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THE EFFECTS OF SOCIAL AND NONSOCIAL CONTEXTUAL STIMULI ON THE RENEWAL OF COCAINE SEEKING

Bree Humburg
University of Kentucky, bhumburg72@gmail.com
Author ORCID Identifier:
https://orcid.org/0000-0002-7223-7117
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Bree Humburg, Student

Dr. Michael T. Bardo, Major Professor

Dr. Michael T. Bardo, Director of Graduate Studies

THE EFFECTS OF SOCIAL AND NONSOCIAL CONTEXTUAL STIMULI ON THE RENEWAL OF COCAINE SEEKING

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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By

Bree Ann Humburg

Lexington, Kentucky

Director: Dr. Michael T. Bardo, Professor of Psychology

Lexington, Kentucky

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https://orcid.org/0000-0002-7223-7117

ABSTRACT OF THESIS

THE EFFECTS OF SOCIAL AND NONSOCIAL CONTEXTUAL STIUMLI ON THE RENEWAL OF COCAINE SEEKING

Those with substance use disorders can undergo craving and relapse when re-exposed to a drug-associated context. This study determined if renewal of cocaine seeking is differentially controlled by contexts consisting of social and/or nonsocial stimuli. Experiment 1, rats self-administered cocaine in Context A which included a social peer and house light illumination. Following self-administration, rats were randomly assigned to an AAA or ABA group for extinction and renewal. For the AAA rats, context was similar to self-administration; for ABA rats, the drug-associated stimuli (peer and house light) were removed (Context B). Following extinction, renewal of cocaine seeking was examined by testing the peer alone, house light alone, or the combination. Experiment 2 was similar, except only a house light (no peer) was used throughout the experiment. Results revealed rats acquired cocaine self-administration and extinguished lever pressing for both experiments. For Experiment 1, ABA rats renewed cocaine seeking to the peer alone and peer+house light but not the house light alone. Experiment 2 found ABA rats renewed cocaine seeking to the house light alone, but the AAA group did not. These data indicate that social peers serve as powerful stimuli that can overshadow nonsocial stimuli in the renewal of cocaine seeking.

KEYWORDS: Renewal, Cocaine, Contextual, Social, Nonsocial

Bree Ann Humburg

(Name of Student)

04/25/2023

Date

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By
Bree Ann Humburg

Dr. Michael T. Bardo
Director of Thesis

Dr. Michael T. Bardo
Director of Graduate Studies

04/25/2023
Date

DEDICATION

To all of the incredible friends I have made along the way, especially Mason Coats.

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CHAPTER 1. INTRODUCTION

1.1 Cocaine

Cocaine is a naturally occurring alkaloid from Erthroxylum coca (coca plant) which is native to the Andean Highlands and northern parts of the Amazon in South America (Drake & Scott, 2018; Iversen, 2009). Cocaine has been around for centuries, beginning with indigenous South Americans chewing on dried coca leaves or soaking the leaves for tea (Jatlow, 1988). There are two forms of cocaine. Pure cocaine (aka- blow, C, snow) is a hydrochloride salt that is water soluble and comes in the form of white powder. This is typically insufflated through the nasal cavity or injected intravenously. "Crack cocaine" (aka- crack, rock) is a free base and is typically a yellow solid that is smoked with a pipe (Drake & Scott, 2018; Iversen, 2009).

Cocaine is readily absorbed into the bloodstream via the routes mentioned above and crosses the blood brain barrier (BBB) easily. When injected or insufflated, intoxication happens within 2-3 minutes. When smoked, the onset of intoxication is even faster, within about 10 seconds. The typical dose of cocaine is around 20-50 mg (Iversen, 2009). One of the first systematic investigations on the different routes of administration of cocaine was performed by Jeffcoat et al. (1989). They investigated intravenous injection, nasal insufflation, and smoke inhalation. They found that cocaine's bioavailability is roughly 80% for nasal insufflation and 57% smoking inhalation. With the different routes of administration, absorption times varied, with nasal insufflation being about 11.7 minutes and smoking inhalation being about 1.1 minutes. For cocaine levels to reach peak concentration, smoking took about 6 minutes, while insufflation took about 45 minutes. It has been reported that the elimination half-lives when insufflated cocaine's elimination

half-life is about 78 minutes, while smoked cocaine's elimination half-life is about 69 minutes (Drake & Scott, 2018; Jeffcoat et al., 1989). Looking further at the metabolism and elimination, Jatlow (1988) reported that about 85-90% of administered cocaine is found as metabolites in the urine. Once eliminated, cocaine as the parent compound is present as only about 1-5% in urine, while roughly 75-90% is ecgonine methyl ester (EME) and benzoylecgonine (BE) combined. Cocaine is rapidly eliminated from the body with a biological half-life of about one hour and total body clearance of about two liters per minute (Iversen, 2009; Jatlow, 1988). However, the metabolites of cocaine EME and BE eliminate slower and have half-lives of about 5 and 8 hours respectively (Jatlow, 1988).

1.1.1 Mechanisms of Action

Before cocaine was studied as a drug of abuse, it was studied and used as a local anesthetic. Back in 1884, cocaine was starting to be used in surgeries as an anesthetic because it numbs the mucous membranes. Based off of that, Dr. Carl Koller used cocaine as an anesthetic to be used for the first painless cataract surgery. It is reported that the anesthetic pharmacodynamic actions are because of an inhibition of voltage-gated sodium channels and the stopping of neuronal potentiation (Drake & Scott, 2018).

Cocaine is similar to the naturally occurring catecholamine neurotransmitters dopamine (DA) and norepinephrine (NE). Cocaine acts by making naturally occurring neurotransmitters more available by blocking/inhibiting their reuptake via the neurotransmitter transporters (Drake & Scott, 2018; Iversen, 2009). The rewarding effect of cocaine is due primarily to its action at the DA synapse, specifically in the nucleus accumbens (NAc). Here, cocaine prolongs dopamine activity by blocking the DA reuptake

mechanism via the transporters (DAT) (Wise, 1984). Extracellular DA binds to a postsynaptic receptor via G protein-coupled receptors (GPCRs) that leads to a response. DA receptors are classified as either D1-like or D2-like, depending on the function and pharmacological criteria. D1-like receptors are coupled to Gs protein which increases adenylyl cyclase activity, whereas D2-like receptors are coupled to Gi protein which decreases adenylyl cyclase activity.

Cocaine is not selective for DAT, however, as it also blocks NE reuptake (Drake & Scott, 2018). Furthermore, cocaine blocks serotonin reuptake (Filip et al., 2005; Matsui & Alvarez, 2018). Recently, researchers have studied the interactive effects of cocaine with serotonin (5-HT) systems. The ventral pallidum (VP) is a part of the basal ganglia circuitry that receives dense serotonergic input from the raphe nucleus and is known to be activated during cocaine self-administration (Sizemore et al., 2000). Until recently, modulation of VP firing was thought to work indirectly through cocaine's actions on NAc DA. However, Matsui & Alvarez (2018) reported that there is selective stimulation of indirect pathway projections to VP neurons which leads to synaptic transmission inhibition by cocaine directly. When these projections are optogenetically stimulated it leads to evoking 5-HT transients and the GABAergic transmission to VP neurons is inhibited (Matsui & Alvarez, 2018). Overall, this study reveals that 5-HT acts as the main neuromodulator rather than DA with regards to cocaine's effects in the VP.

