# INHIBITING REPETITIVE AND RESTRICTED BEHAVIORS IN SPRAGUE-DAWLEY RATS USING PROPRANOLOL: SEX DIFFERENCES

by

Melanie Berry

Liberty University

A Dissertation Presented in Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

Liberty University

May, 2024

# INHIBITING REPETITIVE AND RESTRICTED BEHAVIORS IN SPRAGUE-DAWLEY RATS USING PROPRANOLOL: SEX DIFFERENCES

by

Melanie Berry

Liberty University

A Dissertation Presented in Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

Liberty University

May, 2024

APPROVED BY:

MGopaul_____

Name and degree, Committee Chair

[redacted]_____

Laura Rolen, Ph.D., Committee Member

## ABSTRACT

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that is diagnosed when an individual has persistent deficits in social communication and restrictive repetitive patterns of behaviors, interests, or activities. Restricted repetitive behaviors (RRBs) are typically triggered by anxiety and can cause significant detriment to an individual's life. While there is no cure for ASD, there are pharmacological treatment options for the symptoms caused by ASD. Propranolol, a beta-adrenergic antagonist, is used to treat anxiety and shows promise in reducing many of the symptoms associated with ASD. This study explores the effects of Propranolol on RRBs in a sample size of 24 Sprague Dawley rats. Additionally, this study sought to examine if gender moderates a change in RRBs once Propranolol is administered. This study used a quasi-experimental within-subject design to demonstrate the effectiveness of Propranolol injections on Sprague-Dawley rats while undergoing a marble burying test. I used a Two-Way ANOVA to compare the variance in each group mean to the variance of the dependent variables. My findings showed that Propranolol had a significant effect on RRBs in both male and female Sprague-Dawley rats. These findings imply that there are alternative treatment options that may be more effective and less aversive for the side effects associated with ASD. Additionally, males and females may be affected differently in terms of treatment options, and more research into the symptoms of ASD and treatment options is pertinent.

*Keywords*: autism spectrum disorder, restricted repetitive behaviors, beta-adrenergic blockers, Propranolol

**Copyright Page**

(Optional; centered horizontally and vertically on the page)

## Dedication

I would like to dedicate my work to my grandmother, Dr. Evelina Cross Ph.D., who set an inspiring example for me in education and in life. She instilled in me from a young age a desire to travel and explore, to not be afraid to try something new, and to never stop learning.

**Acknowledgments**

First and foremost, I would like to thank my dissertation committee, Dr. Margaret Gopaul and Dr. Laura Rolen, for their continued patience and efforts in guiding me to completion of my doctoral thesis. This would not have been possible without the continued support and encouragement I received.

I would like to thank my parents, Dr. Scott, and Eve Berry, for standing by my side in every decision I have made throughout my life.

I would also like to thank my fiancé, Joshua Hartley, the rest of my family, and my friends, for their unconditional encouragement, support, and patience throughout my academic career.

Finally, I would like to thank the members of my previous research lab - Dr. Amy Kohtz, for her countless hours of teaching and guiding me through the research process and her invaluable contributions to my education of the data collection and statistical analysis process required in order to complete this project. Additionally, Silvia Kelsen for the knowledge instilled in me through my time working in the research lab and the necessary skills she taught me in animal care and following protocols appropriately.

TABLE OF CONTENTS

## List of Figures

# CHAPTER 1: INTRODUCTION TO THE STUDY

## Introduction

According to the Fifth Edition of the *Diagnostic and Statistical Manual* (American Psychiatric Association, 2013), in order to receive a diagnosis of autism spectrum disorder (ASD), one must have persistent deficits in social communication and social context and restricted repetitive patterns of behaviors, interests, or activities. Restrictive, repetitive behaviors (RRBs) are often triggered by anxiety and can greatly interfere with an individual's ability to interact socially and engage in daily activities (Cuccaro et al., 2003 Song et al., 2022;). With there being many facets to the disorder, a single treatment option is not likely, and the combination of behavioral and pharmacological interventions may be the most effective approach (London et al., 2020; McDougle et al., 2003).

Currently, there are two antipsychotic medications approved by the FDA for the treatment of some of the symptoms of ASD, risperidone and aripiprazole. However, their long-term use has been found to be problematic due to their many adverse side effects (London et al., 2020; Orsolini et al., 2016). Propranolol, a non-selective beta-adrenergic receptor blocker, has been shown to be effective in reducing and reversing impairments caused by stress in non-ASD individuals (Beversdorf, 2020). Additionally, propranolol, along with other beta-blockers, is often prescribed as a therapeutic agent for anxiety, which is often said to trigger RRBs (Song et al., 2022).

Researchers have started to look at the positive effects of propranolol for individuals with ASD, but further research is still needed. Additionally, it has been found that there are not only gender differences in RRBs, but also there are gender differences

in the response to many pharmacological drugs (Antezana et al., 2019; Farkouh et al., 2020). This research will aid in closing the gap in understanding the gender differences found between pharmacology and restricted repetitive behaviors in individuals with ASD.

## Background

The Autism and Developmental Disabilities Monitoring Network (ADDM), a program funded by the Center for Disease Control and Prevention (CDC), is a collaborative network that tracks not only the number but also the characteristics of children with ASD (CDC, 2023). As of 2020, there were 11 network sites across the United States, allowing for the ability to ascertain ASD among children at the age of 8 years old. The ADDM looks specifically at age 8 as it allows for an in-depth analysis of the child's health and education records. Additionally, by age 8, most children can be effectively diagnosed (CDC, 2023).

As of 2020, almost 3%, or 1 in 36, children aged 8 were identified as having a diagnosis of ASD, with males being affected nearly 4 times as often as females (Maenner et al., 2021). ASD is a neurodevelopmental disorder characterized by deficits in social communication and interactions and restricted, repetitive behaviors (RRBs) (Tian et al., 2022). RRBs are often noticed by the caregiver as they can affect not only the quality of life of the individual with ASD, but additionally the family members who are involved in the individual’s life (Leekam et al., 2011; Wolff et al., 2014). While the social deficits are often discussed when referring to ASD, RRBs have gained less attention in terms of recognition, but also treatment.

RRBs discuss a broad category of behavioral patterns. These behaviors may include a more specific interest or knowledge about an item or topic (e.g., vacuum

cleaners or computers) or an adherence to a very specific routine (e.g., insisting on their breakfast being made a certain way every day). RRBs also present as repetitive motor functions (e.g., waving hands), or a preoccupation with certain objects (e.g., hyper-focusing on the wheels on a toy race car (Kim & Lord, 2010). Along with the interruptions, RRBs may also interfere with a child developing adaptive skills and engaging in daily activities (Song et al., 2022).

While the U.S. Food and Drug Administration (FDA) has approved medications for the irritability associated with ASD, there are no medications approved for the management of the core symptoms of ASD (Farmer & Aman, 2011; Wink et al., 2010). Ongoing research has looked at the effects of medications such as oxytocin as a potential treatment option for ASD side effects, but further research is still needed to seek FDA approval (Daniels et al., 2023). There is, however, an FDA-approved drug that may be effective in treating the symptoms of ASD.

There have been small case studies conducted that do show promise of an additional treatment that may be effective, a beta-adrenergic blocking agent, propranolol (London et al., 2020; Sagar-Ouriaghli et al., 2018). Previous research has shown that the noradrenergic system may be a target for effective pharmacological intervention for ASD (Lake et al., 1977; Launay et al., 1987; Zamzow et al., 2015). Propranolol is a non-selective beta-adrenergic antagonist that is used for reducing noradrenergic system activity. Previous research with propranolol and ASD has focused on language and sociability in individuals with ASD, but its proven efficacy with anxiety could potentially produce treatment options for RRBs (Beversdorf et al., 2008; Beversdorf et al., 2011 Ratey et al., 1987).

As previously stated, males are much more likely to be diagnosed with ASD than females, with previous epidemiological studies showing the male-to-female ratio found to be 4:1 (CDC, 2023). While it is often discussed that ASD presents differently in males and females, the research has resulted in very inconsistent findings. It should be noted that in the meta-analysis and literature reviews conducted, there were different measurements used, though inconsistencies exist within each measurement type (Schuck et al., 2019). Further research into the differences between males and females could aid in treatment options, both pharmacological and behavioral, for individuals with ASD.

While the diagnosis of ASD was not present during the time when the Bible was being written, more recent research has proposed that there were individuals in the Bible who potentially suffered from developmental disorders. Mathew and Pandian (2010) have discussed the developmental and neurological disorders associated with many of the Biblical characters of the Old Testament. Although there was not a diagnostic criterion written during the Biblical times for any mental health disorder, the traits of Samson have been found to be identifiable as ASD traits.

Unfortunately, those who suffered from neurological or developmental disorders during that time were often said to be possessed by evil spirits. Mary Magdalene, a prominent figure often mentioned in the Bible as one of Jesus's original followers, was said to have been "cured of evil spirits and diseases...from whom seven demons had come out" (Luke 8:2, New International Version). An individual trained in diagnosing mental health disorders would more likely say she suffered from the psychotic disorder schizophrenia.

As Christians, it is important to not only think Biblically about the normalcies of the secular world but also to educate ourselves and become aware of how the ancient texts of the Bible may speak to contemporary realities, such as a mental health disorder like ASD. Thus, it is the responsibility of the individual to use the text of the Bible, not as a vehicle for documentation but as a means for edification. The preference, therefore, is not to say that individuals with ASD are possessed by an evil spirit but to use the Biblical text as a guide for helping those who suffer from mental health disorders such as ASD. The book of Galatians tells us to "bear one another's burdens, and so fulfill the law of Christ" (Galatians 6:2, New International Version).

## Problem Statement

ASD is characterized by deficits in social communication and social context and restricted repetitive patterns of behaviors, interests, or activities (DSM-5, 2013). The most recent studies show a prevalence of ASD in American children being 1/44 or 2.27% (Maenner et al., 2021). As previously stated, RRBs are a core symptom of ASD and have been shown to greatly affect the quality of life in an individual with ASD. Behavioral intervention has helped with RRBs, but there is not an FDA approved medication to alleviate this symptom (Boyd et al., 2012; London et al., 2020).

Pharmacological treatment for symptoms of ASD is centered around selective serotonin reuptake inhibitors (SSRIs). While possibly beneficial for some side effects associated with ASD, SERTs are often used to target aggression as opposed to perseveration or RRBs. Additionally, SERTs are associated with a number of negative side effects, including increased irritability, nausea and/or weight gain, and insomnia

(Francis, 2005; Leskovec et al., 2008; Sagar-Ouriaghli et al., 2018). With these negative side effects, it becomes essential to explore other pharmacological options.

Researchers have explored the effectiveness of beta-adrenergic blockers, such as propranolol, as a pharmacological treatment for the symptoms of ASD, but not specifically RRBs (Ratey et al., 1987; Ward et al., 2013). Propranolol inhibits the actions caused by noradrenaline and adrenaline, the two hormones responsible for increasing heart rate and blood pressure levels, causing hyperarousal in an individual (Deepmala & Agrawal, 2014; Sagar-Ouriaghli et al., 2018). Alleviating these symptoms that are related to autonomic dysregulation can potentially improve the therapeutic outcomes for individuals struggling with RRBs.

It has been known that ASD and the associated symptoms do not present the same in both males and females, with males being three times more likely than females to be affected by RRBs (Amodeo et al., 2019). The gap in research is surrounding the differences in response to pharmacological treatment options based on the different genders. The problem is that there has yet to be research supporting the hypothesis that males and females will respond differently to the administered doses of propranolol which is clearly supported by current literature.

## Purpose of the Study

The purpose of this quantitative quasi-experimental study was to examine the relationship between propranolol and restricted repetitive behaviors in male and female Sprague-Dawley rats.

## Research Question(s) and Hypotheses

### Research Questions

RQ1: How does the number of restrictive, repetitive behaviors change after the administration of propranolol in an autism model of Sprague-Dawley rats?

RQ 2: Does gender moderate the change in the number of restrictive, repetitive behaviors once propranolol is administered?

**Hypotheses**

Hypothesis 1: The number of restrictive, repetitive behaviors will be inhibited following the administration of propranolol in an autism model of Sprague-Dawley rats.

Hypothesis 2: Gender will moderate the change in the number of restrictive, repetitive behaviors once propranolol is administered.

## Assumptions and Limitations of the Study

It was assumed that the number of RRBs would be inhibited following the administration of the drug propranolol. Propranolol is a drug often administered for anxiety, and RRBs are thought to be triggered by anxiety. Additionally, with behavior presenting differently in males and females with ASD, it was also an assumption that gender will show a clear difference in the RRBs demonstrated.

There are limitations and challenges that should be recognized with this study. The Sprague-Dawley rats that will be used for data collection were either a vehicle model serving as the control or an autism spectrum disorder model. With ASD being diagnosed based on behavior and there being no validated genetic biomarkers, the severity of the ASD in the rat was unknown.

