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OPTIMIZING A HUMAN MODEL OF THE AMPHETAMINE CONDITIONED PLACE PREFERENCE PARADIGM

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List of Abbreviations

ALC	Alcohol
AMP	d-Amphetamine
ANS	Autonomic nervous system
ANOVA	Analysis of Variance
APA	American Psychiatric Association
ARCI	Addiction Research Center Inventory
BP	Blood pressure
CPP	Conditioned place preference
CDR	Conditioned drug response
CR	Conditioned response
CS	Conditioned stimulus
DEQ	Drug Effects Questionnaire
DSM-IV	Diagnostic and Statistical Manual, 4th Edition
ECG	Electrocardiograph
HR	Heart Rate
HRV	High-frequency heart rate variability
ICG	Impedance Cardiograph
NEM	Negative Emotionality
PEM	Positive Emotionality
PEP	Pre-ejection period
PL	Placebo

POMS	Profile of Mood States
PSNS	Parasympathetic nervous system
RMANOVA	Repeated measures analysis of variance
RR	Respiratory rate
RT	Reaction time
SAMHSA	Substance Abuse and Mental Health Services Administration
SEM	Standard error of the mean
SNS	Sympathetic nervous system
TEPS	Temporal Experience of Pleasure Scale
US	Unconditioned stimulus

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Abstract

Humans form powerful associations between drug-taking experiences and the physical environment that surrounds their drug use through a process called contextual conditioning. These drug-associated environments can reinforce drug-taking behavior, and in many cases, can cause a drug addict to relapse, even after years of abstinence. Because of this, contextual conditioning plays a key role in the study of addiction. In animals, drug-associated environments promote the reinstatement of drug-seeking, as well as elicit a range of behavioral and physiological reactions that predict relapse. In clinical settings, empirical and anecdotal evidence show the powerful effect of drug-associated contexts on drug cravings, and also suggest that humans vary in their susceptibility to relapse. However, little is known about how these drug-environment associations develop in humans, and what factors underlie variability in how contextual conditioning is acquired. In animals, the acquisition of contextual conditioning is studied using the conditioned place preference paradigm, wherein animals receive a reward, such as a drug, consistently in the same environment, and it is tested whether they develop a behavioral preference for the reward-paired environment (i.e. whether they spend more time in that environment) over time. Very few human conditioned place preference studies have been completed using drugs of abuse; therefore, it is critical that more controlled studies of contextual conditioning to drug-associated environments take place. Human conditioned place preference studies can help elucidate the subjective, behavioral, and physiological mechanisms that underlie individual differences in the acquisition of contextual conditioning.

The purpose of the first study in this dissertation (Study 1) was to replicate a previous human conditioned place preference study, which used an amphetamine reward, and measured preference using self-reports of explicit room preference. In addition, we aimed to determine whether humans would spend more time in a room in which they previously received AMP after the pairings than beforehand, like in the animal CPP paradigm. We gave healthy human volunteers 20 mg of amphetamine and placebo twice each in either of two rooms. One group (the Paired Group) consistently received amphetamine in one room and placebo in the other room, while the Unpaired Group received amphetamine and placebo in both rooms. We examined the change in preference for the two rooms using both objective and subjective measures. Before and after conditioning, subjects explored the two conditioning rooms for ten minutes, and we measured how much time the spent in each room. We also had them rate on a questionnaire how much they liked and preferred each room. Following conditioning, subjects in the Paired Group demonstrated a significant increase in liking and preference for the amphetamine-paired environment, but they did not exhibit an increase in time spent in that environment. We also found that the degree of subjective conditioning correlated with the magnitude of the subjects' "liking" of the drug. Here, we successfully replicated the subjective preference measures seen previously in humans, but we were unable to reproduce the time spent measure that is used in animals.

Next, in Study 2, we aimed to determine individual differences in the acquisition of conditioning in Study 1, in combination with the study it replicated. Here, we sought to find out whether personality predicts amphetamine conditioned place preference, and whether personality moderates the relationship between the positive subjective effects

of amphetamine and conditioning. Here, we found that Positive Emotionality predicted the increase in subjective preference for the amphetamine-paired, but that Negative Emotionality did not. We also found that amphetamine-induced euphoria predicted conditioning, but that neither Positive Emotionality nor Negative Emotionality moderated the relationship between amphetamine-induced euphoria and conditioning. This analysis demonstrated for the first time that personality predicts conditioned place preference in humans, and that this relationship is independent of the subjective effects of amphetamine.

Finally, because we were unable to elicit an objective place preference in Study 1, we sought to expand and refine our amphetamine conditioning place preference paradigm with the hope of inducing an objective preference for the amphetamine-paired room (Study 3). In this study, we aimed to determine the optimal number of conditioning sessions for inducing the strongest place preference. Secondarily, we aimed to study the subjective, behavioral, and physiological effects of the amphetamine-paired room. Here, all subjects underwent eight conditioning sessions. Paired Group subjects underwent four amphetamine-room pairings, and both groups' subjective and objective preferences for the rooms were measured after two, four, and eight conditioning sessions. Additionally, we measured mood, heart rate, blood pressure, and autonomic nervous system activity during the conditioning sessions as well as after conditioning. In this study, despite experiencing the prototypical acute subjective and physiological responses to the drug, subjects in the Paired Group did not express an increase in subjective or objective preference for the amphetamine-paired room after any number of drug-room pairings. Also, subjects did not express conditioned increases in the subjective, behavioral, or physiological responses to the drug in the drug-paired

environment, or to the drug-paired room in the absence of drug. In this chapter, we discuss the potential implications of the study design on the study's outcome.

The studies presented here provide further evidence that humans will develop a subjective preference for an amphetamine-paired room, but they also demonstrate that this paradigm requires further refinement and expansion. First, we demonstrated the reliability of subjective measures of contextual conditioning in humans. Second, we also showed that this preference is related the subjective effects of amphetamine and personality. Finally, our last study demonstrates the sensitivity of contextual conditioning to parametric changes in the protocol, and therefore, that more steps must be taken to optimize this paradigm. These studies provide a basis for future research into how drug-environment associations develop in humans and what individual differences underlie the susceptibility to acquiring contextual conditioning.

Chapter 1: Introduction

1.1 Summary

Drug dependence is a debilitating chronic illness marked by an uncontrollable craving for drugs and a compromised ability to refrain from drug use despite its destructive consequences It is one of the most prevalent and costly psychiatric conditions in the United States. About 24.6 million Americans currently abuse illicit drugs, and drug addicts account for about 15% of the Americans who have been diagnosed with a psychological disorder in their lifetime (Kessler et al., 2005; SAMHSA, 2014). Alcohol (ALC) abuse by itself is the second most prevalent psychological disorder behind major depression (Kessler et al., 2005). In addition to this substantial health burden, addiction also imposes a significant strain on the United States economy: substance use disorders led to $700 billion in losses to the economy in 2014 as a result of legal and health expenses and lost work productivity (NIDA, 2015). Despite these horrific statistics, most drug addicts do not seek treatment. Even among those who do quit using drugs, however, 40-60% of them relapse, often after years of abstinence (McLellan et al., 2000). Recently the factors that contribute to relapse have become a target for research and treatment (Napier et al., 2013).

Scientists argue that a major instigator of relapse is contextual cues associated with the drug-taking experience. Just as Pavlov's dogs salivated in response to the

environment in which they received food, it is believed that contextual cues associated with past drug use can elicit drug cravings that lead to relapse (Pavlov, 1927; O'Brien et al., 1992). Still, individuals exhibit significant variation in their acquisition of these associations and their response to drug-related contexts. This indicates that there is a potential to predict and prevent the development of these associations on an individual basis by modeling the acquisition and expression of contextual conditioning in the laboratory. Therefore, laboratory models are valuable to study the behavioral neurobiological mechanisms that underlie the development of drug-context associations and the expression of craving responses to drug-related contexts.

Here, I will a) discuss the significance of drug-related contexts in drug craving and relapse in both humans and in animals, b) describe the conditioned place paradigm, the method scientists developed in animals to study the acquisition and expression of drug-context associations c) review the literature regarding animal and human conditioned place preference (CPP) and d) summarize my recent efforts to expand and refine this paradigm in humans.

1.2 The Role of Contextual Cues in Drug Seeking and Relapse

Addiction is a complex disorder, incorporating psychological, biological, and sociological elements, and it is impossible to pinpoint a single cause for why, even after years of sobriety, individuals relapse to drug-taking. Many theories of relapse hinge on the overwhelming influence of drug-related contextual cues on drug craving: it is believed that exposure to contextual cues that have become associated with past drug

use may cause an addict to seek out drugs (Wikler and Pescor, 1967; Stewart and Eikelboom, 1987; Napier et al., 2013). Although discrete, localizable cues such a light or a lever become conditioned to the drug experience in animals, there are reports that broader contexts such as an operant chamber induce strong conditioned responses as well, and even enhance cue-induced cravings (Conklin et al., 2008; Sciascia et al., 2015). In humans, unfortunately, we do not know much about how these drug-context associations develop nor do we understand the mechanisms underlying the conditioned response to a drug-associated context. Drug-related contexts, and the mechanisms by which they develop and affect behavior, continue to complicate both the study and treatment of addiction.

Anecdotal and empirical evidence in humans demonstrates the powerful influence of contextual drug cues on behavior. For instance, recovering addicts report that exposure to drug-associated contextual cues can lead them to seek drugs even after years of abstinence. Anecdotal reports show that drug-related contextual cues such as bar or even an addict's home greatly impact treatment outcomes - often, individuals who have relapsed to drug taking will cite drug-related contexts as the primary cause for their fall (O'Brien et al., 1993). Contextual cues often elicit a return to the compulsive drug-seeking behavior that is characteristic of addicts (Wikler and Pescor, 1967; O'Brien et al., 1993). Empirical evidence suggests that a reward-related context can elicit overindulgence for natural rewards even in young children (Wikler and Pescor, 1967; Birch et al., 1989; O'Brien et al., 1993). In one study, sated pre-school children were given snacks in one room and nothing in another room. Upon re-exposure to these two environments, the children ate more snacks and waited for less time to start eating in the room in which they previously received snacks (Wikler and Pescor, 1967; Birch et al.,

1989; O'Brien et al., 1993). These anecdotal and empirical data show that individuals are highly sensitive to contextual cues associated with rewards.

There is also evidence from laboratory animals showing that drug-associated contexts facilitate drug-seeking. Drug-related contexts are believed to elicit drug seeking behaviors via a Pavlovian mechanism. For example, Pavlov observed that his dogs salivated when they approached the room where the experimental apparatus was housed, even before they were exposed to the discrete food-associated cue (Pavlov, 1927). There are also classic studies by Schuster of monkeys showing anticipatory excitability before their daily morphine injections (Thompson et al. 1964). More recently, researchers have applied this principle to in an animal model of drug relapse by studying reinstatement of drug self-administration by exposure to a drug-paired context. A standardized procedure has been developed to study context-induced relapse in animal models (Bouton and Bolles, 1979). First, a rat is trained to press a lever to receive a dose of drug in a particular environment (Context A), and it learns to associate this environment with the drug reinforcement. Then, the rat is placed in a different environment (Context B), where this association is extinguished. Finally, the rat is placed back in Context A, and the researcher determines if this drug-related context renews the lever-drug association and elicits drug seeking. Using this paradigm, studies have demonstrated context-induced reinstatement of cocaine, heroin, speedball, and nicotine seeking (Crombag and Shaham, 2002; Bossert et al., 2004; Fuchs et al., 2008; Neugebauer et al., 2014). Furthermore, it has been shown that even if, in Context B, lever-pressing is punished with a footshock, re-exposure to Context A will still renew drug-self administration of ALC (Marchant et al., 2013). These studies show that the contextual conditioning principles that Pavlov initially described apply to drug

conditioning: even if an animal spends time in an environment where its drug seeking-behavior has gone unreinforced or even punished, returning to environment in which drug-seeking was originally reinforced can reinstate this behavior. These examples of context-induced reinstatement of drug-seeking aid in our understanding of the behavioral mechanisms underlying relapse in humans. However, the reports in humans and animals discussed so far do not reveal the mechanisms by which initial drug-context associations develop. To study the acquisition of drug-context associations, scientists employ the CPP paradigm.

1.3 CPP in Animals

The CPP procedure is based on the tenets of classical conditioning and is one the most popular methods for studying acquisition and expression of contextual conditioning with drugs and other rewards (Tzschentke, 1998). Having performed the procedure thousands of times over the past half-century, scientists have developed a standardized regimen for inducing a preference for a particular environment by pairing it with a drug of abuse. In a typical CPP procedure, an animal is first placed in three-chamber apparatus, containing two chambers on either end that are distinct from each other and neutral chamber in the middle . The animal is allowed to freely explore the two distinct environments, and the researcher measures how much time the animal spends in each chamber. Then, over the course of several trials, a researcher will inject the animal with either a drug (the unconditioned response; US) or a vehicle and confine it to one of the two chambers (the conditioned response; CS), respectively. Finally, after conditioning, the animal is allowed to freely explore both chambers again, and the time

spent (the conditioned response; CR) in each chamber is assessed. This "time spent" measure is used as an index of preference in animals. Therefore, an increase in time spent in the drug-paired chamber is interpreted as a preference for that chamber. Most studies used a "biased" design, which means that during the conditioning trials, animals are assigned to receive the to-be-conditioned drug in the chamber it initially spends less time in. This method maximizes allows for a greater increase in preference in response to the association develops.

Although standard now, the CPP protocol and its purpose have evolved over time. The CPP paradigm was first created to elucidate the psychobiological mechanisms that underlie morphine addiction (Beach, 1957). Horace Beach was the first scientist to demonstrate that animals will come to prefer an environment paired with a drug over one paired with a placebo; however, his protocol differed slightly from that used today. Beach first gave saline injections to rats and then placed them in a box in at the end of one arm of a y-maze. Then, he administered morphine injections and placed the rats in a box at the end of the other arm of the y-maze. Beach then gave the rats free access to both arms of the y-maze and assessed which arm of the maze the rats would run down most often. To maximize the effect of conditioning, Beach utilized a "biased" design: he paired the morphine injections with the box that the rats initially preferred less. Beach's goal was to demonstrate that rats could be trained to approach the morphine-associated arm more often. Further he used the procedure to demonstrate that the animals exhibited this "preference" whether or not they were in withdrawals during testing. At the time, it was believed that relief of withdrawal was the primary determinant of drug-seeking. Beach demonstrated that rats will choose to enter an environment that was previously paired with morphine, and that the environment was

associated with the post-consumptive effects of the drug regardless of the state of abstinence.

Later, researchers introduced the "time spent" measure of CPP The "time spent" measure was first used by Rossi and Reid to demonstrate that a rat will come to prefer an environment in which it has previously experienced a positive affective state in response to morphine (Rossi and Reid, 1976). In this study, rats were injected with either morphine or saline, and placed in one of two differently colored chambers in a three-chamber apparatus, respectively. They found that when the rats were allowed to freely explore both environments, they would spend less time in their initially more-preferred environment if it were paired with placebo, not but not if it were paired with morphine. However, this effect was only seen if the morphine chamber was paired with the drug at the moment of peak effect, suggesting that the rats spend more time in the morphine-paired chamber because the chamber was associated with the post-consumptive, positive effects of the drug. This study supported Beach's work, demonstrating that the positive effects of morphine contribute to place conditioning. This study did not use a biased design, which is why Rossi and Reid were not able to demonstrate a straightforward increase in time-spent in the drug-paired environment following conditioning. The importance of the biased design was demonstrated many years later using a nicotine CPP paradigm.

Calcagnetti and Schechter (1994) wanted to determine if nicotine was dependent on the methodology of the CPP paradigm itself; more specifically, whether or not a rat's initial preference for a particular chamber influenced conditioning to that chamber. Citing weak, contradictory evidence of CPP with nicotine rats, they sought to determine whether the increase in time spend in the drug-paired chamber was related to

the rat's initial chamber preference. They compared conditioning in rats that received nicotine in their initially more preferred chamber or their initially less preferred chamber, and found that only the rats in the latter group exhibited an increase in time spent in the nicotine-paired chamber (Calcagnetti and Schechter, 1994). This study, as well as several that followed, demonstrated the sensitivity of the paradigm to individual differences in initial preference and speaks to the validity of the biased design (Tzschentke, 2007). These three studies introduced and refined an experimental method that has since become one of the most popular methods for studying reward and motivation using a host of different variations.

Since these seminal works, the animal CPP paradigm has been further refined, and used to study the motivational rewarding properties of numerous rewards, both natural and 'unnatural', in many different animal species. First, parametric analyses of the paradigm have been performed to understand the learning mechanism that underlies CPP. For instance, researchers have altered the length, interval between, and number of the conditioning trials, the length and number of the test trials, the latency between drug administration and placement in the apparatus, and the state of the animal during testing (having drugs on board or not) (Tzschentke, 1998). Researchers have used CPP to study the rewarding value of opiates, stimulants, and ALC, as well as other drugs (Tzschentke, 1998, 2007). They have used the procedure with natural rewards such as food, sex, and social contact, and with many species, including rats and mice, hamsters, goldfish, zebrafish, quail, and flatworms (Tzschentke, 1998). Overall, the CPP paradigm has been widely used in preclinical studies, and its parameters are well understood. On the other hand, few CPP studies have been performed using

human subjects, and little is known about the optimal conditioning parameters or the nature of the conditioned response to explicitly paired contextual cues.

1.4 CPP in Humans

Just over 50 years after the first animal CPP study took place (Beach 1957), our laboratory published the first human CPP studies. We used methods modeled on animal CPP studies to demonstrate that healthy young adults prefer a room where they received compared to a room where they received placebo, and that the degree of this preference correlates with how much individuals like the effects of the drug (Childs and de Wit, 2009, 2013). The subjective measures used in these studies exemplify the unique advantage of using human subjects; that is, we could answer the question originally posed by Beach in 1957 and later by Rossi and Reid in 1976: is the expression of a preference for a drug-related context related to the anticipation of receiving the reward, or is it related to the post-consumptive, positive subjective effects of the drug? Our studies showed that the latter is the case. We then extended these findings by demonstrating that, in addition to reporting a subjective preference for a drug-paired room, subjects will also spend more time in a room paired with ALC than a room paired with PL, and will drink more ALC in an ALC-paired room if given the opportunity to drink *ad libitum* (Childs and De Wit, *in prep*). The objective measures of time spent and self-administration mirror the contextual conditioning measures that are used in animals and imbue face validity upon the human CPP paradigm.

Beyond our laboratory there is a small but growing literature on CPP procedures using other rewards besides drugs, in both real and virtual environments. In the first

human CPP study to use a natural reward as a US, healthy human volunteers developed a preference for a virtual house in which they had previously heard consonant music relative to a virtual house where they heard white noise (Molet et al., 2013). Next, scientists found that humans will also spend more time in a virtual environment paired with a food reward than one paired with no food (Astur et al., 2014). Furthermore, a separate study showed that not only will individuals show an increase in preference for a food-paired environment after conditioning, but they will also exhibit increased craving for and increased salivation in anticipation of receiving the food reward in that environment (van den Akker et al., 2013). Finally, children will spend more time in an environment paired with engaging toys relative to one paired with less engaging toys (Hiller et al., 2015). Thus, there is growing evidence that humans will not only exhibit a subjective preference for real and virtual environments paired with both drug and natural rewards, but they will also spend more time in these environments. These latter findings confirm the reliability of the time spent measure in humans.

1.5 Expanding and Refining the Human CPP Paradigm

Despite the progress in developing the human CPP paradigm, there are still questions about the optimal methods to detect key conditioned behaviors, and to conduct parallel studies with animals. Until now, our CPP studies with amphetamine (AMP) have assessed self-reported room preference, a measure that has no parallel in animals. My current studies focus on developing more objective measures of preference to bring our protocol closer to replicating the behavior that is seen in animals. Childs et al. (2009; 2013) used subjective measures of room liking or room preference to assess

conditioning with AMP. These measures require individuals to reflect on their experiences and apply a cognitive label of "liking" or "preference" to the rooms. Animal CPP, on the other hand, is thought to be driven by Pavlovian approach mechanisms; that is, animals spend time in a drug-paired chamber because it acquires some of the incentive value of the reward with which it was paired (Huston et al., 2013). Therefore, it is possible that our studies measure a different aspect of "preference" than what is measured in animals. In a later study, Childs and de Wit (*in prep*) used the CPP procedure to show that social drinkers also spent more time in a room paired with a moderate dose of ALC, compared to a room whether they received placebo. Time spent in the conditioned context has also been shown with both real and virtual environments paired with natural rewards (Molet et al., 2013). It remained unknown whether the measure of time spent in the conditioned environment would be a sensitive measure of preference for AMP, as it was with ALC.

Here, our goal was to expand and refine the human CPP paradigm with AMP. First, we added the "time spent" measure of conditioning, parallel to the animal CPP studies and similar to the ALC CPP study by Childs and de Wit. In a separate analysis, we examined individual differences in conditioning as a function personality and drug responses. Second, we varied the number of conditioning sessions to determine whether more pairings would lead to stronger conditioning. Third, we substantially increased our number of conditioning measures to more comprehensively characterize the subjective, cognitive, and physiological response to the drug-paired room. The overall goal of these studies was to continue to establish the AMP CPP paradigm in humans by cross-validating the "time spent" measure used in animals, by inducing a stronger and better defined place preference, and by beginning to understand

the mechanisms underlying individual differences in the conditioned drug effects in response to (the conditioned response to) the AMP-paired room.

1.6 Conclusion

Drug addiction imposes a substantial burden on individuals, from both a health and economic standpoint, yet treatments for treating addiction are limited and fairly ineffective. Recovery efforts are often hampered by the tendency to relapse, and most addiction researchers blame relapse on the development and presence of drug-related cues in the addict's environment. While discrete drug-related cues are known to elicit cravings, it is believed that the context surrounding the drug-taking experience imposes a more intense conditioned effect on the individual. However, treatments targeting these drug-context associations, and the conditioned response to a drug-paired environment, are limited. Very little is known about how drug-context associations develop in humans, and how these associations affect behavior; therefore, it is difficult to construct treatments that specifically target this critical aspect of addiction. Anecdotal evidence in humans and empirical evidence in animals, including studies using the CPP procedure, demonstrate the powerful influence of drug-related contexts on the propensity to relapse. For example, many drug addicts have reported relapsing after they were exposed to an environment that they had previously associated with drug use. Reflecting stories of context-induced relapse in humans, animal researchers have demonstrated the effects of drug-associated context on drug-taking behavior. In addition, the CPP paradigm has been widely used in animals to examine the acquisition of these associations. Researchers have refined the animal CPP paradigm over the past

59 years, but the human CPP paradigm is relatively new. We know that discrete drug cues elicit subjective, cognitive, and physiological responses (O'Brien et al., 1993), but we still do not fully understand what responses become conditioned in a drug-paired context. Additionally, we do not know why some individuals condition more readily, or are more susceptible to relapse than others. Overall, the human CPP paradigm has the potential to improve our understanding of conditioned responses. This understanding may lead to improved treatments for addiction and it will help identify susceptible individuals from relapsing.

