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ABSTRACT

Cocaine addiction is associated with an extremely high rate of relapse, the resumption of drug taking behavior following a period of abstinence. Relapse may be induced by exposure to drug-associated cues, stress, or drug challenge. Rodent models of addiction investigate reinstatement, the resumption of drug-seeking behavior following a period of abstinence. This study investigated the necessary procedures for establishing Pavlovian conditioning between a discrete sensory cue and cocaine stimulant effects (15.0 mg/kg, IP). Successful conditioning was indicated by cue induced conditioned hyperactivity. In Experiment 1, a simple discrete visual cue failed to be attributed salience. Cocaine-treated rats showed heightened locomotor activity independent of cue condition, suggestive of contextual conditioning. Experiment 2 replaced the simple visual cue with a compound auditory/visual cue and implemented various procedural adaptations to prevent contextual conditioning; comparable results were observed. Experiment 3 introduced an alternating cue conditioning/no cue conditioning training regimen with 6 drug-cue pairings over 12 days. This alternating training procedure minimized contextual conditioning and resulted in successful attribution of salience to the discrete cue for tests after 3, 14, and 28 days of withdrawal. This study suggests that an alternating drug-cue pairing training procedure can be used to establish conditioned locomotor activity specific to a discrete compound sensory cue in Sprague-Dawley rats.

Keywords: Cocaine, Addiction, Hyperactivity, Pavlovian Conditioning

INTRODUCTION

Social Context

Cocaine is one of the most highly abused stimulant drugs in the world. North America has the highest prevalence of cocaine use in the world, with approximately 6.2 million annual users (United Nations Office on Drugs and Crime (UNODC), 2010). Cocaine is extremely addictive, with 46% of all Americans entering drug treatment programs doing so for cocaine addiction (UNODC, 2010). Moreover, of cocaine addicts receiving outpatient treatment, only 25% completed their treatments (Substance Abuse and Mental Health Services Administration, 2009). Such figures indicate the limited success of current treatments for cocaine addiction. It is imperative that researchers further our inadequate understanding of relapse to establish successful treatments for cocaine addiction.

Cocaine Pharmacology

The mesolimbic dopamine system is implicated as the primary source of cocaine's characteristic locomotor and reinforcing effects, including its characteristic euphoric "high" (Volkow et al., 1999; Sabeti et al., 2003; Dackis and O'Brian, 2005; Thomas et al., 2008). Cocaine acts primarily as an indirect dopamine (DA) agonist, blocking the dopamine transporter (DAT) and preventing DA clearance from the synapse (Ritz et al., 1987; McFarland et al., 2003; Sabeti et al., 2003). Cocaine also affects glutamatergic neurotransmission, reducing basal extracellular glutamate in the nucleus accumbens (NAc) (Hotsenpiller et al., 2001; Kalivas et al., 2003; McFarland et al., 2003; for review of glutamatergic neuroadaptations see Schmidt and Pierce, 2010). Cocaine has also been observed to also affect norepinephrine (NE) and serotonin (5-HT) transporters (Ritz et al., 1990).

Cocaine Relapse and Reinstatement

Recovering cocaine addicts are plagued with a high potential for relapse, the resumption of drug-taking behavior following a period of abstinence. Relapse has a variety of triggers, including exposure to drug-associated cues (See, 2002; Crombag et al., 2008), stress (Erb et al., 1996; Weiss et al., 2001; McFarland et al., 2004; Duncan et al., 2007), and drug re-exposure or "priming" (Brown and Fibiger, 1992; Shalev et al., 2002; McFarland et al., 2003). No animal model directly correlates to relapse in humans, since they cannot accurately measure the subjective characteristics associated with drug administration, such as the human phenomenon of craving. Instead, the majority of animal studies focus on reinstatement, the resumption of drug-seeking behavior following exposure to cues, stressors, or drug after a period of withdrawal (Shaham et al., 2003; Anker and Carroll, 2010).

Craving

Craving is defined as "the desire to experience the effect(s) of a previously experienced psychoactive drug" (UNDCP & WHO, 1992) and is a major contributor to relapse. Craving demonstrates an unusual temporal relationship; it is low during acute withdrawal, it peaks over a span of additional months, and then diminishes, following an "inverted-U" pattern (Grimm et al., 2001; Lu et al., 2004 a,b). Neuroimaging studies have striven to uncover the neural basis of craving. Investigative findings suggest an association between craving and increased activation of the amygdala, anterior cingulate gyrus, prefrontal cortex (PFC), and NAc (Childress et al., 1999; Volkow et al., 1999; Kilts, et al., 2001). Additional studies have implicated increased DA release in the dorsal striatum with craving (Volkow et al., 2006).

Craving-induced neural activation patterns associated with the NAc, PFC, and cingulate cortex may result in an enhanced sensitivity to discrete and contextual drug-associated cues. For

example, contextual cues have resulted in increased metabolic activity in the cingulate cortex, piriform cortex, thalamus, amygdala, and basal ganglia (Brown et al., 1992). Presentation of discrete cues has been observed to increase metabolic activity in the orbitofrontal cortex, insula and PFC regions (Childress et al., 2008).

Drug-Associated Cues and Pavlovian Conditioning

Drug-associated cues are thought to be attributed salience through Pavlovian conditioning, a form of associative learning. Pavlovian conditioning involves simultaneously exposing a subject to an unconditioned stimulus (UCS), which normally results in an unconditioned response (UCR), and a conditioned stimulus (CS). Before conditioning, the CS does not elicit any response. Following repeated pairings, CS exposure results in a conditioned response (CR), which resembles the UCR. In the context of this experiment, cocaine stimulant effects (UCS) resulted in increased locomotor activity (UCR). It is anticipated that following repeated pairing of cocaine exposure with a discrete sensory cue (CS), exposure to the cue alone will elicit hyperactivity, the conditioned response (CR).

Studies investigating cocaine conditioning have used many cue types, including environmental/contextual cues (Brown et al., 1992; Hotsenpiller et al., 2001; Carey et al., 2005b), discrete sensory cues (Panlilio and Schindler, 1997; Hotsenpiller et al., 2002), and pharmacological cues, such as centrally active medications (Carey et al., 2002; Felszeghy et al., 2007). A critical component of Pavlovian conditioning paradigms are their reliance on non-contingent UCS and CS exposures, which prevent the formation of goal-directed behavior. In contrast, self-administration (SA) experiments have CS exposure contingent on an operant response (i.e. successful bar press); the operant behavior results in drug administration and CS exposure (Kruzich et al., 2001; Uslaner et al., 2006). Goal-directed behavior complicates any

reinstatement results due to the presence of both conditioned behavioral responses and response-reward expectancies (Olmstead et al., 2001).

The Self-Administration Model of Reinstatement

Self-administration models are believed to correlate best with human drug taking behavior and the associated addiction phenomenology. In this model, rats are trained to self-administer drug infusions by lever pressing on the "active" lever. Active lever presses are also paired with presentation of a drug-associated cue. "Inactive" lever presses are not reinforced and do not result in cue presentation. Once lever-pressing criteria are met, drug-seeking behavior is extinguished in a series of extinction sessions in which the active lever is not reinforced (both drug and cue are absent). Extinction sessions result in a significant reduction in drug-seeking behavior; extinguished activity measures are frequently used as baseline values for subsequent comparison (Bouton et al., 2006). Reinstatement of drug-seeking behavior is then examined following exposure to a drug-paired environmental or discrete sensory cues, stress, or drug priming. Reinstatement is indicated by a significant increase in "active" lever pressing during these sessions compared to "active" lever responsiveness during extinction (See, 2005). Self-administration studies conclude that exposure to drug-paired environmental or discrete sensory cues, stress, or drug challenge successfully reinstates drug-seeking behavior following extinction (Ciccocioppo et al., 2001; Anker and Carroll, 2010; for summary see Shalev et al., 2002).

Neural Substrates of Cue-Induced Reinstatement in Self-Administration Models

Using the self-administration model, researchers are beginning to understand the neural basis of reinstatement. Cue-induced reinstatement appears to be mediated by the mesocorticolimbic dopamine system (Di Chiara, 1999; McFarland et al., 2003), in particular, the basolateral

amygdala (BLA), PFC and NAc (Reid and Berger, 1996; Ito et al., 2000; Parkinson et al., 2000; Fuchs and See, 2002; See 2002; for review see Kalivas and McFarland, 2003). It has been suggested that glutamate innervation in the NAc is critical for cue-induced reinstatement (Park et al., 2002; Kalivas et al., 2003; Schmidt et al., 2005; Madayag et al., 2010). Furthermore, inactivation of the BLA and dorsomedial PFC attenuate cue-induced reinstatement (McLaughlin and See, 2003), whereas BLA lesions block cue-induced reinstatement (Meil and See, 1997). It has also been observed that stimulation of the medial PFC (mPFC) serotonin 2C receptor (5-HT2CR) attenuates cocaine cue-induced drug-seeking behavior (Pentkowski et al., 2010).

Incubation Effects in the Self-Administration Model

Cocaine addicts show heightened craving and an increased propensity for relapse during protracted withdrawal compared to acute withdrawal. The propensity for relapse dissipates after long withdrawal periods. A similar phenomenon has been observed in rodent reinstatement models; increased cocaine-seeking behavior is associated with intermediate periods of withdrawal (1-4 months, Ciccocioppo et al., 2001; Lu et al., 2004a) compared with shorter (1-60 days, Grimm et al., 2001) or longer (6 months, Lu et al., 2004a) periods of withdrawal. This "inverted-U" shaped function is referred to as the "incubation effect" (Kelamangalath and Wagner, 2009) and models the human phenomenon of craving.

The neural basis of this phenomenon is not fully understood. Moreover, self-administration models indicate the ability for exposure to discrete and contextual drug-associated cues to reinstate cocaine-seeking behavior after prolonged periods of drug-abstinence (Grimm et al., 2001, 2002; Lu et al., 2004 a,b).

Non Self-Administration Models of Reinstatement using Drug-Associated Cues

Pavlovian conditioning models have shown utility in examining the conditional effects of non-contingent drug administration. For example, place conditioning establishes a relationship between contextual cues and the subjective effects of drugs (Mackintosh, 1974). Conditioned locomotor activity paradigms establish a relationship between either contextual or discrete sensory cues and the locomotor activating effects of stimulant drugs (Cervo and Samanin, 1996; Panlilio and Schindler, 1997; Hotsenpiller et al. 2001).

Conditioned Locomotor Activity Paradigms

Conditioned locomotor activity paradigms utilize Pavlovian conditioning parameters with either single or repeated exposure(s) to an unconditioned stimulus (UCS), often the documented effects of the drug of interest. Drug exposures are concurrent with exposure to a specific cue (CS). Following completion of drug-cue pairing, often involving numerous UCS/CS exposures (Panlilio and Schindler, 1997; Hotsenpiller et al., 2001; Hotsenpiller et al., 2002), rats are tested under drug-free conditions to determine whether the cue has been attributed salience. Increased activity in the presence of the drug-associated cue, as compared to its absence, is indicative of successful associative learning (Hotsenpiller et al., 2001). Since this paradigm measures conditioned activity, it is imperative to include habituation sessions to diminish novelty effects as a potential confound. Novelty effects also wield the potential to diminish the success of drug-cue pairing (Cervo and Samanin, 1996; Panlilio and Schindler, 1997; Hotsenpiller et al., 2001). Conditioned locomotor activity provides a direct measure of cue-attributed salience without the confounding variable of goal-seeking behavior and motivation state, both of which are associated with reinstatement models (Olmstead et al., 2001).