1.1.2 Abuse Potential and Clinical Relevance

Worldwide, there is a large concern with the number of drug overdose-related deaths. The opioid crisis is troubling, with fentanyl and its analogs being easily obtainable

and tainting other drugs of abuse. Fentanyl and its analogs have highest reporting of drug related overdose deaths (NIDA, 2022). While this is a serious problem, cocaine-related overdoses should not be ignored, as they continue to increase as well. According to the World Drug Report, about 21.5 million people worldwide have used cocaine at least once within the year of 2020 (UNODC, 2022). The National Institute on Drug Abuse (NIDA) reported that cocaine-related overdose deaths have increased from 3,822 in 1999 to 24,486 in 2021 (NIDA, 2022). Cocaine use disorder (CUD) is associated with high relapse rates, with about 24% of cocaine users relapsing within the first year after seeking treatment (Tackett, 2023).

To help prevent relapse and overdose from cocaine, behavioral therapies are currently the only option, as there are no FDA-approved medications available. Cognitive Behavioral Therapy (CBT) has been shown to help treat substance use disorders (SUDs), including CUD, but the effect sizes are quite modest (Decker et al., 2018). Therefore, finding other forms of treatment is greatly needed.

1.2 Conditioning Factors in Relapse

As many in the field are aware, context plays a significant role in the rates of relapse when it comes to SUDs (Neisewander et al., 2012; O'Brien et al., 1992). When someone with a SUD goes to in-patient rehabilitation and is no longer around the same environment where they used drugs, being reintroduced back into the previous drug-associated environment prior to treatment can lead to craving and relapse. Walking by their drug dealers house or seeing a used needle are both stimuli that can lead to craving and relapse (Jaffe et al., 1989; Robbins et al., 1999; Xue et al., 2012). When it comes to animal models

of relapse, reinstatement is commonly used. With reinstatement, animals are first trained to lever press or nose poke for some kind of reinforcer like food (Grimm & Sauter, 2020), drug (Bossert et al., 2013), or even a social peer (Venniro & Shaham, 2020). After establishment of that behavior, rats begin either forced extinction by removing access to the reinforcer within the operant chamber or forced abstinence in the home where they do not get access to the operant chamber for a certain period of time. In addition to those options, a voluntary abstinence paradigm allows rats to choose an alternative reinforcer over the initial primary reinforcer (e.g., choice of food over drug). Reinstatement testing is then conducted by presenting a drug prime (Ma et al., 2013), a specific drug-associated cue (Namba et al., 2018), or stressful stimulus (Fulenwider et al., 2020) to measure the return of responding for the reinforcer.

Another way to model relapse is by using a related paradigm typically referred to as context-induced reinstatement or "renewal". This preclinical model has good clinical relevance because it focuses on the environmental context in which the drug is experienced. As mentioned above, those with SUD typically use drugs in a distinct context. During treatment at a rehabilitation center, individuals are exposed to a new context in which learning of new behaviors occurs and coping mechanisms are practiced to avoid drug use. Once these individuals are reintroduced into their old drug-taking context, craving and relapse are at a much higher risk. To model this clinical situation, the renewal paradigm involves training an animal to make an operant response (e.g., lever press or nose poke) for drug reward in one context (context A) and then forcing them into extinction where the reward is no longer present in a different context (context B). The renewal test is then conducted by re-presenting context A, with renewal being defined as a return of responding

relative to responding in the extinction context B (Berry et al., 2014; Eddy et al., 2016; Sweeney & Shahan, 2015)

As mentioned above, the ABA renewal design is where an animal responds for a reinforcer in one distinct context (context A), their behavior is extinguished in a different distinct context (context B) and then the animal is re-introduced into the original context (Context A), but without the reinforcer. Context A stimuli can be visual stimuli like wall patterns in the operant chamber (Todd et al., 2014) or light illumination patterns (Podlesnik & Shahan, 2009). Since rats have a well-developed sense of smell, odor manipulations are also often used in this species (Bouton et al., 2011). Context B is typically a different set of stimuli that use the same sensory modality used in context A, e.g., (horizontal vs vertical striped walls; flashing vs constant light illumination; almond vs. banana odor). In addition to visual, tactile, and odor stimuli, researchers have recently started to use social peers as contextual stimuli in the renewal paradigm, as described in the next section.

1.3 Social Influences on Drug Use and Relapse

Previous literature has shown that when it comes to drug use and relapse, social peers are able to serve as protective stimuli by helping decrease the chances of use or relapse (Stevens et al., 2015). By having a good support system and encouraging people around those who are trying to abstain from drugs can be highly beneficial and protective (Panebianco et al., 2016). On the other hand, social peers can serve as contextual stimuli that accelerate the rate of drug use (Smith, 2012) and can lead to relapse (Eitan et al., 2017). For example, social pressures from a friend offering drugs at a party increases the chances of one trying a drug for the first time (Strickland & Smith, 2014). As for relapse, those with

SUD often use drugs around their friends and drug dealers, leading to those peers becoming contextual stimuli; however, there has been little work to confirm that these drug-using peers can trigger relapse directly.

Similar to humans, most mammals, including rats, find social interaction to be rewarding. There are a handful of preclinical models that have examined social reward and how it plays a role in modulating drug self-administration, which will be discussed later. Social play during adolescence is highly important for development. This form of social interaction during adolescence in rats is called "rough and tumble" play (Vanderschuren et al., 2016). In this model, rats are observed when they partake in a behavior like play fighting. Play fighting involves contests in which the nape of the neck is attacked and defended, and where successful contact involves rubbing the snout into the fur (i.e. nosing) (Pellis & McKenna, 1992).

Not only is social play important for normal development, it has been shown to be highly rewarding. The endogenous opioid system is activated when playing occurs, thus producing a form of hedonic pleasure that can function as a reward like food and sex (Kennedy et al., 2012; Lampe et al., 2019; Vanderschuren et al., 2016). Interestingly, changes in social interactions during development can coincide with changes in behavior like increased risk taking and drug seeking. When rats are in these playful interactions, they have specific, positively valenced vocalizations that can produce conditioned place preference (CPP), indicating that rough and tumble play is associated with a positive affective state (Lampe et al., 2019).

1.3.1 Conditioned Place Preference

While most social interactions can be beneficial and rewarding, social stressors also control affective-like behavioral responses across multiple species. In rodents, social defeat stress produces robust depression-like phenotypes that result in anhedonia and social avoidance (Ayash et al., 2020; Carnevali et al., 2020; Golden et al., 2011). In one form of this model, C57BL/6J mice are repeatedly subjected to bouts of social defeat by a larger CD-1 mouse screened for aggressive behavior. While the physical defeat itself lasts about 5-10 minutes, the defeated C57BL/6J mouse is subjected to further psychological stress by being exposed to the dominant CD-1 mouse in the home cage via a clear perforated divider (Golden et al., 2011). After this exposure, the defeated mice develop social avoidance and anxiety-like behaviors, although a small percentage of the population of mice can become "resilient" and do not develop this behavior. Recent studies have targeted this type of resilience and conditioned learning to determine the mechanisms involved in the risk and protective factors associated with this type of stressful behavior (Ayash et al., 2020; Carnevali et al., 2020).