ASD is a disorder that is diagnosed and treated based on a spectrum, and without knowing exactly where the rat falls on the spectrum, we were not able to evaluate individual differences in true language skills or cognitive ability. While it has been well

documented that RRBs present differently in males and females with ASD, future research using a test other than marble burying may allow for more insight into the expression of RRBs in males vs. females (Hiller et al., 2014; Tillmann et al., 2018).

An additional limitation of this study that should be noted is the data collection process. The drugs were mixed and administered by a single researcher, and the marble burying was recorded and analyzed by the same researcher making this a single-blind study. This could allow for opportunity for bias in the data collection process, with the researcher knowing the dosage of propranolol administered for each session.

## Theoretical Foundations of the Study

ASD is diagnosed based on the DSM-5's (2013) criteria stating that an individual must have persistent deficits in social communication and social interaction across multiple contexts, and restricted, repetitive patterns of behavior, interests, or activities. An additional symptom that often co-occurs with ASD is anxiety. By middle childhood, studies show that over 40% of individuals with ASD also have anxiety disorders that are conceptualized as persistently elevated anxiety symptoms leading to significant distress or impairment (APA, 2013; van Steensel et al., 2011).

Leo Kanner, one of the original ASD researchers, referenced children with ASD to have "anxious tenseness" and an "anxiously obsessive desire for the maintenance of sameness" (Kanner, 1943, p. 245). It can be theorized, based on Kanner's original work, along with many other researchers, that anxiety can be a trigger for RRBs in individuals with ASD. It should also be noted that anxiety in individuals with ASD can contribute to mood disorders such as depression and bipolar disorder (Cummings & Fristad, 2012).

The majority of ASD research has focused on characterizing the communicative and social deficits experienced by those affected, while RRBs have received much less attention. RRBs occur early in development and typically interfere with the individual's ability to acquire essential lifestyle skills. Additionally, RRBs severely affect the quality of life of the individual and their caregivers (Leekam et al., 2011; Wolff et al., 2014). The early diagnosis of RRB's in ASD is vital as the symptoms are considered predictive of anxiety in children and adults (Baribeau et al., 2020; Kuzminskaite et al., 2020).

Propranolol is a non-selective beta blocker that works by inhibiting the action of noradrenaline and adrenaline, two hormones often associated with anxiety (Deepmala & Agrawal, 2014). Propranolol has been used for decades to manage anxiety associated with stage fright, exam-related anxiety, and interview-related anxiety (Brantigan et al., 1982; Stone et al., 1973). Thus, it would be appropriate to theorize that propranolol would be an effective therapeutic treatment option for anxiety in individuals with ASD.

An additional consideration that is more recently being researched is the difference in symptomology between males and females with ASD. The majority of epidemiological research studies report the diagnosis of males to females at a ratio of 4:1 (Fombonne, 2009; Lyall et al., 2017). It is not uncommon for females to go undiagnosed or to be diagnosed late, as females seem to require more symptomology to be present in order to be diagnosed (Begeer et al., 2013; Lai et al., 2015; Petrou et al., 2018). Additionally, and more closely pertaining to this research, females with ASD score lower than males on measures of RRBs and are less likely to show restricted interests than males (Hartley & Sikora, 2009; Hiller et al., 2014; Lehnhardt et al., 2016). This research

supports the theory that there will be sex differences shown between the behaviors of the rats with ASD and without.

The need to further study ASD is imperative in order to develop a set of practices to allow for the reading of the Bible constructively in relation to individuals who suffer from ASD. The drastic increase in diagnoses and prevalence of ASD requires Christians to look at the condition and to question what it means to think Biblically about what it means to have such a diagnosis (Macaskill, 2022). This research will potentially aid in a deeper understanding of ASD and what it means to think Biblically about the condition.

## Definition of Terms (the cost of ASD)

The following is a list of definitions of terms that are used in this study.

**Autism and Developmental Disabilities Monitoring (ADDM) Network –** Term one is defined as is a program funded by CDC to collect data to better understand the number and characteristics of children with autism spectrum disorder (ASD) and other developmental disabilities living in different areas of the United States (CDC, 2023).

**Autism Spectrum Disorder (ASD) –** Term two is defined as persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history and restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least two of the following, currently or by history (CDC, 2023).

**Beta adrenergic receptor antagonists –** Term three is defined as a drug that binds to receptors in the lungs and heart causing the heart to beat faster and relaxes the muscles in the airways, allowing them to open up (Cleveland Clinic, 2023).

**Biomarkers –** Term four is a distinctive biological or biologically derived indicator (such as a metabolite) of a process, event, or condition (such as aging, disease, or oil formation) (Merriam-Webster, 2023).

**Diagnostic and Statistical Manual of Mental Health, Fifth Edition, Text Revision (DSM-5 TR) –** Term five is defined as the standard classification of mental disorders used by mental health professionals in the United States (APA, 2023).

**Dizygotic twins –** Term six is defined as twins that develop from two separate eggs (Cambridge Dictionary, 2023).

**Epigenetics –** Term seven is defined as a branch of genetics that studies the chemical reactions that turn genes on and off (Cambridge Dictionary, 2023).

**Food and Drug Administration (FDA)**– Term eight refers to an organization responsible for protecting public health by assuring the safety, efficacy, and security of human and veterinary drugs, biological products, medical devices, our nation's food supply, cosmetics, and products that emit radiation (FDA, 2023).

**Gene –** Term nine is a unit of heredity which is transferred from a parent to offsprings and is held to determine some characteristic of the offspring (Merriam-Webster, 2023).

**Geopolitically –** Term ten is defined as in a way that is connected with political activity as influenced by the physical features of a country or area, or with the study of the way a country’s size, position, etc. Influence its power and its relationships with other countries (Cambridge Dictionary, 2023).

**Infantile –** Term eleven is defined as of or relating to infants or infancy (Merriam-Webster, 2023).

**Insomnia –** Term twelve is defined as a common sleep disorder where a person may have trouble falling asleep, staying asleep or getting good quality sleep (NIH, 2023).

**Monozygotic twins –** Term thirteen is defined as two babies born to the same mother at the same time and develop from just one egg (Cambridge Dictionary, 2023).

**Neurodevelopmental Disorder –** Term fourteen is defined as conditions characterized by impairments in cognition, communication, behavior and/or motor skills resulting from abnormal brain development (AMA, 2013).

**Pathophysiological –** Term fifteen is defined as the functional changes that accompany a particular syndrome or disease (Merriam-Webster, 2023).

**Pharmacological –** Term sixteen is defined as the science of drugs including their origin, composition, pharmacokinetics, therapeutic use, and toxicology (Merriam-Webster, 2023).

**Prenatal –** Term seventeen is defined as occurring, existing, performed, or used before birth (Merriam-Webster, 2023).

**Prolific** – Term eighteen is defined as causing abundant growth, generation, or reproduction (Merriam-Webster, 2023).

**Propranolol** – Term nineteen refers to a class of medications called beta-blockers. It works by relaxing blood vessels and slowing heart rate to improve blood flow and decrease blood pressure (NIH, 2023).

**Restricted Repetitive Behavior (RRB)** – Term twenty is defined as behavioral patterns characterized by repetition, inflexibility, invariance, inappropriateness, and frequent lack of obvious function or specific purpose (Tian et al., 2022).

**Selective Serotonin Reuptake Inhibitors (SSRI)** - Term twenty-one refers to a class of medications most commonly prescribed to treat depression (NIH, 2023)

## Significance of the Study

ASD affects approximately 2% of children in the US, yet effective treatment options are severely lacking (Frye et al., 2019). Children with ASD and their families have to rely heavily on social, educational, and medical systems for support, creating a significant weight for all involved. Anxiety disorders are very common and often co-occurring conditions in individuals with ASD (van Steensel et al., 2011; Vasa & Mazurek, 2015). Anxiety is a well-known associate of RRBs with extensive research suggesting that RRB severity is an indicator of risk for increased anxiety symptoms in ASD (Baribeau et al., 2020; Keating et al., 2023; Sellick et al., 2021)

Restricted and repetitive behaviors affect individuals with ASD strongly and are considered to be disabling symptoms for not only the individual suffering from ASD, but also for the patient's family members (Bishop et al., 2007). Past research shows that early specific RRB symptoms can predict the severity and outcome of ASD, with specific studies indicating that preschool children with ASD who displayed RRBs tended to have worse school-aged language outcomes that those who did not exhibit RRBs (Charman et al., 2005; Miller et al., 2021; Paul et al., 2008 Troyb et al., 2016). The findings of this study will provide further insights into how individuals with ASD respond to certain pharmacological treatment options in order to help alleviate the symptoms associated with ASD.

Additionally, the findings of this study should further the investigation into how ASD and treatment options affect males and females differently. Early evidence supports

the idea that gender plays a significant role in diagnosing, treating, and symptomology of patients with ASD (Comparan-Meza et al., 2021).

## Summary

The primary goal of this study is to characterize how the number of restrictive, repetitive behaviors changes after the administration of propranolol in differing doses in an autism model of Sprague-Dawley rats. Additionally, we will also look at how gender moderates the change in the number of restrictive, repetitive behaviors once the drug is administered. ASD is a complex disorder that is diagnosed on a spectrum and characterized primarily by deficits in social communication and interactions and restricted repetitive behavior (RRB) patterns (APA, 2013; Comparan-Meza et al., 2021). While there are drugs used to treat some of the side effects of ASD in males and females, there is not an FDA approved drug to treat the disorder, and research is lacking in knowing the differing treatment effects on males and females. This research will aid in further discovering not only additional treatment options for the debilitating side effects of ASD, but also to better understand how these treatment options affect the individual whether male or female.

# CHAPTER 2: LITERATURE REVIEW

## Overview

Autism Spectrum Disorder (ASD), first published by Dr. Leo Kanner in the 1940s, is a neurodevelopmental disorder characterized by deficits in social communication and restricted repetitive behavior patterns. ASD affects an individual's biologic, neurologic, cognitive, social, and linguistic traits, making it a complex and crucial disorder to research. Although there are many theories on the cause of ASD, studies have not been conclusive and are often difficult to replicate. Additionally, there are currently no FDA-approved drugs for the treatment of ASD as a whole, and the drugs prescribed to treat the symptoms of ASD often have negative side effects, and long-term efficacy has been brought into question.

Restricted repetitive behaviors (RRBs), a core symptom of ASD thought to be associated with anxiety, currently do not have an FDA-approved drug treatment option; thus, the need for further investigation is imperative. Propranolol is a beta-blocker often prescribed to individuals to treat anxiety-like symptoms and could be a potential treatment option for individuals with ASD. With ASD being a complex disorder, there may also be gender differences not only in the presentation of ASD but also in the treatment options that need further exploration as well.

Although the exploration into ASD is imperative, it is also important to think Biblically about the disorder and to recognize that while it is labeled as a disability, it does not necessarily mean that it needs to be cured. Researching options for alleviating the burden of the symptoms of ASD, such as RRBs, is more beneficial to an individual than a persistent need to find a cure.

## Description of Search Strategy

The search strategy began in 2022 using databases such as PubMed and Google Scholar. A combination of terms was used in the main search strategy, such as autism spectrum disorder restricted repetitive behaviors beta blockers, AND differences AND gender differences anxiety propranolol ASD drug therapies and RRB drug therapies AND anxiety drug therapies AND polypharmacy and ASD AND Oxytocin and ASD AND Cannabis and ASD AND marble burying AND ASD rodent model AND biblical references to mental health.

Additionally, biblical research was conducted with the use of the website Bible Gateway and Google. In each of the databases, the searches were further limited to English language, published between 2019-2023, and peer-reviewed articles. A number of sources were found outside of this time frame as it was somewhat limited to only use in the last five years. Only full-text articles were reviewed and chosen for inclusion based on the information presented in each article.

## Review of Literature

### Autism Spectrum Disorder

#### Origination of ASD

Autism spectrum disorder is a developmental disability that is characterized by deficits in social communication and interactions and restricted repetitive behavior (RRB) patterns (APA, 2013; Comparan-Meza et al., 2021). A psychiatrist by the name of Leo Kanner was the first to publish a description of early infantile autism in the early 1940s. Kanner founded and directed the child psychiatry program at Johns Hopkins University School of Medicine and authored the first textbook on child psychiatry (Harris, 2018). He

is most well-known for his 1943 paper, *Autistic Disturbance of Affective Contact*, the first description of ASD as a neurodevelopmental disorder (Kanner, 1943, 1968).

Thirty years later, Lorna Wing and Judith Gould examined autism using Leo Kanner's diagnostic criteria. They found a large number of children with what was called, at the time, Kanner autism demonstrated difficulties with social interactions, communication, and imagination (National Autistic Society, 2023). It was not until the late 1990s and into the early 2000's that autism started to be discussed on a spectrum and encompassed Autistic Disorder, Asperger's Disorder, Pervasive Developmental Disorder – not otherwise specified (PDD-NOS), Rett Disorder, and Childhood Disintegrative Disorder (DSM-IV, 1994).