In this chapter, I have explored the role that contextual cues play in relapse to drug taking in both humans and animals, I have introduced the CPP paradigm, which is used to study the acquisition and expression of conditioned responses to drug-associated environment, and I have demonstrated the initial human research using this paradigm. My research is designed to expand and refine the human CPP paradigm and to identify the subjective, cognitive, and physiological components of the conditioned responses. I hope to validate the measures used in animal CPP studies, with the goal of identifying individual risk factors, and developing strategies to eliminate drug-context learned responses.

Chapter 2: Amphetamine-Induced Place Preference in Humans: A Replication

2.1 Summary

Associations between drug effects and the context surrounding their use are thought to influence drug-taking. These associations are often studied in laboratory animals using the CPP paradigm. In this paradigm, animals typically display a preference for an environment paired with administration of a rewarding drug, as measured by the time they spend in the environment during a choice test. Recently, using subjective measures of preference, we showed that healthy human volunteers report liking a room paired with AMP over one paired with placebo. However, it is not known whether humans will also spend more time in a room where they received , using an objective index of preference parallel to that used in CPP procedures laboratory animals. Here, we assessed contextual conditioning with d- in healthy humans (n = 37) using both subjective measures of room liking and an objective measure of time spent in the drug-paired room. Subjects completed four conditioning sessions, wherein they received AMP (20 mg) and placebo twice each in a randomized, double-blind fashion. Subjects were randomly assigned to either a paired group (n = 26) who received AMP in one room and placebo in the other, or an unpaired group (n = 11) who received both treatments in each room. Subjective and cardiovascular effects were recorded at

repeated intervals. Before and after conditioning, subjects freely explored the rooms and assigned them subjective ratings. During this exploration phase, we also measured how much time they spent in each room. The amount of time spent in the two rooms determined the room that the paired group would receive AMP or drug. They received drug in the room they spent less time in at pre-test. After conditioning, the paired group liked and preferred the AMP-paired room significantly more than before conditioning. Additionally, the strength of preference for the AMP-paired room was correlated with subjective drug "liking" during conditioning. However, the paired group did not spend more time in the AMP-paired room following conditioning. Overall, the unpaired group showed no changes in preference on any measure. This study replicates previous findings that humans will develop a subjective preference for an AMP-paired room that is related to the subjective effects of the drug, but the subjects' preference did not extend to the objective measure of the amount of time they spent in the drug-paired room.

2.2 Introduction

Current research suggests that contextual conditioning, or the learned association between a context and a reward, strongly promotes drug addiction and relapse to drug taking (Epstein et al., 2009; Carey et al., 2014). Contextual conditioning has long been studied by animal researchers using the CPP paradigm (Tzschentke, 2007). In this paradigm, an animal receives a drug and vehicle in two unique environments, respectively, and then is allowed to move freely in both environments. An increase in time spent in the drug-paired environment is thought to

indicate a drug-related conditioned preference, mediated by a combination of classical and operant conditioning mechanisms (Huston et al., 2013; Carey et al., 2014). Until recently, however, there were no standardized models to study contextual conditioning with drugs in humans. Not long ago, our laboratory developed a human laboratory model to assess the development and expression of conditioned subjective liking and preference for an environment where an individual has previously received a rewarding drug.

Using a paradigm modeled after CPP studies in animals, we have found that human volunteers will develop a preference for a room that was previously paired with a rewarding drug (AMP or ALC) compared to a placebo (Childs and de Wit, 2009, 2013, *in prep*). This preference was measured in two ways: by having subjects rate their liking of the two rooms, or by measuring the amount of time they spend in the two rooms after conditioning trials. In the two studies with AMP (Childs and de Wit, 2009, 2013, *in prep*), only ratings measures were used, and participants reported liking the drug-paired room after conditioning, In the study with ALC (Childs and De Wit, *in prep*), the procedure also included a measure of time spent, and subjects spent more time in the ALC-paired room after conditioning, when given a free choice. The objective measure of time spent in the drug-paired room is particularly important for the cross-species translation of the place conditioning procedure to humans, because time spent in the drug-paired environment is the primary outcome measure used in animal studies (Stephens et al., 2010; Stephens et al., 2013). Therefore, the present study was designed to assess the time spent in a room paired with AMP, compared to a placebo-paired room.

In addition to conditioned preference, we also designed this study to examine conditioned drug effects during the conditioning sessions. In both animals and humans, an AMP-associated context can elicit greater conditioned responses following more drug-context pairings. For instance, if an animal receives AMP repeatedly in a particular environment, it will exhibit greater responses to AMP in than environment with each exposure (Singer et al., 2014). In humans, we showed a similar effect: during place conditioning, subjects exhibited enhanced subjective responses to AMP the second time they received the drug in a particular room relative to the first (Childs and de Wit, 2013). Here, we sought to replicate this finding.

We hypothesized that paired group subjects, but not unpaired group subjects, would report liking and preferring the AMP-paired room after conditioning, as well as spend more time in the AMP-paired room following conditioning than before. We expected that the subjects' ratings of how much they liked the drug during conditioning sessions would predict subjective ratings of room liking, room preference and the increase in time spent. Finally, we also hypothesized that the paired, but not unpaired, subjects would greater subjective responses to the AMP-paired room during the second, compared to the first, pairing.

2.3 Methods

2.3.1 Overall Design

The study used a between-subject design in which healthy young adults were randomly assigned to a paired group (n = 26) or an unpaired group (n=11). Across four conditioning sessions, subjects in the paired group received AMP (20 mg) in one room

and PL in the other room and subjects in the unpaired group received AMP and PL in each room. Participants completed six sessions conducted 2-7 days apart: first a 1.5-hour pre-test session, then four 4-hour conditioning sessions, and finally a 1-hour post-test session. During the pre-test session, they explored two testing rooms (Rooms A and B) for five minutes, and the time spent in each room was recorded. They also rated their liking and preference for the rooms using a questionnaire (*See Dependent Measures*). During the four conditioning sessions, they received AMP or PL on two sessions each, while confined in one of the two testing rooms. During the post-test session, participants completed the same measures as in the pre-test session. The primary outcome measure was the change in time subjects spent exploring Rooms A and B from pre-test to post-test. A secondary measure was the change in the paired group's ratings of how much they liked (on visual analog scales) the AMP- and placebo-paired room from pre- to post-test, and how much they preferred (on a rating scale) the AMP-paired room relative to the placebo paired room. In the paired group, we also examined the relationship between ratings of drug liking of AMP and a) the change liking of the AMP-paired room and b) the change in time spent in the AMP-paired room. Finally, we examined the change in stimulation and reports of feeling "high" in response to and "wanting more" drug in response to AMP from the first to the second AMP session during conditioning.

2.3.2 Participants

Healthy male (n = 28) and female (n = 9) adults between the ages of 18 and 34 were recruited using online advertisements and flyers. After a pre-screening phone interview, qualified participants underwent an in-person screening interview that

included a physical examination, an electrocardiogram (EKG), and a Structured Clinical Interview for Diagnostic and Statistical Manual (DSM-IV) disorders (APA, 2000). Exclusion criteria included lifetime diagnosis of a major axis I DSM disorder, current use of prescribed medication, abnormal EKG, body mass index outside 19-26 kg/m^2, age outside 18-40, less than high school education, lack of English fluency, current night shift work, and pregnancy in women. Women were also excluded if they were not using hormonal birth control, as menstrual cycle phase is known to affect response to AMP (White et al., 2002). Following the screening, qualified participants underwent an orientation session, wherein they signed a consent form that explained the study procedures. They were told that the purpose of the study was to investigate interactions between drugs, mood, and the environment. For blinding purposes, participants were told that they might receive a stimulant/appetite suppressant (e.g. caffeine, AMP), a sedative/tranquilizer (e.g. diazepam, alprazolam), or a PL (sugar pill).

2.3.3 Experimental Procedure

2.3.3.1 Testing Environment

The study was conducted using three separate rooms in a human behavioral laboratory: a neutral room and two testing rooms, labelled Rooms A and B. Drug administration took place in Rooms A and B, and all other experimental procedures were conducted in the neutral room (including informed consent, pre-session screening and baseline measures). Testing rooms A and B were the same size and located next-door to each other along a hallway. Curtains were drawn across the hallway on either side of the testing room doors to create an enclosed testing space during the room exposure tests. The two rooms were furnished with a couch, a coffee table, a side table,

an easy chair, a desk with a computer (for completing questionnaires), a television/VCR, and magazines and books. They had different wall posters (paintings vs. photographs) and accent colors (e.g. couch covers, wood finishes, table cloths, flowers), but they were matched for comfort and desirability. When participants were not completing study measures, they were allowed to relax and watch movies or read magazines or books in their assigned room.

2.3.3.2 Pre-Test Session

A 1.5-hour pre-test session was conducted at least 48 hours before the first conditioning session. Subjects were told about the study aims and informed consent was obtained. They were informed that the study would investigate interactions between drug effects and environments. They were also told that subtle differences in the environment, such as lighting, temperature, and décor can affect responses to drugs, and that they should attend to both drug effects and the environment that they were in during the conditioning sessions. Next, they were familiarized with the testing procedures (completing questionnaires and vital signs). Finally, subjects completed a 10-min Room Exploration Test (RET) to obtain an objective measure of time spent in each room. They were told that they could move back and forth between Rooms A and B as much as they liked and could spend as much time in each room as they liked for 10 minutes. The doors to the rooms were open and curtains were drawn across the hallway to create an enclosed space for exploration. Participants' movements were recorded using overhead video cameras in the rooms to determine the time spent in each room at this pre-test. For the subsequent conditioning sessions, paired group subjects were assigned to receive AMP in the room in which they spent less time during the initial RET

(*See Dependent Measures)*. After the exploration period, participants completed a Room Preference Questionnaire (RPQ) on which they rated their liking of, and relative preference for, Rooms A and B.

2.3.3.3 Conditioning Sessions

The four conditioning sessions were conducted from 9 am to 12:30 pm, 2-7 days apart. First, in a neutral room, subjects provided breath and urine samples to detect recent drug use or pregnancy (females). If they tested positive, their session was rescheduled. Then, they completed baseline mood questionnaires and, 15 minutes later, their baseline heart rate (HR) and blood pressure (BP) were recorded. Next, participants were escorted to one of the testing rooms (A or B), where they consumed capsules that contained either 20 mg AMP or PL. Subjects in the Paired Group always received AMP in the room in which they had spent less time during the pre-test RET (i.e., their initially less preferred room; *See Dependent Measures*). Usually, the amount of time subjects spent in a room corresponded with the room they rated as being preferred. On the rare occasions when participants' subjective ratings of preference on the RPQ was inconsistent with their time spent measure, the room paired with AMP was assigned randomly. Subjects in the unpaired group receive AMP and PL in both rooms on one occasion each. Subjects remained in the testing room for 3 hours. Every 30 min, they completed mood questionnaires and a research assistant recorded their HR and BP. At 12:30 pm, they completed a questionnaire rating their overall reactions to the capsules, and then they were allowed to leave the laboratory.

2.3.3.4 Post-Test Session

The post-test session was conducted 2-7 days after the last conditioning session, and was similar to the pre-test session. Participants again completed the RET and RPQ. Then they were debriefed about the aims of the study and the drug they received.

2.3.4 Dependent Measures

2.3.4.1 Demographics and Drug Use History

Demographic information and past drug use were assessed during the screening interview. Questionnaires were used to record ethnicity and race as well as current use of caffeine, nicotine, ALC, and cannabis and lifetime use of nicotine, ALC, cannabis, sedatives, stimulants, opiates, hallucinogens, ecstasy and related drugs, and inhalants.

2.3.4.2 Drug Effects

Subjective drug effects were measured using three standardized questionnaires including the Profile of Mood States (POMS; McNair et al., 1971), the Addiction Research Center Inventory (ARCI; Haertzen, 1966), and the Drug Effects Questionnaire (DEQ; Johanson and Uhlenhuth, 1980). HR and BP were measured using a digital monitor (767PV, LifeSource, Rosemont, IL).

2.3.4.3 Objective Measure of Room Preference (RET; Time Spent)

The RET provided an objective measure of room preference. We calculated the proportion of time that participants spent in Rooms A and B during the 10 minute test. Subjects in the paired group were assigned to receive AMP in the room where they spent at least 10% less time during the pre-test. If the amount of time that each subject spent

in the two rooms differed by less than 10%, the room in which they were to receive AMP was assigned randomly. To compare the paired group and unpaired group, we used the change in time spent in the initially less preferred room, from before to after the drug sessions, as the primary measure of preference in both groups.

2.3.4.4 Subjective Measures of Room Preference (RPQ)

Subjective ratings of the rooms were assessed using a paper and pencil questionnaire. It consisted of three 100 mm visual analogue scales. The first two questions asked participants to rate how much they liked "Room A" and "Room B," respectively, from "Dislike very much" (at 0 mm) to "Like very much" (at 100 mm). The third question assessed 'room preference': participants rated their *relative* preference for the rooms, from "Prefer Room A" (at 0 mm) to "Prefer Room B" (at 100 mm).

2.3.5 Drugs

AMP sulfate (four 5-mg tablets; Barr Laboratories, Pomona NY, USA) was administered in two opaque gelatin capsules (size 00) with dextrose filler. The PL capsules contained only dextrose.

2.3.6 Data Analysis

2.3.6.1 Demographic Characteristics

Demographic characteristics and drug use history were compared between the paired group and unpaired group using independent samples t-tests for continuous variables and Pearson's chi-squared analysis for categorical variables.

2.3.6.2 Overall Drug Effects

To determine the direct subjective and physiological effects of AMP, we compared area under the curve (AUC) measures of subjective and cardiovascular reactions to AMP and PL. Area under the curve was calculated relative to pre-drug baseline for AMP and PL sessions using the trapezoid method (Pruessner et al., 2003). To account for multiple comparisons within each measure, differences were deemed significant at $p = .025$.

2.3.6.3 Conditioning Measures

Subjective liking and preference for the initially less preferred room, as measured on the RPQ, and time spent in the initially less preferred room, as measured during the RET, were compared between the pre- and post-test sessions in each group using a two-factor (Group × Time) repeated measures analysis of variance (RMANOVA) for each outcome measure. Significant Group × Time interactions were further investigated using *t*-tests to compare pre- to post-test changes within each group.

2.3.6.4 Relationship between Subjective Drug Responses and Conditioning

To examine the relationship between acute drug responses and conditioning measures, a double-difference score was calculated for subjective and physiological responses. We subtracted the mean PL AUC, averaged across the two sessions, from the AMP AUC for each measure. Relationships between the subjective response to AMP and conditioning measures in the paired group, as assessed using the measures of liking and preference, were determined using Pearson correlations. Analyses were conducted

using SPSS Version 22 (SPSS Inc., Chicago, IL, USA). Differences were deemed significant at $P < .05$.

2.3.6.5 Context-Dependent Drug Effects

To examine context-dependent changes in drug effects, we compared measures of subjective and cardiovascular reactions to AMP or PL during the first and second pairs of administration sessions in the paired group and unpaired group using a three-factor Group × Drug × Pair RMANOVA.

2.4 Results

	Paired Group (n = 26)	*Unpaired Group (n=11)*
Sex (male/female)	21 / 5	7 / 4
Age	22.7 ± 4.0	22.6 ± 3.41
Body mass index (kg/m²)	22.8 ± 1.5	22.9 ± 1.8
Race (%)		
White	69	55
Black/African American	19	27
Asian	8	18
Other	4	0
Current Drug Use		
Caffeine consumption (cups/week)	10.2 ± 13	8.6 ± 8
Alcohol consumption (drinks/week)	7.1 ± 6	9.4 ± 8
Cigarette use (cigarettes/week)	1.9 ± 6	0.4 ± 1
Cannabis use (uses/month)	4 ± 7	3.6 ± 5
Past Drug Use (% ever used)		
Marijuana	77	91
Stimulants	35	9
Opiates	54	55
Tranquilizers	19	9
Hallucinogens	42	36
Club Drugs	19	18

Table 2.1: Demographic characteristics of the participants in the Paired and Unpaired Groups. Data represent N's, mean ± SEM, or percent of participants in the group.

2.4.1 Demographic Characteristics

Most participants were Caucasian, light drinkers and cannabis users in their early twenties (See Table 2.1). The paired group and unpaired group did not differ on any demographic measure.

2.4.2 Drug Effects

Participants in both the paired and unpaired groups reported feeling the prototypical subjective effects of AMP. For instance, relative to PL, AMP increased ARCI BG (Paired Group: $t(25) = 4.22$, $p < .001$; Unpaired Group: $t(10) = 4.82$, $p = .001$), DEQ "Feel high" (Paired Group: $t(25) = 3.78$, $p = .001$; Unpaired Group: $t(10) = 2.91$, $p = .015$) and DEQ "want more" (Paired Group: $t(25) = 5.51$, $p < .001$; Unpaired Group: $t(10) = 4.08$, $p = .002$). The two groups did not differ on any of these measures.

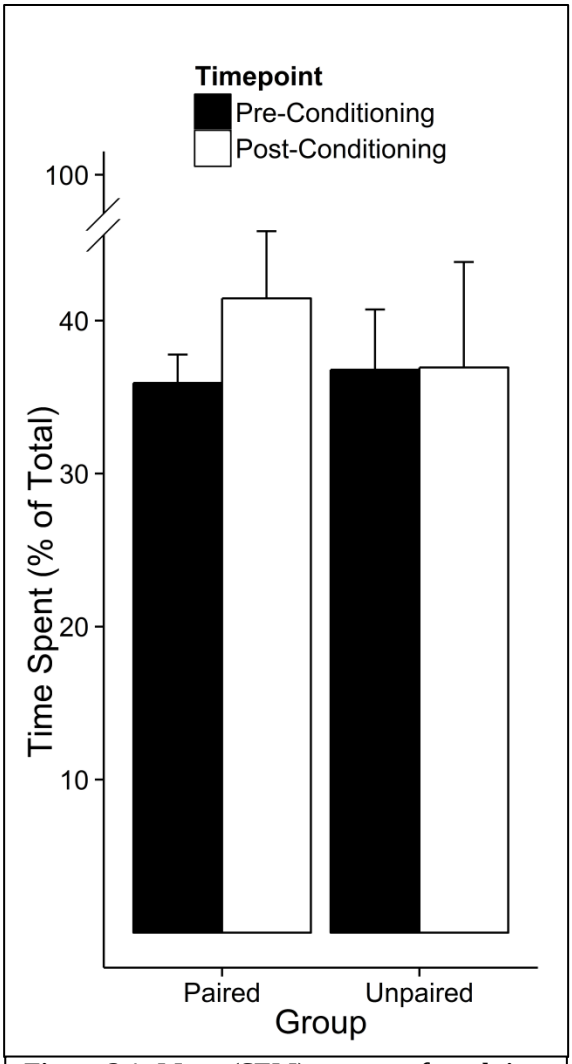

Figure 2.1: Mean (SEM) percent of total time spent in the initially less preferred room during the pre-test and post-test sessions in the PG and UG. Times were calculated as a percentage of the time spent in both rooms. Time spent did not increase significantly in either group.

AMP also produced its prototypical cardiovascular effects. It increased systolic BP (Paired Group: $t(25) = 6.86$, $p < .001$; Unpaired Group: $t(10) = 4.96$, $p = .001$). In the paired group only, the drug increased diastolic BP ($t(25) = 5.36$, $p < .001$) , and HR ($t(25) = 3.50$, $p = .002$) relative to PL as well.

2.4.3 Objective Measure of Room Preference

Overall, the amount of time participants spent in their initially less preferred room on the RET did not change from pre- to post-test, in either group ($F_{1,36} = .531$, $p =$

.471; Figure 2.1). Neither group spent a significantly different amount of time in their initially less preferred room from before to after conditioning. Also, amount of time spent in a room at either pre-test or post-test was not correlated with room liking ratings, and the change in time spent from pre- to post did not correlate with the change in self-reported liking or preference.

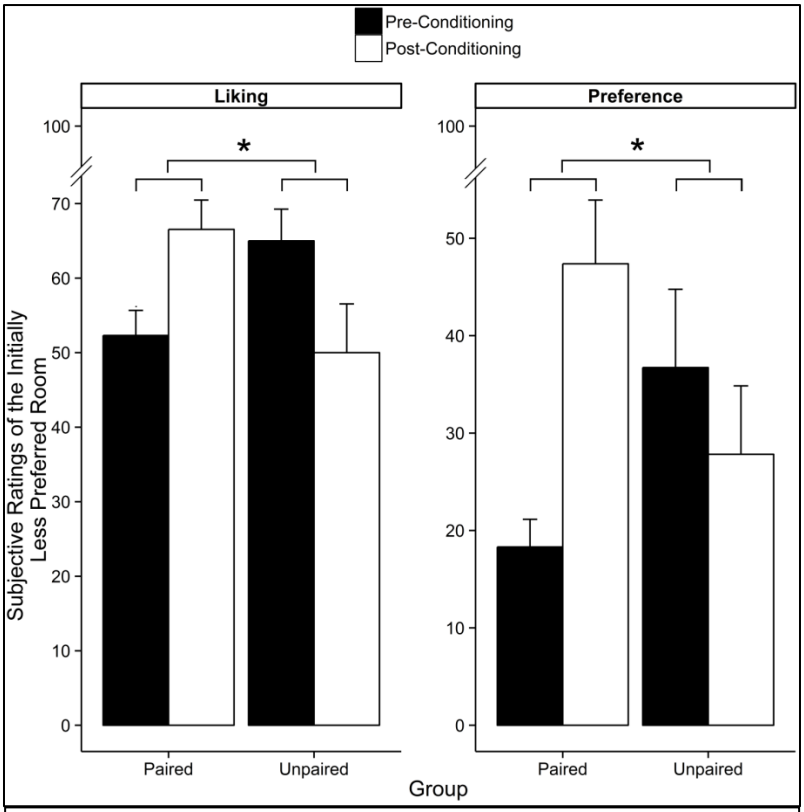

Figure 2.2: Room preference scores for paired group and unpaired group subjects before and after conditioning sessions (filled and open bars, respectively) with 20-mg AMP and PL. The Paired group always received AMP in the room that they initially preferred less, whereas the unpaired group received AMP and PL in both rooms. Bars represent mean ± SEM. Asterisks indicate a significant Group × Time interaction (left; analysis of variance (ANOVA), $P < .05$) and difference between pre- and post-conditioning scores (right; Student's paired t-test, $P < 0.05$).

