CONDITIONED PLACE PREFERENCE IN THE MALE WISTAR-KYOTO RAT

by

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ABSTRACT

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The current set of experiments examined the role of trait anxiety, using the Wistar-Kyoto (WKY) rat, on cocaine- and morphine-induced conditioned place preference. Both four and six pairings of 10mg/kg cocaine during conditioning yielded no significant preference at acquisition, yet a preference at reinstatement was observed after a protracted period of abstinence. The three pairings of 10mg/kg morphine yielded no significant preference for either of the outer choice chambers at any time point, however an increasing preference for the neutral chamber in which no injection was given was observed as extinction continued. Taken together, the data collected from these experiments suggest a model that is driven primarily by the negatively reinforcing properties of drugs (the reduction in anxiety induced by the drug following an extended

period of abstinence). This research adds to the literature investigating the use of this model in the context of drug addiction.

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CHAPTER 1

INTRODUCTION

Substance abuse is a significant problem that not only affects the individual, but also has a great impact on society. The lifetime prevalence in the United States for substance use disorders is 14.6% (Kessler, Berglund et al. 2005). While there are a number of factors that can contribute to the development of a substance use disorder, there is substantial support that brain stress systems play a major part in the various phases of the addiction process (Koob 2008). Not surprisingly, individuals who display anxious behavior have an altered relationship with drugs of abuse. An estimated 18% of individuals with a substance use disorder have a comorbid anxiety disorder (Grant, Stinson et al. 2004). While research investigating the relationship between stress and addiction pathways has started to gain prominence, more information is needed to fully understand the vulnerabilities of developing substance use disorders in individuals who display a trait anxiety.

1.1 Goal-Directed vs. Stimulus-Directed Behavior

In addiction, a transition from goal-directed (response-outcome) behavior to habitual stimulus-response behavior is observed. Initially, drugs of abuse are administered to achieve reward, or a “high”. With continued use of the drug, a shift from goal-directed to habitual use and even compulsion occurs (Mazzucchelli,

Vantaggiato et al. 2002). This transition from goal-directed to stimulus-directed behavior reflects a shift from prefrontal to striatal (more specifically the dorsal striatum) areas of the brain (Everitt and Robbins 2005). Essentially, the dorsal striatum takes over execution of behaviors associated with drug-seeking/administration of the drug, minimizing the role of higher order processing and decision-making. This is fundamentally a hijacked version of the same system responsible for the development of most behavioral "habits". This compulsive response reflects persistence in behavior even after the goal of the behavior has been devalued. In other words, the goal no longer drives the behavior (Robbins and Everitt 1999).

The transition from goal-directed to stimulus-directed behavior is also seen in fear conditioning. A particularly high risk group of individuals are those who display trait-anxiety (Sehlmeyer, Dannlowski et al. 2011). Once a fear response has been conditioned to a particular stimulus, these individuals display a resistance to the extinction of the fear response even after repeated exposure to the cue in the absence of real danger. Some research has indicated that this may be due to a reduced control by the prefrontal cortex, and a lasting transition to networks that regulate habit behavior (Comeau, Stewart et al. 2001; Bishop 2008). Taken together, a propensity to develop compulsive behaviors and a resistance to extinction of the stimulus-response behavior may be indicative of a vulnerability to the development of addictive behavioral patterns.

1.2 Transition from Positive Reinforcement to Negative Reinforcement

Motivational properties of drug seeking can also shift over time. Just as there is a transition from goal-directed to stimulus-directed behavior, there may also be a

transition of the motivational properties of drug seeking from positive reinforcement to negative reinforcement (Koob 2004). As mentioned previously, for certain drugs the initial motivating factor is the anticipation of reward (positive reinforcement). However, as drug use continues, the motivating factor shifts to preventing unpleasant symptoms associated with withdrawal (negative reinforcement). Periods of abstinence that extend beyond acute withdrawal can result in an increase in anxiety behaviors (Koob 2008). The relief of this growing anxiety by administration of the drug becomes the primary factor in drug seeking, rather than the "high" that one receives. This marks the transition from impulsive to more compulsive behavior, and is characterized as the stage in which an "addiction" has been solidly acquired. This particular form of negative reinforcement is driven by the relief of anxiety, or growing internal stress, by the drug of abuse. As such, certain individuals who have an altered reactivity to stress display a stronger response during this phase of addiction. Further, individuals who display a higher sensitivity to anxiety also display a more pronounced vulnerability to the negatively reinforcing properties of drugs of abuse (Koob and Le Moal 2008).

1.3 Additional Trait Vulnerabilities to the Development of Substance Use Disorders

A number of risk factors can contribute to the development of both anxiety disorders and substance use disorders. The presence of behavioral inhibition (a syndrome marked by social avoidance, withdrawal, and general fear of the unknown) in early life is a risk factor for the subsequent development of a number of anxiety disorders, particularly avoidant anxiety disorder (Turner, Beidel Patricia et al. 1996; Biederman, Hirshfeld-Becker et al. 2001; Morgan 2006). An increased prevalence of

behavioral inhibition (BI) is also associated with a behavioral pattern that is hypervigilant, inflexible, and persistent (McDermott, Perez-Edgar et al. 2009). Additionally, recent reports suggest that those with BI are also vulnerable to the development of a comorbid substance use disorder, however more studies are necessary to fully elucidate the nature of this relationship (Eagle, Lehmann et al. 2009; Fox, Shelton et al. 2008).

1.4 The Wistar-Kyoto Rat: A Model of Trait Anxiety

The behaviorally inhibited Wistar-Kyoto rat (WKY) has been recently presented as a vulnerability model for the expression of stress-induced avoidant behavior (Servatius, Jiao et al. 2008). The WKY strain displays a pronounced BI in a number of tasks including low locomotor activity (ambulation scores) in an open field arena and a

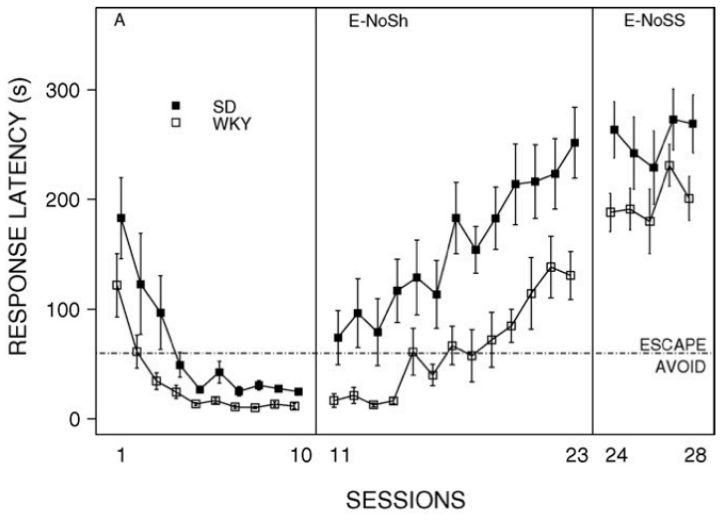

Figure 1.1 WKY rats in an escape/avoidance paradigm.
Shown is the response latency between SD and WKY rats across acquisition (A), Extinction - No Shock (E-NoSh), and Extinction - No Stimulus No Shock (E-NoSS) in an escape/avoidance paradigm. Servatius, R. J., Jiao, X., Beck, K. D., Pang, K. C. H., & Minor, T. R. (2008). Rapid avoidance acquisition in Wistar-Kyoto rats. *Behavioural Brain Research, 192*(2), 191-197.

decreased latency to immobility in a forced swim test (Paré 1994).

Of particular interest is the degree to which active avoidance is prevalent in their behavioral phenotype. In an escape/avoidance paradigm the animal is trained to press a lever to avoid an impending shock or escape the shock once it has started. WKY rats learn to press the lever much more quickly than the Sprague Dawley (SD) control group. Moreover, these rats exhibit a resistance to extinction (where the cue indicating impending shock is displayed but no shock occurs) than SD rats and will persistently continue to press the lever in the absence of a shock (Servatius, Jiao et al. 2008). The compulsivity with which the WKY rats respond suggests that the behavior quickly becomes stimulus-response driven and reflects a striatal-dependent mechanism (Everitt and Robbins 2005). Based on these and other findings, a theory of hypervigilance has been developed to explain the behavior (McAuley, Stewart et al. 2009). To support this theory, additional behavioral tests were performed. WKY rats showed increased scores in a pre-pulse inhibition test (a greater startle response) compared to SD rats. This strain also displayed an enhanced acoustic startle response when no pre-pulse cue was present (McAuley, Stewart et al. 2009). We believe that the observed anxiety behaviors will also modulate the relationship that they have with drugs of abuse.

A small amount of previous research has examined the role of drugs of abuse in the WKY rat. WKY rats consumed significantly greater amounts of alcohol at baseline, and in response to stress (Yaroslavsky and Tejani-Butt 2010; Paré, Paré et al. 1999). Further investigation is needed to determine the exact nature of the vulnerability. Given the compulsive responding seen in the active escape/avoidance paradigm and the few

studies that have been conducted that suggest a susceptibility to excessive consumption of drugs of abuse, we believe that the WKY strain is an excellent model for the investigation of anxiety vulnerability in the context of substance abuse.