In 2013, the DSM-5 was released, and the diagnostic terminology for Autism changed yet again. The DSM-5 removed the separation of the diagnosis and created one continuum known today as autism spectrum disorder, or ASD (Faroy et al., 2016). This change created the need for further exploration into the disorder and the treatment options that would be the most effective for each individual.

**Initial Tracking and Diagnosing- POSSE**

While the initial description of ASD was in the 1940s, the tracking of ASD in the United States only dates back to 1998 following the first CDC study based in Brick Township, NJ. A citizen group named POSSE, Parents of Special Services and Education, living in the town had concerns about a larger than expected number of children with ASD. The main concern was that environmental factors may be playing a role in the increased instances of ASD. The role of environmental factors as a contributor to ASD is still being researched as a theory to this day.

Clinical evaluations by a developmental pediatrician were conducted using the Autism Diagnostic Observation Schedule-G (ADOS-G) along with a battery of tests to assess the language, spatial-cognitive, intellectual, and adaptive functioning of each child. The findings of this study found that the rate of ASD in Brick Township was 4.0 per 1,000 children. These rates were higher than any other rates that had been previously diagnosed in the United States thus far (Bertrand et al., 2001; CDC, 1998). This study led to the broadening of diagnostic criteria and improved recognition for diagnosing and treating ASD.

**Genetic Component of ASD**

Those with ASD face unique challenges in that the negative impacts span adaptive, biologic, neurologic, cognitive, social, and linguistic traits (Hus & Segal, 2021; Hus & Segal, 2021). ASD can be inherited, but there is no specific gene or marker for determining its presence (Hus & Segal, 2021; Charman & Gotham, 2013). It has additionally been found that the prevalence of ASD varies geopolitically, with an extreme rise in occurrence from 1 in 2000 to 1 in 54 in less than 50 years (Hus & Segal, 2021; Edelson, 2021).

A number of researchers have conducted studies that have found factors that could contribute to an ASD diagnosis, including epigenetics, increased parental age, neonatal complications, and environmental exposures, to name a few (Bilder et al., 2009; Gardener et al., 2011; Gregory et al., 2009; Grether et al., 2009; Hu et al., 2006; McCanlies et al., 2012; Newschaffer et al., 2002; Reichenberg et al., 2006; Schmidt et al., 2012; Shelton et al., 2010; Windham et al., 2006). However, these studies have not

concluded significance and are often difficult to replicate, leaving room for much more needed research.

Folstein and Rutter (1977) conducted twin studies and found incidence among siblings was 50x higher than average, with an 80% chance that if one identical twin is diagnosed, the other will also have an ASD diagnosis (Nuwer, 2015). Additionally, it was found that monozygotic twins were more likely to share a diagnosis than dizygotic, further suggesting a genetic influence (Bailey et al., 1995; Rylaarsdam & Guemez-Gamboa, 2019). These findings were groundbreaking in the study of the involvement of genetics with ASD.

Even with the incredibly high and increasing rates of diagnosis in the United States, there are serious limitations when trying to identify, diagnose, and treat ASD. These challenges arise due to the fact that the diagnosis is based solely on observation. There are no true biological measurements to date that can identify the pathophysiological processes that aid in diagnosing or treating ASD (Frye et al., 2019). Additionally, much of the research that is being used is based on parent-observation as opposed to a clinician or specialist. As previously stated though, ASD is heavily and becoming increasingly researched with the steady rise in diagnosis.

Ongoing research is taking place to develop biomarkers to measure the biological abnormalities associated with ASD that will allow for better detection and treatment options for those affected, but much research is still needed to find conclusive results. A biomarker is an objective measure of pathophysiological processes or pharmacologic responses to therapeutic interventions. Biomarkers help to diagnose a disease, classify disease severity, indicate prognosis, or predict or monitor response to type of therapy

(Biomarkers Definitions Working Group, 2001). A genetic biomarker for ASD could potentially change treatment options, both in terms of behavioral and pharmacological.

**Prenatal Risks and ASD**

In recent years, there has been an abundance of research into prenatal factors playing a role in the development of individuals with ASD. There is little awareness of how an infection or fever during pregnancy can affect offspring in terms of mental health issues. It has been estimated that 30% of schizophrenia diagnoses could be prevented with the prevention of viral, bacterial, and parasitic infections during pregnancy (Brown et al., 2010; Patterson, 2011).

While the impact of schizophrenia on offspring is important to note, it has also been found that viral infections have a strong association with ASD. A study that looked at over 10,000 ASD cases found a strong association with maternal viral infection in the first trimester. Additionally, an association was found between bacterial infections during the second trimester and ASD (Atladottir et al., 2010).

Additional research has demonstrated that changes in the composition of the gut microbiome during pregnancy may also contribute to ASD in offspring (Mayer et al., 2014). A pilot study examined children with ASD and the gut microbiome of the mothers. The findings showed a dramatic variation between the mothers of ASD children and the mothers of TD children (Li et al., 2019).

An additional concern for offspring developing ASD due to complications during pregnancy is iron deficiency (ID). A study that used health and population data from the Stockholm Youth Cohort evaluated over 500,000 children to find a correlation between children with ASD and mothers who were diagnosed with anemia during pregnancy. The

results showed that the prevalence of ASD, ADHD, and ID was higher among children born to mothers who received an anemia diagnosis during their first trimester (Wiegersma et al., 2019).

There are many issues during pregnancy that are being found to have correlations with neurodevelopmental disorders. It has been found that something as simple as a fever during pregnancy may be implicated in offspring having ASD. A study conducted in Norway that looked at maternal fever in the second semester found an association with an elevated risk of ASD, and a study in California found second-trimester fever to double the risk of ASD in offspring (Brucato et al., 2017; Hornig et al., 2017).

**The Cost of ASD**

ASD has become one of the more prolifically researched medical disorders of this time due to the number of individuals it affects and the rapidity of its increase in diagnoses. Over the past three decades, the prevalence of ASD is now estimated to affect 2% or more of children in the United States (Christensen et al., 2018; Xu et al., 2019). The disability of an individual with ASD not only affects the quality of life for the individual and the individual's family or caretakers, but it also carries a significant economic burden.

A diagnosis of ASD typically carries a substantial cost spanning the lifetime of the individual and their family members. A case study using parent reports from almost 46,000 children aged 3-17 with an ASD diagnosis was conducted to assess the cost of the diagnosis for the caretakers. The annual per-child cost of ASD relative to a child without an ASD diagnosis was close to 4000$ (Zuvekas et al., 2021). It should also be taken into

consideration that, at times, one family may have more than one individual with an ASD diagnosis, thus adding to the financial strain.

The economic burden does not simply affect the caretakers and family members involved, but it also affects the country as a whole in terms of healthcare costs. In 2015, it was found that ASD cost the US over $260 billion dollars and is projected to cost over $460 billion by 2025 (Rogge & Janssen, 2019; Leigh & Du, 2015). The rising cost of ASD in the U.S. further confirms the need for further research on diagnostic and prevention treatment options.

**Early Recognition and Intervention for ASD**

There are currently both behavioral and pharmacological treatment options available for the symptoms of ASD, but unfortunately, these treatment options do not reach every individual diagnosed. The National Survey of Children's Health found that among 1115 children with a diagnosis of ASD, only 43.3% are treated with behavioral treatment, 6.9% are treated with medication, and an astounding 29.5% receive no treatment at all (Xu et al., 2019).

Among the treatment options are behavioral therapy, nutritional therapy, joint attention therapy, and medication treatment. Unfortunately, ASD is a complex diagnosis and the most effective therapies for one individual with ASD are often not the same for another individual with ASD (NIH, 2023). The best prognosis for a child with ASD has been found to be early intervention paired with prompt and evidence-based intervention strategies (Zwaigenbaum et al., 2015).

Growing evidence supports the idea that ASD can be diagnosed accurately prior to 2 years of age (Chawarska et al., 2007; Guthrie et al., 2013). Additionally, research has

shown that age two is a particularly important year for intervention due to the dynamic brain growth that is occurring and the substantial neural plasticity that is happening, providing the potential to alter the course of development for the individual (Courchesne et al., 2011; Dawson et al., 2008; Lewis et al., 2014).

## Topography of ASD

Individuals with ASD often develop difficult and challenging behaviors. These behaviors are not seen as culturally or socially acceptable, may put the physical safety of themselves or others in jeopardy, limit access to community settings, and/or affect the learning of the individual or those around them (Hong et al., 2018; Matson et al., 2010). Some of the more common topographies of challenging behaviors are repetitive stereotypic vocalizations, elopement, disruptions or tantrums, and self-injury (Horner et al., 2002; Jang et al., 2011).

Research that analyzed challenging behaviors in over 2000 individuals with ASD receiving behavioral intervention found that in most cases, one specific behavior emerges as the dominant behavior (Hong et al., 2018; Stevens et al., 2017). It should be noted, however, that difficult and challenging behaviors are not a part of the ASD diagnosis, and the topography of the behaviors typically is not treated until a diagnosis already persists.

## ASD, Irritability, and Sleep Disturbances

One of the most discussed symptoms associated with ASD is high levels of irritability. Irritability is often associated with adverse responses to certain sensory stimuli, such as a strong smell or a sound (Haimovich et al., 2023). These associated sensory problems are now described in the DSM-5 as presenting restricted, repetitive behavior patterns (American Psychiatric Association, 2013).

Additionally, sensory sensitivities are found to be correlated with sleep disturbances. Sleep disturbances are reported in 44-84% of children with ASD, in contrast to 10-30% in TD children (Hirata et al. 2016., Little et al. 2018). Changes in sleep disturbances could potentially have an effect on the improvement of certain ASD symptoms, such as RRBs.

A study investigated anxiety in ASD individuals and the relation it has to increased ASD symptoms and sleep disturbances. It has been well established that ASD children often have sleep problems, including insomnia, delayed onset of sleep, shorter sleep duration, and increased levels of sleepwalking (Cortesi et al., 2010; Elrod & Hood, 2015; Posar & VIsconti, 2020). Previous research has found that these sleep problems were linked to higher levels of anxiety, but more recent research found that anxiety and sleep disorders may be bidirectional (Adams et al., 2014).

An additional study by Distefano et al. (2023) looked at how sleep disorders affect the core symptoms of ASD and their relationship with clinical symptoms. The results showed that sleep disorders were heavily associated with ASD symptoms such as anxiety, withdrawal, depression, aggression, tantrums, and inattention. Pharmacological therapeutic options to alleviate sleep disorders could potentially result in lower rates of anxiety and anxiety-like behaviors, such as RRBs, in individuals with ASD.

**Non-pharmaceutical ASD Treatment Options**

With the two main symptoms that are used to diagnose ASD both being behavioral, the preferred form of treatment is often centered around behavioral therapy. The most notable behavioral treatment option for ASD is Applied Behavior Analysis (ABA), which encourages desired behavior and discourages unwanted behaviors through

reinforcement (CDC, 2022). The two types of ABA therapy that are the most popular are discrete trial training (DTT) and pivotal response training (PRT) (Pruneti et al., 2023).

DTT and PRT are similar in that they both train an individual to build skills, but DTT is a structured form of skill building, and PRT usually occurs in a more natural environment through play (Chicago ABA Therapy, 2017). Along with the more popular behavioral therapy options for ASD, there are a number of other interventions that are employed as well. The treatment and education of autism and communication handicapped children (TEACCH), picture exchange communication system (PECS), early start Denver model (ESDM), and early intensive behavioral intervention (EIBI) are other recognized behavioral treatment options for individuals with ASD.

Pruneti et al. (2023) conducted research to determine the efficacy of these treatment options for ASD. The comparative analysis showed that all of the previous behavioral methods mentioned were effective in improving the side effects associated with ASD. While behavioral therapy is proven to be effective, there are disadvantages to the implementation of behavioral therapies, such as the selection of the appropriate intervention, the time it takes to implement and to see change, and the cost of the services (Pros and Cons of ABA Therapy, 2023).

## Restricted Repetitive Behavior

### RRBs

Restricted repetitive behavior (RRB) patterns are a core symptom of ASD that have been discussed and described in detail by many specialists dating back to Leo Kanner and Hans Asperger. Both specialists discussed not only the extent to which children appreciate sameness but also the stereotypic movements an individual with ASD would make (Asperger, 1952, p.43; Kanner, 1973, p. 63). RRBs are characterized by high

frequency, repetition in an invariant manner, and a desire for sameness in the environment (Kanner, 1943; Leekam, 2011).

The DSM-5 (2013) divides RRBs into four subtypes: (a) Stereotyped or repetitive movements, use of objects, or speech. (b) Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or non-verbal behavior. (c) Highly restricted, fixated interests that are abnormal in intensity or focus, and (d) Hyper- or hypo-reactivity to sensory input or unusual interests in sensory aspects of the environment. Although there are guidelines for assessing whether an individual is presenting RRBs, they have not received the attention needed with how crucial they are to the diagnosis.

