

2018

CHARACTERIZING THE ROLE OF GLUCOCORTICOIDS IN THE SIGN TRACKING BEHAVIOR OF MALE JAPANESE QUAIL (*COTURNIX JAPONICA*)

Beth A. Rice

University of Kentucky, bethannrice01@gmail.com

Digital Object Identifier: <https://doi.org/10.13023/ETD.2018.173>

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Beth A. Rice, Student

Dr. Chana K. Akins, Major Professor

Dr. Mark Fillmore, Director of Graduate Studies

CHARACTERIZING THE ROLE OF GLUCOCORTICOIDS IN THE SIGN TRACKING
BEHAVIOR OF MALE JAPANESE QUAIL (*COTURNIX JAPONICA*)

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor
of Philosophy in the College of Arts and Sciences at the University of Kentucky

By

Beth Ann Rice

Lexington, Kentucky

Director: Dr. Chana K. Akins, Professor of Psychology

Lexington, Kentucky

2018

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ABSTRACT OF DISSERTATION

CHARACTERIZING THE ROLE OF GLUCOCORTICOIDS IN THE SIGN TRACKING BEHAVIOR OF MALE JAPANESE QUAIL (*COTURNIX JAPONICA*)

A devastating feature of drug-dependence is the susceptibility of relapse (40-60%) after stretches of abstinence. One theory that may account for relapse suggests that drug cues (e.g., paraphernalia) may increase stress hormones, and this may prompt relapse. Repeatedly pairing a neutral cue with a reward is commonly utilized to measure what subjects learn about a cue that is predictive of reward. Research has shown that animals that attend to a cue more than to the reward (sign trackers) may be more vulnerable to drug addiction. Additionally, research has shown that sign tracking is associated with an increase in corticosterone (CORT), a primary stress hormone. PT 150 is a novel glucocorticoid receptor antagonist that attenuates the effects of CORT. Experiment 1 hypothesized that subjects given repeated oral administration of 40 mg/kg PT 150 would reduce sign tracking compared to subjects given placebo. Results of Experiment 1 showed that repeated oral consumption of 40 mg/kg PT 150 decreased sign tracking behavior compared to placebo. In Experiment 2, it was hypothesized that PT 150 (20/40/60 mg/kg) given by subcutaneous (SC) injection would reduce sign tracking dose-dependently, and that sign tracking behavior would correlate with CORT levels. Results of Experiment 2 showed that SC injection of 20 mg/kg PT 150 reduced sign tracking but not 40 or 60 mg/kg. Additionally, the correlation between CORT and the sign tracking for the 20 mg/kg approached significance. Although tentative, the correlation may suggest that elevated plasma CORT concentrations correlate with elevated sign tracking. The current findings extend the current literature by suggesting that the glucocorticoid receptor may be a potential pharmacological target for reducing relapse-like behaviors.

KEYWORDS: Sign tracking, Glucocorticoids, Addiction, Corticosterone, Japanese quail

Beth Ann Rice
4/24/2018

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BEHAVIOR OF MALE JAPANESE QUAIL (*COTURNIX JAPONICA*)

By

Beth Ann Rice

Dr. Chana Akins

Director of Dissertation

Dr. Mark Fillmore

Director of Graduate Studies

April 24, 2018

Date

It is with genuine gratefulness and warmest regard that I dedicate this work to my mentor,
Dr. Chana Akins. Thank you for taking a chance on me. You are a wonderful teacher,
mentor, and friend. I am so grateful to have known you.

ACKNOWLEDGEMENTS

In combination with the mentorship of my advisor, I am grateful to have worked with enthusiastic and supportive committee members; Dr. Peggy Keller, Dr. Kim Nixon, and Dr. Mark Prendergast. Additionally, I would like to thank Lynda Sherrett-Field. Without their guidance and support, this dissertation would not have been nearly as successful.

In addition to my academic mentors, I would also like to thank the following people for their support.

Abigail Katherine Osterholm, my daughter, thank you for putting up with mommy while I wrote this dissertation. I owe you, “100 bouncy balls.”

My friends who have ‘made the magic’ for my daughter during long days; Alex Bybee, Chelsea Gorham and Dana Thomas.

All the family who have supported me in many ways throughout the years while completing this dissertation. Especially, Pam and Conrad Sanders, Angie and Travis Deeds, Michelle and Larry Rice (aka Hoss), Larry Rice Sr., and Wayne Osterholm.

Finally, I would like to thank all the amazing women in the Psychology office who have supported me throughout this dissertation specifically; Meagan Coomes, Jennifer Casey and Kim Reeder.

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Chapter One

INTRODUCTION

The economic cost of illicit drug use in the United States (US) is estimated in the billions (NIDA, 2017). These costs include loss of productivity, increased healthcare, and criminal justice expenses. Additionally, users spend billions per year on illegal substances like heroin and cocaine. An average of 21.5 million Americans are battling drug dependence (NIDA, 2014). Alarmingly, the highest at risk for dependence are youths, with early abuse starting at 12 to 13 years of age (NIDA, 2014). It is estimated that 1 in 12 youths is suffering from a dependence use disorder. Among the individuals that seek treatment, 40-60% relapse, depending on the substance (NIDA, 2014). Because of the alarming rate of relapse, drug dependence is characterized as a chronic, relapsing disease (Leshner, 1997).

It is widely accepted that visual cues in the environment may become associated with drug taking and subsequently, in the absence of drug, cause drug-seeking and ultimately relapse (Childress et al., 1999). Cues (items, places or people) associated with a drug may trigger relapse (Lee, Milton & Everitt, 2006; Saunders & Robinson, 2013; Meyer, Ma & Robinson, 2012). In recent studies with human participants, it has been demonstrated that cues associated with drugs of reward, prompt drug-seeking behaviors (Wang, Shi, Chen, Xu, Li, Sun & Lu, 2013). Specifically, studies investigating cocaine cues established that subjects experience increased craving in response to cocaine-related cues (Fox et al., 2012). In animal models, it has been reliably demonstrated that cues paired with drugs of reward may initiate renewed drug-seeking behavior (Loweth, Tseng & Wol 2013; Pratt & Ford, 2013; Fischer, Houston & Rebec, 2013; Gipson, Khoo, Gibson, Prasad & McNally, 2017). Collectively,

these experiments suggest that cue-induced relapse may be one of the underlying problems with drug dependence.

Incentive Saliience

The theory of incentive saliience postulates that cues associated with rewards may bias attention towards those cues and motivate reward seeking (Morrow, Maren & Robinson, 2011; Robinson & Berridge, 1993). When cues that come to elicit approach through conditioning “become attractive” and motivate actions, they are said to have acquired incentive saliience (e.g., Meyer, Cogan & Robinson, 2014; Saunders & Robinson, 2013; Robinson & Berridge, 2008). Research conducted with rodents indicates that there are individual differences in the propensity to attribute incentive saliience to reward paired cues. For example, when a localizable cue (conditioned stimulus, i.e., CS) becomes associated with the receipt of food reward, for some rodents (sign trackers; STs), the cue itself becomes attractive, eliciting approach and engagement with it (Hearst & Jenkins, 1974). For these rodents, the CS also serves as a potent conditioned reinforcer (i.e., STs will work to get it; Flagel, Akil & Robinson, 2009; Robinson & Flagel, 2009). For other rodents (goal trackers; GTs), the cue is equally predictive of reward (i.e., it serves as an effective CS), but they instead learn to approach the location of reward delivery, and for these rodents the CS is relatively ineffective as a conditioned reinforcer (Robinson & Flagel, 2009; Yager & Robinson, 2010).

The attribution of incentive saliience to an addiction-relevant stimulus (CS) is thought to be responsible for a CS’s ability to reinstate drug-seeking behaviors after bouts of abstinence (e.g., Everitt & Robbins, 2000; Kruzich, Congleton & See, 2001). One possible reason for this theory is that when a person is in the presence of cues that have acquired

incentive salience, they may experience stress, which subsequently instigates renewed drug seeking (i.e., relapse). This may happen because a cue that has acquired incentive salience may activate the brain's reward circuitry, which overlaps with the brain's stress pathway (Cobb & Thiel, 1982; Robinson & Berridge, 1993; Kreek & Koob, 1998). For example, individuals with drug dependency show increased cortisol plasma concentrations (Wilkins, Gorelick & Nademanee, 1982; Wand & Dobs, 1991; Mello & Mendelson, 1997), display increased drug taking that is correlated with elevated stress, and report stress as a cause for relapse (Jacobsen, Southwick & Kosten, 2001). In rodents, a stimulus repeatedly paired with a reward increases plasma corticosterone concentrations (Tomie, Silbberman, Williams & Pohorecky, 2002; Tomie, Tirado & Pohorecky, 2004). Specifically, increases were higher in sign-tracking rodents when compared to goal-tracking rodents (Flagel, Watson, Akil & Robinson, 2008; Tomie, Lincks, Nadarajah, Pohorecky & Yu, 2012). Thus, it is hypothesized that corticosterone contributes to the rewarding effects of drugs and that addiction-relevant cues facilitate stress-like changes in plasma corticosterone concentrations. Therefore, corticosterone may serve as a relatively new therapeutic target for cue-induced relapse because of the potential interactions between stress and cues that have acquired incentive salience.