2.4.4 Subjective Measures of Room Preference

The paired group rated liking and preferring the AMP-paired room more after the conditioning sessions, while the unpaired group did not (Figure 2.2, Group x Time interaction: Liking $F_{1,35} = 10.486$, $p = .003$; Preference $F_{1,35} = 14.07$, $p = .001$). The paired group reported liking the AMP-paired room (i.e., initially less preferred room) more after the conditioning sessions than before [$t(25) = 2.87$, $p = .008$], whereas subjects in the unpaired group reported liking their initially less preferred room *less* after the conditioning sessions ($t(10) = -2.44$, $p = .035$). Further, subjects' relative

subjective preference for their initially less preferred room increased in the paired group ($t(25) = 4.67$, $p < .001$), but not in the unpaired group ($t(10) = -1.84$, $p = .10$).

2.4.5 Subjective Preference and the Acute Subjective Responses to Amphetamine

In the paired group, the subjective response to AMP during conditioning predicted the change in subjective preference for the AMP-paired room. More specifically, subjects who reported higher peak ratings of DEQ drug "liking" after AMP, relative to PL, exhibited a greater increase (from pre to post-conditioning) in self-reported "preference" for the AMP-paired room (r = .40, p=.045; Figure 2.3). On the other hand, in the unpaired group, subjective responses to AMP were unrelated to a change in preference for the initially less preferred room.

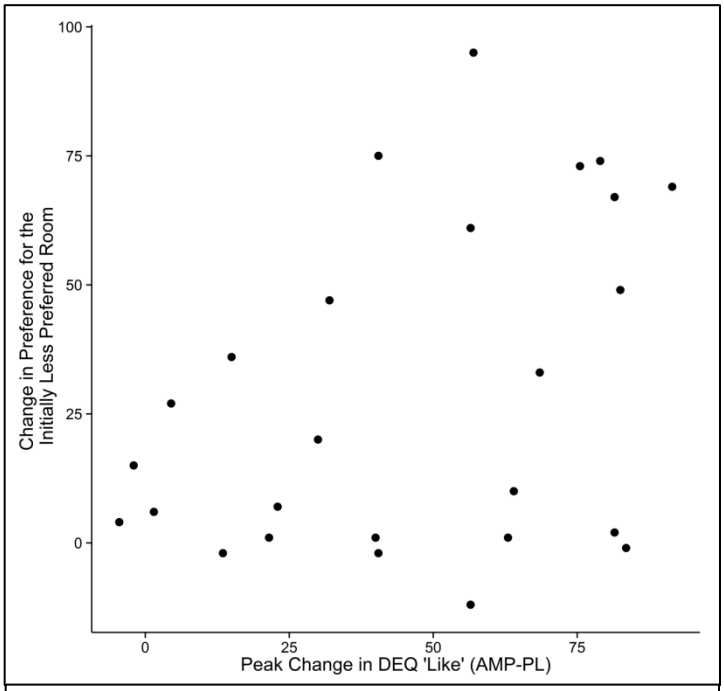

Figure 2.3: Correlation between changes in relative preference for the AMP-paired room (relative to PL-paired room) and peak drug liking ratings (AMP minus PL) in the Paired group. Drug liking significantly correlated with room preference scores. R = .405, p = .036.

2.4.6 Change in Subjective and Cardiovascular Response to AMP across Administrations in the Paired Group and Unpaired Group

Subjective response to AMP declined during the second administration of the drug, in the paired group only. Contrary to our hypothesis, the paired group reported

lower scores on 'wanting more' during the second, compared to the first, administration of AMP [Drug*Group $F_{1,35} = 7.1$, $p = .012$; Figure 2.4]. Relative to placebo, paired group subjects reported lower ratings of DEQ "want more" during the second AMP administration ($t(25) = -2.19$, $p = .038$), while unpaired group subjects tended to score higher on this scale on the second drug administration ($t(10) = 2.16$, $p = .056$). The effects of AMP on the ARCI BG and DEQ "high" scales did not change across the two pairs of sessions in either group. Cardiovascular responses to AMP (systolic and diastolic BP and HR) did not differ across the two administrations (Figure 2.5).

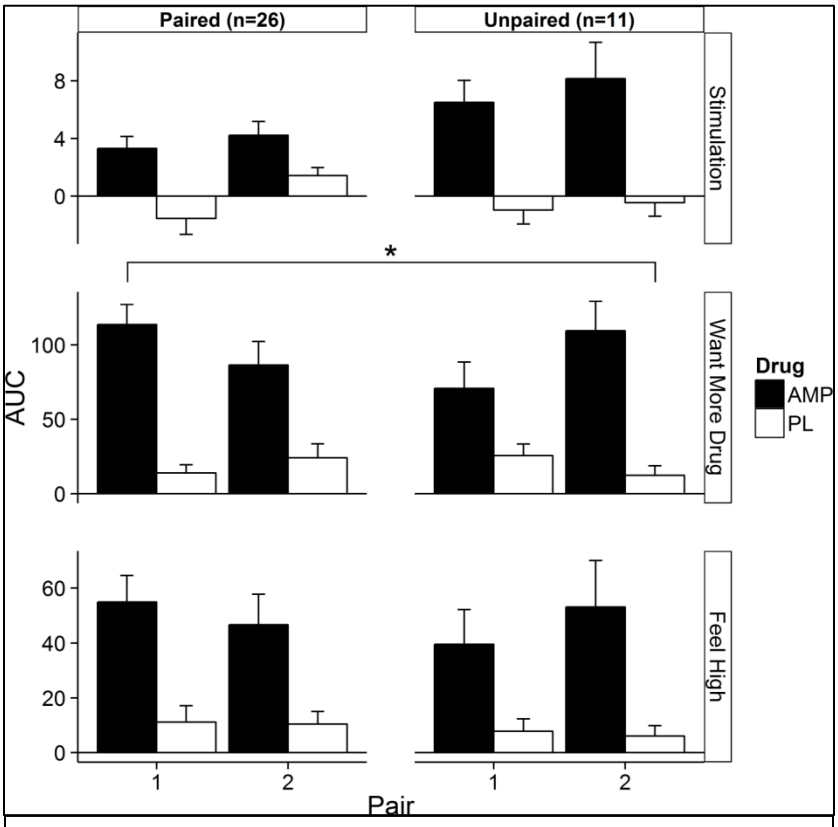

Figure 2.4: Selected subjective effects of *AMP (filled bars)* and PL (open bars), corresponding to area-under-the-curve over the three hours after capsule ingestion, on measures of Stimulation (ARCI BG), "wanting more" drug, and "feeling high" on the first and second pair of exposures to AMP or PL. Compared to PL, ratings of "want more" in response to AMP were significantly lower on the second exposure in the paired group only. Data represent mean ± SEM and asterisk indicates a significant Group × Drug × Pair interaction (*$P < 0.05$)

2.5 Discussion

In this study, we examined the acquisition of CPP for a room paired with a single dose of AMP or PL in healthy young adults, using measures of both time spent in the rooms during a free choice period, and subjective ratings of room liking. Based on our previous studies, we hypothesized that individuals would spend more time in, and develop a subjective liking and preference for, a room in which they received AMP (20 mg oral) compared to one where they received PL, and that the subjective "liking" response to AMP would correlate with "liking" of and time spent in the AMP-paired room. We also expected that subjects would report more positive subjective responses to AMP during the second administration in the paired group. Unexpectedly, we found that the amount of time that subjects spent in the AMP-paired room did not change. This contrasts with a previous study with CPP with doses of ALC, in which volunteers spent more time in the drug-paired room after conditioning (Childs and de Wit, *in prep*). However, we did find

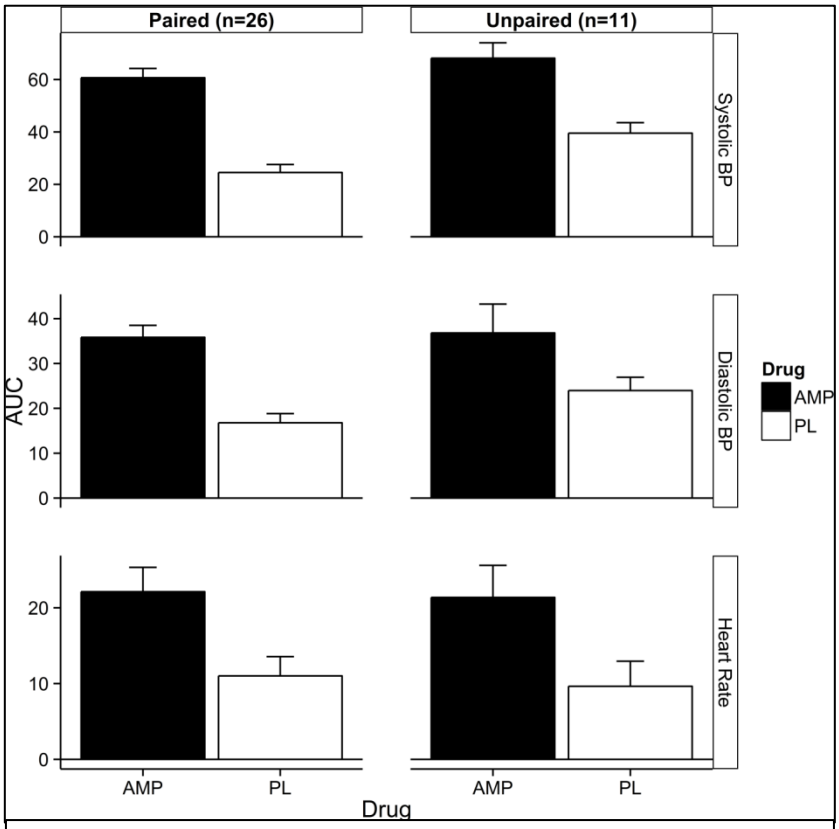

Figure 2.5: Cardiovascular effects of *AMP (filled bars)* and PL (open bars), as measured by area-under-the curve for the three hours after capsule ingestionz, on systolic and diastolic BP, and HR. No changes were found from the first pair of sessions to second pair of sessions in response to AMP and PL on any measures. Data represent mean ± SEM.

that subjects liked the AMP-paired room more than the PL-paired room, and preferred it in a paper-and-pencil preference test. Also, the degree to which paired group subjects liked the drug predicted the change in how much they preferred the drug-paired room. Finally, in contrast to one of our previous studies, the subjective response to AMP did not increase in the paired group during the second exposure to the drug in the same environment. Despite these inconsistencies, this study largely replicated our previous AMP CPP studies (Childs and de Wit, 2009, 2013).

The present results with AMP differ in two ways with our previous findings using a similar protocol. First, in our recent ALC CPP study, subjects spent more time an ALC-paired room after conditioning compared to before (Childs and De Wit, *in prep*), but we did not replicate this finding with AMP. There are several possible reasons for these different findings. First, the dose of AMP used in the present study was low, relative to the dose of ALC used by Childs and de Wit (*in prep*). On ratings of 'feel drug' and 'like drug', the 20 mg dose of AMP yielded peak effects of 43.4 and 60.6, respectively (Childs and De Wit, *in prep*), whereas the 0.8 g/kg dose of ALC resulted in rating of 53.9 and 65.0, respectively. In studies with animals (Spyraki et al., 1982; Risinger and Oakes, 1996; Brabant et al., 2005), the strength of the CPP is directly related to the dose of drug used during conditioning. Second, the two drugs differed in the time to peak effects: In this study, AMP peaked 90 minutes after ingestion of the capsule, whereas the ALC in the previous study peaked after just 30 minutes. These differences in time of onset of effects may contribute to the strength of conditioning, and the 'time spent' measure may be a less robust index of conditioning. Third, there may also be differences in the subject samples in the two studies: Childs and de Wit (*in prep*) recruited moderate drinkers, who may have been predisposed to like the effects of ALC,

whereas our current study used light drug users whose liking of AMP was unknown. It is possible that CPP with AMP might be more robust in heavier drinkers, or heavier users of other drugs. Finally, there were also minor methodological differences between the studies. In the Childs and de Wit (*in prep*) subjects remained in the conditioning rooms for just 90 min, coinciding with the peak stimulant effects of ALC, whereas in the current study, subjects remained in the AMP-paired room for 3 hours. The longer time spent in the conditioning room both before the onset of the drug's effects and during the descending limb of the effects may have weakened the conditioning. Future studies with different parameters or heavier drug users may shed light on the question of whether there are truly differences between the conditioning responses acquired with ALC and AMP.

Second, we observed a context-dependent change in the subjective response to AMP that was opposite to that described previously. In Childs and de Wit (2011), the paired group reported greater stimulation (as measured using the ARCI BG scale), "feeling high", and "wanting more" drug during the second administration compared to the first, whereas in the present study, the paired group reported *lower* ratings of "wanting more" drug during the second AMP session. The reasons for the differential outcomes are not known. There are reports of both increases ('sensitization'; e.g., (Blaser et al., 2010; Eisener-Dorman et al., 2011; Singer et al., 2014) and decreases ('tolerance') in responses to stimulant drugs with repeated administration (Leith and Barrett, 1976; Krebs and Anderson, 2015). The conditions under which sensitization or tolerance develop are not fully understood (Schenk and Partridge, 1997; Zernig et al., 2007). Although the procedures used across studies were very similar, it is possible that random unidentified differences in the subject samples or subtle differences in the

testing conditions could have influenced the results. Thus, we conclude that the failure to replicate the enhanced response to AMP suggests that context-dependent changes in drug effects are subtle and variable across conditions. Further research will determine the nature of the context-dependent enhancement of drug effects, and what contextual variables control this effect.

In the present study, neither subjective ratings of room liking or preference were related to the amount of time spent in the AMP-paired room before conditioning, and the change in these measures from before to after conditioning were not correlated. This calls into question whether humans spend time in an environment because they "like" that environment, or for other reasons. Even in studies with laboratory animals, it has been suggested that an animal spends time in a reward-paired chamber not because it 'likes' the effects of the drug per se, but because that chamber predicts the ability to acquire or experience the associated reward (Spiteri et al., 2000). This motivational response may be biologically discrete from the affective response that produces self-reports of room "liking" in humans (Stephens et al., 2010). Similarly, several theories of drug addiction suggest that drug conditioning leads to separate and distinct responses of hedonic "liking" and biologically-motivated "wanting" for the drug (Robinson and Berridge, 1993; Koob and Le Moal, 2008). The fact that we found a positive association between subjective drug liking and subjective room preference, but not objective room preference, lends support to the hypothesis that drug liking can lead to a positive affective response to a drug-paired environment separate from the tendency to spend time in the environment. Nevertheless, the apparent discrepancy between the findings with laboratory animals and humans remains to be resolved.

2.5.1 Limitations

This study had several limitations, most notably the use of only a single dose of AMP, and aspects of the subject sample. It is possible that more robust conditioned effects would be observed with higher doses of AMP, or AMP administered by a different route. It would also be valuable to determine whether the place preference procedure yields a dose-dependent effect (e.g., higher preference with higher doses), or whether the preference is an all-or-nothing response. The sample was relatively small and homogeneous. A larger sample might reveal stronger place preferences, and inclusion of a broader range of individuals, including those with heavier drug use history or some psychiatric symptomatology, might yield different results. We and others have shown that there are marked individual differences in responses to stimulant drugs (Chait, 1993; Holdstock and de Wit, 2001; Abi-Dargham et al., 2003; Kirkpatrick et al., 2013; Kirkpatrick et al., 2015; Yarosh et al., 2015), and our sample was relatively homogeneous with regard to age, race, light drug use history and minimal psychiatric symptomatology. More robust conditioned effects might develop in other samples e.g. heavier drug users, or with more pairings.

2.5.2 Summary

Overall, the purpose of this study was to replicate and extend our previous findings that individuals come to like and prefer places where they experienced pleasurable drug effects. As in our previous studies (Childs and de Wit, 2009, 2013), subjects liked the AMP-paired room more after conditioning, and subjects who liked AMP the most also preferred the drug-paired room most after conditioning, supporting the idea that the conditioned preference ratings provide an index of drug reward. However, subjects did

not exhibit an increase in time spent in the AMP-paired room, contrasting our previous work with ALC CPP (Childs and De Wit, *in prep*).

2.5.3 Conclusion

Adapting the CPP protocol to humans allows us to investigate questions and assumptions that arise from the CPP procedure in animals. This will not only improve our understanding of the validity of the procedure in animals, but it also serves to bridge the CPP procedure with the phenomenon of contextual cue conditioning in human drug users. In preclinical studies of CPP, rewarding drug effects are inferred from the time spent measure, and no information can be obtained about interoceptive drug effects (Bardo and Bevins, 2000). Human subjects provides the opportunity to study the role of subjective drug effects on conditioning processes and establishment of a CPP, as well as other behavioral and cognitive factors that contribute to the acquisition and expression of CPP. By establishing the "time spent" measure in humans, we can bridge the gap between the objective findings in animals and subjective reports that we collect from humans. Ultimately, using human subjects better equips us to model context–induced drug craving and relapse using the CPP paradigm. By learning more about the mechanisms that underlie CPP, and by refining the human CPP paradigm, we can develop more targeted methods for reducing the influence of a drug-associated context on drug-taking.

Chapter 3: Individual Differences in the Acquisition of Amphetamine Conditioned Place Preference

3.1 Summary

Individuals demonstrate significant variability in their acquisition of AMP CPP; yet the cause of this variability is unknown. Since CPP models drug reward, it is likely that individual differences in the acquisition of AMP CPP predict the abuse liability of AMP in individuals. Therefore, it is integral to elucidate the individual traits that influence the acquisition of contextual conditioning. One potential factor that may contribute to the acquisition of AMP CPP is the positive subjective response to AMP. Another factor that may influence conditioning is personality. Emotional personality traits are associated with the positive subjective effects of drugs, including AMP, so it is possible that the traits Positive Emotionality (PEM) and Negative Emotionality (NEM) may predict AMP CPP, either independently or in combination with the positive subjective effects of AMP. For this analysis, we combined data from two similar AMP CPP studies, and examined individual differences in the acquisition of conditioning. Each subject in this dataset underwent the same CPP protocol: subjects received 20mg AMP in one room twice and PL in another room twice. The subjects always received the AMP in the room they initially preferred less. The change in preference for the AMP-paired room was assessed as an indicator of CPP acquisition. Subsequently, we

examined the data for predictors of this change in preference. Using hierarchical multiple regression (HMR) analysis, we examined the role of AMP-induced euphoria, PEM, and NEM, in the acquisition of conditioning. We first hypothesized that all three of these measures would independently predict conditioning. Second, we hypothesized that the personality traits would moderate the relationship between AMP-induced euphoria and conditioning. That is, we tested whether AMP-induced euphoria would only predict AMP CPP among individuals who exhibited high levels of either PEM or NEM. Similar to previous studies, we found that AMP-induced euphoria predicted CPP. We also found that PEM independently predicted CPP, but NEM did not. Finally, we found that neither PEM nor NEM predicted AMP CPP in combination with AMP-induced euphoria. This analysis confirmed previous reports that the positive subjective effects of AMP predict CPP, and it was the first analysis to directly demonstrate the predictive value of personality in human CPP.

3.2 Introduction

Our results from Study 1, in agreement with past CPP studies in humans, indicate that there is substantial individual variability in the acquisition of the conditioned preference. However, the sources of this variability have not been identified. One possible determinant of individual differences in place preference is the extent to which the individual experiences positive subjective effects for the conditioning drug (US) during the conditioning sessions. Another possible determinant is the personality of the individual, which may influence acquisition either by affecting the subjective drug response, or by affecting conditioning independently of the subjective response to the

drug. Alternatively, individual differences in place conditioning may be related to demographic characteristics such as age, sex or drug use history. Here, using data collected from two AMP CPP studies in humans, we sought to determine a) if individual differences in place conditioning were related to individual differences in the positive subjective effects of AMP, b) if individual differences in place conditioning were related to scores on the PEM and NEM scales on the Multidimensional Personality Questionnaire (MPQ; Patrick et al., 2002) and, c) if the stimulant response to AMP mediated the relationship between our personality measures and conditioning, and d) if PEM and NEM moderated the relationship between the subjective effects of AMP and the level of place conditioning.

We have some evidence that place preference in humans is related to subjective drug effects experienced by the individual. More specifically, individual differences in the subjective response to AMP are related to the degree of preference in the CPP procedure (Childs and de Wit, 2009, 2013). In one study, greater AMP liking and lower AMP-induced anxiety predicted greater liking of an AMP-paired environment (Childs and de Wit, 2009). In another study, AMP liking predicted the *increase* in liking of and relative preference for an AMP-paired room after conditioning (Childs and de Wit, 2013). These studies demonstrate that positive responses to drugs are related to the strength of CPP expression in humans. Additionally, it is also possible that trait variables, such as personality, influence either subjective response to AMP or CPP.

PEM and NEM represent the predisposition to experiencing positive and negative emotions, respectively, in response to stimuli (Patrick et al., 2002), and there is some evidence that these traits, as well as other similar traits, predict subjective responses to AMP. For instance, an individual's propensity to show excitement over engaging in

pleasurable experiences, as measured using the Temporal Experience of Pleasure Scale (TEPS; Gard et al., 2006), predicts greater AMP-induced positive mood (Kirkpatrick et al., 2015). Similarly, Sensation Seeking, as measured using the Zuckerman Kuhlman Personality Questionnaire (ZKPQ; Zuckerman et al., 1991), which is defined as a preference for novel, complex, risky, and emotionally intense experiences, is also related to the positive subjective effects of AMP (Kelly et al., 2006; Stoops et al., 2007). The related trait Novelty Seeking (Zuckerman, 1994), as measured using the ZKPQ, predicts AMP-induced stimulation (Hutchison et al., 1999), and trait reward sensitivity, as measured with the Social Potency scale of the MPQ, predicts AMP-induced euphoria (Kirkpatrick et al., 2013). Surprisingly, individuals who display more negative affect and more anxiety-related general distress, as measured using the Mood and Anxiety Symptom Questionnaire Short Form (Watson et al., 1995) also report more positive mood in response to AMP (Kirkpatrick et al., 2015). Finally, there is also evidence that individuals with a DSM diagnosis of Major Depression (MDD; First, 1994) report more euphoria in response to single doses of AMP, relative to healthy individuals (Tremblay et al., 2002; Tremblay et al., 2005). Thus, several personality traits, both positive and negative, predict the response to AMP. To the extent that a positive subjective response to AMP predicts AMP CPP, this raises the possibility that the positive subjective response to AMP mediates the relationship between personality and conditioning, and that personality moderates the relationship between subjective response to AMP and the strength of conditioning in the AMP CPP paradigm.

Moderators and mediators differ slightly in their role in causal relationships. A mediator explains the relationship between an independent and dependent variable; in other words, a mediator must be present for the relationship between these two

variables to exist (Baron and Kenny, 1986). A moderator, on the other hand, affects the strength or direction of a relationship, but it does not explain the cause of this relationship, and its presence is not necessary for this relationship to exist (Baron and Kenny, 1986). Given the difference between these two effects, we choose to test for both in this study.

