UNIVERSITY OF CALIFORNIA SAN DIEGO

Investigating the Role of Glyoxalase 1 as a Therapeutic Target for Cocaine and Oxycodone Use Disorder

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

Elizabeth Alcantara

Committee in charge:

Professor Abraham A Palmer, Chair
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The Thesis of Elizabeth Alcantara is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2022

DEDICATION

To my family. Your hard work, your support, and love helped me get here. To my past, present, and future self. All the years of hard work and tears led to this moment, and I hope this serves as a reminder to my future self of what I can achieve no matter how small I feel sometimes.

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LIST OF ABBREVIATIONS

BAC	Bacterial Artificial Chromosomes
CPP	Conditioned Place Preference
CUD	Cocaine Use Disorder
D1-MSN	D1 receptor medial spiny neurons
Glo1	Gene for glyoxalase 1
GLO1	Enzyme for glyoxalase 1
I.P.	Intraperitoneal
MG	Methylglyoxal
NAc	Nucleus accumbens
OFT	Open Field Test
OUD	Opioid Use Disorder
pBBG	S-bromobenzyl glutathione cyclopentyl diester
SUD	Substance Use Disorder
VTA	Ventral tegmental area

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To start, I would like to express my gratitude to Dr. Abraham Palmer for being my mentor for the past years and serving as my thesis advisor. I'm so grateful for the opportunity to be in his lab and the fact that he took me under his wing. I've heard from more than one person that the type of PI and mentor they have defines their experience in a lab and I feel incredibly grateful that I ended up in Abe's lab. I've always felt seen and supported under his mentorship as a scientist and a person.

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All chapters are currently being prepared for submission for publication of the material. Alcantara, Elizabeth; Ortez, Clara A; Ilustrisimo, Anne; Stromberg Cole; Barkley-Levenson, Amanda M; Palmer, Abraham. The thesis author was the primary researcher and author of this material.

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Poster: **Elizabeth Alcantara**, Clara Ortez, Anne Ilustrisimo, Cole Stromberg, Amanda M. Barkley-Levenson, Abraham A. Palmer (May 2022) Investigating the Role of Methylglyoxal as a *GABAA* Agonist Through Glyoxalase 1 Manipulation. *International Behavioral and Neural Genetics Society Annual Meeting*, Memphis TN
Poster: **Elizabeth Alcantara**, Clara Ortez, Anne Ilustrisimo, Cole Stromberg, Amanda M. Barkley-Levenson, Abraham A. Palmer (May 2022) Investigating the Role of Methylglyoxal as a *GABAA* Agonist Through Glyoxalase 1 Manipulation. *Biology Undergraduate and Master's Mentorship Program Student Symposium*, La Jolla CA
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ABSTRACT OF THE THESIS

Investigating the Role of Glyoxalase 1 as a Therapeutic Target for Cocaine and Oxycodone Use Disorder

by

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Master of Science in Biology

University of California San Diego, 2022

Professor Abraham A. Palmer, Chair
Professor Matthew Banghart, Co-Chair

Nearly 20,000 people died from cocaine overdoses in 2020 (NIDA, 2020). Similarly, overdose deaths involving opioids reached 70,000 following the pandemic (NIDA, 2021). Despite the impact that these disorders have on society, there are no FDA approved drug treatments for CUD and only three for OUD. Therefore, more effective treatment options are needed.

One possible target for new pharmacotherapies is the GABA-A system. Methylglyoxal, an endogenous product, is a GABA-A agonist (Distler et al. 2012). The enzyme GLO1, metabolizes MG; thus, GLO1 inhibitors increase levels of MG. Studies have shown the co-administration of a GABA-A agonist, Midazolam, with cocaine potentiates the locomotor activation of cocaine (Morris, 2008). Similarly, co-administration of fentanyl with the GLO1 inhibitor, pBBG, increased locomotor activation (Harp et al., 2022). These studies suggest a role for GABA-A in the response to cocaine and oxycodone.

This thesis investigated the potential of GLO1 to alter behavioral responses to cocaine and oxycodone, including locomotor activation and reward seeking behavior, measured by the open field test (OFT) and the conditioned place preference (CPP) test. Results showed that co-administration of pBBG with cocaine, but not oxycodone, potentiates the locomotor activation typical with cocaine. In the conditioned place preference experiments mice showed a robust CPP following conditioning trials, but preference was not affected by pBBG. Although pBBG does not affect drug seeking behavior to cocaine or oxycodone in CPP, the interaction between cocaine and pBBG supports a relationship between GABAergic signaling and the effects of cocaine

INTRODUCTION

Over 5 million people in the United States used cocaine in 2020. Furthermore, over 1 million people suffer from cocaine use disorder (CUD), and almost 20,000 people died from overdoses that involved cocaine in the last year (NIDA, 2022). Cocaine is an addictive stimulant, and its direct action is to alter the mesolimbic dopaminergic system by blocking monoamine reuptake receptors at synapse terminals and effectively increasing dopamine levels. Cocaine is known to influence the brain's reward circuitry, specifically targeting the nucleus accumbens (NAc). Administration of cocaine increases the activity of the D1 receptor medial spiny neurons (D1-MSN) in the NAc, which previous studies relate to an increase in reward and likely contributes to the addictive properties of cocaine (Lobo et.al, 2010). There are no current FDA approved pharmacotherapies for CUD. A few studies have examined the potential of the GABA system to modulate addiction. A previous study by Roberts & Brebner (2000), demonstrated that GABA agonists, such as Baclofen, can modulate cocaine self-administration in rats. In addition, preliminary studies by G. de Guglielmo, et al (unpublished) determined that administration of 15 mg/kg of the GLO1 inhibitor, S-bromobenzylglutathione cyclopentyl diester (pBBG), after 28 days of abstinence following self-administration of cocaine in rats, significantly reduced self-administration in animals with a high addiction index in a contextual drug free environment. These findings point to a possible target for CUD in GABA manipulation.

Oxycodone is a semi-synthetic opioid that is regularly prescribed by physicians to treat pain; however, oxycodone is highly addictive and around three million people in the U.S. have developed an opioid use disorder (OUD) (Huecker et al., 2019). There are currently three modestly effective FDA approved treatments for OUD, two of which are simply agonist replacement therapies, yet OUD has the highest relapse rate of any substance use disorder, with

as many as 91% of patients in recovery relapsing (American Addiction Centers, 2022). Oxycodone, like the other opioids, work by binding to mu, kappa, and delta g-coupled protein receptors then cause adenyl cyclase inhibition, hyperpolarization of neurons, and decreased excitability. Previous studies have demonstrated that oxycodone gets its analgesic properties from its strong inhibition of GABA transmission (Vaughan, 1997). The ventral tegmental area (VTA) projects to and modulates dopamine release from the NAc. GABA interneurons output onto the VTA and inhibit its activity. Oxycodone removes the inhibitory regulation of GABA from the VTA which results in subsequent increased dopamine in the NAc. Opioids, such as oxycodone, also directly target the opioid receptors on the NAc which also results in an increase of dopamine levels (Kibaly, 2021). A study by Harp et al. (2022) indicated that co-administration of pBBG with another opioid, fentanyl, mitigated the locomotor activation caused by fentanyl in mice. In summary, oxycodone can hijack the reward system in the brain through the mechanisms explained above and GABA signaling plays a role in these circuits.

The open field test is a behavioral test that is commonly used to measure the mechanisms that underlie behavior by tracking different types of activity. For example, the activity of animals after the administration of a drug is one common application. Many studies have examined the stimulant effects of substances like cocaine and oxycodone. Administration of these drugs often leads to increased locomotor activity and repeated exposure leads to behavioral sensitization. Previous studies have established that the dopaminergic system in the brain is responsible for locomotion and increases in dopamine levels lead to an increase in locomotor activity (Ryczko, D., & Dubuc, R., 2017) This remains a significant area of interest because sensitization is hypothesized to be related to the development of substance use disorders (SUD) (Smith et al., 2009).

