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SEROTONIN 1B/1A RECEPTOR MODULATION ON BEHAVIORAL FLEXIBILITY IN C57BL/6J MICE

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A Thesis

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by

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ABSTRACT

Pharmacological activation of the 5-HT1B and 1A receptors has been implicated in OCD-like behaviors in rodents such as increased perseverative circling, checking behaviors, and locomotor stereotypy. However, little is understood about the effects of 5-HT1B and 1A receptor activation on behavioral inflexibility, a common symptom associated with OCD. The present study utilized the 5-HT1B/1A receptor agonist RU24969 at 0.01, 0.1, and 1.0 mg/kg to test three hypotheses. The first hypothesis predicted RU24969 would lead to a dose-dependent impairment on behavioral flexibility in C57BL/6J mice. It was also predicted that male C57BL/6J mice would be more inflexible than female C57BL/6J mice following RU24969 administration. The second hypothesis stated that RU24969 would have a dose-dependent increase in locomotor activity. Finally, it was hypothesized that RU24969 would increase anxiety-like behaviors in C57BL/6J mice. Results concluded that male mice had impaired behavioral flexibility at all doses of RU24969 while female mice were only impaired at the 1.0 mg/kg dose. For locomotor activity, male mice exhibited reduced distance traveled at the 1.0 mg/kg dose while RU24969 had no significant effect on female locomotion scores. Finally, male mice exhibited greater anxiety-like behaviors at 0.1 and 1.0 mg/kg while female mice were not significantly affected. Overall, the evidence suggests that 5-HT1B and 1A receptor activation could play a role in the manifestation of learning impairments associated with core OCD symptoms.

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CHAPTER ONE

OBSESSIVE-COMPULSIVE DISORDER

Obsessive-compulsive disorder (OCD) is characterized by insistent urges, persistent thoughts, and/or repetitive behaviors that are resistant to inhibition (APA, 2013). OCD is the fourth most common mental disorder with estimates of 3% of the adult population currently afflicted (Centers for Disease Control and Prevention [CDC], 2019; Kessler et al., 2005). According to the National Institute of Mental Health (NIMH) (2019), it is estimated that OCD affects one in a hundred adults in the United States, with a higher prevalence in women at 1.8% compared to men at 0.5%, although, men are more commonly diagnosed with childhood OCD (APA, 2013). Roughly 85% of adults diagnosed in the United States report experiencing moderate to severe symptoms (Alegria et al., 2007). OCD also has a relatively high comorbidity rate with disorders such as Tourettes (APA, 2013; Browne et al., 2014). According to the most recent 2003 survey of OCD lifetime prevalence rates, OCD was most prevalent in adults (1.5%) aged 18-29 and remains prevalent throughout the lifespan with the lowest rates (0.5%) in adults over the age of 60 (Alegria et al., 2007; NIMH, 2019). Given that obsessive-compulsive symptoms are seen in as high as 20% of the general population (Fullana et al., 2009), continued efforts to understand the mechanisms of OCD are needed.

Current research suggests that genetic factors directly impact the onset of OCD. Studies have shown significantly higher rates of OCD among monozygotic twins compared to dizygotic twins (Browne, et al., 2014; Eley, et al., 2003). The evidence points to genetic factors for the development of OCD because monozygotic twins share the same genetic code; thus, the prevalence of OCD is higher. Amongst families, the likelihood of inheriting OCD is 50% (Browne, et al., 2014; Mataix-Cols et al., 2013), although environmental factors such as early childhood stress (Adams et al., 2018) and trauma (Badour et al., 2012) have been associated with the development of OCD symptoms.

Over time, behavioral theories of anxiety have attempted to explain both the obsessive and compulsive characteristics that underlie the development, maintenance, and treatment of OCD. The current theory suggests that OCD is brought about by the misattribution of fearful thoughts/feelings to an otherwise neutral stimulus (D'Alessandro, 2009; Kagan et al., 2017). Moreover, a fear response to related and objectively non-threatening stimuli is conditioned over time. Compulsions then develop as a behavior to alleviate the anxious feelings toward potential fear-inducing stimuli. At a foundational level, it is evident that OCD behaviors are a learned response in an attempt to alleviate anxiety which is conditioned throughout development, to the extent that maladaptive compulsions and repetitive behaviors are formed. Moreover, the urge to suppress anxious feelings is reinforced by decreasing anxiety.

In the most recent fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), OCD is considered an anxiety-related disorder by which intrusive obsessions can cause an abnormal increase in anxiety or distress (APA, 2013). To suppress obsessive thoughts, urges, or images, the individual will perform a compulsive behavior (thoughts and/or actions). OCD has a direct impact on the quality of life by which obsessions and compulsions create social and/or occupational hurdles that are difficult to overcome (APA, 2013) and treatment options are limited to anti-depressant medications and cognitive behavioral therapy (Hirschtritt et al., 2017). Along with the psychological determinants of OCD, there are also various neurological theories (structural and functional) explaining the onset and maintenance of OCD that have increased in validity. Neuroimaging studies suggest cortico-striato-thalamo-cortical (CSTC) network dysfunction is thought to be primarily responsible for the neurological basis of OCD (Gao et al., 2019; Posner et al., 2014; Tang et al., 2016). This network is made up of many brain areas and thus further study is needed to further elucidate specific areas of the network that are responsible for OCD pathology.

OCD is a debilitating and prevalent disorder. Current theory suggests that OCD's grounding in anxiety is the reason compulsive symptoms are acquired and maintained. OCD harms an individual's quality of life as obsessions and compulsions can not only be maladaptive for the person with the disorder, but for the people around them as well. Treatment options are limited, yet much can be

learned by studying the neural components that influence obsessive and compulsive behaviors. Further study of the neurological mechanisms behind OCD is necessary to inform potential novel treatments of this disorder.

CHAPTER TWO

NEUROLOGICAL BASIS OF OBSESSIVE-COMPULSIVE DISORDER

Many studies have aimed to understand the neurological determinants of OCD. Prior to the utilization of neuroimaging which highlights possible brain areas responsible for OCD symptoms, pathophysiological studies pointed to the orbitofrontal cortex (OFC) and basal ganglia as areas most likely involved with OCD. With measurements of positron emission tomography (PET), magnetic resonance imaging (MRI), and functional MRI (fMRI), previous theories speculated increased activity in the frontal cortex, including OFC, anterior cingulate cortex (ACC), caudate nucleus, and thalamus (Baxter, et al., 1987; Swedo, et al., 1989; Zohar, et al., 1989). There is also evidence to suggest that OCD patients have differing OFC, hippocampus, and amygdala volumes compared to healthy controls (Atmaca et al., 2008; Kwon et al., 2003; Rao et al., 2018; Szeszko et al., 1999). These imaging studies further demonstrate that OCD is most likely attributed to several brain areas or circuits and not a single brain region. More recent evidence suggests the cortico-striato-thalamo-cortical (CSTC) circuit is involved in the pathophysiology of OCD, as this circuit has been implicated in the disruption of response inhibition and goal-oriented behaviors (Rao, et al., 2018). The OFC, caudate, thalamus (Nakao, et al., 2014), and hippocampus (Rao, et al., 2018) are among brain regions that had show

structural changes relating to OCD diagnosis. Thus, these brain regions are of particular interest when investigating the neural correlates of OCD.

The OFC is among the most commonly discussed brain regions implicated in OCD. The OFC is responsible for executive and spatial cognition (Bryden & Roesch, 2015; Robbins, 2000) including flexible learning and goal-directed learning (Lei et al., 2018; Sadacca et al., 2017). The OFC is of particular interest as patients with damage to the OFC show a similar OCD-like phenotype including impairments in cognitive flexibility (Fellows & Farah, 2003; Ghahremani et al., 2010) and excessive rumination (Grados, et al., 2008). In addition, pediatric OCD patients have abnormally large right OFC white matter volume (Macmaster et al., 2010) and in general, have larger white matter volumes throughout the brain (Atamaca, et al., 2008). Imaging studies show that the OFC is hyperactive in patients with OCD compared to healthy controls (Lagemann, et al., 2012). In mice, induced hyperactivity in the OFC is associated with impairments in behavioral flexibility (Longo, et al., 2018). Overall, a large and hyperactive OFC seems to explain symptoms related to OCD from a neurological perspective.

