The Effects of Rimonabant (SR141716) on the Reinstatement of Cocaine-Seeking Behavior via Oral Self-Administration in the Presence of a Tactile Discriminative Stimulus



Adam Joseph Brandner

Submitted to the Neuroscience Department, Allegheny College
in partial fulfillment of the requirements for the degree of
BACHELOR OF SCIENCE
3rd April 2017

The Effects of Rimonabant (SR141716) on the Reinstatement of Cocaine-Seeking Behavior via Oral Self-Administration in the Presence of a Tactile Discriminative Stimulus

A Senior Comprehensive Project
Presented by:
Adam Joseph Brandner

3rd April 2017

Pledge: _____

Approved:

_____

Dr. Rodney Clark, Ph.D.

_____

Dr. Jeffrey Hollerman, Ph.D.

Acknowledgements

Dr. Rodney Clark and Dr. Jeffrey Hollerman for their continual assistance and inspiration.

I would not have chosen any other way to spend my last four years than spending it with The Neuroscience community at Allegheny College.

Table of Contents

Abstract…….....5

Introduction…….....6

Literature Review…….....7

- The Reinstatement Model
- Mechanisms for Drug-Seeking Behaviors
- Modulation of the CB_1 Receptor
- Statement of Purpose

Methods and Materials……...10

- Animal Care
- Apparatus
- Cocaine and Saccharin Preparation
- Rimonabant and Priming Cocaine Preparation
- Experimental Protocol
  - Training
  - Acquisition and Maintenance
  - Extinction
  - Reinstatement
- Data Analysis

Results……15

- Acquisition
- Maintenance
- Extinction
- Reinstatement

Discussion……..19

Conclusion…….23

References……..24

Appendix……....27

- A
- B
- C
- D

Abstract

Cocaine addiction in the United States has reached epidemic proportions. About 30 percent of people above the age of 18 reported using cocaine in 2014 and an increase in overdoses has been observed since 2001. Current rehabilitation techniques still result in a high rate of relapses. Rimonabant has been proposed as a pharmacological treatment for preventing relapse in patients that suffer from cocaine addiction. The present study investigated whether or not Rimonabant can attenuate cocaine-seeking behavior via oral self-administration in the presence of a discriminative stimulus by using the four phase reinstatement model. Six female Sprague-Dawley rats were trained on a variable ratio 5 (VR5) schedule of reinforcement to acquire, maintain, and extinguish the behavior of lever pressing for cocaine. A mixed model analyses of variance (ANOVA) revealed a significant difference in response rate between Rimonabant (1.0 mg/kg) paired with a subthreshold dose of cocaine (1.25 mg/kg) injected intraperitoneally (i.p.) when compared to the response rate with just the i.p. injection of subthreshold dose of cocaine ($p < 0.05$, $p = 0.05$). However, the oral self-administration behavior was not acquired at the beginning of the acquisition phase, therefore, the significance of the reinstatement phase remains inconclusive.

Introduction

Cocaine abuse has reached an epidemic proportion in the United States and about 1.5 million Americans reported using cocaine regularly according to the 2011 survey done by Substance Abuse and Mental Health Services Administration (SAMHSA, 2011). About 30 percent of people above the age of 18 reported using cocaine in 2014, and this trend is expected to increase in later years according to the National Institute on Drug Abuse (NIDA, 2014). A report done by the Centers for Disease Control and Prevention describes that there has been an alarming increase of cocaine overdoses since 2001, specifically, a 42 percent increase in deaths (CDC, 2014). There is a major difference between using cocaine and becoming psychologically addicted to the drug.

Cocaine works by blocking the reuptake of dopamine in the mesocorticolimbic pathway in the brain, and this effect leads to euphoria and a heightened sense of accomplishment and strong susceptibility to rewarding events. A person who abuses cocaine will experience physiological changes in their body and will feel psychologically dependent to the drug (Gawin, 1989). When using cocaine, extreme amounts of positive feedback from the environment reinforces the user. As the user becomes more tolerant to the drug, the amount of cocaine needed to reach the "peak" euphoria increases (Gawin, 1989). Cocaine is a stimulant and consistent and excessive usage will eventually lead to death.

Rehabilitation techniques include removing oneself from the environment cocaine was used in. This means all environmental cues, including people and settings, should be avoided to increase recovery and decrease relapse vulnerability (Walton, A., Reischl, T., & Ramanathan, C., 1995). Despite current rehabilitation techniques, about 28 percent of cocaine abusers attempting to quit relapse after a period of withdrawal (Walton et al., 1995). This study conducted by

Walton and colleagues state that the most important factors that initiate drug-seeking behavior in humans are the environment, stress and the drug itself. Despite current treatment options for drug addiction, rehabilitation techniques still lead to an alarming rate of relapses (1995). A pharmacological drug could be developed in order to resolve this issue.

Literature Review

The Reinstatement Model

Drug addiction constitutes a long term motivation and craving for a drug that the user has had a particular reinforcing history with (De Vries and Schoffelmeer 2005). Currently, research suggests that to satisfy the negative effects of withdrawal, three modes of environmental cues are possible in triggering relapse: the drug itself, stress, and exposure to discriminative stimuli (De Vries and Schoffelmeer, 2005). De Wit and Stewart first reported on increased cocaine-seeking behavior after a period of withdrawal (1981). Based on this evidence, they were able to identify the factors that initiate relapse, or reinstatement of a previously extinguished drug-seeking behavior (Shaham, Y., Shalev, U., Lu, L., De Wit, H., & Stewart, J., 2003). They concluded that stressors and environmental cues would initiate the drug-seeking behavior which ultimately reinstates use of the drug of choice. (Shaham et al., 2002; De Wit and Stewart 1981).

Drug reinstatement protocols are used to model human drug-seeking behavior in animals and constitutes as four phases: acquisition, maintenance, extinction and reinstatement (Bossert, J., Marchant, N., Calu, D. & Shaham, Y., 2013). Shaham et al. consolidated research that uses a wide array of drugs and environmental cues in the reinstatement model (2002). During cocaine maintenance with and without discrete cues, a CB_1 antagonist attenuates cocaine-seeking behavior (Shaham et al., 2002). In clinical studies, increased brain activity is reported in addicts

when shown a discrete cue that was paired with their drug usage (Kalivas and McFarland, 2003). The difference between discrete and contextual cues, as Shaham et al. states in the paper, is a matter of whether the stimuli was classically or operantly conditioned (2003). It may be possible that these two ways of "learning" utilize separate pathways in the brain, and therefore, the effect of a CB_1 antagonist will be different depending on how the stimuli that predicts drug-availability was conditioned (Kalivas and McFarland, 2003).