The conditioned place preference test is a well-established behavioral test that is used to measure drug seeking behavior due to the motivational properties of a substance through context induced cues. The CPP test uses classical conditioning to pair a naturally rewarding stimulus to a neutral stimulus. After animals are conditioned to attribute a drug such as cocaine or oxycodone to a location, their preference to spend time in that location will increase significantly where none existed before. This allows us to measure the motivational aspects of the respective drug by assessing whether a preference was established, and it allows us to measure drug seeking behavior by monitoring how much time animals spend in a drug paired context compared to a neutral one. For example, in order to test the effectiveness of pBBG to affect CPP, moderate doses of cocaine (10 mg/kg) and oxycodone (1 mg/kg) were used that had previously been shown to produce a reliable CPP (Orsini, 2005; Niikura, 2013).

Use of the open field test and the conditioned place preference will allow for the investigation of Glo1 modulation and its effect on various attributes of drug addiction such as locomotor activation, drug seeking behavior, and the motivational properties of cocaine and oxycodone. Based on previous studies, it's probable that Glo1 can play an important role in modulation of addiction or help uncover more about the mechanisms that occur in the brain.

CHAPTER 1: OPEN FIELD TEST WITH COCAINE IN C57BL/6J MICE

Methods

A total of 116 male and 115 female C57BL/6J mice were ordered from Jackson Laboratories at 8 weeks of age. Age at the time the experiment began varied from ages 63-127 days. Mice were allowed to acclimate following their arrival for a minimum of 72 hours before experimental testing began. All animals were ear tagged following arrival and before the beginning of any testing for identification. Mice were housed 5 per cage and had access to food and water ad libitum and maintained on a 12h/12h light/dark cycle with lights on at 06:00.

The animals used for this experiment were either naïve to all drugs and experimental paradigms or had previous exposure to cocaine and pBBG as well as the CPP boxes. Naïve and non-naïve animals were run in separate cohorts. Non-naïve animals were placed in new pBBG/vehicle drug groups to counterbalance previous experience. The length of the OFT depended on previous exposure, naïve animals received a 3 day OFT to compensate for the non-naïve animals' prior exposure to the experimental design while non-naïve animals had a one day OFT.

Cocaine HCl (Sigma Aldrich, CAS # 53-21-4) was dissolved in 0.9% saline to a concentration of 10 mg/kg. The pBBG was synthesized at UCSD and was dissolved in 8% DMSO (Sigma Aldrich), 18% 80Tween (Sigma Aldrich), and 74% Saline (0.9% NaCl) and diluted to different concentrations to yield dosages of 12.5, 25, and 50 mg/kg when administered at a volume of 0.01 mL/g body weight. All solutions were prepared fresh the day they were used. Vehicles for cocaine and pBBG consisted of their respective solvents.

An open field test is a behavioral paradigm that is used to track locomotor activity. The OFT was conducted in an entirely open clear box placed in a white sound attenuating cabinet

equipped with a light and fan for background noise (Figure 1.1) while activity was monitored via photobeams in the VersaMax Legacy Open Field Chamber (Omnitech Electronics Inc.) and recorded through the Fusion program (v6.5 r1198 VersaMax Edition) for 30 minutes. On experiment day, animals were moved from colony room to the experiment room and allowed to acclimate to the room for a minimum of 30 minutes. All animals were weighed, and their tails were marked with a marker for easy and quick identification. pBBG pretreatment consisted of I.P. injections with 12.5, 25, 50 mg/kg pBBG or vehicle, 1.5 hours prior to the beginning of the test. For the single day OFT, animals (n=4-9/sex/drug group) were pre-treated and then immediately before going into the OFT box, animals were injected I.P. with either cocaine (10 mg/kg) or 0.9% saline. For the 3 day OFT (n=15/sex/drug group), the preparation remained the same, but all animals received I.P. saline injections on the first two days. On the third test day, animals were pre-treated with pBBG (12.5, 25, or 50 mg/kg) or pBBG vehicle for 1.5 hours and then administered cocaine before beginning the test. Locomotor activity was measured as total distance traveled in centimeters by tracking centroid body movement using photobeams (Figure 1.2). 10% Isopropyl alcohol was used to clean the OFT box in between subjects. All procedures and tests were approved by the Institutional Animal Care and Use Committee at the University of California San Diego

Results are given as a mean ± standard error mean (SEM). Statistical analyses were done using SPSS (Version 28; IBM Corp, Armonk, NY, USA). The data was analyzed using a mixed model two-way ANOVA with a repeated measure of day given the experiment and between subject factors of distance traveled, sex, and drug group. Significance level was set at $p<0.05$.

Result

Locomotor activity in mice was tested in an open field (Figure 1.1) to measure the effects of co-administration of 10 mg/kg cocaine and pBBG, at several doses. Either a one or three day OFT was used, depending on the cohorts' previous experience with other experimental paradigms. For the one day OFT, the animals used were non-naïve mice who had previous exposure to cocaine, pBBG, and other behavioral protocols. The non-naïve animals all experienced the same amount of previous exposure. A two-way ANOVA showed a significant effect of pBBG dose on cocaine activation (Figure 1.2, $F_{1,3}$= 5.887, p<0.001). Initially the factors sex, pass, and box # were included in the analysis to ensure there was no significant interaction. They were removed for the final analysis after it was determined that they had no significant interactions with other factors and no main effect. As shown in Figure 1.3, pBBG has no effect on locomotor activity on its own, as can be seen when co-administered with saline. However, when pBBG is administered with cocaine there was a significant potentiation of cocaine activation, which increased with increasing dose of pBBG. A one-way ANOVA showed that at every dose of pBBG, there was a significant difference in the distance traveled between animals who receive saline and cocaine, which is an expected result typically seen with cocaine administration ($F_{1,3}$=141.904, p<0.001).

In the three day OFT experiment, the mice were naïve animals and had no prior experience with cocaine, pBBG, or any behavioral testing. A mixed model ANOVA with a repeated measure of day and between group factor of drug was performed, initially including sex, pass, and box # to assure there were no confounding variables. These factors were removed from the final analysis after it was determined that they showed no significant main effects or interactions. The repeated measure one-way ANOVA between day two (saline) and day three (cocaine) showed an expected significant difference between days ($F_{1,2}$=249.245, p<0.001),

which reflects the locomotor stimulation caused by cocaine on Day 3. In this case, day 2 of saline was selected for comparison because day 1 of saline showed an increase in locomotor activity due to novelty of the experience. Therefore, day 2 (saline) allowed for a better comparison of baseline saline locomotor activity to cocaine induced locomotor activation. Based on our prior study, we expected to see a difference in the distance traveled between a saline and cocaine day because of the stimulating effects of cocaine. However, the cocaine activation seen on day three was not affected by any dose of pBBG, as seen in Figure 1.4 ($F_{1,3} = 1.285$, p<0.283)

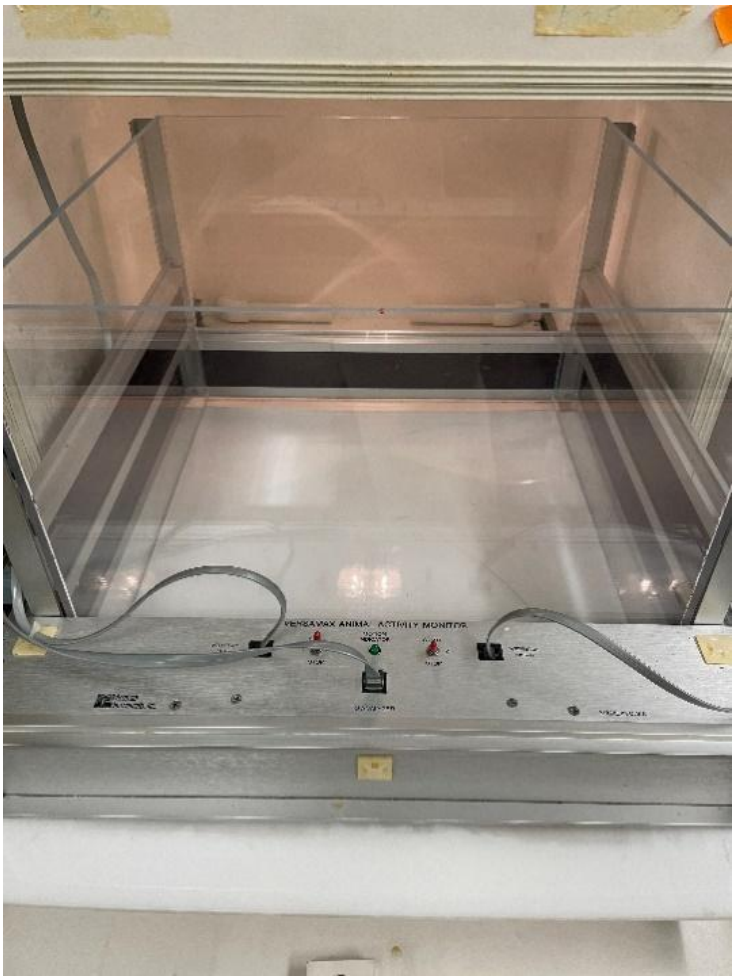

Figure 1.1: Open field test box 42x42x31 cm in size with clear plexiglass walls and flooring