1.5 Preliminary Data

Previous unpublished findings from our laboratory using a conditioned place preference paradigm (CPP) have demonstrated a moderately stable acquisition of drug preference across multiple doses between WKY and SD strains. The only exception was a moderate aversion to cocaine at the 15mg/kg dose by the WKY rats (not directly shown). Given the difficulty of WKY rats in extinguishing stimulus-response behaviors, our hypothesis is that WKY rats will also demonstrate a greater resistance to extinguishing CPP for cocaine. In other words, the WKY rats should continue to seek drug for a longer amount of time than their SD counterparts, in the absence of drug. We would also expect, due to the anxiety-like behaviors that are exhibited by this strain, to see a significant response to a priming dose of drug following a protracted period of abstinence (a greater reinstatement to drug seeking as measured by CPP.

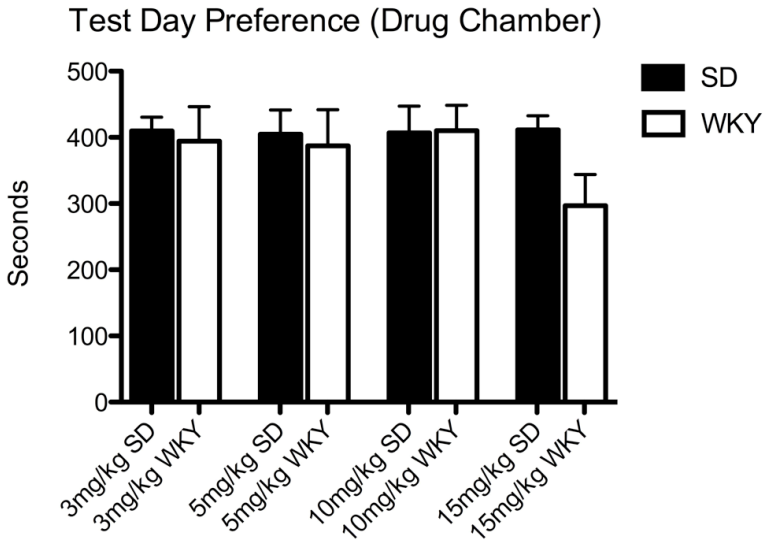

Figure 1.2 Drug-paired chamber for preliminary cocaine CPP.
Conditioned place preference data for the drug-paired box on acquisition test day. Four doses (3, 5, 10, & 15mg/kg) of cocaine hydrochloride are shown comparing SD rats against WKY rats.

Additional biochemical evidence for this hypothesis comes from brain tissue that was collected from the escape/avoidance paradigm outlined previously. Rats were sacrificed immediately after the acquisition phase, before the extinction phase (see Figure 1.1). Western blotting was performed and the samples were probed for the presence of phosphorylated and total extracellular signal-related kinase (ERK). ERK activation on the most basic level indicates the recent activation and subsequent stable sensitization of a neuron (Lu, Koya et al. 2006). A ratio of pERK/ERK was formed to measure the degree of ERK activation resulting from the acquisition of compulsive responding in escape/avoidance acquisition (Figure 3). Results were stable for most of the brain regions between strains with the important exception of the dorsal striatum. Expression

was significantly higher in the WKY rats that received the escape/avoidance training (WKY/t) when compared to both the control of the same strain (WKY) as well as the trained groups of the SD rats (SD/t). This suggests that more robust transition to stimulus-directed behavior (regulated by the dorsal striatum) has occurred in the WKY

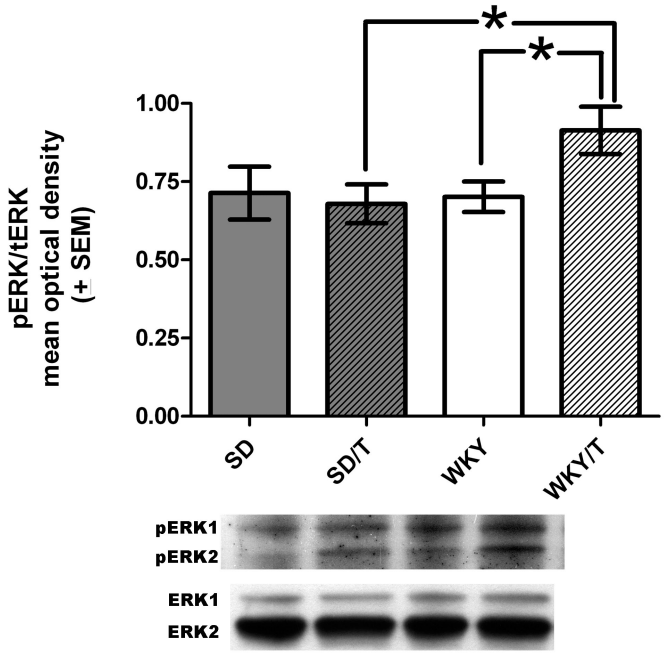

Figure 1.3 Preliminary Western blots for ERK.
Western blot mean optical density values (pERK1+pERK2)/(ERK1+ERK2) shown for the Dorsal Striatum. Control groups are indicated by SD (Sprague Dawley) and WKY (Wistar Kyoto). Experimental groups are indicated by SD/t (Sprague Dawley trained) and WKY/t (Wistar Kyoto trained). An asterisk (*) indicates an significance level of $p < 0.05$.

rats than the SD control rats. This also provides an explanation of the perseveration of responding that is observed by these rats in the escape/avoidance paradigm.

The importance of ERK expression in the addiction process has been well established. Activation of ERK in the striatum is necessary for the establishment of

psychostimulant-induced CPP (Mizoguchi, Yamada et al. 2004). Introducing ERK inhibitors prevents a conditioned response to a drug, and can also abolish previously learned drug associations if in the system when the subjects are once again exposed to the drug cues (Valjent, Corbillé et al. 2006).

In summary, the WKY strain displays a pronounced BI, rapid acquisition of avoidance responding that is resistant to extinction, increased alcohol consumption, and evidence of a dysregulation of striatal-dependent behavior (as confirmed by the western blots). Taken together, these results suggest that the WKY rat is an ideal model for investigating the development of substance use disorders in an anxiety-prone strain.

1.6 Hypotheses

1.6.1 Hypothesis 1

WKY rats will demonstrate a greater resistance to extinction of drug-induced conditioned place preference than the SD control strain.

1.6.2 Hypothesis 2

Protracted abstinence between the acquisition and reinstatement tests in a conditioned place preference paradigm will lead to a greater reacquisition/reinstatement response by WKY rats compared to SD rats.

CHAPTER 2

METHODS

2.1 Drugs

2.1.1 Cocaine

Cocaine Hydrochloride (Sigma-Aldrich, US) was dissolved in 0.9% (wt/vol) saline. Drug was injected at a volume of 1ml/kg.

2.1.2 Morphine

Morphine sulfate (Sigma-Aldrich, US) was dissolved in 0.9% (wt/vol) saline. Drug was injected at a volume of 1ml/kg.

2.2 Animals

Male SD and WKY rats were purchased from Harlan (Houston, TX) at the age of 10 weeks and were housed three to a cage. They were allowed to habituate to their new environment for two weeks, and were handled for one week before any testing took place. Food and water were supplied ad libitum. Rats in the cocaine group were kept on a 12-hour light/dark cycle with the dark cycle starting at 7:00am. Rats in the morphine group were kept on a 12-hour light/dark cycle with the dark cycle starting at 11:00am. All rats were left undisturbed for at least two hours after the beginning of the dark cycle each day, and all experiments were conducted during the dark cycle (the active period for rats).

2.3 Open Field Testing

2.3.1 Open Field Apparatus

The open field arena is a steel tub, 100cm across, and 45cm high. The entire surface is covered in a non-reflective black adhesive. Movements are automatically tracked with a camera above the arena, connected to a computer running Noldus software.

2.3.2 Open Field Paradigm

All rats underwent open field testing prior to the onset of the conditioned place preference paradigm. Rats were placed in the center of the open field arena and allowed to freely explore the arena for 10 minutes. Distance traveled (cm) and velocity (cm/s) was recorded.

2.4 Conditioned Place Preference

The methods outlined below describe three different sets of experiments. The premise of the experiments are very similar, with the only varying properties being the drug administered, the number of conditioning days, the number of extinction days, and the amount of time in each trial (depending on the drug administered). For each strain between all three paradigms n=9 (a total of 54).

2.4.1 Conditioned Place Preference Apparatus

Conditioned place preference (CPP) boxes were purchased from Med Associates Inc. The boxes consist of three distinct chambers. A small neutral grey chamber (4.75” L x 8.25” W x 8.25” H) is separated by two outer choice chambers (26.75” L x 8.25” W x 8.24” H) by manually operated guillotine doors. One outer choice chamber is white

and contains a wire mesh grid floor. The opposing outer choice chamber is black and contains a stainless steel rod floor. Each outer chamber contains six photo beams and the middle neutral chamber contains three. Behavior is determined by breaks in photo beams and recorded by a connected computer loaded with Med PC software.