As mentioned above, however, prosocial interactions in rodents are able to produce CPP, particularly when the interaction is with a non-aggressive peer of the same body weight and sex. CPP is a classical Pavlovian paradigm where animals are conditioned to prefer a context that has been paired with a rewarding stimulus. Typically, this paradigm assesses how rewards like sucrose and drugs of abuse can induce a preference to a certain side of a chamber that was paired with the rewarding stimulus. In contrast, conditioned place aversion (CPA) involves inducing an avoidance to a particular context paired with an aversive stimulus.

Several studies have examined the role of social peer interactions using CPP. For example, Calcagnetti and Schechter (1992) found that isolate-housed rats develop CPP to an environment that has been paired with an active social peer. In a more recent study, Yates et al. (2013) examined social CPP in adolescent and adult rats that were either individually housed or pair housed. Individually housed adolescents showed robust social CPP, but no other group showed significant preference, suggesting that the motivational drive for social play is greatest during adolescence. Interestingly, this same study found that when given the choice between a compartment paired with amphetamine and a compartment paired with a social peer, adolescents that were individually housed preferred the social interaction compartment, whereas adolescents that were pair-housed preferred the amphetamine compartment.

There is also evidence suggesting that limited social contact may be sufficient to produce CPP. Kummer et al. (2011) reported that when adult rats are separated by steel bars from their peer, thus preventing full social play, a robust CPP is still obtained. In contrast, in that same experiment, when the rats were separated by a glass partition or just olfactory stimuli were present, CPP was not obtained. These findings indicate that tactile stimuli are more important than visual and olfactory stimuli in producing social CPP. To take this model a step further, other researchers have found a drug stimulus and a social stimulus paired together produce an additive effect that increases the magnitude of CPP compared to the drug or the peer alone (Kennedy et al., 2012; Reyna et al., 2021; Thiel et al., 2008). This suggests that a drug enhances the rewarding effect of a peer, and vice versa. While this model of social interaction demonstrates the effects of social peers on CPP, it has not typically been used to study relapse.

Considerable work has shown that social influences during development have a profound impact on later drug use and relapse. Environmental social enrichment has been studied as a moderator of drug-related behaviors since the 1970s, starting with Bruce Alexander (Alexander et al., 1981). In this model, rats are housed with 8-12 peers in a large cage enriched with plastic objects that are changed daily. In contrast, non-enriched rats are housed in social isolation in hanging wire metal cages without any novel objects. Researchers have examined the acquisition of drug self-administration, relapse rates, incubation of craving, and the neurobiological mechanisms that mediate the effects of socially enriched vs isolated environments (Garcia & Cain, 2020; Grimm et al., 2016; Imperio et al., 2018). In general, it has been shown that social enrichment is protective against the acquisition, craving and relapse with different drugs of abuse and even with non-drug rewards like sucrose (Cain et al., 2006; Chauvet et al., 2012; Garcia & Cain, 2020; Grimm et al., 2016; Hofford et al., 2014; Imperio et al., 2018). While this model clearly demonstrates the importance of social enrichment during development on vulnerability to drug abuse, it does not directly address the role of social peers during the drug taking process, i.e., in this paradigm, rats are tested without any peers present.

To assess drug seeking behavior as a model of relapse, operant-based drug self-administration can be used. With drug self-administration, animals undergo surgery to receive an indwelling catheter and are able to then nose poke or lever press for a delivery of a drug (Beckmann et al., 2011; Childs et al., 2006; Nicolas et al., 2019). Typically, following a period of extinction, the introduction of a drug-associated stimulus is used to assess the return of the extinguished behavior (drug seeking) as a model for relapse. Typically, these stimuli involve nonsocial stimuli like lights, tones, or odors (Bossert et al.,

2013; Namba et al., 2018). Unfortunately, using a social peer in the same chamber as a rat self-administering drug to induce reinstatement is not possible for at least two reasons. First, the peer might press on the lever, which would be an inaccurate representation of the number of infusions the drug-taking rat received on their own. Second, the rats could also get tangled up with the self-administration leash, which could lead to head/back mount injury to the drug-taking rat. To circumvent these problems, researchers have used a dual-compartment apparatus to separate the two peers, allowing for limited social interaction (Smith, 2012; Venniro & Shaham, 2020).

1.3.2 Self-Administration

A new model of preclinical research has been developed to better understand how social peers can influence behavior during the drug self-administration session. Smith (2012) utilized a custom-built dual-compartment operant chamber where rats were able to take drug in the presence of a peer located in an adjacent compartment (Figure 1). In this apparatus, peers are separated by a wire screen partition that allows limited social contact (tactile, auditory, visual, and olfactory stimuli). In one experiment, Smith (2012) had rats self-administer cocaine in the presence of a peer located in the adjacent compartment, with that peer having access to either cocaine or saline. The results showed that, compared to rats that self-administered cocaine alone, cocaine self-administration was facilitated in the rats that had a peer also responding for cocaine, while it was inhibited in rats that had a peer responding for saline. These results suggest that the peer influences drug taking behavior by either facilitation or inhibition, perhaps because the behavior of the peer is imitated.