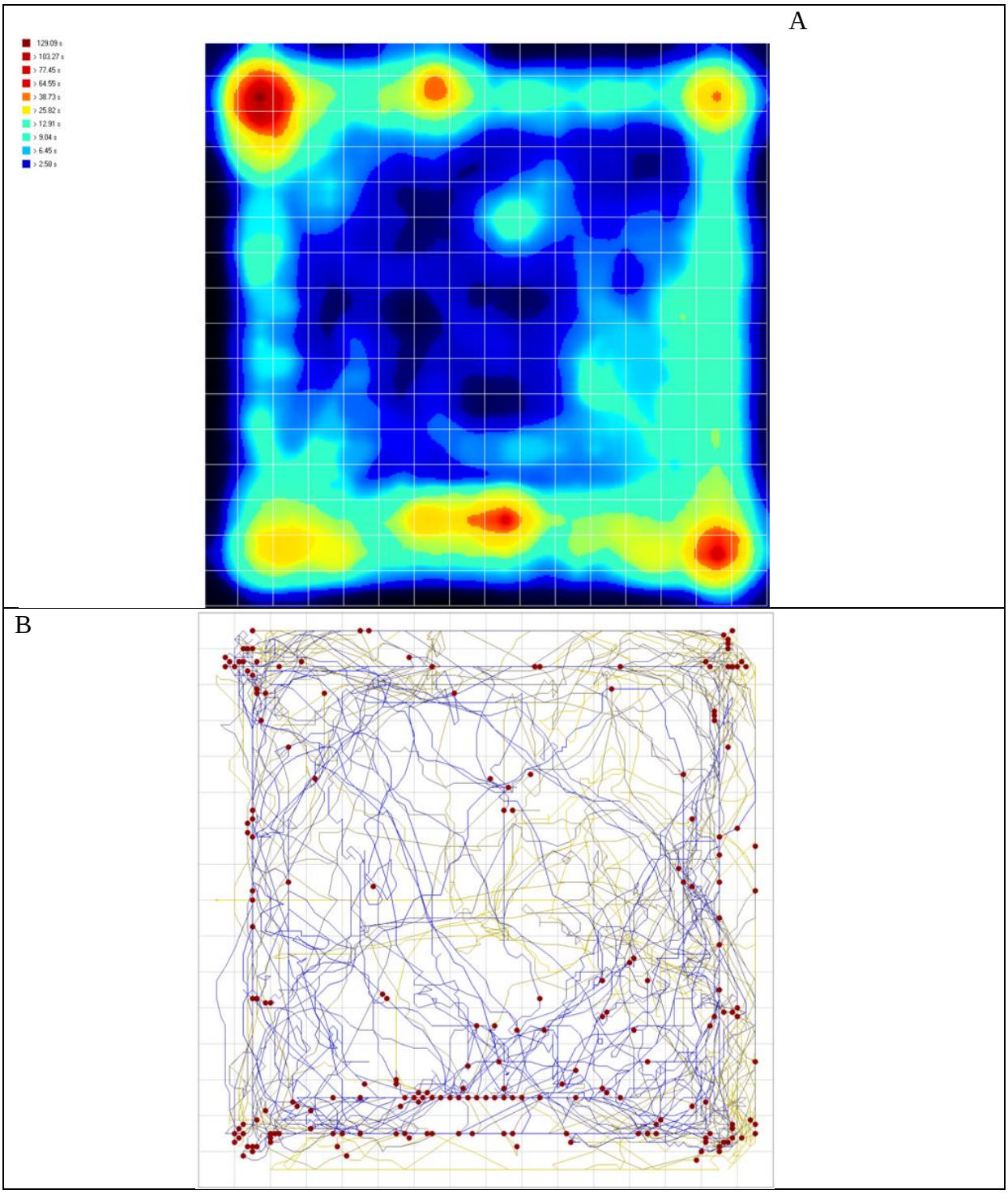

Figure 1.2: The activity tracked by the open field test box. Heat map of activity for a random animal (a) with color intensity denoting time spent. Path of distance traveled for a random mouse (b); lines show time progression from yellow to blue. Total distance in centimeters is tracked by body movement of the mouse.

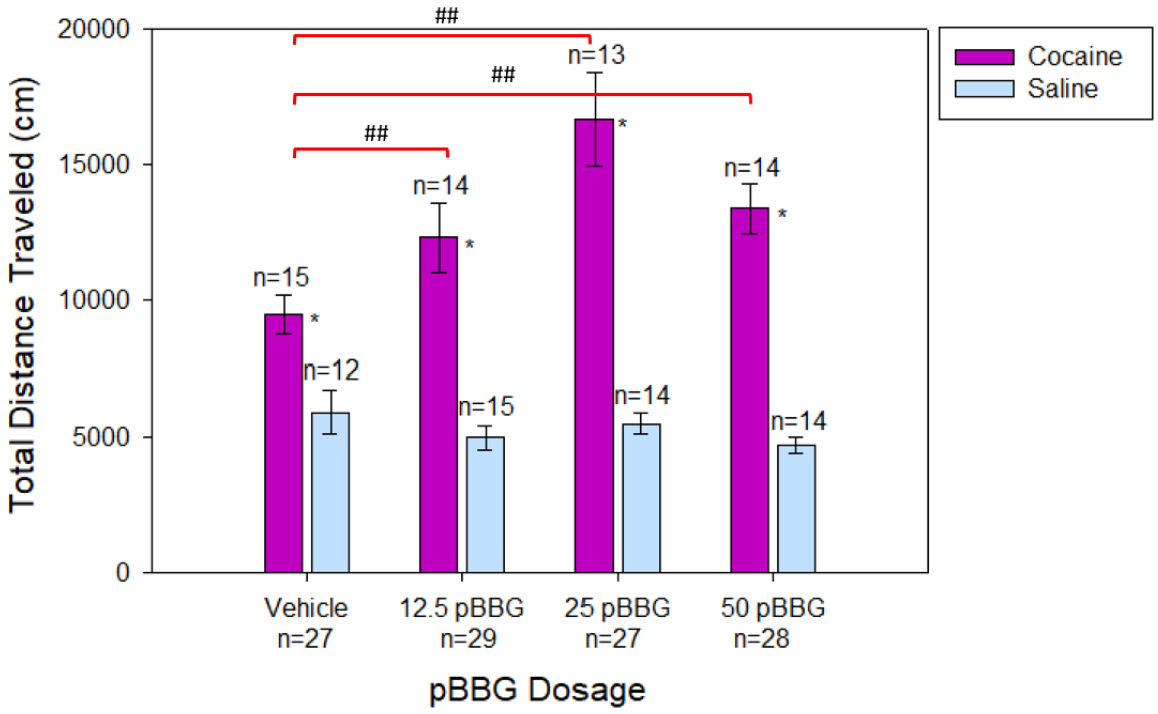

Figure 1.3: One day open field test in non-naïve C57BL/6J mice. The bars represent the mean total distance traveled in the 30-minute test of each group. The error bars denote the standard error mean. The y-axis shows the distance traveled (cm) while the x-axis shows the four doses of pBBG: 0, 12.5, 25, and 50 mg/kg. Each dosage is further split into two groups where the animals received either cocaine (10 mg/kg) or saline. * and ## represent significance $p<0.05$ using a two-way ANOVA with between subject factors of distance traveled, sex, and drug group.