Mechanisms for Drug-Seeking Behaviors

The Ventral Tegmental Area (VTA) can be regarded as part of the reward circuit with projections to the Nucleus Accumbens (NAcc) and glutamatergic neurons from the cerebral cortex that innervate the VTA (Maldonado, R., Valverde, O., & Berrendero, F., 2006). These circuits, which are all connected, play an important role with processing the negative motivational consequences of drug withdrawal and drug relapse. Specifically, glutamatergic neurons project to the NAcc from the cerebral cortex, hippocampus, and amygdala to provide context for the internal physiological processes that occur during drug intake (Maldonado et al., 2006). When rats self-administer cocaine, there is a change in the NAcc firing pattern suggesting that the NAcc is being affected by drug-seeking behavior (Carelli and Deadwyler 1996). All of these pathways interact with the mesocorticolimbic dopamine system in drug-seeking behavior (Ghitza, U., Fabbricatore, A., Prokopenko, V., & West, M. 2004).

Endocannabinoids are neuromodulators that regulate a number of neuronal pathways in the mesocorticolimbic dopamine system which ultimately excite dopaminergic neurons of the NAcc (Xi, Z., Gilbert, J., Peng, X., Pak, A., Li, X., & Gardner, E., 2006; Maldonado et al., 2006). Recently, these endocannabinoids have demonstrated retrograde control of the reward circuit by being released post-synaptically after glutamate and dopamine receptors are activated,

and bind to CB_1 receptors on pre-synaptic cells (Maldonado et al., 2006). The CB_1 receptors are mainly found on glutamatergic and GABAergic neurons that innervate the VTA. Although the mechanism of CB_1 receptors is still unknown, it is still generally understood that drug reinstatement after a period of withdrawal is evident because of increased concentration of endogenous cannabinoids (Xi et al., 2006).

Modulation of the CB_1 Receptor

Two CB_1 antagonists are known to reduce the drug-seeking behavior in animals. In a study done by Rodriguez et al. suggested that the CB_1 antagonist AM251 attenuated methamphetamine self-administration via injection into the nucleus accumbens at high doses (2010). Likewise can be said for the CB_1 antagonist Rimonabant (SR141716), which caused a significant decrease in nicotine self-administration (Cohen, C., Perrault, G., Griebel, G., & Soubrie, P., 2004). Previous studies demonstrated that activating the CB_1 receptor with an agonist can reinstate cocaine-seeking behavior after a period of withdrawal from the drug (De Vries, T., Shaham, Y., Homberg, J., Crombag, H., Schuurman, K., Dieben, J., Vanderschuren, L. & Schoffelmeer, A., 2001). In this study, data suggests that Rimonabant reversed the conditions induced by the CB_1 agonist, HU210. When Rimonabant was administered alone, it had no effect when compared to baseline data with just the vehicle (De Vries et al., 2001).

Evidence suggests that a CB_1 antagonist attenuates self-administration during reinstatement of drug-seeking behavior maintained by a discriminative stimulus. Because of the data presented from previous studies, stimulant-seeking behavior can be attenuated with a CB_1 antagonist. It is important to note that the authors used the reinstatement model of drug-seeking behavior to conduct their respective study.

Statement of Purpose

The evidence above suggests that elevated concentrations of endocannabinoids are directly related to drug-seeking behavior, but drug-seeking behavior can be reinstated in three ways: the drug itself, stressors, or a discriminative stimulus that was previously paired with that drug. Discriminative stimuli, which cue reinstatement of the drug-seeking behavior that was previously extinguished, is linked to elevated endocannabinoid levels in the NAcc. The present study proposes that the CB_1 receptor antagonist Rimonabant (SR141716) will attenuate cocaine-seeking behavior in the presence of a discriminative stimulus that was paired with oral self-administration of cocaine. This study will make use of the four-phase drug-reinstatement model to obtain results that proves the hypothesis correct.

Methods and Materials

Animal Care

Six Sprague-Dawley female rats (*Rattus norvegicus*) from Hilltop Farms in Scottsdale, PA, were used for this study. The subjects were experimentally naïve before experimental protocols commenced and they maintained at least 80% of their body weight for the duration of this experiment. Animals were water deprived for 23 hours prior to each training session and were handled for 20 minutes a day before each training session. Water deprivation was faded out from 23 hours to 0 hours during the acquisition phase of the reinstatement protocol. During maintenance, the animals had free access to food and water. The animals were housed in groups of three in Carnegie's rat colony. During the time of experimentation, they underwent experimentation with minimal light and sound. Vitals were monitored during experimentation. If

the animals appeared to be visibly ill, then the antibiotic Terramycin was dissolved in the water supply.

Apparatus

A standard Lafayette Instruments Co. operant conditioning chamber (model # 80001) served as the apparatus. The chamber was equipped with two levers and two stimulus lights. Scheduling of all experimental events and data collection was accomplished through a Lafayette operant conditioning console (model #81335). Since Allegheny College does not have a self-administration apparatus, the rat orally self-administered the cocaine.

Cocaine and Saccharin Solution Preparation

Two solutions were made and used as a reinforcers during the acquisition and maintenance phases of the reinstatement protocol. A 4 g/L sugar solution was used initially at the start of the acquisition phase and a 0.05 g/L cocaine solution was used at the start of the maintenance phase. The concentration of cocaine used was determined by whether or not rats were able to increase the rate of drinking a cocaine solution with subsequent increases in cocaine concentration (George and Goldberg, 1988). Both cocaine and sugar were dissolved in distilled water to create two solutions. Variable amounts of both solutions were mixed together during the acquisition phase in order to create a fading protocol.