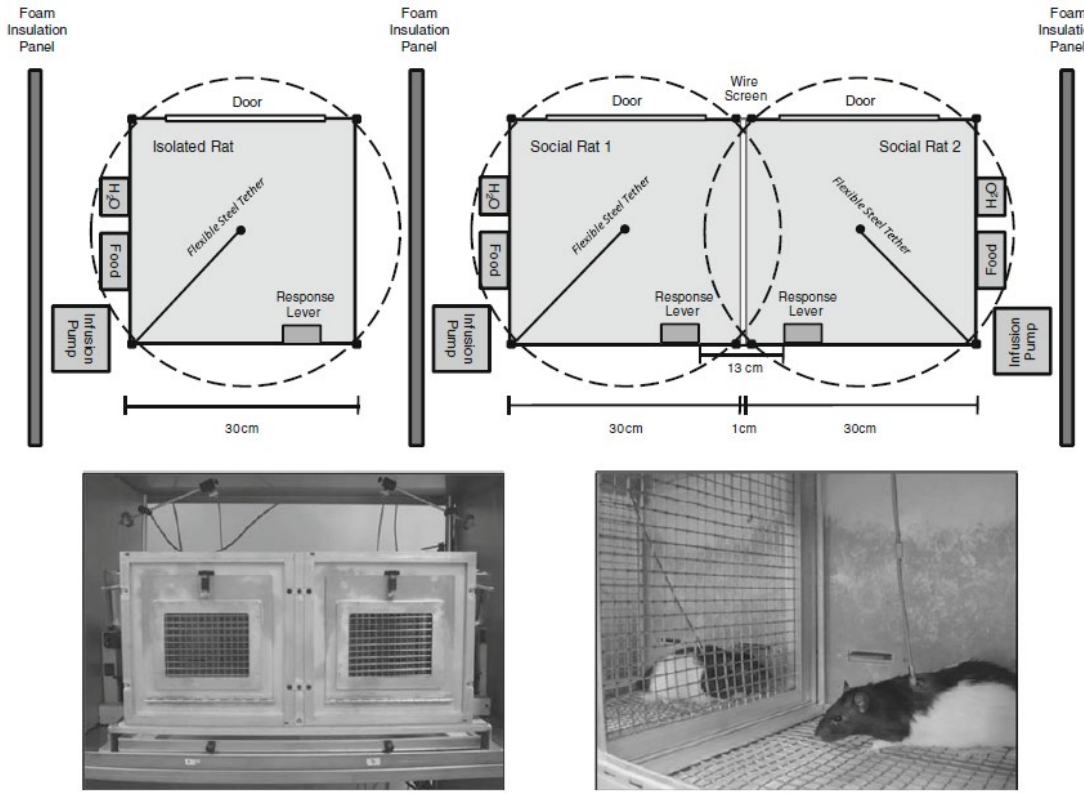

Figure 1-1 Smith, 2012 dual-compartment operant chamber.

To take this a step further, Smith et al. (2016) assessed whether a peer could serve as a stimulus to reinstate cocaine seeking. This experiment used a discrimination procedure where some sessions consisted of cocaine being available in the presence of a cocaine self-administering peer and alternating sessions consisted of saline being available in the presence of a different saline self-administering peer. Following a period of extinction (no peer), neither the cocaine-associated peer nor saline-associated peer elicited a significant change in responding relative to extinction, indicating that peer-induced reinstatement was not obtained. However, responding was greater in the presence of the cocaine-associated

peer compared to the saline-associated peer after extinction. This latter difference may have been obtained because the cocaine-associated peer served as a weak excitatory stimulus to modestly increase responding and the saline-associated peer served as a weak inhibitory stimulus to modestly decrease responding, thus producing a significant between-group difference. While this finding is suggestive, a more robust demonstration of peer-induced reinstatement may have required more conditioning trials and/or a different dose of cocaine than that used by Smith et al. (2016).

One limitation in the study reported by Smith et al. (2016) is that it was not clear if the social peer per se controlled responding or whether the behavior of the peer controlled the responding. This is an important distinction because the cocaine self-administering peer pressed the lever more than the saline self-administering peer and, thus, in addition to the mere presence of the peer, there were additional non-social discriminative stimuli (e.g., lever extension/retraction, sound of infusion pump). As a result, the discriminative stimulus could actually be considered a compound stimulus (social peer + non-social stimuli).

To address this issue, Weiss et al. (2018) used a procedure in which the discriminative stimulus was merely the presence of a passive social peer that did not undergo any training in the adjacent operant conditioning chamber. The purpose was to determine if different peers can serve as discriminative stimuli in a reinstatement test. Acquisition of discrimination training consisted of pairing cocaine self-infusions with one same-sex, same-age peer on some sessions and pairing saline self-infusions with a different peer on alternate sessions. The active lever was kept the same for both saline and cocaine self-administration sessions. In order to ensure robust responding during acquisition, a cue light situated directly above the active lever was illuminated for 20 sec at the beginning of

each cocaine and saline infusion. After self-administration discrimination training, rats were extinguished without a peer, drug, or cue light. The reinstatement tests then consisted of presenting either the cocaine- or saline-associated peer on separate tests in counterbalanced order; cue light illumination was not present during these reinstatement tests. Results from this experiment showed that social peers served as discriminative stimuli for reinstatement of cocaine seeking, as responder rats had significantly higher responding in the presence of the cocaine-associated peer compared to the saline-associated peer.

In another study, Hammerslag et al. (2022) used the same procedure as Weiss et al. (2018) but added a non-social discrete conditioned stimulus (CS; cue light illumination paired with the cocaine infusion). On alternating sessions, rats self-administered cocaine in the presence of one peer (S+) and saline in the presence of a different peer (S-); the non-social CS was presented with each infusion (both cocaine and saline). After self-administration, rats went through extinction (no peer, no cue lights, no infusion) followed by 6 reinstatement tests as follows: (1) extinction session; (2) S+ only; (3) S- only; (4) CS only; (5) S+ and CS; (6) S- and CS. The results showed that only the S+ only and S+/CS tests reinstated responding, indicating that the cocaine-associated peer, rather than the CS, accrued the greatest associative strength.

While the studies by Weiss et al. (2018) and Hammerslag et al. (2022) yielded greater evidence for peer-induced reinstatement of cocaine seeking than originally reported in Smith et al. (2016), there are a couple of limitations to that work from our laboratory. First, the discrimination procedure used during acquisition resulted in a relatively small effect size even with extensive training (14-28 sessions). Thus, a procedure that yields a

more robust behavioral outcome might be more useful for interrogating the potential neural mechanisms of relapse following exposure to drug-associated peers. Second, the use of a cue light CS during acquisition is also problematic because, in contrast to the peer that is present continuously during the session, it is presently transiently as a CS associated with the infusion. Thus, this does not allow for a direct comparison between the abilities of social and non-social stimuli to control responding. The purpose of the current master's thesis was to address these two main limitations in order to provide a robust behavioral model for determining if the neural mechanisms involved in social and non-social triggers for relapse are dissociable.

1.4 Current Proposed Study

To begin assessing the neural mechanisms involved in cocaine relapse triggered by social and non-social stimuli, we adopted a procedure based on that outlined by Browning and Shahan (2018) that demonstrated robust renewal using a sucrose reward. In that study, a dual compartment apparatus was used similar to that described previously. Rats were trained to press a lever for a delivery of a dipper of sucrose in Context A; no peer was present in Context A. After sucrose self-administration was established in Context A, rats were assigned to one of two groups: (1) ABA or (2) AAA. The ABA group underwent extinction in Context B which had a peer rat present in the adjacent compartment and then renewal was tested by re-exposing rats to Context A (no peer). The AAA served as the control group which experienced Context A (no peer) throughout the training, extinction, and renewal phases of the experiment. Results from this experiment showed that lever pressing for sucrose was renewed for the ABA group, but not for the AAA group.

To take this a step further, Browning and Shahan (2018) conducted a second experiment where the manipulations were reversed. In this experiment, rats self-administered sucrose in the presence of a peer (Context B) and were then divided into two groups: (1) BAB or BBB. The BAB group went through extinction where the peer was taken away (Context A) and the BBB group had the peer present throughout extinction. Again, renewal testing of lever pressing was examined, but this time in Context B where the peer was reintroduced. The results showed that the BAB group renewed their lever responding when the peer was introduced, whereas the BBB group did not. Taken together, the results from Browning and Shahan (2018) indicate that social peers can serve as contextual stimuli that renew sucrose seeking following extinction. A key overall goal of the current master's thesis project is to determine if peers can also serve as contextual stimuli to renew cocaine seeking using a similar procedure.