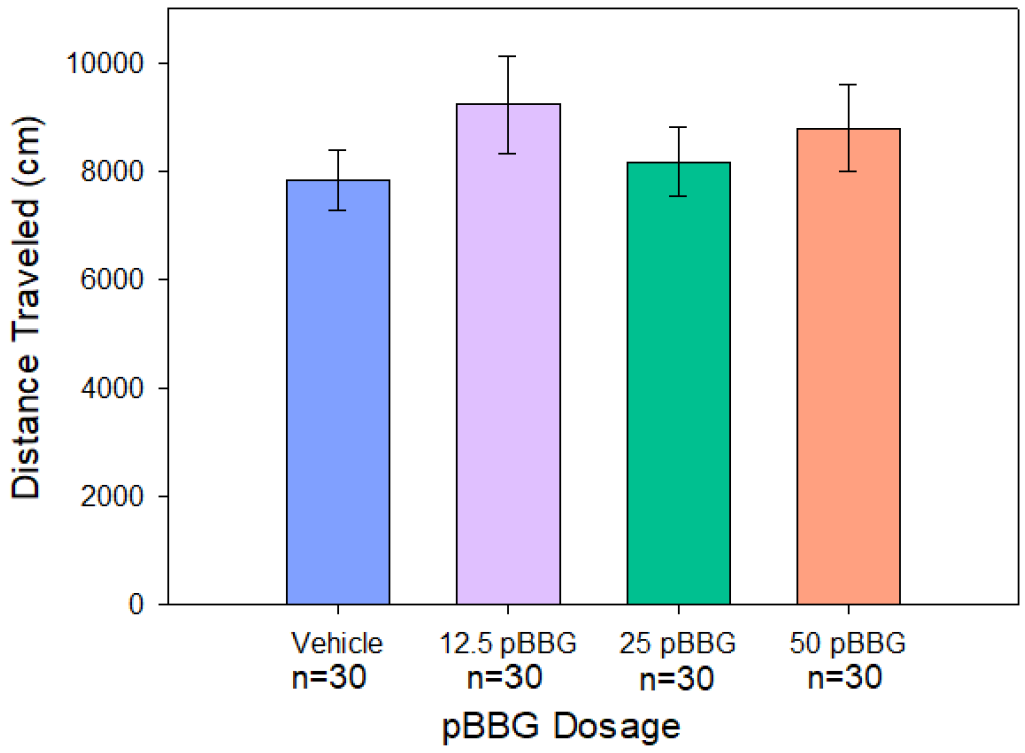

Figure 1.4: Three-day open field test in naïve C57BL/6J mice. This figure shows the mean distance traveled of each group on day 3 of the experiment. Error bars represent the standard error mean. The y-axis shows the distance traveled (cm) and the x-axis shows the four pBBG dosages: 0, 12.5, 25, and 50 mg/kg.

CHAPTER 2: OPEN FIELD TEST WITH OXYCODONE IN C57BL/6J MICE

Methods

In total, 36 male and 38 female C57BL/6J mice were used for this experiment. Animals were ordered from Jackson Laboratories at 8 weeks of age. Experimental age varied from 133-141 days.

All animals were previously used in the experiment described in Chapter 5 and had prior exposure to oxycodone, pBBG, and the behavioral paradigm. The mice were placed in new drug groups to counterbalance for previous exposure to drugs.

Oxycodone HCl was ordered from Sigma Aldrich (CAS # 76-42-6) and dissolved in 0.9% saline to a concentration of 1 mg/kg. The pBBG was the same as described in Chapter 1. All solutions were prepared the day they were used, and animals received injections at a volume of 0.01 mL/g body weight.

A one day long OFT was conducted as a result of the familiarity with the experimental protocol. Animals were prepped for the experiment in the same manner as outlined in Chapter 1. Mice (n=3-6/sex/drug group) were then pre-treated with pBBG (0,12.5, 25, 50 mg/kg) 1.5 hours before the start of the OFT and administered oxycodone immediately before going into the box via I.P. injection. 10% Isopropyl alcohol was used to clean the OFT box in between subjects.

Results are given as a mean ± standard error mean (SEM). Statistical analyses were done using SPSS (Version 28; IBM Corp, Armonk, NY, USA). The data was analyzed using an analysis of variance (two-way ANOVA) and between subject factors of distance traveled, sex, and drug group. Significance level was set at $p<0.05$.

Results

A one day OFT with 1 mg/kg oxycodone was performed to measure the effects of pBBG at four doses on locomotor activation to oxycodone. A one-day test was conducted due to the animal's prior exposure to oxycodone, pBBG, and the behavioral protocol. A two-way ANOVA was performed and concluded that pBBG does not affect the increased locomotor activity caused by oxycodone administration at any dose (Figure 2.1, $F_{1,3}$=1.731, p=0.169). Similarly, pBBG does not affect locomotor activity on its own as seen when co-administered with saline in Figure 2.1. Other factors such as sex, pass, and box # were included in the analysis and then later removed from the final analysis. We did observe the expected difference between the distance traveled by animals that received oxycodone compared to those that received saline consistent with the well-established stimulant effects of oxycodone ($F_{1,3}$=52.253, p<0.001).

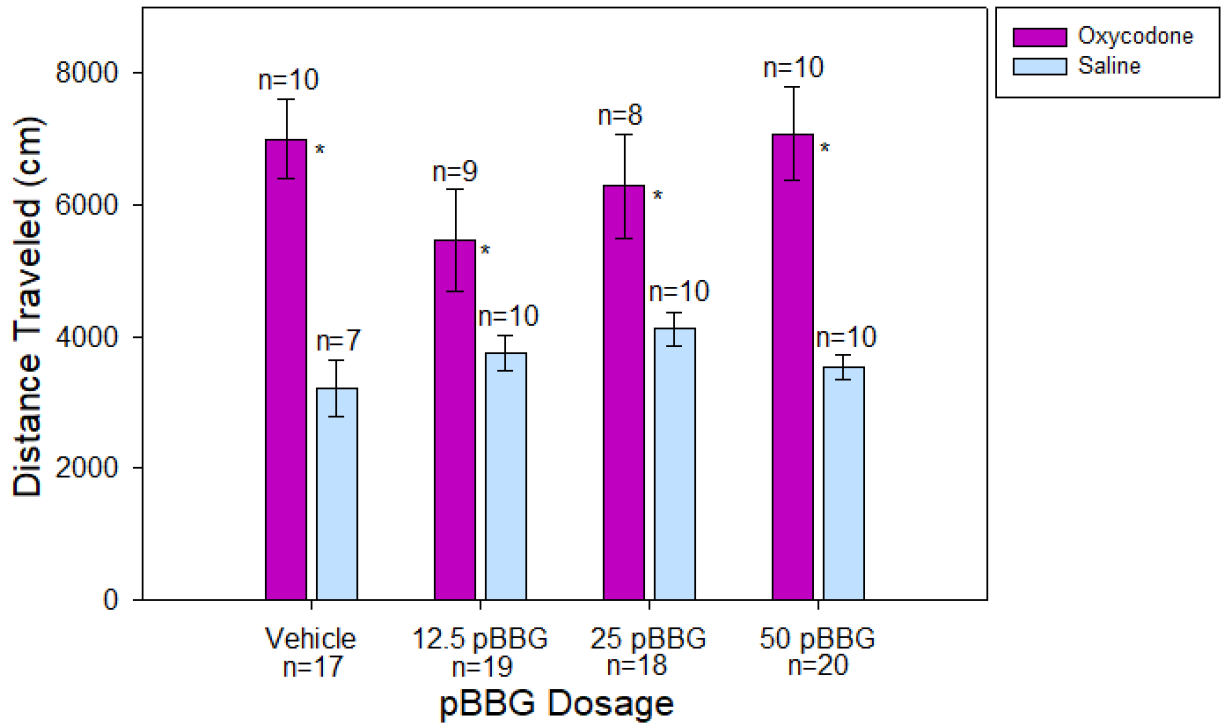

Figure 2.1: One day open field test with oxycodone (1 mg/kg) in non-naïve C57BL/6J mice. The distance traveled in the 30-minute test is shown as a mean for every group and the error bars are indicative of the standard error mean. The y-axis represents the mean total distance traveled (cm) of each group while the x-axis shows the four doses of pBBG: 0, 12.5, 25, and 50 mg/kg. The pink bars denote the group of animals that received BW injections of oxycodone and the blue bars the group that received saline. * shows significance $p<0.05$ and was analyzed using an analysis of variance (two-way ANOVA) and between subject factors of distance traveled, sex, and drug group.