In the present analysis, we combined the data from two human place conditioning studies with AMP (Childs and de Wit, 2013; Study 1), to identify variables that predict stronger CPP. A novel question addressed here was whether personality independently predicts AMP CPP, whether the positive subjective effects of AMP mediate the relationship between personality and conditioning, and whether the personality traits PEM and NEM might moderate the relationship between the subjective response to AMP and CPP expression. In view of the evidence that similar personality traits are related to positive responses to stimulant drugs (Kirkpatrick et al., 2013; Kirkpatrick et al., 2015), and that the positive subjective response to AMP predicts CPP (Childs and de Wit, 2009, 2013; Study 1), we predicted 1) that AMP-induced euphoria, as well as trait PEM and NEM, would independently predict the expression of CPP in the paired group, 2) that AMP-induced euphoria would mediate the relationships between PEM and NEM and CPP, and 3) that AMP-induced euphoria would predict CPP only in individuals who exhibit high PEM or NEM.

3.3 Methods

3.3.1 Participants

This post-hoc analysis was conducted using data from two studies that used the same eligibility criteria and screening (Chapter 2 and (Childs and de Wit, 2013). For these analyses we used only data from subjects assigned to the Paired Groups. The demographic information for the two subject samples are presented in Table 3.1, and the groups did not differ on any measured variables. The final sample included 53 male and 18 female volunteers.

	Study 1	Childs and de Wit (2013)
N	*26*	*19*
Sex (male/female)	21 / 5	13/6
Age	22.7 ± 4.0	23.6 ± 0.9
Body mass index (kg/m²)	22.8 ± 1.5	22.4 ± 0.3
Race (%)		
White	69	53
Black/African American	19	0
Asian	8	16
Other	4	32
Current Drug Use		
Caffeine consumption (cups/week)	10.2 ± 13	5.0 ± 0.9
Alcohol consumption (drinks/week)	7.1 ± 6	5.8 ± 1.1
Cigarette use (cigarettes/week)	1.9 ± 6	6.6 ± 2.4
Cannabis use (uses/month)	4 ± 7	4.8 ± 1.7
Past Drug Use (% ever used)		
Cannabis	77	26
Stimulants	35	26
Opiates	54	21
Tranquilizers	19	0
Hallucinogens	42	21

Table 3.1. Demographic characteristics of the participants in the two datasets analyzed in this study. Data represent N's, mean ± SEM, or percent of participants in each dataset.

3.3.2 Overall Design

The purpose of this study was to determine whether individual differences in either personality or subjective responses to AMP predict conditioned room preference

in the human CPP paradigm, and whether personality moderates the relationship between the subjective effects of AMP and the degree of conditioning. Here, we combined datasets from two studies that used a similar study protocol (Childs and de Wit (2013) and Study 1). All subjects underwent four conditioning sessions, wherein they received AMP (20 mg) in one room and PL in another. Both studies used a biased design: subjects always received AMP in their initially less preferred room. During the conditioning sessions, mood and cardiovascular measures were taken at regular intervals. Before and after the conditioning sessions, subjects explored the two conditioning rooms and rated on a questionnaire how much they liked each room and how much they preferred one room over the other. Conditioning was measured by the change in subjective preference for the AMP-paired room. After all sessions were complete, subjects were debriefed about the study procedures and the purpose of the study.

3.3.3 Study Procedures

First, subjects attended a 30-minute orientation, as described in Chapter 2. Subjects were allowed to explore the two conditioning rooms, and then they reported their liking of and preference for them. In Study 1, we also measured how much time individuals spent in each room during the room exploration test, and this objective "time spent" measure was used to gauge initial preferences; that is, Paired Group subjects in Study 1 were assigned to receive AMP in the room they spent less time in initially. In Childs and de Wit (2013), the amount of time spent in the rooms was not assessed, but instead, rating of room preference was used as the measure of initial preference. Thus, subjects in the Paired Group in Childs and de Wit (2013) received

AMP in the room they reported preferring less. To resolve this difference between the two studies, we removed one subject in Study 1 who reported preferring the room he spent less time in during the pre-conditioning test (that is, his subjective and objective preferences contradicted each other) from further analysis. Therefore, for the purposes of this analysis, all paired group subjects ultimately received AMP in the room they initially reported preferring less.

At least 48 hours after subjects finished the orientation session, they underwent four conditioning sessions followed by a testing session (See Chapter 2). During the testing session, subjects rated their liking of and preferences for the two rooms. Then, they were debriefed and paid.

3.3.4 Drugs

d-AMP sulfate (four 5-mg tablets; Mallinkrodt, Hazelwood, MO, USA) were placed in two red, opaque gelatin capsules (size 00) with dextrose filler. PL capsules were identical to the AMP capsules, but contained only dextrose.

3.3.5 Measures

3.3.5.1 Personality Measures

During the initial screening session participants completed a computerized version of the MPQ (Patrick et al., 2002). The MPQ consists of 155 true/false items, grouped into 11 primary scales (Well-being, Social Potency, Achievement, Social Closeness, Stress Reaction, Alienation, Aggression, Control, Harm Avoidance, Traditionalism, and Absorption). These trait scales are grouped into three superfactors (PEM, NEM, and Constraint). For this analysis, we focused on PEM and NEM because

these traits have been associated with the subjective effects of AMP (Kirkpatrick et al. 2013; Kirkpatrick et al. 2015), and in animals, behavioral indices of these traits, such as anxiety-like behavior and defecation, have been associated with the degree of conditioning in CPP (Nadal et al. 1992; Klebaur and Bardo 1999).

3.3.5.2 Subjective Drug Effects

Mood questionnaires were administered during the conditioning sessions, at baseline, and every 30 minutes for three hours after capsule administration. Subjects completed the POMS (McNair et al. 1971) and the DEQ (Johanson and Uhlenhuth 1980).

3.3.5.3 Subjective Measures of Room Preference (RPQ)

Subjective ratings of room preference were assessed using a paper and pencil questionnaire, as described in Chapter 2.

3.3.6 Data Analysis

3.3.6.1 Overall Data Analysis Strategy

We determined the relationship between the Paired Group's responses on each of our variables with Pearson correlations. Further, to determine the relationship among the subjective effects of AMP, trait personality measures, and the degree of conditioning in the CPP paradigm, we used methods derived from a recently published analysis (Kirkpatrick et al. 2013). Because the subjective effects of AMP in the present analysis were similar to those in a previous analysis (Kirkpatrick et al. 2013), we used the same factors derived in the previous study to create summary measures of the subjective

responses to the drug. Next, to determine whether AMP-induced euphoria or PEM or NEM predicted conditioning, we entered these variables into HMRs. Each analysis is detailed below.

3.3.6.2 Demographic Characteristics

Demographics and drug use history were compared between the Paired Groups in Study 1 and Childs and de Wit (2013). Categorical variables were compared using chi-squared tests and continuous variables were compared using independent-samples t-tests.

3.3.6.3 Acute AMP-Related Subjective Effects

The acute subjective effects of AMP were calculated using the methods employed in Study 1 (See Chapter 2).

3.3.6.4 Reduction of Subjective Effects Measures

To reduce the subjective effects data into factors appropriate for using as predictor measures of conditioning, we combined the subjective effects scales according to a scheme established using a principal components analysis in a previous study (Kirkpatrick et al. 2013). We reduced our subjective effects variables to three components: euphoria, arousal, and dysphoria. The individual items that were assigned to a particular scale were summed to generate a score on that scale.

3.3.6.5 Relationship among Subjective AMP Effects, Personality, and Conditioning

We determined the relationship between the Paired Group's responses on each of our variables with Pearson correlations. Further, to determine whether personality moderated the relationship between the subjective effects of AMP and conditioning in the paired group, we entered PEM, NEM, and AMP-induced euphoria into two HMRs: one using PEM as the moderator variable and one using NEM as the moderator variable. The personality measures were put in block one. In block two, we included AMP-induced euphoria. In block three, we included interaction variables, including the interactions between AMP-induced euphoria and PEM, and the interaction between AMP-induced euphoria and NEM. These interaction variables were constructed by multiplying the values for AMP-induced euphoria with the values for each personality variable, respectively.

3.4 Results

3.4.1 Descriptive Statistics

Means, standard deviations, and bivariate correlation coefficients for all of the variables are presented in Table 3.2. There were no instances of multicollinearity among the variables (all tolerance values ≥ 0.948 and all variance inflation factor values ≤ 1.055).

3.4.2 Mediation Analysis

We were initially interested in whether AMP-induced euphoria mediated the relationship between personality and conditioning. For a mediation to be valid,

however, all three tested variables (the independent variable, the dependent variable, and the mediator) must be correlated with each other (Baron and Kenny, 1986). In this study, though, PEM was not significantly correlated with AMP-induced Euphoria, and NEM was not significantly correlated with either AMP-induced Euphoria or the change in subjective room preference (Table 3.2). Therefore, any mediational analysis that we could perform would be invalid.

	M	**SD**	**1**	**2**	**3**	**4**
1. Euphoria	14648.87	8375.86	1	-.007	.105	.328*
2. PEM	74.24	14.69		1	.013	.444**
3. NEM	25.51	8.58			1	-.011
4. Change in Room Preference	29.84	30.70				1

Table 3.2. Means, standard deviations, and bivariate correlation coefficients of the variables. The "euphoria" measure is the peak change from baseline in response to AMP, averaged across the two AMP sessions. This measure is constructed using the same components as the "Euphoria" measure in Kirkpatrick et al. (2013). *p<.05 **p <.01.

3.4.3 Regression Analyses

Our hypotheses that PEM and AMP-induced euphoria independently predicted CPP strength, and that PEM and moderated the relationship between AMP-induced euphoria and place conditioning, were tested using HMRs. The model incorporated

Predictor	**ΔR^2**	***p***	**Model *F (df)***	***Model p***	**Standardized β**
Block 1. PEM	.197	.002	10.536 (1,43)	.002	.444
Block 2. Euphoria	.110	.013	9.291 (2,42)	.000	.332
Block 3. PEM × euphoria	.002	.756	6.094 (3,41)	.002	.041
Final R^2	.308				

Table 3.3. HMR with PEM and euphoria on the change in preference for the AMP paired room from before to after conditioning.

PEM in block 1, the subjective drug response variable, AMP-induced euphoria, in block 2, and the interaction term, PEM x euphoria, in block 3.

Both independent variables were mean centered. Overall, the model accounted for 30.8% of the total variance in the change in subjective preference for the AMP-paired room (Table 2). Greater PEM accounted for a significant level of variance in the change in preference for the AMP-paired room; greater PEM significantly predicted a greater increase in AMP room preference ($\Delta R^2 = .197$, $p = .002$). Adding AMP-induced euphoria significantly increased the level of variance accounted for in the change in

AMP room preference ($\Delta R^2 = .110$, $p = .013$). However, adding the interaction term (PEM x Euphoria) did not increase the amount of variance explained in the change in AMP room preference. The contribution of each variable to the model is shown in Table 3.3.

Predictor	**ΔR^2**	***p***	**Model *F (df)***	***Model p***	**Standardized β**
Block 1. NEM	.000	.942	0.005 (1,43)	.942	-.011
Block 2. Euphoria	.110	.028	2.595 (1,42)	.087	.333
Block 3. NEM × euphoria	.002	.739	1.731 (3,41)	.176	.051
Final R^2	.112				

Table 3.4. HMR with NEM and Euphoria on the change in preference for the AMP paired room from before to after conditioning.

Our hypotheses that NEM, along with AMP-induced euphoria, independently predicted CPP strength, and that NEM and moderated the relationship between AMP-induced euphoria and conditioning, were tested using the same method as described above, replacing PEM with NEM.

Overall, the model accounted for 12.2% of the total variance in the change in subjective preference for the AMP-paired room (Table 2). Greater NEM did not account for a significant level of variance in the change in preference for the AMP-paired room. But, as expected, adding AMP-induced euphoria did significantly increase the level of variance accounted for in the change in AMP room preference ($\Delta R^2 = .110$, $p = .028$). Adding the interaction term (NEM x Euphoria) did not increase the amount of variance explained in the change in AMP room preference. The contribution of each variable to the model is shown in Table 3.4.

3.5 Discussion

The positive subjective effects of drugs are risk factors for addiction, and emotional personality traits like PEM and NEM may contribute to the development of addiction as well. PEM and NEM may affect the liability to addiction by influencing the subjective response to drugs; therefore, it is possible that these personality traits and the subjective response to AMP may interact to produce individual differences in AMP CPP.

In this analysis, we sought to determine the individual contributions of PEM, NEM, and AMP-induced euphoria to the strength of AMP CPP, as well as whether personality moderates the role of the subjective effects of AMP in eliciting an AMP CPP in humans. We hypothesized that PEM and NEM, as measured on the MPQ, and AMP-induced euphoria, as measured using empirically derived factors from the POMS, ARCI, and DEQ, would independently predict AMP CPP in humans, as measured by an increase in subjective preference for an AMP-paired room. Also, given evidence that PEM and NEM both relate to the positive subjective effects of AMP and CPP

(Kirkpatrick et al. 2013; Kirkpatrick et al. 2015), we hypothesized that PEM and NEM would both moderate the relationship between AMP-induced euphoria and CPP. In agreement with previous studies, we found that AMP-induced euphoria predicted CPP in our sample. Also, PEM independently predicted CPP, but NEM did not. Finally, neither PEM nor NEM moderated the relationship between AMP-induced euphoria and CPP. It is notable that PEM did not correlate with the level of AMP-induced euphoria, as this disagrees with past findings (Kirkpatrick et al. 2013; Kirkpatrick et al. 2015). This may explain why PEM did not moderate the relationship between the AMP-induced euphoria and CPP, despite that both PEM and AMP-induced euphoria predicted CPP.

3.5.1 AMP-induced Euphoria and CPP

Our finding that AMP-induced euphoria predicts AMP CPP confirms previous findings, and also provides evidence for the sensitivity of our measures of AMP-induced euphoria. In two previous analyses (Childs and de Wit 2011; Study 1), we found that preference for the AMP-paired room was related to AMP-induced euphoria using a composite measure of 'euphoria' that included ratings of drug liking. In the present analysis, we observed a similar relationship between AMP-induced euphoria and preference for the room, even though we excluded the measure of 'drug liking' from the calculation of AMP-induced euphoria. Thus, the measure of AMP-induced euphoria was related to place preference whether or not it included the ratings of drug liking.

3.5.2 PEM and CPP

PEM predicts CPP, but PEM did not moderate the relationship between AMP-induced euphoria and conditioning. We expected PEM to predict CPP because the positive subjective effects of AMP predict conditioning (Childs and de Wit 2009; Childs and de Wit 2011), and personality traits related to PEM predict AMP-induced euphoria. For example, lower negative affect predicts greater AMP-induced euphoria (Kirkpatrick et al. 2015). Also, as mentioned previously, Sensation Seeking, Novelty Seeking, and reward sensitivity all predict the positive subjective effects of AMP as well (Kelly et al. 2006; Stoops et al. 2007; Hutchinson et al. 1999; Kirkpatrick et al. 2013). In this study, however, AMP-induced euphoria was not related to PEM, which may explain why PEM did not moderate the relationship between AMP-induced euphoria and CPP. Our results suggest that PEM influences AMP CPP via a mechanism unrelated to the drug's effect on euphoria.

3.5.3 NEM and CPP

Unlike PEM, NEM did not predict CPP. This is surprising, as 1) negative emotional traits are associated with the positive response to AMP in humans, 2) chronic stress and anxiety-like behaviors predict CPP in animals, and 3) NEM predicts drug use in humans. First, individuals with both mild and severe levels of distress show enhanced subjective responses to AMP. For instance, Anxiety-Related General Distress in healthy individuals predicts greater AMP-induced euphoria (Kirkpatrick et al. 2015). Also, individuals with MDD exhibit enhanced subjective responses to AMP (Tremblay et al. 2002; Tremblay et al. 2005). Second, chronic stress, NEM, and anxiety-like behavior predict CPP in animals. For instance, negative emotionality in rats

(as measured by increased defecation) predicts ALC CPP (Nadal et al. 1992). Also, chronic stress-induced anxiety-like behavior predicts both ALC and nicotine CPP (Bahi 2013; Falco et al. 2014). Third, negative emotionality predicts problematic drug use overall. For instance, NEM during childhood predicts general problematic drug use in adulthood (Oliva et al. 2012). Also, NEM during late adolescence predicts DSM-diagnosed substance dependence (Krueger 1999). Additionally, NEM predicts greater ALC dependence during adolescence (Hicks et al. 2012), and when brought on by emotional abuse, NEM predicts greater problematic ALC use during adulthood (Mezquita et al. 2014). Given a) that the CPP is thought to reflect the abuse potential of drugs, b) that there is a strong link between NEM and the subjective response to drugs and problematic drug use, it is surprising that we did not observe a relationship between NEM and CPP. NEM did not predict CPP, yet this does not mean that it could not moderate the relationship between the subjective effects of AMP and CPP.

3.5.4 PEM and NEM as Moderators of the Relationship between AMP-induced Euphoria and CPP

While AMP-induced euphoria predicted CPP, neither PEM nor NEM moderated this relationship. This shows that personality and the subjective response to AMP uniquely contribute to AMP CPP. Our findings were unexpected, yet informative. That is, given that traits related PEM and NEM both predict the subjective response to AMP (e.g. Kirkpatrick et al. 2015), we believed that the subjective effects of AMP would predict AMP CPP only in combination with high levels of PEM or NEM. However, we found that PEM and AMP-induced euphoria are not related in our sample, and are possibly independent predictors of conditioning. Alternatively, it possible that either a)

the presence of other traits associated with AMP-induced euphoria uniquely moderate the relationship between the subjective effects of AMP and CPP, or b) PEM or NEM moderate this relationship in combination with other traits. At this point, we cannot tell whether PEM or NEM are necessary or sufficient for moderating the relationship between the subjective effects of AMP and AMP CPP.

3.5.5 AMP-Induced Euphoria and PEM

In two past studies, positively valenced personality traits predicted AMP-induced euphoria (Kirkpatrick et al. 2013; Kirkpatrick et al. 2015), yet in this study, this relationship was not found. These conflicting results may relate to differences in how positive personality traits were measured among these studies. More specifically, Kirkpatrick et al. (2013; 2015) did not test the influence of PEM directly. Instead, in their studies, reward sensitivity, Anticipatory Pleasure, and lower Negative Affect the predicted AMP-induced euphoria (Kirkpatrick et al. 2013; Kirkpatrick et al. 2015), and these traits may not be related to PEM. For instance, just as we found that PEM and NEM did not correlate in our sample, PEM and Negative Affect are not necessarily opposites. That is, just because someone displays low Negative Affect does not automatically mean that they display high PEM. Next, the PEM scale of the MPQ and the Anticipatory Pleasure scale of the TEPS measure anticipatory and consummatory pleasure, respectively (Patrick et al. Gard et al. 2006), and it has been argued that these two dispositions are supported by distinct personality traits (Klein et al. 1984). Finally, the Social Potency scale, which is the index of reward sensitivity used in Kirkpatrick et al. (2013) is a component of the PEM scale of the MPQ, but the PEM also includes several other components unrelated to reward sensitivity. Overall, although several

positive personality traits relate to the euphorigenic effects of AMP, this does not necessarily mean that PEM itself predicts AMP-induced euphoria.

3.5.6 Limitations

This analysis had several limitations. Our first limitation was our use of a fairly small subject sample. While subjects with similar demographic characteristics have been used to elucidate the relationship between personality and the subjective effect of AMP in the past, those studies used much larger samples (Kirkpatrick et al. 2013; Kirkpatrick et al. 2015). Furthermore, analyzing a sample for moderation effects usually results in even smaller effect sizes (Agunis et al. 2005); therefore, it is likely that we needed a much larger sample to produce significant interaction effects. Our first regression, which included PEM, had a moderate effect size, but this was likely due to the strong relationship between PEM and CPP. On the other hand, our second regression, which included NEM instead, had a very small effect size, and to find a significant result from this regression, we would have to almost triple our sample size to 121 subjects. This potential sample size, in fact, matches those of recent studies that have employed similar analyses (Allen and Gabbay 2013; Roselyn et al. 2015). Therefore, it is likely that our study was underpowered to detect moderator effects of NEM in the relationship between AMP-induced euphoria and conditioning

Our second limitation was the homogeneity of our subject sample. For this analysis, we used data from two studies that used the same eligibility criteria, including a lack of lifetime mental health disorders or SUDs. Hence, this analysis only addresses the susceptibility to conditioning in healthy individuals who may have a low potential to

develop a substance abuse disorder. By limiting our sample to healthy subjects, we may have limited the external validity of our results.

Another limitation was that we only used a single dose of AMP in the supporting studies. It is possible that we could have found a relationship between NEM and CPP if we had used a larger dose of AMP. To understand the relationship between NEM and CPP, it is necessary to perform CPP experiments with multiple doses.

Finally, for this analysis, we used data from two separate AMP CPP studies, performed at two different times by two different individuals, using subjects who were screened by different personnel. While we held the AMP dose and other parameters of the two studies constant, the two datasets could have varied from each other because of confounds that we did not immediately recognize. This could have increased the variance in our subject sample, which would have reduced the power of our regression analysis.

3.5.7 Summary and Conclusion

In summary, we analyzed data from two AMP CPP studies to determine whether PEM, NEM, or AMP-induced euphoria predict the change in preference for an AMP-paired room following contextual conditioning in a human CPP paradigm. We also examined whether PEM or NEM moderated the relationship between AMP-induced euphoria and the change in preference for an AMP-paired room. As expected, we found that AMP-induced euphoria, as well as PEM, predicted AMP CPP. However, NEM did not predict CPP, and neither personality measure moderated the relationship between AMP-induced euphoria and CPP.

While largely confirming past studies examining the relationship between AMP's subjective effects and CPP, this is the first study to show that PEM predicts CPP in humans. These results call for further research into the role of POM and NEM in CPP. While the role of personality in the subjective response to drugs is well understood (e.g. Kirkpatrick et al. 2013; Kirkpatrick et al. 2015; Stoops et al. 2007; Kelley et al. 2006), the role of emotionality in drug addiction is poorly understood. Our novel finding that PEM predicts CPP provides a new avenue through which we can predict individual differences in CPP acquisition.

