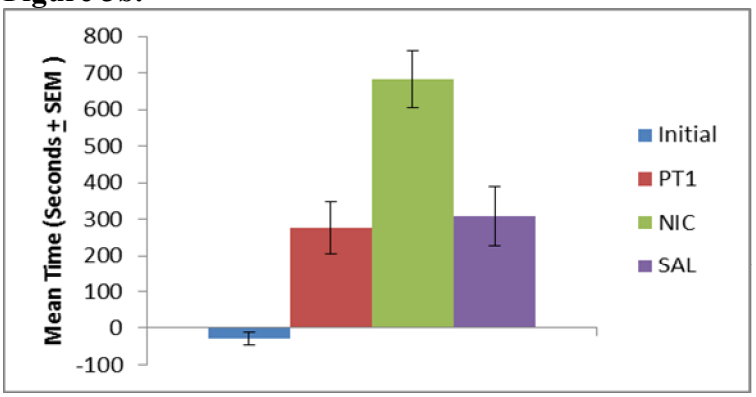

Figure 3c.

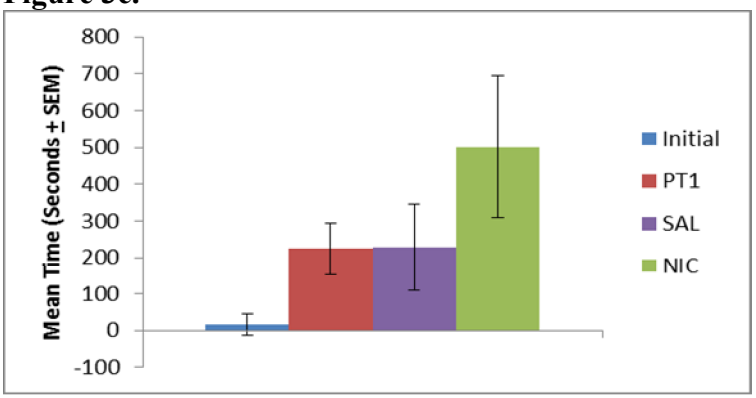

Figure 3. (a) The effect of nicotine (0.4mg/kg SC; -5min) on established cocaine CPP in all animals (n=13), (b) group 1 animals who followed the standard order of injections (NIC/SAL, n=7), and (c) group 2 animals who received counterbalanced injections (SAL/NIC, n=6). Data are represented as the mean difference score (UCS chamber time ï control chamber time; ±SEM) of animals during the full length of the initial preference assessment and each post-conditioning preference test.

Analysis of the relationship between cocaine CPP and the extent to which nicotine enhanced this CPP yielded positive and significant results (R=0.78, p=0.001). These statistics suggest that the ability of nicotine to enhance cocaine CPP depended on the strength of that cocaine CPP displayed by the animals.