**RRBs and Cognition**

Initially, researchers thought that RRBs were a result of executive function (EF) impairment. Executive function was a term first proposed in the mid-20$^{th}$ century to explain the functions of the frontal lobe. The frontal lobe received much attention for needed research following certain studies, such as the case study of Phineas Gage, where it was found that frontal lobe damage led to impairment of planning, organizing, self-regulation, and impaired discrete functions (Demetriou et al., 2019; Harlow, 1868). As science has advanced and neuroimaging has become more of a possibility, many discoveries have been made in understanding the frontal lobe and how it affects behavior (Szczepanski & Knight, 2014).

Multiple researchers have thus looked to the frontal lobe for signs of impairment in individuals with ASD. It has been found that the frontal lobe in ASD patients is overgrown relative to the other regions of the brain (Buxhoeveden et al., 2006). Additionally, it has been found that there are gene expression changes in the cerebral

cortex of individuals with ASD that include distinctions between the frontal and temporal cortices in ASD brains (Voineagu et al., 2011). The distinctions between brain regions could further allude to why individuals with ASD partake in the behaviors they do.

Although RRBs are a core symptom of ASD, understanding why an individual with ASD partakes in these behaviors is still unknown. Ravizza et al. (2013) researched the idea that they could be due to deficits in attention and a lack of motor control. Individuals with ASD typically have selective attention toward one object, interest, or ritual. Multiple studies have found that children with ASD are not able to switch attention as rapidly as those without (Harris et al., 1999; Townsend et al., 1999; Wainwright-Sharp et al., 1993).

**Set-Shifting**

An early focus in researching ASD and RRBs focused on set-shifting. Set shifting is the ability to shift mindset to new concepts. Essentially, individuals who suffer from ASD are impaired in their ability to switch from one learned rule to a new rule when trying to change behavioral contingencies (Corbett et al., 2009; Geurts et al., 2009; Kaland et al., 2008). The inability to set shift could be what leads individuals with ASD to engage in restricted behaviors, affect their level of intelligence, induce aggressive behavior, and affect self-control and level of social activity (Farrelly & Mace, 2015; Memari et al., 2013; Visser et al., 2014).

Researchers over the years have developed strategies and interventions to improve flexibility in individuals with restricted behavior habits. An intervention titled *Unstuck and On Target* (UOT), was developed to target insistence on sameness in individuals with ASD (Kenworthy et al., 2014). UOT used experiments involving videos and

discussion scenarios in an attempt to train children in physical/mental flexibility goal setting, and planning. In comparing the effectiveness of UOT, the results showed significant improvements in the children's ability to remain flexible and set goals.

Saniee et al. (2019) conducted a study of set-shifting improvement tasks (SSIT) in an attempt to improve set-shifting abilities in preschool aged children with ASD. The idea behind the tasks was that if an individual with ASD can learn how to shift, it will aid in perseverating behaviors and a number of emotional and behavioral problems. A home-based computer game was developed, and the participants were to train on the game and see if the learned abilities extended to their daily lives. The results of the study showed a major impact on behavioral and cognitive flexibility.

**When RRBs Present**

RRBs are said to be identifiable as early as 12 months as they are among the earliest detectable behavioral markers of ASD (Elison et al., 2014; Harrop et al., 2014; Ozonoff et al., 2008). Additionally, it was found that infants who had a high familial risk for ASD and later received a diagnosis of ASD have elevated repetitive behaviors early in life (Wolff et al., 2014). This research shows that RRBs could be an effective way for an early diagnosis of ASD and, thus, earlier prevention strategies.

A challenging aspect of RRBs that should be noted is that they are not specific to individuals with ASD. Not only do they present in typically developing children, but they also present across a range of neurodevelopmental disorders (Evans et al., 2016; Hoch et al., 2016). An effective way to distinguish between normative RRBs and those predictive of risk for ASD is to look at the change in RRBs over time (Sifre et al., 2021).

Uljarevic et al. (2017) analyzed data from individuals with RRBs at 15 months of age until 77 months of age. Three different time points were used, 15, 26, and 77 months, and the Repetitive Behavior Questionnaire-2 (RBQ-2) was sent to parents to fill out. The RBQ-2 is a 20-item questionnaire to assess repetitive behaviors occurring in both children with ASD and typically developing (TD) children. The results showed that RRBs were present as young as 15 months and still at 77 months of age, showing consistency across time for individuals with ASD.

**How RRBs Present**

As previously stated, RRBs present a**s** repetitive movements, use of objects or speech, inflexible adherence to routines, or ritualized patterns of verbal or non-verbal behavior, and highly restricted, fixated interests (American Psychological Association, 2013). To date, there is not a clear representation of how RRBs differentiate between TD children and children with ASD. There is also limited research that tracks the trajectory of RRBs and their later outcome for those children (Leekam et al., 2011).

According to the Kennedy Kreiger Institute (2023), RRBs often present in repetitive movements such as hand flapping or lining up items in a row. There may also be an insistence on sameness, such as taking the same route to school every day or doing activities in exactly the same order every time. An individual with ASD may also display repetitive speech, referred to as echolalia, however, there is not much that is known about the group of children with ASD who are minimally verbal. (Harrop et al., 2021).

Researchers have additionally started to subdivide RRBs into two factors known as “lower-order” and “higher-order.” Lower-order RRBs are characterized by stereotyped motor movements, repetitive forms of self-injurious behaviors, and repetitive movement

of objects. Higher-order RRBs are more likely to include echolalia, insistence on sameness, and extreme attachments to objects (Chaxiong et al., 2022). An additional way of categorizing RRBs is to organize them as repetitive sensory motor (RSM) or insistence on sameness (IS) (Hiruma et al., 2021; Uljarevic et al., 2017).

**Sleep Problems and RRBs**

It has previously been found that children with neurodevelopmental disorders, such as ASD, suffer from problems sleeping much more than TD children (MacDuffie et al., 2020; Mannion et al., 2013; Maskey et al., 2013; Reynolds et al., 2019). Additionally, sleep problems have been associated with an increase in RRBs for individuals with ASD Cohen et al., 2014; Goldman et al., 2009; Hundley et al., 2016; Park et al., 2012; Tudor et al., 2012). Previous findings support the idea that the association between RRBs and sleep problems is often due to anxiety in the individual with ASD (Hundley et al., 2016).

A more recent study investigated the course of RRBs in early development in relation to the sleep problems reported by parents of children with ASD. The findings were interesting in that the reported association between sleep and RRBs remained consistent with previous findings until the children were assessed at a single timepoint of age 4. MacDuffie et al. (2020) found that at age 4, anxiety symptoms and sleep problems independently contributed to RRBs. This research may support the idea that individuals with ASD should be assessed in narrower timepoints in early childhood when ASD symptoms initially start to present.

**Anxiety and RRBs**

While RRBs are a well-established symptom of ASD, the specific cause of RRBs for an individual with ASD remains elusive. The onset and continuance of RRBs are seen

as somewhat of a cycle where the individual initiates a repetitive behavior to block out the noises of the environment, signaling the individual to behaviorally adapt. The RRBs, thus, increase in frequency and intensity as a response to the escalating environmental signaling (Cashin et al., 2018; Cashin & Yorke, 2016; Frawley, 2008). It is thought that levels of anxiety and depression escalate once this cycle commences (Cashin, 2016).

Anxiety is found to be one of the most prevalent comorbidities with an ASD diagnosis, with approximately 30% of individuals with ASD also presenting with an anxiety disorder (Hollocks et al., 2019). Additionally, there have been significant positive associations found between RRBs specifically and anxiety in children with ASD (Magiati et al., 2016; Teh et al., 2017).

Kuzminskaite et al. (2020) conducted a study to assess whether social communication difficulties or RRBs were more likely a risk factor for causing anxiety-like symptoms in individuals with ASD. The results indicated that RRBs, higher-order, and lower-order, significantly predicted anxiety symptoms in individuals with ASD. It was additionally found that when RRBs were considered together with social communication difficulties, there was no significant prediction for anxiety. It was only when RRBs were considered alone that there was a positive correlation.

**Pharmaceutical Therapy and RRBs**

Early recognition of RRB symptoms can help predict the severity and outcome of ASD, making RRBs incredibly important to be aware of (Miller et al., 2021; Tian et al., 2022; Troyb et al., 2016). While there are drugs that are approved and used to help in treating ASD symptoms, there are currently no drugs approved by the FDA to target the

symptoms of restricted and repetitive behaviors to date, but that has not stopped researchers from trying (London et al., 2020).

A data analysis was conducted that analyzed the data for 41 drugs across 100 trials and 17 dietary supplements across 43 trials and 7450 participants. The results of this study showed that children with social-communication difficulties showed improvements with risperidone, aripiprazole, folinic acid, probiotics, and bumetanide. There was some crossover for RRBs, with improvements shown with risperidone, aripiprazole, atomoxetine, bumetanide, valproate, and guanfacine. The most significant results for both social communication and RRBs were shown when the individual received risperidone and aripiprazole (Siafis et al., 2022).

An additional study was conducted that assessed the placebo response in relation to pharmacological and dietary supplements in core symptoms of ASD. An analysis of 2360 participants on a placebo was included in the analysis, and both social communication deficits and RRBs were assessed. The results showed that about 20% of the participants significantly improved while taking a placebo compared to a pharmacological or dietary supplement (Siafis et al., 2020). It should be noted that it has been argued that the placebo response may be more significant in children and adolescents than adults. However, this study did not find a difference between age groups (Weimer et al., 2013).

Finding a pharmacologically therapeutic option for treating the symptoms of ASD is not the only hurdle, however. The efficacy and tolerability of the medication that is used to treat the individual also have to be considered. Finding a medication that works for an individual is typically a lengthy process that requires medication changes and

treatment trials throughout a patient's life (Holdman et al., 2022). There are studies that support the efficacy and tolerability of methylphenidate and atomoxetine for ASD, but there is much research still needed to assess the long-term use of the medications (Rodrigues et al., 2021).

## Pharmacokinetics and Pharmacodynamics

### Historical Findings of Pharmacological Testing and ASD

As previously stated, there are currently no drugs that are approved by the FDA for the treatment of ASD as a whole; however, there are some options for the side effects of ASD. The most commonly prescribed medications for ASD are aripiprazole and risperidone for treating irritability associated with ASD (NIH, 2021). The long-term use and the side effects of both of these medications are often called into question, however.

Risperidone has been approved by the FDA to treat the irritability associated with ASD since 2006, but it was initially used to treat schizophrenia (Hutchinson et al., 2023; Mano-Sousa et al., 2021). The efficacy and tolerability have also been assessed in several clinical trials and show a 70% response rate in efficacy for treating ASD symptoms (Maneeton et al., 2018). Additional research has shown that treatment with risperidone was comparable to a placebo, and there was no difference found between the drug and placebo for tolerability (Hutchinson et al., 2023; Maneeton et al., 2018).

While an individual's cognition does not seem to be affected by risperidone, there are other side effects to take into consideration. The physical health of the individual should be assessed by prescribing pharmaceutical treatment options. A meta-analysis that looked at weight gain for long-term and short-term risperidone use showed an increase in

patients' weight when compared to baseline values and to a placebo (Mano-Sousa et al., 2021).

Aripiprazole, the other drug approved by the FDA for ASD side effects, is also used to treat irritability in individuals with ASD (NIH, 2021). Similar to risperidone, aripiprazole was initially used to treat schizophrenia (NIH, 2023). A data analysis that reviewed three trials found that aripiprazole was effective in treating irritability but did cause weight gain, a higher risk for sedation, and tremors (Hirsch & Pringsheim, 2016).

An additional study that looked at olanzapine, risperidone, and aripiprazole use in children with ASD found similar results. While the irritability was decreased, sedation and duration of sleep increased; additionally, weight gain became a problem (Hesapcioglu et al., 2020). It was additionally found that long-term use of aripiprazole and risperidone can cause hyperglycemia, and the importance of monitoring for symptoms such as polydipsia, polyphagia, and weakness (Marcus et al., 2011). While the objective of the medication is to reduce the side effects associated with ASD, the adverse effects should not be ignored.

A noteworthy concern with introducing pharmaceutical therapies for an individual with ASD is that it often leads to polypharmacy or the use of multiple drugs to treat a single ailment (Fieiras et al., 2021). Polypharmacy is found to be predicted by age, time since ASD diagnosis, and psychiatric comorbidities (Croteau et al., 2017; Houghton et al., 2017; Jobski et al., 2017; Spencer et al., 2013). It is found to be common practice, but there is minimal effectiveness for multi-drug treatment options for ASD management (Espadas et al., 2020; Li et al., 2017; Spencer et al., 2013). Additionally, studies show

that polypharmacy is negatively associated with the health-related quality of life and psychological stress of individuals (Wilder et al., 2022).

**Current Research into Pharmaceutical Options**

There has been a substantial amount of research into the pharmacological testing for individuals with ASD, but there is still much research that is needed. While risperidone and aripiprazole are currently, FDA approved and show signs of efficacy for irritability, the use of other drugs is being tested, which may have lesser side effects and long-term outcomes. Additionally, a drug that is used to treat ASD as opposed to simply treating the conditions associated or using multiple medications to treat ASD would be ideal.