Rimonabant and Priming Cocaine Preparation

Ten mg of Rimonabant was obtained from Cayman Chemicals and dissolved in ethanol (96% EtOH), Tween-80, and saline (0.9% NaCl) in a 0.5mL: 0.5mL: 9mL ratio (Crunelle, C., Giessen, E., Schulz, S., Vanderschuren, L., Bruin, K., Brink, W. & Booij, J., 2011). This solution served as a vehicle for Rimonabant. This dosage of Rimonabant will be considered a high dose

compared to other relatively ineffective doses (Rodriguez, J., Boctor, S., Flores, L., Phelix, C., & Martinez, J., 2010). 1.25 mg of cocaine was dissolved in a 10 mL of saline solution (0.9% NaCl) to create a subthreshold dose of cocaine that was used as a priming agent for the reinstatement of the cocaine-seeking behavior (Bossert et al., 2013; Crunelle et al., 2011). The subthreshold dose is based on whether an i.p. injection of cocaine under or over 5 mg/kg primed an animal to respond (Fuchs, R., See, R., & Middaugh, L., 2003).

Experimental Protocol – Training

Animals were placed into operant chambers and trained to respond on CRF (continuous reinforcement) schedule of reinforcement for 30 minutes/day. The schedule of reinforcement was gradually increased from a CRF to VR-3 (variable ratio) as subjects maintained at least 80% correctness on the appropriate lever. Then, the schedule of reinforcement was increased from a VR-3 to a VR-5 as subjects maintained at least 80% correctness on the appropriate lever. Water served as the primary reinforcer. Pressing the front right lever of the operant chamber with at least 20 grams of force constituted as one response in this experiment.

Experimental Protocol – Acquisition and Maintenance of Cocaine Self Administration

Ingestion of cocaine is aversive to the rat, so to ensure proper oral self-administration of cocaine, the sugar substitute and cocaine was faded out/in during the acquisition phase, respectively (Carelli and West, 2014). The fading procedure for cocaine and sugar substitute was adapted from Cunningham and Niehus (1997). The sugar substitute was present at the beginning of the acquisition phase in the reinforcing solution at concentration of 4 g/L. One fourth of the sugar substitute in the reinforcing solution was faded out every 3 days during the acquisition phase. At the same time, ¼ of the cocaine was added to the reinforcing solution until the cocaine

reached a concentration of 0.05 g/L. Water deprivation will also be faded out during the acquisition phase starting from 23 hours down to no water deprivation. We expected to see the rate of responding to decrease during the transition from acquisition to maintenance (Kosten, T., Miserendino, M., Haile, C., DeCaprio, J., Jatlow, P., & Nestler, E., 1997). Rats were placed under a VR-5 schedule of reinforcement for 30 minutes/day. A 4x8 inch rubber tactile mat was placed at the beginning of each session in front of the levers which acted as the discriminative stimulus.

Cocaine maintenance began when the sugar was completely faded out of the reinforcing solution and when the cocaine reached the anticipated concentration of 0.05 g/L. Maintenance used a within subjects session dose-response test and continued until we saw the lever response maintain 80% correctness (Shaham et al., 2003). Animals were placed under a VR-5 schedule of reinforcement for 30 minutes/day. A 4x8 inch rubber tactile mat was placed in front of the levers which acted as the discriminative stimulus. After several successful trials with the rate of responding remaining consistent, experimentation continued with phase three of the reinstatement protocol.

Experimental Protocol – Extinction of Cocaine Self Administration

During the extinction phase, the cocaine solution and tactile mat will be withdrawn from the operant chambers. The rats will be free to respond in the chamber, but no contingency will take place during extinction. Extinction will continue for 30 minutes/day until the rate of responding reaches <1 per 30 minute session for 3 consecutive days (Xi et al., 2006).

Experimental Protocol – Reinstatement of Cocaine Self Administration

Reinstatement of cocaine occurred after extinction criteria was met. Reinstatement proceeded for four days. The first day consisted of the subthreshold dose of cocaine administered intraperitoneally (i.p.) for each animal. In conjunction with the subthreshold dose of cocaine, the animals received 1.0mg/kg of Rimonabant via an i.p. injection laterally to where the cocaine was injected. During the second day of reinstatement, the subthreshold dose of cocaine was only administered to the animals. The process was repeated for four consecutive days, establishing an ABAB schedule of drug administration. Fifteen minutes after each round of injections, the animals were placed in the operant chambers with the tactile mat and water as the reinforcer.

Data Analysis

Data obtained from the acquisition, maintenance, and extinction phases were analyzed using repeated measure and a one-way analysis of variance (ANOVA) on the active levers to compare average response rates within each phase. To measure effect size and main effects, reinstatement data was analyzed using repeated measures and a mixed model (2x2) analyses of variance (ANOVA) on the active levers. The average response rate for both conditions in the reinstatement phase were also analyzed using a paired-samples T-test.

Results

One-way ANOVA - Acquisition

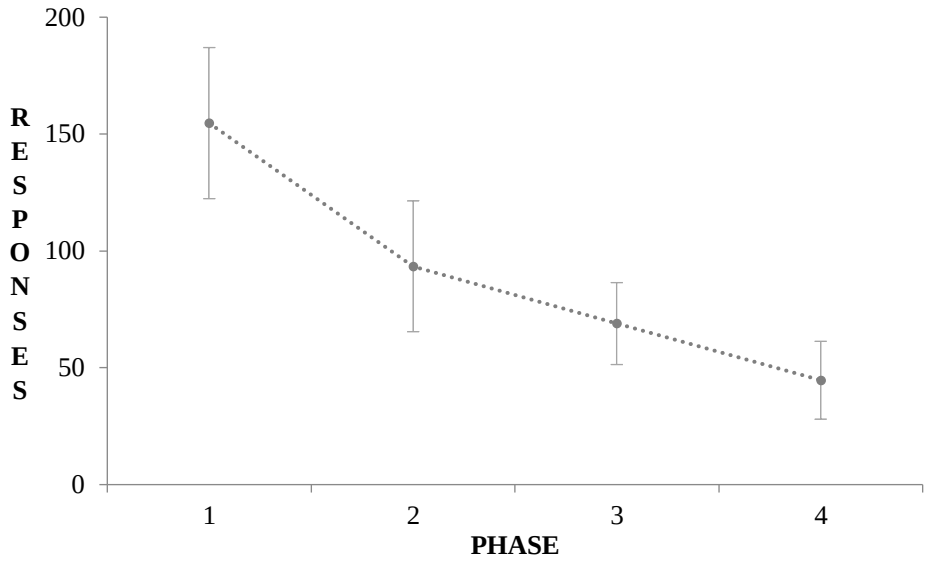


Figure 1: Average responses of all six subjects across the 12 day acquisition period (See Appendix A). Phases 1-4 represent days 1-3, 4-6, 7-9, and 9-12 respectively. In phase one, the rats received full sugar as a reinforcer. Phase 2 contained ¾ of the original concentration of sugar while ¼ of the 0.05 g/L cocaine solution. Phase three reinforced the rats with a solution containing ½ of the original sugar solution and cocaine solution. Phase four reinforced the rats with ¼ sugar solution and ¾ cocaine solution. Water deprivation decreased across all four phases from 23, 16, 12, and then 0 hours. Rats responded on a VR5 schedule of reinforcement while in the presence of the tactile mat.