2.4.2 Cocaine Conditioned Place Preference with Four Cocaine Pairings

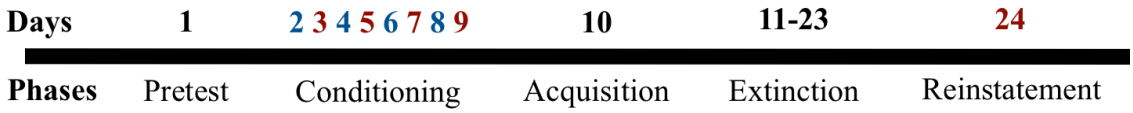

Figure 2.1 Timeline of four pairing cocaine CPP
Timeline depicting the different phases of the conditioned place preference paradigm. Blue numbers indicate days in which saline was administered, red numbers indicate days in which cocaine was administered, and black numbers indicate days in which no injection was given.

Pretest (Day 1): Rats were placed in the center neutral chamber for five minutes to allow for habituation to the CPP apparatus. After five minutes elapsed, the two guillotine doors separating the center chamber from the two outer chambers were opened, allowing the rat free access to the entire apparatus for an additional 15 minutes. Following the test, individual rats were assigned to a particular outer choice chamber for cocaine conditioning, and the opposing outer choice chamber for saline conditioning.

Conditioning (Days 2-9): On even days (2, 4, 6, 8), rats were administered 1ml/kg 0.9% saline via IP injection and confined to the saline-paired chamber for 30 minutes. On the odd days (3, 5, 7, 9), rats were administered cocaine (10mg/kg) via IP injection and confined to the drug-paired chamber for 30 minutes.

Acquisition Test (Day 10): The procedure for the acquisition test was identical to the pretest. Rats were placed in the center neutral chamber and allowed to habituate for five minutes, after which the doors to each outer chamber were opened. The rats were then allowed free access to the entire apparatus for an additional 15 minutes. This is meant to test whether a preference for the drug has been "acquired".

Extinction (Days 11-23): The extinction phase consisted of repeated acquisition tests for 13 consecutive days.

Reinstatement Test (Day 24): Twenty-four hours after the last extinction day each animal went through a reinstatement test. Rats received a priming injection of cocaine (5mg/kg) and were placed in the middle neutral chamber for five minutes, after which the two doors were opened allowing the rats once again 15 minutes of free access to the entire apparatus.

2.4.3 Cocaine Conditioned Place Preference with Six Cocaine Pairings

Figure 2.2 Timeline of six pairing cocaine CPP
Timeline depicting the different phases of the conditioned place preference paradigm. Blue numbers indicate days in which saline was administered, red numbers indicate days in which cocaine was administered, and black numbers indicate days in which no injection was given.

Pretest (Day 1): The same procedure for pretest as above is used.

Conditioning (Days 2-13): On even days (2, 4, 6, 8, 10, 12), rats were administered 1ml/kg 0.9% saline via IP injection and confined to the saline-paired chamber for 30 minutes. On the odd days (3, 5, 7, 9, 11, 13), rats were administered cocaine (10mg/kg) via IP injection and confined to the drug-paired chamber for 30 minutes.

Acquisition Test (Day 14): Same as acquisition test for four parings.

Extinction (Days 15-34): The extinction phase consisted of repeated acquisition tests for 20 consecutive days.

Reinstatement Test (Day 35): Same as above reinstatement test for four parings.

2.4.4 Morphine Conditioned Place Preference with Three Morphine Pairings

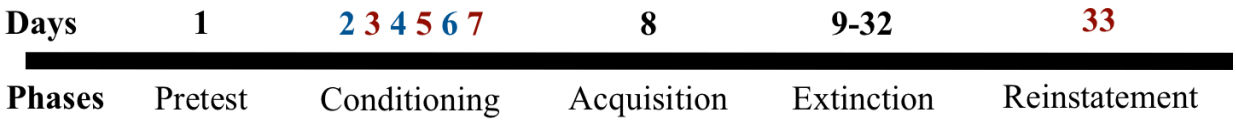

Figure 2.3 Timeline of morphine CPP
Timeline depicting the different phases of the conditioned place preference paradigm. Blue numbers indicate days in which saline was administered, red numbers indicate days in which morphine was administered, and black numbers indicate days in which no injection was given.

Pretest (Day 1): Rats were placed in the center neutral chamber for five minutes to allow for habituation to the CPP apparatus. After five minutes elapsed, the two guillotine doors separating the center chamber from the two outer chambers were opened, allowing the rat free access to the entire apparatus for an additional 30 minutes. Following the test, individual rats were assigned to a particular outer choice chamber

for morphine conditioning, and the opposing outer choice chamber for saline conditioning.

Conditioning (Days 2-7): On even days (2, 4, 6), rats were administered 1ml/kg 0.9% saline via subcutaneous injection and confined to the saline-paired chamber for 60 minutes. On the odd days (3, 5, 7), rats were administered Morphine (10mg/kg) via subcutaneous injection and confined to the drug-paired chamber for 60 minutes.

Acquisition Test (Day 8): The procedure for the acquisition test was identical to the pretest. Rats were placed in the center neutral chamber and allowed to habituate for five minutes, after which the doors to each outer chamber were opened. The rats were then allowed free access to the entire apparatus for an additional 30 minutes.

Extinction (Days 9-32): The extinction phase consisted of repeated acquisition tests for 24 consecutive days.

Reinstatement Test (Day 33): Twenty-four hours after the last extinction day each animal went through a reinstatement test. Rats received a priming injection of morphine (5mg/kg) and were placed in the middle neutral chamber for five minutes, after which the two doors were opened allowing the rats 30 minutes of free access to the entire apparatus.

2.5 Tissue Collection

Immediately after behavior was completed, rats were injected with 400 mg/kg of chloral hydrate for euthanasia. The rats were then intracardially perfused at a rate of

20ml/minute with PBS for 7-10 minutes, and then with 4% paraformaldehyde in PBS for 15 minutes. Brains were extracted and stored in 4% paraformaldehyde in PBS at 4C for 24 hours and then transferred to 20% glycerol in PBS at 4C. Brains will be stored indefinitely as part of a tissue bank for future analysis.

CHAPTER 3

RESULTS

3.1 Cocaine Conditioned Place Preference with Four Cocaine Pairings

3.1.1 Open Field

A t-test was performed to determine differences between WKY rats and SD rats for both distance traveled, and velocity of movement in the open field arena prior to CPP testing. WKY rats (Mean=4234, SEM=188.6, n=9) traveled a significantly shorter distance [$t(16) = 6.943$, $p<.001$] than SD rats (Mean=7547, SEM=438.4, n=9). WKY rats (Mean=8.12, SEM=0.34, n=9) also traveled at a significantly lower velocity [$t(16) = 7.165$, $p<.001$] than SD rats (Mean=13.88, SEM=0.73, n=9), confirming their behavioral inhibition.

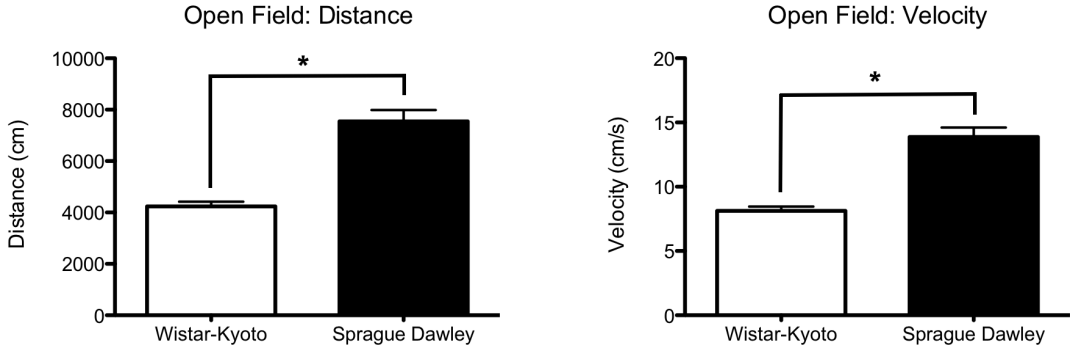

Figure 3.1 Open field for four pairing cocaine group

Open field measures were taken before the onset of the conditioned place preference paradigm to confirm behavioral inhibition by the WKY rats. Distance traveled and velocity of movement were taken as measures. * indicates a $p<.001$.

3.1.2 Conditioned Place Preference

A 2(Strain) x 3(Chamber) x 16(Day) mixed ANOVA was used to analyze the amount of time rats spent in each chamber of the CPP apparatus. A main effect was observed for both day [$F(2, 15) = 14.76$, $p<.01$] and chamber [$F(2, 32) =7.64$, $p<.01$]. A two-way interaction for day x strain was not observed [$F(2,15) = 0.47$, p=ns]. However, a two-way interaction for chamber x strain [$F(2, 15) = 6.10$, $p<.05$] and day x chamber [$F(30, 480) = 3.314$, $p<.01$] was observed. Finally, a three-way interaction for day x chamber x strain [$F(30, 480) = 3.193$, $p<.01$] was observed.