Neural Substrates Underlying Cue-Induced Reinstatement in Non-Self-Administration Models

Studies have observed that exposure to a drug-paired stimulus induces a significant decrease in basal glutamate metabolism compared to controls; subsequent exposure to the drug-paired cue resulted in a significant increased glutamate levels in the NAc (Hotsenpiller et al., 2001). Similarly, Brown and colleagues (1992) observed that a contextual cocaine-associated cue significantly increased locomotor activity and Fos expression in the claustrum, lateral septal nucleus, cingulate cortex, amygdala, paraventricular nucleus, but not in the NAc. Moreover, conditioned locomotor activity to a contextual cue was blocked by inactivation of the NAc or mPFC, but not the BLA (Brown and Fibiger, 1993; Franklin and Druhan, 2000). In addition, administration of MK-801, an NMDA-receptor antagonist, and DNQX, an AMPA/kainite receptor antagonist, during cocaine conditioning blocked conditioned activity (Cervo and Samanin, 1996). In addition, pre-training BLA lesions successfully block acquisition of contextual drug-cue pairing and subsequent place preference (Brown and Fibiger, 1993; Fuchs et al., 2005). Thus, the BLA may be differentially involved in conditioned reinforcement and conditioned activity (Di Ciano and Everitt, 2004), while the medial PFC and NAc may be involved in both conditioned reinforcement and conditioned activity. In addition, infusion of a 5-HT-2A antagonist into the ventromedial PFC has also been seen to attenuate cue-induced reinstatement (Pockros et al., 2011).

Discriminating Conditioning Effects of Contextual versus Discrete Sensory Cues

A major pitfall of Pavlovian conditioning experiments using a discrete sensory cue is the potential for establishing unintended associations between the UCS (drug effects) and a contextual cue (i.e. test chamber). Theoretically, if the discrete sensory cue is attributed sufficient salience, rats will ignore contextual cues in favor of the sensory cue. However, it is

very difficult to negate all potential learned associations between drug effects and the testing environment (Hotsenpiller et al., 2001). Nevertheless, such contextual associations may be minimized using procedural changes that enhance cue salience (Panlilio and Schindler, 1997).

Study Goals and Implications

The primary goal of the current study was to develop a Pavlovian conditioning paradigm that results in successful attribution of salience between cocaine stimulant effects and a discrete sensory cue. Should Pavlovian conditioning between cocaine administration and discrete cue prove successful, the training and testing parameters implemented may be used in a study with multiple experimental components, such as one that implements both conditioned locomotor activity and the 5-Choice Serial Reaction Time Task (5CSRTT), a task that reliably measures impulsivity (Robbins, 2002; Paine et al., 2007; Pattij and Vanderschuren, 2008). Such a multimodal experimental approach could investigate whether re-exposure to a discrete drug-associated cue affects cognitive functioning, specifically impulsive choice, and whether such cognitive changes and associated neural adaptations influence cocaine relapse. It might thereafter be suggested that cue-induced impulsivity is directly associated with cue-induced craving, as per the similarities in active underlying neurological substrates. Alternatively, these cognitive changes may be distinct in their contributions to drug relapse.

MATERIALS AND METHODS

Experiment 1: Testing Drug-Cue Pairing on Conditioned Locomotor Activity Using a Simple Discrete Visual Cue

Goal: To establish a cocaine hydrochloride dosage regimen and conditioning parameters that result in successful Pavlovian conditioning between cocaine stimulant effects (15.0 mg/kg, IP) and a discrete flashing visual cue (flashing red bicycle light) measured by the emergence of cue-induced conditioned locomotor activity under drug-free conditions.

Animals

Drug-naïve adult male Sprague-Dawley rats born and bred at Oberlin College and derived from rats from Hilltop Animal Laboratories (n=8, Scottsdale, PA) or born and bred at Hilltop Animal Laboratories (n=8, Scottsdale, PA) were used in this experiment. Rats were aged approximately 90 postnatal days (PD 90) during initial testing. Rats were housed in pairs in plastic cages (47.5 cm x 27.0 cm x 20.0 cm) with wood shavings on a 14:10 hour light/dark cycle (lights on at 0600, EST). Rats housed together received identical treatments. Rats were maintained on a restricted diet of approximately 18.0 grams (roughly 4 pellets) of food daily (LabDiet 5001 food pellets; PMI, Nutrition International Inc). Rats were fed following completion of daily training/testing or at approximately the same time during incubation periods. Establishing a restricted diet allowed for only slight weight gain (roughly 2-4% per month). Use of a restricted diet allowed for potential future extension with the 5-CSRTT in which rats must be food restricted without first replicating the current experiment under conditions of food restriction. Rats had at least one week to acclimate to housing conditions prior to food restriction and an additional week to acclimate to the restricted diet prior to initiation of the experiment. Water was provided *ad libitum* in the colony room. Rats were removed from the colony room only for experimental

purposes to maintain identical withdrawal conditions across cohorts. All experimental procedures received formal approval by the IACUC of Oberlin College and were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington D.C., USA, 1996).

Drug Information

Cocaine hydrochloride (15.0 mg/kg, dose based on salt weight**,** Sigma-Aldrich, St. Louis, MO) was diluted with physiological saline (0.9% NaCl, Fisher Science, Fair Lawn, NJ). Drug dosage was based on the findings of Hotsenpiller and colleagues (2001) and Todetenkopf and Carlezon (2006). All solutions were stored at 4°C when not in use. All injections were performed intraperitoneally (IP) at 1.0 mL/kg injection volume. It has been established that the lethal dose of cocaine in rodents is 85.0 mg/kg (Derlet et al., 1990); therefore, the selected dosage was likely to be tolerated.

Behavioral Apparatus: Automated Locomotor Activity Chambers

Locomotor activity was assessed in four clear Plexiglas automated locomotor activity chambers (43.2 cm x 43.2 cm, ENV-515, Med Associates Inc., St. Albans, VT) housed in sound attenuating cubicles (66.0 cm x 55.9 cm x 55.9 cm, ENV-017M, Med Associates Inc., St. Albans, VT). Ventilated clear polycarbonate lids were positioned approximately 33.0 cm above the removable stainless-steel collection tray. Sound-attenuating chambers contained ventilating fans that also provided masking noise. Chamber house lights were extinguished throughout the experiment. Three identical 16-beam infrared sensor arrays were mounted on metal frames to each Plexiglas chamber: two perpendicular arrays positioned 2.5 cm above the chamber floor and one array positioned 12.7 cm above the chamber floor. Sensor arrays were connected to a PC

computer running Activity Monitor software (SOF-811 Open Field Activity, 2009, Med Associates Inc., St. Albans, VT). Distance traveled (cm) was used as an accurate measure of behavioral sensitization and drug-induced locomotor activation (Hotseniller et al., 2001; Hotsenpiller and Wolf, 2002; Carey et al., 2005a).

Procedures

All procedures were carried out between 1200-1900 hours during the rats' light phase. Rats were assigned to a specific chamber for the duration of the experiment. Rats were weighed at the start of each session to determine injection volume. Sessions began by placing rats into the center of the chamber, thereby breaking the infrared beams and initiating the recording software. Rats were returned to their home cages upon session completion. Chamber walls, lids, and removable collection trays were cleaned between animals with deionized water followed by 70% ethanol solution to remove potential odorant stimuli.

Habituation: Rats were handled for 5 consecutive days preceding habituation. Rats underwent a single 60-minute habituation session one day prior to training. Rats were injected with physiological saline (1.0 mL/kg, IP) and placed in designated chambers. Total distance traveled (cm) during the habituation session was used as the basis for treatment group assignment (Cocaine, n=8; Saline, n=8).

Training: Thirty-minute training sessions were employed to maximize the probability of successful drug-cue pairing. Maximal behavioral sensitization in cocaine-treated rodents occurs during the initial 30 minutes following drug exposure (Todtenkopf and Carlezon, 2006). Rats underwent ten training sessions with drug-cue pairings. Training sessions were arranged as two consecutive sets of five training sessions followed by two testing days (Cue, No Cue). On each

training day, rats were weighed prior to injection of either cocaine (n=8; 15.0 mg/kg/mL) or saline (n=8; 1.0 mL/kg). Immediately following injection, rats were placed into assigned activity chambers with the simple discrete visual cue, a flashing red light (Spider flasher for bike, Bell-Sports, Inc., Rantoul, IL). The cue was centered horizontally over the middle ventilation hole in the Plexiglas lid. The cue was chosen because alcohol-intoxicated rats successfully attributed drug-related salience to a similar discrete flashing red-light cue (Olmstead et al., 2006). In addition, this cue was easily transferable between different testing environments, a prerequisite for any cue used in subsequent experiments assessing changes in impulsivity following cue exposure to a conditioned cue in 5-Choice Serial Reaction Time Task boxes.

Testing: Rats underwent testing on two consecutive days following completion of five and ten training sessions, respectively. Rats were weighed and placed into activity chambers for 30 minutes without injection. During the two-day testing period, each rat underwent testing under cue present (Cue) and cue absent (No Cue) conditions; the order of cue exposure/absence was randomized and counterbalanced to minimize any effect of cue order.

Incubation Testing: Cue-induced conditioned locomotor activity was examined with increased periods of withdrawal to test for the emergence of an "incubation effect." Rats were retested 7, 14, and 28 days after the final drug-cue pairing session. The order of cue exposure/absence over the two-day test period was alternated after completion of initial testing and after each subsequent 2-day test period. For a procedural timeline, see Figure 1 section I.

Data Analysis: Data are expressed as total locomotor activity (cm) during 60-minute habituation sessions and 30-minute training and testing sessions (Mean ± SEM). One-way analysis of variance (ANOVA) was used to examine differences in habituation activity across assigned

treatment groups [dependent variable: treatment (Cocaine, Saline)]. All two-way and three-way ANOVAs implemented a mixed repeated-measures design. Where appropriate, Greenhouse-Geisser adjustments were used; post-hoc analyses were carried out using an Estimated Marginal Means procedure with Least Significant Difference correction. Training session main effects and interactions were analyzed using 2-way ANOVA [within-subject factor: session (1-10); between-subject factor: treatment (Cocaine, Saline)]. Three-way ANOVAs investigated main effects and interactions between treatment, cue condition and test session [within-subject factors: Cue (Cue, No Cue); session (1-4); between-subject factor: treatment (Cocaine, Saline)]. Conditioned locomotor activity, an increase in total distance traveled (cm) in the presence of the cue versus in its absence, was considered indicative of successful drug-cue pairing. Statistical significance was set at α=0.05 for all analyses.

Experiment 2: Testing Drug-Cue Pairing on Conditioned Locomotor Activity using a Discrete Compound Auditory/Visual Cue

Goal: To establish a cocaine hydrochloride dosage regimen and conditioning parameters that result in successful Pavlovian conditioning between cocaine stimulant effects and a discrete auditory/visual cue (flashing yellow LED light with metronome auditory stimulus) measured by the emergence of cue-induced conditioned locomotor activity under drug-free conditions.

Animals

Drug-naïve male adult Sprague-Dawley rats born and bred at Oberlin College and derived from rats from Hilltop Animal Laboratories (n=8, Scottsdale, PA) or born and bred at Hilltop Animal Laboratories (n=8, Scottsdale, PA) were used in this experiment. Rats were aged approximate

PD 90 days during initial drug-free testing (Test1). Rats received identical housing and dietary conditions as in experiment 1.

Behavioral Apparatus: Y-Maze

A black Plexiglas Y-maze was used in experiments 2 and 3 as a secondary measure of the attribution of incentive value/salience to the compound cue. The Y-maze consisted of three identical rectangular arms (50.0 cm x 17.0 cm x 30.0 cm) with conjoining equilateral triangle center (17.0 cm per side) forming a "Y" shape. For all Y-maze sessions, one arm always served as the designated "starting arm" in which rats were placed at the start of each habituation or testing session. A thin layer of wood shavings (approximately 0.5 cm) was equally distributed in the maze. During Y-maze testing, the visual cue (flashing yellow light) was positioned 33.0 cm above the cue-associated arm through use of a ring stand; the auditory cue (metronome) was located at the base of the ring stand for experiment 2 and atop the visual cue for experiment 3. The ring stand and cue components were moved to identical positions at the end of each cue-associated arm; the selected cue-associated arm was randomized across treatment groups. Between rats, the Y-maze walls were cleaned with 70% ethanol solution and the bedding was redistributed in the maze. All Y-maze sessions were conducted under red-light conditions.

Procedures

Drug information, dosage, and injection procedures were unchanged from experiment 1. The locomotor activity chamber apparatus was described previously.

Habituation: Rats underwent 3 habituation sessions of 60-minute duration prior to the start of training. Average habituation activity (total distance traveled, cm) was used to balance baseline activity across assigned treatment groups (Cocaine, n=8; Saline, n=8).