Chapter 4: Extension of the Amphetamine Conditioned Place Preference Paradigm in Humans

4.1 Summary

Environments associated with previous drug use are known to elicit drug-related responses, including changes in mood, behavior, and physiology that often lead to drug seeking. While researchers have successfully modeled the acquisition and expression of responses to drug-related environments in animal models, research on drug conditioning in humans has primarily focused on the expression of conditioned responses to discrete, rather than contextual, cues. In laboratory animals, drug-environment associations are studied using the CPP paradigm, wherein an animal receives a drug in a particular chamber, and the primary measure of preference is the amount of time spent in the drug-paired chamber following conditioning. In Study 1, we replicated previous work using a human version of the CPP paradigm, showing that if individuals receive AMP and PL twice each in discrete environments, they develop a subjective preference for the environment in which they received AMP. However, the subjects did not spend more time on the AMP-paired environment after conditioning. Thus, the conditioned response was not apparent using the measure

typically used in animal studies to assess conditioning. In Study 3, we increased the number of conditioning sessions to determine whether the time spent measure would emerge with more pairings. We also included several additional measures of conditioning, by measuring mood, behavior and physiology in the conditioned rooms. Twenty-eight healthy volunteers received 20mg AMP and PL four times each, in a combined within and between subjects design. A Paired Group (N=12) received AMP and PL consistently in two rooms across 8 sessions, and an Unpaired Group received the same amount of drug, but received the drug randomly in the two rooms. The within-subject variable was the number of conditioning trials: Room preferences were tested after 2, 4 and 8 sessions, corresponding to 1, 2 and 4 pairings of drug and placebo with each room. Conditioning rooms were assigned according to a biased design: the Paired Group received AMP in the room they initially preferred less. The results of this study failed to replicate previous human drug conditioning studies. The Paired Group did not exhibit an increase in either subjective or objective (time spent) preference for the drug-paired room. In addition, there was no evidence of conditioning on measures of mood, attention, cognitive speed, HR, BP, or autonomic nervous system (ANS) activity during testing in the conditioned room. We discuss reasons for the failure to replicate, including the use of the within-subject design.

4.2 Introduction

As described earlier, the CPP paradigm has been used widely to study the acquisition of contextual conditioning in laboratory animals, but less often in humans. In the study described in Chapter 2, we found that healthy volunteers did

report a subjective preference for a room in which they had received AMP, but they did not spend more in the AMP-paired room following conditioning. This finding contrasted with findings with ALC (Childs and De Wit, *in prep*), in which subjects did spend more time in an ALC-paired room after conditioning trials. In the present study, we again measured time spent in the conditioned context, but extended our previous AMP study in several ways. First, we systematically varied the number of drug-room pairings (2, 4 or 8), to determine whether conditioning would be linearly related to the number of pairings. Second, with more pairings than the study described above, we hypothesized that we might detect a conditioned increase in time spent in the drug-paired room. Third, we measured several additional subjective, cognitive, and physiological responses in the drug paired room.

With many forms of associative conditioning, such as conditioned approach and fear conditioning, the number of conditioning trials directly correlates with the strength of the CR (Risinger and Oakes, 1996; Brabant et al., 2005; Gottlieb and Rescorla, 2010). However, the relationship between the number of sessions on the acquisition of a CPP in humans is unknown. Therefore, in this study we tested conditioned responses after one, two, and four AMP-room pairings, and we predicted that the CPP would be stronger after more pairings.

A second goal was to explore ways in which conditioned drug responses (CDRs) may be expressed in the AMP-paired room. In laboratory animals, CDRs can include physiological responses such as changes in body temperature (Schwarz-Stevens and Cunningham, 1993), locomotor activity (Singer et al., 2009) and conditioned drug-seeking behavior (e.g., in the reinstatement procedure; Crombag and Shaham, 2002). In humans, CDRs include subjective reports of craving (Childs and De Wit, *in*

prep), physiological responses (Everitt et al., 1999) and behavioral measures such as attentional bias (Franken et al., 2000; Vadhan et al., 2007). Whether, or how, these other CDRs contribute to human CPP remains to be determined. It is also recognized that CDRs may be either in the same or opposite direction as the unconditioned, direct drug effect (Eikelboom and Stewart, 1979; Staiger and White, 1988). In human subjects, it is possible to assess multiple CDRs in the same individuals at the same time. Thus, in our CPP procedure, we will assess not only the conditioned preference for the AMP-paired room, but also other CDRs.

AMP produces distinctive subjective, behavioral, and physiological effects, any of which might be conditioned. First, AMP increases positive mood, an effect that can be conditioned to a stimulant-paired contextual cue (Wardle and de Wit, 2012; Depue and Fu, 2013). Contextual conditioning itself can enhance the subjective effects of AMP (Childs and de Wit, 2013). For instance, participants who received AMP twice in the same room reported greater stimulation and drug craving in response to AMP during the second administration than during the first. Second, AMP and similar drugs enhance motor velocity and working memory in humans, and these effects can be conditioned to a context as well (Depue and Fu, 2013). For example, subjects who received methylphenidate twice in the same experimental context demonstrated enhanced motor velocity and working memory upon the second administration relative to the first. Third, AMP and other stimulants activate the SNS (Seiden et al., 1993) and inhibit the PNS (Klemfuss and Adler, 1986) and although contextual conditioning of these metrics has not been measured, it is possible that these effects may also be expressed as CDRs. Studying CDRs in the context of human place preference

conditioning may provide insights into the processes by which conditioned contexts influence drug craving and relapse.

One category of measures that was introduced in the present study was the effect of AMP on the ANS. AMP has known actions on both the sympathetic nervous system (SNS) and parasympathetic nervous system (PSNS), and we reasoned that some of these effects might be conditioned to the contextual cues. To measure activity of the PSNS and SNS, we recorded high-frequency heart rate variability (HRV) and pre-ejection period (PEP) length, respectively (see Dependent Measures). AMP, as well as the stimulants cocaine and 3-4-methylenedioxymethamphetamine, reduce HRV, indicating a reduction in PSNS activity (Klemfuss and Adler, 1986; Vongpatanasin et al., 2004; Frye et al., 2014). Because AMP has sympathomimetic effects, it would also be expected to shorten PEP length, but to our knowledge this has not yet been tested (Robinson et al., 1988; Wardle and de Wit, 2012; Depue and Fu, 2013). Additionally, very few studies have examined conditioned HRV effects. One study showed that cues paired with ALC can reduce HRV (Garland et al., 2012), but to our knowledge the effects AMP-paired contexts on HRV are not known. Also, contexts paired with stimulant drugs such as AMP and methylphenidate have elicited some of the prototypical subjective and physiological indicators of increased SNS (Childs and de Wit, 2013; Depue and Fu, 2013), but like in the case of HRV, the effect of AMP-paired contexts on PEP have not been measured. By recording HRV and PEP in the AMP-paired room, we hoped to determine both the direct effects of AMP on ANS activity, and the emergence of context-induced conditioned effects on ANS activity.

In summary, the purpose of this study was to a) determine the relationship between the number of AMP-room pairings and the strength of conditioning and b)

investigate other indices of conditioned responses to an AMP-paired room in a human CPP paradigm. We hypothesized that the increase in both time spent and subjective preference would directly correlate with the number of conditioning sessions. Also, we expected the strength of conditioning to correlate with the positive subjective effects of the drug. Next, we expected the AMP-paired environment to induce subjective, behavioral, and physiological effects that either mimic or contrast the effects of the drug. Finally, in addition to exhibiting these responses during extinction in the AMP-paired room, we also anticipated that the reaction to AMP itself would be enhanced when it was administered in a consistent context, because this might indicate acquisition of a conditioned response.

4.3 Methods

4.3.1 Participants

Healthy male (n = 18) and female (n = 7) adults, aged 18-31, were recruited using flyers and online advertisements. Participants were recruited and screened as described for Study 1 (Chapter 2).

4.3.2 Overall Design

The primary purpose of this study was to determine whether the number of contextual conditioning sessions affects the strength of CRs in a human CPP paradigm. Using a within- and between- subjects design, subjects underwent eight conditioning sessions, wherein they received either AMP (20mg) or PL in one of two rooms. Subjects were assigned to either a paired group (N=11), who always received

AMP in one room and PL in another, or an unpaired group (N=14), who received AMP and PL in both rooms. This study also used a biased design: Paired Group subjects always received AMP in their initially less preferred room. Subjects' conditioned preferences for the rooms were assessed after two, four, and eight sessions.

Secondarily, to determine whether individuals developed conditioned subjective, cognitive, and physiological responses, we examined several responses to AMP over the course of the conditioning trials, as well as conditioned responses to the testing rooms following conditioning. The metrics that we assessed included: mood, cognitive processing speed, attention lapses, HR, BP, HRV, PEP duration, and respiratory rate (RR). We measured these responses during conditioning because we were interested in investigating whether or not the drug-paired room would enhance the effect of the drug. Next, we measured these effects during the final test session, in the AMP-or PL-paired rooms, because we wanted to determine if paired group subjects would exhibit a conditioned response to the AMP-paired room in the absence of drug.

This study differed from the study described in Chapter 2 in several ways. First, and most importantly, the subjects underwent 8 conditioning sessions instead of 4. Also, we reduced the length of the free exploration period during the room preference test from 10 minutes to 5 minutes for three reasons a) to reduce the amount of pre-exposure to the CS during the pre-test at orientation, b) to reduce the potential for extinction between conditioning sessions, and c) to reduce the potential for boredom among the subjects. A post-hoc analysis of the exploration test in the study described in Chapter 2 revealed that the time subjects spent in each room did not differ significantly between the first five minutes and the whole ten minutes of the RET; therefore, we predicted that shortening the free exploration period would not affect our measures of

preference. Next, we also attempted to recruit participants who had previously used stimulant drugs for nonmedical purposes at least once, since previous stimulant users, as compared to stimulant-naïve individuals, showed an increase in time spent in the AMP-paired room in the first study. We determined our N based on our results from Study 1. Since we were able to induce a subjective preference for the AMP-paired room, we predicted that we would generate the same result in this study with a similar N. In addition, since we recruited a higher proportion of past stimulant users in this study, we predicted that the probability of detecting an objective preference for the AMP-paired room with the same N would be greater.

4.3.3 Drugs

d-AMP sulfate (four 5-mg tablets; Mallinkrodt, Hazelwood, MO, USA) placed in two red, opaque gelatin capsules (size 00) with dextrose filler. PL capsules contained only dextrose.

4.3.4 Study Procedures

4.3.4.1 Orientation

First, subjects attended a 30-minute orientation which was the same as that described in Chapter 2. As in Study 1, we measured subjects' initial self-reported liking of and preference for the rooms, and also how much time the subjects spent in each room during five minutes of free exploration. The time spent in each room was used to assign the drug room in the paired group: paired group subjects were designated to receive AMP in the room they spent less time in during the initial room preference test.

4.3.4.2 *Conditioning Sessions*

Subjects underwent eight 3.5-hour conditioning sessions, conducted from 9am to 12:30pm, 2-7 days apart. Baseline measures were taken in a neutral room, like in Study 1 (Chapter 2). In addition, subjects were linked to a mobile impedance cardiograph to measure heart rhythms and thoracic impedance. Most of the procedures on the conditioning sessions were the same as in Study 1 (Chapter 2). However, on sessions 3 and 5, subjects completed a 5-min room preference test (described below) before going to their assigned testing room for that day. At 9:30 the subject was escorted to one of the two conditioning rooms where they received capsules containing either 20mg AMP or PL. Every half hour between 10:00 and 12:30, mood and physiological measures were taken, and HRV measures were obtained continuously. At 11:00, subjects completed two cognitive tasks (described below), and at 12:00 the mobile impedance cardiograph was removed. Between scheduled experimental events, subjects were allowed to read and watch movies, and at 12:30, they completed an end of session questionnaire. Then, if their HR and BP had returned to baseline, they were allowed to leave.

4.3.4.3 *Final Test Session*

The final test session took one hour to complete, and occurred at any time during the day (between 9:00am and 5:00pm), within 7 days of the last conditioning session. In this session, we first placed the mobile impedance cardiograph on the subjects and then took baseline mood and cardiovascular measures. They then completed a final room preference test and then underwent conditioned response testing in both rooms. For conditioned response testing, subjects completed mood questionnaires and both cognitive tasks, had their HR and BP taken, and had their other

physiological responses (HRV and PEP) measured for five minutes, in each room. Once a subject finished conditioned response testing in one room, they were immediately moved to the other room for testing. The subjects spent about 10 minutes in each room, and the order in which subjects were placed in the two testing rooms was counterbalanced between subjects. Finally, after the subjects were tested in each room, they were informed of the purpose of the study and, they were allowed to ask any questions they wanted about the study procedures, and they were paid.

4.3.5 Dependent Measures

4.3.5.1 Demographics and Drug Use History

Demographic information and past drug use were assessed during the screening interview using the same protocol as in Study 1 (Chapter 2).

4.3.5.2 Drug Effects

i. Mood. We measured subjective drug effects using the same mood questionnaires as in Study 1 (Chapter 2).

ii. Physiological Measures. HR and BP were measured using a digital monitor (BP786, Omron Healthcare, Lake Forest, IL). We measured HRV, PEP length, and RR using a mobile impedance cardiograph (Model 50-2303-00, MindWare Technologies, Gahanna, OH). The mobile impedance cardiograph measured SNS and PSNS activity and RR by simultaneously recording heart rhythms with an electrocardiogram (ECG) and thoracic impedance with an impedance cardiogram (ICG). First, SNS activity is indirectly determined by measuring the duration of a specific phase of the heartbeat termed the PEP (van Dijk et al., 2013). The PEP begins when the ventricles of the heart

contract and ends when the aortic valve opens. Increased SNS activity causes the ventricles to contract more forcefully, which causes the aortic valve to open quicker; therefore, SNS tone negatively correlates with the duration of the PEP. These events are detected using ECG and an ICG, respectively. More specifically, the ECG detects when the sinus node depolarizes, which signals the contraction of the left ventricle, while the ICG picks up the sharp drop in thoracic impedance that is caused by the opening of the aortic valve (van Dijk et al., 2013). Monitoring these two events simultaneously allows us to determine the length of the PEP, and in turn, the degree of SNS activity. PSNS activity, on the other hand, is measured by recording heart contractions and respiration using an ECG and ICG. The vagus nerve, the primary motor output nerve of the PSNS, drives HRV, or fluctuations in the HR that correspond with changes in lung volume. HR is positively correlated with and is synchronized with lung volume, therefore, like lung volume, HR fluctuates in cycles (Yasuma and Hayano, 2004). The amplitude of this fluctuation, or variability, is directly related to activity of the vagus nerve. Therefore, by measuring the change in HR as a function of respiration over time with an ECG, we can determine the degree of HRV, and in turn, vagal tone, and the level of PSNS activity. Here, the data will be reported as respiratory sinus arrhythmia (RSA), of which high frequency HRV is a component.

Finally, the impedance signal captured by the ICG also reveals RR. As one inhales, the lungs fill with air. The electrical signal traveling through the body between the ICG electrodes travel more easily through blood than through air, so when one's lungs fill with air, thoracic impedance increases. When one exhales, the lungs release air, and thoracic impedance decreases. By measuring thoracic impedance, we can indirectly track tidal volume, and in turn, measure RR.

Overall, the primary physiological measures were BP, HR, RSA, PEP duration, and RR. First, we were interested in response to AMP during conditioning (compared to PL). We calculated the difference between the response to AMP and the response to PL in both the paired and unpaired groups, and then we compared these differences between the groups. Second, we were also interested in response to the AMP-paired room during the final testing session. For this, we calculated the difference between the two testing rooms (the room in which subjects initially spent less time vs. the room in which subjects initially spent more time) during the final test session in both the paired and unpaired groups. Then, we compared these differences between the groups.

iii. Cognitive Measures. We measured attention lapses using the simple reaction time task (SRT; Leith and Barrett, 1976). This task is used to measure of lapses in attention based on variation in reaction times (RTs) during a simple visual response time task. A simple stimulus (a star) was presented briefly on the computer screen at random intervals, and the subject had to press the spacebar on a keyboard as quickly as possible following the appearance of each stimulus. The primary outcome measure was the mean deviation from the mode, or the mean of the difference between each RT and the mode.

Cognitive processing speed was measured using the Digit Symbol Substitution Test (DSST; Hindmarch, 2004). The DSST, a subtest of the Wechsler Adult Intelligence Scale, is used to measure working memory and cognitive efficiency. In this paper-and-pencil task, subjects are required to match symbols with numbers as quickly as possible. The primary outcome measure is how many numbers are matched with the correct symbol after 90 seconds.

4.3.5.3 Objective Measure of Room Preference

Subjects completed an objective preference test during the orientation (pre-test), immediately before the 3rd and 5th sessions, and during the post-test. Thus, preference for the rooms was measured after two, four, and eight conditioning sessions, or after one, two, and four AMP-room pairings in the paired group (post-test). In this test, explored the two testing rooms for 5 minutes, and were free to move freely between them. We calculated the proportion of time each subject spent in each room and used this metric as our primary measure of room preference. As in Study 1 (Chapter 2), the drug-room assignments were based on the room in which subjects spent less time at pre-test (i.e., biased procedure).

4.3.5.4 Subjective Measures of Room Preference

Immediately after each room exploration test, subjects completed the same paper-and-pencil room preference questionnaire as in Study 1 (Chapter 2).

4.3.6 Data Analysis

4.3.6.1 Demographic Characteristics

Demographic and drug use history were analyzed using the same method as in Study 1 (Chapter 2).

4.3.6.2 Overall Drug Effects

The direct subjective and physiological effects of AMP (including HRV and PEP) were determined using the same statistical methods as in Study 1 (Chapter 2).

4.3.6.3 Conditioning Measures

Subjective liking and preference for the room in which subjects initially spent less time, as reported on the RPQ, and time spent in the initially less preferred room, as recorded during the RET, were compared among all four tests using the same methods as in Study 1 (Chapter 2).

4.3.6.4 Relationship between Subjective and Physiological Drug Responses and Conditioning

We determined the relationship between acute drug responses and the conditioning measures using a double-difference score for each subjective and physiological response. We averaged the AUC values for all four AMP sessions and for all four PL sessions, then subtracted the PL AUC value from the

	Paired Group (n = 11)	*Unpaired Group (n=14)*
Sex (male/female)	9 / 2	9 / 5
Age	22.9 ± 4.1	22.5 ± 3.7
Body mass index (kg/m^2)	23.0 ± 1.7	21.9 ± 2.0
Race (%)		
White	67	67
Black/African American	17	0
Asian	8	13
Other	17	20
Current Drug Use		
Caffeine consumption (cups/week)	8.2 ± 7	8.8 ± 6
ALC consumption (drinks/week)	9.7 ± 15	9.8 ± 8
Cigarette use (cigarettes/week)	0.7 ± 2	2.5 ± 9
Cannabis use (uses/month)	7.6 ± 9	8.1 ± 14
Past Drug Use (% ever used)		
Marijuana	92	93
Stimulants	67	80
Opiates	25	14
Tranquilizers	25	7
Hallucinogens	75	73
Club Drugs	75	87

Table 4.1. Demographic characteristics of the participants in the Paired and Unpaired Groups. Data represent N's, mean ± SEM, or percent of participants in the group.

AMP AUC value. In the paired group, relationships between the subjective and physiological effects of AMP and the conditioning measures (the subjective measures of "liking" and "preference" and the objective measure of time spent) were calculated using Pearson correlations. Analyses were conducted using SPSS Version 22 (SPSS Inc., Chicago, IL, USA). Alpha was established at $p < .05$.

4.3.6.5 Context-Dependent Drug Effects

To examine context-dependent changes in drug effects, we compared measures of subjective, cognitive, and physiological reactions to AMP or PL across each of the four pairs of administration sessions between the paired and unpaired groups using a three-factor Group × Drug × Pair repeated measures RMANOVA. To examine context-dependent conditioned responses to the rooms, we compared measures of subjective, cardiovascular, and physiological reactions to the two rooms using paired-samples t-tests. Alpha was set at $p < .05$.

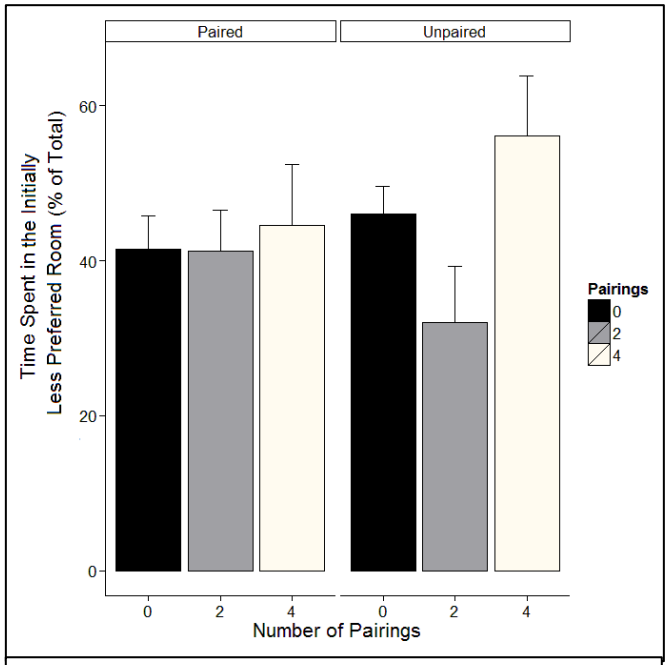

Figure 4.1. Proportion of time spent in the initially less preferred room before conditioning ("0") and after two and four AMP sessions, for the paired group and unpaired group subjects with 20-mg AMP and PL. The paired group always received AMP in the room that they initially preferred less, whereas the unpaired group received AMP and PL in both rooms. Bars represent mean ± SEM.

4.4 Results

4.4.1 Demographic Characteristics

The subjects were mostly white, male, and in their early to mid-twenties. They consumed caffeine and ALC regularly and most had used cannabis and stimulant drugs in the past (See Table 4.1). The paired and unpaired groups did not differ on demographic characteristics or in drug use history.

4.4.2 Objective Measure of Room Preference

The change in the proportion of time spent in the initially less preferred room, as compared to the change in the proportion of time spent in the more preferred room, did not differ between the groups (Group × Room × Time RMANOVA: $F_{3,69} = .957$, p = .418; Figure 4.1).

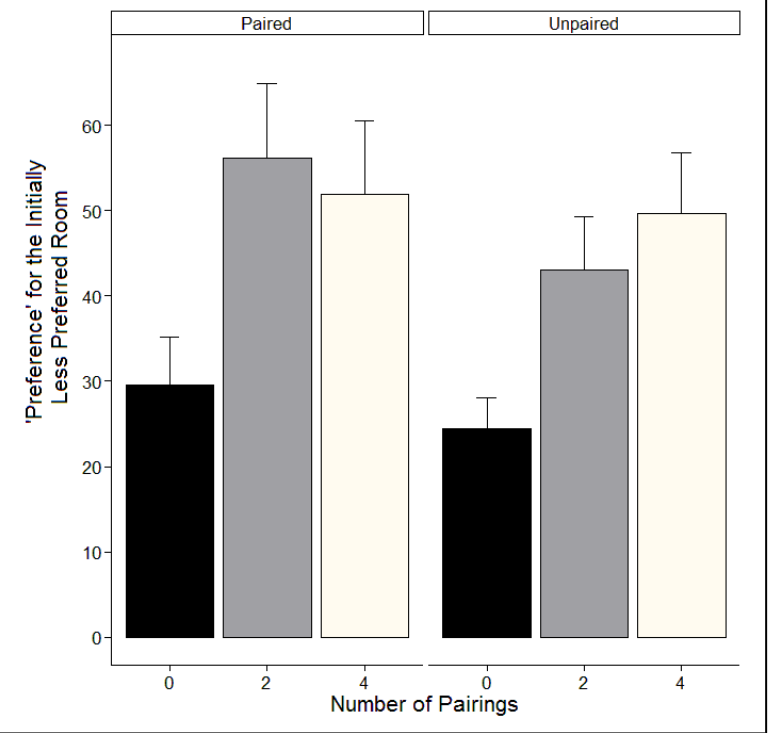

Figure 4.2. Subjective preference for the initially less preferred room at each RET, after zero, two and four AMP sessions, for the paired group and unpaired group subjects. Bars represent mean ± SEM. Asterisks indicate a significant difference between time points (Student's paired t-test, $P < 0.05$).