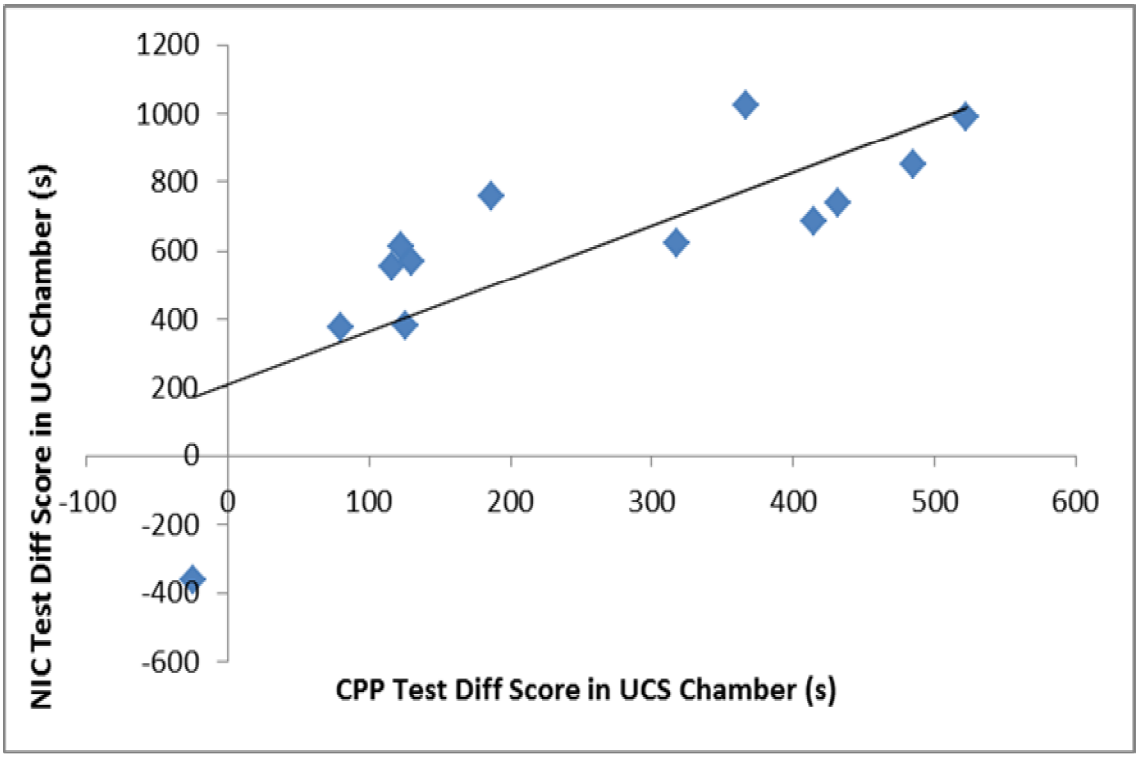

Figure 4. The linear relationship between the cocaine CPP and its enhancement by nicotine (0.4mg/kg SC; -5min). Data are plotted as each animalôs individual difference score (UCS chamber time ï control chamber time from post-conditioning preference test 1 versus its individual difference score from the post-conditioning preference test preceded by nicotine. Difference scores reflect the behavior of the animals during the full length of the 20-minute preference tests.

4.0 DISCUSSION

The principal aims of the present experiment were to 1) establish sucrose and cocaine CPPs in adult male rats and 2) determine whether or not nicotine could enhance these expressed CPPs. It was expected that after repeated associations with either sucrose or cocaine, the UCS chamber would become a conditioned stimulus (CS) for animals with no initial chamber preference. Resultantly, this CS would produce the conditioned response (CR) of more time spent in the UCS chamber during post-conditioning preference test 1 (i.e. animals would show a CPP). It was also hypothesized that a one-time, acute injection of SC nicotine given prior to a subsequent preference test would lead to an enhanced CR (i.e. even more time spent in the UCS chamber). The results of this study support these hypotheses. Both sucrose and cocaine CPPs were demonstrated, and a SC nicotine injection enhanced these CPPs relative to the saline and injection-free post-conditioning preference tests which, as was predicted, were statistically similar. Furthermore, animals in the cocaine paradigm showed that this trend can be expressed regardless of injection order. Overall, these results provide validation for the reinforcement-enhancing effect of nicotine within a paradigm not previously used to study the phenomenon.

By demonstrating this enhancing action of nicotine outside the commonly used operant paradigm, this experiment serves to support the existence of the phenomenon and emphasize its

importance in understanding nicotine reinforcement. It also helps counter the arguments of reinforcement-enhancement effect opponents. For instance, in response to a study by Donny et al. (2003), Frenk and Dar (2004) made claims challenging both the primary and reinforcement-enhancing properties of nicotine. They argued that rather than producing these effects, nicotine merely accelerated the animalsô ongoing operant behavior by increasing locomotor activity, an effect commonly produced by stimulants. From their point of view, once the active lever became a CR through repeated pairings with a food reward, the animals continued to press the active lever due to the strength of this association. The introduction of nicotine only served to increase the locomotor frequency of this behavior. The results of the present study refute these assertions made by Frenk and Dar. If nicotine only stimulated locomotor activity, the animals in both paradigms would not have displayed an enhanced preference for the UCS chamber. More likely, they would have shown increased movement throughout the entire CPP apparatus and less time spent in the UCS chamber relative to the other two post-conditioning preference tests. Thus, the results of this experiment appear to provide more support for the reinforcement-enhancement phenomenon.

Though these findings seem to uphold those of operant studies investigating the reinforcement-enhancing effect of nicotine, it would be imprudent to attempt to directly compare the results without first comparing the conditions necessary for the expression of a pronounced effect within the operant and CPP contexts. In a comprehensive review of the dual-reinforcement model of nicotine, Caggiula et al. (2009) described some of the aspects now known to be crucial for the reinforcement-enhancing effect within the operant paradigm. Consistent with the studies in the Caggiula et al. overview, the stimuli utilized in this study appear to possess the qualities

necessary to elicit the reinforcement-enhancing actions of nicotine. The effect is said to be more robust when the environmental stimulus is moderately reinforcing on its own (Chaudri et al., 2006; Palmatier et al., 2007b). Moreover, this robust effect can also be expressed with stimuli that have been established as conditioned reinforcers by non-nicotine primary reinforcers (Chaudri et al., 2006a; Olausson, Jentsch, & Taylor, 2004). In the present study, the UCS chamber was established as a potent conditioned reinforcer after multiple pairings with sucrose or cocaine as can be seen in the CPP displayed in preference test 1. In this way, the nature of the key stimulus in this study met the standards of those used in its operant counterpart studies.