Toward this goal, our laboratory has published one experiment using a renewal procedure, i.e., Experiment 1 in Weiss et al. (2018). In that experiment, rats self-administered cocaine on a terminal fixed ratio (FR) 5 schedule for 14, 1-hr sessions in the presence of a same-sex, same-age peer in the adjacent compartment. During self-administration, the rats also had the house light on continuously and were presented with 20-sec cue light illumination (CS) delivered at the beginning of each cocaine infusion in order to promote reliable responding. After self-administration training, rats underwent extinction with the peer and cue light CS removed, but the house light remained illuminated. Renewal testing was then conducted in the presence of the peer and house light, but with no cue light CS; 3 additional extinction sessions intervened between each renewal test. The results revealed that rats renewed responding to the first presentation of

the peer, but not upon the second presentation of the peer, thus indicating a transient peer-induced renewal effect. As noted previously, however, one problem with this study is that exposure to the CS during acquisition of cocaine self-administration, but not during the renewal test, may have weakened the renewal effect.

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CHAPTER 2. BEHAVIORAL EXPERIMENTS

2.1 Introduction

As mentioned above, Browning and Shahan (2018) and Weiss et al. (2018) demonstrated renewal of cocaine and food seeking when presented with a social peer after extinction. While these results are important, nonsocial stimuli typically occur at the same time as social stimuli, thus forming a social+nonsocial compound context, rather than one or the other alone. Typically, when someone is using drugs, there are multiple stimuli present that can be paired with the drug taking experience. For example, at a bar there are many nonsocial stimuli like the tables, lights, and sounds, but rarely is there a bar without people, so we have social stimuli present as well. In this case, social and nonsocial stimuli are experienced together during drug taking.

The key goal of this thesis is to determine if one stimulus element (social vs non-social) is more important than the other when both are presented simultaneously as a compound. That is, when it comes to craving and relapse, is drug seeking triggered more by the nonsocial context or the social context, or do they both need to be present? Moreover, when social and nonsocial stimuli are presented together as a compound, are they both processed together as a single configuration or do social and nonsocial stimuli get processed as separate elements during conditioning, each taking on associative strength to produce relapse? One way to test this is to determine if each element alone elicits a relapse or if both are needed in combination to elicit a relapse. In these experiments, it was hypothesized that the social element would have more saliency when compared to the nonsocial element and, thus, would show greater renewal of cocaine seeking when presented alone.

2.2 Experiment 1: Renewal of Cocaine Seeking Using a Compound Social + Nonsocial Context

2.2.1 Animals

Both male and female young adult Sprague-Dawley rats (approximately 50-55 days of age, N=15) were used. The rats were housed individually in a temperature-controlled colony room on a 12:12 h light schedule (lights on at 0800 h) with testing occurring during the light phase. Rats had ad libitum access to food and water in their home cage, except when food was restricted for initial lever press training and the first day of acquisition. All procedures were in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council (U.S.), 2011) and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

2.2.2 Apparatus

Food pre-training occurred in a single operant conditioning chamber (28 x 24 x 21 cm; ENV-008CT; Med Associates, St. Albans, VT, USA) containing two levers on the same wall which were retracted before and after the session. There was a central food tray on the same wall as the levers, into which food pellets could be dispensed from a food hopper. A cue light was located above each lever and a house light was mounted over the center of the opposite wall.

Acquisition of cocaine self-administration occurred in a custom-built apparatus consisting of two operant chambers joined together and separated by a wire screen partition

(1.27 cm, 19 gauge) which allowed animals to interact with a peer (see Figure 2-1). One side of the chamber was used as the self-administration training chamber and the adjacent chamber was used for presentation of the social and nonsocial contextual stimuli. Each chamber contained two retractable levers, with a 28-V cue light located 6 cm above each lever, that were placed opposite of one another on the side of the chamber closest to the wire screen partition. A house light was present at the top of each chamber, serving together as the nonsocial stimulus. In the non-self-administration chamber, the levers were always retracted. This apparatus was located within a sound attenuating cabinet.

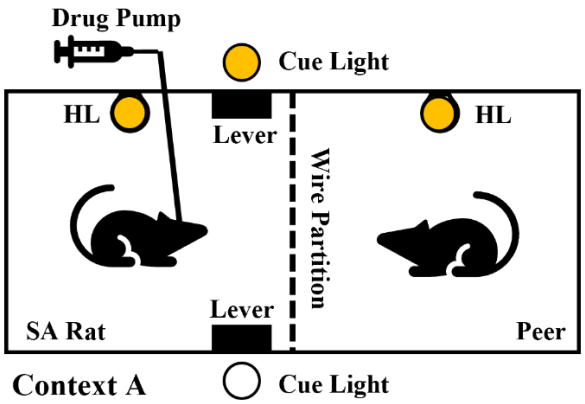


Figure 2-1 Schematic of renewal apparatus. HL = House light, SA = Self-administration

2.2.3 Food Pre-Training

Rats were handled for 3 days after arrival to the laboratory and on the last day of handling the rats were moved to food restriction (12-15 g/day). The next day, rats began food-reinforced pretraining in the single operant conditioning chamber that was distinctly different from the dual-compartment chamber. Presses on the active lever resulted in a food pellet (Bio-Serv Dustless Precision Pellet 45mg, Product # F0021) delivery on a FR 1 schedule of reinforcement. There was no house light, cue light, or time out period present

during these sessions. Rats were trained to lever press for food pellets until they earned at least 50 pellets during the 1-hr session. 3-5 sessions of food training were sufficient to learn and meet the criteria. Following food-reinforced pretraining to press a lever, rats were taken off food restriction and allowed ad libitum access to food in the home cage.

2.2.4 Surgery

On the day after the end of food pretraining, rats underwent indwelling jugular catheter surgeries. Rats were anesthetized with a ketamine (75mg/kg) + xylazine (7.5 mg/kg) mixture (males also received acepromazine; 0.75 mg/kg) and received a chronically indwelling jugular catheter that exited from an acrylic head mount attached to the skull with jeweler screws. Following catheter surgery, rats were allowed to recover for 7 days and then began acquisition of cocaine self-administration.

2.2.5 Cocaine Self-Administration Training

The day before acquisition, indwelling catheters were flushed with saline, then hooked up to an infusion pump tether and the rat was placed into the chamber where they were trained to self-administer cocaine. When the active lever was pressed, an infusion of cocaine (0.75 mg/kg/infusion; 0.1 ml delivered over 5.9 sec) was given with illumination of the cue light above the active lever signaling a time out (TO) period of 20 sec. Pressing the active lever during the TO period was recorded, but no infusion was delivered. Pressing on the inactive lever at any point during the session was also recorded but did not result in any programmed consequence.

