THE EFFECT OF A 5-HT2A INVERSE AGONIST ON ANIMAL MODELS OF OBSESSIVE-COMPULSIVE DISORDER: MARBLE BURYING TASKS

by

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Dedication

For my mother and father, who always pushed me to always strive to be the best you can be. For not only raising me believing education was the biggest privilege for self-betterment, but also that no matter where you start, you can always improve. I owe all my love for knowledge to them.

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ABSTRACT

THE EFFECT OF A 5-HT2A INVERSE AGONIST ON ANIMAL MODELS OF OBSESSIVE-COMPULSIVE DISORDER: MARBLE BURYING TASKS

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Compulsive persistent, non-functional behavior is a staple characteristics of obsessive-compulsive disorder (OCD) in humans. It is commonly studied in rodents through defensive burying of harmless objects. Multiple serotonergic drugs have affected marble burying in rodents. The inclusion of other kinds of interaction with marbles has been suggested as an additional model for compulsive behavior not motivated by anxiety or reward. This study hypothesized that marble interactions, like marble burying will persist or increase over days despite not being motivated by anxiety. It was also hypothesized that pimavanserin, an FDA-approved 5-HT2A serotonin receptor inverse agonist, will decrease the amount of marble interaction over four consecutive days. There was a 20 minute baseline observation with all rats injected with isotonic saline. An experimental group of Lewis rats received 1 mg/kg intraperitoneally of pimavanserin in isotonic saline, daily for four subsequent days. Control rats were injected daily with isotonic saline alone. On each day, there were injections, habituation to the test room and a 20 minute

observation with the rat in a tub containing 16 marbles on rodent litter. The baseline scores were subtracted from each rat's subsequent test score. These change scores were than averaged over all four test days. There was very little deliberate marble burying. In fact review of video recordings show that most "buried" marbles had simple simply been accidently stepped upon by the rats. The control rats persisted in other marble interactions over time, despite showing no aversion to the marbles. This suggests compulsive-like behavior. There was an increase from baseline of marble interactions in the placebo group but a slight decrease in the pimavanerin group. This difference was statistically significant. There were no significant effects on activity level or bouts of inactivity to explain this difference. Thus pimavanserin may serve to decrease compulsive behaviors, and reducing 5HT2A receptor activation might be a novel strategy for managing obsessive-compulsive behaviors.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

CHAPTER 1: INTRODUCTION

Obsessive-Compulsive Disorder

Obsessive-Compulsive Disorder (OCD) is a restrictive psychiatric disorder that was recognized as early as the seventeenth century. The symptomology of OCD was described in Burton's *The Anatomy of Melancholy* published in 1621 (as cited in Parmar & Shah, 2014). OCD falls into two major categories of symptoms: obsessive thoughts and compulsive behaviors. Obsessive thoughts are defined as being recurrent, unwanted, and intrusive, whereas compulsion comes from repetitive or ritualistic behavior to suppress the obsessive thoughts. The two categories interact with each other through what can be considered a feedback loop. People with OCD are unable to stop their behavior owing to their belief that the behavior prevents some ill effect. This leads to them having significant trouble holding down a job or fulfilling personal responsibilities (American Psychiatric Association, 1994). The behavior is usually followed without an ill effect; therefore, it reinforces the individual's preconceived notions and increases the likelihood of them repeating the behavior.

The classification of the disorder in the *Diagnostic and Statistical Manual of Mental Disorders (DSM-5; 5th ed.;* American Psychiatric Association, 2013) focuses on the operational definitions of compulsions and obsessions, the resulting clinically significant stress, the timing of the symptoms, and the symptoms are not attributed to a substance or alternative disorder. Obsessions and compulsions are not mutually exclusive, and an individual is not required to have both for a diagnosis. However in most cases, they occur together. The operational definition of obsession involves urges that cause anxiety or distress with attempts to avoid or suppress the urges through behaviors. It is important to note that intrusive thoughts, disturbing or repulsive thoughts or images,

are not uncommon and are universal (Rachman & de Silva, 1978). Those with OCD believe these thoughts are abnormal and need an act to prevent something harmful from happening (Salkovskies et al., 1995). These intrusive thoughts could be something as distressing to the individual such as wanting to put themselves or another person in harm's way.

Behaviors become compulsive when they are repetitively aimed specifically at reducing or preventing distress from a situation. The feedback loop of disturbing results not occurring after an act fuels the compulsion. It is not uncommon for the affected individual to understand how illogical the behaviors are, which the *DSM-5* defines as good or fair insight, especially when they do not seem to have any direct effect on the situation at hand. Some actions even become emotionally reassuring to the individual, and treatment becomes a difficult task. Behaviors can become dangerous, even despite a healthy origin, such as excessive hand washing leading to skin irritation and damage. Cleaning and checking locks may start as a harmless task- as such tasks are performed by most individuals – but can gradually become ritualistic. Repetitive mental acts can also be considered a compulsion, such as constant counting or praying to the point that it impacts normal functioning. Counting how many people enter a door would not be significantly stressful; however, refusing to enter a building until a certain number of people enter could affect someone's ability to run errands or perform a job. The *DSM-5* also observes that obsessions and compulsions are either time-draining or severe enough to impair an individual in social, occupational or other areas of functioning (American Psychiatric Association, 2013).

The prevalence rate of OCD in the United States population is around 1%, according to the National Institute of Mental Health (2016), with the *DSM-5* rating it at around 1.2% prevalence in the United States and as high as 1.8% internationally. Women

have a slightly higher rate (American Psychiatric Association, 2013). Age of onset typically occurs around 20 years of age, with males beginning symptoms at a slightly earlier age than women and approximately a quarter of men with an onset before age 10. Males not only developed symptoms earlier in age, but they more frequently had an insidious development compared to the more acute onset in later years that females have (Bogetto, Venturello, Albert, Maina, & Ravizza, 1999). The prevalence of obsessions in children with OCD may be underestimated because of the lack of introspection as to why their compulsions occur. However, many children do meet the criteria for both obsessions and compulsions (American Psychiatric Association, 2013).

Before discussing the epidemiology and risk factors for OCD, it is important to understand what much of the research considers as the subtypes of the disorder. The *DSM-5* does not include these subtypes in their diagnostic criteria, but the subtypes can vary in treatment effectiveness. Baer (1994) helped develop the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) Symptom Checklist to differentiate factors involved in OCD. Using this scale, which was applied to 107 patients in his study, Baer found that three main factors held up over time when scoring symptoms: symmetry/hoarding, contamination/cleaning, and pure obsessions – intrusive thoughts without compulsive behaviors. It was hoped that categorizing symptoms could further individualize therapy. A stratified meta-analysis broke down the symptoms in four factors instead of three: Forbidden Thoughts – which could be aggressive, sexual, or religious; Symmetry – repeating and counting; Cleaning – which also included contamination; and Hoarding (Rosario-Campos et al., 2006). It should also be noted that while the Dimensional Yale-Brown Obsessive-Compulsive Scale (DY-BOCS) is used for both adults and children, there are some differences in the factors based on age: Obsessions were higher in forbidden thoughts for adults, and cleaning was higher for children (Leckman, Bloch, &

King, 2009). There is also a high comorbidity rate with 30% of OCD patients having tic-related disorders, especially in males (American Psychiatric Association, 2013).

While no direct cause has been identified for disorder development, there are multiple risk factors, both environmental and biological, that attribute to the disorder. There tends to be a significant correlation between childhood upbringing and risk for obsessive-compulsive symptoms. Social isolation and physical abuse are both associated with adult OCD diagnosis. Poor childhood motor skills predicted risk for behaviors in the checking dimensions. Perinatal insult injuries were linked to adult behaviors in both symmetry and shameful thought dimensions (Grisham, Fullana, Cols, & Moffitt, 2011). Harm avoidance in children, due to negative life events, also has a possible association with OCD and other anxiety disorders in adolescents and young adults (Gothelf, Aharonovsky, Horesh, Carty, & Apter, 2004). Harm avoidance found in first-degree relatives of those who had OCD was more common than in relatives of the control groups (Ettelt et al., 2008). A 2004 study looked at the interaction of parental rearing style to OCD rates and found that hoarding could be somewhat predicted by low emotional warmth from parents. There also was a trend that showed that individuals with OCD had a history of rejection by fathers but did not occur in all individuals (Alonso, Sola, Real, Segalas, & Menchon, 2004).