The four parts of acquisition (Phase) did have a significant effect on response rate, $F(1, 5) = 10.09$, $p = 0.001$, $\acute{\eta}^2_p = 0.67$. One-way analysis of variance (ANOVA) revealed that the response rate of Phase 1($M = 154.67$, $SD = 45.77$) was significantly greater than the response rate of Phase 4 ($M = 44.67$, $SD = 30.98$), $p = 0.005$.

One-way ANOVA - Maintenance

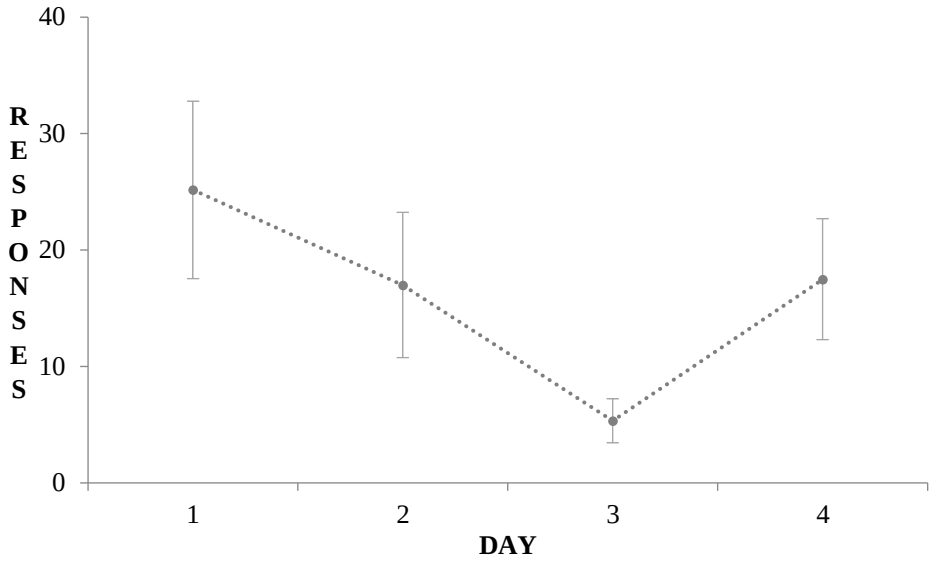


Figure 2: Average responses of all six subjects across a four day period of maintenance (See Appendix B). The rats received a drop of 0.05g/L cocaine solution with each completed variable ratio run. Rats responded on a VR5 schedule of reinforcement while in the presence of the tactile mat.

The 4 days of maintenance did not have a significant effect on responses, $F(1, 5) = 2.27$, $p = 0.12$, $\acute{\eta}^2_p = 0.31$. One-way analysis of variance (ANOVA) revealed that the response rate of the first day of maintenance ($M = 25.17$, $SD = 18.67$) was significantly greater than the response rate of last day of maintenance ($M = 17.50$, $SD = 12.72$), $p = 0.003$.

One-way ANOVA - Extinction

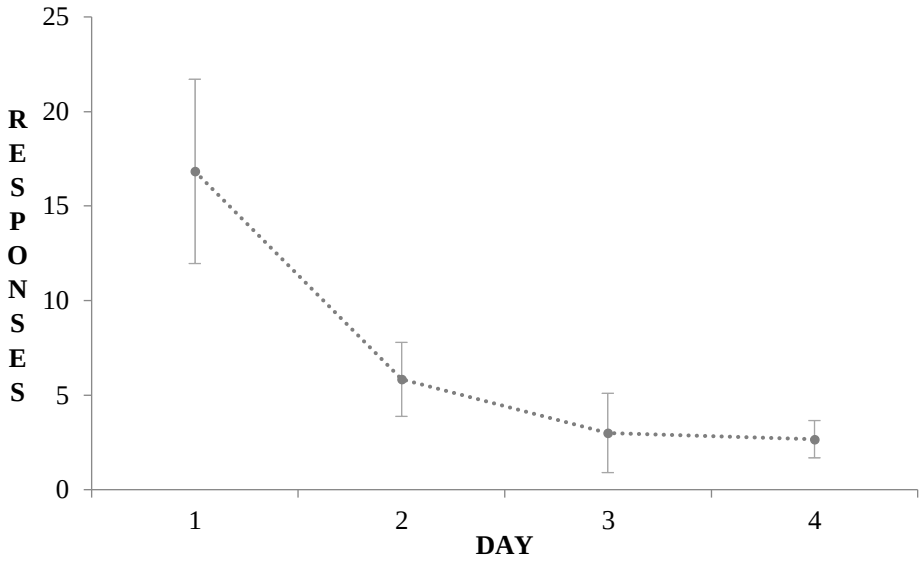


Figure 3: Average responses of all six subjects across a four day period of extinction (See Appendix C). Subjects had no opportunity for reinforcement, and the tactile mat that served as the discriminative stimulus was removed from the operant chamber.

The four days of extinction did have a significant effect on response rate, $F(1,5) = 5.03$, $p = 0.01$, $\acute{\eta}^2_p = 0.5$ One-way analysis of variance (ANOVA) revealed that the response rate of the first day of extinction ($M = 16.83$, $SD = 11.94$) was significantly greater than the response rate of the last day of extinction ($M = 2.67$, $SD = 2.42$), $p = 0.003$.