Training: Training consisted of 5 drug-cue pairing sessions spanning 5 consecutive days. Training days had two components: an initial 30-minute baseline session, followed by treatment-specific injection and subsequent 30-minute conditioning session. At the start of each training day, rats were weighed then placed in activity chambers, initiating the software for the baseline session. Upon completion of baseline sessions, rats were injected with either cocaine (n=8; 15.0 mg/kg) or saline (n=8; 1.0 mL/kg). Rats were then returned to activity chambers for the 30-minute conditioning session with the compound cue. The compound cue consisted of a visual cue (flashing yellow LED bicycle light, Flashlight with 5 Yellow LEDs, Ventura LED) and an auditory cue (metronome, AM-701 Clip-on Metronome, Aroma Music Co., LTD., China, 120 BPM, 4 beats per measure, sixteenth notes). The visual cue was centered on the transparent chamber lid, as in experiment 1; the auditory cue was positioned inside the sound-attenuating chamber adjacent to the Plexiglas enclosure.

Testing: After five days of training, rats underwent two days of testing under varied cue conditions (Cue, No Cue; Test 1). The order of cue exposure was counterbalanced across test days to randomize cue exposure between the two groups of rats (2 x n=4) trained simultaneously on a given day. The two groups were tested approximately 90 minutes apart due to equipment limitations. Rats were tested in assigned chambers, but all rats tested together received identical cue conditions to minimize inter-chamber cue-related 'noise' pollution.

Incubation Testing: Rats were re-tested 7, 14, and 28 days following final drug-cue pairing session (Tests 2, 3, 4). Incubation testing parameters and procedures were identical to those described previously for Test 1.

Y-Maze Habituation: Following completion of daily testing, rats were habituated to a Y-maze. Rats were individually placed into the starting arm of the Y-maze and allowed 5 minutes of unimpeded exploration. The Y-maze was cleaned with 70% ethanol solution between rats.

Y-Maze Testing: Y-maze testing was conducted following completion of Test 1. During Y-maze testing, one of the two non-starting arms was associated with the compound cue in a randomized order across treatment groups. Each rat was tested for five minutes following placement into the starting arm. Y-maze data were recorded as number of entries in each arm and the total time spent in each arm. An arm entry was defined by having all four paws in an arm. Total time spent in each arm was tabulated from the duration of each arm entrance with a minimum threshold for recorded entries of 1 second. For experiment 2 timeline, refer to Figure 1 section II.

Data Analysis: Data are expressed as total locomotor activity (Mean ± SEM) for 60-minute habituation sessions and 30-minute training or testing sessions. All two-way and three-way ANOVAs implemented a mixed repeated-measures design. Where appropriate, Greenhouse-Geisser adjustments were used; post-hoc analyses were carried out using an Estimated Marginal Means procedure with Least Significant Difference correction. For habituation data, a two-way ANOVA was employed [within-subject factor: session (1, 2, 3); between-subject factor: treatment group (Cocaine, Saline)]. The effects of treatment and training session were analyzed separately for baseline training sessions and for conditioning training sessions with two-way ANOVAs [within-subject factor: session (1-5); between-subject factor: treatment (Cocaine, Saline)]. Separate three-way ANOVAs examined the main effects and interactions between treatment, cue condition, and session for baseline sessions and for test sessions [within-subject factors: Cue (Cue, No Cue), Session (1, 2, 3); between-subject factor: treatment (Cocaine,

Saline)]. Conditioned locomotor activity, an increase in total distance traveled (cm) in the presence of the cue compared to activity in its absence, was considered indicative of successful drug-cue pairing. Y-maze testing was analyzed using one-way ANOVAs. Separate analyses compared the total duration in cue-associated versus no-cue arm and the number of arm entries into cue versus no-cue arm [within-subject factor: treatment (Cocaine, Saline); between-subject factor: arm (Cue, No Cue)]. A preference for the cue-associated versus no-cue Y-maze arm for both measures was considered indicative of successful attribution of salience to the sensory cue. Statistical significance was set at α=0.05 for all analyses.

Experiment 3: Testing Drug-Cue Pairing on Conditioned Locomotor Activity with a Discrete Compound Auditory/Visual Cue with Alternating Baseline/Conditioning Training Schedule.

Goal: To establish a cocaine hydrochloride dosage regimen and conditioning parameters that result in successful Pavlovian conditioning between cocaine stimulant effects and a compound discrete auditory/visual environmental cue (flashing yellow LED light combined with metronome auditory cue) measured by the emergence of cue-induced conditioned locomotor activity under drug-free conditions using an alternating baseline/conditioning training procedure.

Animals

Sixteen drug-naïve adult male Sprague-Dawley rats born and bred at Oberlin College and derived from animals from Hilltop Animal Laboratories (Scottsdale, PA) were used in this experiment. Rats were aged approximate PD 90 days during training. Rats received identical housing and dietary conditions as in previous experiments.

Procedures

Experiment 3 procedures were based on the methodologies implemented by Panlilio and Schindler (1997) and Hotsenpiller and colleagues (2001). Drug information and injection procedures were the same as in experiment 2. Rats were handled extensively for one day preceding the first habituation session.

Habituation: All rats received three 60-minute habituation sessions. Average activity across habituation sessions was used to balance treatment group baseline activity.

Training: The training period lasted 12 days, during which drug animals received a total of 6 drug-cue pairings (Cue). Rats underwent similar drug-cue pairing sessions as in experiment 2 on the first training day and every second day afterward. Both treatment groups underwent identical procedures on interim days: all animals were weighed before a 30-minute baseline session followed by saline injection and a 30-minute conditioning session without cue exposure (No Cue). Following completion of the 12-day training period, rats remained in the colony room for one day prior to testing (For timeline, see Figure 1 section III).

Training procedures for cue conditioning sessions were mostly unchanged from Experiment 2; the auditory cue settings were changed to better align the rates rate of visual and auditory cue stimulation (77 BPM, 2 beats per measure, quarter notes). In addition, the metronome was relocated alongside the visual cue on the activity chamber lid.

Testing: All rats received injections of physiological saline (1.0 mL/kg) between baseline and conditioning sessions, allowing for greater procedural consistency between training and testing

sessions. Rats were tested in the same groups of four animals trained at a given time. The two groups were tested approximately 90 minutes apart due to equipment limitations.

Y-Maze Habituation: Y-maze habituation occurred on the day after completion of locomotor activity testing. Y-maze habituation consisted of two cycles of the five-minute habituation session. Session procedures are outlined previously for experiment 2.

Y-Maze Testing: Y-maze testing occurred on the day after Y-maze habituation. Y-maze testing procedures only deviated from those used for experiment 2 by having the auditory cue relocated on top of the visual cue extended into the cue-associated arm via ring-stand. The auditory cue settings were the same as used for experiment 3 locomotor activity testing.

Incubation Testing: Rats underwent incubation testing 14 and 28 days following final drug-cue pairing (Tests 2, 3). Rats remained in the animal facility except during incubation testing. Testing parameters and procedures were identical to those used for test 1. The order of cue presentation was alternated for each subsequent round of testing (cue order was identical for tests 1 and 3).

Data Analysis: Locomotor activity, measured as total distance traveled (cm) (Mean ±SEM) was compared across cue condition (Cue, No Cue) via within-subject and between-treatment group analyses to discern successful drug-cue pairing. Conditioned locomotor activity, an increase in total distance traveled (cm) in the presence of the discrete cue versus in its absence, was considered indicative of successful drug-cue pairing. A mixed repeated-measures, two-way ANOVA was performed to discern differences across habituation sessions [within-subject factor: session (1, 2, 3); between-subjects factor: treatment group (Cocaine, Saline). Four, two-way

mixed repeated-measures ANOVA analyses were performed on training data. Baseline training sessions and conditioning training sessions were analyzed using separate ANOVAs: two ANOVAs were conducted to analyzed training day sessions with cue exposure (Baseline Sessions, Cue Conditioning Sessions) [within-subject factor: session (1, 3, 5, 7, 9, 11); between-subjects factor: treatment (Cocaine, Saline)] and two ANOVAs for no-cue training days (No Cue Baseline Sessions, No Cue Conditioning Sessions) [within-subject factor: session (2, 4, 6, 8, 10, 12); between-subjects factor: treatment (Cocaine, Saline)].

Y-maze data were analyzed with two, two-way mixed repeated-measures ANOVA analyses for number of arm entries [within-subject factor: arm (Cue, No Cue); between-subject factor: treatment (Cocaine, Saline)] and for duration in a non-starting arm [within-subject factor: arm (Cue, No Cue); between-subject factor: treatment (Cocaine, Saline)]. Post-hoc analyses were performed using an Estimated Marginal Means procedure with Least Significant Difference correction. Greenhouse-Geisser adjustments were used when appropriate. Statistical significance was set at α=0.05 for all statistical analyses.

RESULTS

Experiment 1

Habituation: Locomotor activity measures during the habituation session were used to balance activity measures across treatment groups. As expected, there was no significant difference in baseline activity across treatment groups (Cocaine: 4478.13 ± 728.12 cm, Saline: 4067.23 ± 534.04 cm; $F_{(1,15)}$=0.656, p>0.05).

Training: Two-way ANOVA analysis of training sessions revealed a significant main effect of treatment with cocaine treatment inducing robust hyperactivity compared to saline-induced

activity ($F_{(1,14)}$=30.321, p<0.001, Figure 2). This finding was indicative of cocaine's characteristic stimulant effects. No other main effects or interactions were significant (all F<1.365, p>0.05).

Testing: There was a main effect of test session on locomotor activity ($F_{(4,56)}$=11.492, p<0.001). Post-hoc analyses revealed no significant difference in activity between Test 1 and Test 2. This suggested that inclusion of five additional five training sessions had no effect. Activity during Tests 1 and 2 were significantly lower than activity exhibited during Tests 3-5 (p<0.05, p<0.001, respectively; Figure 3). No other main effects or interactions were significant (all F<1.668, p>0.05).

Experiment 2

Habituation: Rats showed reduced locomotor activity across habituation sessions ($F_{(2,28)}$=6.968, p<0.05, Figure 4). The expected reduction in activity was observed following one session with the second and third session showing an unexpected leveling-out of activity. No main effects or treatment interactions yielded significance (F<0.116, p>0.05).

Baseline Training: Baseline training was used to reduce chamber novelty and any attribution of salience to the chamber environment. Activity decreased across sessions ($F_{(4,56)}$=2.491, p=0.05, Figure 5A). Post-hoc analysis revealed significantly diminished activity for the session 5 relative to activity for session 1 (p<0.05). No significant treatment interactions were observed (all F<0.381, p>0.05).

Conditioning: Compared to activity following saline treatment, cocaine induced robust hyperactivity ($F_{(1,14)}$=81.453, p<0.001, Figure 5B). No other main effects or interactions were significant (all F<1.997, p>0.05).

Baseline Testing: Data from the fourth round of testing (28 days post-final drug exposure) were excluded due to missing data attributed to computer malfunction. No significant main effect of treatment was observed ($F_{(1,14)}$=0.258, p>0.05). A significant main effect of test emerged ($F_{(2,28)}$=26.721, p<0.001). Post-hoc analysis revealed that tests 2 and 3 showed significantly greater overall activity compared to test 1, independent of treatment or cue conditions (both p<0.001). Comparable activity measures were observed for tests 2 and 3 (p>0.05). This was likely due to increased chamber novelty following each two-week incubation period.

Testing: Data from the fourth round of testing (28 days post-final drug exposure) were excluded due to missing data attributed to computer malfunction. No significant main treatment effect emerged (p>0.05). However, a very significant test x treatment interaction emerged ($F_{(2,28)}$=9.449; p=0.001), independent of cue exposure. Post-hoc analysis indicated no difference in activity measures across tests for cocaine-treated animals. Unexpectedly, controls showed heightened locomotor activity during tests 2 and 3 relative to activity measures from test 1 (p<0.05 and p<0.001, respectively). A slight trend emerged suggestive of heightened locomotor activity during test 3 compared to activity during test 2 for controls ($F_{(2,28)}$= 9.449, p=0.07, Figure 6). All main treatment effects and interactions failed to yield significant results (all F<2.766, p<0.05).

Y-Maze: Y-maze testing failed to yield significant results for any main effects or interactions (*data not shown*, all $F<2.543$, $p>0.05$; all $F<0.697$, $p>0.05$; analysis of entries and duration, respectively).