OCD rates are about two times higher among those with a first-degree relative with the same disorder. If that relative developed symptoms in early childhood, that rate increases to 10-fold (American Psychiatric Association, 2013). One study took eight probands from OCD clinics and found that their relatives had an 11.7% prevalence rate compared to 2.7% in unrelated subjects with a higher preference of obsessions over compulsions (Nestadt et al., 2000). Carey and Gottesman (1981) found that there was a concordance rate of 87% in monozygotic twins and 47% in dizygotic twins, but a number

of more recent studies have found slightly lower genetic rate. In a study of 1,224 OCD subjects, using the YBOCS-CL battery followed by factor analyses, a heredity link was found for the contamination and symmetry/hoarding factors. Genetic variance was also linked with symptom severity (Katerberg et al., 2010). The overall evidence suggests a strong genetic influence for the disorder prevalence as well as the specific symptoms.

While there is no one specific gene responsible for the development of the disorder, there has been significant progress in the past 30 years in learning how genes and chromosomes play a collaborative role. To assess genetic links, a group of universities and hospitals banded together to create the OCD Collaborative Genetics Study (OCGS). This has collected the largest data bank including subjects from UCLA and Johns Hopkins, Brown, Columbia and Harvard Universities, as well as the National Institute on Mental Health (NIMH). Multiple genetic links have been addressed each correlating with a specific phenotype. Using data from the OCGS, one study found a strong possible linkage of the compulsive hoarding phenotype to chromosome 14 at marker D14S588 for those with two or more hoarding relatives. For those who had fewer hoarding relatives, chromosome 3 at marker D3S2398 shows some promise (Samuels et al., 2007). This may be limited to one phenotype, but that could possibly explain why there are individual differences in OCD behavioral phenotypes. Hanna et al. (2007) found possible evidence of OCD association on the markers of chromosome 10p15, specifically in the region of adenosine deaminase.

Autistic children often present similar OCD-like behaviors. There is an increased rate of autistic children with severe OCD-like behaviors whom often had parents with high OCD behaviors. Because of this there has been some focus linking the two disorders genetically. Autism Spectrum Disorder (ASD) shares many common behavioral symptoms of OCD, such as ritualistic or repetitive behaviors. A study found there was a

shared peak score on chromosome 1, at marker D1S1656. This suggests a slight linkage between the two disorders which may in time explain the similar behaviors (Buxbaum et al., 2004). No clear genetic markers have been found for the onset of OCD, however, the scientific community is still searching and will hopefully reach a better conclusion in the future.

Biological risk factors include structural and neuronal differences. There is a possible connection between hyperactivity and differing blood flow patterns in the orbitofrontal-subcortical circuits, specifically the striatopallidal pathways (Saxena, Brody, Schwartz, & Baxter, 1998). A more recent 2004 study further emphasized the orbitofrontal cortex, owing to its role in action consequences and decision making. The anterior cingulate cortex was also a point of concern because of its role in error reduction. The abnormalities in these areas might plausibly clarify how repetitive thoughts and behavior are maintained (Aouizerate et al., 2004). PET scans of those with OCD have also revealed consistent differences in the orbital gyrus and caudate nucleus (Whiteside, Port, & Abramowitz, 2004). Higher OCD symptom severity was reflected in brain scans through smaller gray matter in the BA6 area in the dorsal cortical region (Gilbert et al., 2008). Gray matter amounts correspond to the amount of nerve cell bodies, or somata, in that area of the brain; the thicker the gray matter, the more somata and dendrites are associated with the area.

Previously listed in the *DSM-4* (4th ed., text rev.; *DSM-IV-TR*; American Psychiatric Association, 2000) under anxiety disorders; OCD now has its own chapter in the newer *DSM-5* showcasing the distinction between specified anxiety disorders and the ritualistic nature of OCD. Many of the distinctions between anxiety disorders and OCD were distinguished by which medication types alleviate symptoms in human and animal subjects. The first-line medications for the most symptom relief of OCD are selective

serotonin reuptake inhibitors (SSRIs) and atypical antipsychotics, while norepinephrine reuptake inhibitors or dopamine antagonists are found to be less effective. The effectiveness of SSRIS suggest that serotonin is the prominent neurotransmitter connected to OCD (Micallef & Blin, 2001). Fluvoxamine, an SSRI commonly used to treat OCD symptoms, was found to have very significant results in reducing symptoms when compared to a placebo (Hollander et al., 2003). The use of SSRIs also is beneficial because of the high comorbidity of OCD and depression. The lifetime diagnosis of depression in those with OCD is as high as 63% for affective disorders such as major depressive disorder and bipolar disorder, with the former being more common at around 41%. This goes hand-in-hand with the high prevalence of suicidal thoughts (50%) and suicidal attempts (25%) in individuals with OCD (American Psychiatric Association, 2013). Depression often reflects abnormality of the serotonergic system, same as with theories on OCD, and there tends to not be any negative impact of depression on the effective treatment of OCD with SSRIs (Boer, 1997).

There are two generations of antipsychotics: typical and atypical. Typical antipsychotics were first introduced for schizophrenia and tic-type disorders, but were soon replaced by atypical antipsychotics due to their alarming side effects. A prominent side effect of typical antipsychotics is tardive dyskinesia, involuntary and repetitive movement. Many describe this side effect as Parkinson-like symptoms, which is also a reason research started focusing on the sites of action of these typical antipsychotics to treat Parkinson's disease. Atypical antipsychotics became more commonly used because of their lessened side effects. The use of atypical antipsychotics is commonly for those who had no symptom reduction on SSRIs, treatment-resistant OCD. For these cases, an atypical antipsychotics is used in conjunction with an SSRI. Atmaca, Kuloglu, Tezcan, & Gecici (2002), studied this interaction of joint medication and found that those who

received both medications had at least 60% or greater improvement on the Y-BOCS and Clinical Global Impression scales. Combination therapy was found to be more beneficial to those with symmetry/ordering and hoarding symptoms, than other subtypes of OCD (Matsunaga, Nagata, Jayashida, Ohya, Kiriike, & Stein, 2009).

Treatment studies also have focused with medication using other neurotransmitter systems. Not all obsessions and compulsions, such as hoarding, are associated with a positive response to SSRIs (Mataix-Cols, Rauch, Manzo, Jenike, & Baer, 1999). Compared to a placebo, pramipexole, a dopamine receptor antagonist, was found to decrease exaggerated cingulate error signals in OCD individuals, decreasing difficulty in self-regulating behavior (Murray et al., 2017). Serotonin-norepinephrine reuptake inhibitors (SNRIs), which block the reuptake of both serotonin and norepinephrine, also have been prescribed to treat OCD symptoms, as well as depression and chronic pain. Pigott and Seay (1999) conducted a meta-analysis of treatments for OCD and found that clomipramine, a widely prescribed SNRI, had a higher efficacy than SSRIs in multiple studies. However, the adverse effects, such as anticholinergic side effects, can outweigh the benefits for some subjects; thus, SSRIs are more commonly prescribed. New innovative treatments are currently proposed aside from medication. Recently, Fluvoxamine was given to 23 mild to moderate OCD participants. A similar number received saffron, a type of spice from the Middle East. The Y-BOCS was used to test the individuals periodically and at the end of the study both the SSRI group and the saffron group achieved similar symptom reduction (Esalatmanesh et al., 2017).

Behavioral techniques are often co-administered to those on OCD medication to build coping mechanisms for the compulsive nature of the disorder. Similar to how some compulsions are resistant to certain medications, the same can be said for behavioral treatments. Cleaning and checking compulsions respond better to behavioral treatment.

This is not as effective for symmetry, hoarding, or multiple compulsions (Ball, Baer, & Otto, 1996). Cognitive-behavioral therapy (CBT) has become the chief behavioral treatment for multiple mental disorders in the past couple of decades, including OCD. CBT focuses on short-term goals and hands-on psychotherapy to change patterns of thinking or behavior. For OCD, exposure to response prevention (ERP) has shown efficacy in constructive treatment. The International OCD Foundation page on ERP (2018) defines ERP as exposing the OCD individual to the thoughts that initiate one's obsessions and forcing the individual to not follow through with the compulsive behavior. This can break the feedback loop. Long-term treatment with ERP has shown constant effectiveness, suggesting that ERP benefits are highly durable over time (McKay et al., 2015).