Planned comparisons with a Sidak correction for each strain, at every time point between the drug-paired chamber and saline-paired chamber were made. At acquisition ($p<.05$, $SE=56.80$) and extinction D1 ($p<.05$, $SE=57.26$), the SD rats spent significantly more time in the cocaine-paired chamber than the saline-paired chamber. No significant differences between the two outer choice chambers were observed for any of the additional extinction days, or for the reinstatement test.

The WKY rats displayed no significant difference at acquisition, and showed a significant aversion of the cocaine-paired chamber at extinction D2 ($p<.05$, SE=53.55) and extinction D12 ($p<.05$, SE=82.25). However, this aversion was reversed during reinstatement, with a significant preference for the drug-paired chamber over the saline-paired chamber ($p<.05$, $SE=88.46$).

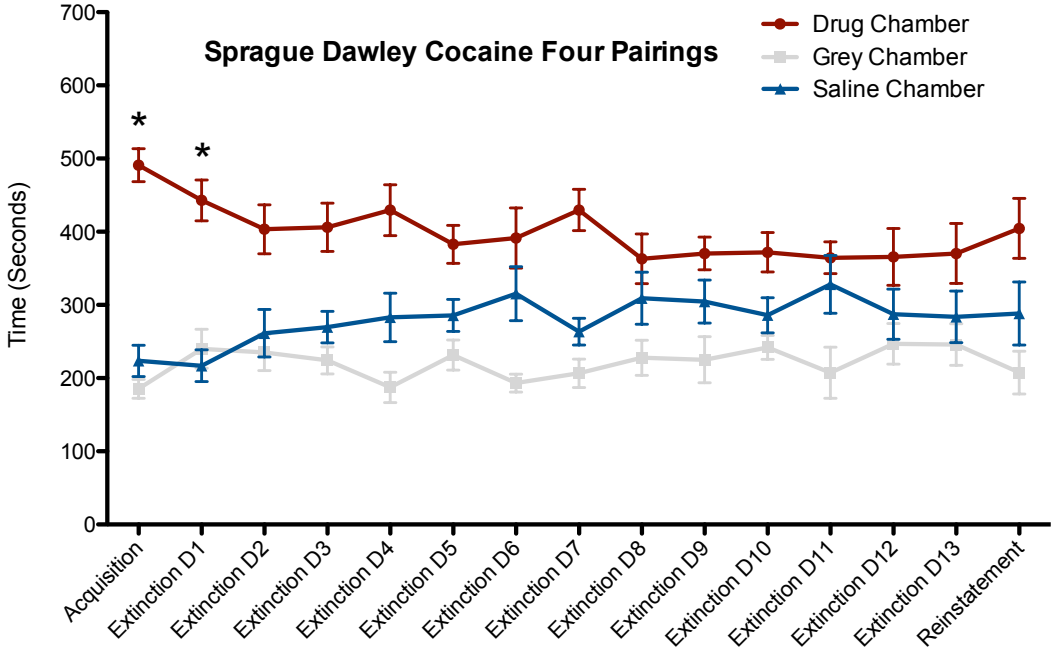

Figure 3.2 Sprague Dawley time scores for cocaine four pairings
The amount of time spent in each box on each of the test days is plotted for SD rats from the conditioned place preference paradigm. * indicates a significant difference ($p<.05$) between the drug-paired and saline-paired boxes. n=9 for every chamber across all days.

Table 3.1 Sprague Dawley time score mean and SEM for cocaine four pairings

	Drug-Paired Chamber Mean	Drug-Paired Chamber SEM	Neutral Chamber Mean	Neutral Chamber SEM	Saline-Paired Chamber Mean	Saline-Paired Chamber SEM
Acquisition	491.024	± 27.846	185.377	± 15.293	223.599	± 30.893
Extinction D1	442.872	± 35.352	240.116	± 27.634	217.012	± 27.777
Extinction D2	403.402	± 26.061	235.201	± 33.625	261.397	± 36.330
Extinction D3	406.046	± 32.533	224.261	± 20.822	269.693	± 32.954
Extinction D4	429.578	± 34.958	187.418	± 23.354	283.004	± 40.765
Extinction D5	382.812	± 32.103	231.517	± 22.814	285.671	± 39.035
Extinction D6	391.407	± 34.366	193.252	± 23.190	315.341	± 34.997
Extinction D7	429.777	± 29.324	206.590	± 33.317	263.633	± 40.762
Extinction D8	362.968	± 38.827	227.791	± 28.207	309.241	± 40.682
Extinction D9	370.390	± 43.953	225.061	± 32.611	304.549	± 36.148
Extinction D10	372.014	± 38.114	241.998	± 26.006	285.988	± 37.095
Extinction D11	364.458	± 28.095	207.410	± 37.534	328.132	± 35.515
Extinction D12	365.583	± 38.643	247.011	± 40.366	287.406	± 51.999
Extinction D13	370.376	± 38.165	245.903	± 31.507	283.721	± 34.748
Reinstatement	404.614	± 50.528	207.654	± 28.338	288.286	± 42.018

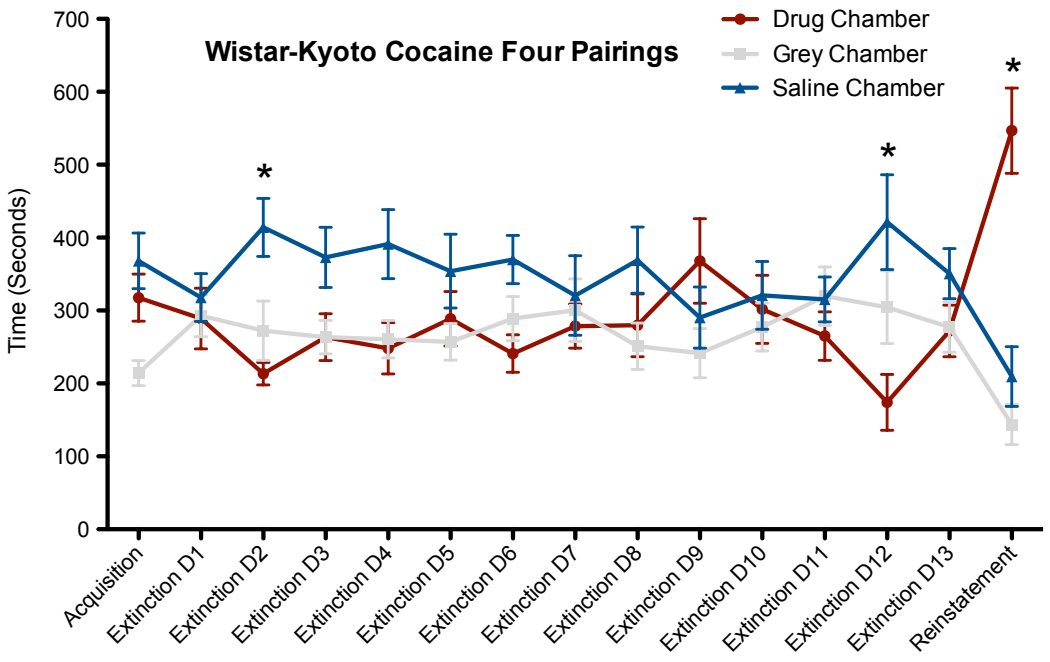

Figure 3.3 Wistar-Kyoto time scores for cocaine four pairings

The amount of time spent in each box on each of the test days is plotted for WKY rats from the conditioned place preference paradigm. * indicates a significant difference ($p<.05$) between the drug-paired and saline-paired boxes. n=9 for every chamber across all days.

Table 3.2 Wistar-Kyoto time score mean and SEM for cocaine four pairings

	Drug-Paired Chamber Mean	Drug-Paired Chamber SEM	Neutral Chamber Mean	Neutral Chamber SEM	Saline-Paired Chamber Mean	Saline-Paired Chamber SEM
Acquisition	317.672	± 27.846	214.229	± 15.293	368.099	± 30.893
Extinction D1	289.119	± 35.352	292.951	± 27.634	317.930	± 27.777
Extinction D2	213.540	± 26.061	272.377	± 33.625	414.083	± 36.330
Extinction D3	263.426	± 32.533	263.631	± 20.822	372.943	± 32.954
Extinction D4	248.228	± 34.958	260.623	± 23.354	391.149	± 40.765
Extinction D5	288.781	± 32.103	257.129	± 22.814	354.090	± 39.035
Extinction D6	241.056	± 34.366	288.932	± 23.190	370.012	± 34.997
Extinction D7	278.694	± 29.324	300.639	± 33.317	320.667	± 40.762
Extinction D8	279.819	± 38.827	251.091	± 28.207	369.090	± 40.682
Extinction D9	367.941	± 43.953	241.609	± 32.611	290.450	± 36.148
Extinction D10	301.722	± 38.114	277.437	± 26.006	320.841	± 37.095
Extinction D11	265.024	± 28.095	319.771	± 37.534	315.204	± 35.515
Extinction D12	174.047	± 38.643	304.614	± 40.366	421.339	± 51.999
Extinction D13	272.060	± 38.165	277.186	± 31.507	350.754	± 34.748
Reinstatement	546.813	± 50.528	143.660	± 28.338	209.527	± 42.018

3.2 Cocaine Conditioned Place Preference with Six Cocaine Pairings

3.2.1 Open Field

A t-test was performed to determine differences between WKY rats and SD rats for both distance traveled, and velocity of movement in the open field arena prior to conditioned place preference testing. WKY rats (Mean=3867, SEM=214.4, n=9) traveled a significantly shorter distance [$t(16) = 8.056$, $p<.001$] than the SD rats (Mean=6331, SEM=218.1, n=9). WKY rats (Mean=7.07, SEM=0.39, n=9) also traveled at a significantly lower velocity [$t(16) = 8.158$, $p<.001$] than SD rats (Mean=11.50, SEM=0.38, n=9), confirming their behavioral inhibition.