**Cannabis as a Treatment Option**

Medical cannabis, or medical marijuana, is derived from the plant *Cannabis sativa*, one of the world's oldest propagated plants (Agarwal et al., 2019). There are many controversies surrounding the use of cannabis both recreationally and medically. The U.S. Drug Enforcement Agency (DEA) currently has cannabis categorized as a Schedule I controlled substance, having a high potential for abuse and lacking the safety data needed for the use under medical supervision (Bridgeman et al., 2017).

Cannabis has also often been cited as a *gateway drug*, simply meaning that it is likely to precede the use of other licit and illicit substances (NIH, 2023). A three-year study that looked at the relationship between cannabis use and alcohol use disorder (AUD) found that there was a significantly increased risk for AUD following the use of cannabis. Additionally, the use of cannabis predicted the persistence of the AUD within three years of use (Weinberger et al., 2016).

Despite its controversial standing legally, in 2014, approximately 22.2 million Americans reported current recreational cannabis use (Substance Abuse and Mental Health Services Administration, 2016). Additionally, cannabis has been gaining popularity for medicinal use in treating a number of ailments, including but not limited to the conditions associated with ASD (Agarwal et al., 2019). According to the World Population Review (2023), as of March of 2023, 41 states and 13 countries have legalized marijuana for medicinal purposes.

A case study review that assessed cannabis use for medicinal purposes found that while cannabis could potentially show promise, the benefits of the use of cannabis for ASD are insufficient (Agarwal et al., 2019). It is also important to note that the long-term physical and mental health effects of cannabis on an individual are still unknown (Vigil et al., 2022). Several studies have found an association between recreational cannabis use in adolescence and cognitive impairment lasting up to 28 days following use (Bolla et al., 2002; Medina et al., 2007; Pope et al., 2003).

A study conducted on the population of individuals in Northern Finland with a sample size of 6534 individuals yielded interesting results pertaining to cannabis use and psychosis. The results showed that the use of cannabis in adolescent years was associated with psychosis, and the use of cannabis at least five times or more was positively associated with a psychosis diagnosis (Mustonen et al., 2018). While the use of cannabis may show promise in treating conditions associated with ASD in the short-term, the long-term outcomes are still being researched.

**Oxytocin as a Treatment Option**

Another drug that has been shown to be beneficial but is still not an FDA-approved drug to prescribe to children with ASD is oxytocin. Oxytocin is a regulatory hormone that is crucial to early life social learning and lifelong social behavior (DeMayo et al., 2019). It has previously been shown in a number of studies to improve social responsiveness and attachment styles in children with ASD compared to a placebo (Bernaerts et al., 2020; Daniels et al., 2023; Yamasue et al., 2020).

A study that looked at the effects of oxytocin on social interaction when used as a nasal spray yielded interesting results. The study looked at the effects of oxytocin on social development in children (aged 3 to 12 years) with ASD. Unfortunately, there was no overall benefit of using oxytocin as a treatment option for children with ASD as a whole, but there was evidence showing that there was a benefit when used with younger children aged 3-5 Guastella et al., 2022).

Additional research has looked at combining oxytocin with other drug therapies as a treatment option for ASD. Although ASD is typically seen as neurodevelopmental and behavioral, gut microbiome composition and inflammation have been reported to also affect individuals with ASD (Alam et al., 2017). There is evidence that demonstrates a connection between changes in behaviors with changes in the gastrointestinal (GI) tract via the gut-microbiome-brain axis (Fung et al., 2017; Kong et al., 2019).

Kong et al. (2021) conducted a clinical trial that combined the use of oxytocin and probiotics in patients with ASD. The study explored the two treatment options, both alone and in combination with each other, against a placebo control group. The results showed a trend of improvement in both social and behavioral measures, but only when the two drug therapies were used in combination. While these results are promising, the

risk of polypharmacy should not be forgotten when treating individuals with multiple drug therapies at once.

**Propranolol as a Treatment Option**

While there are a number of drug therapies in trial phases to treat ASD, the search for one that will receive FDA approval and prove efficacy and long-term safety has yet to be found. Propranolol is a beta-blocker that is prescribed to individuals to treat high blood pressure, tremors, and heart rhythm disorders (MedlinePlus, 2023). It is a commonly researched drug as it is a beta-blocker that crosses the blood-brain barrier, exerting its effects on the central nervous system in addition to its peripheral activity (Steenen et al., 2016).

Along with heart problems, propranolol has also been used to help with the physical symptoms of anxiety (NHS, 2023). According to past research, propranolol has been shown to be effective in lowering emotional arousal, eradicating stage fright, and alleviating anxiety-related cognitive dysfunction (Brantigan et al., 1982; Faigel et al., 1991; Grillon et al., 2004; Szeleszczuk et al., 2022). As previously stated, anxiety is found to be one of the most prevalent comorbidities with an ASD diagnosis (Hollocks et al., 2019). Additionally, it is thought that RRBs are triggered by anxiety.

In the late 1980s, propranolol was tested to show improvements in language and sociability in the context of ASD (Ratey et al., 1987). Additionally, propranolol has been explored to test how it affects verbal abilities, working memory, facial scanning, and functional connectivity (Berversdorf et al., 2008; Berversdorf et al., 2011; Bodner et al., 2012; Narayanan et al., 2010; Zamzow et al., 2014). While this research is beneficial in further understanding the effects of beta-blockers on an ASD individuals' symptoms,

there is a lack of research surrounding how it affects an important side effect of ASD, specifically RRBs.

## Gender Differences

### Gender Differences for ASD Detection

In the most recent report on ASD prevalence, the CDC estimated that 1 in 36 children has an ASD diagnosis, with 4% being males and 1% being females (Maenner et al., 2023). Detecting ASD in females has also been said to be more difficult because females are more likely to behave in a quiet and timid manner in comparison to males. A study conducted on children in India found that females without intellectual disability (ID) were diagnosed significantly later than males with ASD (Malhi & Singhi, 2023). An additional study supporting Malhi and Singhi's study found that females without ID were diagnosed almost two years later than males (Goin-Kochel et al., 2007).

A commonly researched idea for why there are more males than females is often credited to a phenomenon called "camouflaging" (I.e., females masking their autistic symptoms) (Hull et al., 2017). The idea of camouflaging suggests that the prevalence of ASD in females may be higher than what is estimated and that it is simply harder to detect in females than in males (Schuck et al., 2019). While camouflaging can happen in both males and females, it is found to be much more likely in females.

An additional theory was proposed in 1981 by a researcher at the University of Michigan in Ann Arbor named Luke Tsai. Tsai found that females with autism had more relatives with autism on average than males. This finding alluded to the idea that girls need to inherit more factors related to autism than boys do in order to show traits of ASD (Tsai et al., 1981).

Along with behavioral differences that are shown to be different in males and females with ASD, neuroimaging studies have also demonstrated frontal lobe abnormalities in male patients with ASD that were not present in female patients (Meng-Chuan et al., 2013; Zeestraten et al., 2017). Additionally, there were significant gender differences found in the motor system area of the brain, and fetal testosterone levels were found to be correlated with gender differences as well (Baron-Cohen et al., 2011). In addition, research is still needed to conclude what physiological differences are found between males and females.

**Gender Differences in RRB presentation**

Previous research has found gender differences not only in the detection of ASD but also in how the symptoms present. It should be noted that RRBs are commonly observed in TD infants and toddlers, but persistence over time is what distinguishes RRBs in ASD from what is observed in TD children (Matson et al., 2009). As previously stated, early diagnosis and intervention are incredibly beneficial for the individual and the caregivers when discussing ASD.

Due to an intricate history of the use of female subjects in research, females are rarely studied independently from males in research. Harrop et al. (2015) conducted a study with 29 males and 29 females to investigate whether males and females with ASD show similar types and rates of RRBs in early childhood. While the study showed that children older than six had suggested higher rates of RRBs in males with ASD, between the ages of two and five, there were equivocal rates of RRBs shown between males and females (Harrop et al., 2015).

It has been previously found that males with ASD more frequently externalize behavior problems such as aggression and hyperactivity, while females deal with greater internalizing issues such as non-verbal communication and prosocial behavior patterns (Rynkiewics et al., 2016; Antezana et al., 2019). Additionally, research has found increased scores of stereotyped and restricted behaviors in males vs. females (Antezana et al., 2018). While the research into gender differences is receiving recognition, there is still much-needed research to have a better understanding of recognizing RRBs in males and females.

**Gender Differences and Treatment Options**

The exploration of gender differences in the diagnosis and appearance of ASD has been gaining popularity in recent years. Historically, the study of women's health was given the nickname "bikini medicine" due to medical professionals thinking that the only difference between males and females biologically could be covered by a bikini (Talesnik, 2018). It was not until 1993 that the NIH mandated including women in clinical trials that were NIH-funded, but many investigators were still hesitant to follow this policy (Clayton, 2016; Geller et al., 2018.).

The lack of inclusion with females in research also extends to preclinical drug discoveries, where male cells and male animal models were predominantly used (Danska, 2014; Mauvais-Jarvis et al., 2017). In 2001, the U.S. Government Accountability Office report found that the majority (80%) of drugs that were withdrawn from market use exhibited greater adverse effects in women than in men (U.S. Government Accountability Office, 2001). These findings could be due to a lack of research including females in clinical drug trials.

Without a deeper understanding of how an individual is affected and presents with a disorder such as ASD, there will be a lack of understanding surrounding how to approach and treat the individual based on their gender. Along with gender differences in ASD, it has been found that there are differences in the influence of gender on the pharmacokinetics and pharmacodynamics of drugs (Mauvais-Jarvis et al., 2021). A deeper understanding into the gender differences surrounding pharmacology is also needed in order to effectively treat individuals with ASD and other disorders.

As previously stated, propranolol is a beta-blocker that crosses the blood-brain barrier, meaning that there is an effect on the central and peripheral nervous systems. Propranolol is metabolized by a cytochrome in the liver that is different from men, and that has a lower activity in women than in men (Labbé et al., 2000; Tanaka & Hisawa, 1999). Additionally, propranolol reaches plasma levels that are up to 80% higher in women compared with men due to females having more adipose tissue than males (Soldin et al., 2011).

Along with the physiological differences shown between genders, the optimal effect of beta blockers may also be achieved with lower doses in women than in men (Eugene, 2016). A study performed across 11 European countries suggested that women with some heart conditions might require lower doses of beta blockers than men for optimal effects (Santemaetal., 2019).

The differences in pharmacokinetics and pharmacodynamics of beta blockers, such as propranolol, could aid in further understanding more appropriate pharmacological therapeutic treatment options for ASD (Farkouh, 2020). The gender related difference research that is lacking is the difference in how a beta-blocker, such as propranolol, will

not only affect the gender differently overall, but how it will affect each gender's response to RRBs which have also been found to be gender specific (Loftin et al., 2008).

## Sprague-Dawley ASD Model and Subsequent Testing

### Pesticide Exposure

Organophosphate (OP) compounds are chemicals that are today the most widely used as insecticides, flame retardants, fuel additives, lubricants, plasticizers, and pharmaceuticals (Richardson & Makhaeva, 2014). OPs are used in commercial agriculture to control pests on fruit and vegetable crops. They are additionally used in home gardens and occasionally for flea control on pets. OPs were also previously used in homes to control ants and termites but were discontinued for this use in 2001 due to fear of toxicity (CA.gov, 2023).

The CDC (2023) notes that some OP insecticides actually work by damaging an enzyme in the body called acetylcholinesterase, which is critical for controlling nerve signals in the body. The damage to this enzyme can potentially lead to unwanted side effects if humans are exposed (CDC.gov, 2023). There is a growing body of evidence suggesting that there is an association between gestational exposure to OPs and ASD (Ongono et al., 2020).

Chlorpyrifos (CPF) is one of the most widely used OPs in the world. CPF is a type of OP that has often been associated with an ASD diagnosis in humans and ASD-like behaviors in rodents. Rats that are prenatally exposed to CPF have been shown to have severe ASD-like symptoms and, thus, have been used to serve as a model for ASD research (Fernandez et al., 2022). One of the main ASD-like symptoms shown in a rat

following prenatal exposure to CPF are behaviors that are the most similar to RRBs in ASD.

### Marble Burying

A behavioral model often used in pre-clinical research for testing repetitive and compulsive behavior is the marble-burying behavior test (Dixit et al., 2020). This test allows researchers to fully measure compulsive behavior from an ASD rodent model by observing their burying practices over a period of time. The rodent is often filmed while in a polypropylene cage with clean glass marbles evenly spaced on the sawdust (Angoa-Perez et al., 2013; de Brouwer et al., 2019; Hoffman, 2016).

Marble burying was initially used to test anxiety-related behaviors due to the observation that the burying was reduced following the use of anxiolytics. It was later found that the number of marbles buried depends on the intensity of the rodent's digging behavior. These findings led authors to maintain that the marble-burying test is more accurate for repetitive or compulsive behavior (Hoffman, 2016).