The hippocampus is also viewed as having a mediating role in the neurological mechanisms of OCD, although, neuroimaging studies show conflicting results regarding the neural correlates in OCD. It has been demonstrated that there is increased left hippocampal volume in patients with OCD compared to healthy controls (Kwon, et al., 2003; Rao, et al., 2018). Also,

recent evidence suggests a negative correlation between left hippocampal volume and rates of compulsivity, with larger volumes associated with higher compulsivity ratings (Rao, et al., 2018). In opposition, reductions in bilateral hippocampal volume in OCD patients compared to controls have been reported in various studies (Atamaca, et al., 2008; Szeszko, et al., 1999). These conflicting findings may be due to differences in pharmacological effects or unknown comorbidity with other disorders. One meta-analysis points out that patients with OCD and reduced hippocampus volume were also shown to simultaneously be receiving pharmacological treatment before neural testing (Boedhoe et al., 2016). This may suggest that larger hippocampal volumes are attributed to untreated OCD and that a reduction in hippocampal volume in OCD individuals is due to pharmacological treatment. In mice, lesions of the hippocampus are shown to increase behavioral rigidity as found with impaired reversal learning performance in different spatial tests (Bardgett, et al., 2003; Kleinknecht, et al., 2012; Rossi, et al., 2012) suggesting that the hippocampus is important for cognitive flexibility and that abnormalities to the hippocampus can increase habit formation.

In fMRI tests, the ACC and caudate have also been implicated in OCD etiology. The dorsal ACC (dACC), which is responsible for reward processing and decision making (Bush, et al., 2000), is shown to have increased functional connectivity to the caudate in unmedicated OCD patients. The dACC has also been shown to be positively correlated with compulsion scores on the Yale-

Brown Obsessive-Compulsive Scale (Y-BOCS) (Zhang, et al., 2017). Higher connectivity between the dACC and caudate compared to healthy controls may be a clinical indicator of OCD. While it has also been reported that there is increased activation between the dACC and prefrontal cortex (PFC) (Yun, et al., 2017). Both network pathways are included in the CSTC circuit which has been repeatedly implicated in the expression of OCD symptoms. Also, activation of the dACC is shown to facilitate fear-conditioned learning (Phelps, et al., 2004) which is thought to significantly contribute to OCD compulsions, while lesions in humans lead to a reduction in OCD symptoms (Dougherty, et al., 2002) further implicating the dACC in OCD pathology. Overall, the results indicate that abnormalities in the circuitry between the ACC and caudate are a part of the neural alteration that contributes to OCD.

Neurobiological models of OCD continue to suggest that a malfunctioning CSTC network is involved in the manifestation of OCD and its symptoms (Menzies, et al., 2008; Saxena, et al., 1998). The CSTC circuit includes the OFC, thalamus, and striatum (Saxena, et al., 1998), all of which communicate during goal/reward-oriented learning (Bradfield & Balleine, 2017). Using optogenetics, repeated activation over days of the OFC and ventromedial striatum has been shown to elicit OCD-like behaviors in mice; specifically, repetitive grooming behaviors (Ahmari, et al., 2013). However, it is worth noting that acute activation did not increase grooming behavior and that to elicit increases in grooming behavior repeated activation was required (Ahmari, et al., 2013). This suggests

that the onset of OCD could be the result of chronic repeating hyperactivation. There is also evidence to suggest that severing the connection between the thalamus and dorsomedial striatum (DMS) impairs reversal learning in rats (Bradfield & Balleine, 2017). Rats with lesions to the thalamus-DMS pathway after the acquisition of a spatial task performed worse after the initial acquisition yet performed similarly to control rats after a second training session (Bradfield & Balliene, 2017). This indicates that the thalamus-DMS pathway may modulate behavioral flexibility. Thus, the CSTC network is important for behavioral flexibility and a dysfunctional CSTC network may lead to impairments in behavioral flexibility which could explain one of OCD's core symptoms.

The CSTC is commonly divided into two networks called "loops." These are the direct and indirect CSTC loops, in which studies have demonstrated that OCD symptoms can be attributed to each loop (Mataix-Cols & van den Heuvel, 2006; Saxena et al., 1998; Saxena & Rauch, 2000). Specifically, the direct loop is responsible for the initiation and continuation of behaviors, while the indirect loop is responsible for inhibiting and changing between behaviors (Mataix-Cols & van den Heuvel, 2006). Therefore, difficulty inhibiting repetitive behaviors may stem from issues with the CSTC circuit. In mice, activation of the CSTC circuit using a soluble cytokine receptor agonist-induced repetitive head "bobbing" and increased locomotor behaviors (Patel et al., 2012). This is supported by the later finding from Ahmari, et al. (2013) that optogenetic stimulation of the CSTC regions increased repetitive grooming behaviors in mice. Hyperactivation of the

CSTC neural loop seems to be a factor for increased OCD-like behaviors in rodents, although, little is understood about the relationship between this CSTC network and behavioral flexibility.

The neurological basis for OCD is quite complex. What is known is that several brain regions and circuits are involved. Irregularities in the CSTC network seem to have the most influence on the manifestation of OCD symptoms, such as excessive grooming and impaired reversal learning in rodents. This, in part, seems to be due to the CSTCs involvement with goal-directed learning. Thus, issues with the CSTC network, such as hyperactivation, could contribute to some of the goal-directed learning impairments like behavioral inflexibility. Overall, structural and functional abnormalities to the CSTC network of brain areas seem to be heavily involved in the neural representation of OCD and must be investigated further.

CHAPTER THREE

SYMPTOMS AND TREATMENTS OF OBSESSIVE-COMPULSIVE DISORDER

OCD presents itself in many ways and symptoms that range in severity. It is believed that obsessions and compulsions do not co-occur; instead, they occur one after the other (Laposa et al., 2019). Also, the DSM-5 indicates that some patients with OCD can experience solely obsessions or compulsions, while both do not need to be present for diagnosis (APA, 2013). Research looking into the symptomology of OCD has identified five separate dimensions of symptoms: obsessions with contamination/cleaning, symmetry/ordering, doubt/checking behaviors, intrusive and unacceptable thoughts, and hoarding (APA, 2013; Brakoulias et al., 2013). Due to underlying anxiety, symptoms are often debilitating and individuals with clinically diagnosed OCD often require pharmacological and/or psychological interventions. Treating OCD is sometimes difficult due to the wide range of symptoms and the lack of available interventions. The most common treatment for OCD is co-administration of a selective serotonin reuptake inhibitor (SSRI) to treat the underlying anxiety and cognitive behavioral therapy (CBT) to address maladaptive and habitual behaviors that impair everyday functioning (Hirschtritt et al., 2017). However, this treatment combination is lacking, as it is a general treatment option for most anxiety-related disorders and thus does not specifically address the obsessive and compulsive symptoms solely attributed to OCD.

Maladaptive fear conditioning leads to obsessive behaviors and excessive habit formation, which are some of the core symptoms of OCD. Habits are formed from repeating behavioral actions over time. Habits can become excessive, leading to disruptions in daily living, which is a core symptom of OCD (APA, 2013). Excessive habit formation can have negative implications for individuals inflicted with OCD and can be seen in both humans and animals (Gillan & Sahakian, 2015; Hadjas et al., 2019). Under normal circumstances in healthy individuals, habits result in automatic responses to various stimuli even if the response does not provide a favorable outcome (Dickinson, 1985). This automatic response can cause individuals with OCD to disregard goal-oriented behaviors in favor of appetitive behaviors (Gillan et al., 2011) which is thought to be a result of the underlying anxiety that OCD entails (Eysenck et al., 2007). It is demonstrated that patients with OCD are more prone to habit formation of avoidance behaviors compared to controls (Gillan et al., 2014). Since habit formation and behavioral flexibility are modulated by the same brain areas, issues with excessive habit formation may create impairments in flexibility. Excessive habit formation is also seen in genetic mouse models of OCD compared to healthy wild-type littermates (Hadjas et al., 2019). Also noted was an impairment in behavioral flexibility, which was believed to be a result of excessive habit formation (Hadjas et al., 2019). Tasks like the Wisconsin Card Sorting Task (WCST) and other reversal learning tasks recruit the OFC to help adapt to differing contingencies (Bechara et al., 2000). Compulsivity scores have

been linked with a lack of connectivity throughout the OFC (Meunier et al., 2012) and since patients with OCD often display neurological abnormalities in the OFC (MacMaster et al., 2010), deficits in behavioral flexibility have been associated with compulsivity (Izquierdo & Jentsch, 2012). Also, rodent studies have shown deficits in the OFC and striatum to induce behavioral inflexibility (Izquierdo & Jentsch, 2012). Overall, behavioral flexibility utilizes areas of the brain that are shown to be dysfunctional in OCD suggesting that compulsivity and impairments to behavioral flexibility are related. The evidence seems to indicate that excessive habit formation creates problems with the ability to be flexible in cognition and behavior.

Compulsions are an attempt to alleviate anxious feelings that are related to the obsessive symptoms in OCD and usually include a ritual that is performed repetitively (APA, 2013; Laposa et al., 2019). Repetitive and maladaptive behaviors are often non-voluntary in which the patient feels forced to carry out a behavior (Robbins et al., 2012). It is also noted that in some cases, compulsive behaviors manifest first in which anxiety becomes a byproduct while in other cases, anxiety is the precursor to compulsivity (Kashyap et al., 2012; Robbins et al., 2012). OCD's underlying anxiety symptoms seem to vary, making it difficult to understand whether anxiety is the root cause or a result of OCD. Much is still unknown about the impact of OCD on cognition. Understanding the types of cognitive deficits related to OCD assists with discovering treatment options for individuals afflicted with this disorder.