Hypothalamic-pituitary-adrenal axis (HPA)

The HPA has significant involvement in stress and reward (Tomie, Grimes, Pohorecky, 2008). In sign tracking, it is hypothesized that reward-predicting cues activate the HPA axis which stimulates the hypothalamus (Flagel et al., 2008). The hypothalamus when stimulated releases a hormone, corticotrophin releasing factor (CRF) (Peeters, Ruist, Craighead & Kitchner, 2008). CRF in turns signals to the pituitary gland to release

adrenocorticotrophic hormones (ACTH) into the bloodstream (Iverson, Iverson, Bloom & Roth, 2009). ACTH travels in the bloodstream to the adrenal glands where it stimulates the release of glucocorticoids. The release of glucocorticoids is a self-regulating system, in that as levels of glucocorticoids increase, a built-in feedback mechanism shuts off CRF. Specifically, this feedback system turns CRF off when the steroid receptors in the hypothalamus register excess glucocorticoids (Ivesron et al., 2009). In rodents, the predominant glucocorticoid is corticosterone (CORT), and it is released following the activation of the HPA axis (Raubenheimer, Young, Andrew & Seckle, 2006).

CORT, Glutamatergic Plasticity, and Sign tracking

Sign tracking is a possible behavioral consequence of associative learning resulting from Pavlovian conditioning (Beckmann & Chow, 2015). Research has shown that glutamatergic receptors (i.e., AMPA) increase in response to Pavlovian conditioning (Mead & Stephans, 2003). Learning involves glutamatergic plasticity resulting from the transmission of glutamate receptors (e.g., NMDA and AMPA) that have critical roles in long-term potentiation (LTP), (Mead & Stephans, 2003). LTP is a strengthening of synaptic plasticity as a result of learning (Rioutl-Pedotti, Friedman & Donoghue, 2000). Because sign tracking is a learned phenomenon and has been associated with increased CORT (Flagel et al., 2008), it is possible that increased sign tracking is the result of CORTs enhancement of glutamatergic plasticity (Calandreau et al., 2011).

Glucocorticoid Receptor (GR) antagonists

Glucocorticoids such as CORT exert their effect by binding to the GR (Peeters et al., 2008). When CORT binds to the GR, the GR forms a GR-CORT complex that enters the nucleus and exerts its effects by the transcription of new genes. GR antagonists block

glucocorticoids from binding to the GR, thus reducing the effect of CORT. The natural hormone CORT is not specific for the GRs (Peeters et al., 2004). For example, traditional antagonists of the GR (RU 486) also act on progesterone receptors (Vegeto et al., 1992). The affinity of RU 486 for the progesterone receptor can result in pregnancy termination. As a result, RU 486 is not a viable treatment option for half the population (i.e., females). Finding alternatives to RU 486 is a significant research endeavor because of CORT's role in metabolic disorders and addiction (Arnaldi et al., 2003; Sinha & Jastreboff, 2013).

The GR antagonist, 11, 21-Bisphenyl-19-norpregnane derivative (PT 150) is a novel antagonistic that competes with CORT for GR binding in the cytoplasm (Peters et al., 2008). When PT 150 binds to the GR, the GR inhibitory complex is not dislocated. Therefore, the signaling cascade stops and CORT's effects cannot be realized. Most importantly, PT 150 has a 6-fold lower affinity for the progesterone receptor when compared to RU 486, making it a useful alternative (Peeters et al., 2004). In addiction research, PT 150 attenuated ethanol-increased plasma CORT in rodents (Reynolds et al., 2015). For these reasons, PT 150 may be of value in addiction research in that it can attenuate the effects of CORT without affecting the progesterone receptor.

Avian Model of Sign tracking

It is widely accepted that visual cues in the environment (e.g., paraphernalia, drug taking confederates, and places) may become associated with drug taking and later, in the absence of drug, renew drug-seeking behaviors and ultimately lead to relapse (e.g., Childress, et al., 1999; Carter & Tiffany, 1999). Because of the ability of visual cues to reinstate drug taking, birds extend the study of sign tracking, as they are primarily visual, whereas rodents largely depend on olfaction (Johnson & Whittow, 2000; Crombag, Badiani, Maren &

Robinson, 2000). Specifically, Japanese quail are a visually oriented bird species with color vision and high visual acuity (Mills, Crawford, Domjan & Faure, 1997). The study of sign tracking can benefit from an animal model that has a similar visual system to that of humans. Importantly, sign and goal tracking behaviors have already been identified in quail (e.g., Burns & Domjan, 1996; 2000; 2001).

In humans, the diurnal rhythm of plasma stress hormones (i.e., cortisol) peak in the morning and slowly declines into the night (Cauter, Leproul & Kupper, 1996). Similar to rodents, the bird's predominant glucocorticoid is corticosterone (CORT) (Ellestad, Puckett & Porter, 2015). Birds, including Japanese quail, have a similar diurnal rhythm of plasma CORT, where plasma CORT increases at the start of darkness, with a maximum CORT concentration at first light (Kovack, 1983; Joseph & Meier, 1972). Conversely, rodent's diurnal rhythm is the opposite, with the lowest point of plasma CORT concentration being at first light (the inactive period for these nocturnal animals) and the peak at the onset of darkness (the active period) (Ulrich-Lai, Arnold & Engleland, 2005). Because studies of sign tracking with rodents are conducted in the day, when their CORT is the lowest, this may make quail a better translational model when studying stress hormone effects, in that the diurnal rhythm of CORT in quail may be more representative of humans.

The Current Experiments

The overall goal of the present research was to attenuate sign tracking by reducing the effect of CORT. To assess the effect of CORT on sign tracking, PT 150 was administered after the acquisition of sign tracking in male quail. We hypothesized that repeated administration of PT 150 would decrease sign tracking compared to placebo. Moreover, it

was hypothesized that PT 150 would dose-dependently decrease sign tracking compared to placebo.

Chapter Two

A GLUCOCORTICOID RECEPTOR ANTAGONIST REDUCES SIGN-TRACKING BEHAVIOR IN MALE JAPANESE QUAIL

(Rice B.A., Eaton S.E., Prendergast M.A. & Akins C.K., 2018)

Decades of substance abuse research have demonstrated the parallels between drug addiction behaviors and behaviors elicited from Pavlovian conditioning (for review, Anselme, 2016; Lamb, Schindler & Pinkston, 2016). For example, in Pavlovian conditioning, environmental cues that become associated with a reward come to elicit approach to the cue following conditioning. When these cues become attractive and motivate actions, they are said to have acquired incentive salience (Meyer, Cogan & Robinson, 2014; Saunders & Robinson, 2013; Robinson & Berridge, 2008). Research conducted with rodents indicates that there are individual differences in the propensity to attribute incentive salience to cues that become associated with reward. For example, when a localizable cue (conditioned stimulus, i.e. CS) becomes associated with the receipt of food reward, for some rodents (sign trackers; STs), the cue itself becomes attractive, eliciting approach and engagement with it (Hearst & Jenkins, 1974). For STs, the CS also serves as a potent conditioned reinforcer (Flagel, Akil & Robinson, 2009; Robinson & Flagel, 2009). For other rodents (goal trackers; GTs), the cue is equally predictive of reward (i.e., it serves as an effective CS), but they instead learn to approach the location of reward delivery, and for these rodents the CS is relatively ineffective as a conditioned reinforcer (Robinson & Flagel, 2009; Yager & Robinson, 2010).

The identification of animals that have the propensity to sign track is important because attribution of incentive salience to cues is associated with addictive behavior. In rats,

STs that attribute incentive salience to a cue are more likely to self-administer cocaine compared to GTs (Beckmann, Marusich, Gipson & Bardo, 2011). Additionally, STs show greater cocaine-induced behavioral sensitization (Flagel et al., 2008), a greater cocaine-induced conditioned place preference (Meyer, Ma, & Robinson, 2012), and have more robust rates of reinstatement to drug-paired cues compared to GTs (Saunders & Robinson, 2010). Overall, these studies show that STs may be more vulnerable to drug addiction.

In rodents, high levels of corticosterone (CORT), a predominant stress hormone, correlate with the propensity to sign track (e.g., Flagel et al., 2008). Additionally, the presentation of a CS that predicts reward increases CORT (Tomie, Silbberman, Williams, & Pohorecky, 2002; Tomie, Toreadp, Yu, & Pohorecky, 2004) and this increase is greater in sign tracking rodents that attribute incentive salience to the cue when compared to subjects that do not sign track (i.e., goal trackers) (Flagel et al., 2008). These findings suggest that CORT may be a potential target for reducing sign tracking behaviors.

It is widely accepted that visual cues in the environment may become associated with drug taking and subsequently, in the absence of drug, cause drug-seeking and ultimately relapse (Childress et al., 1999). Therefore, studying substance abuse with an avian species may be of an additional benefit because birds are primarily visual whereas most rodents largely depend on olfaction (Carsia & Harvey, 2000; Crombag, Badiani, Maren & Robinson, 2000). Japanese quail are a visually-oriented bird species with color vision and high visual acuity (Mills, Crawford, Domjan & Faure, 1997). Numerous drug studies have been conducted with Japanese quail (Levens & Akins, 2004; Geary & Akins, 2007; Akins & Geary 2008; Bolin & Akins, 2012; Gill, Rice & Akins, 2015; Gill, Madison & Akins, 2015).

These studies demonstrate that the drug responses in quail are largely conserved relative to rodents.

The HPA axis involves an auto-regulated feedback system that includes 2 types of adrenal steroid receptors, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) (Bachmann, Linthorst, Holsboer & Reul, 2003). This dual receptor system is responsible for homeostasis of the HPA axis. GR antagonists (e.g., PT 150) have been shown to increase MR expression (Bachmann et al., 2003). When the MR/GR balance shifts toward an increase in MRs, there is a decreased corticosterone response to stress (de Kloet, 1991). Therefore, PT 150 may be a potential target for reducing the stress hormone corticosterone.

11, 21-Bisphenyl-19-norpregnane derivative (PT 150) is a GR antagonist that competes with the binding of CORT in the cytoplasm (Peeters, Ruigt, Craighead & Kitchener, 2008). A similar compound to PT 150 is RU 486 which is a methylenedioxy phenyl analog of PT 150 (Peeters et al., 2008). However, in addition to competing with the GR, RU 486 modulates the progesterone receptor (PR) (Gagne, Pons & Philibert, 1985). The compound PT 150 is a novel compound in that, unlike RU 486, PT 150 has minimal effect on the progesterone receptor (PR). PT 150 has a 500-fold greater affinity for the GR while RU 486 has a 5.4-fold affinity for the GR over the PR (Gebhard, Van Der Voort, Schuts & Schoonen., 1994).