Not only the strength but the timing of the stimulus presentation also plays a significant role in the expression of nicotineôs enhancing effects. The results of a study by Palmatier et al. (2007b) highlight the importance of the temporal relationship between nicotine and the stimulus. In comparing the effects of pretest injections of nicotine against those of posttest injections on responding for a moderately reinforcing stimulus (the offset of the house light), it was found that only animals who received pretest injections displayed enhanced responding. This finding suggested that the effect depends on the initial rather than the long-term pharmacological effects of nicotine. In other words, the initial effects of nicotine must coincide with access to the stimulus of interest. In the present experiment, animals were permitted to explore the entire CPP apparatus while the initial pharmacological effects of nicotine were ongoing. Therefore, the need for the simultaneous availability of the key stimulus and the initial actions of nicotine was met by the design of this CPP study.

Though this CPP study met two of the criteria necessary for the expression of the reinforcement-enhancing action of nicotine in the operant paradigm, there is at least one

significant difference between the two models: the amount of time necessary for the effect to develop. In the present study, the effect was significantly produced after a one-time, acute nicotine injection. However, for the effect to be potently expressed in the operant model with IV or SC nicotine, it requires multiple test days and repeated infusions or injections (Caggiula et al., 2009; Chaudri et al., 2006; Donny et al., 2003; Liu et al., 2007; Palmatier et al., 2006; Palmatier et al., 2007a,b). This incongruence raises a key question: if prior experience with nicotine is necessary for the expression of the enhancing actions of nicotine in operant paradigm, why did it not appear to be necessary in this CPP study? Before examining this problem, one must first try to understand why the phenomenon develops over time with repeated nicotine exposure in operant models.

Two possible reasons explaining this operant process were proposed and investigated in the aforementioned experiment by Palmatier et al. (2007). While testing the effects of pretest and posttest nicotine injections on responding for the offset of the house light, the pretest injection group displayed a gradual increase in responding over 9 sessions. It was suggested that this steady development over time indicated either 1) an increase in the effects of nicotine over time or 2) learning of the new value of the stimulus in the presence of nicotine. To determine which process was taking place, a second experiment was conducted in which half of the posttest injection animals were switched to receive pretest injections while all other animals maintained the same conditions. Animals in the original pretest injection group continued to show the same trend of enhanced responding while the animals that continued receiving posttest injections continued their trend of minimal responding. As for the switched posttest injection group, instead of displaying the same gradual increase in responding initially shown by animals in the pretest

injection group, these animals showed an immediate, synergistic increase in responding. These results seemed to indicate that the synergy in responding was not dependent upon the learned association between nicotine and the offset of the house light, but rather it was dependent upon the animalsô previous exposure to nicotine. Through these repeated exposures, it is possible that animals experienced nicotine sensitization ï an increased sensitivity to the effects of nicotine ï or developed a tolerance to the initial aversive and motor suppressing effects of nicotine.

With the absence of previous nicotine exposure in the present study, it is not possible for animals to have experienced sensitization or tolerance to nicotine. However, looking specifically at the data from the cocaine CPP paradigm, one might suggest the role of cross sensitization in the outcomes. Cross-sensitization occurs when repeated exposures to one drug increases behavioral or physiological sensitivity to another drug (Palmatier, Fung, & Bevins, 2003). Since nicotine and cocaine are psychostimulants, they both produce similar pharmacological and physiological effects such as increased blood pressure, heart rate, and dopamine levels in the brain (Jones, Garrett, & Griffiths, 1999). In this sense, one might argue that instead of there being an interactive relationship between the reinforcing properties of nicotine and the UCS in the present study, nicotine merely generalized the effects of cocaine, causing the animals to spend more time in the cocaine-associated chamber. The results of the correlational analysis between cocaine CPP and the extent to which nicotine enhanced this CPP seem to support this argument. Animals that displayed higher levels of CPP showed greater enhancement of this CPP by nicotine (Figure 4). It is difficult to make this claim, however, because though nicotine and cocaine are stimulants that produce similar physiological effects, they differ in terms of the degree to which they produce certain effects. In a clinical study comparing the subjective and