Social support is also found to be significant for the treatment for OCD. This is particularly seen in family accommodation, where family members help the patient avoid triggering situations or help the individual complete activities to reduce stress. Family accommodation treatment focus has led to a decrease of scores on the Y-BOCS with a faster treatment response than for those on ERP alone (Thompson-Hollands, Abramovitch, Tompson, & Barlow, 2015). Social support can be less successful for some subtypes of OCD individuals who score high on the Pragmatic Rating Scale (PRS). Interestingly, those with high PRS scores tend to have a different chromosomal trait than those with low PRS scores (Samuels et al., 2014 It is important to note that because OCD is a chronic condition, not receiving treatment usually results in low remission rates (20% at 40 years later) compared to those who receive treatment (American Psychiatric Association, 2013).

Animal Models of OCD

Rodent models have been used for the past several decades to further specify what receptors are responsible for impulsive behaviors. While rats may not provide much insight into obsessive thoughts, which is mostly a unique human quality, animal models offer multiple ways to analyze compulsive behaviors. Animal research has been important to the medical and psychological fields because it is a way to test drugs and treatments safely before testing on human subjects. Rodents are especially beneficial due to their fast breeding cycle, access of multiple strains, and ability to control their environment. Of course, validity limitations exist in the behavioral study of rodents. A meta-analysis on animal models determined that to receive higher validity in comparison to human traits, multiple test batteries in animal models should be used to define a single behavioral phenotype in humans. There is also a need for observable response patterns, such as fear or anxiety, rather than a specific symptom to form endophenotypes (Steimer, 2011). Interaction with marbles and burying behavior serve as a definable operations of compulsive behavior, rather than relying on marble-burying on its own.

Validation criteria for animal models are important factors for proper testing technique. Willner (1986) highlighted three specific types of validity that all animal researchers should attempt to account for to mimic human behavior: face validity, construct validity, and predictive validity. Face validity is defined as the phenomenological similarity of the animal model and the human symptoms. In animal models of OCD, obsessions cannot be studied but compulsive behaviors can. Constructive validity focuses on how animal behavior mechanisms are similar to human behavior mechanisms. Anxiety is an easier subject to model than depression because anxiety symptoms are easily observable in both animals and humans. The final category is predictive validity. The alleviation of specific symptoms through one treatment should

then lead to symptom alleviation in a similar treatment. Alonso et al. (2015) believed predictive validity was highly important because it can influence the rate of constructive validity. While animals do not necessarily model human symptoms of mental disorders, there is validity in behavioral similarities, such as excessive grooming or inability to stop binge eating or drinking (Alonso et al., 2015).

Despite OCD models in animals being a newer field of study, psychologists have developed multiple ways of assessing compulsive behavior similar to what they have done with anxiety models. Albelda and Joel (2012) compared the leading behavioral models of OCD in animals and what pharmacological interventions effectively alleviate symptoms in rodents. One of the most prominent models of OCD in rodents is the signal attenuation model. The idea of this model is that there is a flaw in the feedback of goal-directed responses. The rodent is trained to press a lever for food along with a stimulus, the stimulus is then presented without the reward of food. The rodent continues to press the lever because the stimulus is continued. Therefore, normal extinction does not occur. The rodent would be considered non-compulsive if the lever response goes through an extinction phase despite the conditioned stimulus still being presented. The validity of the signal attenuation model is based on the decrease in compulsive lever-pressing after doses of medication used to treat OCD symptoms (Albelda & Joel, 2012).

There also may be a chemical component to OCD-type behavior. Kriess et al., 2013 found that using meta-chlorophenylpiperazine (mCPP), a serotonergic agonist, to produce ritualistic behavior such as excessive chewing or specific pauses in searching rituals. Another inducement of compulsive behaviors is when rodents are given tricyclic antidepressants in combination with excessive sucrose pellets. The study by Freund et al. (2015) found that Sprague-Dawley rats binge-ate sucrose pellets when given fixed-ratio feeding for five days without any food deprivation. The binge eating is believed to mimic

the compulsive habits of repetitive human behaviors that are not due to resource availability. The study also found that the female were two-to-six times more likely to binge than male rats (Freund et al., 2015).

A D2 and D3 receptor agonist, quinpirole, caused a compulsive behavior in rats that was similar to binge eating. Frederick and Cocuzzo (2017) injected rats with quinpirole and left them in a cage with adequate water that they had been earlier trained to press a lever for. Compared to the control rats that did not receive the dopamine agonist, the medicated rats would drink an exaggerated amount of water. The same rat groups were then trained to know that water would only come after every other lever press. The rats that received the dopamine agonist learned the new response more quickly than the control group. This is considered contrafreeloading in literature and can be traced back to a study by Inglis, Forkman, and Lazarus (1997). The behavior is seen as adaptive because the source of food, or in this case, water, might not be available later (Inglis, Forkman, & Lazarus, 1997). The ability to compare contrafreeloading to human behaviors might point to OCD developing from environmental contingency changes (Frederick & Cocuzzo, 2017).

Another prominent animal model for OCD, and what the present study uses, is defensive burying. Boer and Koolhaas (2003) define defensive burying as moving bedding material by forepaw pushing movements directed at a threat. Defensive burying was first discovered by Hudson (1950), who noticed that rats buried harmful objects under bedding. He was also able to replicate the same finding with squirrels, mice, and hamsters. Despite Hudson first noting the behavior, the phrase was not coined until 1978 when Pinel created the shock-probe defensive burying paradigm. This method consisted of startling the mice with a probe, which would, in turn, increase extensive burying. Londei, Valentini, and Leone (1998) tested how invasive a stimulus needed to be to

trigger defensive burying. He first started by placing a noxious live small scorpion in the cage with the mice, who then attempted to bury the scorpion as a defensive mechanism. Then they wanted to test a non-noxious stimulus, something that posed no threat. For this, he placed glass marbles in the cage, which the mice ended up burying without prompting, even over multiple exposures to the marbles. This could suggest that the action stopped being investigative and started being compulsive (Londei et al., 1998).

Defensive burying became a model of interest for different anxiety studies. One study wanted to further the shock-probe paradigm by assessing the marble burying behavior over an extended period. They used a single shock exposure on a rat and then set up a marble-burying task 28 days later. The rats that had been traumatized through shock became hyper-vigilant and readily buried the unfamiliar marbles (Mikics, Baranyi, & Haller, 2007). The idea that marble burying was an anxious response to a stimulus was perpetuated over the years by multiple drug studies. As far back at 1985, diazepam (Valium), a benzodiazepine drug used to treat anxiety and seizures, decreased defensive burying of a novel stimulus (Treit, 1985). Multiple other anxiolytic drugs also were found to decrease defensive buying. However, because many anxiety drugs fall under the category of SSRIs, it is important to note the medication could be treating the compulsive symptoms. Anxiolytics and anticonvulsant drugs do not always alleviate OCD symptoms in humans, meaning that defensive burying does not fall completely under an OCD. Atypical antipsychotics have been more recently tested in rat OCD models because they consistently resulted in decreasing compulsive behavior (Matsushita et al., 2005; Takeuchi, Yatsuhi, & Yamaguchi, 2002).

Even though anxiety medication decreases marble burying in multiple studies, there are also several reasons the rodents' behavior is more compulsive than it is anxious. Because rodents are not afraid to approach the marbles, it may not be an anxious

approach, but an innate behavior. It could be suggested that the behavior might be species-specific. Categorizing defensive burying as an anxiety model met with some difficulties as studies continued. Not only did pre-housing a rodent with the marbles have no decreasing effect on marble interaction, habituation to the marbles over multiple occasions did not decrease interaction either. Pharmaceutically, diazepam increased marble burying, but inversely, agents that normally increase anxiety did not decrease burying (Njung'e & Handley, 1990). Thomas et al. (2009) put together an extensive test of marble burying with different scenarios of testing. They found that repeated exposure to the marble-burying task over five days did not decrease the number of marbles buried, and they found that repeated exposure of the task on the same day slightly increased over time before decreasing at the fourth trial. A common animal model to test anxiety disorder is the open-arm task in which a rodent would stay in the covered area as much as possible to decrease anxiety (Thomas et al., 2009). But when Thomas et al. gave mice larger space, so as to not have to interact with the marbles, they did not avoid marbles as they would with noxious stimuli

Researchers have also been interested in related behaviors besides just burying. This started when research started focusing on rats instead of mice. Hayashi, Kuratani, Kinoshita, and Hara (2010) created five parameters to further expand the marble-burying task: digging, latency to digging, exploration around the marbles, rearing, and locomotor activity. The drugs their study used were fluvoxamine (an SSRI), bupropion (a noradrenaline and dopamine reuptake inhibitor), imipramine (a tricyclic antidepressant), and diazepam (a benzodiazepine). Each of the drugs was found to impact the non-burying parameters, but they did not always impact burying activity (Hayashi, Kuratani, Kinoshita, & Hara, 2010). Medication that decreases burying does not always decrease other interactions with the marbles.