In the current study, we used a Pavlovian conditioning paradigm that measures individual differences in acquired incentive salience to cues that become associated with reward (Domjan, Lyons, North & Bruell, 1986; Beckmann & Bardo, 2012; Flagel et al., 08). To assess the ability of a GR antagonist to attenuate sign tracking, PT 150 was administered

after acquisition of sign tracking in male quail. We hypothesized that PT 150 would decrease sign tracking compared to placebo following repeated administration.

Methods

Subjects

Twenty-two (N = 22) adult male Japanese quail (*Coturnix japonica*) were hatched from eggs (Georgia Quail Farm (GQF), Savannah, GA) and were raised in mixed sex groups until approximately 6 weeks of age. Quail were housed in individual wire mesh cages (supplied by GQF Manufacturing, Savannah, GA) and maintained on a 16:8 hr light cycle. At 10 months of age, twenty-two male quail were randomly assigned to one of 2 groups (drug or placebo; ns = 11). The number of subjects per group was chosen based on a power analysis using Gpower (Faul & Erdfelder, 1992; Erdfelder, Faul & Buchner, 1996) with power set at 0.80, an effect size at 0.50, and $\alpha = 0.05$ for two tailed F tests.

All experimental procedures were conducted according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky and by the standards outlined in the 8th edition of the Guide for the Care and Use of Laboratory Animals (National Research Council, 2010). These entities adhere to the standards of the APA ethical standards for treatment of animals.

Glucocorticoid receptor antagonist (PT 150)

The study was designed to ensure that birds would voluntarily consume PT 150 because forced administration (e.g. gavage in liquid vehicle) might have induced stress. Thus, a solid vehicle that birds found highly palatable, peanut butter, was used to assure consumption of the drug. Use of highly palatable solid foods (e.g., Peanut butter and Nutella)

are a common means of assuring voluntary oral consumption of pharmacological substances (Goldkuhl, Hau & Abelson, 2010; Berger & deCatanzaro, 2007; Cope et al., 2005).

Capsules containing drug (150 mg) or placebo were opened and added to Peanut Butter (PB) (150 mg/2g). The mixture was measured out at 0.53 mg/kg, resulting in a 40 mg/kg dose, and was given orally one hour before Pavlovian conditioning on days 11-15. As a control measure, 0.53 mg/kg of PB was provided on Pavlovian conditioning days 1-10. The dose of PT 150 was chosen based on previous research with rodents (Sharrett-Field, 2013).

Apparatus

Behavioral testing was conducted in a standard conditioning chamber (Med Associates Inc., Georgia, VT; 22 cm × 19 cm × 13 cm) that had an acrylic hinged loading door, stainless steel side panels, and an acrylic back panel. The chamber was located in a sound- and light-attenuating cabinet equipped with a fan that provided continuous ventilation. A white noise generator provided low-level background noise, and a white house light provided illumination. Two key lights (red and green) were mounted at one end of the chamber 12.7 cm above the metal rod floor, and a food magazine (ENV-205m MED Associates) was located between the key lights. The key lights and food magazine were illuminated with LED lights when activated.

Procedures

Food restriction

During Pavlovian conditioning, food restriction was used to ensure motivation of reward (i.e., grain). Male quail were maintained at 85% body weight, where food was available from 3 PM to 6 PM daily. This body weight matches previously defined avian

methods of food deprivation (Duval, Cassey, Miksik, Reynolds & Spencer, 2013a; Duval et al., 2013b; Shousha, Nakahara, Nasu, Sakamoto & Murakami, 2007). Quail were weighed daily, and following the establishment of their mean free-feeding body weight, 18% of their mean body weight was calculated to provide minimum caloric intake in grams of feed to sustain 85% body weight throughout conditioning (e.g., Lejeune & Nagy, 1986).

Conditioning

A Pavlovian-conditioned approach (PCA) procedure was used to measure the attribution of incentive salience to a CS. Before conditioning, birds were shaped with successive approximation until they reliably retrieved grain from the hopper. Shaping was followed by 15 days of Pavlovian conditioning. During conditioning, each trial was composed of presentation of an 8-sec green key light (CS+) followed by immediate 8-sec access to grain (unconditioned stimulus, US) in a food hopper. The pairing of the CS and US was on a variable time interval (VT) of 90 sec resulting in an average of a 30 min session. An 8-sec red key light (CS-) was presented unpaired with the US and used as a control, and thus was not predictive of food. Twenty trials were conducted each day for 15 days for a sum of 300 trials each for CS+ and CS-. Time spent near the CS to both key lights was measured during CS presentation on days 6, 10, 11, and 15. Day 6 was chosen because previous studies have shown that sign tracking is evident by trial 125 of conditioning (e.g., Flagel et al., 2008) which corresponds to day 6 in the current experiment. Day 10 was the last day before PT 150 was administered, day 11 was the first day PT 150 was administered, and day 15 was the last day PT 150 was administered.

Time spent near the CS was scored when subjects had both feet inside the zone (15.24 cm x 7.62 cm) marked off in front of the CS light and were oriented toward the CS. Goal

tracking was scored as time spent near the food hopper when subjects had both feet inside of the zone (15.24 cm x 8.89 cm) marked off in front of the hopper. Video recordings were scored by visual observation. Researchers scoring the videos were blind to the treatment condition of the subjects.

Statistical Analysis

Sign and goal tracking

Repeated-measures ANOVAs were conducted with treatment as a between-subjects factor and day (collapsed across trials) as a repeated measure to investigate changes in sign tracking to the CS+ and the CS-. A repeated-measures ANOVA with treatment as a between-subjects factor was conducted for day 10 (the last day before treatment) and day 15 (the last day of treatment) to determine the effects of repeated PT 150 administration. A posthoc analysis was conducted on day 15 to examine changes in sign tracking as a result of treatment. To determine the behavioral specificity of PT 150, a repeated-measures ANOVA with treatment as a between--subjects factor was conducted on goal tracking for days 10 and 15.

Response bias

A response bias analysis was used to determine the relative amounts of sign and goal tracking and then to investigate whether drug effects were the same regardless of extent of sign tracking. A similar response bias analysis has been used in previous research (e.g., Meyer et al., 2012; Paolone, Angelakos, Meyer, Robinson & Sarter, 2013). Response bias was defined as the difference between time spent near the food hopper and the CS+ light during CS+ presentation. The calculation was expressed as [(time spent at CS+ minus time spent at hopper) / (time spent at CS+ plus time spent at hopper)]. Quail were considered to be

expressing a response bias of sign tracking if they obtained scores ranging from +0.4 to +1.0 and were considered to have a response bias of goal tracking if they obtained scores ranging from -0.4 to -1.0. Quail with intermediate scores (ITs) were not considered to be showing a response bias of either sign or goal tracking. The response bias analysis was conducted on day 10 because it was the last day before treatment.

Difference score

To evaluate the change in sign tracking across days 10 and 15 in relation to the extent of sign tracking (response bias), a difference score was calculated as time spent near the CS+ on day 15 minus time spent sign tracking on day 10 during the CS+ presentation. A positive difference score indicated increased sign tracking from day 10 to day 15, while a negative score difference indicated decreased sign tracking from day 10 to day 15.

To determine whether PT 150 had a similar effect regardless of the extent of sign tracking, the relationship between the response bias and the difference score was analyzed with a Pearson's r correlation with treatment as a factor.

For all statistical analyses, alpha was set at $p < 0.05$.

Results

Figure 1.1A shows mean sign tracking across days (1, 6, 10 and 15) for each treatment group. A repeated-measures ANOVA revealed a main effect of Day [$F(3, 60) = 12.25, p < 0.05$], demonstrating that sign tracking (time spent at CS+) increased over conditioning days, regardless of treatment. There was no significant Day x Treatment interaction [$F(3, 60) = 2.24$], nor a main effect of Treatment [$F(1, 20) = 2.6, ps > 0.05$].

The repeated-measures ANOVA conducted to investigate the effect of PT 150 on sign tracking behavior on day 10 and 15 revealed a Day x Treatment interaction [$F(1, 20) = 5.68$]

and a main effect of Treatment, $[F(1, 20) = 5.93]$, $ps < 0.05$. A posthoc analysis indicated a significant main effect of Treatment on day 15 $[F(1, 20) = 5.42]$, $p < 0.05$. Subjects that received PT 150 ($M = 3.35$, $SEM = 0.3$) spent significantly less time sign tracking than subjects that received the placebo ($M = 4.33$, $SEM = 0.3$).

To determine whether PT 150 had an effect on time spent near the CS-, a repeated-measures ANOVA was conducted for days 6, 10, 11 and 15 for placebo and drug treatments (see Figure 1.1B). There was no significant Day x Treatment interaction $[F(3, 60) = 2.13]$, indicating that treatment groups did not change in time spent near the CS- across days. There were also no main effects of Treatment or Day, F_s were between 0.38 to 2.13, $ps > 0.05$.

To explore the individual differences of sign and goal tracking a response bias was calculated. The analysis showed that 12 out of 22 quail had a response bias toward sign tracking. Ten out of the 22 quail had neither a sign nor goal tracking response bias.

There was no positive correlation between the difference score and the response bias ($r = 0.52$), nor between treatment groups and the response bias ($r = 0.06$), ns , 22 , $ps > 0.05$. Therefore, the extent of sign tracking did not correlate with the difference score. However, there was a negative correlation between treatment groups and the difference score. Treatment with PT 150 was correlated with decreased sign tracking, ($r = -0.47$, $p < 0.05$). Thus, regardless of the extent of sign tracking, sign tracking appeared to be associated with treatment effects rather than the degree of the response bias.