CHAPTER 3: OPEN FIELD TEST WITH COCAINE IN *GLO1 OVEREXPRESSING* MICE

Methods

For this experiment a total of 38 male and 37 female transgenic *Glo1* overexpressing mice on a FVB background were used. The transgenic *Glo1* overexpressing mouse line was created in 2012 in the Palmer lab using a BAC containing *Glo1* with 35 copies of the gene which increased GLO1 mRNA expression 17-fold (Distler et al., 2012). The transgenic mouse line has been maintained since then by breeding heterozygous males to wildtype females obtained from Jackson Laboratories. Mice were housed 2-5 per cage based on litter counts at time of weaning. All animals were given ad libitum access to food and water and maintained on a 12h/12h light/dark cycle with lights on 06:00. Animals were ear tagged following weaning age (P21) for identification and tissue sample was used to genotype. Experimental age varied from 63-92 days. This cohort consisted of heterozygous animals and their wildtype littermates (n=35-40/genotype).

The mice used in this experiment were naïve to all drugs and behavior paradigms. A 3 day long OFT protocol was performed as detailed in Chapter 1, except there was no pBBG pre-treatment on day 3 of the experiment.

Results are shown as mean ± standard error mean (SEM). Statistical analyses were done using SPSS (Version 28; IBM Corp, Armonk, NY, USA). The data was analyzed using an analysis of variance (two-way ANOVA) with a repeated measure of day given the experiment and between subject factors of distance traveled, sex, and genotype.

Results

In this cohort, animals that over expressed Glo1 and their wildtype litter mates were tested in a three day OFT with 10 mg/kg cocaine to assess the effects of cocaine in transgenic

animals overexpressing *Glo1* which is expected to result in a decrease of GABA transmission in contrast to the effects of pBBG treatment. The three-day paradigm was chosen for this cohort due to a lack of previous exposure to any elements of the experiment. A repeated measures two-way ANOVA was conducted, originally including sex, pass, and box #; however, those variables were removed from the final analysis because they were not involved in any significant interactions. The analysis showed there were no genotype differences between the wildtype littermates and animals who overexpressed *Glo1* on day 2 (saline) or day 3 (cocaine; Figure 3.1, $F_{1,73}$= 0.007, p=0.933). There did not appear to be differences in overall locomotor activity on the days in which animals received saline only across genotypes. Although activity increased on day three after cocaine administration, this was an expected result and there remained no differences between overexpressing animals and their littermates.

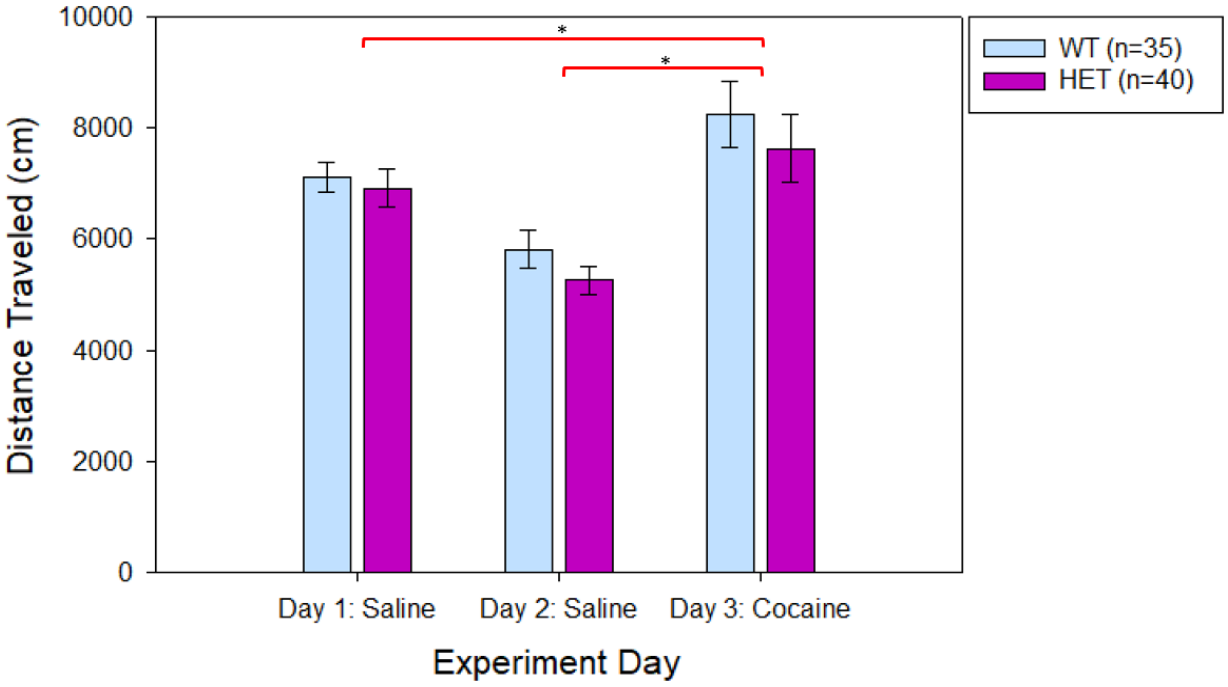

Figure 3.1: Three-day open field test with cocaine (10 mg/kg) in *Glo1* overexpressing mice and their wildtype littermates on an FVB/NJ background. The y-axis represents the total distance traveled (cm) by each group and the x-axis shows the individual days of the experiment and what solution they received via I.P. injections. The data is shown as means with error bars representing the standard error mean. The blue bars represent wildtype littermates (n=35) while the pink bars represent the animals that are heterozygous for *Glo1* overexpression. * denotes significance $p<0.05$. The data was analyzed using an analysis of variance (two-way ANOVA) with a repeated measure of day given the experiment and between subject factors of distance traveled, sex, and genotype

CHAPTER 4: CONDITIONED PLACE PREFERENCE TEST WITH COCAINE IN C57BL/6J MICE

Methods

In total, 56 male and 58 female C57BL/6J wildtype mice that were obtained from Jackson Laboratories were used. The animals were ordered at 8 weeks of age. Experimental age varied from 65-79 days.

This cohort of animals (n=5-9/sex/drug paired compartment/drug group) had no prior exposure to any drug or behavioral paradigms. Preparation of animals on test days were the same as outlined in Chapter 1. The conditioned place preference test is a behavioral paradigm used to measure the motivational properties of an experience, in this case the subjective effects of cocaine. The CPP test was performed in a two-compartment box with both visual and tactile cues (Figure 4.1). One compartment has white horizontal lines and a smooth flooring while the other has black vertical lines and a ridged floor. The two compartments were separated by a black divider with a dome shaped opening that allows movement between the two compartments. First, all mice underwent a pretest day where they received a saline injection and were placed in the box for 30 minutes with free access to both sides. Time spent on each side was recorded to measure any pre-existing compartment bias. Subsequently animals were placed in conditioning groups to have unconditioned bias by pseudo randomly assigning animals to receive drug on either the white or black compartment while maintaining that each group has an overall equal preference for both compartments. On days 1 and 3 of conditioning, half of the animals received I.P. cocaine injections and the other half received saline while being restricted to the black compartment for 30 minutes. On conditioning days 2 and 4, half of the mice receive cocaine and the other half saline while being restricted to the white compartment for 30 minutes. On day 5, test day, mice were pre-treated with pBBG (0, 12.5, 25, 50 mg/kg) for 1.5 hours before the start

of the test. Immediately before going into the box and starting the test, animals were given I.P. injections of saline and then allowed free access of both compartments for 30 minutes. Preference is measured as time spent in each compartment in seconds and movement is measured by centroid body movement on day 5. CPP boxes were wiped down in between each subject using 10% Isopropyl alcohol.

Results are given as a mean ± standard error mean (SEM). Statistical analyses were done using SPSS (Version 28; IBM Corp, Armonk, NY, USA). The data was analyzed using an analysis of variance (two-way ANOVA) with between subject factors of time spent in drug paired compartment, drug paired compartment, and drug group. Significance level was set at $p<0.05$.