The most common pharmacological intervention for treating OCD is the use of SSRIs. According to the American Psychiatric Association, they are used as a treatment and have demonstrated efficacy in many patients by reducing symptom severity (Koran et al., 2007). A more recent meta-analysis analyzing placebo-based clinical trials using SSRIs shows that SSRIs begin to be effective around week six of treatment with most patients reporting SSRIs effectiveness by at least week 12 of treatment (Issari et al., 2016). A commonly used SSRI also used to treat depression, fluoxetine, has shown in a clinical trial to remain effective at reducing OCD symptoms (Tural et al., 2019). Improvements in OCD-like symptoms such as repetitive grooming have also been shown in genetically induced mouse models of OCD (Ahmari et al., 2013; Ullrich et al., 2018). Chronic fluoxetine treatment has also been shown to alleviate obsessive-compulsive behaviors in mice with pharmacologically induced OCD (Ho et al., 2016; Woehrle et al., 2013). SSRIs seem to be the most effective pharmacological treatment currently available. However, these treatments are not perfect solutions as SSRIs target the brain's serotonin levels as a whole instead of acting on specific neural targets. It is shown in rodents that certain 5-HT receptor targets may be responsible for OCD-like behaviors. For example, one study concluded that activation of the 5-HT1B receptor was responsible for inducing impairments in delayed alternation, a test of spatial working memory, and that administration of fluoxetine was able to alleviate the deficits as a result (Woehrle et al., 2013). This example demonstrates that specific serotonergic receptors may be implicated in

some of the cognitive impairments associated with OCD in humans and that the SSRI fluoxetine may alleviate deficits in cognition by inhibiting 5-HT1B receptors.

OCD has many different symptoms, some of which impact cognition in various ways. However, some of these cognitive deficits related to OCD are not widely understood. Since pharmacological options like SSRIs are an important tool used alongside behavioral therapy for treating OCD, the need for novel treatments is high. Further, OCD does not have specific pharmacological therapies designed to treat its unique symptomatic profile. SSRIs are a common pharmacological therapy for various psychiatric disorders including depression, schizophrenia, and autism spectrum disorder (ASD) which leaves much to be gained from discovering novel pharmacological therapies that are specific to OCD and other related disorders. To do this, further investigation of the serotonergic neurotransmitter system would be beneficial so that patients with OCD have a more targeted approach to their treatment.

CHAPTER FOUR

SEROTONIN IN OBSESSIVE-COMPULSIVE DISORDER

As discussed in Chapter Three, the most common pharmacological therapy for treating OCD is a selective serotonin reuptake inhibitor (SSRI). SSRIs are primarily used as an anxiolytic or antidepressant. These compounds work by inhibiting the process of serotonin reuptake to effectively increase serotonin in the brain. While SSRIs like fluoxetine are effective at reducing OCD symptoms, they cannot target specific receptors that may be responsible for some of OCD's most prominent symptoms. A more effective pharmacological approach would be to target specific serotonin receptors that are implicated with specific OCD impairments. The current study focuses on typical memory impairments associated with OCD such as behavioral inflexibility, as well as anxious and repetitive behaviors, and how they are affected by serotonin receptor modulation. It is necessary to identify specific serotonergic targets related to OCD so that future pharmacology can develop novel treatments for OCD-related symptoms.

Serotonergic neurons are relatively limited throughout the brain, and in general, seem to have a large influence on mood, emotion, and sleep (Bear et al., 2016), as well as various cognitive functions (Vadodaria et al., 2018). Serotonin (5-hydroxytryptamine, 5-HT) is a ligand in the amine group of neurotransmitters and is derived from the amino acid tryptophan which enters the brain via blood. Ultimately, tryptophan enters the bloodstream via the gut, as it is consumed via various meats and dairy products (Bear et al., 2016; Vadodaria et

al., 2018). Tryptophan is converted to 5-hydroxytryptophan which is then converted to 5-hydroxytryptamine (Jonnakuty & Gragnoli, 2008). This process is essential for providing the central nervous system with serotonin. Once 5-HT is excreted into the synaptic cleft during synaptic transmission, excess 5-HT is transported back into the neuron to be recycled and used in future synaptic transmissions (Bear et al., 2016). The process of 5-HT reuptake is important for efficiency and to prevent wasted neurotransmitter. However, excessive reuptake of 5-HT can have negative implications.

5-HT is an important neurotransmitter in the brain, and since there is such a small amount in the central nervous system, small alterations to 5-HT levels can have significant effects. Some of the implications of altered 5-HT levels are negative. A lack of 5-HT is thought to be associated with increased anxiety symptoms such as a lack of perceived control and increased interfering thoughts (Hood et al., 2017).

This suggests that decreased 5-HT is related to anxiety, but not depression. Since SSRIs increase overall serotonin levels in the brain, SSRIs are a common treatment option for individuals with anxiety, especially OCD-related anxiety (Albert et al., 2019; Hirschtritt et al., 2017; Romanelli et al., 2014). SSRIs are an important and effective treatment option for individuals afflicted by anxiety disorders like OCD. However, due to the pharmacodynamics of SSRIs, the full extent of their effectiveness is unknown. Consequently, there are mixed findings on the effectiveness of SSRIs when treating anxiety.

The anxiolytic effects of SSRIs seem to differ in the literature. Some results indicate SSRIs act as an anxiolytic. Specifically, rats given fluoxetine had reduced immobility time in the tail suspension test (Kamei et al., 2003). Also, mouse pups separated early from the dam exhibited less anxiety-like symptoms as evidenced by decreased ultrasonic vocalization from the pups treated with fluoxetine (Fish et al., 2004). However, another study demonstrated that increased levels of anxiety in rats (such as someone with an anxiety disorder) resulted in anxiogenic effects following treatment with the SSRI escitalopram as evidenced by increased startle response in an acoustic startle paradigm (Pettersson et al., 2015). In another study, escitalopram and fluoxetine both showed an anxiogenic effect in rats as evidenced by increased latency in an open field during a novelty suppressed feeding test and fluoxetine administration acted as an anxiogenic as evidenced by less time spent in the open arm during an elevated plus-maze task (Turcotte-Cardin et al., 2019). Although, Turcotte-Cardin et al. (2019) found anxiogenic effects of SSRI administration in 5-HT1A receptor knockout rats which suggested that 5-HT1A receptors are somewhat responsible for the paradoxical effects related to SSRI and anxiety. While SSRIs are shown to have mixed effects in reducing anxiety symptoms, it is important to understand the cognitive effects of SSRIs as well.

The cognitive effects of SSRIs are important as many psychiatric disorders including OCD result in cognitive impairment. It has been noted that OCD specifically can result in impairments to behavioral flexibility (Hadjas et al.,

2019; Izquierdo & Jentsch, 2012). Therefore, effective pharmacological treatment for OCD would assist in reducing cognitive impairment. However, the SSRI fluoxetine was shown to impair reversal learning in attention deficit hyperactivity disorder (ADHD) patients but not ASD patients compared to controls; specifically, patients with ADHD receiving fluoxetine committed more perseverative errors than controls (Chantiluke et al., 2015). SSRIs may be treating only certain symptoms and exacerbating others. These findings further support the need to investigate 5-HT receptor modulation as a novel pharmacological treatment for OCD.

One potential target is the 5-HT1A receptor. The 5-HT1A receptor has been identified as a potential 5-HT receptor subtype that is involved in the effectiveness of anti-obsessive-compulsive drugs (Lesch et al., 1991). The 5-HT1A receptor is inhibitory causing downregulation of 5-HT neuron activity and becomes desensitized following chronic SSRI usage (Turcotte-Cardin et al., 2019). Essentially, the inactivation of 5-HT1A receptors should increase neuronal activity in 5-HT neurons. Also, 5-HT1A post-synaptic receptors are mostly located in the hippocampus (Lesch et al., 1991) but they are also expressed in the PFC (Puig & Gulledge, 2011) which may suggest that modulation of 5-HT1A receptors could have cognitive effects such to that of behavioral flexibility, a core symptom of OCD.

Effective pharmacological treatment for OCD would ideally target multiple receptor sites attributed to core symptoms like behavioral flexibility and anxiety.