Another rat study was having trouble replicating marble-burying behaviors seen in mice. The researchers noticed that their rats were interacting and playing with the marbles rather than burying them. This type of marble interaction behavior had not been noted in other mice marble burying studies. Because of this unusual behavior, they developed a secondary scoring of the marble burying exercise to further assess marble related phenotyping. The behaviors viewed were exploration of the cage, marble interaction, self-grooming, inactive periods, and digging (Ku et al., 2016). Another reason these extra parameters were used in their scoring was the acknowledgment that covered marbles could have been caused by the rats walking on top of them and pushing them under unintentionally. The Ku et al. (2016) study found differences of Sprague-Dawley rats interacting with the marbles more than Long-Evans rats did. This thesis has decided to use Lewis rats due to their more reactive and fearful personality compared to other strains of rats (Ramos et al., 2002).

The layout for marble-burying tasks is almost completely consistent to studies researched for this thesis. Most studies have focused on mice, and in more recent times, rats. Marble burying has become a chief study of OCD because of its ease of access and low financial resources needed. Locomotion is tested in most studies to verify whether a difference in the number of marbles buried is due to symptom reduction or sedative effects of the drugs. Unlike other anxiety disorder studies, it is important to not startle the rats and to increase handling to decrease symptoms of anxiety. Of the studies assessed, 30 minutes has bene the general amount of time used for rats in the testing cage. Previous experiments usually included habituation periods to the room or the testing cage to further reduce anxiety and increase of familiarity to the testing situation (Angoa-Perez et al, 2013; Matsushita et al.; Ichimaru, Egawa, & Sawa, 1995.; Saadat et al, 2006).

Habituation is a primary focus in marble-burying tasks to compare it to a compulsive behavior rather than an anxious one. Because a rat is exposed to the same stimuli each test period, overtime the stimulus should become less novel and more non-threatening (Thomas et al., 2009). Pre-tests and post-tests were not found in local literature when assessing drug efficacy. This current study uses a pre-test baseline to focus on individual differences. An anxious response would show an instant fear or avoidance of marbles. To clarify, when rodents in one study were given the marble burying tasks for 10 consecutive days, most marbles were still being buried throughout the entire week. This could suggest that the burying is not determined by novelty (Poling, Cleary, & Monaghan, 1981). Multiple exposures to the marbles have not shown any decrease in burying or digging due to time only (Londei et al., 1997; Njung'e et al., 1990). A common aspect of human compulsive behavior is the lack of decrease in unnecessary actions, despite proof that there is no logical threat. Rodents continuing to bury marbles despite continuous stimulus exposure follows this behavioral pattern in humans. Londei's study found that burying and digging behaviors stayed stable throughout the week and marble interactions only decreased over time with the female rats (Londei et al., 1997).

Consensus over the past couple decades of research is that, for the most part, defensive burying produces reproducible results. But where marble-burying is strong in replication, it does lack in its ability to operationally differentiate between compulsive and anxious behavior. For instance, not all medication that is useful against OCD symptoms in humans, such as riluzole, effects defensive burying behavior (Albelda & Joel, 2012). Burrowing behaviors may also alter the effects of marble-burying. Because mice and rats are naturally burrowing creatures, burying marbles through digging may simply be fulfilling the innate drive that rodents have, more than because they are a novel

stimulus (Thomas et al., 2010). Whether anxiety and antidepressant drugs relieve marble burying still requires more research on the task and a more specific drug route on receptors studied in both animals and humans.

The Serotonergic System

Serotonin, 5-hydroxytryptamine (5-HT), is a well-known monoamine neurotransmitter that plays multiple roles in the central and peripheral nervous system. Synthesized from the amino acid tryptophan, 5-HT plays roles throughout the entire body. While the present study focuses primarily on the 5-HT in the brain, it is important to take into account the role of 5-HT in the periphery. Most serotonergic receptors are found outside the central nervous system. Almost 90% are found in enterochromaffin cells of the gastrointestinal track. There, it helps regulate intestinal movements (Berger, Gray, & Roth, 2009). When serotonin is released from the enterochromaffin cells, it filters into the blood and is stored on blood platelets. There it has vasoconstrictive properties on vascular smooth muscles (Vanhoutte, 1987). Serotonin has more recently been found to regulate bone mass. In an animal study, mice were depleted of 5-HT in the gut, resulted in an increase in bone density (Modder et al., 2010). A final important role outside of the central nervous system is in the blood. Blood platelets store 5-HT from which they are involved in three different roles. Serotonin promotes the release of nitric oxide, which plays a role in insulin secretion and angiogenesis (McDuffie, Motley, Limbird, & Maleque, 2000). The stored 5-HT is also activated when platelets bind to damaged tissue to stop bleeding. Platelet 5-HT plays an additional role of fibrocyte mitotic growth factors to aid in healing (Marieb, 2009).

In the central nervous system, the raphe nuclei are the primary source of 5-HT release in the brain (Frazer & Hensler, 1999). The raphe nuclei are found along the midline of the brainstem and make up the center of the reticular formation, an important

brain area for waking, alertness, pain, cardiovascular control, and motor control. This is also where habituation to stimuli occurs (Binder & Hirokawa, 2009). While there are nine raphe nuclei, they are typically grouped in literature for purpose of discussion. Raphe nuclei are broadly grouped in the caudal raphe that projects 5-HT to the brain stem and spinal cord, and the rostral raphe project 5-HT to the forebrain (Jacobs & Azmitia, 1992). Having 15 receptors subtypes helps explain some of the multiple roles 5-HT plays in behavioral and emotional effects. Because of this wide range of function, medications that alter serotonergic systems are often used to treat mental disorders such as anxiety, depression, and schizophrenia. Serotonin is also involved in the regulation of multiple hormonal secretions. For instance, the hypothalamus uses 5-HT for partial control of adrenocorticotropic hormone (ACTH), prolactin, and growth hormone that is released from the pituitary (Frazer & Hensler, 1999). It promotes wakefulness and is important for suppressing rapid eye movement (REM) sleep. REM sleep is inhibited through the activation of inhibitory 5-HT_{1A} receptors in the ventrolateral preoptic area (VLPO) (Gallopin et al., 2000). Serotonin inhibits REM sleep through post-synaptic inhibition of brain stem cholinergic neurons (Boutrel, Monaca, Hen, Hamon, & Adrien, 2002). It is also a precursor for melatonin which plays an important role in circadian rhythms. In a final note, 5-HT is inhibitory in the effects of light in the lateral geniculate complex, which in turn resets the electrical rhythm of those cells (Frazer & Hensler, 1999).

The receptors for 5-HT are found on the cellular membrane of nerve cells. All receptors are coupled to a G-protein, except for one (5-HT_3) which operates a ligand-gated ion channel. The G-protein coupled receptors are activated when a ligand, 5-HT or a drug that mimics 5-HT, binds to the receptor and activates a G protein through hydrolysis bonds transforming GDP (guanosine diphosphate) to GTP (guanosine triphosphate). The G protein will split and trigger a signal pathway within the post-

synaptic cell that either increases or decreases intracellular levels of cyclic adenosine monophosphate (cAMP) or inositol trisphosphate (IP_3) and diglyceride (DAG) in the case of 5-HT_2 receptors. The process is imperative to understand because a drug can act as an antagonist by either binding to the active site (competitive) or to a different site that determines the magnitude of the response (non-competitive), also called allosteric antagonism (Neubig, Spedding, Kenakin, & Christopulos, 2003). Inverse agonists have a similar effect even on unstimulated cells. An agent is considered an inverse agonist when it binds to the same receptor as an agonist but causes a response opposite to the natural ligand (Nutt et al., 2017).