Experiment 3

Habituation: As expected, rats showed reduced locomotor activity across habituation sessions ($F_{(2,28)}=20.46$, $p<0.001$; Figure 7). Rats were significantly more active during the first habituation session compared to the second and third habituation sessions (both $p<0.01$). No other main effects or interactions were significant (all $F<0.023$, $p>0.05$).

No Cue Training: Two-way ANOVA for No Cue baseline training sessions revealed a significant main effect of session ($F_{(5,70)}=2.371$, $p<0.05$; Figure 8B). Post-hoc analysis revealed a significant reduction in activity for the second and fourth No Cue baseline sessions (B4, B8) compared to initial baseline activity ($p=0.001$, $p<0.05$, for sessions 2 and 4, respectively). A trend emerged suggestive of reduced activity for the third and fifth baseline sessions (B6, B12; $p=0.07$, $p=0.09$, respectively). There were no other significant effects (all $F< 0.875$, $p>0.05$).

Activity differed across No Cue training sessions ($F_{(5,70)}=5.55$, $p<0.001$, Figure 8B); activity was significantly reduced for session 6 (ND12) relative to activity measures for initial No Cue training (ND2) ($p<0.05$). No other effects or interactions were significant (all $F<0.635$, $p>0.05$).

Cue Training: Two-way ANOVA analysis of Cue baseline sessions revealed a significant main effect of session ($F_{(5,70)}=3.098$; $p<0.05$; Figure 8A). Post-hoc analysis indicated a significant reduction in baseline activity for the second, fifth, and sixth (B3, B9, B11) sessions relative to

session 1 (all $p<0.05$). A trend emerged suggestive of reduced baseline activity for the third session (B5) relative to session 1 ($p=0.07$, Figure 8A).

Cocaine induced robust hyperactivity during Cue training sessions ($F_{(1,14)}=46.38$, $p<0.001$, Figure 8A). There were no other significant main effects (all $F<1.251$, $p>0.05$).

Baseline Testing: Three-way ANOVA of baseline sessions (cue x session x treatment) indicated a significant main effect of session ($F_{(2,28)}=15.31$, $p<0.001$; Figure 9A); baseline activity increased for tests 2 and 3 (14 and 28 days after final drug-cue pairing) relative to baseline activity for test 1 (both $p<0.001$). No other main effects or interactions for baseline testing reached significance (all $F<2.161$, $p>0.05$).

Testing: Test session analysis comparing cue conditions (Cue (CS+), No Cue (CS-)) revealed a significant Cue x treatment interaction ($F_{(1,14)}=13.651$, $p<0.05$, Figure 9B); cocaine-treated rats exhibited heightened locomotor activity during Cue sessions compared to No Cue sessions. Conversely, saline-treated animals did not exhibit any difference in activity during Cue and No Cue sessions ($p>0.05$). A significant main effect of treatment was observed ($F_{(1,14)}=4.47$, $p=0.05$). No other main effects or interactions for conditioning testing reached significance (all $F<2.161$, $p>0.05$).

Y-Maze: Y-maze testing revealed a significant main effect of cue for both number of arm entries ($F_{(1,12)}=4.820$, $p<0.05$; Figure 10A) and duration of time in each arm ($F_{(1,12)}=11.504$, $p<0.05$; Figure 10B). Post-hoc analysis revealed that rats made more entries into the cue-associated arm and spent more time in the cue-associated arm than the no-cue arm, suggesting of the cue not being aversive.

DISCUSSION

The goal of this experiment was to determine the procedures and stimulus parameters necessary to establish successful Pavlovian conditioning between cocaine stimulant effects and a discrete sensory cue. Significant cue-induced hyperactivity for cue exposure relative to no cue conditions was considered indicative of successful conditioning. A secondary goal of this study was to investigate the possible emergence of an "incubation effect." This phenomenon has been characterized in self-administration paradigms as an increase in cue-induced activity for protracted versus acute withdrawal (Tran-Nguyen et al., 1998; Neisewander et al., 2000; Lu et al., 2004a).

Non-contingent cocaine administration induced a robust stimulant effect in all three experiments. The magnitude of cocaine-induced hyperactivity was consistent across training sessions. An inconsistent drug effect would signify the presence of a sensitization effect. Sensitization, a form of reverse tolerance, may have adversely influenced behavioral responses by changing rats' individual vulnerability to conditioning, thereby reducing data accuracy (Todtenkopf et al., 2002; Todtenkopf and Carlezon, 2006).

Discriminating Between Contextual and Discrete Cue Conditioning

Conditioning studies are confounded by the difficulty of distinguishing the specific targets of UCS-attributed salience. Consequently, studies investigating cue-specific conditioned locomotor activity must account for the complex interplay between contextual and discrete stimuli. To minimize the ability for rats to attribute the UCS (cocaine stimulant effects) with any random cue, experimenters manipulate the training and testing parameters to target the cue of interest, namely the simple discrete visual cue in experiment 1 and the compound discrete cue in experiments 2 and 3. Consequently, researchers must minimize the potential attribution of

salience to undesired stimuli present during conditioning sessions, while also maintaining CS neutrality. Should the CS not be neutral, the results are likely to show an effect of goal-directed behavior, a confounding variable that is otherwise absent from conditioned activity paradigms (Carey and Gui, 1997).

Experiment 1: Findings and Implications

Olmstead and colleagues (2006) observed that inebriated Long-Evans rats retain the ability to discriminate a discrete red flashing light and attribute it salience. In designing experiment 1, it was hypothesized that it might be possible to extend such findings to a Pavlovian conditioning paradigm between cocaine stimulant effects and exposure to a comparable discrete flashing red visual cue. However, experiment 1 failed to demonstrate cue-specific conditioned activity, indicated by the lack of cue-specific changes in activity in cocaine-treated rats. Instead, cocaine-treated rats showed heightened overall activity measures relative to controls, independent of cue condition. Although the observed treatment group difference was not significant, it was suggestive of contextual conditioning, that is, conditioned hyperactivity associated with exposure to the test chamber environment.

The most probable explanation for such unanticipated results was that rats failed to distinguish between cue conditions (Cue, No Cue). Although the Olmstead study implemented a very similar discrete visual cue to the one chosen for experiment 1, it tested Long-Evans rats, whereas this study used Sprague-Dawley rats. Although frequently used in behavioral research, the albino Sprague-Dawley shows dramatically reduced visual acuity relative to pigmented rats, such as Long-Evans rats (Prusky et al., 2002). In addition, Sprague-Dawley rats show qualitative and quantitative movement differences relative to Long-Evans rats (Whishaw et al., 2003). These strain differences suggest that conditioned locomotor activity studies should be replicated across

rat strains to determine whether specific study findings are strain-specific or whether they may be generalized. It may therefore be concluded that the lack of a cue-specific treatment interaction may have resulted from poor cue selection in relation to the intrinsic limitations in visual acuity associated with the chosen animal model.

However, if the rats were unable to distinguish the red-light cue, it follows that the non-contingent cocaine administration (15.0 mg/kg, IP) should have lead to attribution of salience to the chamber environment, as observed in numerous place-conditioning studies (Brown and Fibiger, 1992; Cervo and Samanin, 1996). Although cocaine-treated rats exhibited increased locomotor activity during the first two test sessions, independent of cue condition, the differences in overall activity were not significant across treatment groups. This suggests that some contextual conditioning may have transpired, but that sufficient salience was not attributed to the contextual cue. It is likely that replication of experiment 1 with an increased sample size would result in a stronger trend in the data.

Habituation Effects and Procedural Modifications in Experiments 2 & 3

To overcome the contextual conditioning observed in experiment 1, experiments 2 and 3 included two additional habituation sessions preceding drug-cue pairing sessions. Habituation sessions were intended to reduce test chamber novelty, thereby improving the accuracy of subsequent activity measures (Carey et al., 2005b). Average locomotor activity during habituation session(s) was used to balance average activity measures across treatment groups for all experiments to minimize group differences preceding training sessions.

In experiments 2 and 3, significant reductions in activity were observed after a single 60-minute habituation session, after which average activity measures stabilized. This presents the

possibility that including additional habituation sessions was unwarranted, but this remains to be proven.

Procedural changes implemented for experiment 2 were based on the methodologies of two studies (Panlilio and Schindler, 1997; Hotsenpiller et al., 2001). Changes included a delay between the termination of training and the start of testing, the incorporation of 30-minute baseline sessions preceding drug-cue pairing sessions, and the use of a compound cue consisting of both auditory and visual cues rather than the simple cue used in experiment 1. The delay between training and testing was intended to account for potential negative confounding effects associated with acute cocaine withdrawal. Baseline sessions served to reduce any latent chamber novelty, while also making subsequent cue exposure more explicit. The compound cue consisting of a flashing yellow visual cue and an auditory cue was chosen to enhance cue salience. Sprague-Dawley rats were more likely to discriminate the yellow visual cue; frequent studies of conditioned activity have used a paired light-tone stimulus for its enhanced salience (Hotsenpiller et al., 2001).

Experiment 2 testing failed to distinguish any cue-specific conditioned hyperactivity in cocaine-treated rats versus controls, although controls showed an unexpected significant increase in overall activity across test sessions. This increase was not associated with cue condition and may have resulted from novelty effects following reintroduction to the activity chamber following prolonged withdrawal. However, the procedural changes introduced in experiment 2 successfully eliminated the contextual conditioning observed in experiment 1, possibly through the discrete cue enhancement.

Experiment 3 elaborated upon the protocol used in experiment 2 by introducing an alternating training regimen. After three habituation sessions, rats underwent 12 training days of

alternating conditioning session type. All training days consisted of an initial 30-minute baseline session without cue exposure. On alternating days, rats were either administered cocaine or saline followed by a 30-minute conditioning session in which the cue was present (odd sessions), or alternatively, rats were administered a saline injection with subsequent 30-minute conditioning period under no-cue conditions (even sessions). It was theorized that rats would be more likely to associate cocaine administration with cue exposure if they experienced a neutral interim day between drug-cue pairings. The alternating training schedule was thought to be functioning by neutralizing any attributed salience to the chamber environment, or any other contextual cues. The final procedural change was to alter the frequency of the auditory cue. The auditory cue frequency was reduced to better align the visual and auditory rates of stimulation. It is possible that the high frequency auditory stimulation used for experiment 2 impaired drug-cue pairings, possibly due to auditory overstimulation.

Experiment 3 testing revealed the desired cue-specific conditioned hyperactivity. That is, cocaine-treated rats exhibited increased activity in the presence of the cue compared to its absence. This conditioned effect serves to validate the parameters and procedures used in experiment 3 as establishing successful Pavlovian conditioning.

Y-Maze Testing

Rats demonstrated a significant preference for the cue-associated arm of the y-maze, independent of treatment. The observed Y-maze results were likely an effect of contextual novelty. Rats show a preference for novel versus familiar environments (Bevins and Bardo, 1999). Therefore, it is not surprising that following Y-maze habituation, in which all arms are comparable to the no-cue condition, rats showed increased interest in the novel cue-associated arm. This biased preference for novelty limited the utility of Y-maze testing, since any additional exposures to the

conditioned discrete cue would serve to enhance the extinction effects on cue salience, a confound of critical importance. In addition, the treatment-independent preference for the cue-associated arm suggests that the compound cue was not aversive, an important quality of any conditioned cue.

Incubation Effect

Previous studies using self-administration models (Ciccocioppo et al., 2001; Lu et al., 2004 a,b) describe an increase in conditioned activity following prolonged withdrawal periods compared to acute or extended (6+ months) withdrawal. This inverted-U function, referred to as the incubation effect, was not observed for any of these experiments, likely due to extinction effects. Extinction effects refer to the reduction in salience attributed to a conditioned cue following repeated cue exposure in the absence of reinforcement (UCS). The magnitude of extinction effects increases with each subsequent CS exposure, such that a conditioned cue may lose all attributed salience after repeated non-reinforced pairings (De Wit and Stewart, 1981; Barr et al., 1983; Hotsenpiller et al., 2001).

However, it must also be recognized that the observed heightened locomotor activity for tests 2 and 3 in experiment 3 may have resulted from increased chamber novelty following prolonged incubation periods, as indicated by the increase in overall activity across baseline test sessions. Future studies should incorporate short, pre-incubation exposures one day prior to incubation testing to attenuate, if not remove, this novelty effect. However, the incorporation of baseline test sessions attempted to control for this effect during subsequent testing.