Another target worth investigating is the 5-HT1B receptor. The 5-HT1B receptor has been identified as a modulator of anxiety disorders and impulsive behaviors (Kent et al., 2002). More importantly, 5-HT1B receptors are dispersed throughout the midbrain with a significant concentration in the hippocampus and caudate (Bonaventure et al., 1997; Kent et al., 2002). Using PET imaging, the 5-HT1B receptor has been shown to have increased binding in OCD patients that displayed deficits in pre-pulse inhibition (Pittenger et al., 2016). This suggests that some of OCDs symptoms could stem from excess 5-HT1B receptor binding. Also, chronic SSRI treatments reduce OCD-like behaviors induced by 5-HT1B receptor activation in the OFC of mice (Shanahan et al., 2011). Further, 5-HT1B receptor activation can induce OCD-like deficits such as hyperlocomotion which was attenuated by chronic SSRI treatment (Shanahan et al., 2009). This evidence adds support for the investigation of the 5-HT1B receptor since SSRIs seem to rescue 5-HT1B receptor activation-induced OCD-like behaviors in rodents.

Overall, the serotonergic system is implicated in the pathophysiology of OCD in both humans and animals. While SSRIs are the pharmacological treatment of choice for patients with OCD, SSRIs can have unpredictable effects, and in some cases, can exacerbate impairments to cognitive abilities such as behavioral flexibility. The paradoxical effects of SSRIs make novel pharmacological treatments a primary concern. As mentioned, modulation of 5-HT1A and 1B receptors demonstrate an association with OCD symptoms which

makes them interesting targets for investigation. To study the behavioral effects of 5-HT1A and 1B modulation, animal models are necessary to draw parallels to OCD symptomology.

CHAPTER FIVE

ANIMAL MODELS OF OBSESSIVE-COMPULSIVE DISORDER

Understanding the altered neural function behind OCD would enable more effective treatment options to become available. Current treatment options are limited, and their effectiveness is not reliable. OCD is commonly studied in humans using existing and/or novel test measurements that probe individuals' levels of OCD behaviors and associate these measurements with other variables like regional brain activity and structural brain imaging. Essentially, the research attempts to find brain differences in patients with distinctive OCD subtypes (clinical OCD, non-clinical OCD, self-diagnosed OCD, etc.) compared with healthy non-OCD individuals. Clinical trials enable researchers to evaluate novel pharmacological treatments for OCD and measure whether the treatments effectively reduce OCD symptoms. Certain species express translational behaviors that are widely accepted as analogs to OCD in humans. This allows for the utilization of animal models of OCD as a proxy for understanding OCD in humans.

Animal models of OCD are an effective method of understanding the neural and biological mechanisms behind OCD. Behavioral inflexibility is one symptom of OCD that is commonly measured in both humans (Gruner & Pittenger, 2017; Lucey, et al., 1997) and animals (Boom, et al., 2019; Eilam, et al., 2012). One prominent measure of behavioral flexibility in humans is the

Wisconsin Card Sorting Task (WCST) which specifically measures the ability to adapt behavior in response to shifting rules and patterns (Bizon, et al., 2012). Different studies across age groups using the WCST have shown that individuals with OCD make more perseverative errors and require more trials compared with healthy matched controls (Lucey, et al., 1997; Min-Sup, et al., 2008; Yazdi-Ravandi, et al., 2018) and that increases in obsessional beliefs tend to exacerbate errors (Bradbury, et al., 2011). In addition, a genetic rodent model of OCD similarly showed that flexibility was impaired as evidenced by impaired reversal learning performance (Boom, et al., 2019). The parallels between individuals with OCD and rodent models of OCD in behavioral flexibility support further examinations utilizing rodent models of OCD. While there are apparent similarities in flexibility between humans and rodents, another symptom of OCD that is important to address is anxiety.

Pharmacological models of OCD most commonly use dopaminergic or serotonergic system modulation to elicit OCD-like behaviors in rodents as a method of understanding behavioral outcomes linked to these systems. There are a variety of possible 5-HT receptor targets useful for understanding OCD at a neurobiological level. Acute administration of the 5-HT1A receptor agonist 8-OH-DPAT reduced alternation ratios in male C57BL/6J mice (Odland, Jessen, Fitzpatrick, et al., 2019; Odland, Jessen, Kristensen, et al., 2019) which suggests that activation of 5-HT1A receptors induces compulsive-like behaviors. Also, using an open-field test, 8-OH-DPAT was shown to increase compulsive-like

checking behaviors in male rats (Alkhatib et al., 2013). Rats that received the 5-HT1A agonist would repeatedly return to specific areas of the open field which is similar to OCD-like checking behaviors in humans. Therefore, 5-HT1A receptor activation may be responsible for compulsive behaviors in male rodents. Studies showing the effects of 5-HT1B agonism on OCD-like behaviors in rodents are lacking. However, 5-HT1B agonist-induced mouse models of OCD are also seen to be effective.

One relatively novel 5-HT receptor target for investigating OCD behaviors in mice is the 5-HT1B receptor. Serotonin 1B receptor activation is shown to increase locomotor behaviors and produce deficits to PPI and delayed alternation (Shanahan et al., 2009, 2011; Woehrle et al., 2013), all of which are relevant OCD-like behaviors. Acute treatment with the 5-HT1B/1A receptor agonist RU24969 in female C57BL/6J mice increases PPI (Dulawa & Geyer, 2000). Also, acute administration of RU24969, a 5-HT1B/1A receptor agonist, at relatively high doses (1-10 mg/kg) is shown to increase the total distance traveled in an open field test in female C57BL/6J mice (Ho et al., 2016; Shanahan et al., 2009). However, the direct effects of 5-HT1B/1A activation on behavioral flexibility are not understood. 5-HT1B and 1A activation seem to induce OCD-like behaviors in mice making them novel targets of interest for studying the neurobiology of higher-order executive function measured by probabilistic learning in mice.

CHAPTER SIX

SUMMARY AND HYPOTHESES

OCD's symptomology includes excessive habit formation which could lead to impairments in goal-directed learning and behavioral flexibility (Gruner & Pittenger, 2017). Neuroimaging studies have highlighted the involvement of the OFC in the expression of OCD symptoms (Menzies et al., 2008). OFC lesion studies have also found impairments to probabilistic reversal learning, a measure of behavioral flexibility in animals (Chang, 2014; Chase et al., 2012). Previous studies using direct co-administration of a 5-HT1B agonist and antagonist into the OFC of mice demonstrated that the 5-HT1B receptors in the OFC are necessary to produce perseverative circling behaviors in an open field (Shanahan et al., 2009, 2011). The 5-HT1B/1A receptor agonist RU24969 has been shown to induce OCD-like behaviors in mice, such as increases in locomotor stereotypy and grooming behaviors (Ho et al., 2016). Together these findings suggest 5-HT1B receptor modulation may impact behavioral flexibility as well as locomotor activity.

The current study examined the effects of acute, systemic administration of the 5-HT1B/1A receptor agonist RU24969 (0, 0.01, 0.1, and 1.0 mg/kg) in both male and female C57BL/6J mice. To examine the impact of 5-HT1B/1A receptor activation on behavioral flexibility mice were tested on a spatial probabilistic reversal learning task following administration of RU24969. It was predicted that

RU24969 would cause a dose-dependent impairment in spatial probabilistic reversal learning. Specifically, acute RU24969 administration at 0.01, 0.1, and 1.0 mg/kg would significantly impair probabilistic reversal learning in C57BL/6J mice with 1.0 mg/kg eliciting the greatest impairment. To test this, all mice underwent an initial acquisition of spatial discrimination following vehicle treatment. Twenty-four hours later mice received either 0, 0.01, 0.1, or 1.0 mg/kg RU24969 before being tested on the reversal phase of a spatial probabilistic reversal learning task. The results from this task were used to determine if RU24969 impairs reversal learning performance in C57BL/6J mice. Previous studies utilizing RU24969 have found increased locomotor effects using relatively high doses of 1, 3, 5, and 10 mg/kg (Ho et al., 2016), as well as lower doses of 0.625, 1.25, 2.5, and 5 mg/kg (McDougall et al., 2020). Since hyperlocomotion is considered to be an OCD-like behavior in rodents induced by activation of the 5-HT1B receptor (Ho et al., 2016), it is important to understand whether the 5-HT1B/1A agonist RU24969 administered at the low doses of 0.01, 0.1, or 1.0 mg/kg induces hyperlocomotion. It was predicted that RU24969 will have a dose-dependent increase in locomotor activity. To test this, mice were placed in an open field and recorded for one hour following injection of 0, 0.01, 0.1, 1.0 mg/kg RU24969. Total distance traveled as well as the percent time spent in the center of the open field versus the perimeter was recorded to determine potential treatment effects. It was also predicted that there would be a dose-dependent response for the percent time spent in the center of the open field. Specifically,

higher doses of RU24969 would decrease the percentage of time spent in the center. Finally, current research examining the sex differences in behavioral flexibility and locomotor activity with 5-HT1B/1A activation is scarce. Thus, this study aims to identify potential sex differences in behavioral flexibility and locomotor activity. It was predicted that male mice would become more impaired in behavioral flexibility following administration of RU24969 compared to female mice.