To investigate the behavioral specificity of PT 150, a repeated-measures ANOVA was conducted on days 10 and 15 with goal tracking as the dependent variable. Results showed that there was no main effect of Day $[F(1, 20) = 0.30]$, $p > 0.05$, indicating that time spent goal tracking did not change from day 10 to 15. There was also no significant Day x

Treatment interaction [$F(1, 20) = 0.09, p > 0.05$], indicating that the drug did not affect goal tracking. Overall, these results indicate that the behavioral effects of PT 150 may have been specific to sign tracking.

Discussion

Similar to rodents, the quail in the current experiment demonstrated an increase in sign tracking toward a cue that had been paired with reward across days (e.g., Flagel, Akil & Robinson, 2009). Most importantly, repeated oral consumption of PT 150 resulted in a decrease in sign tracking behavior when compared to placebo. This reduction of sign tracking may have been the result of PT 150 blocking the glucocorticoid receptor (GR).

Administration of the GR antagonist PT 150 has been shown to reduce CORT in rodents that consumed alcohol (Reynolds et al., 2015). This reduction in CORT was thought to occur because PT 150 inhibits the nuclear translocation of the GR that indirectly regulates the release of CORT (Peeters et al., 2008). While the findings of the current experiment suggest that reduced sign tracking may have been a result of a blockade of the GR, it is unknown whether the GR is antagonized in the same manner in birds as in rodents.

There are changes in the brain that are related to sign tracking. For example, repeated activation of the reward pathway results in heightened behavioral responses that have been determined to be a predictor of drug addiction (Tomie, Grimes & Pohorecky, 2008). The hypothalamic-pituitary-adrenal (HPA) axis is involved in the activation and management of stress responses and has been shown to contribute to a heightened reward pathway (Piazza & Moal, 1998). In Pavlovian conditioning, this heightened response (e.g., elevated pecking, sniffing, and licking) is often referred to as sign tracking (e.g., Tomie et al., 2008). Similar to stress, sign tracking also disrupts the hypothalamic-pituitary-adrenal (HPA) axis and this may

result in alteration of the HPA axis' negative feedback system, (e.g., Fligel et al., 2008; Tomie et al., 2008). Therefore, in the current study, the reduction in sign tracking may have been associated with dysfunction of the HPA axis.

The current study extends previous research by demonstrating reduced sign tracking as a result of repeated administration of a GR antagonist, suggesting that sign tracking may be associated with an increase in CORT and mediated via the GR. The link between sign tracking and CORT is particularly interesting in that CORT could be a biomarker for future pharmacological treatments in individuals suffering from substance use disorders. However, further research is needed for validating the reduction of CORT by PT 150 and its relationship to sign tracking.

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Figure 1.1

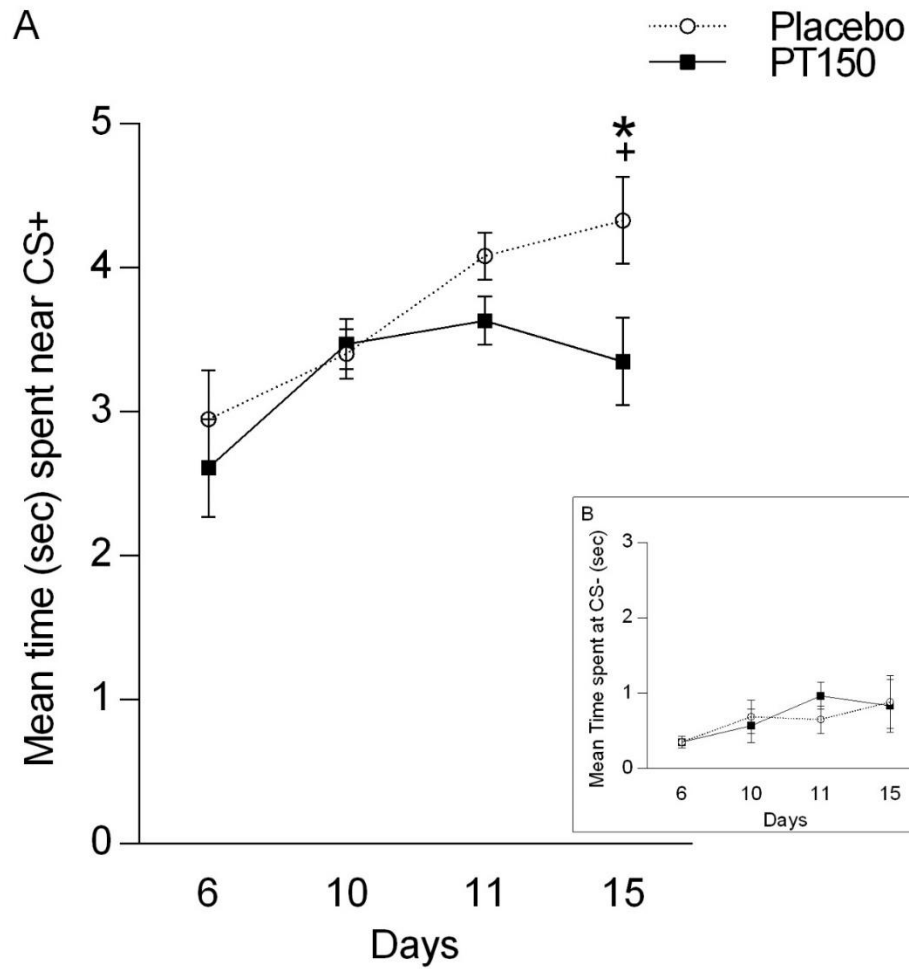


Figure 1.1. Mean time (sec) near the CS+ (Figure 1.1A) and CS- (Figure 1.1B) across days 6, 10, 11, and 15 for placebo and drug treatments. * Significant difference between day 10 and 15, + significant difference between treatment groups on day 15.

Chapter Three

REPEATED BLOCKADE OF THE GLUCOCORTICOID RECEPTOR WITH PT 150 HAS DOSE-DEPENDENT EFFECTS ON SIGN TRACKING IN MALE JAPANESE QUAIL

In both animal (e.g., Davis & Smith, 1976, de Wit & Stewart, 1981) and human (for review, Carter & Tiffany, 1999) research, it has been demonstrated that cues (e.g., paraphernalia) associated with drug reward can instigate renewed drug taking. These cues may be items used to consume the drug, and people, and places they take drugs around. One reason this may occur is that in the presence of these cues, the person experiences a stress response that prompts the individual to seek out drugs to moderate the stress response. There are individual differences in response to drug cues. It has been established that non-human animals that show a stress response to reward-predictive cues exhibit heightened reward behaviors to cocaine, a psychostimulant (Flagel, Watson, Akil & Robinson, 2009). As a consequence, these subjects (i.e., Sign trackers, STs) may be more vulnerable, than subjects that do not sign track to the rewarding effects of drugs. Therefore, the cooperative interaction between stress and a drug-cue association may be a therapeutic target for the treatment of cue-induced relapse.

Individual differences in response to drug cues can be measured in traditional animal models. A Pavlovian conditioned approach (PCA) paradigm, in which a cue predicts a reward (e.g., sex, drug, and food) is typically used to measure these differences (Domjan, Lyons, North, Bruel & Bardo, 1986; Beckmann & Bardo, 2012; Flagel et al., 2008). Animals that spend the majority of the time near a cue that predicts reward (i.e., sign trackers, STs) have been shown to exhibit higher rates of drug-taking behaviors (Beckman et al., 2012) and

increased locomotor activity (i.e., sensitization) in response to cocaine (Flagel et al., 2008). Consequently, STs are thought to be more vulnerable to drug addiction and are utilized as a model of cue-elicited drug-taking behaviors. Sign tracking behaviors have been demonstrated with a key light in pigeons (e.g., Brown & Jenkins, 1968, Papini & Overmier, 1984) bobwhite quail (Gardner, 1969), and Japanese quail (Crawford & Domjan 1993). The behavior exhibited by birds have a corresponding response, similar to the rodent lever press (i.e., key peck). In previous research, we have measured sign tracking in Japanese quail using a similar method as rodent models (Rice, Eaton, Prendergast & Akins, 2018).

Sign tracking is associated with elevated stress hormones (Flagel et al., 2009, Tomie et al., 2002). Previous research has shown that a stress hormone corticosterone (CORT) is elevated after sign tracking behavior, and administration of CORT increases the expression of sign tracking (Flagel et al., 2007). Conversely, the removal of the adrenal glands, which release CORT, reduces the ability of a subject to display sign tracking behavior (Carroll, Campbell & Heidman, 2001). Together, these studies suggest that stress hormones may facilitate sign tracking and the interaction of CORT and sign tracking may be a potential target for pharmacological therapies.

CORT is the primary stress hormone in animals, and its effects are a result of the activation of the glucocorticoid receptors (GRs). GR antagonist, 11, 21-Bisphenyl-19-norpregnane derivative (PT 150) is a novel antagonistic that competes with CORT for GR receptor binding in the cytoplasm (Peeters et al., 2004). The traditional antagonist of CORT (RU 486) is not viable for half the population of drug users (i.e., females). Specifically, RU 486 cannot be used in females as it causes pregnancy termination through its action on the progesterone receptor (Vegeto et al., 1992). Therefore, PT 150 may be a better alternative

than RU 486 because PT 150 is a pure, competitive antagonist with minimal effect on the progesterone receptor.

The current study was designed to test three doses of PT 150 to investigate dose-dependent effects of PT 150 on sign tracking behavior. To examine the effect of PT 150 on CORT concentrations, blood was taken before the first day of conditioning and on the first and last day of PT 150 administration.