2 x 2 Mixed Model ANOVA - Reinstatement

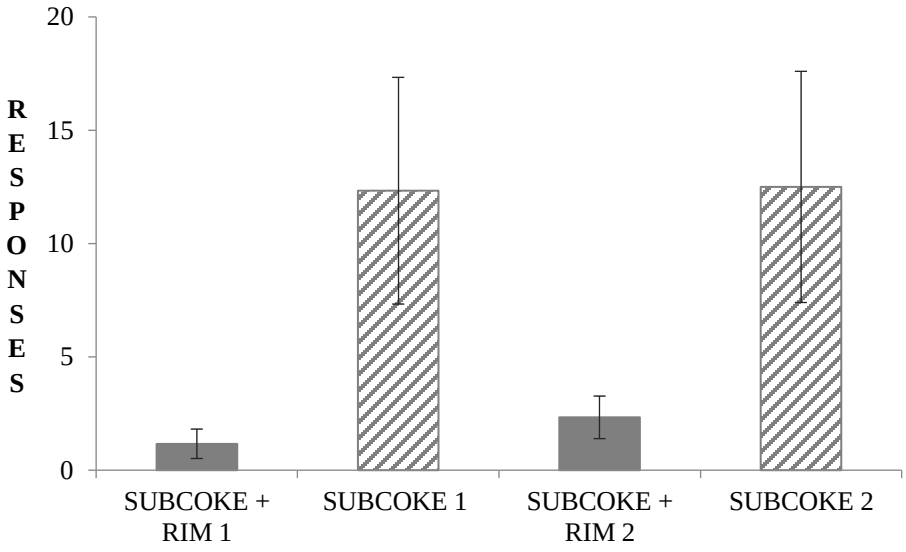


Figure 4: Average responses of all six subjects across 4 days of reinstatement (See Appendix D). The subjects were systemically injected with 1.25 mg/kg of cocaine (SUBCOKE) and 1.0 mg/kg of Rimonabant (RIM) on the first day (1.17 ± SEM 0.65). Subjects were then systemically injected with 1.25 mg/kg of cocaine (SUBCOKE) on the second day (12.33 ± SEM 5.03). The first and second day protocols were repeated to create an ABAB drug administration protocol. On the third day, 1.0 mg/kg of Rimonabant and 1.25 mg/kg of cocaine was administered again (2.33 ± SEM 0.94). The fourth day consisted of 1.25 mg/kg of cocaine (12.5 ± SEM 5.1).

Data was analyzed in a 2 x 2 model ANOVA. The main effect of Rimonabant when paired with a subthreshold dose of cocaine (SUBCOKE + RIM) did have a significant effect on response rate, F (1, 5) = 6.39, $p = 0.05$, $\acute{\eta}_p^2 = 0.56$ (Fig. 4). The response rate of the first day of reinstatement with Rimonabant (SUBCOKE + RIM) (M = 1.17, SD = 1.6) was not significantly different than the response rate of the second day of reinstatement with Rimonabant (SUBCOKE + RIM) (M = 2.33, SD = 4.76) (Fig 4).

The main effect of reinstatement with just the subthreshold dose of cocaine (SUBCOKE) did have a significant effect on response rate, F (1, 5) = 6.39, $p = 0.05$, $\acute{\eta}_p^2 = 0.56$ (Fig. 4). The response rate of the first day of reinstatement with just cocaine (SUBCOKE) (M = 12.33, SD = 8.98) was not significantly different than the response rate of the second day of reinstatement

with just cocaine (SUBCOKE) (M = 12.5, SD = 12.81) (Fig. 4). The interaction of SUBCOKE + RIM x SUBCOKE did have a significant effect on response rate, F (1, 5) = 6.39, $p = 0.05$, $\acute{\eta}_p^2 = 0.56$.

Paired Samples T-test - Reinstatement

The response rate of both days of reinstatement with Rimonabant and cocaine (M = 1.75, SD = 2.2) was significantly less than the response rate of reinstatement with just cocaine (M = 12.42, SD = 9.55), t (5) = -2.6, $p = 0.05$.

Discussion

The goal of the present study was to determine the effectiveness of Rimonabant in its ability to attenuate cocaine-seeking behavior in the presence of a discriminative stimulus. The reinstatement model of drug-seeking behavior has been assessed for its validity over the past couple decades. In order for the reinstatement model to appropriately parallel drug-seeking behavior in humans, this model's criterion validity must be assessed. Reinstatement in laboratory subjects is only possible because of the conditions reported by humans that make them relapse which are environmental cues, stressors, or the drug itself (Epstein, D., Preston, K., Stewart, J. & Shaham, Y., 2006; De Vries and Schoffelmeer, 2005). The model's ability to reinstate drug-seeking behavior in abstinent patients in clinical trials remains unseen, and probably will not occur due to ethical constraints. Thus, the reinstatement model is only pertinent when trying to introduce a therapeutic agent to attenuate the drug-seeking behavior for medical purposes (Epstein et al., 2006). Therefore, the application of the reinstatement model in this study made pharmacological sense.

Most research that employs the reinstatement model for experimental purposes use an apparatus that is crucial for the transmission of the drug to the subject. A self-administration

apparatus involves implanting catheters intravenously into the subject's jugular vein. This method of administration maximizes the dose available to the subject with each successive completion of the schedule of reinforcement they are responding under (Bossert et al., 2013). Intravenous self-administration creates an excessive amount of stress for the subject, which is one of many methodological difficulties when using the reinstatement model. Stress acts as an extraneous variable when studying the effect of an environmental cue on drug-seeking behavior (Adriani, W., Macrì, S., Pacifici, R., & Laviola, G., 2002). For this reason, the study conducted in this paper decided to use oral self-administration.

Many challenges were faced when using oral self-administration in the present study. Cocaine is naturally bitter when suspended in saline solution. This bitterness is quite aversive to the rat and they will not be inclined to consume the solution without any history to the drug (Carelli and West, 2014). A protocol was determined to effectively produce positive increases in response rate during the acquisition phase and establish a strong preference for the 0.05 g/L solution of cocaine used as reinforcement (Cunningham and Niehus, 1997). A sugar substitute solution was initially used during the first phase of acquisition to produce a strong response rate. Once that response rate was maintained across all six subjects, ¼ of the original sugar substitute solution was removed, and ¼ of the cocaine solution was faded into the reinforcing solution. This continued for 12 days until the reinforcing solution was at the anticipated final concentration of cocaine, which was 0.05 g/L. Carelli and West explain that response rate starts to decrease for a natural reward when that lever press comes under the control of an abused drug, such as cocaine (2014).

Theoretically, the present study's fading protocol should have established a strong preference for cocaine over the original sugar substitute solution. The last day of the acquisition

phase did not have a significantly greater response rate than the first day, showing a decreased preference for the gradually increasing concentration of cocaine solution (Fig. 1). One-way analysis of variance (ANOVA) revealed a significant difference in response rate from the first and last days of acquisition ($p < 0.05$, $p = 0.001$), but the reinstatement model requires a positive increase in response rate to conclude the subjects acquired the cocaine-seeking behavior.