CHAPTER SEVEN

METHODS

Subjects

A total of 64 C57BL/6J mice (32 male, 32 female) were tested on the probabilistic reversal learning task and 64 C57BL/6J mice (32 male, 32 female) were used to assess locomotor activity in an open field. All mice were bred and housed at California State University, San Bernardino (CSUSB). Mice were housed in groups of four with same-sex littermates in plastic cages (28cm wide x 17cm long x 12cm high) in a humidity (30%) and temperature (22-23 ° C) controlled room with a 12-hour light/dark cycle (lights on at 0700 hours) on a ventilated rack. For the probabilistic learning task, mice were food-restricted until reaching 85% of their free-feeding weight with no restrictions to water. Testing was completed in a separate room and during the animal's light phase. Animal care and use was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2021) and was approved by the Institutional Laboratory Animal Care and Use Committee at CSUSB. All mice began testing at eight weeks of age.

Treatment

RU24969 (5-Methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1H-indole) is a selective 5-HT1B agonist with a co-affinity for the 5-HT1A receptor (Aronsen et al., 2014). Mice were randomly assigned to receive either 0, 0.01, 0.1, or 1.0

mg/kg of the 5-HT1B/1A selective agonist RU24969 (Tocris Bioscience) dissolved in 50% DMSO solution. These doses were selected based on previous findings that show RU24969 can increase locomotor activity in mice and rats at doses between 0.625 and 10mg/kg (Ho et al., 2016; McDougall et al., 2020; Shanahan et al., 2009). Given that the probabilistic reversal learning task is a spatial task, low doses of 0.01, 0.1, and 1.0 mg/kg were chosen to prevent confounding effects in the spatial probabilistic reversal learning task of increased locomotion. Treatment was injected intraperitoneally (i.p.) at a 5 ml/kg injection volume. Control mice were injected with vehicle (50% DMSO). All mice received injections ten minutes before behavioral testing to ensure complete chemical metabolism. During the training days of the probabilistic reversal learning task, no injections were given.

Behavioral Testing

Spatial Probabilistic Reversal Learning

The spatial probabilistic reversal learning task was conducted in three phases: spatial discrimination training, probabilistic acquisition, and probabilistic reversal learning. All phases of the probabilistic reversal learning task were conducted in a T-maze with equal-sized arms (36cm long x 12cm wide x 12cm tall). Once animals reached 85% of their free freed weight (4-6 days), they began spatial discrimination training. Mice required two to four days of training prior to testing of spatial acquisition. For spatial discrimination training, mice were placed in the starting arm of the T-maze closed by a guillotine door that was

perpendicular to the two choice arms. After one minute had elapsed, the start door was opened, and the mouse was allowed to freely navigate the choice arms and consume a ½ piece of cereal from a food well located at the end of each choice arm. The initial arm choice was recorded for each trial. After both cereal pieces were consumed, the start door was opened allowing the mouse to re-enter the start area. The door was closed, the food wells re-baited, and the next trial began. This procedure was repeated for 15 minutes. Mice were considered habituated to the T-maze once they completed seven or more trials in a 15-minute session across two consecutive days.

Twenty-four hours after the mouse was trained, they began spatial acquisition in the same T-maze. Before the acquisition, every mouse was injected with the vehicle treatment. This was done to be consistent in mice receiving an injection before each test phase. At the beginning of the acquisition phase, each mouse was placed in the start area for one minute. Next, the door was opened allowing the mouse to choose between the two choice arms. Only one of the food wells was baited with a ½ cereal piece. Before testing, one arm was chosen as the “correct” spatial location and will contain a ½ piece of cereal on 80% of trials. The “incorrect” arm was baited during the other 20% of trials. The “correct” arm was animal-specific and was chosen based on the animal’s least initially chosen arm during training. This was done to ensure that animals are not choosing the arm out of habit from training. The first two trials were always be baited as “correct” choices. The learning criterion was considered

achieved when a mouse chose the “correct” arm for six consecutive trials. If a mouse chose the “correct” arm, it was allowed to consume the cereal piece and the guillotine door was raised allowing the mouse to return to the start area. If the “incorrect” arm was chosen, the mouse was allowed to investigate the un-baited food well, the start door was opened, and the mouse was returned to the start area. Every five trials, the maze was cleaned with a 10% alcohol and water solution to minimize the use of odor cues.

Lastly, the reversal learning phase was conducted 24 hours following the acquisition phase. Ten minutes prior to testing, mice were injected with either 0, 0.01, 0.1, or 1.0 mg/kg RU24969 to allow for complete chemical metabolism. Before reversal learning, a retention test was given to ensure that each mouse starts the reversal phase at a similar recall level from the discrimination learned in the acquisition phase. This means that mice were tested using the same “correct” and “incorrect” arm choices used during acquisition. The retention criterion was reached once the mouse successfully chooses the “correct” arm from acquisition on five out of six trials. Once the retention criterion was met, the reversal learning test immediately began. The reversal learning test was identical to the acquisition phase except the “correct” and “incorrect” arms were switched to the opposite arms respective to acquisition. For instance, if the “correct” arm choice was the left arm in the acquisition phase, the new “correct” arm choice in reversal was the right arm. The reversal criterion was met when mice made six consecutive correct choices. Both acquisition and reversal phases were timed on

a digital stopwatch. The treatment groups consisted of the following for both males and females: [acquisition-reversal] vehicle-vehicle (*n*=8), vehicle-RU24969 0.01 mg/kg (*n*=8), vehicle-RU24969 0.1 mg/kg (*n*=8), and vehicle-RU24969 1.0 mg/kg (*n*=8).

The probabilistic reversal learning task required that a mouse choose one of the T-maze arms while considering previously made choices. To ensure that RU24969 at 0.01, 0.1, and 1.0 mg/kg did not induce hyperlocomotion, time-per-trial was calculated (overall time divided by the number of trials) in minutes. Also, win-stay and lose-shift conditional probabilities were analyzed. A win-stay indicated when a mouse made a correct arm choice, received a food reward, and subsequently made another correct arm choice. A lose-shift indicated when a mouse made a correct arm choice but was not rewarded with food, and then choose the incorrect arm in the subsequent trial. If mice exhibited a high win-stay and a low lose-shift, it was more likely that the mouse was making deliberate choices. If there is a high win-stay and high lose-shift, meaning the mouse was simply chasing the food reward and not analyzing the probability aspect of making the arm choice that is baited 80% of the time, then it is more likely that the mouse was not making deliberate choices. This high win-stay high lose-shift scenario could suggest that RU24969 was inducing hyperlocomotion and the mouse was simply running through the maze and not learning the task itself.

Open Field Test

Locomotor activity, as well as anxiety-like behavior, was measured using an open field. The open field was conducted in a square chamber with a solid white floor and black plastic walls (60 cm long x 60 cm wide x 30 cm in height). The testing chamber was divided into four equal quadrants to measure locomotor activity in four mice at a time in individual fields. Mice were tested for a total of 60 minutes and recorded using Ethovision 3 video tracking system (Noldus, Leesburg, VA) to measure total distance traveled, time spent in the center, and time spent on the perimeter of the open field. To determine if RU24969 induced an anxiolytic-like effect, the percent time spent in the center of the open field was calculated by dividing the time spent in the center by the time spent in the center plus the time spent on the perimeter. Following the open field test trial, mice were placed back in their home cage and the open field was cleaned with a 10% alcohol solution. The treatment groups were as follows for male and female mice: vehicle (*n*=8), 0.01 mg/kg (*n*=8), 0.1 mg/kg RU24969 (*n*=8), and 1.0 mg/kg RU24969 (*n*=8).

Statistical Analysis

The effects of the 5-HT1B/1A receptor agonist RU24969 on reversal learning were analyzed utilizing separate two-way sex (female & male) by treatment (0, 0.01, 0.1, & 1.0 mg/kg RU24969) analyses of variance (ANOVAs) tests to determine potential sex and treatment differences. The trials to reach the criterion for the initial spatial acquisition, retention, and reversal learning tasks

were examined by separate two-way ANOVAs. Additionally, two-way (sex x treatment) ANOVAs were conducted to analyze perseverative errors, regressive errors, win-stay, and lose-shift conditional probabilities. To determine if locomotor activity had an effect on performance during the reversal learning task, locomotor activity was analyzed using a two-way sex by treatment (0, 0.01, 0.1, & 1.0 mg/kg RU24969) ANOVA for the total distance traveled and percent time spent in the center of the open field. Dunnett post-hoc analyses were utilized when appropriate for multiple comparisons and statistical significance was determined at $p < 0.05$.