Since selective serotonin reuptake inhibitors are an important class of drug used in the treatment of OCD, understanding the functional roles of 5-HT is important in determining the mechanisms of OCD. SSRIs can alleviate obsessions, while other psychotropic medications do not. Therefore, 5-HT likely plays some type of biological role in the disorder. Repetitive behaviors and thoughts in adults with Autism Spectrum Disorder have also been reduced by SSRIs such as fluvoxamine, moreso than selective norepinephrine reuptake inhibitors such as desipramine (McDougle, Kresch, & Posey, 2000). Four different SSRIs – clomipramine, fluoxetine, fluvoxamine, and sertraline – all alleviated OCD symptoms more than placebo, despite different 5-HT receptors being affected (Greist, Jefferson, Kobak, Katzelnick, & Serlin, 1995). While decreasing 5-HT uptake to increase duration of synaptic actions plays a beneficial role in treatment, excess 5-HT can actually increase symptoms. The drug mCPP, a 5-HT agonist, exacerbated symptoms of compulsive behaviors compared to placebo, and even those not diagnosed with OCD also exhibited behavioral changes after mCPP (Zohar, Mueller, & Insel, 1987).

The relationship between OCD and 5-HT_{2A} has been found during the past several decades to be relatively important. The 5-HT_{2A} receptor is a mostly excitatory one and is

a key target in the study of psychedelic drugs such as LSD. This also led to the discovery of the same activity in 5-HT_{2C} (Egan, Herrick-Davis, Miller, Glennon, & Teitler, 1998). Because 5-HT_{2C} plays a similar role to 5-HT_{2A} when it comes to LSD reactions, there may also be a similarity between the two in compulsive behaviors. In mice, the 5-HT_{2A} receptor has been found to inhibit conflict anxiety paradigms but not depression or fear conditioning, both of which do not occur in OCD (Weisstaub et al., 2006). Furthermore, in impulsivity studies, antagonists of 5-HT_{2A} have shown to decrease impulsive behaviors in rats (Winstanley et al., 2004), which could be relevant to impulsive behaviors acted out by OCD patients. 5-HT_{2A} is also found to have a role in the regulation of mood, sex drive, appetite, and even disorders such as Alzheimer's disease and schizophrenia (Herth & Gitte, 2018).

Because of 5-HT_{2A} prominence in the hippocampus, studies on cognition and memory have been of interest. When Zhang and Stackman (2015) studied the cognitive role of this receptor, they found that the activation of 5-HT_{2A} receptors elicited visual hallucinations and facilitated fear-extinction. Therefore, if marble-burying was a fear or anxiety response, we would expect that increasing 5-HT_{2A} activation would decrease their fear and that blocking these receptors would increase fear and anxiety response. The blockage of this receptor also decreased memory retrieval in rats, which could possibly be related to novelty habituation (Bekinschtein, Renner, Gonzalez, & Weisstaub, 2013).The 5-HT_{2A} receptor also seems to play a role in the autonomic nervous system, especially in the nucleus tractus solitarius and the cardiac sympathovagal system (Kermorgant, Pavy-Le Traon, Senard, & Arvanitis, 2018).

There appears to be a wide localization of 5-HT_{2A} in the cerebral cortex, with some areas showing more prominence in healthy individuals than others. Upper regions of the CNS such as the entorhinal and piriform cortex showed larger localization, as well

as the olfactory bulb. There are intermediate density clusters in the limbic system and basal ganglia (Raote, Bhattacharya, & Panicker, 2007). The limbic system receptors have been a point of interest because of their influence on emotional regulation; however, some studies found no significant difference in receptor density there in OCD patients compared to healthy controls (Simpson et al., 2011). PET scans of those OCD patients found a significant reduction of 5-HT_{2A} receptor availability in the frontal, dorsolateral, and medial frontal cortex and well as the temporal associative cortex. Clinical severity seemed to be linked to availability in the orbitofrontal and dorsolateral frontal cortex (Perani et al., 2008). Dense clusters of 5-HT_{2A} receptors are found on the dendrites of pyramidal cells of the frontal cortex in other studies. Astrocytes, with a possible role in schizophrenia, also have an abundance of 5-HT_{2A} receptors (Xu & Pandey, 2000).

Agonists of the 5-HT_{2A} receptor, such as psychedelic drugs create hallucinogenic effects through the pyramidal cells of the prefrontal cortex. They also enhance dopamine binding, which can alter attention and learning (Bortolozzi et al., 2005). The 5-HT_{2A} receptor agonists activate receptors mostly in the frontal cortex, which are important for planning behaviors, another common deficit in OCD patients (Nichols, 2009). In studying how these receptors are involved in behavioral problems, agonists are not as commonly investigated because of their psychotomimetic or LSD-like properties. For instance, the HIV antiretroviral drug, Efavirenz, causes night terrors and hallucinations (Gatch et al., 2013). More research is currently being directed toward LSD-similar drugs and their impact on drug-resistant depression. Inverse agonists, agents that bind to a receptor and cause an opposite reaction than a normal ligand, have many uses in the medical industry. Because of an inverse agonist property to decrease activity below basal levels, these drugs have been used for stopping overactivity in certain area of the brain. Naloxone has been used for decades for opioid overdoses, as well as in combination with opioids to

reduce the risk of misuse (American Society of Health-System Pharmacists, 2018). Inverse agonists have been commonly used in conjunction with other medication as well.

Antagonism to 5-HT_{2A}, as well as dopamine receptors, defines how atypical antipsychotic drugs play a role in increased dopamine release and 5-HT_{1A} activation (Ichikawa et al., 2001). In the past, these drugs have successfully treated therapy-resistant schizophrenia, especially the negative symptoms. An additional group of atypical antipsychotic drugs antagonizes 5-HT_{2A} receptors. YM992 is a newer atypical antipsychotic that inhibits 5-HT uptake and blocks activity of the 5-HT_{2A} receptor. When tested with rats, this medication decreased marble burying without affecting locomotion. Up to 15mg/kg could be used without inhibition qualities; the larger the dose, the fewer marbles buried (Takeuchi, Yatsugi, & Yamaguchi, 2002). Another atypical antipsychotic, perospirone, which is a D2 receptor and 5-HT_{1A} receptor agonist and a 5-HT_{2A} antagonist, also decreased marble-burying behavior without impacting locomotor activity; however, that might be due to the 5-HT_{1A} receptor (Matsushita et al., 2005).

Inverse agonists of the 5-HT_{2A} rector were originally studied for effects on sleep disorders. Nelotanserin, or APD- 125, made its way into phase two of clinical drug trials in 2008, but development ceased when it wasn't reaching the appointed endpoints (Rosenberg, Restifo, Yang, Morgan, & Dudley, 2008). Around the same time, Sanofi Aventis was testing eplivanserin (SR-46,349) for insomnia as well, but it did not progress beyond a phase two trial (Teegarden, Shamma, & Xiong, 2008). Volinanserin (MDL-100,907), a drug with 100-fold or greater selectivity at 5-HT_{2A} receptors, was created by Sanofi Aventis to treat insomnia and went through phase three trials before development was closed. During the trial, the drug was used to assess other conditions. When provided in conjunction with the SSRI fluoxetine, antidepressant activity was noted, as indicated by higher reinforcement and response rates, at a higher rate than either drug alone

(Marek, Martin-Ruiz, Abo, & Artigas, 2005). Volinanserin also seems to alleviate nicotine withdrawal syndrome, as shown by Gadam (2016). When rats were given a dose of 1mg/kg, withdrawal signs such as gasps, teeth chattering, and tremors decreased significantly.

Acadia Pharmaceuticals developed their own 5-HT_{2A} inverse agonist, called pimavanserin (ACP-103). The drug is different than other antipsychotics because it both has a 40-fold selectivity for 5-HT_{2A} but also has no significant affinity to 5-HT_{2B} receptors or dopamine receptors (Friedman, 2013). Some studies have found that it increases release of dopamine in the medial prefrontal cortex (Ichikawa, Huang, Prus, & Dai et al., 2005). Pimavanserin was evaluated in clinical trials for treating psychosis in those with Parkinson's disease. The clinical drug trials were so effective that the United States Food and Drug Administration (FDA) designated pimavanserin a Breakthrough Therapy status to expedite drug development. When the drug was approved by the FDA in 2016, they added approval for the treatment of schizophrenia, which correlated with previous literature on schizophrenic symptoms associated with the 5-HT_{2A} receptor. Pimavanserin was also the first 5-HT_{2A} inverse agonists to show benefits for sleep cycles (Abbas & Roth, 2008).