4.4.3 Subjective Measures of Room Preference

The paired and unpaired groups did not differ in the change in their subjective preference for the initially less preferred room (Group × Time RMANOVA: $F_{2,22} = .606$, $p = .555$; Figure 4.2). All subjects showed an increase in preference for the initially less preferred room over successive sessions ($F_{3,66} = 7.98$; $p < .001$).

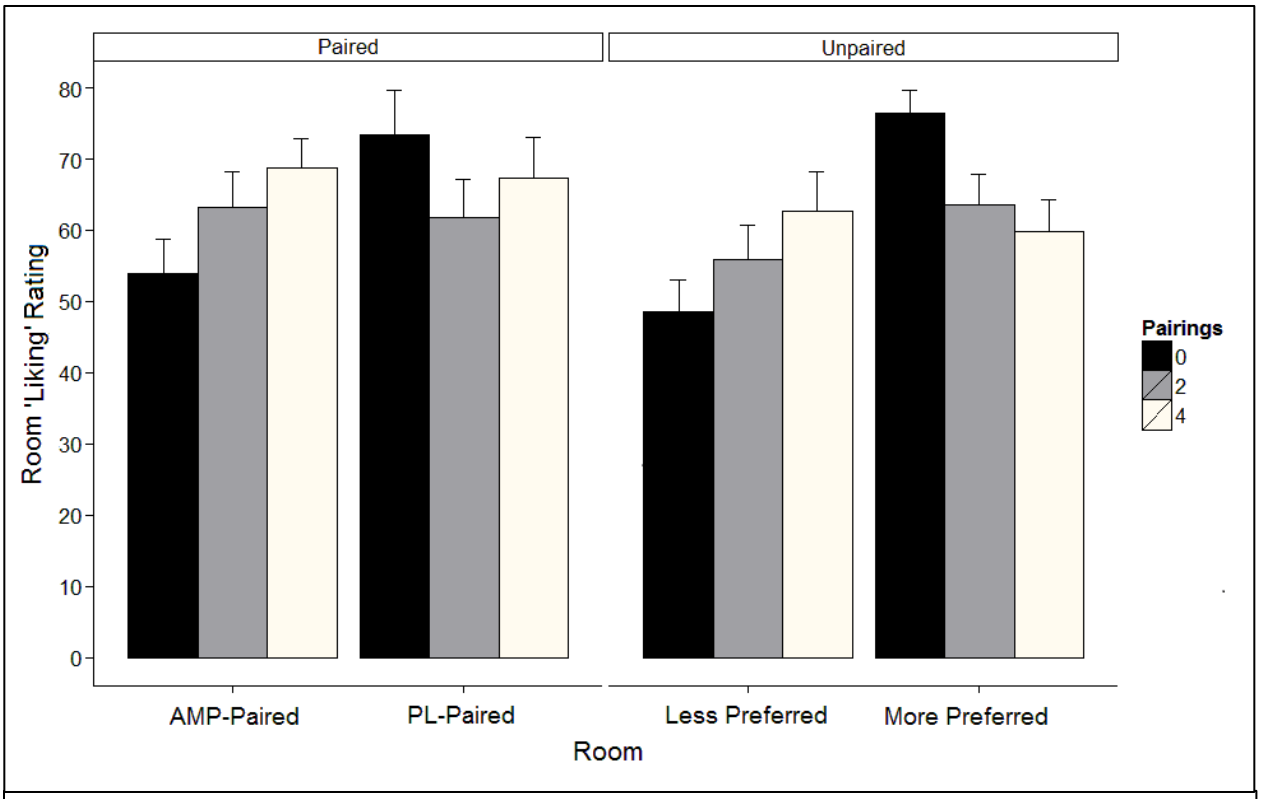

Figure 4.3. Subjective liking of the initially less preferred room at each RET, before conditioning ("0" pairings) and after two and four sessions with either AMP or PL, for the paired group and unpaired group subjects. Bars represent mean ± SEM. Asterisks indicate a significant difference between time points (Student's paired t-test, $P < 0.05$).

The two groups also did not differ in the change in their liking of the two rooms (Group × Room × Time RMANOVA: $F_{2,46} = .703$, $p = .500$; Figure 4.3). Collapsing across groups, however, the change in room liking over time did differ between the two rooms (Room × Time RMANOVA: $F_{2,22} = 14.58$, $p < .001$). More specifically, subjects reported a significant increase in liking of the initially less preferred room over

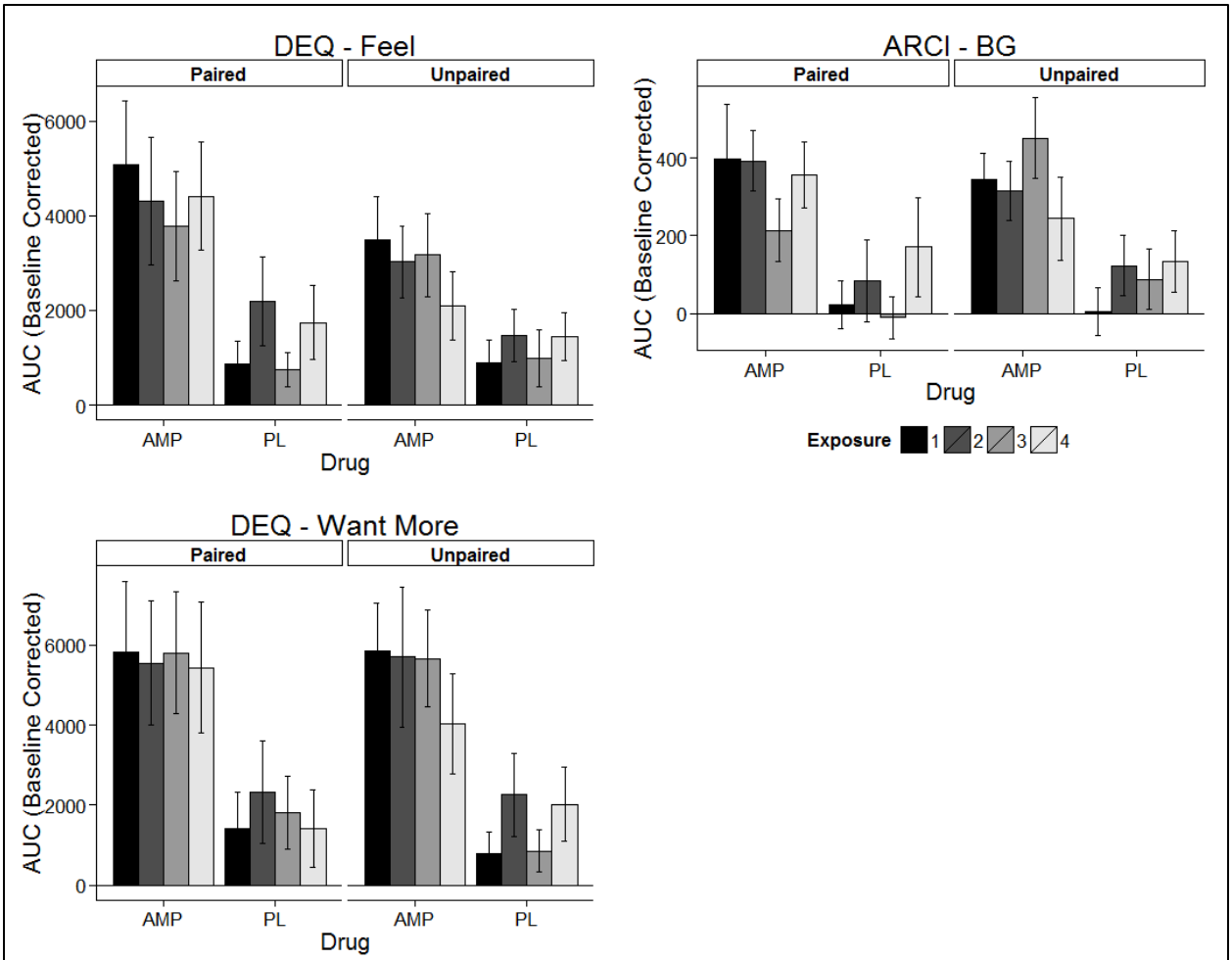

Figure 4.4. Selected subjective effects of AMP and PL, corresponding to area under the curve over the three hours after capsule ingestion, on measures of "feeling" the drug, stimulation (ARCI BG), and "wanting more" drug on each exposure to AMP or PL. The paired group received AMP in one room and PL in another room, and the unpaired group received AMP and PL in both rooms. Data represent mean ± SEM. Responses to AMP did not change systematically with repeated exposures to the drug.

successive sessions (RMANOVA: $F_{2,48} = 9.84$, $p < .001$) and a significant decrease in liking of the initially more preferred room (RMANOVA: $F_{2,48} = 9.86$, $p < .001$). Finally, ratings of room liking were not correlated with time spent, either before or after conditioning (i.e., at pretest or after 2, 4, or 8 sessions).

4.4.4 The Direct Effects of AMP on Mood, Cognition, and Cardiovascular Function, and their Change across Conditioning Sessions

Participants in both the paired and unpaired groups reported feeling the prototypical subjective effects of AMP. Relative to PL, AMP increased scores on ARCI BG scale [paired group: $t(11) = 4.05$, $p = .002$; unpaired group: $t(15) = 4.60$, $p < .001$], DEQ "Feel" scale [paired group: $t(11) = 4.53$, $p = .001$; unpaired group: $t(15) = 4.10$, $p = .001$], and "DEQ Want More" scale [paired group: $t(11) = 4.03$, $p = .002$; unpaired group: $t(15) = 5.75$, $p < .001$]. The changes in ARCI BG, DEQ Feel, and DEQ Want More scores after successive sessions did not differ between the groups [BG: $F_{3,48}$=.812, p = .494; Feel: $F_{3,48} = .293$, $p = .831$; Want More: $F_{3,48} = .210$, $p = .889$; Figure 4.4].

AMP induced its prototypical effects on HR and BP, and these effects did not

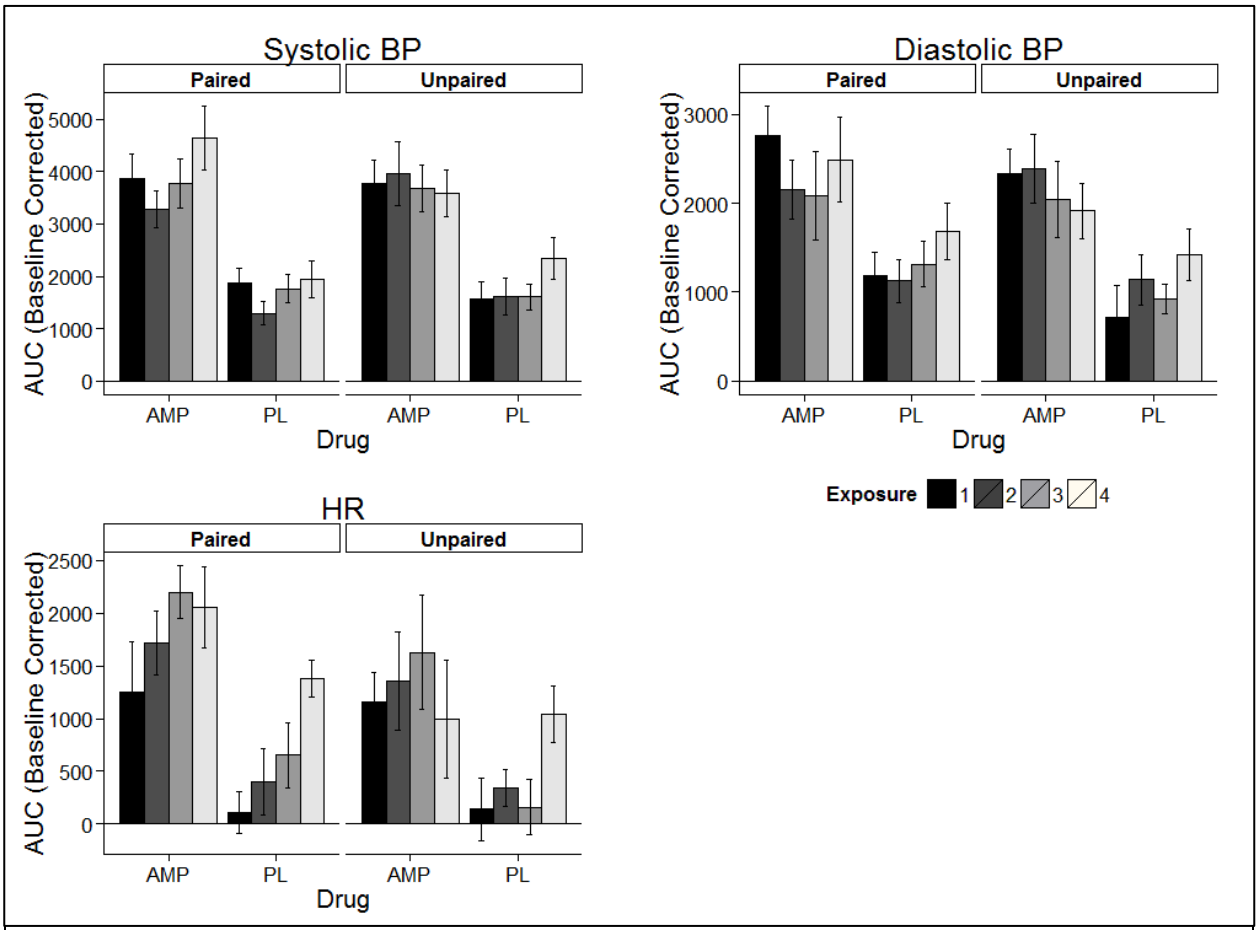

Figure 4.5. Effects of *AMP a*nd PL, as measured by area under the curve for the three hours after capsule ingestion, on systolic and diastolic BP, and HR on each exposure to AMP or PL. Data represent mean ± SEM.

differ between the paired and unpaired groups. Compared to PL, it increased systolic BP [paired group: $t(11) = 7.04$, $p < .001$; unpaired group: $t(15) = 6.72$, $p < .001$] and diastolic BP [paired group: $t(11) = 3.73$, $p = .003$; unpaired group: $t(15) = 3.97$, $p = .001$], as well as HR [paired group: $t(11) = 5.02$, $p > .001$; unpaired group: $t(15) = 3.43$, $p = .004$]. However, the groups did not differ in the changes in BP or HR across successive sessions [Systolic BP: $F_{3,78} = 1.32$, $p = .273$; Diastolic BP: $F_{3,78} = .180$, $p = .909$; HR: $F_{3,78} = .063$, $p = .979$; Figure 4.5].

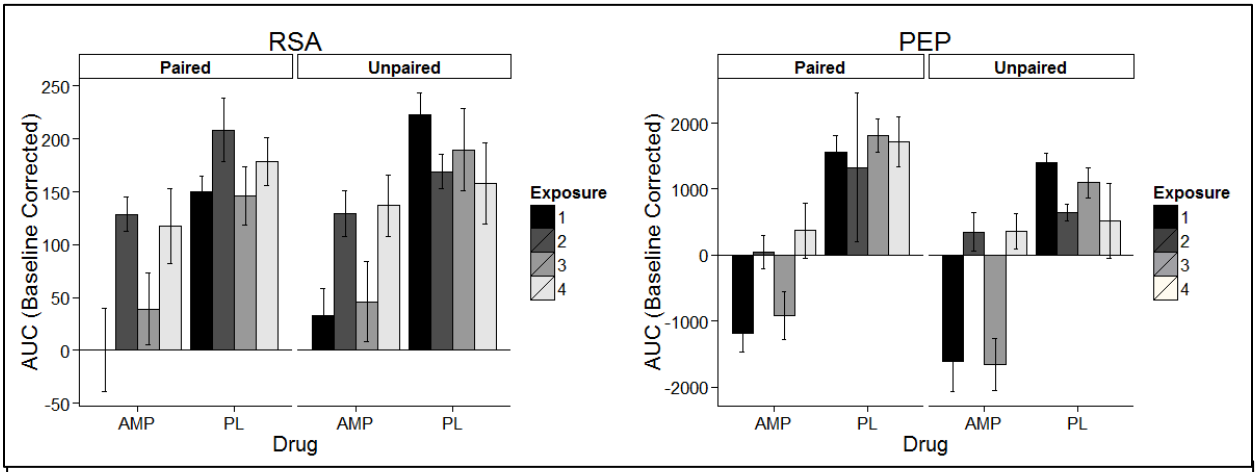

Figure 4.6. RSA and PEP duration in response to AMP and PL, as measured by area under the curve for the three hours after capsule ingestion, on each exposure to AMP or PL. Data represent mean ± SEM.

AMP also induced its predicted effects on RSA and PEP duration, and this did not differ across the two groups (Figure 4.6). Compared to PL, AMP significantly reduced RSA in both groups [paired: $t(11) = -4.72$, $p = .001$; unpaired: $t(15) = -4.88$, $p < .001$] and reduced PEP duration in both groups [paired: $t(9) = -4.05$, $p = .003$; unpaired: $t(12) = -5.03$, $p < .001$]. AMP did not affect RR in either group [paired: $t(11) = -.143$, $p = .889$; unpaired: $t(12) = -.505$, $p < .621$]. The effects of AMP on RSA relative did not vary across the four AMP sessions relative to the four PL sessions in either group

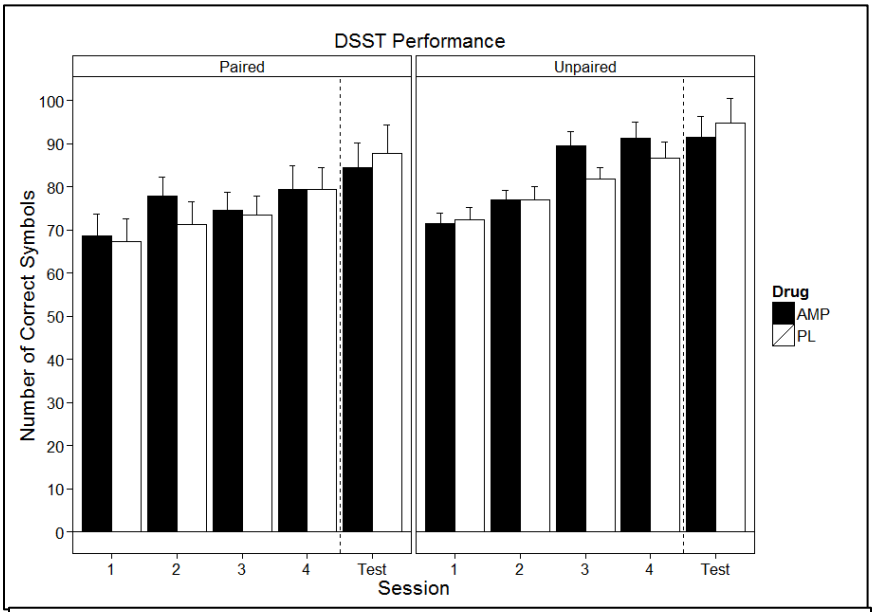

Figure 4.7. DSST performance during each AMP and PL exposure and during the final test session in the two conditioning rooms (no drug administered; AMP and PL represent the AMP- and PL-paired rooms (paired group) and the initially less and more preferred rooms (unpaired group). Data represent mean ± SEM.

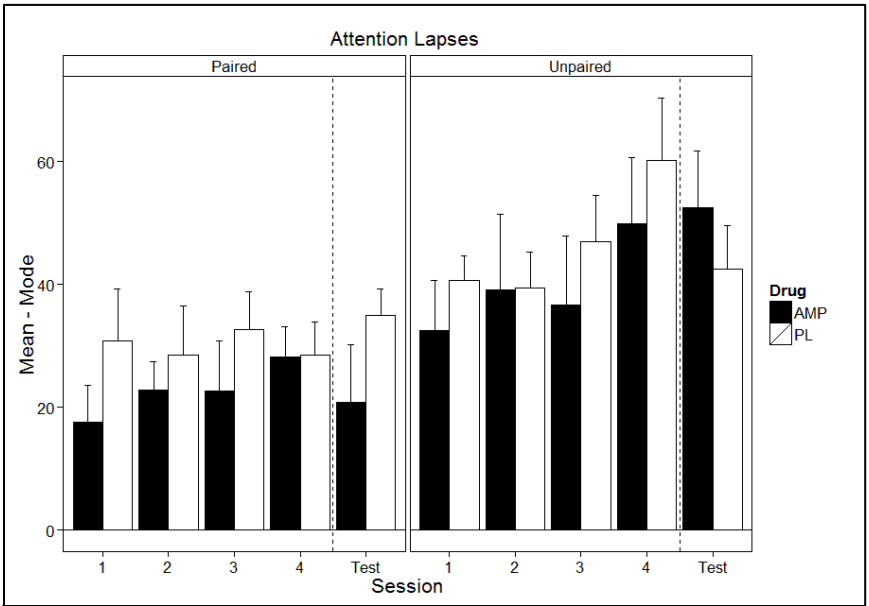

Figure 4.8. Attention lapses during each AMP and PL exposure and during the final test session in the two conditioning rooms (no drug administered; AMP and PL represent the AMP- and PL-paired rooms (paired group) and the initially less and more preferred rooms (unpaired group). Data represent mean ± SEM.

(Figure 4.6). After removing outliers, there was not sufficient data to analyze the changes in PEP duration.

To determine the direct of AMP on the two measures of cognitive performance, first we compared performance during the first AMP session and PL sessions. AMP did not improve performance on the DSST in either group, but it did reduce attention lapses in the unpaired group only ($t(13) = -3.42$, $p = .005$; Figure 4.7; Figure 4.8). DSST performance neither changed across successive sessions nor differed between the two groups (Group × Room × Time RMANOVA: $F_{3,19} = 1.12$, $p = .365$; Figure 4.7). Also, AMP-

induced reductions in attention lapses during the SRT did not change across successive sessions (Group × Room × Time RMANOVA: $F_{3,18}$ = .1.12, p = .369; Figure 4.8).