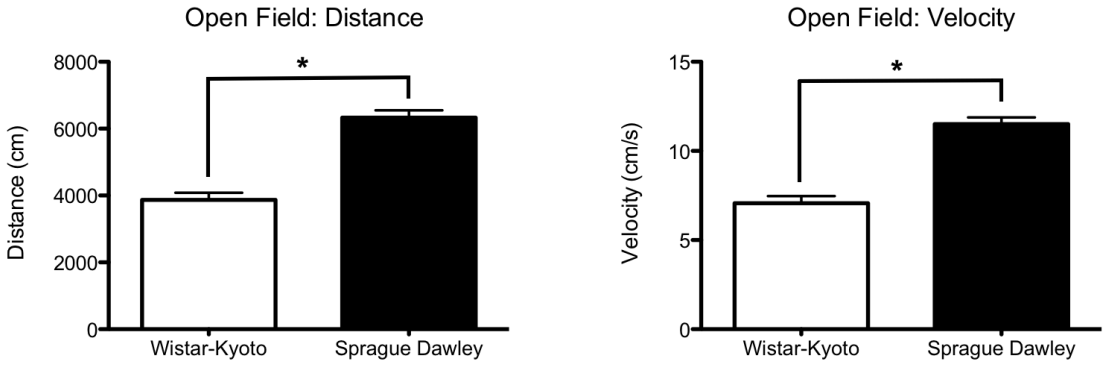

Figure 3.4 Open field for six pairing cocaine group
Open field measures were taken before the onset of the conditioned place preference paradigm to confirm behavioral inhibition by the WKY rats. Distance traveled and velocity of movement were taken as measures. * indicates a $p<.001$.

3.2.2 Conditioned Place Preference

A 2(Strain) x 3(Chamber) x 22(Day) mixed ANOVA was used to analyze the amount of time rats spent in each chamber of the CPP apparatus. A main effect for day

[F(22, 352) = 277.437, p<.001] and for chamber [F(2,32) = 4.676, p<.05] was found. Two-way interactions for chamber x strain [F(2,32) = 9.794, p<.01] and day x chamber [F(44, 704) = 1.451, p<.05] were found. No three-way interaction was observed.

Planned comparisons with a Sidak correction for each strain, at every time point between the drug-paired chamber and saline-paired chamber were made. SD rats spent significantly more time in the drug paired chamber than the saline-paired chamber during acquisition test (p<.05, SE=57.89), extinction D2 (p<.05, SE=60.93), and reinstatement (p<.05, SE=64.80). No other significant differences between the two outer choice chambers were observed.

WKY rats displayed no significant preference for any of the outer choice chambers at acquistion or any of the extinction days, with with exception of a preference for the drug-paired chamber on extinction D10 (p<.05, SE=51.825). However, as with the WKY group that received four cocaine pairings, a significant preference for the cocaine-paired chamber at reinstatement is observed (p<.05, SE=46.641).

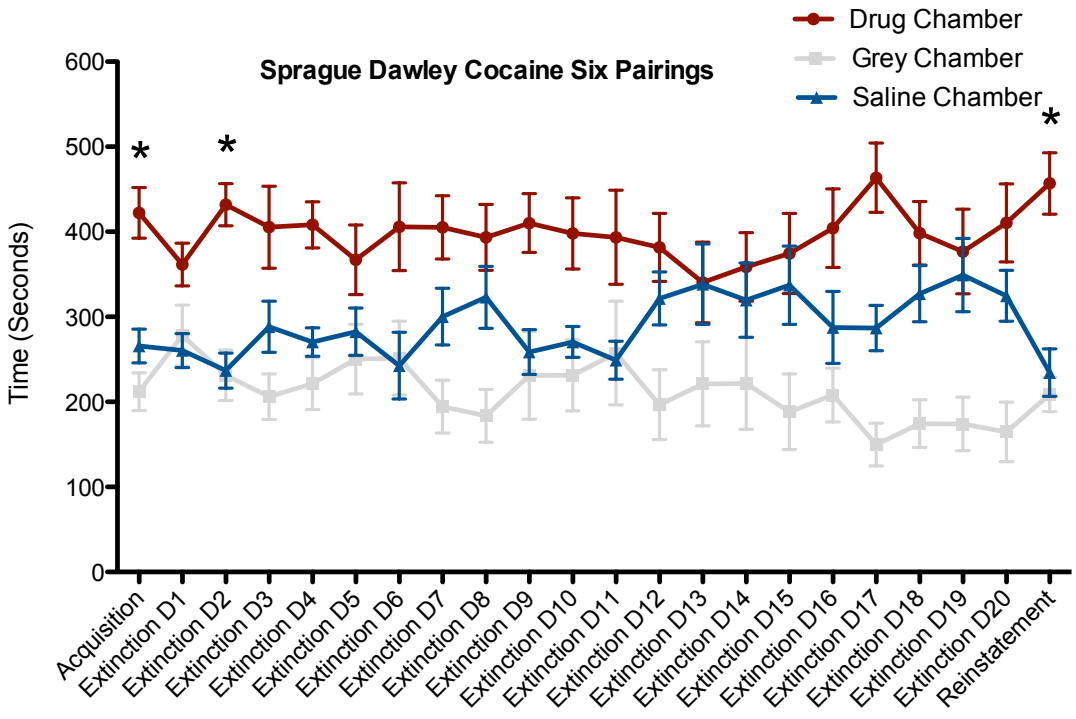

Figure 3.5 Sprague Dawley time scores for cocaine six pairings

The amount of time spent in each box on each of the test days is plotted for SD rats from the conditioned place preference paradigm. * indicates a significant difference ($p<.05$) between the drug-paired and saline-paired boxes. n=9 for every chamber across all days.

Figure 3.3 Sprague Dawley time score mean and SEM for cocaine six pairings

	Drug-Paired Chamber Mean	Drug-Paired Chamber SEM	Neutral Chamber Mean	Neutral Chamber SEM	Saline-Paired Chamber Mean	Saline-Paired Chamber SEM
Acquisition	422.330	± 35.507	212.009	± 27.154	265.661	± 27.988
Extinction D1	361.543	± 36.837	278.121	± 29.986	260.336	± 29.549
Extinction D2	431.931	± 43.495	231.253	± 36.660	236.816	± 25.225
Extinction D3	405.413	± 44.817	206.169	± 33.998	288.418	± 34.749
Extinction D4	408.171	± 38.258	221.402	± 33.701	270.427	± 30.675
Extinction D5	367.150	± 39.224	250.310	± 40.521	282.540	± 33.391
Extinction D6	405.910	± 41.188	251.511	± 45.701	242.579	± 36.880
Extinction D7	405.286	± 35.559	194.411	± 36.569	300.303	± 31.103
Extinction D8	393.431	± 40.609	183.626	± 29.127	322.943	± 32.808
Extinction D9	410.237	± 49.888	231.150	± 51.551	258.613	± 35.205
Extinction D10	398.114	± 46.561	231.459	± 48.924	270.427	± 19.283
Extinction D11	393.542	± 60.649	257.474	± 57.587	248.983	± 25.968
Extinction D12	381.717	± 55.768	196.727	± 51.335	321.557	± 35.132
Extinction D13	340.449	± 53.673	221.194	± 56.068	338.357	± 41.839
Extinction D14	358.626	± 47.916	221.659	± 64.513	319.716	± 34.362
Extinction D15	374.417	± 50.654	188.376	± 46.422	337.208	± 43.674
Extinction D16	404.232	± 50.111	208.224	± 46.276	287.543	± 42.643
Extinction D17	463.511	± 46.098	149.790	± 40.132	286.699	± 40.234
Extinction D18	398.341	± 41.204	174.451	± 44.528	327.208	± 44.031
Extinction D19	376.827	± 59.222	174.088	± 56.384	349.086	± 42.648
Extinction D20	410.498	± 51.253	164.738	± 45.646	324.764	± 32.711
Reinstatement	456.821	± 37.069	208.740	± 21.293	234.439	± 30.854