Other studies have explored whether pimavanserin could be used for disorders outside of Parkinson's Disease. Gardell et al. (2007) found that pimavanserin increased the potency of haloperidol, an antipsychotic used for the treatment of schizophrenia, and decreased head-twitching. However, another study had trouble replicating the haloperidol interaction, but did find that a similar effect with another antipsychotic, risperidone (Meltzer et al., 2012). Another neurological disorder that sometimes induces psychosis is Alzheimer's disease. In rat model of Alzheimer's disease, pimavanserin prevented head twitches (Price, Bonhaus, & McFarland, 2012). Because of pimavanserin's sedative

features in psychosis, it could be considered a possible treatment for anxiety-like symptoms. Hughes (2017) used an elevated plus maze to measure anxiety symptoms in rats and was unable to find significant relief from anxiety symptoms in rats after injections of 3 mg/kg. Because OCD is no longer believed to be solely an anxiety disorder, it was important to assess the role 5-HT_{2A} plays in compulsive behavior

Hypothesis

The purpose of this study was to assess pimavanserin's role in marble burying, a widely used OCD animal model. Since similar 5-HT_{2A} antagonists decreased the amount of marbles buried over time in previous literature (Takeuchi, Yatsugi, & Yamaguchi, 2002), it is hypothesized that a 5-HT_{2A} inverse agonist will cause a similar trend and decrease the number of marbles buried compared to a placebo group. Additionally, interactions with marbles will be measured as well, since more recent research indicates these behavioral measures can represent compulsive behaviors (Ku et al., 2016)

The current study hypothesizes that 1) pimavanserin will decrease marble burying, compared to placebo groups, and 2) pimavanserin will decrease the number of marble interactions over time, including sniffing, playing with and lifting marbles.

CHAPTER II: METHODS

Animals

The subjects were 16 male Sprague-Dawley rats (150-300g). All animals were housed in a climate-controlled animal facility with food and water available *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Houston- Clear Lake, and were in compliance with the Guide for the Care and Use of Laboratory Animals, 8th edition.

Drug

Rats were randomly assigned to a control group or an experimental group Experimenters conducting the behavioral task were blinded to the group assignments on days two through five. The experimental group received pimavanserin, ACP-103, (Acadia Pharmaceuticals, San Diego, CA, USA) a selective inverse agonist of the 5-HT$_{2A}$ receptor, at a dose of 1 mg/kg in isotonic saline, injected subcutaneously one hour before introducing the rats to the marble-burying tasks each day. The control group received an injection of equal volume of saline vehicle. Both groups were given a saline-only injection to assess baseline on day 1.

Handling

All of the rats were handled daily for 3 days to decrease the anxiety caused by being held and moved. Rats were habituated to injections by needle pokes two times to decrease anxiety due to injections. After each session, the rats were gently handled before being placed back in their home cages.

Habituation

Two rats were tested simultaneously in groups of two each night, four from each experimental group. Fifty minutes following injections, rats were moved from the injection room to the testing room where they remained in a housing cage for 10 minutes. After 10 minutes, rats were placed in the experimental tub with marbles already present.

Defensive Marble Burying and Related Behaviors

Defensive burying and interaction with unfamiliar objects, marbles in this experiment, were used to evaluate the intensity of compulsive behavior. Two clear 64 liter tubs at a time, 68cm x 45 cm x 29cm, were used for testing, with a cardboard portion separating the two rats from seeing each other. Each tub contained 16 dark blue glass marbles, 2.5 cm diameterevenly spaced at 12.7 cm in between, and placed completely on top of the bedding.

Soft granule animal bedding (Kaytee Products, Chilton, WI) was used. Bedding was reused by the same rat throughout the week to reduce stress caused by novel bedding. Red lights were used so that animals were still visible on camera. Based on results of a pilot study, the typical 30-minute time frame was reduced to 20 minutes, since activity and interaction with marbles markedly decreased following the first 20 minutes.

The experiment was conducted over a period of five days since resistance to habituation to the marbles is one of the reasons this task is considered a compulsive behavior instead of an anxiety-driven behavior. Day one was a baseline data to access the

pre-existing behavior of each rat. Days two through five were experimental days during which the groups received different injections.

Rodent behavior was recorded using an overhead video camera attached to a standard laptop computer. Video files were stored for later for offline analysis.

Scoring

Scoring was done in three separate ways (see Appendix A). The first measure was marble burying. A marble was considered buried if they were 2/3 covered by bedding. There was a key procedure that differentiated this experiment from previous studies. A marble was not counted as "buried" unless the actual burying behavior could be observed on videotape. Marbles that were simply stepped on or pushed under the bedding because of non-deliberate movements, were not counted. The second type of scoring was a behavioral tally that included tallies for specific actions that occurred during 15 second intervals. The parameters scored were (Ku et al., 2016): 1) Exploration – actively exploring the cage, rearing, and looking for escape; 2) Marble Interaction – playing, rolling, lifting, and sniffing the marbles; 3) Inactivity – in which the animal either sleeps or stays in a specific down position for at least 15 seconds; 4) Grooming – cleaning or scratching self for at least 15 seconds; and 5) Digging – using paws or face to either dig into the bedding.

All scoring was completed before the experimental groups were unblinded. Each video was observed and scored twice for behavioral tallies by the same investigator. Marble burying was scored by two independent investigators.

CHAPTER 3: RESULTS

Extremely few marbles were actively buried, when accidental burying merely by rats stepping or lying on the marbles were not counted. However, there were many other interactions with the marbles, including rolling or picking up the marble, unburying a submerged marble, and sniffing the marbles for more than a second of time.

The hypothesis was that pimavanserin would reduce preoccupation with the marbles as measured by time spent interacting with them. To test the acute effect of pimavanserin, the interaction data was analyzed in terms of each animal's change from baseline. This was done to control for pre-existing individual difference in preoccupation with the marbles.

The average change from baseline over the four subsequent trials was compared between the two drug groups (see Figure 3.1). One outlier subject was removed from each treatment group, since its score was located in the extreme 5% of the distribution of scores for its group. The average scores of the control and pimavanserin groups were significantly different, $t(12) = 1.88$, $p = .042$.

To test the acute effect of pimavanserin, the change in seconds spent interacting from baseline to the next day was analyzed by independent subjects t-test. There was a trend for more increase in the controls compared with the pimavanserin group. The control group increased by 17.87 ±10.51 seconds, while the pimavanserin group decreased by 1.62 ± 5.92 seconds. This difference approached significance, $t(14) = 1.62$,

p = .064. When analyzed by the non-parametric Mann-Whitney test, the p value was .052. The daily seconds each rat interacted with marbles was averaged for each experimental group (see Figure 3.2).

There were no significant group difference in the other scoring parameters between groups in grooming, exploration, or digging behavior.