On the first 1-hr acquisition session, rats did not have a peer present or illumination of the house lights; this procedure helped ensure that the rats would learn to self-administer cocaine without distraction from the peer on the first day. The next 7 daily sessions were like the first session, except a same-sex, same-age peer was placed in the adjacent chamber before the self-administering rat was placed in the chamber and the house lights in both chambers were illuminated; presence of both the peer and house light illumination was continuous throughout the session (Context A). The response requirement was then increased to FR3 for 7 sessions, followed by a terminal FR5 for 7 more sessions. The self-administering rat had the same peer present across all 21 sessions of acquisition. The program that controlled each session was started immediately after the self-administering rat was hooked to the infusion tether and the doors of the sound-attenuating cabinet were closed. Each self-administration session was 1-hr and was signaled by both house lights illuminating and extension of both the active and inactive levers into the self-administration chamber. At the end of the session, all lights shut off and levers were retracted. The self-administering rats were taken out first, had their catheters flushed and were put back to the home cage before the peers were removed from the apparatus.

2.2.6 Extinction and Renewal

After the last self-administration session (session 22), rats were then placed into extinction training. Rats were randomly assigned into 2 equal-sized groups: (1) AAA (control) and (2) ABA (renewal). During extinction, the AAA group had the same context as during acquisition, i.e., same peer and house light illumination (Context A), along with signaled TO on FR5, but no drug delivery. In contrast, the ABA group was extinguished

without the peer or house light illumination (Context B), but with the signaled TO still present on an FR5 with no drug delivery. In both groups, rats received 10 1-hr daily extinction sessions before renewal testing.

Renewal testing began the next day after the last extinction session. Since context A consisted of two distinct elements (peer and house light illumination), both groups were tested for renewal with the compound, as well as each element alone, on separate 1-hr sessions in counterbalanced order as follows: (1) peer alone; (2) house light alone; or (3) peer + house light compound; there was also a control test that was simply another extinction session for each group. The test ordering was counterbalanced within groups via a Latin square design and there were 3 additional sessions of extinction training intervening between each test to return extinction responding back to baseline. As during acquisition and extinction, the cue light TO was presented on an FR5.

2.2.7 Data Analysis

All results were evaluated with analyses of variance (ANOVAs), collapsed across sex, using a rejection criterion of $p < 0.05$. Analysis of cocaine self-administration acquisition was conducted using a 2 x 3 (lever x FR) ANOVA to determine the mean difference between total active and inactive lever presses collapsed across sessions. For extinction, a 2 x 10 (group x session) ANOVA was used to determine the mean difference of lever presses across sessions. Renewal testing was analyzed using a 2 x 2 x 2 (group x peer x house light) ANOVA to evaluate mean differences in total active lever presses between the two groups across each renewal test condition. In all cases, significant

interactions were probed with posthoc comparisons between means using Tukey's HSD or Šídák's multiple comparisons tests.

2.2.8 Results

The results from the acquisition, extinction, and renewal phases for AAA and ABA groups are illustrated in Figure 2-2. For acquisition, with the data collapsed across group and session, a 2-way ANOVA revealed a significant main effect of lever ($F(1,42) = 89.09$, $p<0.0001$), FR ($2,42) = 10.59$, $p=0.0002$), and a lever x FR interaction ($F(2,42) = 17.21$, $p<0.0001$). A Šídák's multiple comparisons test revealed that active lever presses were significantly higher than inactive lever presses on the FR3 (95% CI [-53.21, -0.2197], $p<0.05$) and FR5 (95% CI [-105.8, -52.82], $p<0.0001$), but not on the FR1.

For extinction, a 2-way group x session ANOVA revealed only a main effect of session ($F(2.520,32.76) = 8.548$, $p<0.0001$), with the mean number of active lever presses decreasing across extinction sessions for both groups. A Šídák's multiple comparisons test revealed by session 10, lever responding was significantly decreased compared to session 1 (95% CI [22.99, 104.6], $p<0.01$).

For renewal testing, a 3-way ANOVA revealed main effects of group ($F(1,13) = 39.15$, $p<0.0001$), peer ($F(1,13) = 13.23$, $p<0.001$), as well as a group x peer interaction ($F(1,13) = 8.848$, $p=0.0108$). Subsequent post hoc analysis using a paired t-test revealed that, regardless of the house light condition, responding to the peer was greater in the ABA group compared to the ABA extinction test ($t(7) = 3.715$, $p<0.01$). Further, an unpaired t-test to assess differences between groups under each condition revealed that, regardless of

the house light condition, the ABA group had significantly more active lever presses than the AAA group when the peer was present ($t(13) = 4.587$, $p<0.001$).

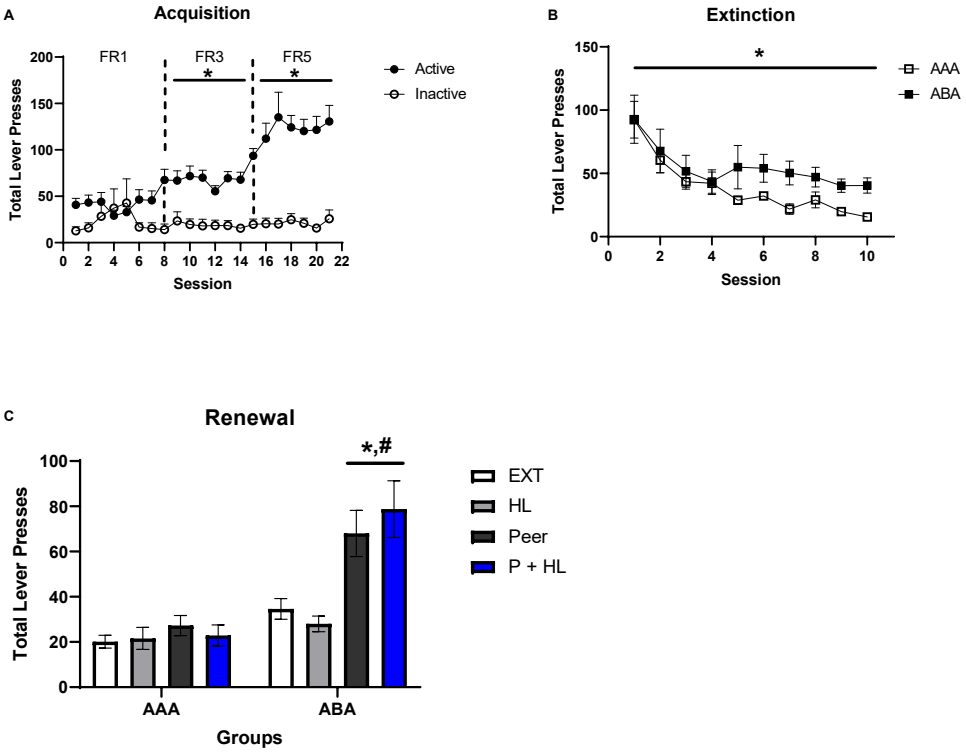


Figure 2-2 A) Acquisition of cocaine self-administration in context A collapsed across groups, showing active and inactive lever presses. *Indicates significant difference between active and inactive presses, collapsed across sessions, $p < 0.05$. B) Extinction of active lever pressing in AAA group in context A and in ABA group in context B. *Indicates significant decrease in presses across sessions collapsed across groups, $p < 0.05$. C) Renewal of active lever pressing in AAA and ABA groups under the different test conditions. *Indicates significant within-group difference compared to test with no peer (EXT and HL), and #indicates a significant between-group difference from AAA group tested with the peer (Peer and P + HL), $p < 0.05$. All data presented as ±SEM.