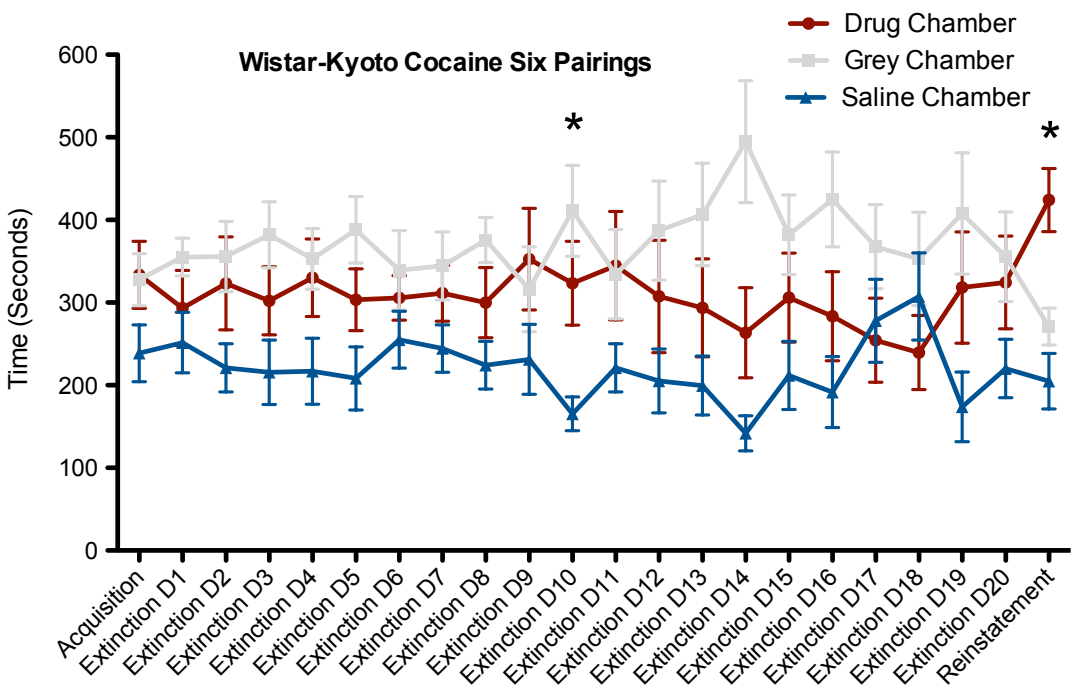

Figure 3.6 Wistar-Kyoto time scores for cocaine six pairings

The amount of time spent in each box on each of the test days is plotted for WKY rats from the conditioned place preference paradigm. * indicates a significant difference ($p<.05$) between the drug-paired and saline-paired boxes. n=9 for every chamber across all days.

Table 3.4 Wistar Kyoto time score mean and SEM for cocaine six pairings

	Drug-Paired Chamber Mean	Drug-Paired Chamber SEM	Neutral Chamber Mean	Neutral Chamber SEM	Saline-Paired Chamber Mean	Saline-Paired Chamber SEM
Acquisition	333.590	± 35.507	327.738	± 27.154	238.672	± 27.988
Extinction D1	293.342	± 36.837	355.067	± 29.986	251.591	± 29.549
Extinction D2	323.204	± 43.495	355.850	± 36.660	220.946	± 25.225
Extinction D3	302.240	± 44.817	382.088	± 33.998	215.672	± 34.749
Extinction D4	330.084	± 38.258	352.900	± 33.701	217.016	± 30.675
Extinction D5	303.588	± 39.224	388.136	± 40.521	208.277	± 33.391
Extinction D6	305.667	± 41.188	339.224	± 45.701	255.109	± 36.880
Extinction D7	311.256	± 35.559	344.364	± 36.569	244.380	± 31.103
Extinction D8	300.024	± 40.609	375.748	± 29.127	224.228	± 32.808
Extinction D9	352.606	± 49.888	316.132	± 51.551	231.262	± 35.205
Extinction D10	323.510	± 46.561	411.122	± 48.924	165.368	± 19.283
Extinction D11	344.699	± 60.649	334.270	± 57.587	221.031	± 25.968
Extinction D12	307.583	± 55.768	387.302	± 51.335	205.114	± 35.132
Extinction D13	293.731	± 53.673	406.594	± 56.068	199.674	± 41.839
Extinction D14	263.436	± 47.916	494.784	± 64.513	141.780	± 34.362
Extinction D15	306.069	± 50.654	382.067	± 46.422	211.864	± 43.674
Extinction D16	283.507	± 50.111	424.807	± 46.276	191.687	± 42.643
Extinction D17	254.417	± 46.098	367.546	± 40.132	278.038	± 40.234
Extinction D18	239.597	± 41.204	352.980	± 44.528	307.423	± 44.031
Extinction D19	318.247	± 59.222	407.900	± 56.384	173.853	± 42.648
Extinction D20	324.404	± 51.253	355.420	± 45.646	220.176	± 32.711
Reinstatement	424.171	± 37.069	270.969	± 21.293	204.860	± 30.854

3.3 Morphine Conditioned Place Preference with Three Morphine Pairings

3.3.1 Open Field

A t-test was performed to determine differences between WKY rats and SD rats for both distance traveled, and velocity of movement in the open field arena prior to conditioned place preference testing. WKY rats (Mean=3899, SEM=137.2, n=9) traveled a significantly shorter distance [$t(16) = 9.979$, $p<.001$] than the SD rats (Mean=6235, SEM=189.7, n=9).

WKY rats (Mean=7.15, SEM=0.24, n=9) also traveled at a significantly lower velocity [$t(16) = 8.762$, $p<.001$] than SD rats (Mean=11.63, SEM=0.45, n=9), confirming their behavioral inhibition.

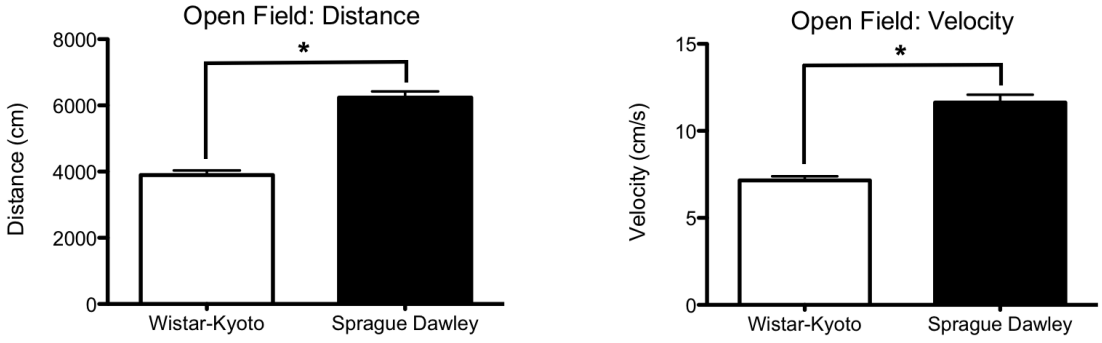

Figure 3.7 Open field for morphine group
Open field measures were taken before the onset of the conditioned place preference paradigm to confirm behavioral inhibition by the WKY rats. Distance traveled and velocity of movement were taken as measures. * indicates a $p<.001$.

3.3.2 Conditioned Place Preference

A 2(Strain) x 3(Chamber) x 26(Day) mixed ANOVA was used to analyze the amount of time that the rats spent in each chamber of the CPP apparatus. A main effect for chamber was observed [$F(2, 32) = 4.178$, $p<.05$]. A two-way interaction was observed for both chamber x strain [$F(2, 32) = 10.253$, $p<.001$] and chamber x day [$F(52, 832) = 1.717$, $p<.01$]. The three-way interaction was only marginally significant [$F(52, 832) = 1.309$, $p=.076$].

Planned comparisons with a Sidak correction for multiple comparisons were performed. When comparing the two outer choice chambers, SD rats preferred the drug-paired chamber over the saline paired chamber at acquisition ($p<.05$, SE=171.147), extinction D2 ($p<.050$, SE=138.504), extinction D3 ($p<.01$, SE=139.314), extinction D4 ($p<.01$, SE=140.887), extinction D9 ($p<.05$, SE=132.282), extinction D14 ($p<.01$, SE=117.089), extinction D18 ($p<.01$, SE=87.403), extinction D19 ($p<.01$, SE=120.010), extinction D20 ($p<.05$, SE=145.363), extinction D22 ($p<.05$, SE=100.156), extinction D23 ($p<.01$, SE=106.866), and extinction D24 ($p<.01$, SE=108.389).

When comparing the two outer choice chambers for the WKY rats at each day, no significant results were found. However, when comparing the neutral chamber to either the drug-paired or saline-paired chamber significant differences were found. These rats spent significantly more time in the neutral chamber than the drug-paired chamber on extinction D10 ($p<.05$, SE=199.956), extinction D12 ($p<.05$, SE=171.634), extinction D13($p<.01$, SE=138.583), extinction D14 ($p<.05$, SE=126.253), extinction D15 ($p<.05$, SE=212.221), extinction D16 ($p<.05$, SE=215.353), extinction D18 ($p<.01$,

SE=167.954), extinction D19 ($p<.05$, SE=237.388), extinction D21 ($p<.01$, SE=126.858), extinction D22 ($p<.05$, SE=178.663), and extinction D23 ($p<.01$, SE=186.997). These rats spent significantly more time in the neutral chamber than the saline-paired chamber at extinction D1 ($p<.05$, SE=127.821), extinction D12 ($p<.05$, SE=130.470), extinction D13 ($p<.05$, SE=133.522), extinction D15 ($p<.01$, SE=167.107), extinction D16 ($p<.05$, SE=181.336), extinction D19 ($p<.05$, SE=178.008), and extinction D23 ($p<.01$, SE=165.424).