## Biblical Foundations of the Study

As followers of Christ, we receive strict instructions to love and care for all children, including those with special needs. Luke 18:16 states, “But Jesus called them to him saying, ‘Let the children come to me, and do not hinder them, for to such belongs the kingdom of God’” (Luke 18:16, ESV). Individuals with disabilities are used in powerful ways in the Bible, and it is our duty as followers to not see limitations but to see the potential someone has.

There is much speculation surrounding the disabilities of the people of the Bible. Researchers such as Mathew and Pandian (2010) took it upon themselves to discuss the

neurological diseases among biblical characters of the Old Testament. In their writing, Samson was found to have autism, but there is no true way to confirm this information. There were no diagnostic criteria or neurological diseases mentioned in the Bible as a reference.

It was often thought that those who would now be diagnosed with a neurological or developmental disorder were possessed by evil spirits or demons (Macaskill, 2022). With this being said, it is imperative to try and change the narrative of how those with developmental disorders, such as ASD, are viewed. Macaskill (2022) delves into the importance of understanding what it means to think Christianly about something as controversial as ASD. He discusses the importance of recognizing that while it is labeled a disability, it is not necessarily something that needs to be cured.

Thinking Biblically about ASD could mean finding a way to meet the individuals with the disorder where they are. In researching ways to alleviate the symptoms that could distract them or potentially ostracize them from the church could aid in bringing those individuals back to their Lord and Savior without distraction or fear of ostracism.

## Summary

The continuation of research into ASD is imperative with the soaring diagnosis rates and unknown origin of the disorder. There are many theories into the cause of ASD including genetics, prenatal risks, and environmental factors, but the research has yet to be conclusive. There are also a number of treatment options available for individuals with ASD, but they are costly and timely, causing a great burden to the caregivers of those with ASD. Additionally, very few FDA approved medications exist to treat the symptoms of ASD, such as social impairments and RRBs. There is a need to further understand not

only the disorder but also how it affects the individual, such as males and females, separately. Research into the most effective treatment options for individuals with ASD and the side effects associated is imperative for the best outcome for those who suffer from the disorder and their family members. Matthew 10:8 tells us, “Heal the sick, raise the dead, cleanse those who have leprosy, drive out demons. Freely you have received; freely give” (Matthew 10:8, New International Version).

# CHAPTER 3: RESEARCH METHOD

## Overview

This quasi-experimental study focused on an ASD model of Sprague-Dawley rats and propranolol as a pharmaceutical treatment option for RRBs. Additionally, this study further explored whether gender moderates a change in the number of RRBs once propranolol was administered. My sample size for this research included 24 Sprague-Dawley rats, 12 vehicle-controlled rodents, and 12 ASD model rodents. I conducted a marble-burying behavioral test with vehicle and ASD rodents who received different doses of the beta-blocker, propranolol, for each session. The process was recorded, and data was analyzed at a later date. This chapter discusses the methods and data collection process, along with the data analysis, and finally the assumptions, delimitations, and limitations of this study.

## Research Questions and Hypotheses

### Research Questions

RQ1: How does the number of restrictive, repetitive behaviors change after the administration of propranolol in an autism model of Sprague-Dawley rats?

RQ 2: Does gender moderate the change in the number of restrictive, repetitive behaviors once propranolol is administered?

### Hypotheses

Hypothesis 1: The number of restrictive, repetitive behaviors will be inhibited following the administration of propranolol in an autism model of Sprague-Dawley rats.

Hypothesis 2: Gender will moderate the change in the number of restrictive, repetitive behaviors once propranolol is administered.

## Research Design

Data was collected as part of a quantitative, static-group quasi-experimental design. A static-group quasi-experimental research design is utilized often when the outcome of interest is measured only once and when it is not feasible to conduct a randomized controlled trial. A randomized controlled trial is the most beneficial design if the efficacy of the intervention has not been established. This study incorporates a well-established intervention that is widely accepted in previous literature, making a quasi-experimental design the superior choice to a randomized control trial (Harris et al., 2006).

This type of research design was beneficial for this study as it evaluated the association between an intervention (propranolol administration), and an outcome (the number of RRBs), without the need for random assignment (Schweizer et al., 2016). A quasi-experimental research design was additionally advantageous in that it met some requirements for causality (Shadish et al., 2002). Determining causality in this research allowed for the discovery of key insights through exploring how propranolol and gender had an impact on RRBs in individuals with ASD (Bojinov et al., 2020).

Previous studies of similar nature have been published using this type of design as it would not be beneficial in determining the difference between an autism model rat and a control group rat if the drug administration were randomly assigned. Additionally, it would not be helpful in determining whether gender moderates change in RRBs once propranolol has been administered if assignments were random. Ethical concerns could also potentially exist if the design is randomized in that intervention in this study involves exposure to a drug treatment (Mauldin, 2020).

## Participants

Female (200–250g, n=12) and male (275-325g, n=12) Sprague Dawley rats (Envigo RMS LLC) were singly housed under a reversed 12 h/12 h light/dark cycle (lights off at 0600 h); all experiments were conducted between 10 am and 2 pm, Monday through Friday. A total of 24 rats were used for this experiment, with 12 serving as an autism spectrum disorder model (6 males and 6 females) and 12 serving as control subjects (6 males and 6 females). Rats had free access to food and water and were housed in the animal facility at the University of Mississippi Medical Center. All experiments were approved by the institutional animal care and use committee (IACUC # 2022-1170) and conducted in accordance with the National Institutes of Health specifications outlined in their Guide for the Care and Use of Laboratory Animals.

The rats that were used for this study were approximately seven to eight weeks old. This age range was chosen as it ensures that the rodents will be of adult age and their central nervous system myelination is complete (Downes & Mullins, 2013; Jackson et al., 2017). An a-priori power analysis was conducted using G*Power version 3.1.9.7 to determine the minimum sample size required to test this study's hypotheses (Faul et al., 2007). The results indicated the required sample size to achieve 80% power for detecting a medium effect, at a significance criterion of a = .05, obtaining a sample size to adequately test this study's hypotheses.

## Study Procedures

### Securing IRB Approval

The procedures for this research project were conducted at the University of Mississippi Medical Center (UMMC) which is my current employer as a research technician under the direct supervision of the primary investigator, Dr. Amy Kohtz. An

application was submitted to the Institutional Review Board (IRB) in order to ensure the protocols for this project met guidelines set forth by the federal Office of Human Research Protections. Once the application was submitted and approved with appropriate protocols included, I requested a memorandum of understanding (MOU) between UMMC and Liberty University (LU) to be created as seen in *Appendix A.* The MOU ensured that LU and UMMC both approved the protocol. Once the MOU was signed and approved by both institutions, the study procedures began.

**Obtaining Subjects**

Previous studies in Kohtz lab have exposed pregnant female rats to chlorpyrifos (CPF) in order to create offspring that served as an ASD model. Twelve of the offspring that were exposed to CPF in utero were used in this study as an ASD model with anxiety-like behaviors, along with 12 rats that were not exposed in utero to serve as the control group.

**Trainings for the Implementation of the Procedures**

UMMC conducted injection training classes in order to teach individuals how to inject rats in the appropriate way for this study. The UMMC behavior corps instructed on appropriate equipment and training for the marble burying procedure. Additionally, protocols as shown in *Appendix B* were used as a guideline for the marble burying procedure.

**Propranolol Injections and Data Collection**

All 24 rats were given injections of S/R-propranolol (S-prop; combined *β*-adrenoceptor and 5-HT1A/1 β receptor antagonist; Pazos et al, 1985) or vehicle (saline) at doses of 0, 5 mg/ml, 10 mg/ml, and 20 mg/ml prior to a 15-minute marble burying

procedure. The sessions were recorded with a video camera and watched later using the Clicker V1.12 software application on the computer to collect the data.

**Marble-Burying**

Rats were habituated to a testing cage (12" x 8.25" x 8.25") filled with clean bedding for 15 minutes. Rats returned to their home cage for 30 min while the bedding in their testing cage was leveled and 15 marbles were arranged in a 3×5 grid. Rats were injected with S/R-propranolol (S-prop; combined *β*-adrenoceptor and 5-HT1A/1 β receptor antagonist; Pazos et al., 1987) or vehicle (saline) at doses of 0, 5 mg/ml, 10 mg/ml, and 20 mg/ml immediately after habituation and tested for marble burying behavior 30 minutes later (males, N = 12; females, N = 12)**.**

The rats were placed in their clean testing cage filled with rows of marbles (3 x 5) for 15 min. Testing was recorded using a Hamilton Buhl 2.7K high definition 30.0 mega pixel still image video recorder, and videos were analyzed for locomotor activity using the Clicker V1.12 application software**.** The rat's burying and grooming percentage, average, total and total time burying or grooming were recorded on an excel data spreadsheet. Marble burying was an effective method for measuring repetitive behaviors due to its history of having an ease of use, the spontaneity of how the rodent display marble burying behavior, and accuracy in the scoring of marble burying. Additionally, video-taping the procedure and scoring for locomotor activity such as marble burying and grooming provided information on levels of anxiety, particularly if using rodents with manipulations that are anxiogenic (Angoa-Perez et al., 2013).

## Instrumentation and Measurement

**Video Recording**

Testing was recorded using a Hamilton Buhl 2.7K high definition 30.0 mega pixel still image video recorder. The length of the recording was 15 minutes. Behavioral observations of burying and grooming was documented using the Clicker V1.12 application software on a laptop while the video of the marble burying procedure was being watched. Two rats in individual cages were viewed and data was recorded simultaneously. The "Q" and "A" keys were utilized for grooming and burying behavior in the cage on the left, and "L" and "P" were utilized for grooming and burying behavior in the cage on the right. The software program presented the data in an excel spread sheet when the 15-minute procedure was complete.

**Grooming vs. Burying**

Grooming behavior was documented when the rat was visibly creating friction movements with their forepaws directed to the nose, face, head, and ears. Body grooming was also recorded and included cleaning actions with the paws and mouth as described by Estanislau et al. (2019) as standard grooming procedure in rodents. The "Q" key was held down for the extent of the grooming process for the animal on the left, and the "P" key was held down for the animal on the right.

Burying behavior was documented when the rodent visibly hovered over and buried a marble under bedding. Additionally, burying behavior was documented when the rodent kicked and pushed bedding on top of a marble in accordance with the marble burying procedure described by Angoa-Perez et al (2013). The "A" key was held down for the extent of the grooming process for the animal on the left, and the "L" key was held down for the animal on the right.

## Operationalization of Variables

**Burying Percentage** – This variable is a ratio variable and was measured by the use of the Clicker application software. Each time the rodent partook in burying, the letter "Q" or "P" on the keyboard was pressed and held down for the extent of the burying. The Clicker application software recorded the time spent burying.

**Grooming Percentage** – Variable two is a ratio variable and was measured by the use of the Clicker application software. Each time the rodent partook in burying, the letter "A" or "L" on the keyboard was pressed and held down for the extent of the burying. The Clicker application software recorded the time spent burying.

**Total Time Burying** – Variable three is a ratio variable and was calculated by taking the burying percentage, dividing it by 100 to create a whole number, and multiplying it by 900 (number of seconds in 15 minutes).

**Total Time Grooming** – Variable four is a ratio variable and was calculated by taking the grooming percentage, dividing it by 100 to create a whole number, and multiplying it by 900 (number of seconds in 15 minutes).

## Data Analysis

### Hypothesis 1

Hypothesis 1 stated that the number of restrictive, repetitive behaviors would be inhibited following the administration of propranolol in an autism model of Sprague-Dawley rats. The data for hypothesis 1 was analyzed and graphs were plotted using GraphPad software (Prism 10). Data was first examined with a Two-way Analysis of variance (ANOVA). In all cases, statistical significance was indicated by $*p < 0.05$.

A Two-Way ANOVA compared the variance in each group mean to the overall variance of the dependent variable. It should be used when we need to know how two

factors affect a response variable, or whether there is an interaction between the two factors on the response variable. The response variable for hypothesis 1 was the number of restricted repetitive behaviors and the factors that affected the response variable was the propranolol administration.

**Hypothesis 2**

Hypothesis 2 stated that gender would moderate the change in the number of restrictive, repetitive behaviors once propranolol was administered. Data was analyzed, and graphs were plotted using GraphPad software (Prism 10). Data was first examined with a Two-way Analysis of variance (ANOVA). In all cases, statistical significance was indicated by $*p < 0.05$.

As previously stated, a Two-Way ANOVA compared the variance in each group mean to the overall variance in the dependent variable. The response variable for hypothesis 2 was the number of restricted repetitive behaviors and the factors that affected the response variable was the rat's gender and propranolol administration. The questions that I wanted answered were whether propranolol affected the number of RRBs, and whether gender had an effect on RRBs once propranolol was administered. Additionally, if there was an interaction between propranolol administration and the rat's gender.

**Assumptions**

In order for the results of the two-way ANOVA to be valid, normality, homogeneity of variance, and independence assumptions had to be met. The results had to show that the dependent variable values followed a bell curve shape, the variation

around the mean for each group was similar, and the independent variables were not dependent on one another, i.e., they were obtained by random sample.