2.3 Experiment 2: Renewal of Cocaine Seeking Using a Nonsocial Stimulus Only

2.3.1 Pre-training and Self-Administration Training

Food pretraining and acquisition of cocaine self-administration were conducted as described in Experiment 1, except that only house light illumination in both compartments (no peer) was used as Context A and no house light illumination was used as Context B.

2.3.2 Extinction and Renewal Testing

Like Experiment 1, following the last cocaine self-administration session, rats began extinction. During extinction, rats were randomly assigned to 2 groups, AAA and ABA, where the AAA group had the same context as acquisition (house light illumination but no drug) and the ABA group had both the house light illumination and drug omitted; response-contingent cue light illumination as the TO signal was continued on an FR5 as during acquisition. Rats extinguished for 10 1-hr sessions before they began their renewal testing. Renewal of cocaine seeking was then tested in the presence of Context A (house light illumination) for both groups; another extinction test used for comparison, with the order of the tests counterbalanced within groups. There were 3 additional days of extinction intervening between each test. Response-contingent cue light illumination as the TO occurred on an FR5 on each renewal test, just as it did during acquisition and extinction.

2.3.3 Data Analysis

Analysis of cocaine self-administration acquisition was conducted using a 2 x 3 (lever x FR) ANOVA to determine the mean difference between total active and inactive

lever presses collapsed across sessions. For extinction, a 2 x 10 (group x session) ANOVA was conducted. Renewal testing was analyzed using a 2 x 2 (group x house light) ANOVA to determine the mean difference in active lever presses between the two groups across each renewal test condition. In all cases, significant interactions were probed with posthoc comparisons between means using Tukey's HSD, Šídák's, and Fisher's LSD multiple comparisons tests.

2.3.4 Results

The results from the acquisition, extinction, and renewal phases for the AAA and ABA groups are illustrated in Figure 2-3. For acquisition, with the data collapsed across group and session, a 2-way ANOVA revealed a significant main effect of lever ($F(1,42) = 567.0$, $p<0.0001$), FR ($2,42) = 36.73$, $p<0.0001$), and a lever x FR interaction ($F(2,42) = 77.74$, $p<0.0001$). A Šídák's multiple comparisons test revealed that active lever presses were significantly higher than inactive lever presses comparing on the FR3 (95% CI [-28.00, -10.33], $p<0.0001$) and FR5 (95% CI [-61.26, -43.59], $p<0.0001$), but not on the FR1.

For extinction, a 2-way ANOVA revealed a main effect of group ($F(1,13) = 14.81$, $p<0.01$) and session ($F(1.926,25.04) = 11.52$, $p<0.0001$). There was a significant group x session interaction ($F(9,117) = 2.017$, $p<0.05$). When probing the interaction, a Fisher's LSD multiple comparisons test revealed that for the ABA rats, their responding was significantly lower beginning at session 3 (95% CI [11.62, 55.27], $p<0.01$) compared to session 1. In contrast, the AAA group did not show a significant decrease in responding until session 7 (95% CI [2.813, 51.19], $p<0.05$) compared to session 1.

For renewal testing, a 2-way ANOVA revealed a main effect of group ($F(1,13) = 28.98$, $p=0.001$) and test ($F(1,13) = 4.736$, $p<0.05$). The interaction was close to significance ($F(1,13) = 4.222$, $p=0.06$). Despite the lack of a significant interaction, a Šídák's multiple comparisons test revealed that the AAA group showed no significant difference in number of presses on the extinction and house light sessions, (95% CI [-22.14, 20.81], $p=0.996$), whereas the ABA group showed more presses during the house light test compared to the extinction test (95% CI [-40.76, -5.687], $p=0.011$).

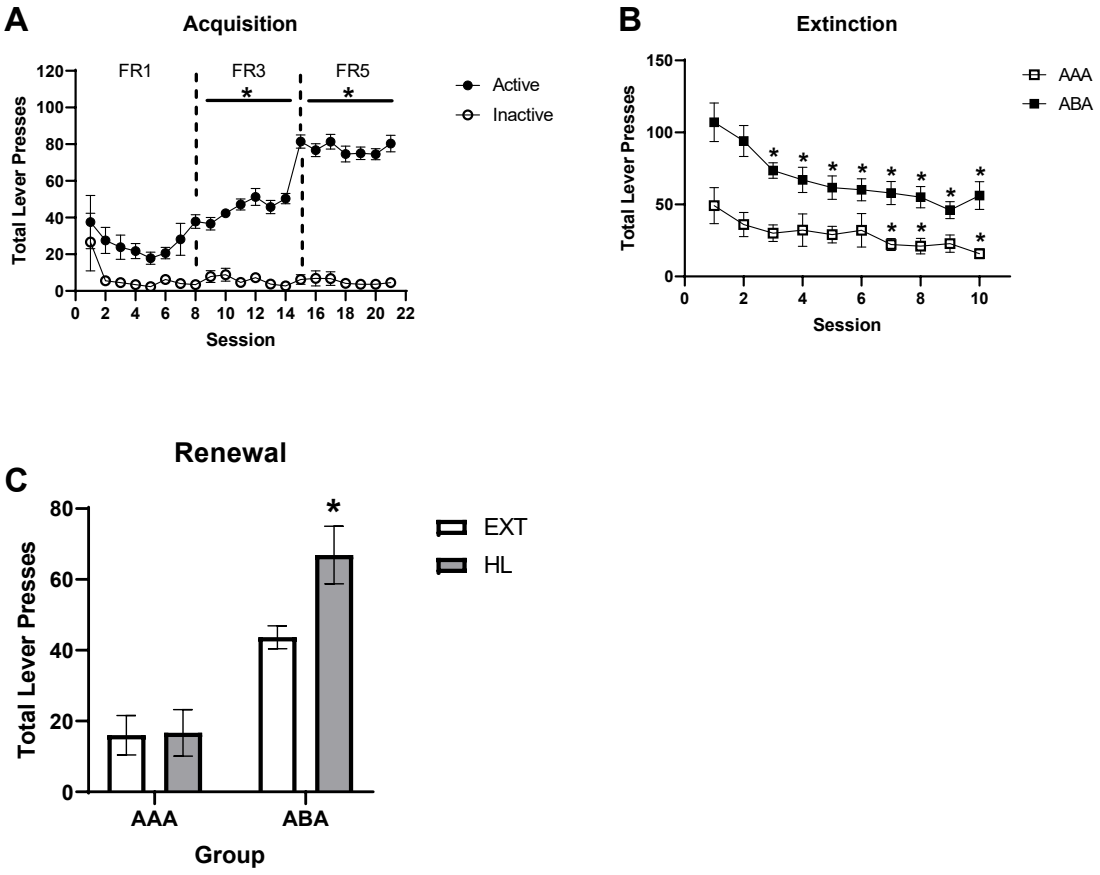


Figure 2-3 A) Acquisition of cocaine self-administration in context A collapsed across groups, showing active and inactive lever presses. *Indicates significant difference between active and inactive presses, collapsed across sessions, $p < 0.05$. B) Extinction of active lever pressing in AAA group in context A and in ABA group in context B. *Indicates significant decrease in presses compared to session 1, $p < 0.05$. C) Renewal of active lever pressing in AAA and ABA groups under the different test conditions. *Indicates significant difference from EXT in ABA group, $p < 0.05$. All data presented as ±SEM.