Methods

Subjects

Thirty-nine (N = 39) adult male Japanese quail (*Coturnix japonica*) were hatched from eggs (Northwest Gamebirds, Kennewick, WA). Quail were raised in mixed-sex groups until approximately six weeks of age and were housed in individual wire mesh cages (supplied by GQF Manufacturing, Savannah, GA) and maintained on a 16:8 hr light cycle. Before the current experiment, male quail were randomly assigned to one of four treatment groups. Data from one bird were not included because the bird died before completion of conditioning. Therefore, thirty-eight quail were used as subjects in the analyses of the experiment.

All experimental procedures were conducted according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky and by the standards outlined in the 8th edition of the Guide for the Care and Use of Laboratory Animals (National Research Council, 2010).

Apparatus

Behavioral testing was conducted in two standard Pavlovian conditioning chambers (Med Associates Inc., Georgia, VT) that had an acrylic hinged loading door, stainless steel

side panels, and an acrylic back panel (interior, 30.5 x 24.1 x 21 cm). The chambers were located in a cabinet equipped with a fan that provided continuous ventilation. The top of the cabinet was removed to illuminate the apparatus with a white fluorescent light. A white noise generator provided low-level background noise. Two key lights (red and green) were mounted at one end of the chamber 12.7 cm above the metal rod floor, and a food hopper was located between the key lights. The key lights and hopper were illuminated with LED lights when activated.

Drug

PT 150 was dissolved in 30% dimethyl sulfoxide (DSMO)/70% polyethylene glycol (PEG) – 300), similar to previously established methods (Johnson, Grant, Ingram & Gartside, 2007). On days 1-5 of the PCA trials, quail received a subcutaneous (SC) injection of vehicle (DMSO-PEG), 30 min before testing (1mL/kg of body weight). On days 6-10 of testing, quail received one of four SC injections of PT 150 (20 mg/kg n=9, 40 mg/kg n=10, or 60 mg/kg n=10) or vehicle (1 ml/kg of body weight, n=9). These doses were selected based on previous rodent literature that used doses ranging from from 20 mg/kg to 60 mg/kg (Bechmann, Linhurst, Hulsbuer & Reul, 2003; Reyonlds et al., 2015).

Procedures

Experiment 2 was conducted in two replications. Twenty animals were tested in replication 1 and the remaining 18 in replication 2. Replication 2 followed immediately after the last conditioning day of replication 1. There were no significant differences between the two replications ($F(3,102) = 1.112, p > 0.05$) so the replications were combined for all analyses.

Food Restriction

During Pavlovian conditioning, food restriction was used to ensure motivation of reward (i.e., grain). Male quail were maintained at 85% body mass where grain was provided 2-3 hours after conditioning. The resulting body mass closely matches previously defined bird methods of food deprivation (Duval et al., 2013a; Duval et al., 2013b; Shousha, Nakahara, Nasu, Sakamoto & Murakami, 2007). Quail were weighed daily and following the establishment of their mean free-feeding body weight, 18% of their body weight was calculated to provide minimum caloric intake in grams of feed to sustain 85% body mass throughout conditioning (e.g., Lejeune & Nagy, 1986). Remaining grain was removed before lights would come on the following morning.

Conditioning

Procedures for Pavlovian conditioning were similar to previously conducted research in our laboratory (Rice et al., 2018) as described in Experiment 1. Additionally, the current experiment recorded additional measures. Time spent measures (sec) were recorded automatically in zones (15.24 x 8.89 cm) that were in front of both key lights and a zone (15.24 x 6.35 cm) that was in front of the hopper. Key pecks and hopper entries with automated equipment were also recorded. There were a total of ten days comprised of 20 trials a day resulting in 200 trials of conditioning.

Locomotor Activity

Distance traveled (meters) was recorded on day 10 for each treatment group.

Corticosterone Procedures

On days 1, 5, 6 and 10 of conditioning, blood was collected via the wing vein, and plasma was separated and frozen for later analysis. Those days were chosen to investigate the

effects of sign tracking (days 1 and 5) and the drug PT 150 (days 6-10) on CORT plasma concentrations. Because of the diurnal rhythm of quail plasma CORT concentrations, testing was delayed until 10 AM and ended prior to 6 PM to maximize the natural nadir levels of CORT. An enzyme immunoassay (EIA) kit (Arbor Assay; K017-H1) was used to analyze CORT concentrations. This particular kit is well suited for quail, as it has a high sensitivity, detecting plasma CORT concentrations as low as 0.07 ng/g (Arbor Assay; K017-H1). Sensitivity is of particular importance as Japanese quail have been reported to have low baseline CORT concentrations, with CORT concentrations as low as 1ng/g (Hayward, Satterlee & Wingfield, 2005; Cockrem, Candy, Castille & Satterlee, 2010).

Statistical Analysis

Automated behavioral analysis

Session events were maintained and collected with Med PC software (ENV013; MED Associates Inc, St. Albans, VT) and videotaped by ANY-MAZE video tracking software (San Diego Instruments, San Diego, CA) for sign and goal tracking behavior. All of the procedures were based on previous rodent and quail studies, (e.g., Meyer et al., 2012; Beckmann & Bardo, 2012, Rice et al., 2018).

Assessment of sign and goal tracking

Sign tracking was scored as two measures; frequency of pecking on the key light and time (sec) spent in the key light zones (CS). The pecking response was scored as the frequency of pecks toward the CS+ or CS- when the CS was activated. Time spent at the CS, and pecking to the CS, while the CS was on, were added together as the sign tracking response for both the CS- and the CS+. These scores were added together to better represent rodent sign tracking measures that include approach to the lever and lever contacts as indices

of sign tracking behavior, resulting in a single sign tracking score for each CS-US presentation (e.g., Flagel et al., 2008; Meyer et al., 2012). These indices are thought to be conditioned responses (CRs) that are similar to appetitive and consummatory responses of the reward (i.e., food) (Flagel et al., 2008).

Goal tracking was measured as time spent in the zone in front of the hopper during the CS+. Additionally, hopper entry frequencies were scored during the CS+ presentation. Time spent at the hopper and hopper entries while the CS+ was activated were added together as the goal tracking response, similar to sign tracking behavior.

Response Bias

The difference between sign and goal tracking behaviors was calculated as a response bias, similar to previous measures published in our lab (Rice et al., 2018) and based on rodent literature (e.g., Flagel et al., 2008, Meyer et al., 2012). The response bias was calculated as the difference between sign and goal tracking responses during the CS+ presentation. The calculation was expressed as $[(\text{Sign tracking} - \text{Goal tracking}) / (\text{Sign tracking} + \text{Goal tracking})]$. Quail were considered to be expressing a response bias of sign tracking if they obtained scores ranging from +0.4 to +1.0 because these scores indicate that sign tracking was more than twice as likely as goal tracking. Quail were considered to have a response bias of goal tracking if they obtained scores ranging from -0.4 to -1.0. Birds with intermediate scores (intermediates; ITs) were not considered to be showing a response bias of either sign or goal tracking.

A repeated-measures ANOVA with treatment as a between-subjects factor was conducted for day 1 (first day of conditioning), day 5 (the last day before treatment), day 6 (first day of treatment) and day 10 (the last day of treatment), to determine the effects of

repeated PT 150 administration on sign and goal tracking behavior. A posthoc analysis was conducted on day 10 to examine changes in sign tracking as a result of treatment.

CS control

For each subject, sign tracking at both the CS+ and CS- were recorded for all 20 trials of 4 days (1, 5, 6 and 10). A t-test was used to analyze mean time spent on day 5 for the CS+ versus CS- to ensure CS+ control before administration of treatments. Day 5 was chosen because it is traditionally utilized for classification of sign tracking in rodent models (e.g., Flagel et al., 2008; Meyer et al., 2012; Paolone et al., 2013).

Locomotor Analysis

To investigate the effects of PT 150 on locomotor behavior, a one-way between subjects ANOVA was conducted to compare the effect of treatment on distance traveled for day 10.

Corticosterone Analyses

A repeated-measures ANOVA with treatment as a between-subject factor was conducted for days 1, 5, 6 and 10 to determine treatment effects on plasma CORT concentrations across days. Individual Pearson's r correlations were computed to assess the relationship of the response bias on day 10 and CORT concentrations for each treatment group.

All statistical analyses were conducted using IBM Statistical Package for the Social Sciences (SPSS) Version 21 Software (IBM Corporation, 2014). The alpha was set at $p < 0.05$ for all analyses.

Results

Figure 2.1 illustrates the mean sign tracking responses for day 5 for all subjects. Day 5 was chosen because it was the last conditioning day before treatment, and day 5 is traditionally used in rodent models for sign tracking characterization. A paired sample t-test indicated a significantly greater amount of sign tracking to the CS+ (Mean = 40.7486, SEM = 10.81) compared to the CS- (Mean = 4.20, SEM = 1.00), [$t(37) = -3.516, p < 0.05$]. These results demonstrated that quail learned that the CS+ was predictive of reward and not the CS-

Figure 2.2 shows the response bias across days for each treatment group. A repeated-measures ANOVA revealed a main effect of Day, $F(3, 96) = 8.818, p < 0.05$, demonstrating that the response bias changed across days. There was also a significant interaction of Day X Treatment, $F(9, 96) = 3.331, p < 0.05$, indicating that the change in the response bias across days was different depending on treatment. To probe the interaction further, one-way ANOVAs were conducted to investigate the difference in the response bias between treatments for each day. There were no treatment differences in the response bias for days 1, 5 or 6, with F 's ranging from .491 to 1.34, $p > 0.05$. However, there was a treatment difference in the response bias on day 10, $F(3, 36) = 11.334, p < 0.05$. Post hoc comparison of the response bias on day 10, using the Tukey HSD test, indicated that the mean score for the 20 mg/kg PT 150 group ($M = -0.40, SEM = 0.21$) was significantly different from placebo group ($M = 0.31, SEM = 0.14$), the 40 mg/kg ($M = 0.70, SEM = 0.18$) and 60 mg/kg ($M = 0.90, SEM = 0.24$) PT 150 groups.