The subjects in the present study needed to acquire the cocaine-seeking behavior first before maintenance commenced. Maintenance phase began with the full concentration of cocaine, which was delivered to the rats for four days at a concentration of 0.05 g/L. The averages of all six subjects' response rates suggests a weak preference for the full 0.05 g/L cocaine solution and the response rate decreased after the first day (Fig. 2). The response rate of the first day of maintenance did significantly differ from the last day of maintenance ($p < 0.05$, $p = 0.003$). Response rate of the first and last days should not significantly differ from each other, as it did in the present study. As the name suggests, the maintenance phase should show a consistent response rate during the course of all trial days.

The drug-seeking behavior of all six subjects was put under extinction the day after maintenance ended. In the present study, the extinction phase followed typical trends usually observed when the response contingency is interrupted; an initially high response rate that decreases as the extinction phase continues (Fig. 3). The first day of extinction significantly differed from the last day of extinction, showing a decrease in response rate ($p < 0.05$, $p = 0.003$). Despite obtaining a response curve resembling that of extinction, the present study was not successful at extinguishing drug-seeking behavior because it was not previously acquired and maintained. It is important to note, however, that evidence reported in previous studies show an initial increase in response rate when drug-seeking behavior is extinguished. Shaham et al.

reported that the least amount of responding occurred during the first day of extinction when trying to reinstate cocaine-seeking behavior motivated by drug associated cues and observed an increase in response rate as the period of extinction progressed (2003). Drug-seeking behavior in the reinstatement model parallels that of human behavior (Katz and Higgins, 2003; Shaham et al., 2003). Based on this evidence, it can be concluded that the withdrawal did not occur in the present study.

Reinstatement of cocaine-seeking behavior in the present study was not a success. Drug reinstatement constituted as a four day drug reinstatement process where Rimonabant and a subthreshold dose of cocaine was administered on days 1 and 3, and only the subthreshold dose cocaine was administered on days 2 and 4 (Fig 4). A 2 x 2 mixed model analysis of variance (ANOVA) revealed that the average of responses on days 1 and 3 were significantly less than the average of responses on days 2 and 4 ($p < 0.05$, $p = 0.05$). While it seems like Rimonabant attenuated cocaine-seeking behavior, the results are inconclusive because of the insignificant data obtained during the acquisition phase.

Despite the present study's findings, the novelty of using a tactile environmental cue and implementing an oral self-administration protocol needs to be addressed. To date, no present studies have used a tactile discriminative stimulus to cue drug availability. Most studies use a discriminative cue that has a priming effect of drug-seeking behavior which is either a large dose of the drug, or agonist of said drug. The present study compounded a subthreshold dose of cocaine along with a tactile mat to cue the animal that reinforcement is available. Cocaine has been proven to act as a reinforcer via oral self-administration for rats, but very few studies take advantage of the rat model when compared to oral self-administration studies done with rhesus monkeys (Meisch, 2001).

The difficulty in the present study was to reinforce the rats with enough cocaine solution in the operant chamber to adequately addict the subjects to cocaine. Future prospects of this study would be to have the animals properly acquire the cocaine-seeking behavior in the first place. Meisch proposed schedule-induced polydipsia to induce drinking the reinforcing solution at a much higher rate or to use a fading procedure similar to the one used in the present study for a longer duration of time (2001). Another possible prospect in further developing this hypothesis is to use a different demographic of rat. The present study utilized adult females to conduct the experiment. Previous studies have reported a higher success rate when acquisitioning the rats to the drug-seeking behavior by using rats right before they reach puberty. Periadolescent rats have proven to be more spontaneous in their behaviors and reportedly have a lower behavioral and hormonal response to stress (Adriani et al., 2002). Stress caused by handling the adult female rats could be the reason the cocaine-seeking behavior was not acquired.

Conclusion

Using oral self-administration, the present study attempted to pair a tactile rubber mat with a subthreshold dose of cocaine that resulted in the attenuation of cocaine-seeking behavior by Rimonabant in the reinstatement phase. Statistical analyses revealed significant differences in average responses from i.p. injections of Rimonabant and the subthreshold dose of cocaine when compared to the average responses for i.p. injections of just the subthreshold dose of cocaine. Despite following specific protocols for drug preparation and acquisition of the oral self-administration of cocaine, attenuation of the cocaine-seeking behavior by Rimonabant remains inconclusive because of failure for the subjects to acquire oral self-administration behavior during the acquisition phase.

References

Adriani, W., Macrì, S., Pacifici, R., and Laviola, G. (2002). Peculiar Vulnerability to Nicotine Oral Self-administration in Mice during Early Adolescence. *Neuropsychopharmacology.* 27(2), pg. 212-224

Bossert, J., Marchant, N., Calu, D. and Shaham, Y. (2013). The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. *Psychopharmacology.* 229, pg. 453–476.

Carelli, R.M. and Deadwyler, S.A. (1996). Dose-dependant transitions in nucleus accumbens cell firing and behavioral responding during cocaine self-administration sessions in rats. *The Journal of Pharmacology,* 277(1), 385-393.

Carelli, R. and West, E. (2014). When a good taste turns bad: Neural mechanisms underlying the emergence of negative affect and associated natural reward devaluation by cocaine. *Neuropharmacology.* 76, pg. 360-369

Cohen, C., Perrault, G., Griebel, G., Soubrie, P. (2004). Nicotine-Associated Cues Maintain Nicotine-Seeking Behavior in Rats Several Weeks after Nicotine Withdrawal: Reversal by the Cannabinoid (CB1) receptor Antagonist, Rimonabant (SR141716). *Neuropsychopharmacology*, 30(1), 145-155.

Crunelle, C., Giessen, E., Schulz, S., Vanderschuren, L., Bruin, K., Brink, W. and Booij, J. (2011). Cannabinoid-1 receptor antagonist Rimonabant (SR141716) increases striatal dopamine D2 receptor availability. *Addict. Biol.* 18(6), pg. 1-5.

Cunningham, C and Niehus, S. (1997). Flavor preference conditioning by oral self-administration of ethanol. *Psychopharmacology.* 134, pg. 293–302

De Vries, T. and Schoffelmeer, A. (2005). Cannabinoid CB_1 receptors control conditioned drug seeking. *Trends in Pharmacological Sciences,* 26(8), 420-426.