### Data Analysis Process

The rats were recorded during their marble burying sessions for 15 minutes. The videos were transferred to a laptop with the Clicker application software program on it. The videos were watched, and data was collected in real time using the software. Once the data collection session was complete, the Clicker application software program created an excel spreadsheet with the data on it.

The excel data sheet recorded burying and grooming percentage, burying and grooming average, and burying and grooming total. These numbers were then transferred to a separate excel spread sheet containing a formula for calculating the total time burying and total time grooming. The sum of the calculation for the total time burying and total time grooming were then transferred to Graphpad Prism 10 where the statistical tests and graphs were completed.

## Delimitations, Assumptions, and Limitations

### Delimitation

A delimitation of the selected group of rodents for this experiment was the supplier location. All of the rats that were used in this experiment were Sprague-Dawley rats from Envigo RMS LLC, in order to get the most reliable research results. Additionally, all of the rats that were used fell within the same age range of 7-8 weeks old upon starting the study.

### Assumptions

The assumptions of this study were directly related to the hypotheses stated previously. First, the number of restrictive, repetitive behaviors should be less following the administration of propranolol. The assumption is that the higher the dose of propranolol, the less repetitive behavior of marble burying will occur. Additionally, it was assumed that gender would moderate the change in the number of restrictive, repetitive behaviors once propranolol was administered. It is thought that males would partake in RRBs more often than females as previous studies have found that ASD and RRBs are more often recognized in males versus females.

**Limitations**

This study had some limitations. First, it has been extensively researched and documented that ASD occurs at a variety of levels for individuals affecting no two people alike. The rats that were used as an ASD model could not accurately be assessed for their level of ASD.

Second, the research design was quasi-experimental, thus, not randomized. Nonrandomized designs do not meet all of the requirements to determine causality in an experiment. Additionally, due to using this type of design, there was potentially a lack of internal validity.

## Summary

This chapter focused on the research design, study procedures, and data analysis related to the effects of propranolol on RRBs in an ASD rodent model. As previously stated, it is well-known that ASD and the associated symptoms do not present the same in both males and females. The gap in research is surrounding the differences in response to pharmacological treatment options based on the different genders. The purpose of this

study was to aid in closing the gap in understanding how propranolol and gender independently affect RRBs in rats with ASD. Using a static group quasi-experimental research design, multiple marble-burying sessions were administered between vehicle and ASD model rodents, and males and females. Data was collected and analyzed for statistical significance supporting my two hypotheses. Chapter IV focuses on the results of the research.

# CHAPTER 4: RESULTS

## Overview

The purpose of this quantitative quasi-experimental study was to examine the relationship between propranolol and restricted repetitive behaviors in male and female Sprague-Dawley rats. The animals were recorded during marble burying sessions, and the videos were transferred to a laptop with the Clicker application software program on it where the videos were watched, and data was collected in real-time using the Clicker software.

Burying and grooming percentage, burying and grooming average, and burying and grooming total were recorded in order to calculate the total time burying and total time grooming. The sum of the calculations for the total time burying and total time grooming were then transferred to GraphPad Prism 10, where a two-way ANOVA was used to test for statistical significance.

The questions that were asked were: will the number of restrictive, repetitive behaviors be inhibited following the administration of propranolol in an autism model of Sprague-Dawley rats? Additionally, will gender moderate the change in the number of restrictive, repetitive behaviors once propranolol is administered?

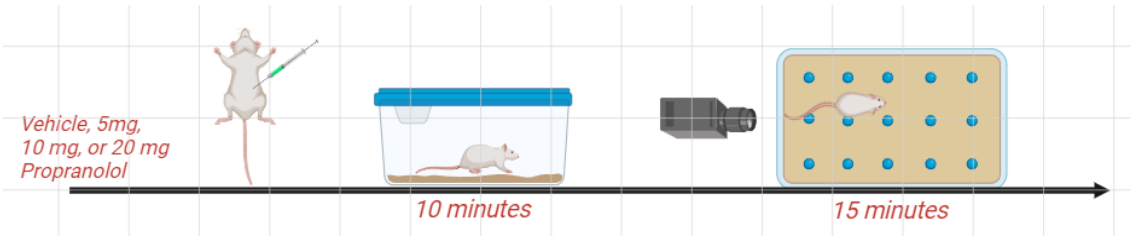



**Figure 1.** Schematic representation of the marble burying and marble grooming behavioral test using different doses of the beta-blocker, propranolol, for each session.

## Descriptive Results

The animals that were used to conduct this research were 24 Sprague-Dawley rats, 12 ASD rodent model (6 males/6 females), and 12 non-ASD rodent model (6 males/6 females). The ASD rodent model was the offspring of female rats that had been exposed to an insecticide called Chlorpyrifos in utero. Additionally, 12 rats that were not exposed in utero served as the control group.

## Study Findings

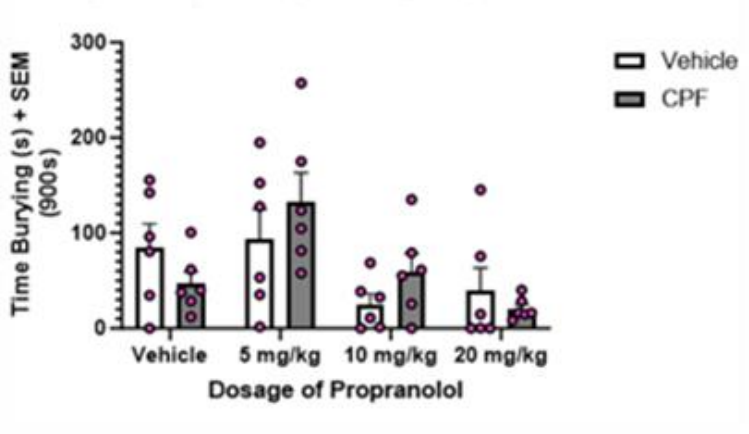
### Propranolol's Effect on RRBs in the ASD Rodent Model

The first hypothesis that I investigated was that the number of restrictive, repetitive behaviors will be inhibited following the administration of propranolol in an autism model of Sprague-Dawley rats. A two-way ANOVA was used to examine the total time spent burying or grooming following propranolol administration at differing doses in an ASD rodent model.

The findings showed that there was a significant main effect for propranolol dose to impact marble burying in females (Fig 2 ($F(3,40) = 5.945$, $p = 0.0019$) and grooming behavior in females (Fig 3 ($F(3,40) = 0.4991$, $p = 0.0442$). In males, there was also significant main effect for propranolol dose to impact burying (Fig 4; ($F(3,40) = 31.80$, $p < 0.0001$), and grooming behavior (Fig 5; ($F$ ($F(3,40) = 7.030$, $p = 0.0007$). These findings support my alternate hypothesis that propranolol has an inhibitory effect on the number of RRBs following propranolol administration. Due to these results, I failed to reject the null hypothesis.

**Figure 2**

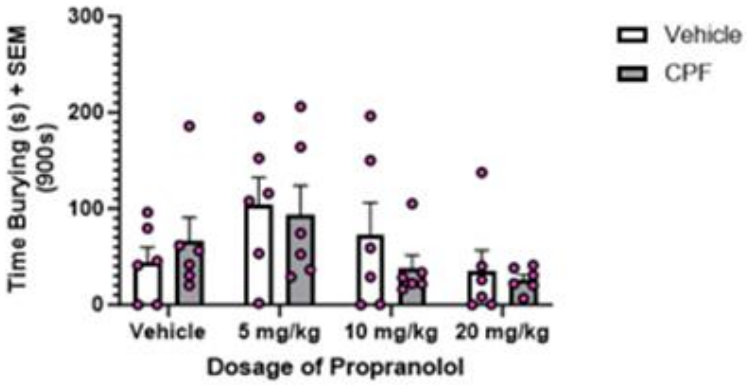
*Time Spent Marble Burying Following a Propranolol Administration- Females*




*Note*. This figure demonstrates the interaction found between female rodents and marble burying behavior following the administration of propranolol. There was a significant main effect for propranolol to effect marble burying in female rodents. Comparisons were made with two-way ANOVA, $p = 0.0019$.

**Figure 3**

*Time Spent Grooming Following a Propranolol Administration- Females*

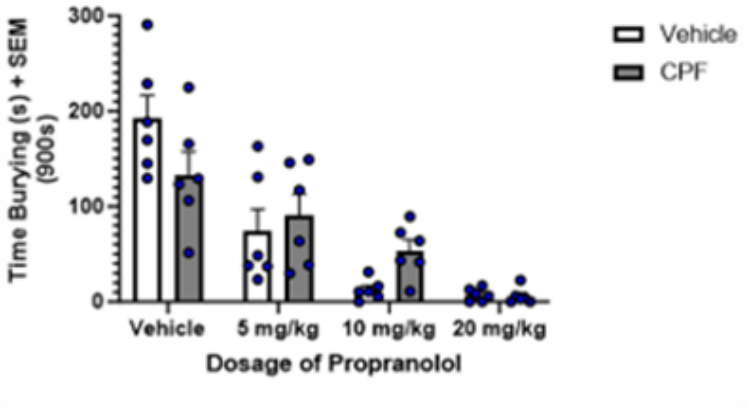



*Note*. This figure demonstrates the interaction found between female rodents and grooming behavior following the administration of propranolol. There was a significant

main effect for propranolol to effect marble burying in female rodents. Comparisons were made with two-way ANOVA, $p = 0.0442$.

**Figure 4**

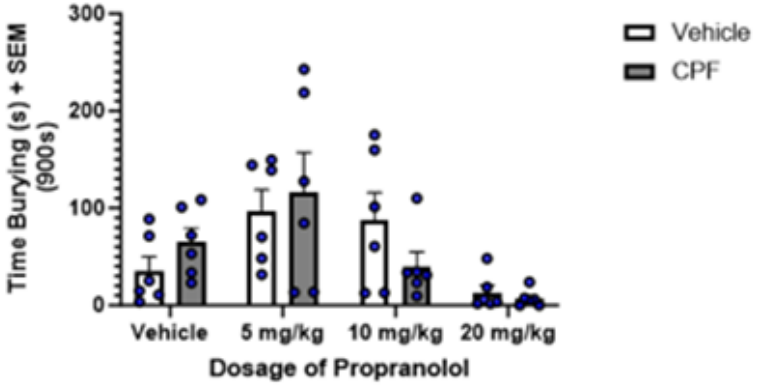
*Time Spent Marble Burying Following a Propranolol Administration- Males*




*Note*. This figure demonstrates the interaction found between male rodents and marble burying behavior following the administration of propranolol. There was a significant main effect for propranolol to effect marble burying in male rodents. Comparisons were made with two-way ANOVA, $p < 0.0001$.

**Figure 5**

*Time Spent Grooming Following a Propranolol Administration- Males*



*Note*. This figure demonstrates the interaction found between male rodents and grooming behavior following the administration of propranolol. There was a significant main effect for propranolol to effect marble burying in male rodents. Comparisons were made with two-way ANOVA, $p < 0.0007$.

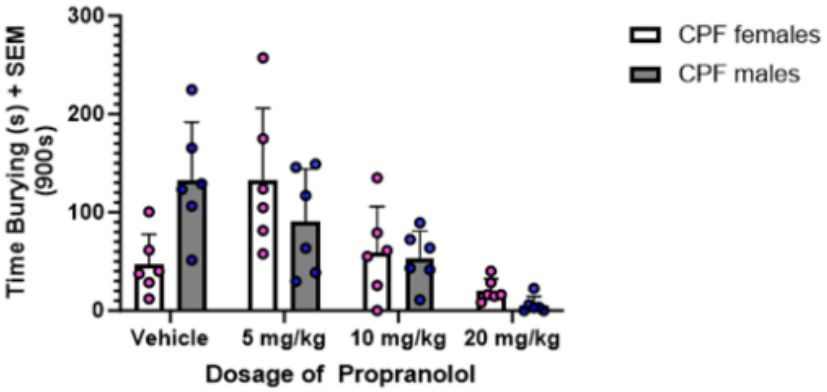
**Genders Effect on RRBs Once Propranolol is Administered**

My second hypothesis was that gender moderates the change in the number of restrictive, repetitive behaviors once propranolol is administered. A two-way ANOVA was used to analyze the total time spent burying and grooming between males and females in an ASD rodent model and non-ASD rodent model.

For burying behavior in the ASD rodent model, I found a significant interaction between propranolol dose on burying between males and female rats (Fig 6; $F(3,40) = 4.842$, $p = 0.0057$. For grooming behavior in the ASD rodent model, I also found a significant interaction between males and female rats once propranolol had been administered (Fig 7; $F(3,40) = 6.123$, $p = 0.0016$. These findings support my second alternate hypothesis that gender will moderate the change in the number of RRBs once propranolol is administered. Due to these results, I failed to reject the null hypothesis.