physiological effects of nicotine and cocaine in cigarette-smoking cocaine abusers, participants generally reported that very different doses of cocaine and nicotine (40mg/70kg and 1.5mg/70kg respectively) produced comparable physiological effects. Even at these doses, cocaine was reported to have produced more positive effects while nicotine was reported as creating more negative effects (Jones, Garrett, & Griffiths, 2009). Furthermore, the existing preclinical literature seems ambiguous with regard to the cross-sensitization effects of nicotine on later response to cocaine. Research has shown that nicotine pre-exposure facilitates the acquisition of cocaine self-administration, indicating that nicotine can sensitize animals to the primary reinforcing effects of cocaine (Horger, Giles, & Schenk, 1991). However, varying test results make it unclear as to whether or not nicotine sensitizes animals to the locomotor effects of cocaine (Collins & Izenwasswe, 2004; Horger, Giles, & Schenk, 1991; McQuown, Dao, Belluzzi, & Leslie, 2009; Schenk, Snow, and Horger, 1990). To determine whether or not cross-sensitization played a role in the cocaine paradigm of the present study, more research needs to be conducted to understand the sensitization effects of cocaine on response to nicotine, especially in relation to the reinforcement-enhancing effect.

Even if pre-exposure to cocaine did sensitize animals to the enhancing effects of nicotine, this particular explanation does not account for the enhancement exemplified by the animals in the sucrose CPP paradigm. Since these animals could not have experienced cross-sensitization to the effects of nicotine, what could have happened to explain the results from this paradigm? In preclinical studies with rats, initial exposure to nicotine tends to result in the suppression of locomotor activity (Bevins & Palmatier, 2003; Palmatier, Fung & Bevins, 2003; Stolerman, Garcha & Mirza, 1995). Adult rats also tend to display more sensitivity to these effects than

adolescent rats (Vastola, Douglas, Varlinskaya & Spear, 2002). In light of this aversive effect, one might postulate that in the present experiment, animals went to the chamber with the most positive associations (the UCS chamber) and remained in that chamber after succumbing to the motor suppressing effects of nicotine. While this hypothesis could account for the lack of a correlation between sucrose CPP and the extent to which nicotine enhanced this CPP, it does not explain the amount of time animals spent in the other two chambers during testing. Furthermore, research has shown that the locomotor movement should not necessarily be generalized to include general motor movement. In one study observing the effects of nicotine on locomotor activity (running on an activity wheel) versus general activity (movement throughout a photocell chamber), it was found that initial exposure to nicotine suppressed locomotor but not general activity (Bryson, Biner, McNair, Bergondy & Abrams, (1981). For these reasons, it is not likely that the locomotor suppressing effects of nicotine explain the sucrose CPP results.

One might instead suggest that nicotine increased the animalsô general appetite or craving for sucrose. This elevated craving or appetite would have led animals to seek a means to satiate that hunger. As animals learned they could receive sucrose solution from the UCS chamber only, they logically would spend more time in this chamber waiting for the sucrose that could quench their hunger. This hypothesis is unlikely, however, as numerous studies have reported nicotine producing anorectic effects in rats (Bellinger, Cepeda-Benito, & Wellman, 2003; Bellinger et al., 2005; Blaha, Yang, Meguid, Chai, & Zadak, 1998; Donny, Caggiula, Weaver, Levin, & Sved, 2011; Grunberg, Winders, & Popp, 1987; Miyata, Meguid, Varma, Fetissov, & Kim, 2001; Winders & Grunberg, 1998). What appears more likely is that nicotine enhanced the salience or reward value of the UCS chamber through its reinforcement

enhancement mechanism. Currently, there is not enough evidence to determine why this was able to occur in the CPP model without the previous exposure to nicotine or cross-sensitization necessary to elicit the effect in the operant model. Future research should investigate this matter further.

The design and model used for this experiment had a number of advantages over those of an operant model. As was stated earlier, one of the major differences between the present experiment and studies employing operant designs is the number of nicotine exposures necessary to produce a reinforcement-enhancing effect. Not only did this paradigm save time in producing this effect, but it also provided information about one-time (as opposed to chronic) nicotine experiences in nicotine-naïve animals. Relating this back to smoking, the use of chronic nicotine in the operant paradigm provides more insight into the effects of nicotine in a chronic smoker while the single, acute nicotine injection in the present experiment may reveal more about the effects of nicotine in first-time smokers. In this sense, this experiment could shed light on the possible consequences of a first exposure to nicotine.