4.4.5 Change in Mood, Cognition, and Cardiovascular Function in Response to the Conditioning Rooms at the Post-Test

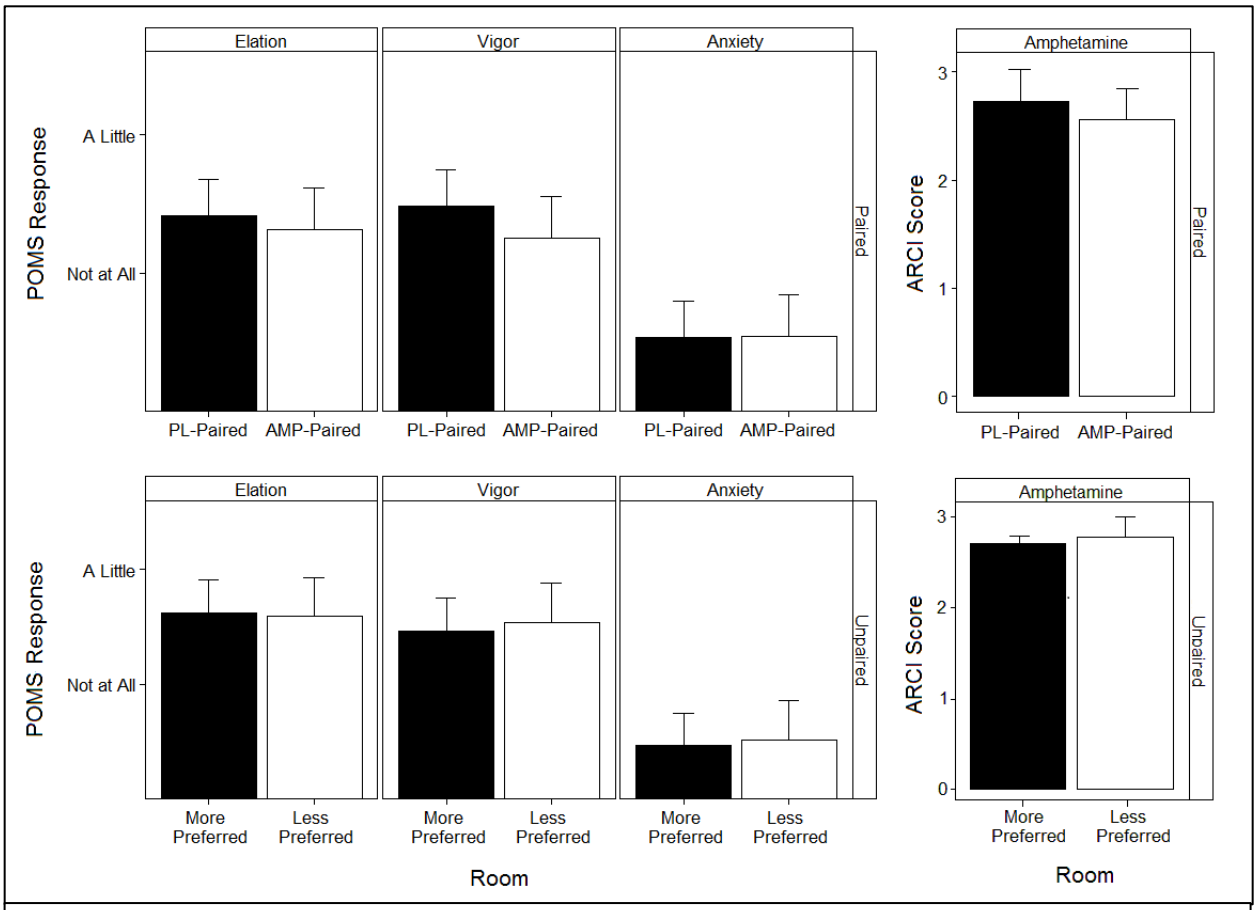

Figure 4.9. Mean ratings of POMS Elation, Vigor, and Anxiety scores (left) and ARCI Amphetamine scores (right) in the Paired and Unpaired Groups, when tested after 8 trials, in either AMP-paired room (i.e., Paired Group) or the initially less preferred room (Unpaired Group). Data represent mean ± SEM.

Following conditioning, subjects rated their mood states during a brief test in each of the two conditioning rooms without receiving any capsules. The paired group subjects did not exhibit differential ratings of mood states (i.e., anxiety, elation, vigor, or

AMP-like subjective effects) in the two rooms, and similar to the unpaired group (Figure 4.9).

Cardiovascular measures, DSST performance and attention lapses also did not differ in the two rooms after conditioning, in either group (Figures 4.10, 4.7, and 4.8, respectively).

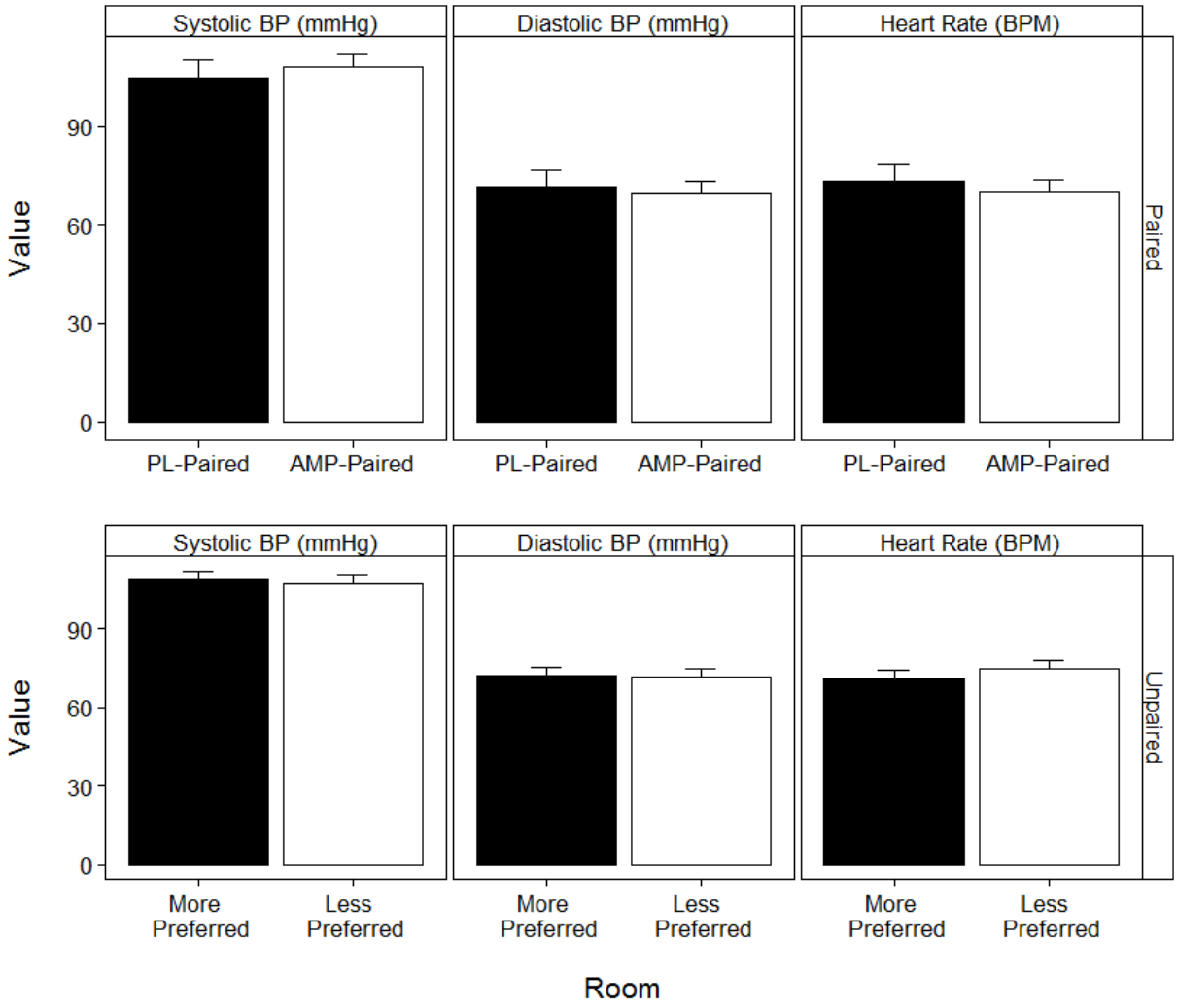

Figure 4.10. The effect of the two conditioning rooms during the post-test session on systolic and diastolic BP and HR, as measured using raw scores. Data represent mean ± SEM.

Finally, compared to the initially more preferred room, the initially less-preferred room elicited significantly different reactions on the RSA measure in the two groups ($F_{1,14}$ = 4.93, p = .043; Figure 4.11); however, within the context of our other results, we believe this to be a spurious effect. Neither group experienced a significant change in RSA between the two rooms [paired group: $t(6)$ = 1.33, p = .233; unpaired group: $t(8)$ = -1.92, p = .091), but unpaired subjects trended towards a higher level of RSA in the initially less preferred room, while RSA in the paired group remained stagnant (Figure 4.11). The effect of the initially less-preferred room on PEP duration did not differ between the two groups (Figure 4.11).

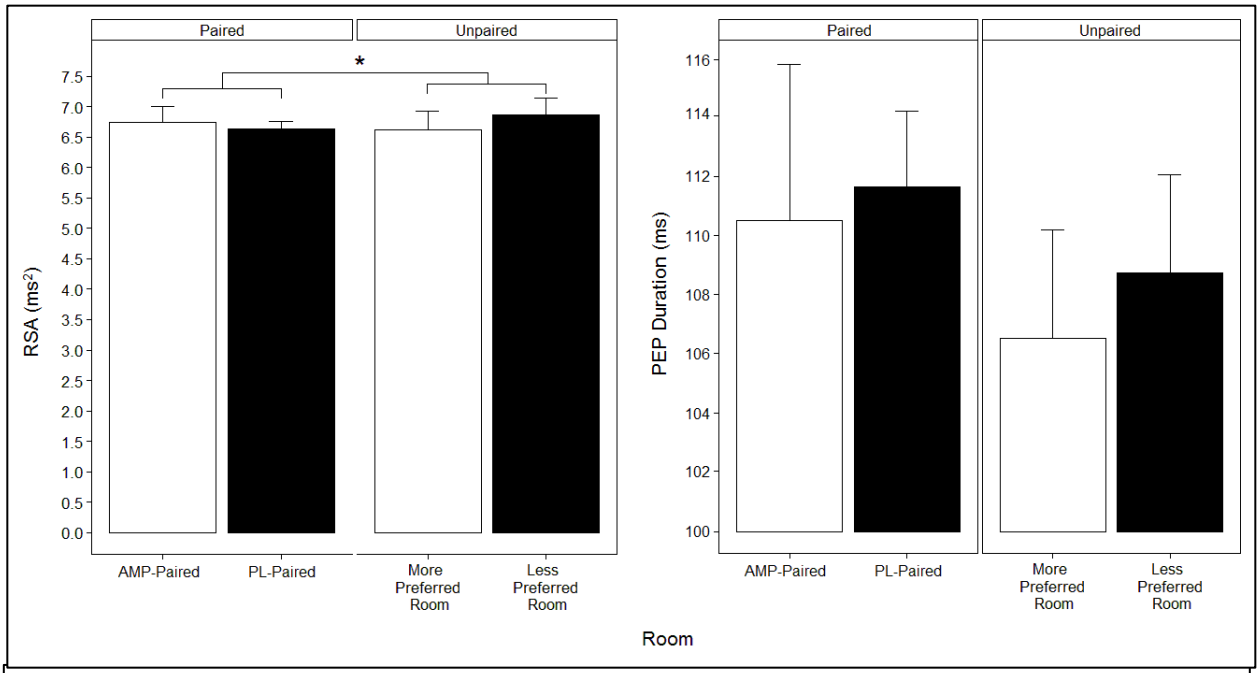

Figure 4.11. The effect of the two conditioning rooms during the post-test session on RSA (left) and PEP duration (right), as measured using raw scores. Asterisk indicates a significant Group x Room interaction (ANOVA, $P < 0.05$). Data represent mean ± SEM.

4.5 Discussion

In this experiment, we studied the acquisition of a CPP with a moderate dose of AMP (20 mg) to determine the relationship between the number of drug-room pairings and the strength of conditioning. We also investigated other potential CDRs to the AMP-paired room, including subjective, cognitive, and physiological responses. We used a combined within- and between-subjects design to assess preference for the AMP-paired room after one, two, and four AMP-room pairings, in participants who received the drug either paired or not paired with distinctive rooms. We also measured potential conditioned responses in the AMP-paired room on mood, cognition, and physiology, both over the course of conditioning and during final the post-conditioning test. Based on evidence in animals (Risinger and Oakes, 1996; Brabant et al., 2005), we hypothesized that individuals would display stronger conditioning following more drug-room pairings, as assessed using both subjective measures of preference and the objective measure of time spent in the drug-paired room.

Unexpectedly, there was no evidence of conditioning on any measure, including subjective room preference, room liking, time spent in the rooms, or any other measure of conditioned drug effects. The drug produced its expected subjective and physiological effects, which were comparable to numerous previous studies (Heishman and Henningfield, 1991; Jayaram-Lindström et al., 2004; Stoops et al., 2004; Wardle and de Wit, 2012). Yet, there was no evidence of CPP: paired group subjects did not express either a subjective preference for or an increase in time spent in the AMP-paired room. These findings contrast a large body of research in laboratory animals (Tzschentke, 1998, 2007), as well as the few existing studies with humans (Childs and de

Wit, 2009, 2013, *in prep*). It is especially notable that our participants did not develop a subjective CPP, as these measures have been reliable in past AMP CPP studies in humans (Childs and de Wit, 2009, 2013, *in prep*; Study 1). Thus, using measures that were previously sensitive to CRs, conditioning did not occur in the present study. There are several possible reasons for this failure to replicate previous findings, including: 1) that this study's within-subjects design may have elicited unexpected effects on our subjects' behavior, and 2) that uncontrolled factors such as subtle differences in participant characteristics, testing procedures, instructions or extra-experimental factors (e.g., time of year, characteristics of research assistants) masked a conditioning effect. Whatever the reason, the lack of conditioning in this study suggests that the AMP CPP paradigm may not be robust in humans, and that it is sensitive to situational variables.

4.5.1 The Within-Subjects Design

The first reason why subjects may not have acquired a subjective preference for the AMP-paired room, in contrast to our previous studies (Childs and de Wit, 2009, 2013, *in prep*), is because we used a within-subjects preference testing protocol, which may have blocked conditioning. That is, the subjects' room preferences were tested after 1, 2, or 4 AMP-room pairings, whereas in previous studies, room preferences were tested on only a single occasion, following 2 pairings. Thus, it is possible that the 5-minute RET after the first two sessions (i.e., at the beginning of the third conditioning session) weakened or even blocked the conditioning that might have been seen after 4 sessions (two pairings). Furthermore, it is possible that the RETs conducted after sessions two and four weakened or blocked the subjective conditioning that might have been seen

after 8 sessions (four pairings). Within-subject designs have been used successfully to demonstrate the relationship between the number of conditioning trials and CPP in animal models (Risinger and Oakes, 1996; Brabant et al., 2005). For this reason, and because our RETs were brief, we expected that the conditioning tests would not interfere with acquisition of CPP. At this point, we cannot confirm that the subjective CPP failed to develop because of these brief conditioning tests.

A related behavioral process that may have contributed to our lack of conditioning is 'assessment reactivity,' wherein the action of assessing or calling attention to a behavior during an experiment can affect the expression of that behavior (Schrimsher and Filtz, 2011). With a wide range of behaviors, it has been found that merely asking about a behavior can affect its expression (Kinmonth et al., 2008; McCambridge and Kypri, 2011). In the current study, subjects were asked to explore the testing rooms four times, with relatively few conditioning sessions in between, and this repeated room testing may have affected their responses on the subjective preference questionnaire. Some evidence in support of this idea of assessment reactivity comes from the patterns of exploratory behavior

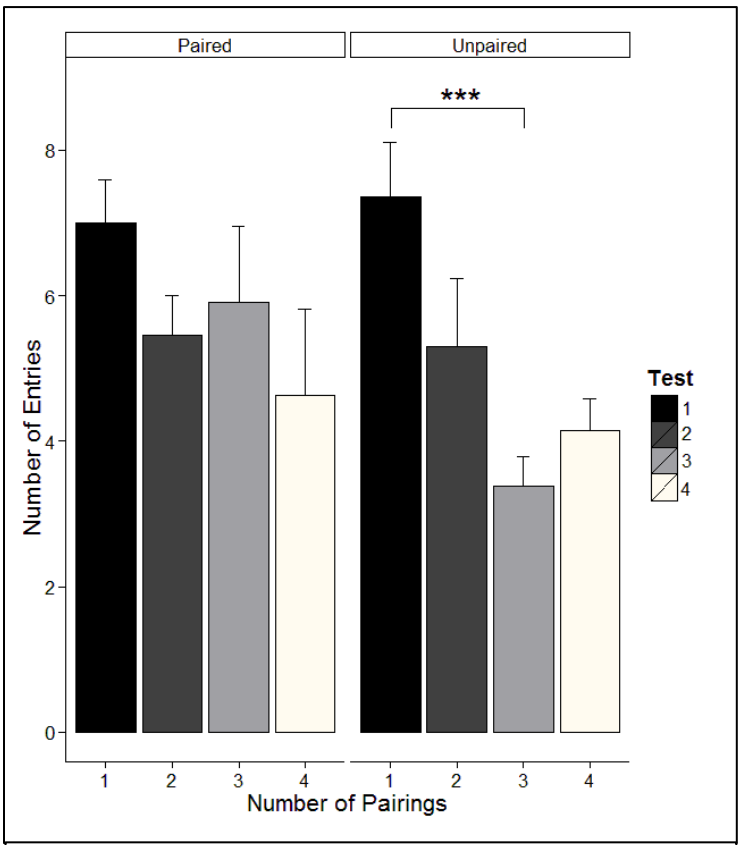

Figure 4.12. Number of entries into the two testing rooms combined during each RET in the Paired and Unpaired Groups. Data represent mean ± SEM. ***$p < .001$.

during conditioning: subjects spent less time exploring the testing rooms with each successive exploration test (Figure 4.13). Although this decline was observed with exploratory behavior, if it was related to "assessment reactivity," there may have been a comparable decline in the effort devoted to assessing their subjective responses to the rooms.

Another behavioral process that may account for the lack of conditioning is 'regression to indifference', related to the fact that we assigned subjects to rooms based on their initial lack of preference. Regression to indifference is a specific example of regression to the mean, a statistical phenomenon wherein each subsequent response on a particular measure will be closer to the mean than each previous response (Barnett et al., 2005). In this study, participants were first assigned to rooms based on their less preferred room, and then on subsequent assessments their ratings of liking of and preference for that room increased, approaching 'neutral'. This shift from an extreme toward neutral occurred in both the paired and unpaired groups, and is consistent with a regression towards indifference. The regression to indifference may also be related to the assessment reactivity described in the previous paragraph. Both of these phenomena are consistent with the idea that repeated testing may have contributed specifically to the lack of expected increase in preference in the AMP-paired room.

The increase in subjective preference and liking from before to after drug administrations was especially surprising in the Unpaired Group. We examined the possibility that the increase in subjective preference in the unpaired group was related to the order in which they received AMP and PL relative to the room tests. Half the subjects received AMP in both rooms during the first two conditioning sessions, and half received placebo on these two sessions, in both rooms. We compared these subjects to

determine whether the order of these conditions (AMP-AMP or PL-PL) influenced preference and liking ratings. It did not, and so the most parsimonious explanation for the apparent increase in preference in the unpaired group is a form of regression to indifference.

4.5.2 Comparison to Previous Studies

We examined the data from this study to previous studies with AMP, to ensure that the subject samples were similar, and that the drug produced its expected effects. We compared the data specifically from the two previous AMP CPP studies (Childs and de Wit, 2013; Study 1). The subjects in all three studies were comparable in age, gender, and ethnicity (Table 4.2). Interestingly, however, the participants in the current study reported more nonmedical use of stimulants and hallucinogens than most of the previous groups. Thus, it is possible that this difference in drug use history contributed to the absence of a conditioned drug effect in the present study. In fact, it is known that prior exposures to a US, in the absence of a CS, reduces the acquisition of a CR (McLaren and Mackintosh, 2000; Kwok and Boakes, 2012).

The acute effects of AMP on mood, HR, and BP in the present study were also largely comparable to the effects observed in our previous AMP studies (Table 4.3). Except for one study (Childs and de Wit, 2013) in which AMP did not affect systolic BP or HR in one of the groups, the effects of AMP were comparable across studies, suggesting that there was not a difference in acute drug effects (see Table 4.3). There was, however, a notable difference in the effects of AMP on a measure of performance, the DSST.

Study	Childs and de Wit (2011)		Study 1		This Study	
Group	*Paired Group (n = 19)*	*Unpaired Group (n=15)*	*Paired Group (n = 26)*	*Unpaired Group (n=11)*	*Paired Group (n = 12)*	*Unpaired Group (n=15)*
Sex (m/f)	13/6	12/3	21 / 5	7 / 4	10 / 2	10 / 5
Age	23.6 ± 0.9	23.4 ± 0.9	22.7 ± 4.0	22.6 ± 3.41	22.9 ± 4.1	22.5 ± 3.7
Body mass index (kg/m^2)	22.4 ± 0.3	22.5 ± 0.5	22.8 ± 1.5	22.9 ± 1.8	23.0 ± 1.7	21.9 ± 2.0
Race (%)						
White	53	67	69	55	67	67
Black/African American	0	7	19	27	17	0
Asian	16	13	8	18	8	13
Other	32	13	4	0	17	20
Current Drug Use						
Caffeine consumption (cups/wk)	5.0 ± 0.9	8.4 ± 1.8	10.2 ± 13	8.6 ± 8	8.2 ± 7	8.8 ± 6
Alcohol consumption (drinks/wk)	5.8 ± 1.1	7.1 ± 2.0	7.1 ± 6	9.4 ± 8	9.7 ± 15	9.8 ± 8
Cigarette use (cigarettes/wk)	6.6 ± 2.4	0.2 ± .1	1.9 ± 6	0.4 ± 1	0.7 ± 2	2.5 ± 9
Cannabis use (uses/mo)	4.8 ± 1.7	3.7 ± 2.0	4 ± 7	3.6 ± 5	7.6 ± 9	8.1 ± 14
Past Drug Use (% ever used)						
Cannabis	26	60	77	91	92	93
Stimulants	26	20	35	9	67	80
Opiates	21	13	54	55	25	14
Tranquilizers	0	7	19	9	25	7
Hallucinogens	21	20	42	36	75	73

Table 4.2. Comparison of demographic characteristics and past and current drug use in the Paired and Unpaired Groups in three separate AMP CPP studies. Data represent N's, mean ± SEM, and the percent of participants in the group

Compared to other studies, DSST performance was poorer in this study, both at baseline and after AMP administration (Table 4.4). Subjects in the present study completed fewer correct symbols on PL in this study than in another large study (N=386) conducted in this laboratory (Wardle et al., 2013; $t(407) = 2.52$, $p = .012$; Table

4.4). Further, in the present study AMP did not improve DSST performance, whereas in the Hart et al study AMP improved performance placebo vs AMP $t(384) = 8.35$, $p < .001$; Table 4.4). For example, subjects overall did not perform as expected on the DSST in response to AMP, or even at baseline (in the absence of drug). It is not clear why AMP did not improve DSST performance in this study, considering that numerous previous studies have shown that AMP improves attention, psychomotor function, and cognitive speed (Wachtel and De Wit, 1999; Silber et al., 2006), as well as DSST performance itself (Ward et al., 1997; Wachtel and De Wit, 1999; Stoops et al., 2006; Makris et al., 2007; Lile et al., 2011). Clearly, the DSST, as administered in this study, was not a valid measure of cognitive performance. We do not know why DSST performance differed from previous studies, either at baseline, or why after AMP.