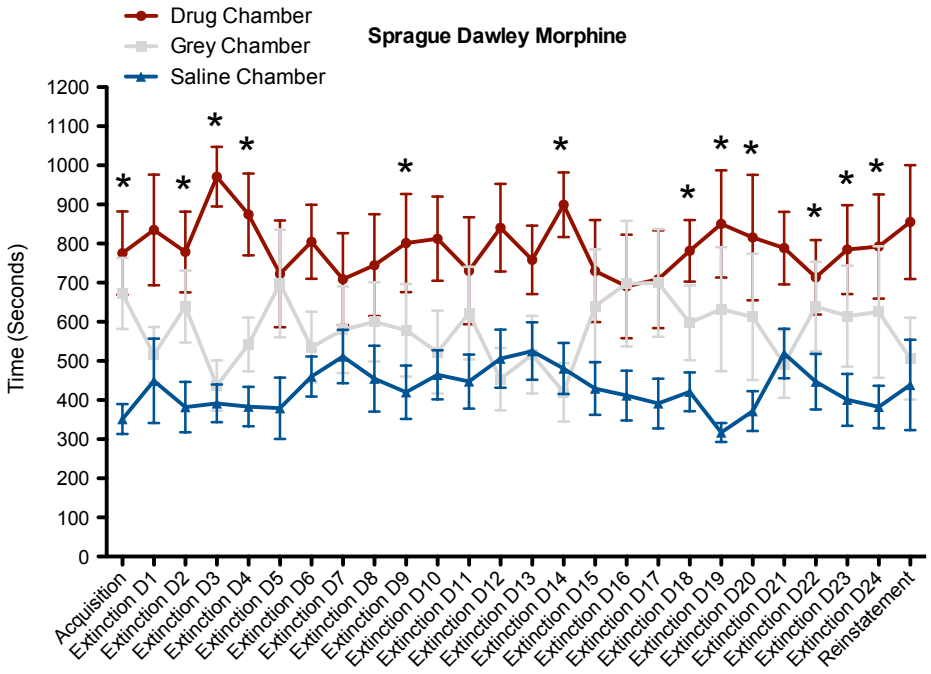

Figure 3.8 Sprague Dawley time scores for morphine pairings

The amount of time spent in each box on each of the test days is plotted for SD rats from the conditioned place preference paradigm. * indicates a significant difference ($p<.05$) between the drug-paired and saline-paired boxes. n=9 for every chamber across all days.

Table 3.5 Sprague Dawley time score mean and SEM for morphine

	Drug-Paired Chamber Mean	Drug-Paired Chamber SEM	Neutral Chamber Mean	Neutral Chamber SEM	Saline-Paired Chamber Mean	Saline-Paired Chamber SEM
Acquisition	775.780	± 94.327	672.644	± 90.423	351.576	± 69.691
Extinction D1	834.950	± 110.459	515.700	± 83.822	449.350	± 85.109
Extinction D2	778.621	± 95.864	639.270	± 84.497	382.109	± 63.015
Extinction D3	971.100	± 68.381	437.271	± 66.995	391.629	± 85.278
Extinction D4	874.504	± 85.925	542.158	± 89.156	383.338	± 80.721
Extinction D5	722.667	± 106.386	698.132	± 124.850	379.201	± 99.089
Extinction D6	804.484	± 74.634	535.280	± 94.155	460.236	± 87.201
Extinction D7	709.049	± 91.469	579.684	± 118.221	511.267	± 87.066
Extinction D8	744.954	± 102.181	600.139	± 131.011	454.907	± 112.903
Extinction D9	801.379	± 93.877	578.514	± 117.996	420.107	± 83.053
Extinction D10	812.569	± 84.516	522.557	± 135.633	464.874	± 105.493
Extinction D11	730.546	± 103.118	622.128	± 105.109	447.327	± 78.617
Extinction D12	840.644	± 97.112	453.540	± 89.113	505.816	± 72.701
Extinction D13	758.538	± 68.142	516.154	± 83.973	525.308	± 64.686
Extinction D14	899.339	± 62.592	419.821	± 85.796	480.840	± 81.349
Extinction D15	729.948	± 100.603	640.364	± 120.856	429.688	± 66.458
Extinction D16	690.598	± 98.294	697.823	± 126.920	411.579	± 71.859
Extinction D17	708.923	± 92.589	699.822	± 115.358	391.254	± 82.849
Extinction D18	781.444	± 73.609	597.622	± 102.546	420.933	± 60.492
Extinction D19	850.261	± 110.466	632.477	± 134.030	317.262	± 63.092
Extinction D20	815.602	± 115.521	612.487	± 125.065	371.911	± 70.997
Extinction D21	788.447	± 67.350	492.282	± 80.452	519.271	± 76.967
Extinction D22	713.926	± 78.761	639.112	± 111.608	446.962	± 70.996
Extinction D23	784.604	± 85.252	614.676	± 112.173	400.720	± 68.802
Extinction D24	792.452	± 109.520	625.120	± 137.537	382.428	± 57.773
Reinstatement	855.174	± 129.288	505.942	± 91.501	438.883	± 129.111

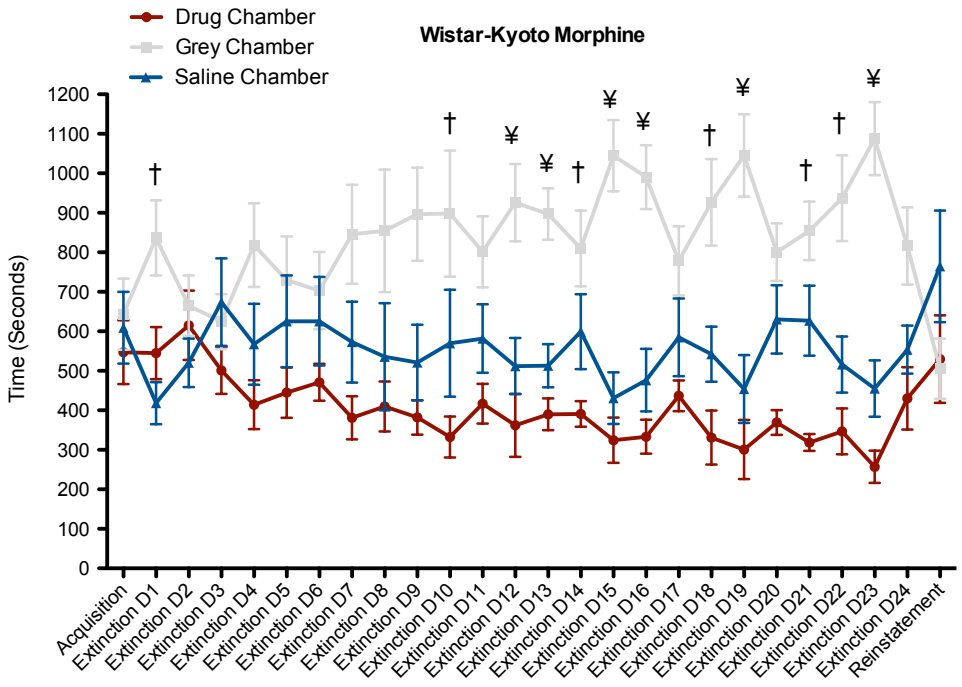

Figure 3.9 Wistar-Kyoto time scores for morphine

The amount of time spent in each chamber on each of the test days is plotted for WKY rats from the conditioned place preference paradigm. † indicates a significant difference between the grey chamber and one of the outer choice chambers ($p<.05$). ¥ indicates a significant difference between the grey chamber and both of the outer choice chambers ($p<.05$). n=9 for every chamber across all days.

Table 3.6 Wistar-Kyoto time score mean and SEM for morphine

	Drug-Paired Chamber Mean	Drug-Paired Chamber SEM	Neutral Chamber Mean	Neutral Chamber SEM	Saline-Paired Chamber Mean	Saline-Paired Chamber SEM
Acquisition	547.192	± 94.327	643.581	± 90.423	609.227	± 69.691
Extinction D1	545.051	± 110.459	836.576	± 83.822	418.373	± 85.109
Extinction D2	615.301	± 95.864	664.901	± 84.497	520.352	± 63.015
Extinction D3	501.259	± 68.381	624.734	± 66.995	674.007	± 85.278
Extinction D4	414.331	± 85.925	818.218	± 89.156	567.451	± 80.721
Extinction D5	444.956	± 106.386	729.587	± 124.850	625.458	± 99.089
Extinction D6	471.021	± 74.634	703.321	± 94.155	625.658	± 87.201
Extinction D7	381.152	± 91.469	845.929	± 118.221	572.919	± 87.066
Extinction D8	409.828	± 102.181	854.604	± 131.011	535.568	± 112.903
Extinction D9	382.052	± 93.877	896.649	± 117.996	521.299	± 83.053
Extinction D10	332.634	± 84.516	897.981	± 135.633	569.939	± 105.493
Extinction D11	416.766	± 103.118	801.336	± 105.109	581.899	± 78.617
Extinction D12	362.153	± 97.112	925.729	± 89.113	512.118	± 72.701
Extinction D13	390.056	± 68.142	896.997	± 83.973	512.948	± 64.686
Extinction D14	390.970	± 62.592	809.769	± 85.796	599.261	± 81.349
Extinction D15	324.472	± 100.603	1044.582	± 120.856	430.946	± 66.458
Extinction D16	333.300	± 98.294	990.378	± 126.920	476.322	± 71.859
Extinction D17	436.829	± 92.589	778.271	± 115.358	584.900	± 82.849
Extinction D18	331.199	± 73.609	926.534	± 102.546	542.267	± 60.492
Extinction D19	300.940	± 110.466	1045.121	± 134.030	453.939	± 63.092
Extinction D20	369.258	± 115.521	800.468	± 125.065	630.274	± 70.997
Extinction D21	318.688	± 67.350	854.440	± 80.452	626.872	± 76.967
Extinction D22	346.796	± 78.761	937.173	± 111.608	516.031	± 70.996
Extinction D23	257.109	± 85.252	1087.850	± 112.173	455.041	± 68.802
Extinction D24	430.288	± 109.520	815.976	± 137.537	553.737	± 57.773
Reinstatement	529.723	± 129.288	505.501	± 91.501	764.776	± 129.111