Study Limitations

This study used a repeated-measures design, although this design has an intrinsic flaw: any repeated test results are intrinsically skewed due to extinction effects. Additional theories have posited that this phenomenon results from increased habituation effects and subsequent reduction in associated conditioned response (McSweeney and Swinnell, 2002). Most recently, Brenhouse and colleagues (2010) have theorized that extinction effects result from the formation of new memories that override previous conditioning memories.

Therefore, an independent groups design may prevent this confound. This is supported by the observation that a significant cocaine-reinforced conditioned place preference was maintained after four weeks of withdrawal (Mueller and Stewart, 2000). In addition, the inclusion of a cocaine-unpaired or pseudocontrol group would further enhance the strength of this study. An unpaired treatment group serves to control for any nonspecific metabolic or other physiological effect of repeated cocaine administration, since this group is administered cocaine in the home cage following conditioning sessions. Including this group would also mandate inclusion of an additional saline injection for all other groups to control for total daily injections, but this is not likely to induce a significant stress response or any other confound. However, equipment limitations prevented the inclusion of this additional treatment group in the current study.

The present study may serve as a basis for a plethora of future studies utilizing the established Pavlovian conditioning paradigm detailed in experiment 3. These studies could prove extremely influential, such as through the investigation of the potential cognitive changes, such as impulsivity, following re-exposure to a discrete drug-associated cue. Impulsivity is a heterogeneous term encompassing numerous behavioral phenomena, including failure to inhibit

one's actions and difficulties in inhibition of behaviors involved in decision-making (Pattij and Vanderschuren, 2008). Such impairments include the phenomenon of impulsive choice or "delay aversion," the abnormally high tendency of addicts to choose smaller immediate rewards over larger delayed rewards (Rachlin and Green, 1972; Cardinal et al., 2004), as well as poor awareness of errors associated with impaired self-monitoring (Hester et al., 2007). By this definition, relapse, the resumption of drug taking following a period of abstinence in humans, may be considered an impulsive act. It may be claimed that addicts are unable to see or value the long-term gains of abstinence in comparison to the immediate gratification obtained through drug taking.

Interestingly, there exists a substantial overlap between the neural substrates associated with both relapse and impulsivity, specifically the medial PFC, ACC, BLA and the NAc, all of which are involved in Pavlovian conditioning (Jentsch and Taylor, 1999). It may be suggested that an association exists between impulsive behavior and cocaine relapse due to their mutual underlying neural substrates and neurotransmitter systems. However, it has yet to be determined whether drug-associated cues induce relapse through conditioned attribution of salience alone, or whether they induce neural changes that result in impulsive behavior. Using the Pavlovian conditioning procedures established in experiment 3, such an investigation could be performed using a paradigm such as the 5-Choice Serial Reaction Time Task.

If drug-associated cues increase impulsive behavior, this could have profound implications for the treatment of drug addiction and relapse**.** For example, it would suggest that abstinent cocaine addicts are prone to relapse due to cue-induced impulsivity, rather than or in addition to the phenomenon of cue-induced craving. Positive findings might also lead to potential new forms of treatment to directly target discrete sensory cues to prevent relapse in

former cocaine addicts. It may be possible that administration of a non-stimulant medication (e.g. atomoxetine) currently approved by the Food and Drug Administration to treat Attention Deficit Hyperactivity Disorder (ADHD) could function to minimize relapse by minimizing, if not disrupting, the cognitive effects of re-exposure to discrete drug-associated cues.

CONCLUSION

Pavlovian conditioning between cocaine stimulant effects and a discrete sensory cue requires the direct attribution of salience to a cue of sufficiently explicit character. In addition, the implementation of specific conditioning procedures, specifically the use of an alternating Cue conditioning/No Cue conditioning training regimen is critical in minimizing confounding contextual conditioning effects, while also enhancing the synced auditory/visual cue salience. Future study is warranted to further examine the potential implementation of the established Pavlovian conditioning procedure described here in a multi-paradigm study examining changes in cognition associated with exposure to a discrete drug-associated cue.

REFERENCES

Anker, J., & Carroll, M. (2010). Reinstatement of cocaine seeking induced by drugs, cues, and stress in adolescent and adult rats. *Psychopharmacology*, *208*(2), 211-22.

Barr, G., Sharpless, N., Cooper, S., Schiff, S., Paredes, W., & Bridger, W. (1983). Classical conditioning, decay and extinction of cocaine-induced hyperactivity and stereotypy. *Life Sciences*, *33*(14), 1341-51.

Bevins, R., & Bardo, M. (1999). Conditioned increase in place preference by access to novel objects: antagonism by MK-801. *Behavioural Brain Research*, *99*(1), 53-60.

Brenhouse, H., Dumais, K., & Andersen, S. (2010). Enhancing the salience of dullness: behavioral and pharmacological strategies to facilitate extinction of drug-cue associations in adolescent rats. *Neuroscience (Oxford)*, *169*(2), 628-36.

Bouton, M., Westbrook, R., Corcoran, K., & Maren, S. (2006). Contextual and temporal modulation of extinction: behavioral and biological mechanisms. *Biological Psychiatry*, *60*(4), 352-60.

Brown, E., & Fibiger, H. (1992). Cocaine-induced conditioned locomotion: absence of associated increases in dopamine release. *Neuroscience (Oxford)*, *48*(3), 621-9.

Brown, E., Robertson, G., & Fibiger, H. (1992). Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. *Journal of Neuroscience*, *12*(10), 4112-21.

Brown, E., & Fibiger, H. (1993). Differential effects of excitotoxic lesions of the amygdala on cocaine-induced conditioned locomotion and conditioned place preference. *Psychopharmacology*, *113*(1), 123-30.

Cardinal, R., Winstanley, C., Robbins, T., & Everitt, B. (2004). Limbic corticostriatal systems and delayed reinforcement. *Annals of the New York Academy of Sciences*, *1021*, 33-50.

Carey, R., & J. Gui. (1997). A simple and reliable method for the positive identification of Pavlovian conditioned cocaine effects in open-field behavior. *Journal of Neuroscience Methods*. *73*, 1-8.

Carey, R., DePalma, G., & Damianopoulos, E. (2002). 8-OHDPAT effects upon cocaine unconditioned and conditioned behaviors: a role for drug stimulus effects. *Pharmacology, Biochemistry & Behavior*, *72*(1-2), 171-8.

Carey, R., DePalma, G., Damianopoulos, E., & Shanahan, A. (2005a). Stimulus gated cocaine sensitization: interoceptive drug cue control of cocaine locomotor sensitization. *Pharmacology, Biochemistry & Behavior*, *82*(2), 353-60.

Carey, R., DePalma, G., & Damianopoulos, E. (2005b). Acute and chronic cocaine behavioral effects in novel versus familiar environments: open-field familiarity differentiates cocaine locomotor stimulant effects from cocaine emotional behavioral effects. *Behavioural Brain Research*, *158*(2), 321-30.

Cervo, L., & Samanin, R. (1996). Effects of dopaminergic and glutamatergic receptor antagonists on the establishment and expression of conditioned locomotion to cocaine in rats. *Brain Research*, *731*(1-2), 31-8.

Childress, A., Mozley, P., McElgin, W., Fitzgerald, J., Reivich, M., & O'Brien, C. (1999). Limbic activation during cue-induced cocaine craving. *American Journal of Psychiatry*, *156*(1), 11-8.

Childress, A., Ehrman, R., Wang, Z., Li, Y., Sciortino, N., Hakun, J., O'Brien, C. (2008). Prelude to passion: limbic activation by "unseen" drug and sexual cues. *PLoS One*, *3*(1), e1506.

Ciccocioppo, R., Sanna, P., & Weiss, F. (2001). Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: reversal by D(1) antagonists. *Proceedings of the National Academy of Sciences*, *98*(4), 1976-81.

Crombag, H., Bossert, J., Koya, E., & Shaham, Y. (2008). Context-induced relapse to drug seeking: a review. *Philosophical Transactions: Biological Sciences*, *363*(1507), 3233-43.

Dackis, C., & O'Brien, C. (2005). Neurobiology of addiction: treatment and public policy ramifications. *Nature Neuroscience*, *8*(11), 1431-6.

De Wit, H., & Stewart, J. (1981). Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology*, *75*(2), 134-43.

Derlet, R., Albertson, T., & Rice, P. (1990). The effect of SCH 23390 against toxic doses of cocaine, d-amphetamine and methamphetamine. *Life Sciences*, *47*(9), 821-7.

Di Chiara, G. (1999). A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. *Journal of Psychopharmacology*, *12*(1), 54-67.

Di Ciano, P., & Everitt, B. (2004). Direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. *Journal of Neuroscience*, *24*(32), 7167-73.

Duncan, E., Boshoven, W., Harenski, K., Fiallos, A., Tracy, H., Jovanovic, T. et al. (2007). An fMRI study of the interaction of stress and cocaine cues on cocaine craving in cocaine-dependent men. *The American journal on addictions / American Academy of Psychiatrists in Alcoholism and Addictions*, *16*(3), 174-82.

Erb, S., Shaham, Y., & Stewart, J. (1996). Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. *Psychopharmacology*, *128*(4), 408-12.

Felszeghy, K., Espinosa, J., Scarna, H., B., érod, A., Rost., et al. (2007). Neurotensin receptor antagonist administered during cocaine withdrawal decreases locomotor sensitization and conditioned place preference. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *32*(12), 2601-10.

Franklin, T., & Druhan, J. (2000). Involvement of the nucleus accumbens and medial prefrontal cortex in the expression of conditioned hyperactivity to a cocaine-associated environment in rats. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, *23*(6), 633-44.

Fuchs, R., & See, R. (2002). Basolateral amygdala inactivation abolishes conditioned stimulus- and heroin-induced reinstatement of extinguished heroin-seeking behavior in rats. *Psychopharmacology*, *160*(4), 425-33.

Fuchs, R., Evans, K., Ledford, C., Parker, M., Case, J., Mehta, R. et al. (2005). The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, *30*(2), 296-309.

Grimm, J., Hope, B., Wise, R., & Shaham, Y. (2001). Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature (London)*, *412*(6843), 141-2.

Grimm, J., Shaham, Y., & Hope, B. (2002). Effect of cocaine and sucrose withdrawal period on extinction behavior, cue-induced reinstatement, and protein levels of the dopamine transporter and tyrosine hydroxylase in limbic and cortical areas in rats. *Behavioural Pharmacology*, *13*(5-6), 379-88.

Hester, R., Sim, ões-Franklin, C., & Garavan, H. (2007). Post-error behavior in active cocaine users: poor awareness of errors in the presence of intact performance adjustments. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, *32*(9), 1974-84.

Hotsenpiller, G., Giorgetti, M., & Wolf, M. (2001). Alterations in behaviour and glutamate transmission following presentation of stimuli previously associated with cocaine exposure. *European Journal of Neuroscience*, *14*(11), 1843-55.

Hotsenpiller, G., Horak, B., & Wolf, M. (2002). Dissociation of conditioned locomotion and Fos induction in response to stimuli formerly paired with cocaine. *Behavioral Neuroscience*, *116*(4), 634-45.

Hotsenpiller, G., & Wolf, M. (2002). Extracellular glutamate levels in prefrontal cortex during the expression of associative responses to cocaine related stimuli. *Neuropharmacology*, *43*(8), 1218-29.

Ito, R., Dalley, J., Howes, S., Robbins, T., & Everitt, B. (2000). Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. *Journal of Neuroscience*, *20*(19), 7489-95.

Jentsch, J., & Taylor, J. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology*, *146*(4), 373-90.

Kelamangalath, L., & Wagner, J. (2009). Effects of abstinence or extinction on cocaine seeking as a function of withdrawal duration. *Behavioural Pharmacology*, *20*(2), 195-203.

Kalivas, P., & McFarland, K. (2003). Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology*, *168*(1-2), 44-56.

Kalivas, P., McFarland, K., Bowers, S., Szumlinski, K., Xi, Z., & Baker, D. (2003). Glutamate transmission and addiction to cocaine. *Annals of the New York Academy of Sciences*, *1003*, 169-75.