CHAPTER EIGHT

RESULTS

Spatial Probabilistic Reversal Learning

Performance on the acquisition phase was comparable between sex and treatment groups (Figure 1A). For acquisition, there was no main effect of sex [$F(1,56) = 0.44$, $p = 0.51$], treatment [$F(3,56) = 0.49$, $p = 0.69$], and no significant sex by treatment interaction [$F(3,56) = 0.43$, $p = 0.74$]. Also, retention of the initial acquisition phase was comparable between sex and treatment groups (Figure 1C). There was no main effect of sex [$F(1,56) = 0.48$, $p = 0.49$], treatment [$F(3,56) = 1.87$, $p = 0.15$], and no significant sex by treatment interaction [$F(3,56) = 0.81$, $p = 0.50$] for trials to reach criterion. For reversal learning, there was no main effect of sex [$F(1,56) = 2.10$, $p = 0.15$] and no significant interaction between sex and treatment [$F(3,56) = 1.93$, $p = 0.14$] for trials to reach criterion. However, there was a main effect of treatment for trials to reach criterion during the reversal learning task [$F(3,56) = 35.56$, $p < 0.001$]. Separate Dunnett's post hoc multiple comparisons were conducted for each sex and determined that there was no significant difference in trials to reach criterion for reversal learning in female mice treated with RU24969 at 0.01 mg/kg ($p = 0.99$) or 0.1 mg/kg ($p = 0.08$) while female mice that were treated with the 1.0 mg/kg ($p < 0.001$) dose required significantly more trials to reach criterion for reversal learning compared to vehicle treated female mice. However, male mice treated with 0.01 ($p = 0.04$), 0.1 ($p < 0.001$), or 1.0 ($p < 0.001$) mg/kg RU24969 all required significantly more

trials to reach criterion compared to vehicle treated male mice (Figure 1B). Therefore, RU24969 impaired reversal learning performance in female mice at the highest dose only (1.0 mg/kg) while performance was impaired for male mice at every dose.

An analysis of errors committed by the mice was conducted to determine the effect of sex and treatment on perseverative errors, regressive errors, win-stay probabilities, and lose-shift probabilities. There was no main effect of sex [$F(1,56) = 0.27$, $p = 0.61$] and no significant interaction [$F(3,56) = 1.75$, $p = 0.17$] between sex and treatment for perseverative errors. However, there was a main effect of treatment [$F(3,56) = 10.49$, $p < 0.001$] on perseverative errors committed. Post hoc Dunnett multiple comparisons conducted for each sex revealed that female mice treated with 1.0 ($p < 0.001$) mg/kg RU24969 committed more perseverative errors compared to female vehicle controls. There was no significant difference in the number of perseverative errors committed in female mice treated with 0.01 ($p = 0.99$) or 0.1 ($p = 0.24$) mg/kg RU24969 compared with female controls. Male mice treated with 1.0 ($p = 0.03$) mg/kg also committed significantly more perseverative errors than vehicle-treated males. Similar to the females, there was no significant difference in the number of perseverative errors committed between male mice treated with 0.01 ($p = 0.63$) or 0.1 ($p = 0.06$) mg/kg RU24969 and vehicle-treated males. Thus, RU24969 treatment at 1.0 mg/kg impaired the ability to inhibit the previously learned spatial discrimination in both females and males (Figure 2A).

Regressive errors showed a similar trend in that there was no main effect of sex [$F(1,56) = 0.71$, $p = 0.40$] and no significant interaction between sex and treatment [$F(3,56) = 0.98$, $p = 0.41$]. There was a significant main effect of treatment on regressive errors [$F(3,56) = 5.76$, $p < 0.01$]. Dunnett post hoc multiple comparisons for each sex revealed that there was no significant difference between the number of regressive errors committed for female controls and females treated with 0.01 ($p = 0.99$), 0.1 ($p = 0.93$), or 1.0 ($p = 0.25$) mg/kg RU24969. However, male mice treated with 1.0 mg/kg RU24969 committed significantly more regressive errors compared to vehicle treated males ($p < 0.001$). There were no significant differences in regressive errors committed between vehicle treated males and males treated with 0.01 ($p = 0.83$) or 0.1 ($p = 0.13$) mg/kg RU24969. Thus, RU24969 treatment at 1.0 mg/kg reduced the ability to maintain the new choice pattern once it was selected in males only (Figure 2B).

Finally, win-stay and lose-shift probabilities were analyzed. For win-stay errors, there was no main effect of sex [$F(1,56) = 1.23$, $p = 0.27$] or treatment [$F(3,56) = 2.23$, $p = 0.10$], and no significant interaction between sex and treatment [$F(3,56) = 0.42$, $p = 0.74$] (Figure 3A). Similarly, for lose-shift probabilities, there was no main effect of sex [$F(1,56) = 1.68$, $p = 0.20$] or treatment [$F(3,56) = 0.80$, $p = 0.50$], and no significant interaction between sex and treatment [$F(3,56) = 0.67$, $p = 0.57$] (Figure 3B).

Open Field Test

A two-way ANOVA with sex (female & male) by treatment (0, 0.01, 0.1, 1.0 mg/kg RU24969) analysis was conducted to determine mean differences in total distance travelled as well as the percent of time spent in the center of the open field. Analysis revealed a significant main effect of treatment on total distance traveled [$F(3,56) = 6.21$, $p = 0.001$] and no significant main effect of sex [$F(1,56) = 0.06$, $p = 0.80$] or interaction between sex and treatment [$F(3,56) = 0.89$, $p = 0.45$]. Dunnett post hoc analysis conducted for each sex revealed that there was no significant difference in total distance travelled between female mice treated with 0.01 ($p = 0.59$), 0.1 ($p = 0.33$), or 1.0 ($p = 0.63$) mg/kg RU24969 and vehicle treated females. However, males treated with 1.0 mg/kg RU24969 exhibited less locomotor activity in the open field compared to vehicle treated males ($p = 0.007$). Males treated with 0.01 ($p = 0.99$) and 0.1 ($p = 0.12$) mg/kg RU24969 demonstrated similar locomotor activity to vehicle treated males. Overall, RU24969 only impaired locomotor activity in males treated with 1.0 mg/kg RU24969 and had no effect on females (Figure 4A).

When analyzing anxiety-like behaviors in the open field test, the analysis showed a significant main effect of treatment on percent time spent in the center of the open field [$F(3,56) = 8.57$, $p < 0.001$], although, there was no main effect of sex [$F(1,56) = 1.74$, $p = 0.19$]. Dunnett multiple comparisons conducted for each sex revealed that there was no significant difference between the percentage of time spent in the center of the open field for female mice treated with 0.01 ($p =$

0.21), 0.01 (p = 0.99), or 1.0 (p = 0.99) mg/kg RU24969 and vehicle-treated female mice. Although, male mice treated with 0.1 (p = 0.002) and 1.0 (p < 0.001) mg/kg RU24969 spent significantly less percentage of time in the center of the open field compared to vehicle-treated males (Figure 4B). There was also a significant interaction between sex and treatment for time spent in the center of the open field [$F(3,56) = 4.69$, p = 0.005]. Using Bonferroni multiple comparisons, it was determined that male mice treated with vehicle spent a greater percentage of their time in the center of the open field compared to female mice treated with vehicle (p = 0.003). However, there were no significant differences between the percent of time spent in the center of the open field between female and male mice treated with 0.01 (p = 0.99), 0.1 (p = 0.99), or 1.0 (p = 0.44) mg/kg RU24969. Overall, male mice that received the two highest doses of 0.1 and 1.0 mg/kg RU24969 spent less time in the center of the open field compared to male mice that received vehicle treatment. RU24969 did not affect the percentage of time spent in the center of the open field in female mice. While the interaction between sex and treatment for percent time spent in center was significant, there was only a significant difference in the percentage of time spent in the center of the open field between females and males that were treated with vehicle while there was no difference between females and males receiving RU24969 (Figure 4C).