CHAPTER 4

DISCUSSION

The present study was conducted to investigate the role of trait anxiety in the addiction process using the WKY rat. The majority of individuals who try drugs do not develop an addiction. While no one factor has been isolated to determine who will, and who will not develop an addiction, there is a growing body of evidence that implicates anxiety as a vulnerability factor for the sustained abuse of a drug (Koob and Le Moal 2008). It is, therefore, important to further characterize the role of trait anxiety in the addiction process. The WKY rat presents a model system sensitive to anxiety and potentially useful in the study of addictive behaviors. While others have used the WKY rat as a possible model of comorbid addiction in the context of alcohol consumption (Paré, Paré et al. 1999; Yaroslavsky and Tejani-Butt 2010), ours is the first study to address the role of environmental associations on preference for both cocaine and morphine.

The major findings of this study are that 1) WKY rats do not acquire cocaine-induced CPP at acquisition; 2) After extinction training WKY rats demonstrate a potentiated reacquisition of cocaine CPP when given an interoceptive cue; 3) WKY rats do not acquire morphine-induced CPP; 4) During extinction of morphine CPP, WKY rats exhibit an avoidance to both the previously paired injection contexts.

4.1 Cocaine Conditioned Place Preference

Data from the four-pairing WKY cocaine group were initially counter-intuitive. A non-significant aversion to the cocaine-paired chamber was observed at acquisition and was sustained for the majority of the extinction days. This conflicted with our original hypothesis that WKY rats would display an initial preference following conditioning, and exhibit a resistance to extinction of that preference. However, a priming dose of cocaine (5mg/kg) elicited a robust reversal of preference to the previously cocaine-paired chamber. These results indicate that a demonstrated preference for the cocaine-paired chamber at acquisition is not necessary for drug associations to be learned. This also suggests that the positively reinforcing properties of cocaine may not be the primary drive for drug seeking behavior in these rats. Biochemical studies investigating the availability of dopamine D1 receptors in the striatum of WKY rats found a lower binding to these receptors when compared to Wistar rats(Novick, Yaroslavsky et al. 2008). This dopaminergic deficit may account for what seems to be a lack of preference during the phase of addiction that is generally associated with positive reinforcement.

Withdrawal from a drug can initiate a transition to negative reinforcement from positive reinforcement. Instead of seeking out drug for the rewarding properties, the motivation shifts to that of relieving the withdrawal symptoms (Koob 2008). The administration of drug relieves these symptoms, thereby increasing the likelihood of continued use. A protracted period of abstinence that extends beyond acute withdrawal induces a state of increased anxiety, which is also relieved by administration of the drug

(Koob 2008). It is possible that the robust drug-induced reinstatement that we observed is due to the relief of this growing anxiety. This idea is consistent with the literature suggesting that individuals with trait-anxiety are more vulnerable to the negatively reinforcing properties of drugs of abuse (Comeau, Stewart et al. 2001).

Six-pairing cocaine groups were added to the study to investigate the role that increased drug experience may play in the initial acquisition test. The additional pairings that the WKY rats received with cocaine did change their response at acquisition. Instead of the non-significant aversion seen with four pairings, they displayed a non-significant preference. The behavioral inhibition that these rats display also extends to a fear of novelty (Paré 1994). It is possible that the additional experience of the six pairings was enough to attenuate the fear of the novel drug-induced state. At the same time the preference was still not statistically significant. Consistent with the four-pairing WKY group overall significant differences between the two choice chambers during extinction were not observed, yet these rats displayed a significant preference for the cocaine-paired chamber at reinstatement. These data further contribute to the findings observed in the four-pairing group, and add to the idea that negative reinforcement is likely the primary motivating factor in the behavior of the WKY rat.

Brains from previous experiments involving WKY and SD rats that were sacrificed immediately following acquisition are available and will be used to biochemically compare the differences in protein expression between both the two strains and the two time points.

4.2 Morphine Conditioned Place Preference

Because the findings from the cocaine experiments seem to support a negative reinforcement hypothesis, we decided to investigate morphine CPP. Our original impression was that perhaps the lack of timely acquisition of cocaine CPP was due to the stimulant effects of the drug on this already anxiety-prone strain, and that they would more readily develop morphine-induced CPP. However, while the SD control strain showed an overall preference for the morphine-paired chamber across time, the WKY strain did not develop a preference for the morphine-paired chamber over the saline-paired chamber at any time point. Additionally, an interesting pattern of behavior emerged during the last half of the extinction phase. The amount of time that the WKY rats spent in the neutral chamber rose to levels significantly greater than one or both of the outer choice chambers. In other words, the WKY rats are again, exhibiting stronger negatively reinforced behavior during this abstinence period from morphine. By demonstrating a preference for the context never associated with any injection the WKY rats are exhibiting a learned avoidance response, suggesting they have developed an aversion to both the previously paired injection contexts; in contrast the SD rats still exhibited a preference for the previously paired morphine context.

This could be explained by a number a factors. The most substantive explanation is based in the anxious nature of this strain (Paré 1994; Servatius, Jiao et al. 2008; McAuley, Stewart et al. 2009). No manipulation is performed in the neutral chamber. This is the only chamber out of the three in which the rats received no injection (a potentially stressful experience for these rats). It is suspected that as the

period of abstinence progressed, the growing negative affect due to the lack of drug led to the preference of an environment in which no anxiety-provoking event had taken place. Additionally, the priming dose of morphine at reinstatement was not enough to elicit a preference for the morphine-paired chamber, yet the amount of time spent in the neutral chamber was decreased. This could indicate a relief of growing anxiety that was building up due to the prolonged abstinence from the drug. As with the cocaine paradigm, no support for the "resistance to extinction" hypothesis was found.

4.3 Overall Discussion

The original hypothesis suggesting that this model of rat may display a continued perseverance of preference through extinction above and beyond the control strain was found to be lacking in overt evidence in all three studies. However, a more interesting pattern of behavior has arisen in its place. In the case of cocaine, both studies failed to show a statistically significant preference during acquisition test, yet paradoxically displayed a preference during reinstatement. Clearly caution has to be made when comparing acquisition directly against reinstatement, as acquisition is given in a drug-free state, and reinstatement is not. However, the dose given during reinstatement is half of the original, and serves principally as an interoceptive cue or "prime". Although no preference is shown at acquisition (where we would naturally expect a preference), this by no means indicates that a failure to learn the drug associations took place. In fact, we see what appears to be a potentiated response at reinstatement. This is interesting because it supports the idea that negative reinforcement may be the primary motivating factor by which these animals (and in turn

the population that they are modeling) seek drug. Although the morphine group did not follow the same profile as the cocaine groups, the growing preference for the chamber in which no aversive event took place is indicative of the anxious nature of this strain. The reduction in time spent in the neutral chamber when drug was administered at reinstatement also suggests that the negative affect associated with abstinence from the drug could be relieved by administration of the drug. Taken altogether, these data suggest the presence of a unique model for the investigation of a stable vulnerability to trait anxiety in the context of drug addiction.

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BIOGRAPHICAL INFORMATION

The primary areas of interest in research for Torry Dennis involve the investigation of the mechanisms underlying addiction and drug seeking behaviors. Of particular interest is the interaction and overlap between stress and addiction pathways in the brain, as well as hormonal influence on the addiction process. During his undergraduate career, he joined a laboratory that studied the effects of stress on immune function and disease progression. While there, he was immersed in an environment that taught him a great deal about the neuroscience of learning, memory, stress, and addiction. In 2008 he graduated from Texas A&M University with a Bachelors of Science in Psychology and minors in Business and Neuroscience.

Since joining the Health Psychology and Neuroscience program at the University of Texas at Arlington, he has become more intimately involved with the scientific approach to addressing problems, and has acquired the laboratory skills necessary to solve those problems. He has worked on a great number of projects investigating both the differential response to drugs of abuse between sexes, and the role of anxiety/stress on the addiction process. Future plans include more focused research on the role of hormone fluctuation on the multiple stages of the addiction process.