Results

This experiment measured whether GLO1 inhibition with pBBG could block CPP to cocaine. All groups showed a conditioned preference to 10 mg/kg cocaine as shown in Figure 4.2 (one-way ANOVA,$F_{1,112}$=52.714, $p<0.001$). However, a two-way ANOVA showed the preference for the compartment where they received cocaine was not changed by any dose of pBBG ($F_{3,1}$=0.822, $p<0.484$). Originally sex, pass, and box # were included in the analysis, but they were removed from the final analysis after no significant interactions were found. At all doses of pBBG, mice spent significantly more time in the compartment where they received cocaine during training, indicating a strong preference.

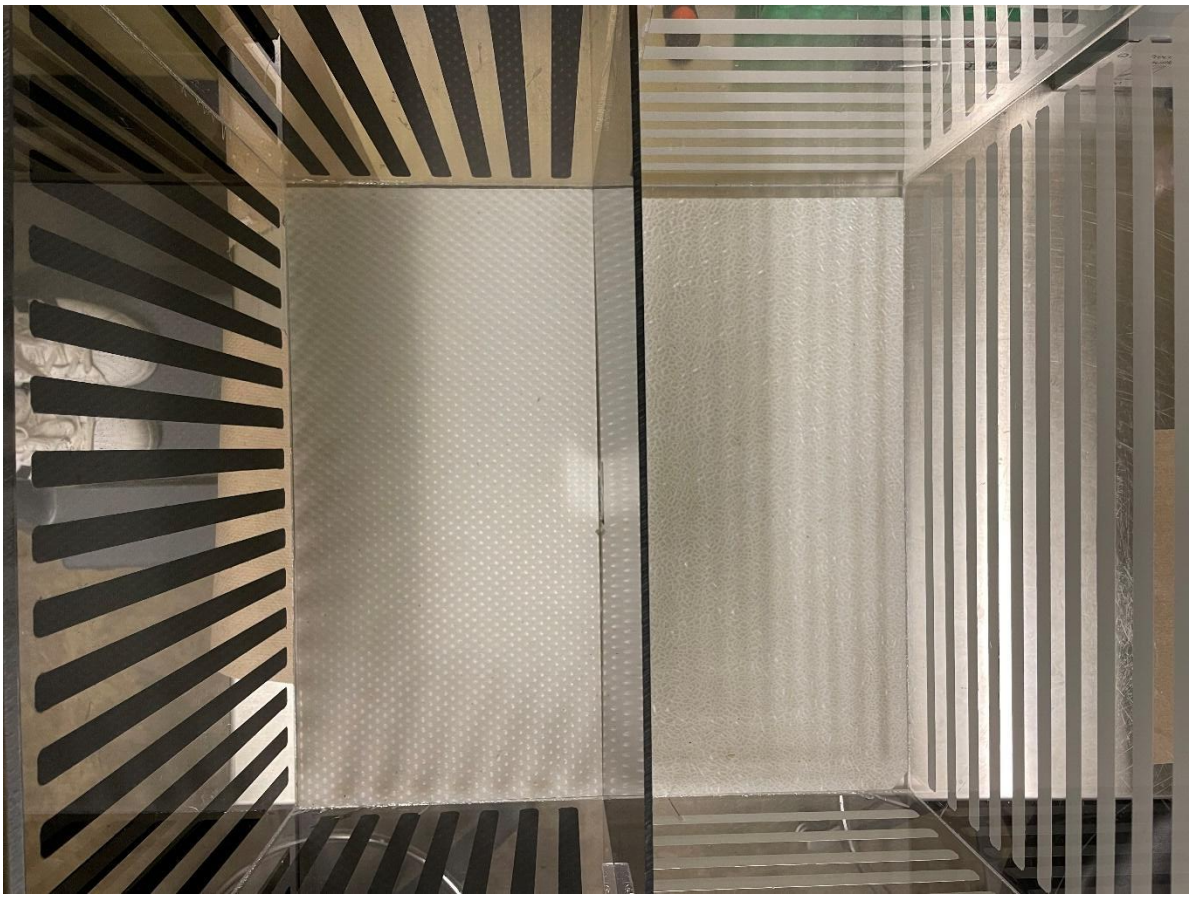

Figure 4.1: The conditioned place preference box with the dimensions 42x42x31 cm made from plexiglass. The left side shows black vertical tape with a rough textured flooring while the right side has white horizontal taping and a smooth textured floor. There is a black removable divider between the two sides with a dome shaped opening in the center for free access to both sides.

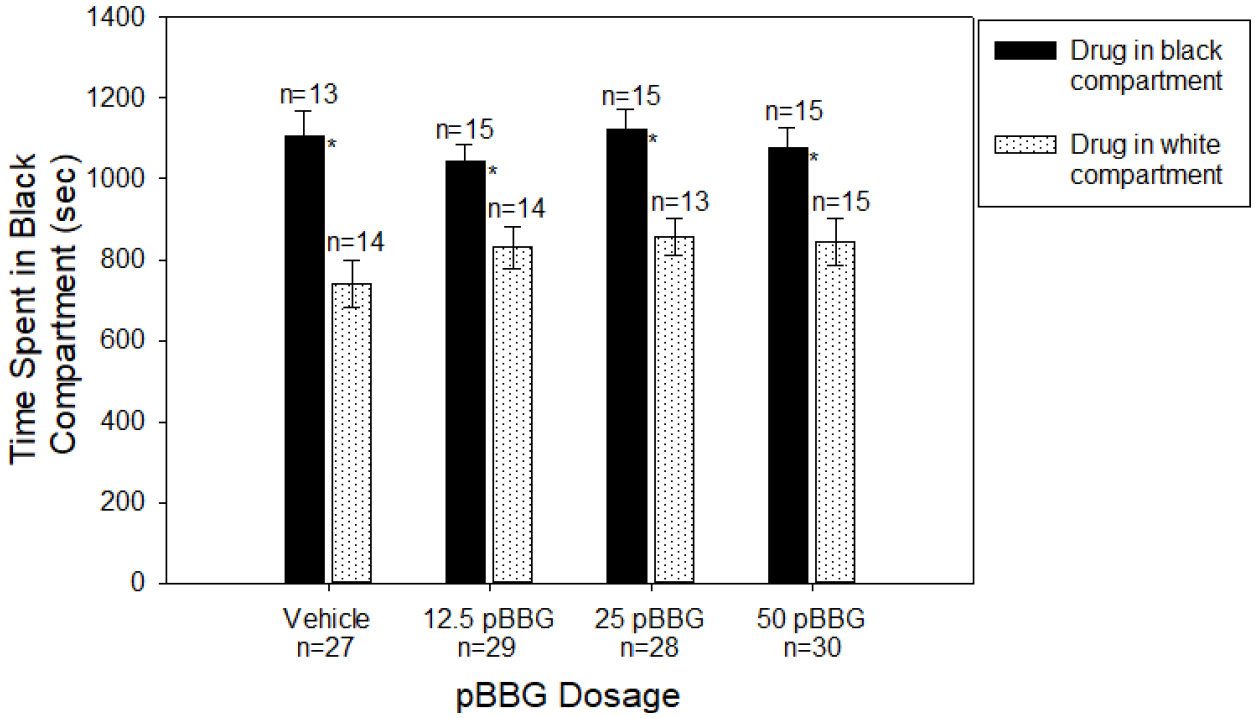

Figure 4.2: Conditioned place preference test in C57BL/6J mice. The y-axis shows the time spent in the drug paired compartment, in this case black, in seconds while the x-axis shows the four doses of pBBG: 0, 12.5, 25, and 50 mg/kg. The bars represent the mean time of each group, and the error bars signify the standard error mean. The black bars show the activity of animals who received cocaine (10mg/kg) in the black compartment and the white dotted bars show the activity of animals who received cocaine in the white compartment. * shows significance $p<0.05$ using a two-way ANOVA with between subject factors of time spent in drug paired compartment, drug paired compartment, and drug group.

CHAPTER 5: CONDITIONED PLACE PREFERENCE TEST WITH OXYCODONE IN C57BL/6J MICE

Methods

Overall, 47 male and 44 female C57BL/6J mice were used for this experiment. Mice were ordered from Jackson Laboratories at 8 weeks of age and given a minimum of 72 hours after arrival to acclimate before any testing began. Experimental age varied from n-n days.