Study	**Childs and de Wit (2011)**		**Study 1**		**This Study**	
Group	*Paired Group (n = 19)*	*Unpaired Group (n=15)*	*Paired Group (n = 26)*	*Unpaired Group (n=11)*	*Paired Group (n = 12)*	*Unpaired Group (n=15)*
POMS Arousal (0-8 scale)	1.26 ± .49	1.78 ± .73	1.53 ± .46	2.12 ± .45	1.49 ± .62	1.13 ± .49
POMS Positive Mood (0-8 scale)	.707 ± .14	.716 ± .52	.697 ± .46	1.18 ± .48	1.18 ± .46	1.05 ± .34
ARCI Amphetamine (0-10 scale)	3.16 ± .83	4.00 ± 1.0	3.31 ± .69	3.95 ± 1.16	4.08 ± .96	3.75 ± 1.12
DEQ Feel (0-100 scale)	21.1 ± 6.3	45.7 ± 8.3	35.3 ± 5.7	23.3 ± 8.67	34.6 ± 9.45	25.9 ± 6.8
DEQ High (0-100 scale)	23.3 ± 6.4	41.8 ± 8.3	28.4 ± 5.8	21.3 ± 8.26	31.5 ± 9.32	27.1 ± 7.3
Systolic BP	-5.22 ± 6.6	4.27 ± 9.6	22.7 ± 5.1	17.0 ± 3.51	18.4 ± 5.59	25.4 ± 6.7
HR	-3.16 ± 4.2	4.87 ± 3.4	5.77 ± 3.1	4.09 ± 5.0	10.5 ± 6.50	11.1 ± 5.4

Table 4.3. Comparison of the subjective and cardiovascular reactions to the first dose of 20mg AMP - the first PL dose in the Paired and Unpaired Groups in three separate AMP CPP studies. Data represent the difference in the mean peak change from baseline ± SEM.

AMP also did not reduce lapses of attention on the SRT in this study, in contrast to previous studies (de Wit, 2009; Weafer and de Wit, 2013; Table 4.4). At baseline, and after the first administration of AMP, the results of this study were similar to previous studies (Table 4.4).

In summary, AMP in this study produced effects on mood and cardiovascular function that were similar to those in previous studies, but for reasons that are not understood, the drug did not produce its prototypic effects on cognitive measures.

Study	**Hart et al. 2013**		**This Study**	
Drug	*PL*	*AMP (difference from PL)*	*PL*	*AMP (difference from PL)*
Number of Correct Symbols (DSST)	77.4 ± .68	5.16 ± .68	70.2 ± 2.70	-3.08 ± 4.01
Deviation from the Mode (SRT)	42.8 ± 2.02	-17.5 ± 3.03	36.7 ± 4.11	-9.93 ± 4.78

Table 4.4. Comparison of DSST and SRT performance at baseline (PL) and after 20mg AMP (difference from PL) in two separate studies. PL data represent the mean score during the first PL session ± SEM. AMP scores represent the mean difference between the score during the first AMP session and the first PL session ± SEM.

4.5.3 Robustness of the Animal CPP Paradigm

We can also consider these findings in light of CPP studies with laboratory animals. That is, despite its widespread use in studies of drug reward (Tzschentke, 1998), there are also numerous reports that the CPP findings in animals depend on species, strain, dose, and other testing conditions. For example, CPP with ALC develops readily in mice but not in rats (Cunningham et al., 1993). Further, different strains of mice vary in sensitivity to the length of the conditioning trial in CPP with cocaine (Cunningham et al., 1999). Also, the strength of cocaine CPP depends on the cocaine

dose (Risinger and Oakes, 1996; Brabant et al., 2005). Finally, in studies with cocaine CPP, cocaine can induce a preference, an aversion, or no effect at all, depending on the interval between the drug injection and placement in the chamber (Ettenberg et al., 1999; Pliakas et al., 2001). Thus, as in CPP studies with laboratory animals, the CPP procedure in human subjects may depend greatly on the conditions under which testing occurs, in ways that are as yet unknown.

4.5.4 Summary

In this study, we first aimed to determine whether the number of conditioning sessions affected the strength of the conditioned response in an AMP CPP paradigm, and secondly, we aimed to measure more facets of the conditioned response to the AMP-paired room. In contrast to previous findings, subjects in the Paired Group did not report increases in subjective preference for and liking of the AMP-paired room, nor did they spend more time in the AMP-paired room following conditioning. Therefore it was difficult to test the hypothesis that more pairings would lead to stronger preference, either subjective or objective (time spent in drug-paired room). Subjects also did not demonstrate any context-dependent changes in the response to AMP, nor did they exhibit any context-dependent drug responses during the final post-test session in the absence of drug. Differences in design (within- vs between-subjects), drug use histories of the participants, or unusual cognitive responses to AMP may have contributed to these results.

4.5.5 Future Directions

Future studies should incorporate methodological changes that will increase the probability of inducing a CPP in humans. This can be accomplished by reducing the influence of repeated testing, and by preventing issues associated with drug dose and subject demographics. First, to avoid confounds associated with repeated testing in a within-subjects design, such as assessment reactivity and regression towards indifference, studies should test the effect of the number of conditioning sessions on conditioning *between* subjects. That is, they should include, for example, a two session group, a four session group, and an eight session group. In this way, each subject will be less susceptible the effects of repeated testing and therefore may exhibit less assessment reactivity and regression towards indifference. Reducing the influence of these behavioral confounds will help uncover any true conditioning effects. Second, future studies should incorporate multiple doses of AMP in order to capture the dose that elicits the highest rewarding effects. As mentioned previously, subjects vary in their subjective response to AMP, and this is related to conditioning (Childs and de Wit, 2009, 2013; Study 1). It is necessary to understand the relationship between AMP dose and CPP to determine the optimal methods for producing a CPP. Finally, future studies should use a more varied subject sample. Using subjects with varying degrees of past drug use and who vary on demographic measures will improve the chances of determine what factors underlie conditioning, and thereby will better inform future researchers on what individuals are more susceptible to CPP. Overall, if future studies use a between-subjects design, multiple doses, and a more heterogeneous subject sample, this will improve the chances of eliciting a strong CPP.

4.5.6 Conclusion

This study demonstrates that the AMP CPP paradigm in humans needs further refinement. This protocol was limited by many factors. Future studies would benefit from a closer examination of the protocol to prevent methodological issues associated with a within-subjects design, such as assessment reactivity, regression to indifference, and the timing of preference tests, as well as with using a single dose of AMP in a relatively homogenous sample. The human CPP literature continues to grow, confirming that it is indeed possible to establish a CPP in humans. Given the overwhelming influence drug-paired contexts on relapse to drug taking (Wikler and Pescor, 1967; Stewart and Eikelboom, 1987), it is integral that we continue to refine the human CPP paradigm with humans. Eventually, we hope to use this protocol to test ways to ameliorate drug-context associations and to prevent relapse.

Chapter 5: Final Comments

5.1 Aims of this Project

The purpose of this thesis project was to replicate and extend a novel AMP CPP paradigm in humans. In an effort to validate the standard animal CPP paradigm, we aimed to establish an objective measure of conditioned preference; namely, we sought to determine whether individuals would who consistently received AMP in one room would choose to spend more time in that room after conditioning. In addition, we examined individual differences in the strength of conditioning in these individuals to determine if factors such as personality or the acute subjective response to drugs influence conditioning. Finally, we aimed to expand this paradigm to determine the optimal methods for inducing the strongest conditioned preference. In that study, we tested whether the number of conditioning trials affected the overall preference for the AMP-paired room. Also, we adopted a multidimensional approach to studying the CR to the AMP-paired room to define the factors that may underlie the preference that develops for this room. We hope that these studies provide cause to continue refining the human CPP paradigm with drugs of abuse. Once researchers are able to establish a strong, objective, multifaceted CR in a drug-paired environment using the human AMP CPP paradigm, others can determine the factors that underlie individual differences in

drug reward and conditioning, and ultimately develop methods to preventing context-induced drug-seeking.

5.2 Summary of Findings

In Chapter 2 (Study 1), we successfully replicated a previous AMP CPP study in humans, wherein they used subjective measures of conditioning, yet we were unable to evoke an objective preference for the AMP-paired room, like what is often measured in animals (Childs and de Wit, 2013). In this study, healthy human volunteers who received AMP twice in the same room (the Paired Group) exhibited an increase in their subjective liking of and relative preference for this room after conditioning, and this increase in subjective preference correlated with the degree to which they reported liking the effects of the AMP. Even though the subjects *reported* an increase in preference for the AMP-paired room, they did not exhibit an objective preference for this room; that is, they did not spend more time in the AMP-paired room after conditioning, relative to before conditioning.

For Chapter 3 (Study 2), we combined the data from Study 1 and the study it replicated (Childs and de Wit, 2013) and analyzed the Paired Group for individual differences in the expression of conditioning in the CPP paradigm. We calculated whether the personality traits PEM and NEM moderated the relationship between the positive subjective effects of AMP and the change in subjective preference for the AMP-paired room. We found that AMP-induced euphoria and PEM both independently predicted AMP CPP, but neither PEM nor NEM moderated the relationship between AMP-induced euphoria and conditioning.

The purpose of the study in Chapter 4 (Study 3) was to expand and refine this AMP CPP paradigm to a) strengthen the CR to the AMP-paired room and b) detect more facets of the CR. Here, Paired Group subjects underwent four AMP-room pairings, and we measured the CRs to the rooms at several points throughout conditioning. Like in Study 1, we measured the subjective liking of, relative preference for, and time spent in the AMP-paired room, but in addition, we also measured several other subjective, cognitive, and physiological responses to the room. We anticipated that subjects would not only express CRs to the AMP-paired room in the absence of drug, but also that their acute responses to the AMP would become greater with more exposures in the same room. In this study, Paired Group subjects showed neither a subjective nor objective preference for the AMP-paired room after any number of conditioning sessions. Also, they did not exhibit any consistent, enhanced subjective, cognitive or physiological responses to either AMP after repeated exposures in the AMP-paired room or to the AMP-paired room after conditioning.

From our results in Study 1 and Study 2, it seems that a strong predictor of AMP CPP is the subjective response to AMP. To the extent that positive subjective responses to a drug are predictive of abuse liability, CPP may also predict drug abuse liability in humans, as it appears to do in nonhuman animals. In the first study, self-reported AMP "liking" was correlated with the increase in subjective preference for the AMP-paired room, and in the second study, AMP-induced euphoria independently predicted this preference. In Study 3, however, the subjective response to AMP did not predict the degree of preference, but this is likely because conditioning did not occur. Overall, our data support previous findings that the positive subjective effects of AMP predict AMP

CPP (Childs and de Wit, 2009, 2013). This relationship may relate to individual differences in the drug abuse liability, as it has been shown that positive subjective responses to drugs predict an enhanced neural response to drug cues and an increased probability of future drug use (de Wit and Phillips, 2012; Courtney and Ray, 2014). It can be determined whether contextual conditioning provides additional information about future drug use, independently of the relationship between the subjective response to drugs and future drug use. Related to this question, it may also be possible to determine whether drug-seeking or craving behavior increases in the drug-paired environment following conditioning in the CPP paradigm.

Additionally, our data show that personality is also a strong predictor of contextual conditioning with AMP, but that personality does not moderate the relationship between AMP's positive subjective effects and the degree of conditioning. PEM independently predicted the change in preference for the AMP-paired room in Study 2, but it appears that the strength of the relationship between the positive subjective response to AMP and CPP is not affected by PEM. This is surprising, given that positively valenced personality traits predict the subjective response to AMP in healthy humans (Kirkpatrick et al., 2013; Kirkpatrick et al., 2015), which would suggest that that high PEM would strengthen the relationship between AMP's subjective effects and conditioning. However, despite that positive personality traits are known to predict AMP-induced euphoria, we discovered that PEM itself does not related to AMP-induced euphoria. This may explain why PEM did not moderate the relationship between AMP-induced euphoria and conditioning. Future studies can determine whether other personality traits are necessary or sufficient for moderating the relationship between the subjective effects of AMP and conditioning.

In Study 1 we also compared subjects' responses to AMP during the two administrations of AMP (sessions 1,2 or 3,4 in the Paired Group). In contrast to our previous finding that subjects reported *enhanced* stimulation and greater wanting *more* drug during the second administration (Childs and de Wit, 2013), in Study 1 we found that subjects reported *lower* ratings of "wanting more" AMP during the second administration. It is unclear why different patterns were observed in Study 1 and Childs and de Wit (2011). In our CPP procedure, as in other conditioning paradigms, there are two possible sources of information about contextually conditioned drug effects. One is the change in response to the drug during repeated administrations in the same context, and the other is responses to the drug-paired context in the absence of the drug. The two measures may reflect a single underlying conditioning effect, or they may develop separately. The present findings, of a conditioned response at post-conditioning test, without a change in response during conditioning, would suggest that the two processes may be independent.

It was surprising that subjects did not spend more time in the AMP-paired room following conditioning in Study 1, despite exhibiting a subjective preference for this room following conditioning. This is especially notable because in a previous CPP study with alcohol, subjects both preferred and spent more time in the ALC-paired room following conditioning (Childs and De Wit, *in prep*). Why subjects spent more time in an ALC-paired environment, but not an AMP-paired environment, after conditioning may relate to the subjective effects of the two drugs. Compared to the subjective effects of ALC, the subjective effects of AMP are modest (See 2.5). It is possible that the process of acquiring subjective and objective CRs recruits discrete neurobiological mechanisms that may rely on different procedural parameters to develop (Stephens et

al., 2010; Stephens et al., 2013). In other words, our AMP CPP protocol may be optimal for evoking a subjective preference for a drug-paired room, but not for evoking an objective preference. The best method for determining whether a strong subjective drug response is sufficient for inducing a significant increase in time spent in a drug-paired room, regardless of drug, would be to perform the CPP procedure with multiple doses of AMP and ALC.

In summary, these studies show that humans can develop a subjective preference for an AMP-paired environment, and that this preference is related to personality and the subjective effects of the drug. However, questions remain as to why we were unable to elicit an objective preference for the AMP-paired room. These studies establish a demand for further research into the personality and subjective factors that influence conditioning, as well as into the methods that are necessary for inducing a strong CPP.

5.3 Individual Differences in Human CPP

The human CPP procedure allows us to more closely examine individual differences in conditioning, and even from the limited number of human CPP studies performed so far, we have learned a great deal about what factors contribute to a strong CPP. For instance, in Study 1 and Study 2, we found that AMP CPP is related to individual differences in the positive subjective response to the drug. Perhaps related to the effect of the acute subjective response to drugs on conditioning, evidence suggests that one's susceptibility to contextual conditioning is also related to their predisposition to the rewarding effects of the US.

The present studies add to our observation that sensitivity to the rewarding effects of the US is important for evoking a CPP. For instance, the ALC CPP study, which is the only study wherein individuals have shown an increase in time spent in a drug-paired room following conditioning, used a population that was predisposed to experiencing positive subjective responses to the ALC (Childs and de Wit, *in prep*). This study used moderate drinkers, a group who presumably liked the effects of ALC more than the general population, and therefore were more sensitive to its effects (King et al., 2002; Courtney and Ray, 2014). The subjective response to ALC in that study was indeed robust compared to the subjective response to AMP in the current studies (e.g., see 2.5). In a similar scenario, in a CPP study with a candy reward, subjects only spent more time in the candy-paired environment if they were hungry (Astur et al., 2014). In a related study, preference for the candy-paired room was stronger in subjects who were dieting (Astur et al., 2015). These studies demonstrate the sensitivity of contextual conditioning to one's predisposition. Taken even further, data suggests that personality influences contextual conditioning as well.

Finally, human CPP studies have revealed that personality predicts CPP. For example, we showed that PEM predicts AMP CPP. Another study showed that the strength of CRs to a food-paired room is related to impulsivity. While limited in number, these studies demonstrate that individual differences in personality predict one's susceptibility to contextual conditioning, and call for further research into to the factors that influence one's response to a reward-paired environment. Overall, all of these studies demonstrate the unique advantages of the human CPP paradigm, in that it makes it easier to study individual differences in conditioning.

5.4 The Potential of the Human CPP Paradigm

Humans provide many advantages over nonhuman animals in the CPP paradigm, and may lead to new insights in the study of contextual conditioning in humans. The biggest advantage in studying humans is the ability to capture self-report measures. With this ability, we confirmed for the first time that the strength of the conditioned preference for an AMP-paired room is related to how much one likes the subjective effects of the drug (Childs and de Wit, 2009, 2013; Study 1). We also showed that robust subjective responses to AMP are not sufficient to evoke a CPP. Finally, also using self-reports, we learned that PEM predicts acquisition of a CPP (Study 3). It would be impossible to observe any of these findings in animals.

Future CPP studies in humans could exploit even more of the unique advantages that humans provide. For instance, for nonhuman species, outward behavior, and in some cases, physiology, are the only indicators that we have of their internal emotional state. In humans, since we can measure subjective responses, behavior, and physiology concurrently, we can learn how these three domains relate to each other in the context of CPP. For instance, a pressing question in the animal literature is why animals choose to spend time in a chamber in which they previously received a reward (Spiteri et al., 2000; Stephens et al., 2010; Huston et al., 2013; Stephens et al., 2013). Researchers are still unsure as to whether animals stay in the reward-associated chamber because a) they are looking for more of the reward, b) they are waiting to receive more of it, or c) because they experience the reward's subjective effects in that environment. By combining the behavioral measures used in animals with subjective measures in humans, we can begin to understand why animals choose to spend time in a drug-

associated chamber. In addition, applying physiological measures may reveal the etiology of these behavioral and subjective CDRs. In a future iteration of the CPP paradigm, where robust conditioning occurs, researchers can apply subjective, behavioral, and physiological measures to elucidate the mechanisms that underlie conditioning.

5.5 Implications within the Field

Study 1 confirmed the validity of the AMP CPP paradigm, as we replicated findings from a previous AMP CPP study (Childs and de Wit 2011) that showed that individuals will increase their subjective preference for an AMP-paired environment. Additionally, our analysis of individual differences in CPP (Study 2) showed that there are definite personality traits that predict the magnitude of AMP CPP. Overall, these studies showed that the human CPP procedure can be used to elicit cognitive associations between a drug effect and a room, and that the development of these associations can be predicted by individual differences in personality. In the future, we can, in theory, learn how these associations relate to context-induced drug seeking, and ultimately, we can develop methods for breaking these associations and preventing context-related drug use. Study 3 showed that this paradigm is still in need of improvement. Nevertheless, all of these studies contributed information to the field that will be useful for creating a stronger human CPP paradigm using drug rewards.

The studies presented here offer just one small addition to the greater effort to translate the CPP paradigm, one of the most reliable animal models of contextual conditioning in animals, to humans (Tzschentke, 2007). As evidenced by the few drug

CPP studies completed in humans so far (Childs and de Wit, 2009, 2013, *in prep*), as well as the ones presented here, this paradigm is still underdeveloped. Hence, it would be premature to form conclusions regarding the reliability of this paradigm overall. The inconsistencies in our results show that much more work must be done to fully understand the intricacies of the CPP paradigm in animals and how to model preference behavior in humans with the greatest possible fidelity. The studies presented here should inform future researchers about what methods help and hurt the effort to produce contextual conditioning with AMP in humans. While we did not make any major advancements through our discoveries here per se, we did put forth the substrate on which future researchers will develop a more efficacious CPP paradigm. For instance, we are now aware of the hazards of repeated testing, and of using a single dose of AMP in a small, homogenous sample. These small steps provide an impetus to continue studying the CPP paradigm in humans. Our hope is that from these three studies, future researchers will derive methods on which they can build to create a better, more reliable AMP CPP paradigm for humans. Regardless of these methodological issues, however, it is still questionable, whether the CPP paradigm is relevant to humans in general, and whether the human CPP paradigm is worthy of further attention.

Humans differ in many ways from animals that may reduce their susceptibility to place conditioning. Humans are capable of thinking in ways that rodents and lower animals cannot, and human cognition could potentially interfere with place conditioning with drugs. For example, animals are not aware that they will not receive drug during the test session, which may affect their motivation to seek out more of the drug effect by exploring the reward-paired environment (Huston et al., 2013). Humans,

however, are aware of this contingency, and therefore, the fundamental expectations that could be leading an animal to spend time in a reward-paired environment might not exist in humans. A second issue is that exploratory behavior is not clearly defined in humans, and exploring a moderately familiar environment may even feel unnatural in the context of conditioned place preference, suggesting that humans and animals use different strategies for learning about and expressing interest in particular environments. Finally, we cannot control for a human's past experience before they enter the CPP paradigm in our laboratory. Unlike animals, who live their whole lives in a controlled laboratory environment, humans are exposed to unique combinations of drugs and cues before they enter the laboratory that may affect conditioning. For these reasons, among others, the CPP paradigm may not be relevant in human subjects.

5.6 Final Comments

Standard practice in pre-clinical psychopharmacology research is to model human behavior and disease using nonhuman paradigms. This approach in itself is fraught with challenges, as it is near impossible to completely mimic the complexities of human behavior and pathology in another species (Stephens et al., 2013). Here, we took one example of this approach, the CPP paradigm, and turned it around: we attempted to replicate an animal behavior representative of a human cognitive process, in humans. It is still poorly understood how the behavior seen in animals in the CPP apparatus applies to drug-seeking behavior in humans; much less, we are only beginning to determine how to model this uniquely animal behavior in human subjects. We are still far from understanding the animal CPP paradigm to the extent that we can create a reliable

human version of the paradigm. Nonetheless, using human subjects is advantageous for many reasons, and while we did not find much success in this project, the human CPP is worth continuing to pursue in the long term.

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