De Vries, T., Shaham, Y., Homberg, J., Crombag, H., Schuurman, K., Dieben, J., Vanderschuren, L. and Schoffelmeer, A. (2001). A cannabinoid mechanism in relapse to cocaine seeking. *Nature Medicine.* 7(10), pg. 1151-1154.

De Wit, H., Stewart, J. (1981). Reinstatement of cocaine reinforced responding in the rat. *Psychopharmacology*, 75(2), 134-143.

Epstein, D., Preston, K., Stewart, J. and Shaham, Y. (2006). Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology*. 189, pg. 1–16.

Fu, K., Lin, H., Miyamoto, Y., Wu, C., Yang, J., Uno, K., Nitta, A. (2015) Pseudoginsenoside-F_{11} inhibits methamphetamine-induced behaviors by regulating dopaminergic and GABAergic neurons in the nucleus accumbens. *Psychopharmacology*.

Fuchs, R.A., See, R.E., Middaugh, L.D. (2003) Conditioned stimulus-induced reinstatement of extinguished cocaine seeking in C57BL/6 mice: a mouse model of drug relapse. *Brain Research*, 973(1), 99-106.

Gawin, F.H. (1989) Cocaine Dependence. *Annual Reviews Medicine*, 40, 149-161.

George, F. and Goldberg, S. (1988). Genetic Differences in Responses to Cocaine. *National Institute of Drug Abuse.* 88, pg. 239-249.

Ghitza, U.E., Fabbricatore, A.T., Prokopenko, V.F., West, M.O. (2004). Differences between accumbens core and shell neurons exhibiting phasic firing patterns related to drug-seeking behavior during a discriminative-stimulus task. *Journal of Neurophysiology*, 92, 1608-1614.

Jackson, M.E., Moghaddam, B. (2004). Stimulus-specific plasticity of prefrontal cortex dopamine neurotransmission. *Journal of Neurochemistry*, 88, 1327-1334.

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

Katz, J. and Higgins, S. (2003). The validity of the reinstatement model of craving and relapse to drug use. *Psychopharmacology.* 168, pg. 21–30.

Kosten, T.A., Miserendino, M.J.D., Haile, C.N., DeCaprio, J.L., Jatlow, P.I., Nestler, E.J. (1997). Acquisition and maintenance of intravenous cocaine self-administration in Lewis and Fischer inbred rat strains. *Brain Research*, 778(2) 418-429.

Maldonado, R., Valverde, O., Berrendero, F. (2006). Involvement of the endocannabinoid system in drug addiction. *Trends in Neuroscience*, 29(4), 225-232.

Meisch, R. (2001). Oral drug self-administration: an overview of laboratory animal studies. *Alcohol.* 24(2), pg. 117-128.

National Institute on Drug Abuse. Trends & Statistics Retrieved from https://www.drugabuse.gov/related-topics/trends-statistics on May 1, 2016

Rodriguez, J.S., Boctor, S.Y., Flores, L.C., Phelix, C.F., Martinez, J.L. (2010) Local pretreatment with the cannabinoid CB1 receptor antagonist AM251 attenuates methamphetamine intra-accumbens self-administration. Neuroscience Letters, 489, 187-191.

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, 3-20.

Substance Abuse and Mental Health Services Administration (SAMHSA). Results from the 2011 National Survey on Drug Use and Health: Summary of National Findings. Rockville, MD: Substance Abuse and Mental Health Services Administration; 2012.

Walton, M. A., Reischl, T. M., & Ramanathan, C. S. (1995). Social settings and addiction relapse. Journal of Substance Abuse, 7(2), 223-233

Xi, Z.X., Gilbert, J.G., Peng, X.Q., Pak, A.C., Li, X., Gardner, E.L. (2006). Cannabinoid CB_1 receptor antagonist AM251 inhibits cocaine-primed relapse in rats: role of glutamate in the nucleus accumbens. *The Journal of Neuroscience,* 26(33), 8531-8536.

Appendix A

Acquisition Phase Response Rate

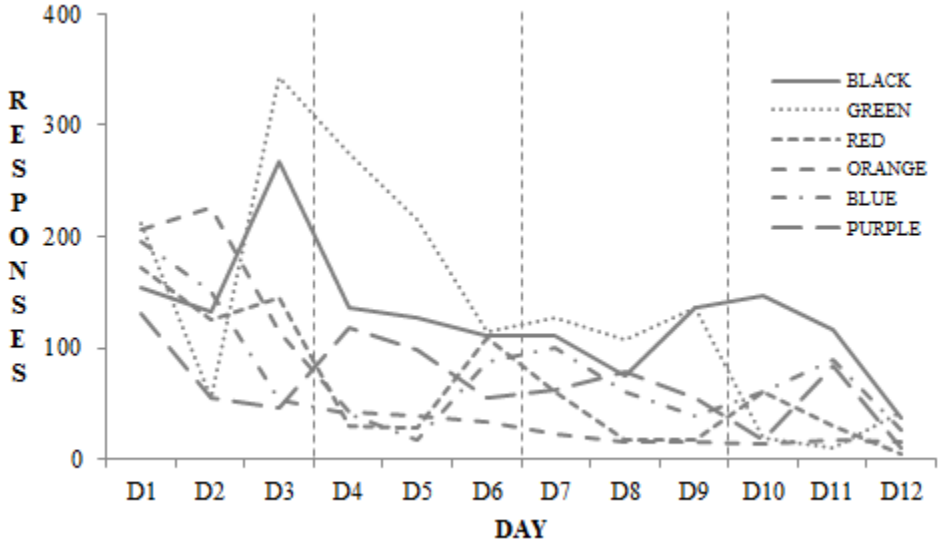


Figure 5: Responses of all six subjects across the 12 day acquisition period. Phases 1-4 are divided by dashed lines representing days 1-3, 4-6, 7-9, and 9-12 respectively. In phase one, the rats received full sugar as a reinforcer. Phase 2 contained ¾ of the original concentration of sugar while ¼ of the 0.05 g/L cocaine solution. Phase three reinforced the rats with a solution containing ½ of the original sugar solution and cocaine solution. Phase four reinforced the rats with ¼ sugar solution and ¾ cocaine solution. Water deprivation decreased across all four phases from 23, 16, 12, and then 0 hours.