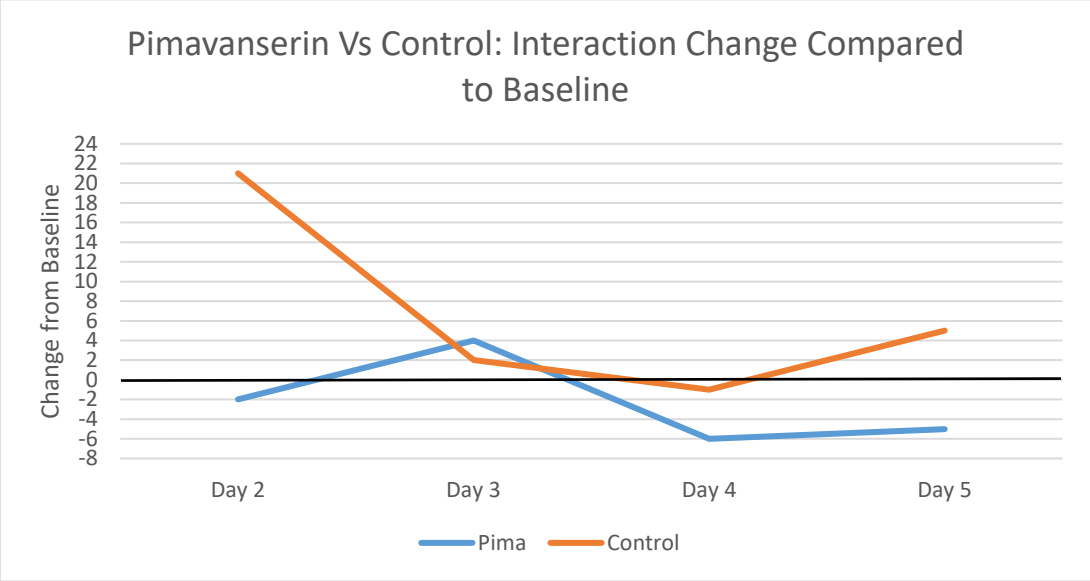

Figure 3.1 Change of Marble Interaction Compared to Baseline

Average change from baseline (horizontal black line) for rats injected i.p. with 1 mg/ml pimavanserin (blue line) and control rats receiving isotonic saline injection vehicle alone (orange line).

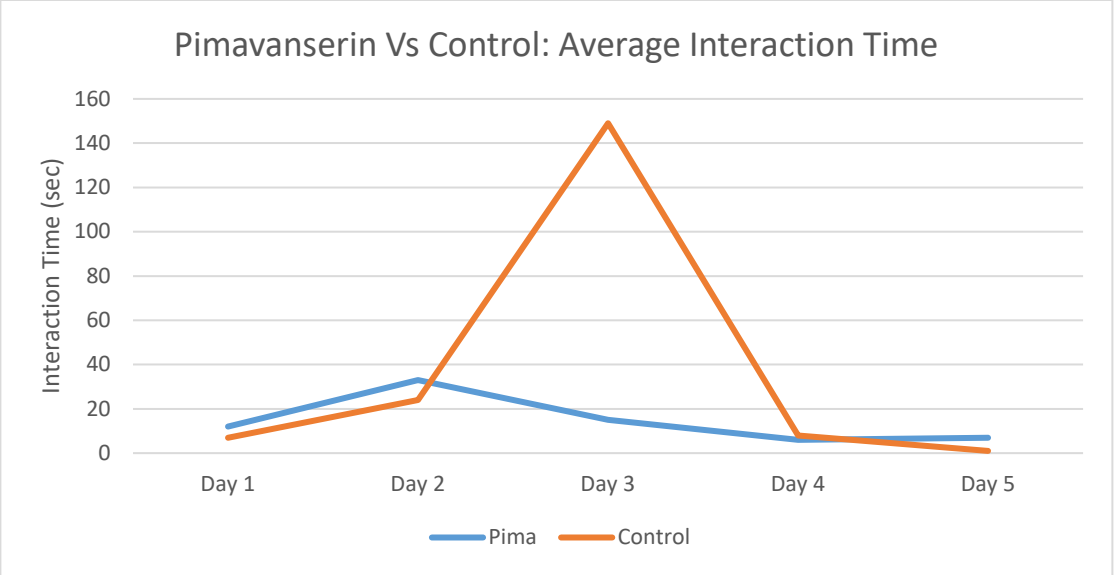

Figure 3.2 Average Time of Marble Interaction

Mean time rats spent interacting with marbles for rats injected i.p. with 1 mg/ml pimavanserin (blue line) and control rats receiving isotonic saline injection vehicle alone (orange line).

CHAPTER 4: DISCUSSION

The study was unable to replicate the magnitude of marbles buried that previous studies have found. Given the small amount of burying data, no statistically significant effect of pimavanserin could be demonstrated. However, there was a consistent trend in the amount of other behavioral interactions with the marbles. Pimavanserin was found to decrease the time spent interacting with the marbles while the control group increased their interaction. While there was no consistent trend of inactivity in the control group, the rats injected with pimavanserin decreased their inactive activity over time. Marble interactions appears to be a more efficient measure in this sort of study for assessing compulsive behaviors in rats compared to marble-burying. One key feature of the current study is the video observation of each rat. This led to the conclusion that most submerged marbles were accidentally, rather than deliberately, buried.

Effects of Pimavanserin on Marble Interaction

What might account for the lack of marble-burying in the present experiment, as compared with previous studies. Multiple factors such as age of animals or environment could be a reason for this, marble-burying might not be as accurate a measure in rats as with mice. The much heavier rats may more readily submerge the marbles accidentally. Burying is considered a compulsive behavior because it is an innate response that does not decrease through repeated exposure to the non-noxious stimulus (Thomas et al., 2009; Njung'e & Handley, 1990; Londei, Valentini, & Leone, 1997).

Is marble interaction a stable measure of a compulsive trait? There was a significant difference between the two groups. Compared to rats in the experimental group, the vehicle-injected control rats actually increased their marble interaction over time. This is consistent with past literature on marble-burying (Thomas et al., 2009), as well as another measure of compulsive behavior in Sprague-Dawley rats (Ku et al., 2016). However, there was no fear response to the marbles in either group; during baseline, none of the rats avoided being near the marbles, in fact, a majority ignored the marbles while exploring their surroundings. When scoring the videos at the end of the week, fewer marbles seemed to be covered toward the end of each 20 minutes observation, although there was not enough consistency to conclusively demonstrate a trend. It could possibly be that fewer marbles were accidently walked upon because of the decreased exploration as rats' habituate to the environment.

Effects of Pimavanserin on Activity Time

Locomotor reactions to the medication we measured by the amount of time each rat spent in exploration and inactivity. Because the time was video-recorded and scored manually, the study scored time rounded to 15-second intervals. Inactivity included lack of locomotion, even if looking around while remaining in one spot, as well as periods of sleep. Rats in the control group showed no significance of being more explorative, nor did they show a more increased form of inactivity. While there was no significance of inactivity within the group, compared to those in the control group, they did have an overall shorter inactive time on average. The experimental group that received

pimavanserin displayed a small significant move toward less inactivity. The lack of inactivity was not correlated with increased exploration or increased grooming.

Implications of Results

When animals and humans are introduced to a noxious stimulus, the autonomic nervous system quickly alerts the body into a fight-or-flight mode. Mice and rats react to threatening stimuli through multiple methods, such as avoidance, freezing, and the use of defensive burying (Vicens-Costa et al., 2011; Njung'e & Handley, 1991). The study in this thesis found none of these reactions. From first introduction to the marbles, the rats showed no avoidance, nor attraction, to the marbles. In fact, most ignored them on the first round and did not attempt to walk around them while exploring their surroundings. In previous open-field experiments, anxious rats commonly froze in their setting and avoided the open areas (Prut & Belzung, 2003). Because the bin being used was translucent around the whole perimeter, the test can be seen as an inescapable open-field setting. If the rats were anxious, they would have had less explorative activity and spent more time in a frozen position.

What separates defensive burying from marble-burying models of OCD, is the perceived lack of threat from the stimulus. The marbles did not carry a threatening smell or taste, nor did they change over time to become more novel. While there was not much deliberate burying occurring in either group, even on baseline, multiple rats became preoccupied with the marbles early on. Instead of being fearful, the rats would roll the marbles around, sniff them periodically, unbury them when they were pushed under the bedding, and even picked them up to move them to a different location. The marble

served no useful or threatening role for the rats, yet interactions in the control group persisted and tended to increase above baseline.

Compared to baseline data, rats that received pimavanserin tended to show less interest in the marbles. There were no significant differences in activity level or episodes of inactivity. Therefore the significant difference in marble interactions suggests that blocking the 5-HT_{2A} receptor decreases repetitive, persistent purposeless behavior. A human behavior that this discovery could implicate is how to possibly reduce compulsive behaviors that do not have a logical purpose. Therapies for fixated cleanliness are far more successful than behaviors with no possible benefits, such as having to touch every street light a person walks past. Interacting with the marbles serves no purpose for the rats that would benefit their survival. There is no way to study obsessive thoughts in rats. However, one might possibly compare their compulsive behavior to that of humans who do not have a reason for their behavior. Just as marble burying does not have a specific phenotype which correlate with human behavior, the same can be said for rodents' marble interactions. However, the differences between the two groups do show somewhat of a connection to the 5-HT_{2A} receptor and studying the 5-HT_{2A} would be a logical step to focus for other OCD animal models.