In order to analyze whether PT 150 changed sign tracking from day 6 to day 10, within-subject contrasts were conducted. These analyses revealed that there was a difference in change of the response bias between treatments from day 6 to day 10, $F(3, 32) = 8.256, p$

< 0.05. Post hoc comparison of the response bias across days 6 and 10, using the Tukey HSD test, indicated that the change in response bias was between the 20 mg/kg PT 150 group and all other treatment groups, $p < 0.05$. Additionally, a paired sample t-test for the 20 mg/kg treatment group for days 6 and day 10 revealed that the 20 mg/kg treatment group switched from a sign tracking response bias score ($M = 0.67$, $SEM = 0.09$) on day 6, to a goal tracking response bias score ($M = -0.40$, $SEM = 0.21$) on day 10, $t(8) = 4.348$, $p < 0.05$.

Figure 2.3 shows the plasma CORT concentration across days, a repeated measures ANOVA indicated a main effect of Day, $F(3, 99) = 83.92$, $p < 0.05$, demonstrating that plasma CORT concentrations (i.e., ng/ml) changed across conditioning days. Pairwise comparisons revealed significant differences between day 5 ($M = 51.18$, $SEM 4.8$) and day 6 ($M = 37.70$, $SEM = 3.80$) and between days 5 and 10 ($M = 39.427$, $SEM = 4.26$), $p < 0.05$. There was no significant Day X Treatment interaction, $F(9, 99) = 0.91$, nor a main effect of Treatment, $F(1, 33) = 0.40$, indicating that the change in CORT across days was not due to treatment.

Figure 2.4 illustrates the correlation between plasma CORT concentrations and the response bias for each treatment group. There was no significant association between the placebo, 40 mg/kg, and 60 mg/kg PT 150 treatment groups and their response biases on day 10; r 's ranged from -0.30 to 0.15, $p > 0.05$. The correlation between the response bias for the 20 mg/kg group ($M = -0.35$, $SEM = 0.21$) and their plasma CORT concentration on day 10 ($M = 32.90$, $SEM = 6.83$) was nearly significant, $r = 0.67$, $n = 9$, $p = .05$.

Figure 2.5 shows the mean distance traveled on day 10 for all groups. There was no significant effect of treatment on distance traveled for day 10 ($F(3, 37) = 1.031$, $p > 0.05$), indicating that the drug did not affect locomotor behavior.

Discussion

Similar to previous research in rodents, quail showed significant sign tracking after five days of conditioning (e.g., Flagel et al., 2009; Rice et al., 2018). The steady increase in sign tracking from the first to last day of conditioning was similar to both rodent (e.g., Flagel, Akil, & Robinson, 2009) and other quail (Burns & Domjan, 1996; 2000; 2001) models of sign tracking. The significant increase in sign tracking was specific to the paired CS (CS+), indicative of robust CS control. Pecking to the CS+ and not the CS- is suggestive of subjects learning the predictive value of the CS+ (e.g., Tomie, Silberman, Williams & Pohorecky, 2002). Additionally, PT 150 did not appear to alter locomotor behavior in the current experiment. This might further implicate the selective nature of PT 150. The selective nature of PT 150 may be important in that these results support a reduced likelihood of behavioral side effects from administration of PT 150.

In the current experiment repeated administrations of PT 150 reduced sign tracking, a drug addiction-like behavior, at a low dose (20 mg/kg PT 150) but not at 40 mg/kg or a 60 mg/kg dose. The reduction in sign tracking in the 20 mg/kg PT 150 group may have been a result of reduced CORT binding to the GR receptor. Reducing the effect of CORT has been shown to attenuate sign tracking behaviors previously in both rodents (Thomas & Papini, 2001) and quail (e.g., Rice et al., 2018). The reduction in CORT's effects, via the administration of PT 150, is thought to occur because GR antagonists like PT 150 reduce CORT's ability to bind to the GR (Peeters, Ruigt, Craighead & Kitchener, 2008). With reduced binding to the GR, CORT's effects may not be actualized.

One possible explanation for why PT 150 did not block sign tracking at the higher doses (i.e., 40/60 mg/kg PT 150) is that PT 150 may have bound to other receptors such as

the mineralocorticoid receptor (MR). PT 150 reduces the effects of CORT by blocking CORT's ability to bind with the GR however, previous research has shown that chronic treatment of PT 150 enhances MR expression (Bachmann et al., 2003), implicating an upregulation of MRs. CORT has a higher affinity for MR compared to the GR (Peeters et al., 2004). Therefore, it is probable that at higher doses of PT 150, there is an upregulation of MRs where CORT can bind and exert its effects. Therefore, the possibility that CORT was binding nonspecifically to the MR at higher doses may be the reason that higher doses did not attenuate sign tracking behavior.

The current study did not show any changes in circulating plasma CORT concentrations across conditioning days. This conflicts with previously reported rodent sign tracking literature. Tomie and colleagues reported an increase in CORT in rodents that received a paired cue when compared to animals that received an unpaired cue (et al., Tirado, Yu & Pohorecky, 2004). Additionally, an increase in CORT was found in rodents that sign tracked compared to rodents that did not (Flagel et al., 2008). The difference between the current results and the rodent studies may be a species difference. However, it is more likely that the procedures used to obtain plasma for CORT analysis were different and may have increased stress in the current study. Research has shown that circulating plasma CORT concentrations increase from blood sampling in as little as 3 min (Romero & Romero, 2002). In rodent models, the blood was taken quickly by tail nip or trunk blood, immediately after conditioning (Tomie et al., 2002; 2004; Flagel et al., 2008). In the current study, blood collection took approximately 5-7 min. The differences in collection techniques may account for these conflicting results.

The response bias for the 20 mg/kg PT 150 treatment group exhibited an approaching significant correlation of the response bias and plasma CORT concentrations on day 10. It appears there was a trend, where higher sign tracking response biases correlated with higher plasma CORT concentrations. Conversely, there was a trend of lower levels of plasma CORT concentrations having a nearly significant association with goal tracking response biases. These tentative results correspond to research in rodent literature where STs display higher CORT concentrations compared to GTs (Flagel et al., 2009).

Sign tracking is thought to be a measure of cue-induced relapse. Because relapse is considered one of the most significant problems in drug dependence, animal models of sign tracking are important. Therefore, biomarkers for reducing behaviors that are associated with cue-induced drug relapse may be relevant in addiction research. The current findings extend previous research by suggesting that the GR may be a potential pharmacological target for reducing relapse-like behaviors. Further research is needed to identify pharmacologically relevant doses of PT 150, as well as potential side effects and long-term effects on behavior.

Figure 2.1

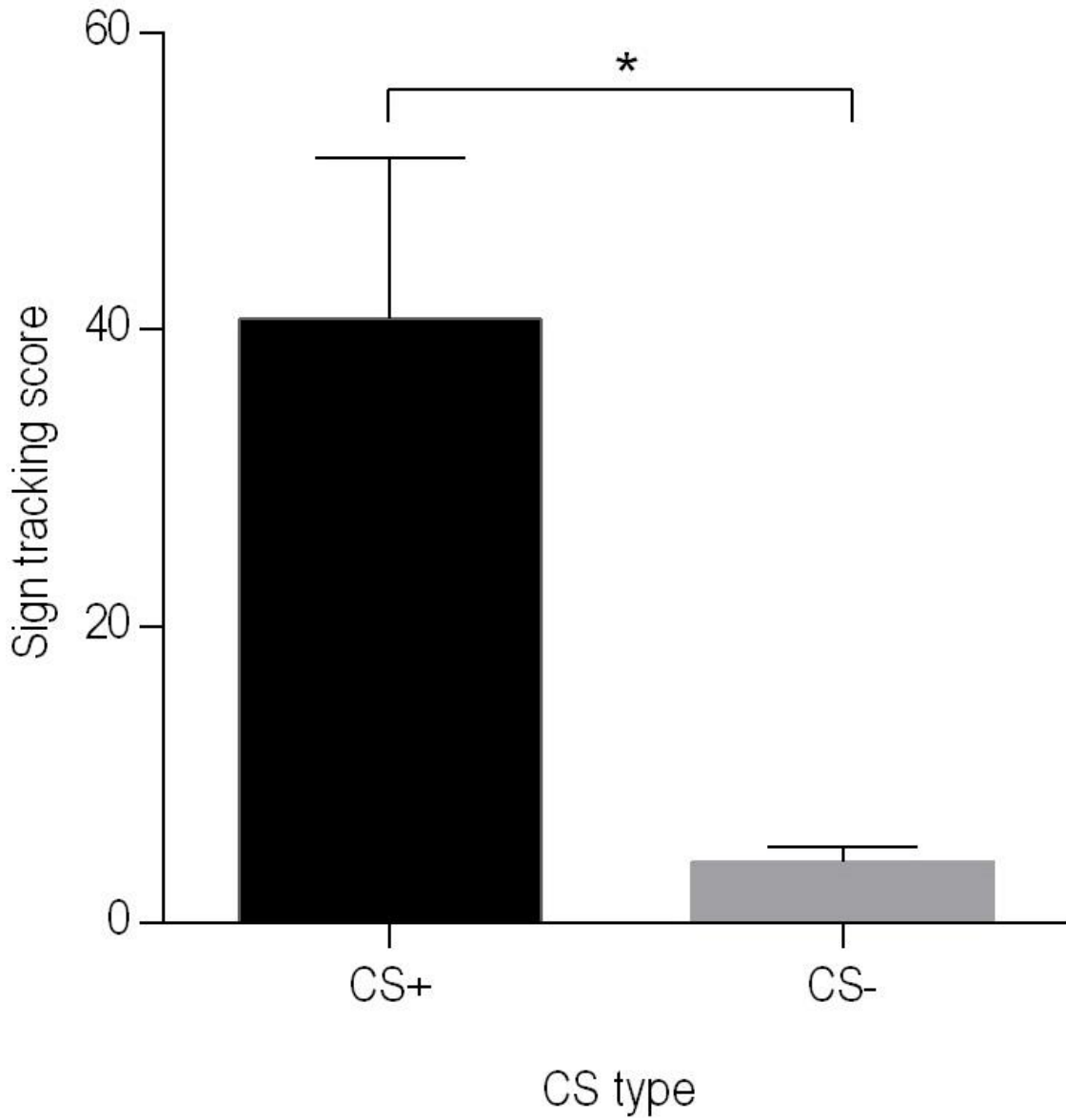


Figure 2.1. Mean sign tracking responses for day 5 for all subjects. * Significant difference in sign tracking responses to CS+ compared to CS- collapsed across all trials.