The mice (n=4-12/sex/drug paired compartment/drug group) in this experiment were naïve and had no previous exposure to any drugs or behavioral paradigms. The CPP test was conducted as outlined in Chapter 4 with no deviations.

Results are given as a mean ± standard error mean (SEM). Statistical analyses were done using SPSS (Version 28; IBM Corp, Armonk, NY, USA). The data was analyzed using an analysis of variance (two-way ANOVA) with between subject factors of time spent in drug paired compartment, drug paired compartment, and drug group. Significance level was set at $p<0.05$.

Results

This experiment measured the ability of GLO1 inhibition through pBBG to affect CPP to oxycodone. After four conditioning trials, each group of animals spent significantly more time in the compartment where they received oxycodone on the test day as seen in Figure 5.1 (one-way ANOVA, $F_{1,89}$=31.432, $p<0.001$). But administration of pBBG did not block this effect at any dose, as seen with a two-way ANOVA analysis ($F_{1,3}$= 0.462, $p<0.710$). There was no overall effect of pBBG on time spent in the drug paired compartment, but further analysis showed there was no significant difference in time spent in drug paired and non-drug paired compartments for the group of animals that received 50 mg/kg pBBG. The original analysis included sex, pass, and box # but were excluded from the final when no significant interactions were found.

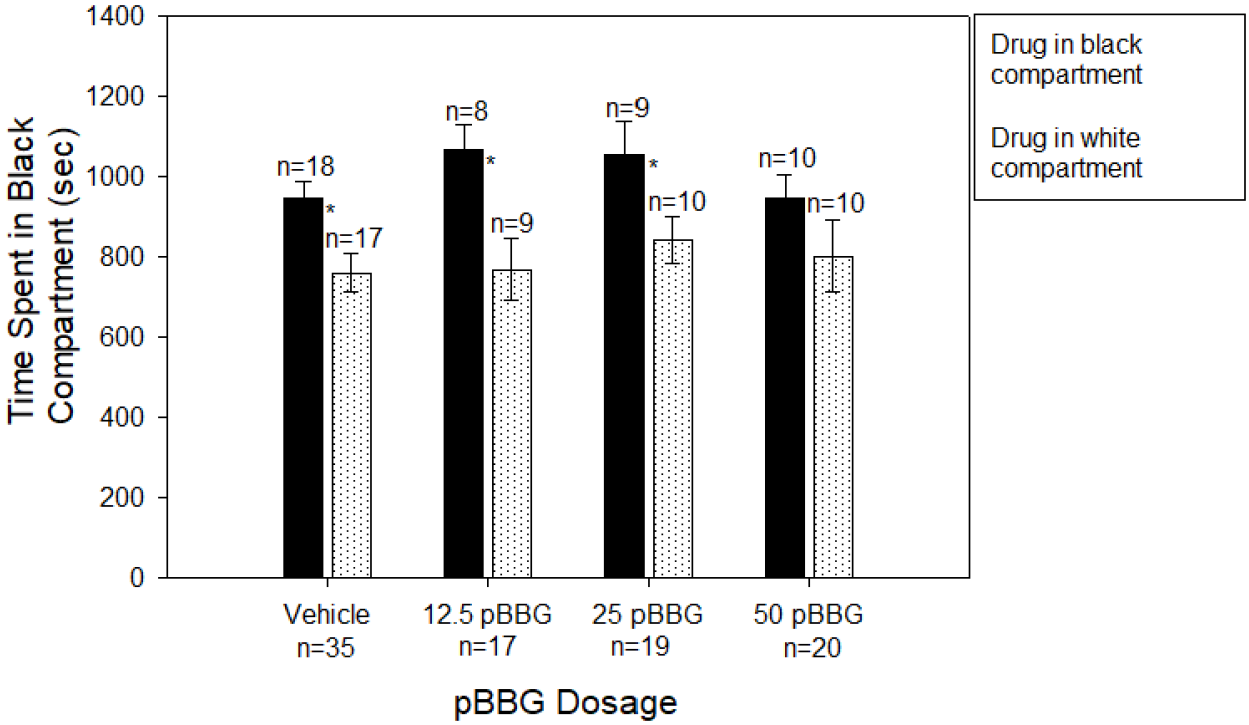

Figure 5.1: Conditioned place preference test with oxycodone (1 mg/kg) in C57BL/6J mice. The total time spent in the drug paired compartment, in this case black, is shown as a mean for each group and the error bars represent the standard error mean. The y-axis represents the time spent in the drug paired compartment while the x-axis shows the four pBBG dosages: 0, 12.5, 25, and 50 mg/kg. The black bars are the animal whose drug paired compartment is black while the white dotted bars represent the animals whose drug paired compartment was the white side. This configuration allows for the visualization of preference for the drug paired compartment. * signifies significance $p<0.05$ using a two-way ANOVA with between subject factors of time spent in drug paired compartment, drug paired compartment, and drug group.

CHAPTER 6: CONDITIONED PLACE PREFERENCE TEST WITH COCAINE IN *GLO1 KNOCKDOWN* MICE

Methods

This cohort consisted of 4 male and 23 female transgenic *Glo1 Heterozygous Knockdown* mice on a C57BL/6J background. The *Glo1 Knockdown* mouse line was generated by Dr. Michael Brownlee in his lab at Albert Einstein College of Medicine, Bronx, NY on a C57BL/6J background. They have been reported to have a 45-65% reduction in *Glo1* gene expression (Osta et al. 2008). The mouse colony has been maintained since then by selectively breeding heterozygous male animals to wildtype C57BL/6J females ordered from Jackson Laboratories. Animals were housed 2-5 per cage depending on weaning dates and were allowed access to food and water ad libitum. Experimental age varied from 90-105 days.

The CPP procedure and all prep steps remained the same as outlined in Chapter 4 with the exception that no pBBG pre-treatment was done on the test day (day 5).

Results are given as a mean ± standard error mean (SEM). Statistical analyses were done using SPSS (Version 28; IBM Corp, Armonk, NY, USA). The data was analyzed using an analysis of variance (two-way ANOVA) with between subject factors of time spent in drug paired compartment, drug paired compartment, and genotype. Significance level was set at $p<0.05$.

Results

This experiment measured the effect of genetic manipulation of *Glo1* on conditioned place preference to cocaine. *Glo1* knockdown animals are expected to have a decrease in GLO1 levels, therefore an increase in MG and GABA signaling similar to the GLO1 inhibitor, pBBG. After four conditioning trials, *Glo1* knockdown animals and their littermates were compared to measure preference for the compartment in which they received 10 mg/kg cocaine (Figure 6.1).

A two-way ANOVA showed a strong preference for the compartment in which they received cocaine ($F_{1,23} = 31.290, p < 0.001$), however, there was no significant effect of genotype on the time spent in the drug paired compartment ($F_{1,23}$=0.399, p<0.534). The original analysis included sex and box# but was removed from the final analysis when no interaction was found.