Relation to Previous Studies

Rat models of OCD have focused on compulsive behaviors because of the inability to assess obsessive thoughts in animals. Marble-burying is one the three staple tests used to measure compulsive behaviors in rats. Our findings were similar to Ku et al. (2016), in that our rats demonstrated less burying of marbles and more interactions with

the marbles. Our rats were rolling the marbles around, placing them in different areas of the bin, digging up previously covered marbles, and even digging themselves under the bedding around the marbles. Because of the supplementary parameters Ku et al. (2016) fashioned, we were able to add on to their research in regards to marble interactions and digging. Our study's use of Lewis strained rats can be compared to their focus on Long-Evans and Sprague-Dawley rats. Their study noted that Sprague-Dawley rats tended to play and interact with marbles more than Long-Evans rats. During their 10-minute baseline, their 12 Sprague-Dawley rats demonstrated approximately the same average of interactions as our own 16 Lewis rats. It is possible that Sprague Dawley rats might be a more efficient strain for marble interaction behaviors than Lewis rats in future studies. The non-pimavanserin control rats in our study persisted in and tended to increase their marble interactions over five 20-minute trials. This demonstrates the persistent, compulsive nature of the marble interaction behavior.

If we consider marble interactions to be a compulsive behavior like their counterpart, marble-burying, we can compare our results to previous drug interactions with marble-burying tasks. Hayashi et al. (2010) used interaction and exploration around the marble in mice to show what behaviors were sensitive to pharmaceutical regulation. Most SSRIs decrease OCD symptoms in humans and have been studied in animal models of OCD. This study also found that when marble burying and digging decreased through use of fluvoxamine and imipramine, so did other marble interactions. Fluvoxamine, buspirone, and diazepam were analyzed in a different mouse study, and all the

medications decreased marble-burying in rats and mice (Ichimaru, Egawa, & Sawa, 1995). This suggests roles for the serotonin and GABA systems in compulsive behavior.

Many atypical antipsychotics have been serotonin/dopamine antagonists used in conjunction with SSRIs (serotonin reuptake inhibitors) for treatment-resistant OCD (Atmaca et al., 2002; Matsunaga et al., 2009). There were two medications that focus on the 5-HT_{2A} receptor and their interaction with marble-burying tasks. Matsushita et al. (2005) found that perospirone decreased the amount of marbles buried in ICR mice without affecting locomotor activity. Perospirone has one site of action at the D2 receptor and blocks the 5-HT_{2A} receptor. This is similar to pimavanserin except that pimavanserin does not affect dopamine receptors. YM992, a specific SSRI that also antagonizes the 5-HT_{2A} receptor, decreased marble-burying behavior as well, without influencing dopamine receptors. To determine whether 5-HT_{2A} is an important contributor in compulsive behaviors, the current study used pimavanserin. This drug has a 40-fold higher affinity for the 5-HT_{2A} receptor than any other receptors. Pimavanserin has been approved by the FDA for Parkinson's disease psychosis. The present results raise the possibility that it might also be useful for obsessive-compulsive behavior.

Limitations of Study

As with most studies, a larger sample size would have been beneficial for this study to lower error rate and pinpoint a more accurate significance in the results. If the sample size was larger, different doses of the drugs could have been assessed to find a lower dose equivalence. Another limitation was the age of the rat. Most literature has used younger rats than those used in the current study. Because of the age, the box size

should have been increased, as some rats attempted to climb out of the cage before cardboard reinforcements were added. Using more assistants to help score would have increased accuracy of results. Using 15-second tallies for actions performed by the rats is less accurate than a computer program analysis and would be highly recommended in future studies. A final limitation would have been the lack of using a locomotor tracker. Locomotion was assessed by time interval tallies of inactivity and exploration, but an electronic system would have been more beneficial and time relieving as well.

Future Studies

Because more recent studies are using repetitive behaviors, such as marble interaction and digging, to assess compulsive behaviors, there needs to be a more specific operational definition for each behavior. Our study focused on parameters already set by Ku et al. (2016), but a separate of each individual behavior would be beneficial for animal models of OCD. Our study struggled noticing marble burying behaviors, and it became a rare occurrence, even in the pilots, compared to previous literature. Future studies might screen a larger sample of rats to select those with a predisposition to bury marbles in their baseline should be used Rats that showed no interest in the marbles would be excluded from the study. We could not do this in our own study due to a small sample size. A handful of studies have focused on male and female rats in marble burying. However, there have not been many studies including marble interaction and digging actions. It also would be helpful to include a comparison between rats and mice, to determine the species difference in burying behavior between these rodents.

Marble interactions and burying are just two of the many animal models of OCD. Other forms of OCD models should be investigated. There are many SSRI drugs that decrease compulsive behaviors in other OCD models that have also been shown to decrease marble burying. For instance, Joel, Ben-Amir, Doljansky, & Flashier (2004) found that both paroxetine and fluvoxamine attenuated or weakened non-rewarded lever-pressing in rats. However, not as much research has been done on OCD models using atypical antipsychotics. Because atypical antipsychotics have been shown to be beneficial for treatment-resistance OCD (Atmaca et al., 2002), there is an important need for more of these drug classes with other OCD models.

Another promising model is “signal attenuation.” A stimulus signals that lever pressing will be rewarded by food. When the food reward is discontinued, many rats persist in responding to the stimulus by lever pressing. It would be interesting to see if pimavanserin would reduce this effect.

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APPENDIX A: SCORING PARAMETERS

Action	Description	Tally
Exploration	Exploring at least 15 seconds (Rearing, looking around, assessing walls)	
Marble Interaction (In Seconds)	Rolling, pushing, lifting, sniffing	
Self-Grooming	At least 15 seconds	
Inactive	At least 15 seconds	
Digging (Tally)	Digging or burying marble or self	
Amount of Marbles Buried	Marbles buried at least 3/4 under	

SECONDS			
Exploration	Marble Interaction	Grooming	Inactivity

Amount of Marbles Under Bedding

APPENDIX B: RAW DATA OF MARBLE INTERACTIONS

Control Group Marble Interactions

Rat	Day 1 (BL)	Day 2	Day 3	Day 4	Day 5
Black Rat 1	3	5	1	1	22
Black Rat 3	13	34	6	5	9
Black Rat 5	4	12	46	11	2
Black Rat 7	7	5	0	8	0
Blue Rat 1	9	6	114	23	0
Blue Rat 2	1	10	11	8	1
Blue Rat 5	9	97	13	1	3
Blue Rat 8*	14	34	58	175	68

Pimavanserin Group Marble Interactions

Rat	Day 1 (BL)	Day 2	Day 3	Day 4	Day 5
Black Rat 2	9	37	51	2	17
Black Rat 4*	11	15	35	136	22
Black Rat 6	12	7	0	0	3
Black Rat 8	18	0	5	6	0
Blue Rat 3	30	3	18	3	0
Blue Rat 4	5	14	4	16	5
Blue Rat 6	4	5	3	11	25
Blue Rat 7	5	0	27	1	1

* Outlier rats removed in SPSS analysis

APPENDIX C: RAW DATA OF MARBLE INTERACTIONS COMPARED TO BASELINE

Control Group

Rat	Day 2 Change	Day 3 Change	Day 4 Change	Day 5 Change
Black Rat 1	2	-2	-2	19
Black Rat 3	21	-7	-8	-4
Black Rat 5	8	42	7	-2
Black Rat 7	-2	-7	1	-7
Blue Rat 1	-3	105	14	-9
Blue Rat 2	9	10	7	0
Blue Rat 5	88	4	-8	-6
Blue Rat 8*	20	44	161	54

Pimavanserin Group

Rat	Day 2 Change	Day 3 Change	Day 4 Change	Day 5 Change
Black Rat 2	28	42	-7	8
Black Rat 4*	4	24	125	11
Black Rat 6	-5	-12	-12	-9
Black Rat 8	-18	-13	-12	-18
Blue Rat 3	-27	-12	-27	-30
Blue Rat 4	75	0	15	0
Blue Rat 6	1	-1	7	21
Blue Rat 7	-5	22	-4	-4

* Outlier rats removed in SPSS analysis