Kilts, C., Schweitzer, J., Quinn, C., Gross, R., Faber, T., Muhammad, F. et al. (2001). Neural activity related to drug craving in cocaine addiction. *Archives of General Psychiatry*, *58*(4), 334-41.

Kruzich, P., Congleton, K., & See, R. (2001). Conditioned reinstatement of drug-seeking behavior with a discrete compound stimulus classically conditioned with intravenous cocaine. *Behavioral Neuroscience*, *115*(5), 1086-92.

Lu, L., Grimm, J., Dempsey, J., & Shaham, Y. (2004a). Cocaine seeking over extended withdrawal periods in rats: different time courses of responding induced by cocaine cues versus cocaine priming over the first 6 months. *Psychopharmacology*, *176*(1), 101-8.

Lu, L., Grimm, J., Hope, B., & Shaham, Y. (2004b). Incubation of cocaine craving after withdrawal: a review of preclinical data. *Neuropharmacology*, *47 Suppl 1*, 214-26.

Mackintosh, N.J. (1974). The Psychology of Animal Learning. Academic Press, London.

Madayag, A., Kau, K., Lobner, D., Mantsch, J., Wisniewski, S., & Baker, D. (2010). Drug-induced plasticity contributing to heightened relapse susceptibility: neurochemical changes and augmented reinstatement in high-intake rats. *Journal of Neuroscience*, *30*(1), 210-7.

McFarland, K., Lapish, C., & Kalivas, P. (2003). Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *Journal of Neuroscience*, *23*(8), 3531-7.

McFarland, K., Davidge, S., Lapish, C., & Kalivas, P. (2004). Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. *Journal of Neuroscience*, *24*(7), 1551-60.

McLaughlin, J., & See, R. (2003). Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. *Psychopharmacology*, *168*(1-2), 57-65.

McSweeney, F., & Swindell, S. (2002). Common processes may contribute to extinction and habituation. *Journal of General Psychology*, *129*(4), 364-400.

Meil, W., & See, R. (1997). Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. *Behavioural Brain Research*, *87*(2), 139-48.

Mueller, D., & Stewart, J. (2000). Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. *Behavioural Brain Research*, *115*(1), 39-47.

Neisewander, J., Baker, D., Fuchs, R., Tran-Nguyen, L., Palmer, A., & Marshall, J. (2000). Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. *Journal of Neuroscience*, *20*(2), 798-805.

Olmstead, M., Lafond, M., Everitt, B., & Dickinson, A. (2001). Cocaine seeking by rats is a goal-directed action. *Behavioral Neuroscience*, *115*(2), 394-402.

Olmstead, M., Hellemans, K., & Paine, T. (2006). Alcohol-induced impulsivity in rats: an effect of cue salience? *Psychopharmacology*, *184*(2), 221-8.

Paine, T., Tomasiewicz, H., Zhang, K., & Carlezon W.A, Jr. (2007). Sensitivity of the five-choice serial reaction time task to the effects of various psychotropic drugs in Sprague-Dawley rats. *Biological Psychiatry*, *62*(6), 687-93.

Panlilio, L., & Schindler, C. (1997). Conditioned locomotor-activating and reinforcing effects of discrete stimuli paired with intraperitoneal cocaine. *Behavioural Pharmacology*, *8*(8), 691-8.

Park, W., Bari, A., Jey, A., Anderson, S., Spealman, R., Rowlett, J. et al. (2002). Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *Journal of Neuroscience*, *22*(7), 2916-25.

Parkinson, J., Willoughby, P., Robbins, T., & Everitt, B. (2000). Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. *Behavioral Neuroscience*, *114*(1), 42-63.

Pattij, T., & Vanderschuren, L. (2008). The neuropharmacology of impulsive behaviour. *Trends in Pharmacological Sciences*, *29*(4), 192-9.

Pentkowski, N., Duke, F., Weber, S., Pockros, L., Teer, A., Hamilton, E., & Neisewander, J. (2010). Stimulation of medial prefrontal cortex serotonin 2C (5-HT(2C)) receptors attenuates cocaine-seeking behavior. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, *35*(10), 2037-48.

Pockros, L., Pentkowski, N., Swinford, S., & Neisewander, J. (2011). Blockade of 5-HT2A receptors in the medial prefrontal cortex attenuates reinstatement of cue-elicited cocaine-seeking behavior in rats. *Psychopharmacology*, *213*(2-3), 307-20.

Prusky, G., Harker, K., Douglas, R., & Whishaw, I. (2002). Variation in visual acuity within pigmented, and between pigmented and albino rat strains. *Behavioural Brain Research*, *136*(2), 339-48.

Rachlin, H., & Green, L. (1972). Commitment, choice and self-control. *Journal of the Experimental Analysis of Behavior*, *17*(1), 15-22.

Reid, M., & Berger, S. (1996). Evidence for sensitization of cocaine-induced nucleus accumbens glutamate release. *NeuroReport*, *7*(7), 1325-9.

Ritz, M., Lamb, R., Goldberg, S., & Kuhar, M. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science (New York, N.Y.)*, *237*(4819), 1219-23.

Ritz, M.C., E.J. Cone & M.J. Kuhar. (1990). Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure–activity study. *Life Sci.* 46: 635–645.

Robbins, T. (2002). The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology*, *163*(3-4), 362-80.

Sabeti, J., Gerhardt, G., & Zahniser, N. (2003). Individual differences in cocaine-induced locomotor sensitization in low and high cocaine locomotor-responding rats are associated with differential inhibition of dopamine clearance in nucleus accumbens. *Journal of Pharmacology and Experimental Therapeutics*, *305*(1), 180-90.

Schmidt, H., Anderson, S., Famous, K., Kumaresan, V., & Pierce, R. (2005). Anatomy and pharmacology of cocaine priming-induced reinstatement of drug seeking. *European Journal of Pharmacology*, *526*(1-3), 65-76.

Schmidt, H., & Pierce, R. (2010). Cocaine-induced neuroadaptations in glutamate transmission: potential therapeutic targets for craving and addiction. *Annals of the New York Academy of Sciences*, *1187*, 35-75.

See, R. (2002). Neural substrates of conditioned-cued relapse to drug-seeking behavior. *Pharmacology, Biochemistry & Behavior*, *71*(3), 517-29.

See, R. (2005). Neural substrates of cocaine-cue associations that trigger relapse. *European Journal of Pharmacology*, *526*(1-3), 140-6.

Shaham, Y., Shalev, U., Lu, L., De Wit, H., & Stewart, J. (2003). The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology*, *168*(1-2), 3-20.

Shalev, U., Grimm, J., & Shaham, Y. (2002). Neurobiology of relapse to heroin and cocaine seeking: a review. *Pharmacological Reviews*, *54*(1), 1-42.

Substance Abuse and Mental Health Services Administration, Office of Applied Studies. (April 23, 2009). *The TEDS Report: Treatment Outcomes among Clients Discharged from Outpatient Substance Abuse Treatment*. Rockville, MD.

Thomas, M., Kalivas, P., & Shaham, Y. (2008). Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. *British Journal of Pharmacology*, *154*(2), 327-42.

Todtenkopf, M., Carreiras, T., Melloni, R., & Stellar, J. (2002). The dorsomedial shell of the nucleus accumbens facilitates cocaine-induced locomotor activity during the induction of behavioral sensitization. *Behavioural Brain Research*, *131*(1-2), 9-16.

Todtenkopf, M., & Carlezon W.A. Jr. (2006). Contribution of drug doses and conditioning periods to psychomotor stimulant sensitization. *Psychopharmacology*, *185*(4), 451-8.

Tran-Nguyen, L., Fuchs, R., Coffey, G., Baker, D., O'Dell, L., & Neisewander, J. (1998). Time-dependent changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *19*(1), 48-59.

UNDCP and WHO Informal Expert Committee on the Craving Mechanism: Report (1992). United Nations International Drug Control Programme and World Health Organization technical.

United Nations Office on Drugs and Crime (UNODC). (2010). *World Drug Report 2010* (United Nations Publication, Sales No. E.10.XI.13). Retrieved from UNODC website: http://www.unodc.org/unodc/en/data-and-analysis/WDR.html

Uslander, J.M., Acerbo, M. J., Jones, S. A., and Robinson, T. E. (2006). The attribution of incentive salience to a stimulus that signals an intravenous injection of cocaine. *Behavioural Brain Research, 169*, 320-324.

Volkow, N. D., Fowler, J. S., & Wang, G. J. (1999). Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. *Journal of Psychopharmacology*, *13*(4), 337-345.

Volkow, N., Wang, G., Telang, F., Fowler, J., Logan, J., Childress, A. et al. (2006). Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *Journal of Neuroscience*, *26*(24), 6583-8.

Weiss, F., Ciccocioppo, R., Parsons, L., Katner, S., Liu, X., Zorrilla, E. et al. (2001). Compulsive drug-seeking behavior and relapse. Neuroadaptation, stress, and conditioning factors. *Annals of the New York Academy of Sciences*, *937*, 1-26.

Whishaw, I., Gorny, B., Foroud, A., & Kleim, J. (2003). Long-Evans and Sprague-Dawley rats have similar skilled reaching success and limb representations in motor cortex but different movements: some cautionary insights into the selection of rat strains for neurobiological motor research. *Behavioural Brain Research*, *145*(1-2), 221-32.

FIGURES

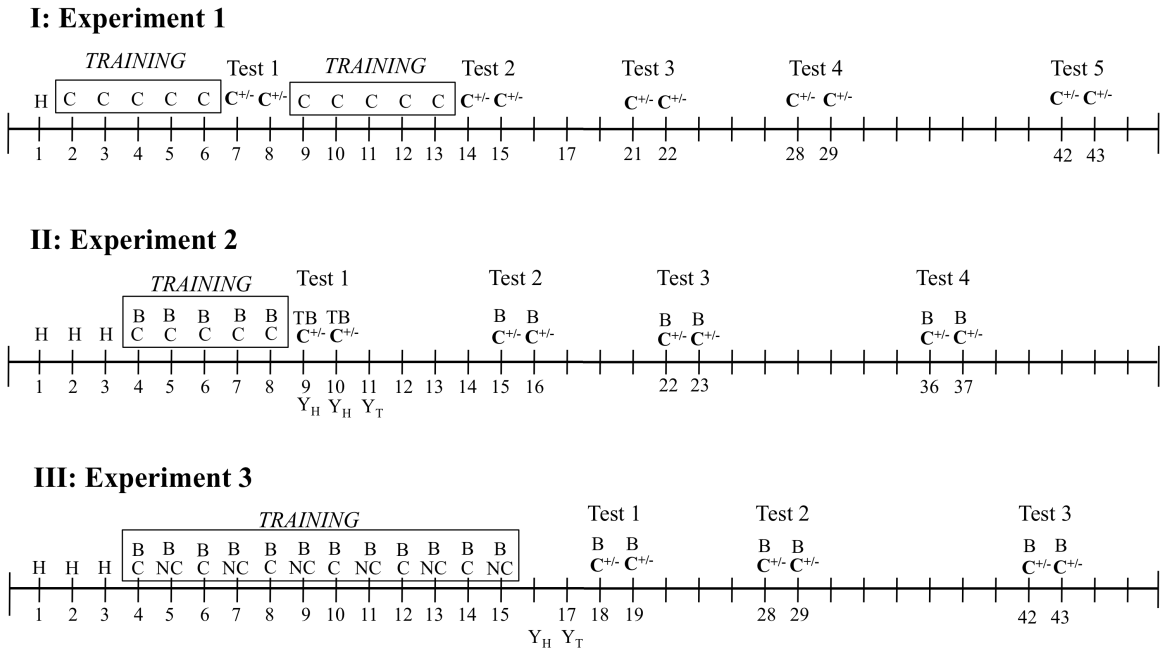

Figure 1. Daily experimental procedures for each experiment. ***I) Experiment 1***: **H**=Habituation Session with saline IP injection and subsequent 60 minute session (no cue exposure). **C**= conditioning session in which rats received IP injection of either Cocaine (15.0 mg/kg) or Saline (1.0 mg/kg) with subsequent exposure to the discrete visual cue for 30 minutes. $\mathbf{C^{+/-}}$= 30-minute test session with either cue exposure ($\mathbf{C^{+}}$) or no cue exposure ($\mathbf{C^{-}}$). Cue condition was counterbalanced across treatment groups. No injections were given on test days. The order of cue presentation was switched after tests 1 and 2. ***II/III) Experiments 2 and 3:*** Experiments 2 and 3 introduced 3, 60-minute habituation sessions (H) identical to those used in experiment 1. Data were used to assign rats to treatment groups. Training sessions were divided into Baseline (B) and Conditioning (C) sessions. **B**=30-minute session in which rats were placed in activity chambers without cue exposure (No Cue). Rats were administered Cocaine (15.0 mg/kg, IP) or Saline (1.0 mL/kg, IP) before conditioning (C) sessions. **C**=30-minute session with cue exposure (Cue). In experiment 3, no cue conditioning sessions (NC) were introduced. All rats were administered saline (1.0 mL/kg, IP) preceding NC sessions. **NC**=30-minute conditioning period without cue exposure (No Cue). Test days had a similar structure to training days with 30-minute baseline sessions (**B**) preceding test sessions ($\mathbf{C^{+/-}}$). Test sessions deviated from experiment 1 only in having all rats receive saline prior to $C^{+/-}$ tests. $\mathbf{Y_H}$=Y-maze habituation session. For experiment 2 this involved placing rats in the starting arm of the Y-maze and allowed 5 minutes of unimpeded exploration. For experiment 3, rats experienced two cycles of the 5-minute habituation procedure with maze exposures separated by approximately 60 minutes. $\mathbf{Y_T}$= Y-maze testing session. Rats were placed into the starting arm of the y-maze with one arm randomly associated with the sensory cue (CS+). The CS+ arm was counterbalanced across treatment groups.