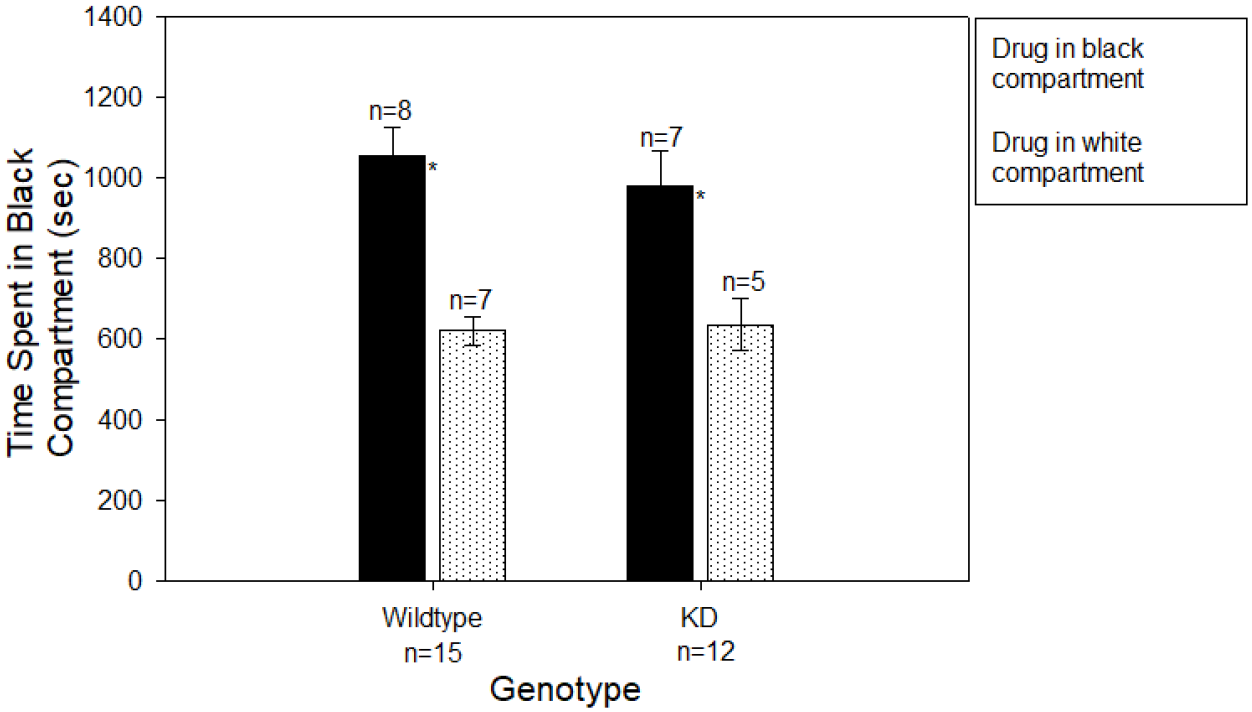

Figure 6.1: Conditioned place preference with cocaine (10 mg/kg) in *Glo1* knockdown animals and their littermates on a C57BL/6J background. The data is shown as a mean of the total time spent on the drug paired compartment, in this case black and the error bars show the standard error mean. The y-axis is the time spent in the drug paired compartment in seconds while the x-axis is the genotype. The black bars represent the group of animals that received drugs on the black compartment while the dotted white bars are the groups of animals who received cocaine on the white compartment. * denotes significance p<0.05 using a two-way ANOVA with between subject factors of time spent in drug paired compartment, drug paired compartment, and genotype.

DISCUSSION

Drug addiction is a prominent problem that has a significant impact on society. We still lack effective and reliable treatment options for many substance use disorders due to a limited understanding of the mechanisms and intricate relationships that many drugs of addiction have in the brain. This thesis aimed at further understanding the mechanisms that underlie addiction to cocaine and oxycodone using mice models and two behavioral paradigms that illustrate some of the behaviors typically seen with drug abuse in humans. The open field test tracks locomotor activation caused by the drugs which has been attributed to sensitization and its addictive qualities. On the other hand, the conditioned place preference test was used to measure the motivational properties of the drugs as well as to parallel drug seeking behavior in mice.

In the first set of experiments, I used the open field test to investigate any potential relationship between the GLO1 inhibitor, pBBG, and cocaine or oxycodone separately. Two OFT protocols were conducted, either a one- or three-day test depending on the animal's previous exposure to cocaine, oxycodone, or pBBG. The results show that when pBBG is co-administered with cocaine, but not oxycodone, it potentiates the locomotor activation typically seen with drugs of abuse (Figure 1.3). On the other hand, animals who underwent the three day OFT, and were naïve to all drugs and behavioral testing, did not show any differences in locomotion in response to cocaine when co-administered with any dose of pBBG (Figure 1.4). The results of the experiment that showed no effect of pBBG on oxycodone induced locomotor activation was different from what was previously seen in the study done by Harp et al. (2020) who saw a locomotor potentiation following the co-administration of pBBG and fentanyl. This was surprising but can perhaps be explained by the differences in experimental design. The animals used in this experiment were not naïve to oxycodone or behavioral testing while the

group of animals used in the Harp et al. study were naive. Furthermore, to habituate the animals to the box, researchers in the Harp et al. study allowed the mice to roam the OFT box for 20 minutes prior to administering fentanyl and beginning the 30-minute test. This approach was different than the one outlined in this thesis. It's plausible that all these differences created changes that were substantial enough to warrant different results as was shown. Moreover, the three day OFT was done in an attempt to investigate the drug interactions between cocaine and pBBG without having other variables such as previous exposure to drugs or behavioral testing confound the results. The intent of the three day OFT was to compensate for previous exposure to testing elements in the other cohort by allowing the mice to habituate to the protocol and the environment for two days before testing for drug interactions. However, by doing so, the one day OFT became more of a novel experience than the three day OFT. Although the one-day cohort had been previously used in the CPP experiment and had experience with similar behavioral testing, cocaine, and pBBG, the previous exposure occurred weeks to months prior to OFT testing. In addition, the CPP boxes are comparably different from the OFT boxes visually and texturally. The three day OFT allowed the animals to habituate and removed some of the novelty. This idea can be reinforced by looking at the mean distance traveled following cocaine administration and pBBG vehicle in the one and three day OFT. The mean distance traveled following cocaine injections was 9487.69 ±710.88 cm for the one day OFT and 7814.96 ±553.03 cm for three day OFT. This could signify that the reason pBBG was shown to potentiate cocaine activation in the one day OFT and not the three day OFT is the novelty of the experience. Another thing of importance to note is that the animals in the one day OFT had previous exposure to cocaine at least a month prior to testing and previous research has shown that repeated exposure to cocaine can lead to sensitization of cocaine that lasts for months (Balda et

al., 2009). The mice used in the one day OFT had repeated exposure to cocaine in the conditioning trials of CPP. It's reasonable to assume that this previous exposure to cocaine played a role in the differences between the results shown in the one day OFT compared to the three day OFT. Co-administration of cocaine and pBBG is related to increased locomotion and affects sensitization to cocaine under novel circumstances. In other words, increasing GABA-A transmission, through MG manipulation with the GLO1 inhibitor, pBBG, potentiates cocaine sensitization. Sensitization to drugs of abuse is well studied because of its perceived role in addiction. Although the drug interaction between cocaine and pBBG did not persist under all conditions, future studies should investigate how pBBG could play a role in sensitization and thus elucidate more about the mechanisms that underlie addiction.

On the other hand, the results of the conditioned place preference test with cocaine and oxycodone showed that neither pharmacological nor genetic manipulation of GLO1 was able to disrupt the motivational aspects of either drug of addiction. Upon further review, a previous paper by van Zessen et al. (2012) explained how VTA GABA activation disrupts reward consumption but not reward predictive cues. Since the conditioned place preference measures reward through contextual cues whereas the self-administration paradigm measures reward through consumption, this provides a possible explanation as to why pBBG affects drug seeking behaviors in the preliminary studies done by Dr. Guglielmo but not in the CPP tests. In addition, Dr. Guglielmo's study was done in a group of genetically diverse rats and results saw an effect of pBBG on a specific subset of animals who expressed a high addiction index in self administration while CPP was in genetically identical C57BL/6J mice. The possibility exists that the CPP mice don't fall under the subset of a high addiction index animals and therefore the effects of pBBG are not apparent. To rephrase, increasing GABA-A signaling through an

increase of its agonist, MG, with the GLO1 inhibitor did not affect drug seeking behavior to cocaine or oxycodone. Similarly, it did not prevent CPP acquisition to cocaine as shown with the *Glo1* knockdown animals. Although GLO1 manipulation did not affect drug seeking behavior as measured by CPP, the role of *Glo1* in addiction remains a possible therapeutic target and further studies should investigate its role in different reward circuitries.

Further analysis into the data could attempt to differentiate two groups of mice with stronger and weaker preferences for their respective drugs in order to create similar subsets shown in Dr. Guglielmo's study. The effects of pBBG could be re-evaluated between the groups and any effect that may have been shadowed could be uncovered. Additionally, given the data already collected from the open field test we can analyze how and if cocaine and oxycodone administration, respectively, affect time spent in the center of the field which is an aspect of the OFT that is used to measure anxiety in animal models. We can investigate how drugs of abuse affect anxiety and thus elucidate more about the addictive nature of them.

All chapters are currently being prepared for submission for publication of the material. Alcantara, Elizabeth; Ortez, Clara A; Ilustrisimo, Anne; Stromberg Cole; Barkley-Levenson, Amanda M; Palmer, Abraham. The thesis author was the primary researcher and author of this material.

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