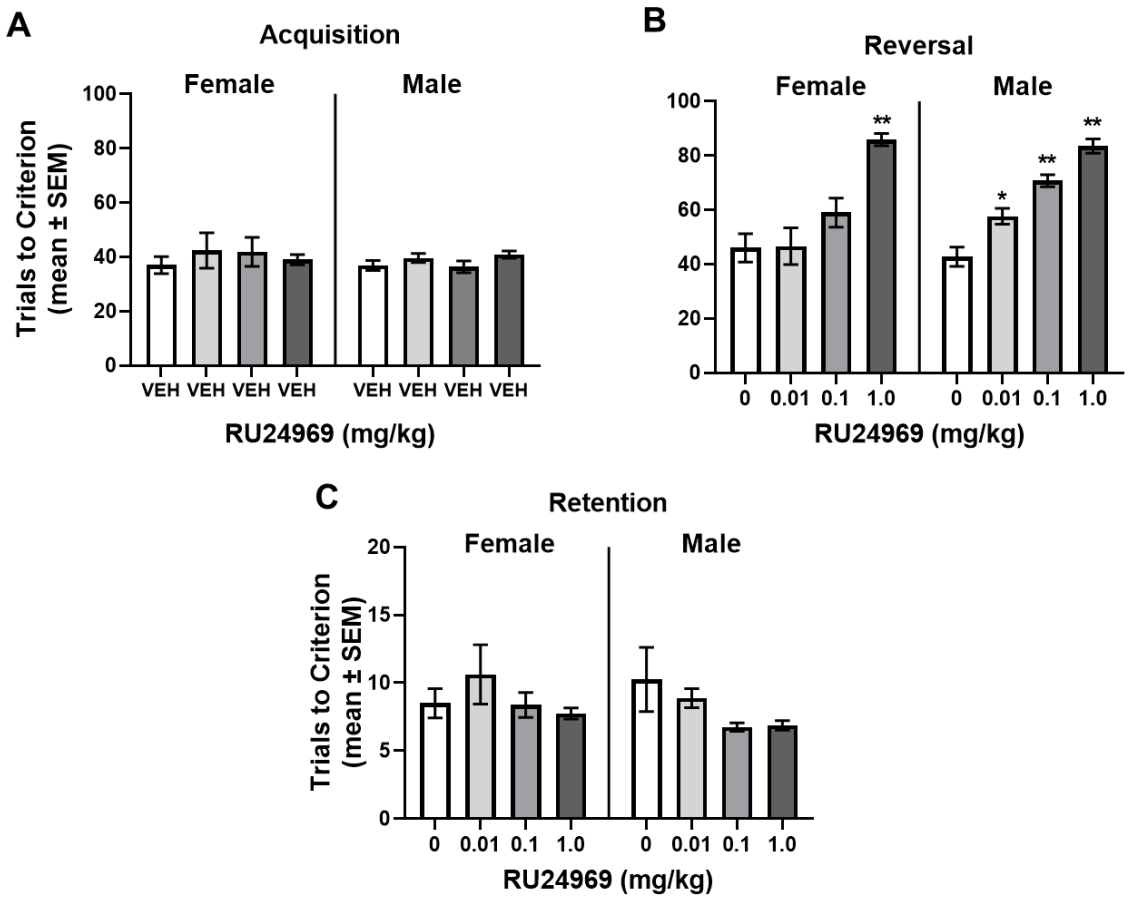

Figure 1. Trials to Reach Criterion for Acquisition, Reversal, and Retention

A) Trials to reach the criterion for the initial spatial acquisition task were similar between all treatment groups for both female and male C57BL/6J mice. All mice were treated with vehicle (VEH) ten minutes before testing on the acquisition phase. **B)** Female mice that received 1.0 mg/kg RU24969 required more trials to reach the criterion compared to vehicle-treated females. Male mice that received 0.01, 0.1, or 1.0 mg/kg RU24969 required more trials to reach criterion compared to vehicle-treated males. Reversal learning was measured immediately following retention. **C)** Trials to reach the criterion for retention were similar across all treatment groups for both females and males. Retention of the initial spatial acquisition was tested before the reversal learning phase and ten minutes before receiving RU24969 treatment. *$p < 0.05$, **$p < 0.01$ vs. vehicle within the same sex group.

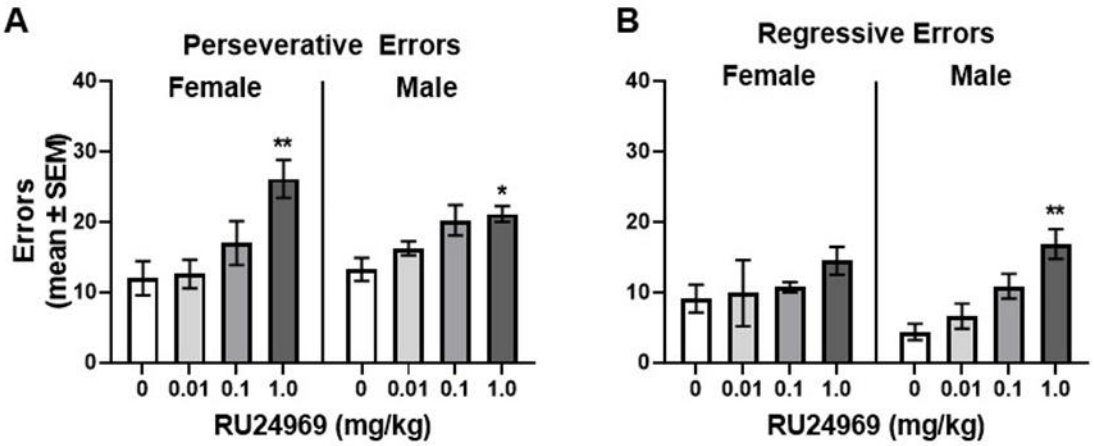

Figure 2. Perseverative and Regressive Errors Committed

A) RU24969 increased perseverative errors in both female and male mice. Perseverative errors committed were similar for both females and males that received 0.01 or 0.1 mg/kg RU24969 compared to same-sex vehicle-treated mice. **B)** Male mice treated with 1.0 mg/kg RU24969 committed more regressive errors compared to vehicle-treated male mice. Regressive errors committed were similar for male mice treated with 0.01 and 0.1 mg/kg RU24969 compared to vehicle-treated males. For the females, there was no difference between the number of regressive errors committed between all doses. *$p < 0.05$, **$p < 0.01$ vs. vehicle within the same sex group.

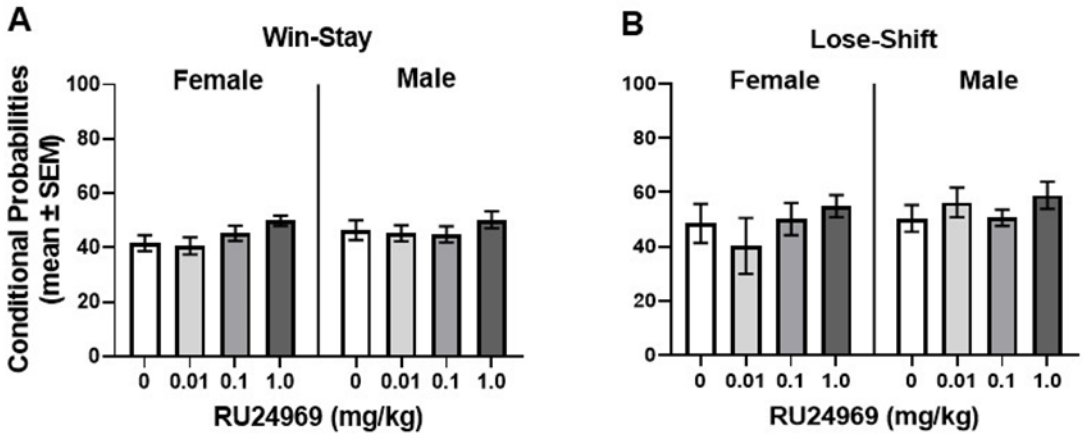

Figure 3. Win-Stay and Lose-Shift Conditional Probabilities

A) Female nor male mice expressed differing win-stay probabilities. **B)** Female nor male mice expressed differing loss-shift probabilities.

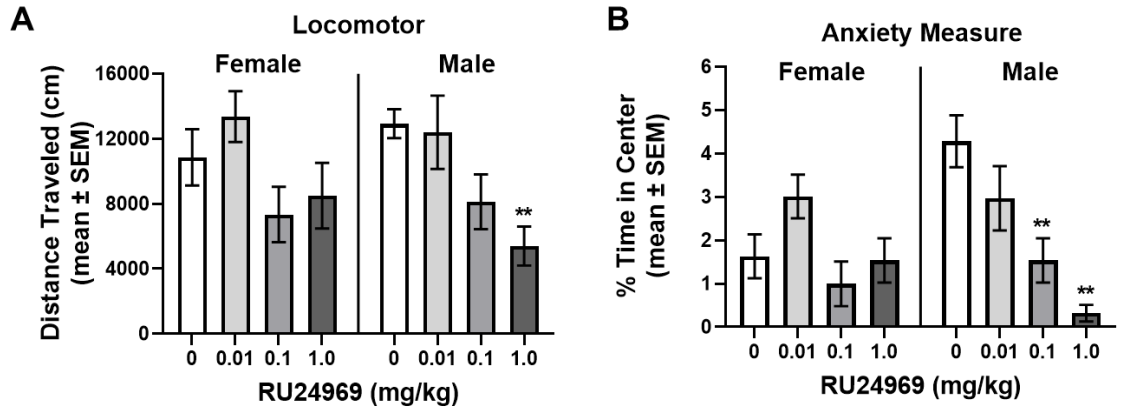

Figure 4. Open Field Test of Locomotor Activity and Anxiety-Like Behavior

A) Male mice that received 1.0 mg/kg RU24969 had reduced locomotor activity compared to vehicle-treated males. There was no difference in locomotor activity between males that received 0.01 or 0.1 mg/kg RU24969 and vehicle-treated males. There was no difference in locomotor activity between female treatment groups. **B)** Male mice that received 0.1 or 1.0 mg/kg RU24969 spent less time in the center of the open field compared to vehicle-treated males. Males treated with 0.01 mg/kg RU24969 spent a similar amount of time in the center of the open field compared to vehicle-treated males. There was no difference in time in the center between female treatment groups. ** $p < 0.01$ vs. vehicle within the same sex group.