Experiment 1

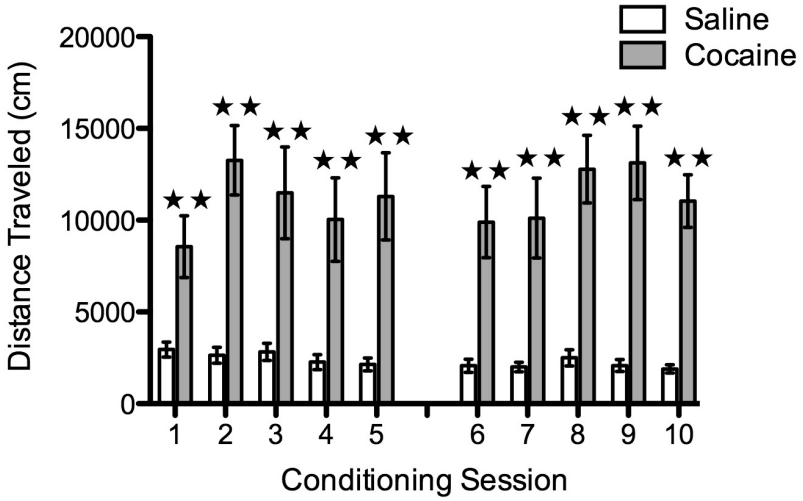

Figure 2. Cocaine stimulant effects on locomotor activity compared to saline-treated controls. Average locomotor activity (Mean ± SEM) during training sessions reveals that cocaine (15.0 mg/kg, IP) induced significant hyperactivity ($p<0.001$). Between-subject comparisons across sessions 1-5 and 5-10 indicated no treatment x session interaction ($p>0.05$) but a significant main effect of treatment ($p<0.001$). ★★ denotes a significant difference from saline-induced activity measures, $p<0.001$.

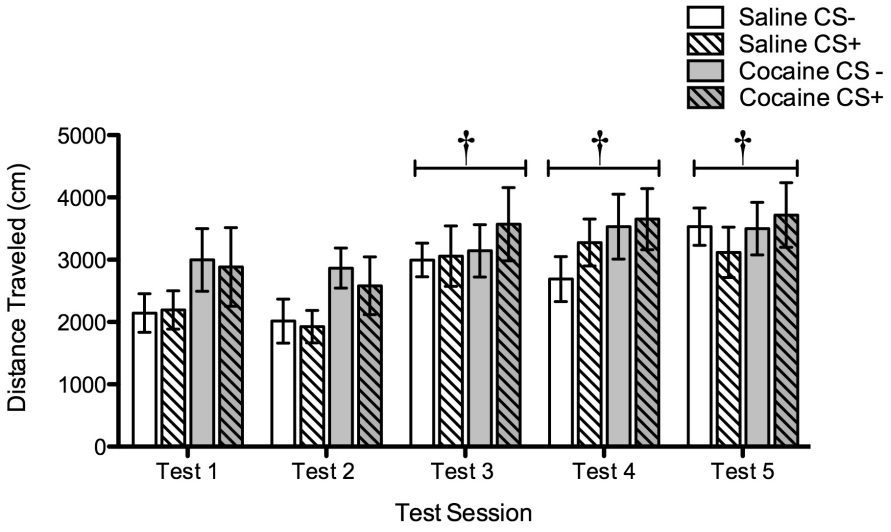

Figure 3. Conditioned locomotor activity associated with re-exposure to discrete visual cue (Mean ± SEM). Following completion of 5 training sessions (Test 1) and 10 training sessions (Test 2), rats underwent 2, 30-minute testing sessions over two consecutive days, one test session with exposure to the discrete cue (CS+) and one test session without cue exposure (CS-) in a counterbalanced order. Rats underwent an identical 2-day test period following increasing periods of cocaine withdrawal (7 days, 14 days, 28 days, for Tests 3, 4, and 5, respectively). Drug withdrawal period was defined as number of days following final drug administration. The order of cue presence/absence was counterbalanced across treatment groups and across animal cohorts. Activity during tests of cue exposure (CS+) did not significant differ from activity during baseline tests (CS-). No incubation effect was observed. † Denotes a significant difference in activity relative to Test 1 activity, $p<0.05$ and relative to Test 2 activity, $p<0.001$ for within-subject comparison.

Experiment 2

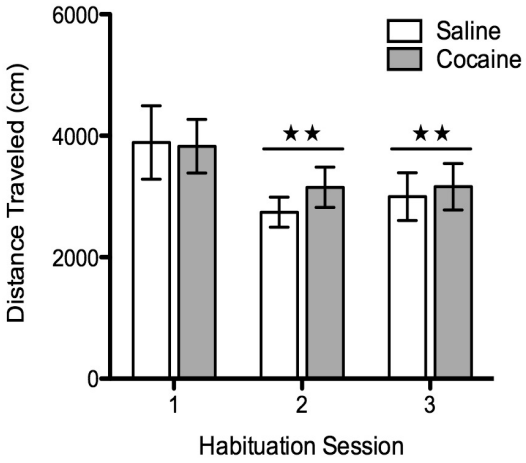

Figure 4. Effect of repeated habituation to activity chamber on average locomotor activity. Data are presented as average locomotor activity (Mean ± SEM). Rats underwent three identical habituation sessions preceding training. Rats were administered saline injections (physiological saline, 1.0 mL/kg, n=16) preceding insertion into assigned activity chambers for each 60-minute session. Average locomotor activity across habituation sessions was used to balance treatment group assignment (Cocaine, n=8; Saline, n=8). A significant main effect of session was observed ($p<0.05$). Post-hoc analysis by Least Significant Difference with Estimated Marginal Means correction indicated a significant reduction in activity for the second and third sessions compared to session 1. Sessions 2 and 3 showed comparable activity. No other main effects or interactions were significant ($p>0.05$). ★★ denotes significant difference in activity relative to session 1, $p<0.001$.

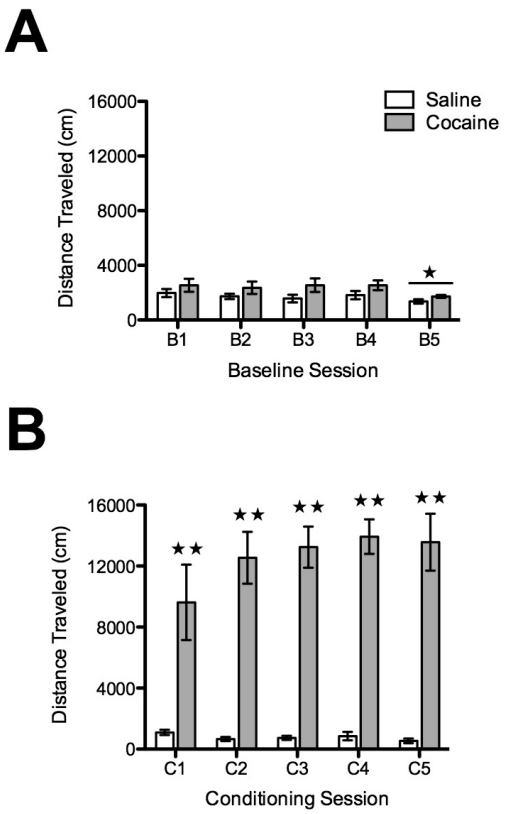

Figure 5. Locomotor activity during baseline and conditioning training sessions. Average activity measures (Mean ± SEM) are presented for 30-minute baseline sessions and 30-minute conditioning sessions. Rats underwent an initial 30-minute baseline session in activity chambers without cue exposure (A). Following session completion, rats were injected with either saline (1.0 mL/kg, IP, n=8) or cocaine (15.0 mg/kg, IP, n=8) and subsequently returned to chambers for a 30-minute conditioning session with cue exposure (B). No significant effect of treatment was observed for baseline training sessions ($p>0.05$). However, average activity for baseline session 5 was significantly reduced relative to baseline session 1 activity, independent of any treatment effect ($p<0.05$). Cocaine treatment induced robust hyperactivity ($p<0.001$). Analysis of conditioning sessions yielded no significant main effect of session ($p>0.05$). # indicates a significant difference in average activity compared to baseline session 1 measures, independent of treatment ($p<0.05$). ★★ denotes a significant difference from saline-induced activity, $p<0.001$.

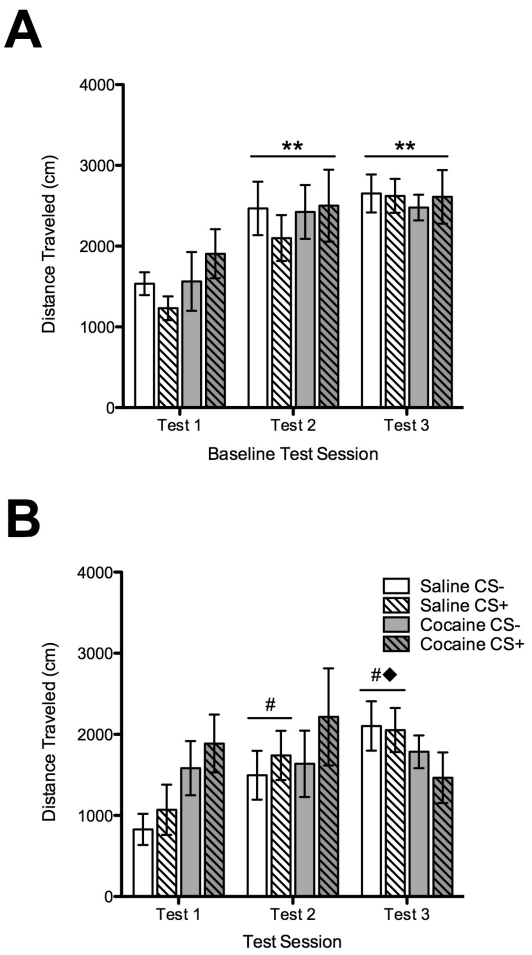

Figure 6. Effect of re-exposure to a conditioned discrete cue on locomotor activity. Rats previously underwent drug-cue pairing between drug treatment (Cocaine, 15.0 mg/kg, IP, n=8; Saline, n=8) and exposure to a compound discrete auditory/visual cue. On test days, rats were habituated to the activity chamber for a 30-minute baseline session (A) prior to saline injection (0.9% NaCl, 1mg/mL, IP) and subsequent 30-minute test session (B) either with the cue (CS+) or without the cue (CS-). Cue order was randomized. Cue exposure (CS+) did not induce any significant change in average locomotor activity (Mean ± SEM) compared to No Cue (CS-) test sessions (within-subjects analysis, $p>0.05$). A significant main-effect of test session was observed ($p<0.001$). Post-hoc analysis by Estimated Marginal Means with a Least Significant Difference correction indicated a significant increase in baseline activity with increasing withdrawal. Pairwise comparison indicated a significant increase in activity from initial testing to incubation testing 1 ($p<0.001$) and from initial testing to incubation test 2 ($p<0.001$). No significant difference was observed between baseline activity for each round set of incubation tests ($p>0.05$). Data from incubation test 3 (4 weeks of drug withdrawal) were excluded from analysis due to data acquisition error associated with computer malfunction. # $p<0.05$ Denotes a significant difference from Test 1 activity. ♦ $p=0.07$ Denotes a trend towards a difference in activity measures for Test 3 versus Test 2. ** $p<0.001$. Denotes significant difference in average activity compared to Test 1 Baseline measures.