Appendix B

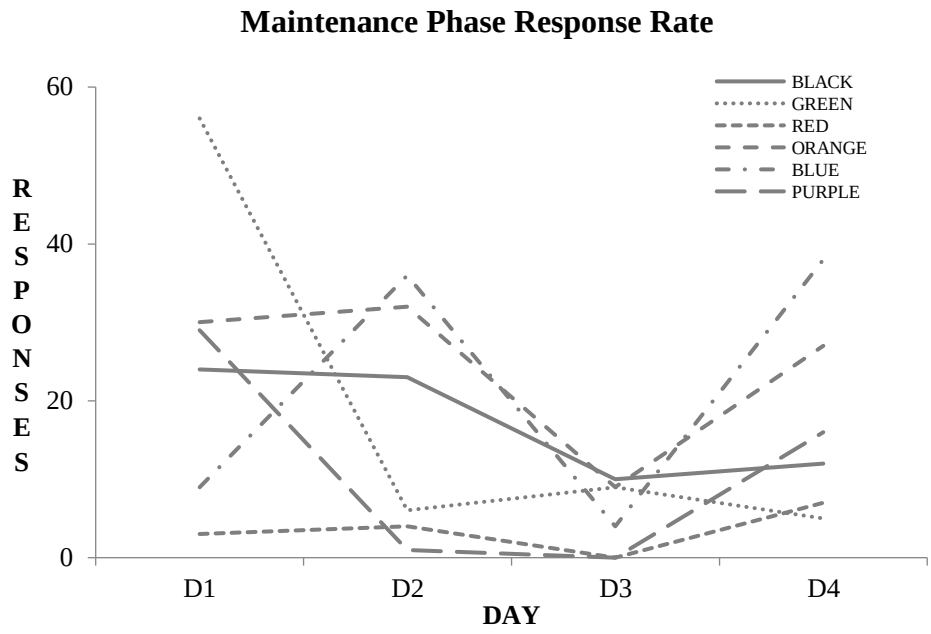


Figure 6: Responses of all six subjects across a four day period of maintenance. The rats received a drop of 0.05g/L cocaine solution with each completed variable ratio run.

Appendix C

Extinction Phase Response Rate

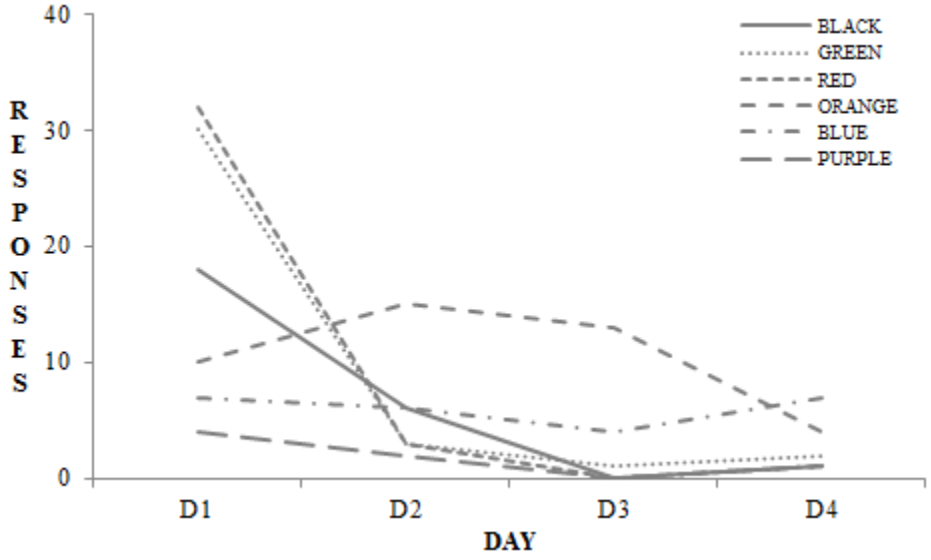


Figure 7: Responses of all six subjects across a four day period of extinction. Subjects had no opportunity for reinforcement, and the tactile mat that served as the discriminative stimulus was removed from the operant chamber.

Appendix D

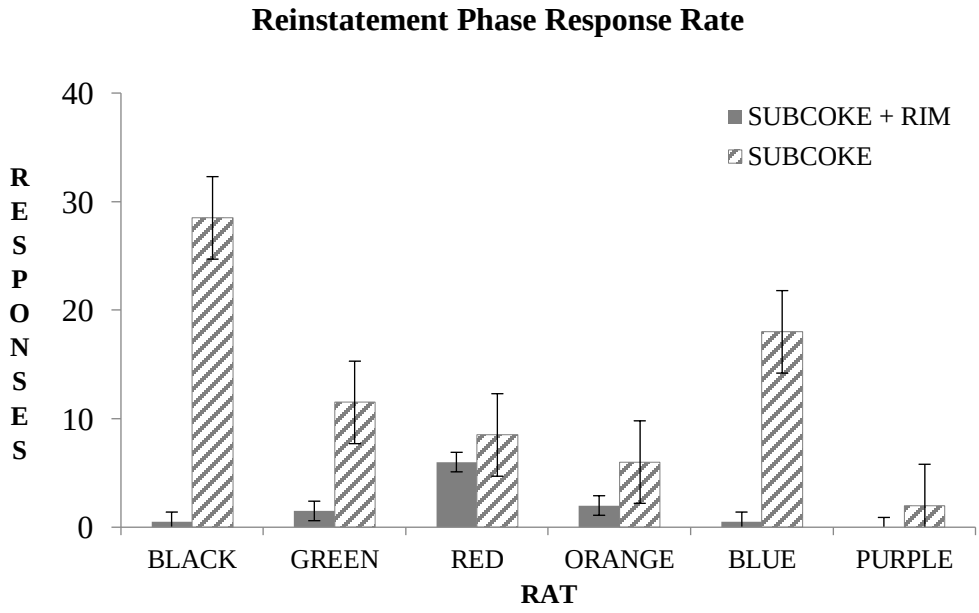


Figure 8: Responses of all six subjects with the average of both conditions of reinstatement. The subjects were systemically injected with 1.25 mg/kg of cocaine (SUBCOKE) and 1.0 mg/kg of Rimonabant (RIM) for the first condition (2.2 ± 0.9). Subjects were then systemically injected with 1.25 mg/kg of cocaine (SUBCOKE) for the second condition (9.5 ± 3.8).