CHAPTER NINE

DISCUSSION

The current experiment examined the effects of simultaneous 5-HT1B and 5-HT1A receptor activation on behavioral flexibility, locomotor activity, and anxiety-like behavior in C57BL/6J mice. It was originally hypothesized that activation of 5-HT1A and 5-HT1B receptors would elicit a dose-dependent impairment in behavioral flexibility at 0.01, 0.1, and 1.0 mg/kg. Overall findings suggest that RU24969 elicited a dose-dependent response in male mice at 0.01, 0.1, and 1.0 mg/kg impairing behavioral flexibility as shown by an increase in trials to reach criterion compared to vehicle-treated controls. Impairments in behavioral flexibility were only observed in female mice that received the highest dose of 1.0 mg/kg RU24969. Both female and male mice that received 1.0 mg/kg RU24969 also exhibited more perseverative errors compared to vehicle-treated controls. Regardless of sex, the highest dose led to an inability to inhibit the initial choice pattern established during acquisition. Although, only male mice treated with 1.0 mg/kg RU24969, demonstrated more regressive errors compared to vehicle-treated controls.

It was also hypothesized that locomotor activity would be significantly increased by RU24969 at 0.01, 0.1, or 1.0 mg/kg. Results showed that only male mice treated with 1.0 mg/kg RU24969 exhibited reduced locomotor activity when compared to vehicle-treated males. Finally, it was predicted that there would be a

dose-dependent reduction in the percentage of time mice spent in the center of an open field for 0.01, 0.1, and 1.0 mg/kg RU24969. It was determined that RU24969 at 0.1 and 1.0 mg/kg reduced the percentage of time male mice spent in the center of an open field and had no effect on female C57BL/6J mice. In addition, vehicle-treated males spent a greater percentage of time in the center of the open field compared to vehicle-treated females. Thus, RU24969 at the two higher doses increased anxiety-like behaviors in only male C57BL/6J mice.

Spatial Probabilistic Reversal Learning

As we have previously found, mice treated with the vehicle on acquisition showed comparable trials needed to reach criterion in male and female C57BL/6J mice (Amodeo et al., 2012, Amodeo et al., 2019). Thus, all mice were comparable on the acquisition of the spatial discrimination before moving onto the reversal phase. Male mice treated with 0.01, 0.1, and 1.0 mg/kg RU24969 displayed deficits in behavioral flexibility. RU24969 treated male mice required more trials to reach completion criterion in the spatial probabilistic reversal learning task compared to vehicle-treated male mice. It has previously been established that 5-HT1A receptor activation using 8-OH-DPAT reduced alternation ratios in male C57BL/6J mice, thus increasing behavioral rigidity (Odland, Jessen, Fitzpatrick, et al., 2019; Odland, Jessen, Kristensen, et al., 2019). In addition, 8-OH-DPAT has been shown to increase compulsive-like checking behavior in male rats (Alkhatib et al., 2013). Together, 5-HT1A activation contributes to an increase in compulsive-like behaviors in male rodents

and may explain the increase in trials to reach criterion with RU24969 treatment male mice, but also the increase in perseverative and regressive errors.

In the current study, both female and male mice treated with 1.0 mg/kg RU24969 committed more perseverative errors than same-sex controls while only male mice treated with 1.0 mg/kg RU24969 committed more regressive errors. Regardless of sex, the highest dose tested led to an impaired ability to inhibit the previously learned discrimination when contingencies were reversed. Thompson and Dulawa (2019) similarly found that RU24969 led to perseverative locomotor patterns. This inability to flexibly adapt in the face of changing reward contingencies can be found in OCD individuals (Deepthi et al., 2021). To our knowledge, this is the first study that has looked at the direct effects of acute RU24969 administration on behavioral flexibility in both female and male mice.

Open Field Test

The current study found that simultaneous activation of 5-HT1B and 1A receptors using RU24969 reduced locomotor activity in male mice treated with 1.0 mg/kg. RU24969 did not influence locomotor activity for female mice at any dose. Although, previous research has demonstrated the opposite effect using RU24969. Locomotor activity has been shown to be increased in female C57BL/6J mice using RU24969 at 1.0, 3.0, 5.0, and 10.0 mg/kg (Ho et al., 2016), while we did not find a significant change in female mice treated with 1.0 mg/kg. Another study testing the much higher 10.0 mg/kg RU24969 dose found an

increase in locomotor activity over 20 minutes in an open field test in male mice (O'Reilly et al., 2021). Interestingly, preweaning female and male Sprague Dawley rats treated with 0.625, 1.25, 2.5, or 5.0 mg/kg RU24969 exhibited increased locomotor activity compared to controls (McDougall et al., 2020, 2021), which may highlight possible maturation changes in 5-HT1B/1A receptor functioning.

Past research has also shown that activation of 5-HT1B receptors with several different compounds at 3.0 and 30.0 mg/kg doses increased locomotor activity in male mice (O'Neill et al., 1997). Another study found no change in locomotor activity following a 5.0 mg/kg injection of RU24969 using male 5-HT1B knockout mice (Malleret et al., 1999). This suggests that the 5-HT1B receptor is responsible for changes in locomotor activity. A possible explanation for the contrasting results between previous research and the current study is that mice treated with high doses of a 5-HT1B/1A agonist respond with increased locomotor activity while lower doses like the ones used in the present study have the opposite effect and reduce locomotor activity. These paradoxical findings highlight the need to further investigate the effects of 5-HT1B and 5-HT1A activation on locomotor activity using a wide range of doses to get a clear understanding of the associated dose-dependent response.

Anxiety-like behaviors were also measured in the open field test. Male mice that received 0.1 and 1.0 mg/kg RU24969 spent less time in the center of the open field suggesting that simultaneous activation of 5-HT1B and 1A

receptors increases anxiety-like behaviors in males only. One previous study showed no change in anxiety-like behaviors using RU24969 in food or water motivated conflict tests (Gardner, 1986). In tasks such as the Vogel conflict drinking test and elevated plus-maze test, 5-HT1B receptor activation using CP94253 resulted in anxiolytic-like effects in male mice (Tatarczyńska et al., 2004). A 5-HT1B antagonist SB 224289 was found to have anxiogenic-like effects in male rats as evidenced by an increase in latency to leave the starting area of an open field compared to controls (Hoplight et al., 2005). These findings suggest that 5-HT1B receptors indeed modulate anxiety-like behaviors and that activation of 5-HT1B should reduce anxiety while blockade should increase anxiety. Instead, we found competing results. The reduced percentage of time spent in the center of the open field observed following RU24969 treatment could be the result of an overall reduction in locomotor activity. Thus, male mice spent less time exploring the center of the open field compared to the perimeter because locomotor activity was significantly reduced in males as well, therefore further studies are needed.

A limitation of the present study is the lack of co-administration of a 5-HT1A or 1B receptor antagonist with RU24969 in hopes of teasing out the effects of specific receptor activation. Co-administration of a 5-HT1A or 1B receptor antagonist would highlight the specific receptor effects on probabilistic reversal learning. In addition, we need to consider whether the increase in anxiety-like behaviors was simply the result of reduced locomotor activity. The inclusion of

other measures of anxiety, such as the elevated plus-maze, needs to be tested and compared alongside the open field test using the same doses of RU24969 to determine if the anxiogenic effect found in the current study was due to increased anxiety or reduced locomotion.

Conclusion

The present study sought to understand whether simultaneous activation of 5-HT1B and 1A receptors using the 5-HT1B/1A receptor agonist RU24969 would elicit OCD-like behaviors in mice. The OCD-like behavioral inflexibility was examined by applying a spatial probabilistic reversal learning task while locomotor activity and anxiety-like behaviors were also measured in the open field. Results showed that RU24969 impaired reversal learning in both females and males suggesting that activation of 5-HT1B and 1A receptors induces the OCD-like symptom of behavioral inflexibility. Administration of RU24969 resulted in reduced locomotor activity as well as increased anxiety-like behaviors in male mice. These findings suggest that 5-HT1B and 1A receptor activation increases anxiety in male mice. Overall, the evidence suggests that 5-HT1A and 1B receptors could play a role in the manifestation of some of OCD’s core symptoms.

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