Experiment 3

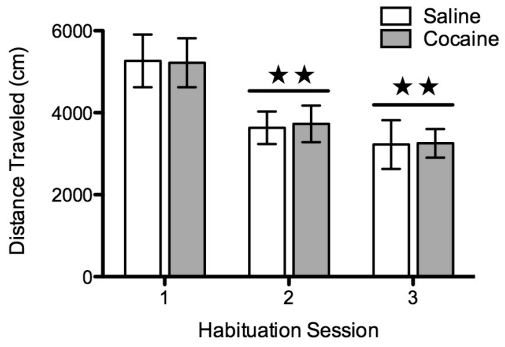

Figure 7. Reduced locomotor activity with repeated habituation to chamber environment (Mean ±SEM). Rats received saline injection (1.0 kg/mL, IP) followed by 60-minute habituation sessions in which rats were exposed to the testing chamber environment in the absence of the discrete cue. Repeated habituation sessions induced reduced average locomotor activity with significant reductions in average activity observed for all animals during the second and third habituation sessions compared to activity measures during initial habituation ($p<0.001$ for both). ★★ $p<0.001$, Denotes a significant difference compared to initial habituation session average activity measures.

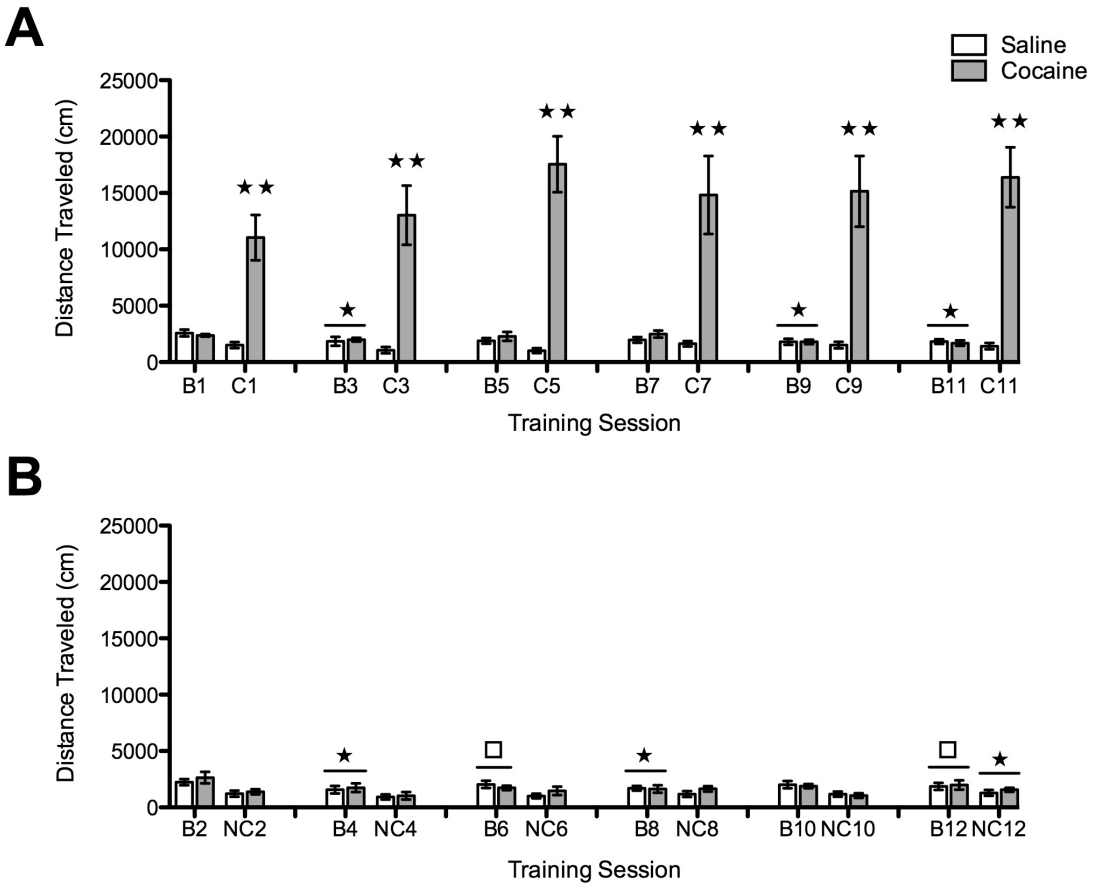

Figure 8. Locomotor activity during baseline and conditioning training sessions. Data are presented as average activity measures per treatment group for baseline, conditioning, and no-drug conditioning sessions (Mean ± SEM). On alternating days, rats underwent identical 30-minute baseline sessions followed by either cocaine administration (15.0 mg/kg, IP, n=8) or saline administration (1.0 mL/kg, IP, n=8) and a 30-minute conditioning session (C) with cue exposure (A). On interim days, all rats had a 30-minute baseline session and subsequent saline injection (1.0 mL/kg, IP, n=16) followed by a 30-minute no-drug conditioning session (NC) without cue exposure (B). Cocaine induced robust hyperactivity during conditioning sessions ($p<0.001$). No treatment-specific differences were observed for baseline sessions ($p>0.05$). Independent of treatment, rats showed significant reductions in average activity for baseline sessions B3, B9, and B11 compared to average activity for session B1 ($p<0.05$). Similarly, baseline sessions preceding no-drug conditioning sessions showed a treatment independent reduction in activity for sessions B4 and B8 compared to activity for session B2 ($p<0.05$). Trends suggestive of reduced activity were observed for sessions B6 and B12 compared to B2 (☐ $p=0.07$, $p=0.09$, respectively). ★ $p<0.05$ Denotes a significant overall difference in activity compared to initial baseline sessions for Cue or No Cue Baseline Sessions. ★★ $p<0.001$ Denotes a statistically significant difference in average activity relative to saline-induced locomotor activity.

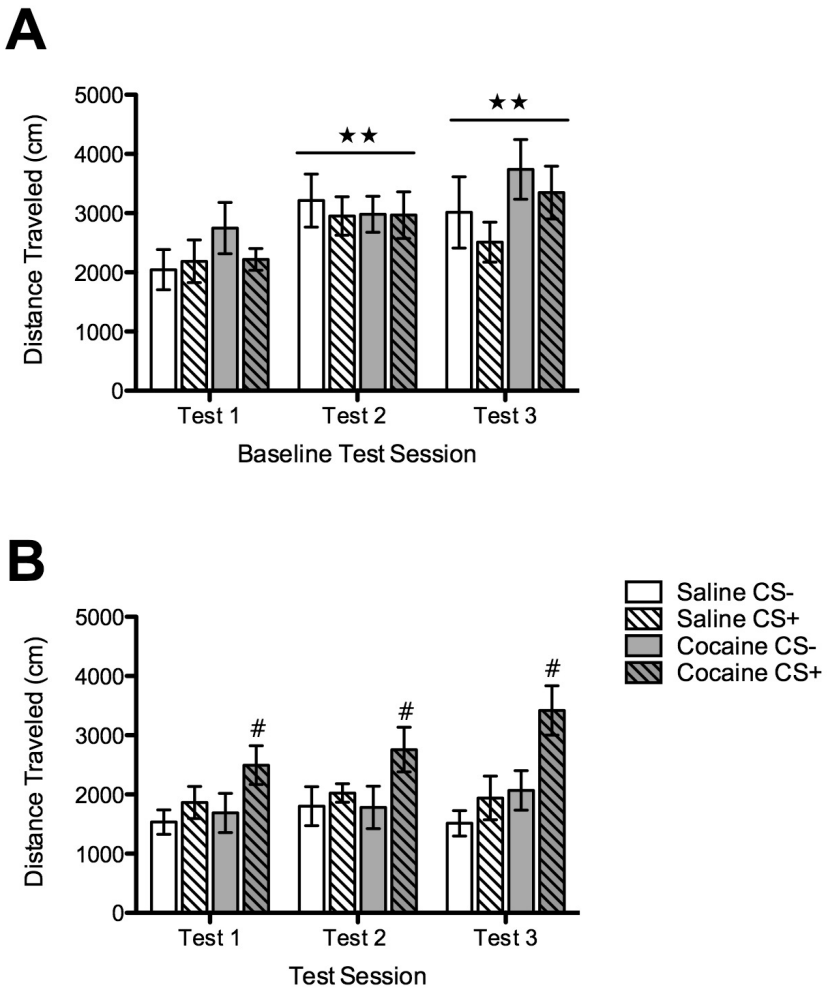

Figure 9. Difference in locomotor activity associated with re-exposure to drug-associated compound cue. Data are presented as average (Mean ± SEM) locomotor activity measures for baseline (A) and testing (either cue present (CS+) or cue absent (CS-) conditions; B). Exposure to the discrete compound cue resulted in significant cue-induced hyperactivity across all three tests for the difference between cocaine cue (CS+) and no cue (CS-) conditions (Test 1 was 3 days after final drug-cue pairing; Test 2 was 14 days after final drug-cue pairing; Test 3 was 28 days after final drug-cue pairing). There was no difference in saline animals for cue (CS+) and no-cue (CS-) test conditions ($p<0.05$ for all three tests) # denotes a significant difference in activity measures between cocaine cue (CS+) and no cue (CS-) conditions ($p<0.05$). ★★ $p<0.001$ denotes a significant difference in activity measures compared to Test 1 measures.

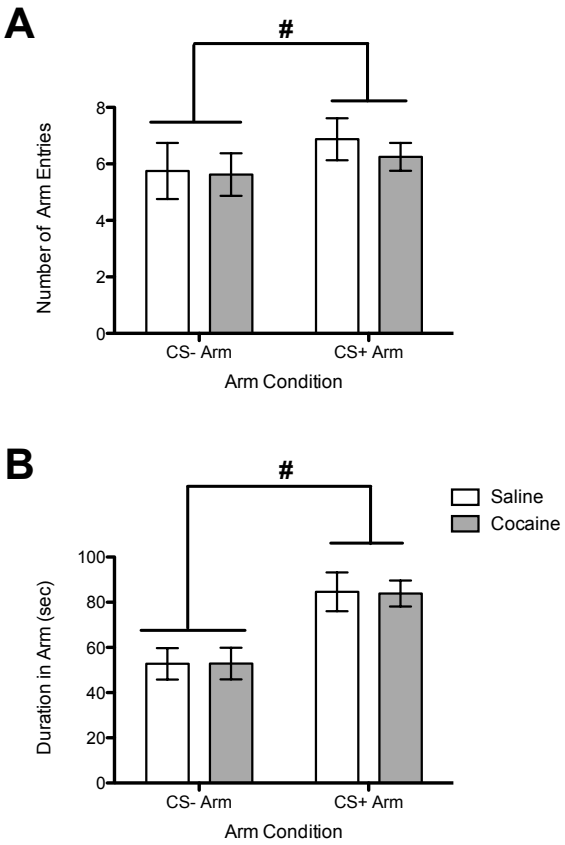

Figure 10. Y-Maze Testing. Rats were placed into the starting arm of the Y-maze for a 5-minute test session in which behavioral measurements were recorded for total arm entries (cue-associated arm (CS+) or no cue arm (CS-); A) and duration of test spent in each arm (cue-associated arm (CS+) or no cue arm (CS-); B). Data are presented as the average per treatment group for both measures (Mean ± SEM). Rats showed a significant preference for the cue-associated arm versus no cue arm as per increased total number of entries and duration of test period spent in the cue-associated arm ($p<0.05$ for both) independent of previous treatment (Cocaine, n=8; Saline, n=8; $p>0.05$)
# denotes significant differences across cue conditions ($p<0.05$).

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I affirm that I have adhered to the Honor Code in this assignment. Jonathan Miller Wachtel