Figure 2.2

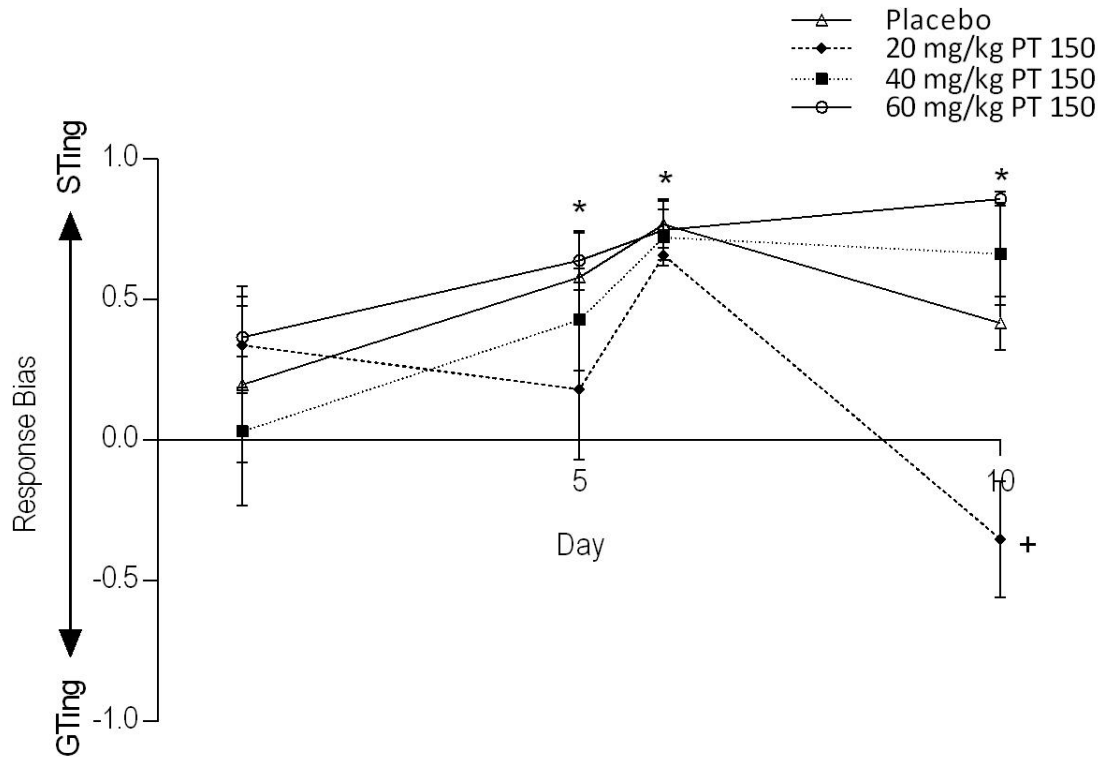


Figure 2.2. Mean response bias across days 1, 5, 6 and 10 for placebo, and treatment groups.

* Significant difference between day 1 and 5, 5 and 6, and 6 and 10. + Significant difference between 20 mg/kg treatment group and placebo, 40 and 60 mg/kg groups on day 10. STing = sign tracking response bias score, GTing = goal tracking response bias score

Figure 2.3

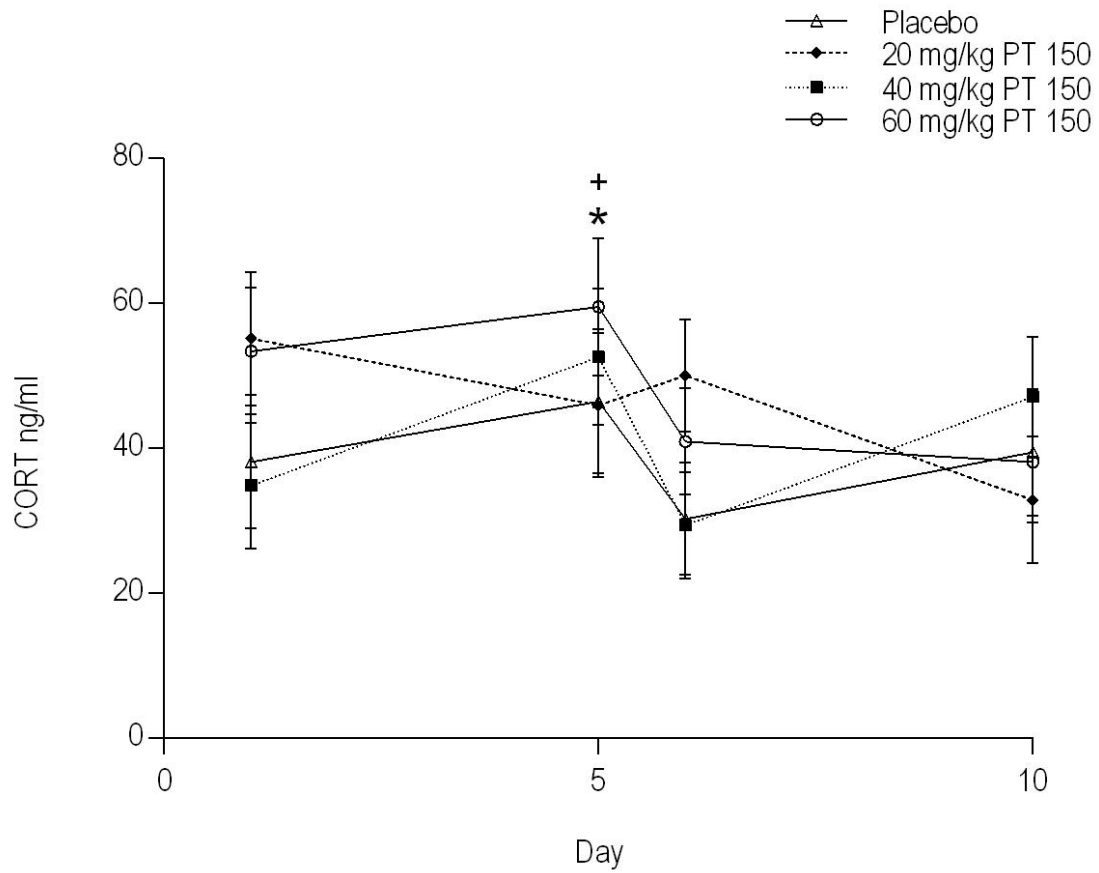


Figure 2.3. Mean plasma CORT concentrations (ng/ml) across days 1, 5, 6 and 10 for placebo, and treatment groups. * Significant difference between days 5 and 6. + Significant difference between days 5 and 10.

Figure 2.4

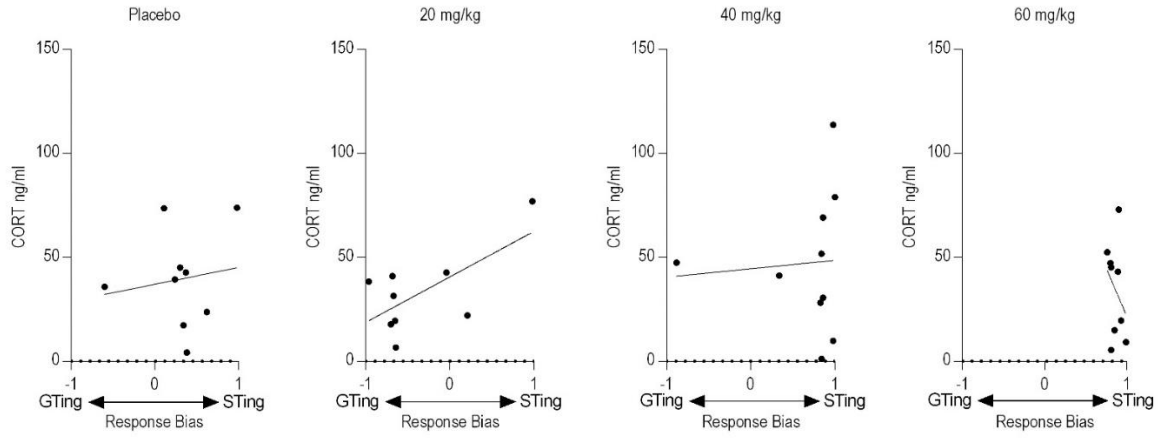


Figure 2.4. Correlations between the response bias and plasma corticosterone (CORT) concentrations (ng/ml), on day 10 for each treatment group. G Ting = goal tracking response bias, STing = sign tracking response bias