The short length of the conditioning and testing sessions constituted another advantage of this experiment over its operant counterparts. Because UCS and control conditioning sessions were short enough to occur in the same day, it was possible to cut the length of the study in half. This aspect was most important as the study design underwent extensive alteration and development. Had the trials for this study taken the same amount of time as operant studies, it would have taken substantially longer to identify the flaws in the design and the final design utilized in this study may not have been reached for a very long time.

Though the condensed nature of the study created the advantage of a shorter study, it also led to the main limitation of this experiment: the small sample size used in each paradigm. Since data were only analyzed within-subjects for the sucrose paradigm, the small sample size is not extremely problematic. However, the two crossover groups within the cocaine paradigm were tested between-subjects with six animals in one group and seven in the other. These small sample sizes make it difficult to generalize the results from the between-subjects analysis of the cocaine paradigm. Future replications of this study should use larger samples, particularly where crossover injection groups are concerned, to give the data more statistical power and improve the external validity of the experiment. Another limitation was the lack of crossover injection groups in the sucrose paradigm. Though statistical analysis for the cocaine paradigm indicated that injection order did not impact the outcomes of the tests, one cannot assume that the same would hold for the sucrose paradigm. For this reason, replications of this experiment should also divide animals in the sucrose paradigm into crossover injection groups for post-conditioning testing.

4.1 IMPLICATIONS FOR FUTURE RESEARCH

The most intriguing and provocative aspect of the present study was the apparent expression of the reinforcement-enhancing effects of nicotine in animals with no previous exposure to nicotine. The fact that this pre-exposure is crucial for expression of this phenomenon in operant models raises questions about what kind of effect pre-exposure to nicotine may have had on the results of this study. A future study could investigate this question. By exposing animals to nicotine

prior to initial preference assessment and incorporating the design used in the present study, such an experiment could shed some light on the effects of nicotine pre-exposure on the expression of a sucrose and cocaine CPP. More importantly, it would show whether or not pre-exposure to nicotine alters the ability of nicotine to later enhance these CPPs.

Despite the apparent stability of the associative learning shown in the post-conditioning preference test comparisons, it is difficult to determine how stable the post-conditioning effects actually were without a baseline control group to compare these outcomes. Such a study would provide a point of comparison for all other studies using injection manipulations and allow for better interpretation of the results of the present study. Assuming that CPP could remain stable for at least four post-conditioning preference tests, another variation of the present experiment could be conducted in which all the steps and conditions of the present experiment are repeated but with the addition of a fourth preference test preceded by a SC nicotine injection. For animals that receiving a saline injection before the final post-conditioning preference test, this test could be used to see if the enhancement effect can be reinstated.

Currently, there is no way of knowing how repeated nicotine injections over multiple post-conditioning test days would have affected the behavior of the animals. It is possible that over repeated injections, the observed enhancement effect could have increased, decreased, or remained stable. Depending on how long it would take for the learned association between the UCSs and the UCS chamber to extinguish, this subject could be investigated in a future variation of this experiment. This type of experiment could provide more information regarding the strength of the enhancement of CPP after repeated nicotine exposures. It could also allow for more sound comparisons of the results between the operant and CPP models.

Still another study could look at the effects of nicotine on the acquisition of a sucrose and/or cocaine CPP. Unlike the study completed by Thiel et al. (2009) in which nicotine was discretely paired with the UCS chamber, such a study would require that animals receive nicotine injections before each UCS and control conditioning session to ensure that nicotine is not associated with a specific chamber. Comparing the results to saline control animals could demonstrate the extent to which nicotine enhances the acquisition of CPP. The results of all these hypothetical studies (and the present studies) could be compared to better understand the effects of non-associative nicotine in different stages of CPP.

4.2 CONCLUSION

Though the reinforcement-enhancing effect of nicotine has been studied at length in the operant paradigm, little research has looked into how the phenomenon generalizes over to other conditioning models. In displaying the reinforcement-enhancing effect of nicotine in two CPP paradigms, the present experiment helps validate this secondary action of nicotine and provides more insight about the effect in other contexts. More research needs to be conducted to understand why this effect can be seen in the CPP paradigm without the previous nicotine exposure that is crucial in operant models. However, as it stands, the present experiment and its future variations can serve to shed more light on the consequences of a first-time exposure to the nicotine. Understanding these data could contribute to an improved understanding of how

nicotine interacts with salient environmental stimuli after an initial exposure and how this initial spark can lead animals and humans down a path toward nicotine addiction.

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