2.4 Discussion

There were several key findings in these two experiments. First, in both experiments, it was found that rats self-administered cocaine and learned to make more responses on the active lever than the inactive lever. Second, in both experiments, rats extinguished their lever responding over the course of 10 sessions. Third, and most importantly, results from Experiment 1 showed that rats trained with a peer + house light compound (Context A) and then extinguished without the compound (Context B) displayed renewal of cocaine seeking only when a peer was present. In addition, results from Experiment 2 showed that rats trained with the house light alone (Context A) and then extinguished without the house light (Context B) displayed renewal of cocaine seeking, indicating that the house light element alone was sufficient to engender renewal. As expected, the AAA control group failed to show renewal in either experiment. Taken together, these results indicate that, while social and nonsocial stimuli typically occur together in the drug taking context, social

stimuli may overshadow nonsocial stimuli in controlling drug seeking, at least under the conditions used the current experiments.

Social peers and their role in drug taking are an important aspect of drug abuse research. Social peers have been used in preclinical models of drug reward using either CPP or self-administration in dual-chamber operant boxes. Browning and Shahan (2018) found that with food self-administration, social peers can renew food seeking behaviors in an ABA renewal paradigm. Weiss et al. (2018) showed further that peers can be used to renew cocaine seeking behavior after a period of extinction; however, in that experiment, both the peer and the cue light TO used during the acquisition phase were both omitted during extinction. In the current study, we maintained the cue light CS throughout all phases of the experiment and instead used a continuous house light to examine social and nonsocial stimuli in a renewal paradigm. With this procedure, we found robust renewal to the peer.

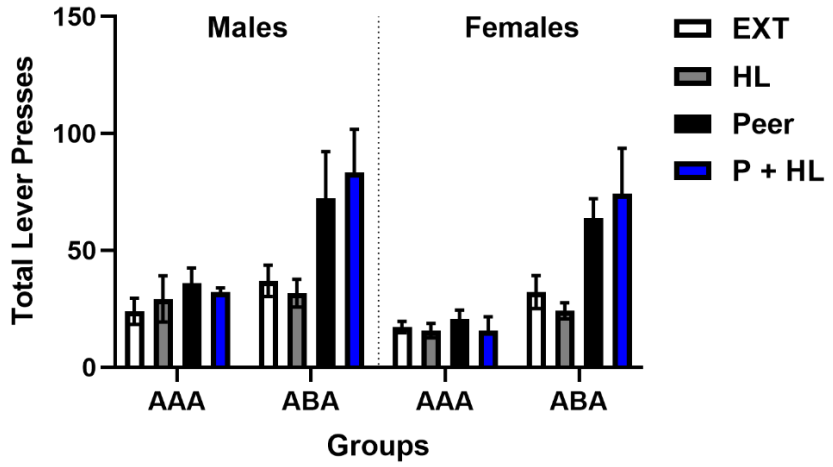
As for the question about whether the peer and house light were processed as independent elements or as a configural stimulus, the results were mixed. If the peer and house light contexts were processed as individual elements, we would expect to see renewal of cocaine seeking to both the peer alone and house light alone. In contrast, if the peer and house light contexts were processed as a configuration, we would expect to see renewal of cocaine seeking to only the peer + house light compound. Instead, we found that the peer alone and the peer + house light compound both renewed similar responding, which partially supports elemental processing. This finding is consistent with a previous study by Honey et al. (2014) which concluded that learned associations to patterns of stimulation require both elemental and configural learning. Importantly, our results showed

that the house light alone, while sufficiently salient to support renewal when tested alone (Experiment 2), accrued no associative strength when combined with the peer. Thus, while both social and nonsocial contextual stimuli may play a role in relapse, the peer seems to have greater control over renewed responding, perhaps due to greater incentive salience in this given paradigm.

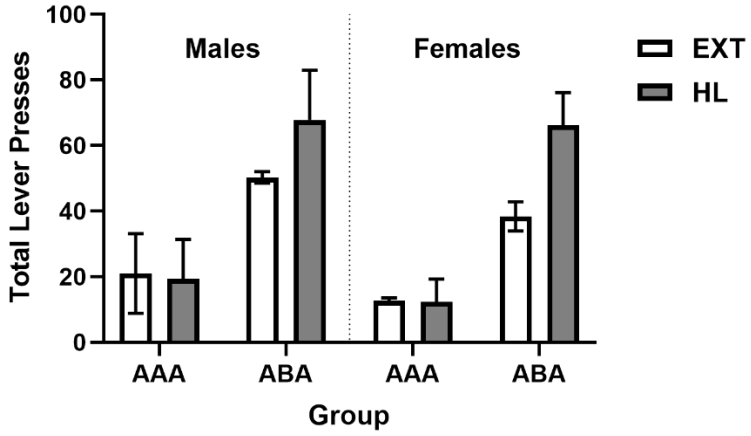
APPENDIX

Exploratory Analysis of Sex Differences in Renewal of Cocaine Seeking.

In Experiment 1, we ran an exploratory analysis adding sex into our model. Using a mixed effect ANOVA (sex x group x house light x peer), there was no significant main effect of sex; $F(1,11.680) = 1.747$, $p = 0.2115$, nor was there a significant interaction involving sex as a factor. A summary of those results, broken down into male and female are shown below:



In Experiment 2, we ran an exploratory analysis adding sex into our model. Using a mixed effect ANOVA (sex x group x house light), there was no significant main effect of sex $F(1,6) = 0.9412$, $p = 0.3694$, nor was there a significant interaction involving sex as a factor. A summary of those results, broken down into male and female are shown below:



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VITA

Degrees Awarded:
Kansas State University, B.S. in Psychology 2018

Professional Positions:
Teaching Assistant
University of Kentucky, Department of Psychology 2020-2021
Research Assistant
University of Kentucky, Department of Psychology 2021-2023

Scholastic and Professional Honors:
NIDA T32 DA035200: PI- Dr. Craig Rush, UKY July 2021-June 2023
Spring GSC Travel Award, UKY Spring 2022
Doreen Shanteau Undergraduate Research Fellowship, KSU Spring 2017
College of Arts and Sciences Student Travel Funding, KSU Fall 2017

Publications:

1. **Humburg, B.A** & Bardo, M.T. (in progress). Renewal of Cocaine Seeking using Social and Nonsocial Contextual Stimuli.
2. Hammerslag, L. R., **Humburg, B. A**., Malone, S. G., Beckmann, J. S., Saatman, K. E., Grinevich, V., & Bardo, M. T. (2022). Peer-induced cocaine seeking in rats: Comparison to nonsocial stimuli and role of paraventricular hypothalamic oxytocin neurons. Addict Biol, 27(5), e13217. https://doi.org/10.1111/adb.13217
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Bree Ann Humburg