Figure 2.5

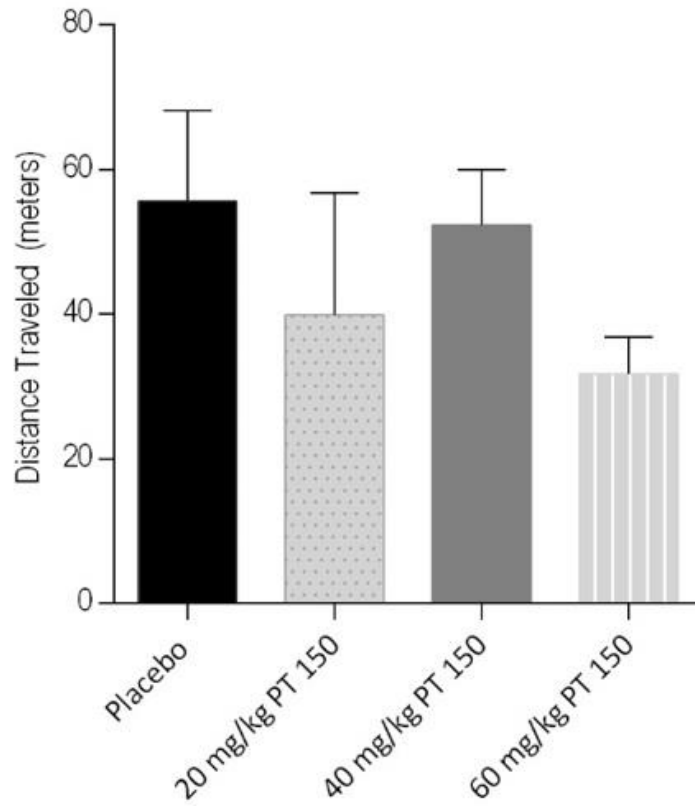


Figure 2.5. Total distance traveled activity (mean meters \pm SEM), collapsed across all trials on day 10 for placebo and treatment groups.

Chapter Four

GENERAL DISCUSSION

Sign tracking has been shown to be associated with an increase in drug taking behaviors, (e.g., Beckman & Bardo, 2012; Saunders & Robinson, 2013). For example, sign trackers learn to self-administer cocaine more quickly (Beckmann, Marusich, Gipson & Bardo, 2011), express heightened behavioral sensitivity to psychostimulants (Flagel et al., 2007), and develop more robust rates of reinstatement to drug paired cues (Saunders & Robinson, 2010) when compared to subjects that do not sign track. For these reasons, sign tracking is utilized as an animal model of a drug dependence like behavior. Additionally, sign tracking has been shown to be facilitated by the stress hormone, corticosterone (CORT) (Campbell & Carroll, 2001; Flagel et al., 2007; Tomie et al., 2002; 2004). The current experiments aimed to reduce sign tracking with a glucocorticoid receptor (GR) antagonist, which has been shown to block the behavioral effects of alcohol induced CORT in a rodent model (Reynolds et al., 2015). In Experiment 1, sign tracking was reduced with an oral administration of 40 mg/kg of the GR antagonist PT 150. In Experiment 2, sign tracking was reduced with a subcutaneous (SC) injection of 20 mg/kg PT 150 but not a 40 or 60 mg/kg dose. Additionally, Experiment 2 investigated the relationship of CORT and sign tracking. In Experiment 2, there was an approaching significant association between the response bias and plasma CORT concentrations. This correlation suggests that there may be an association between higher response biases (sign tracking) and higher plasma CORT concentrations. This finding, although tentative, is similar to what has been found in rodents (Flagel et al., 2009).

Experiments 1 and 2 resulted in differences of PT 150 doses that reduced sign tracking behavior. These differences may be because of the differences between the two experiments in administration of PT 150. Experiment 1 used an oral route of administration and found 40 mg/kg of PT 150 to be effective at reducing sign tracking behavior. Experiment 2 used a SC route of administration and found 20 mg/kg of PT 150 to be effective at reducing sign tracking and not 40 or 60 mg/kg doses. Routes of administration have a critical role in the bioavailability of a drug in the body. An oral route of administration undergoes first-pass metabolism whereby the drug is greatly reduced before reaching circulation (Verma, Thakur, Deshmukh, Jha & Verma, 2010). Experiment 2 used a SC administration, which absorbs directly into the blood, thus bypassing first-pass metabolism (Verma et al., 2010). SC administration likely has a higher bioavailability if compared to the bioavailability of an oral route of administration. This possible increased bioavailability may be why the lower dose (20 mg/kg) reduced sign tracking behavior in the 2nd Experiment.

Another potential reason for the difference between the doses that reduced sign tracking between experiments may be because birds have a crop. The function of the crop is to store ingested feed (Kierończyk et al., 2016) and not a place of digestion (Savory, 1985). Typically, feed bypasses the crop when feed is readily available. However, when food is scarce, quail will store feed in their crop. Feed was restricted in both experiments, thus it is likely that quail stored feed in their crops. Consequently, it is probable that the bioavailability of an orally administered dose of 40 mg/kg PT 150 was affected by both the crop and first pass metabolism.

There were differences in sign tracking behavior across conditioning days within some treatment groups between the two experiments. In Experiment 1, all groups exhibited a

steady increase in sign tracking across conditioning days. However, in Experiment 2, two groups (placebo and 40 mg/kg PT 150) exhibited fluctuating sign tracking behavior. These differences may be because of different characterizations of sign tracking utilized between the two experiments. In Experiment 1, sign tracking was recorded, as orientation to the CS+ and time spent in the CS+ zone. In Experiment 2, sign tracking was recorded as a response bias that took into consideration pecking behavior, time spent in the CS+ zone, and goal tracking behavior. It is possible that orientation to the CS in Experiment 1 inflated the characterization of sign tracking. Additionally, it is possible that pecking measures, taken in Experiment 2, fluctuate more than time spent behaviors. However, the fluctuation of sign tracking seen in Experiment 2 is more likely a result of the response bias taking into account goal tracking behaviors. Subsequently, the differences in sign tracking behavior across conditioning days between the two experiments are likely due to these dissimilarities in how sign tracking was recorded.

Similar to rodents, birds have demonstrated individual differences in sign and goal tracking (Burns & Domjan, 2001; Flagel et al., 2008). However, in the current experiments, birds exhibited robust sign tracking behavior and little or no goal tracking behavior after conditioning and before treatment. These differences may be due to differences in the cues between our model (key light) and those of rodent (lever) and other quail research (wood block) (Burns & Domjan, 2001; Flagel et al., 2008). The salience of a cue has long been shown to effect the conditioned responding to a cue (e.g., Kamin, 1965). Therefore, it is likely that in the current experiments, quail had heightened salience to the cue (key light), when compared to previous models that used levers or wood blocks. Because quail rely on visual cues (Johnson & Whittow, 2000) and rodents rely primarily on olfactory cues

(Crombag, Badiani, Maren & Robinson, 2000), visual cues may be more salient to quail when compared to levers in rodent models, and this may be facilitating increased salience to the cue. Increased salience of a cue has been shown to increase sign tracking behaviors (Robinson & Berridge, 2008).

The current research is the first research to our knowledge to explore the effects of PT 150 on sign tracking, an addiction-like behavior. The main findings from these studies are that the GR receptor antagonist PT 150 reduces sign tracking behavior dose-dependently. Novel pharmaceutical interventions such as PT 150 may reduce the effects of corticosterone in the presence of addiction-relevant stimuli that may instigate relapse. Future research is needed to investigate a therapeutic dose and the long-term effectiveness of PT 150 on sign tracking behavior.

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Beth Ann Rice

BIBLIOGRAPHICAL DATA

EDUCATION

Degree Awarded: **Master of Arts**, Experimental Psychology
December 2015 University of Kentucky

Degree Awarded **Bachelor of Science**, Experimental Psychology
May 2012 Indiana University Southeast

FELLOWSHIPS, HONORS AND AWARDS

2016 - 2018 National Institute on Drug Abuse Pre-Doctoral Training Fellowship(s)
Department of Behavioral Science, University of Kentucky College
of Medicine PI: Craig R. Rush, Ph.D. (T32DA035200)

2017 Behavior, Biology, and Chemistry conference pre-doctoral poster
presentation award in Translational Research in Addiction

2017 - 2018 Behavior, Biology, and Chemistry: Translational Research in Addiction
conference, travel award (s)

2016 - 2017 Robert Lipmann Research Fellowship, Department of Psychology

2013 - 2018 Research Challenge Trust Fund travel award(s), University of Kentucky

2015 - 2016 Outstanding Teaching Assistant Award, Department of Psychology

2013 - 2016 Graduate Student Congress travel award(s), University of Kentucky

2013 - 2016 Lyman T. Johnson Academic Year Fellowship(s), University of
Kentucky

PUBLICATIONS

Rice, B.A., Eaton, S.E., Prendergast, M.A. and Akins, C.K. (2018). A Glucocorticoid Receptor Antagonist Reduces Sign-Tracking Behavior in Male Japanese Quail. *Experimental & Clinical Psychopharmacology*, (in press).

Rice, B.A., and Akins, C.K., (2017). Appetitive responding. In Vonk, J. (Ed) & Shalkelford, T.K. (Ed), *Encyclopedia of Animal Cognition and Behavior*. New York, NY: Springer.

Rice, B.A., **Tariq, R.*** and Akins, C.K. (2017). Intramuscular Route of Administration Increases Potency in Eliciting Cocaine-Induced Behavioral Sensitization. *Current*

Psychopharmacology, 6(1), 36-42.

Gill, K., Rice, B.A. and Akins, C.K. (2015). Cocaine induces state-dependent learning of sexual conditioning in male Japanese quail. *Physiology & Behavior*, 138, pp. 150-153.

***student mentee**

PEDAGOGICAL ACTIVITIES

Course Instructor and Director

Indiana University Southeast (IUS), 2018 (spring) , 2017 (fall)

University of Kentucky (UK), 2015 (summer)

Laboratory Supervisor

UK), 2015 (spring, fall), 2013-2016 (fall, spring)

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