**Figure 6**

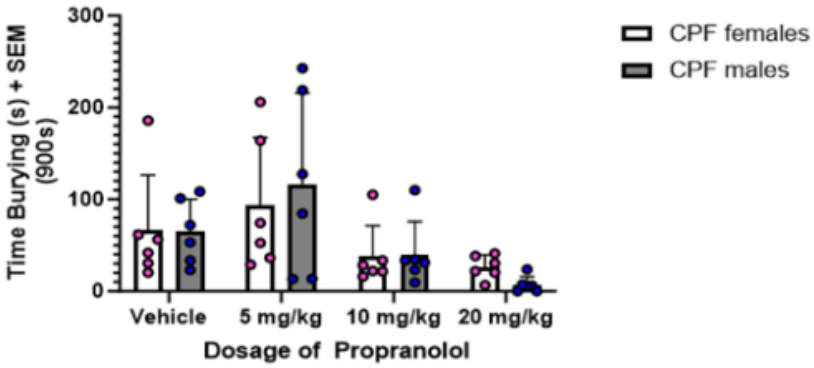
*Time Burying: CPF Females vs. CPF Males*




*Note*. This figure demonstrates the interaction found between males and females once propranolol had been administered for marble burying behavior. Comparisons were made with two-way ANOVA, $p < 0.0001$.

**Figure 7**

*Time Grooming: CPF Females vs. CPF Males*




*Note*. This figure demonstrates the interaction found between males and females once propranolol had been administered for grooming behavior. Comparisons were made with two-way ANOVA, $p = 0.0016$.

## Summary

This chapter focused on stating the overall results of the study. The findings showed that there was a significant main effect for propranolol dose to impact marble burying and grooming behavior in the ASD rodent model in both males and females. For burying and grooming behavior in the ASD rodent model, I additionally found a significant interaction between propranolol dose on burying between males and females once propranolol had been administered.

These findings support both hypotheses, that propranolol has an inhibitory effect on the number of RRBs and that gender will moderate the change in the number of RRBs once propranolol is administered. Chapter 5 will further discuss the findings of the research presented.

# CHAPTER 5: DISCUSSION

## Overview

The purpose of this quantitative quasi-experimental study was to examine the relationship between propranolol and restricted repetitive behaviors in male and female Sprague-Dawley rats. This chapter summarizes and discusses the study's theoretical and empirical findings from my research as well as the study's theoretical, empirical, and practical implications. Additionally, this chapter discusses how a Christian worldview informs an interpretation of the findings of my study. Finally, this chapter discusses recommendations for future research that emerged from the findings of the current study.

## Summary of Findings

### Hypothesis 1

My first hypothesis states that the number of restrictive, repetitive behaviors will be inhibited following the administration of propranolol in an autism model of Sprague-Dawley rats. Following the administration of propranolol and collecting data on the RRBs revealed that there was a significant main effect for propranolol dose to impact marble burying and grooming behavior in females and male rats.

The results of the marble burying showed that following a propranolol injection, the male and female rats either buried less marbles or did not bury the marbles as deeply as they had without a propranolol injection. Additionally, the male and female rats did not partake in grooming behaviors, such as licking their paws or brushing the hair on their noses with their paws as much as without an injection. It should also be noted that the increase in propranolol dosage, i.e. 10mg vs. 20 mg, did have a significant effect on

both grooming and burying in that there was a negative correlation for propranolol dose and burying or grooming.

### Hypothesis 2

My second hypothesis stated that gender will moderate the change in the number of restrictive, repetitive behaviors once propranolol is administered. These results were analyzed using the data collected for my first hypothesis. There was a significant interaction found between propranolol dose on burying and grooming between males and females once propranolol had been administered. These findings suggest that the pharmaceutical treatment option for individuals with ASD may be sexually dimorphic.

## Discussion of Findings

### Empirical Findings

This study found that propranolol, a beta-adrenergic receptor antagonist, does influence RRBs in male and female Sprague-Dawley rats. To date, risperidone and aripiprazole are the only two FDA approved medications that are used for treating the symptoms of ASD. This research has provided insight into the effects of a pharmacological intervention, propranolol, on RRBs in individuals with ASD. Additionally, this research aids in showing how gender may play a role in propranolol's effects.

### Theoretical Findings

It has previously been theorized that anxiety can be a trigger for RRBs. Propranolol is a treatment option that inhibits noradrenaline and adrenaline, the two hormones often associated with anxiety. This study further theorizes that treating anxiety in individuals with ASD will limit the number of RRBs one engages in. Additionally, this

study adds to the theory that more research is needed into the symptomology between males and females with ASD and how the treatment options may be sexually dimorphic.

## Implications

### Theoretical Implications

The implications of this research suggest that there are better pharmaceutical options available for individuals who suffer from the side effects associated with ASD. Additionally, there needs to be further consideration into researching the symptomology between males and females, and how ASD affects them individually. It could additionally be theorized that beta-adrenergic receptors play a larger role in individuals with ASD than what has previously been recognized.

### Empirical Implications

The empirical findings of this research suggest that propranolol, a beta-adrenergic receptor antagonist, could treat RRBs associated with ASD. Additionally, the findings suggest that males and females may be affected differently in terms of treatment options for the symptoms associated with ASD.

### Practical Implications

As previously discussed, the pharmaceutical options for treating the symptoms of ASD are somewhat aversive. There is a need for further research into finding less aversive pharmacological treatment options for individuals with ASD. This research can serve as a reference point for clinical researchers, physicians, counselors, and policy makers when advocating for better treatment options for those with ASD. Additionally, the findings of this research should provide hope and optimism for the caregivers of individuals with ASD who suffer from the side effects that impact their day-to-day lives.

### Christian Worldview

The findings of this research further imply that there is a relationship between science and faith. Additionally, the affirmation of the idea that God is beyond nature becomes evident. While human beings are created in God's image, there should still be an acquisition in science and understanding of the natural world. Proverbs 3:13-14 states, "Blessed are those who find wisdom, those who gain understanding, for she is more profitable than silver and yields better returns than gold" (Proverbs 3:13-14, New International Version).

## Delimitations and Limitations

Delimitations were placed on this study in order to improve reliability and validity, and to ensure that the research is objective and unbiased. This study focused solely on RRBs; one symptom listed in the diagnostic criteria for ASD. This was done to ensure that the study's findings were focused on a target that was measurable using a rodent model. Additionally, the data collection process was conducted by one researcher to confirm validity and reliability of the findings. Finally, this study used only one beta-adrenergic receptor antagonist, propranolol, to ensure that the study remained focused and clear on finding an effective treatment option without aversive side effects.

This study had some limitations as previously stated. First, it has been extensively researched and documented that ASD occurs at a variety of levels for individuals affecting no two people alike. The rats that were used as an ASD model could not accurately be assessed for their level of ASD. Second, the research design was quasi-experimental, thus, not randomized. Nonrandomized designs do not meet all of the

requirements to determine causality in an experiment. Additionally, due to using this type of design, there was potentially a lack of internal validity.

## Recommendations for Future Research

This research revealed that propranolol could be used as an effective treatment option for RRBs. Additionally, it was revealed that male and females may be affected differently by treatment options for ASD. On this basis, future research should examine additional beta-blockers and their effects on the symptoms of ASD. Additionally, future research should investigate the role of beta-adrenergic receptors in individuals with ASD.

## Summary

This chapter summarizes and discusses the research findings of this study. Additionally, implications, limitations, and recommendations for future research were discussed. The two most important findings of this study were that there are not only other pharmaceutical treatment options available for ASD, but there are also better options available. With there being limited, and somewhat aversive, pharmacological treatment options for the symptoms of ASD, further options need exploration.

A second finding that was equally as important is that ASD and the treatment options associated are sexually dimorphic. Males and females' response to the symptoms associated and the treatments should be researched and explored as separate entities and not with a 'one size fits all' approach. Additionally, future research would benefit from investigating additional beta-adrenergic receptor antagonists in addition to the role that beta-adrenergic receptors play in ASD.

ASD is a complex neurodevelopmental disorder that affects many individuals' lives and the lives of those around them. The continuation of research into ASD is

imperative with the soaring diagnosis rates and unknown origin of the disorder. There are several treatment options available for individuals with ASD, but they are costly and timely, causing a great burden to the caregivers of those with ASD. Additionally, very few FDA approved medications exist to treat the symptoms of ASD, such as social impairments and RRBs.

# References

_ Deepmala, & Agrawal, M. (2014). Use of Propranolol for Hypersexual Behavior in an Adolescent With Autism. *Annals of Pharmacotherapy*, *48*(10), 1385–1388. https://doi.org/10.1177/1060028014541630

Adams, H. L., Matson, J. L., Cervantes, P. E., & Goldin, R. L. (2014). The relationship between autism symptom severity and sleep problems: Should bidirectionality be considered? *Research in Autism Spectrum Disorders*, *8*(3), 193–199. https://doi.org/10.1016/j.rasd.2013.11.008

Agarwal, R., Burke, S. L., & Maddux, M. (2019). Current state of evidence of cannabis utilization for treatment of autism spectrum disorders. *BMC Psychiatry*, *19*(1). https://doi.org/10.1186/s12888-019-2259-4

Alam, R., Abdolmaleky, H. M., & Zhou, J.-R. (2017). Microbiome, inflammation, epigenetic alterations, and mental diseases. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *174*(6), 651–660. https://doi.org/10.1002/ajmg.b.32567

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). American Psychiatric Publishing.

American Psychiatric Association. (2022). *Diagnostic and Statistical Manual of Mental Disorders (DSM-5-TR)*. Psychiatry.org; American Psychiatric Association. https://www.psychiatry.org/psychiatrists/practice/dsm

Amodeo, D. A., Pahua, A. E., Zarate, M., Taylor, J. A., Peterson, S., Posadas, R., Oliver, B. L., & Amodeo, L. R. (2019). Differences in the expression of restricted repetitive behaviors in female and male BTBR T + tf/J mice. *Behavioural Brain Research*, *372*, 112028. https://doi.org/10.1016/j.bbr.2019.112028

Andersson, G. W., Gillberg, C., & Miniscalco, C. (2013). Pre-school children with suspected autism spectrum disorders: Do girls and boys have the same profiles? *Research in Developmental Disabilities*, *34*(1), 413–422. https://doi.org/10.1016/j.ridd.2012.08.025

Angoa-Pérez, M., Kane, M. J., Briggs, D. I., Francescutti, D. M., & Kuhn, D. M. (2013). Marble Burying and Nestlet Shredding as Tests of Repetitive, Compulsive-like Behaviors in Mice. *Journal of Visualized Experiments*, *82*. https://doi.org/10.3791/50978

Antezana, L., Factor, R. S., Condy, E. E., Strege, M. V., Scarpa, A., & Richey, J. A. (2018). Gender differences in restricted and repetitive behaviors and interests in youth with autism. *Autism Research*, *12*(2), 274–283. https://doi.org/10.1002/aur.2049

Baird, G., Douglas, H. R., director, a., & Murphy, M. S. (2011). Recognising and diagnosing autism in children and young people: summary of NICE guidance. *BMJ*, *343*(oct21 1), d6360–d6360. https://doi.org/10.1136/bmj.d6360

Baribeau, D. A., Vigod, S., Pullenayegum, E., Kerns, C. M., Mirenda, P., Smith, I. M., Vaillancourt, T., Volden, J., Waddell, C., Zwaigenbaum, L., Bennett, T., Duku, E., Elsabbagh, M., Georgiades, S., Ungar, W. J., Zaidman-Zait, A., & Szatmari, P. (2020). Repetitive Behavior Severity as an Early Indicator of Risk for Elevated Anxiety Symptoms in Autism Spectrum Disorder. *Journal of the American Academy of Child & Adolescent Psychiatry*, *59*(7), 890-899.e3. https://doi.org/10.1016/j.jaac.2019.08.478

Baron-Cohen, S., Lombardo, M. V., Auyeung, B., Ashwin, E., Chakrabarti, B., & Knickmeyer, R. (2011). Why Are Autism Spectrum Conditions More Prevalent in Males? *PLoS Biology*, *9*(6), e1001081. https://doi.org/10.1371/journal.pbio.1001081

Begeer, S., Mandell, D., Wijnker-Holmes, B., Venderbosch, S., Rem, D., Stekelenburg, F., & Koot, H. M. (2012). Sex Differences in the Timing of Identification Among Children and Adults with Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders*, *43*(5), 1151–1156. https://doi.org/10.1007/s10803-012-1656-z

Bernaerts, S., Boets, B., Bosmans, G., Steyaert, J., & Alaerts, K. (2020). Behavioral effects of multiple-dose oxytocin treatment in autism: a randomized, placebo-controlled trial with long-term follow-up. *Molecular Autism*, *11*(1). https://doi.org/10.1186/s13229-020-0313-1

Bertrand, J., Mars, A., Boyle, C., Bove, F., Yeargin-Allsopp, M., & Decoufle, P. (2001). Prevalence of Autism in a United States Population: The Brick Township, New Jersey, Investigation. *PEDIATRICS*, *108*(5), 1155–1161. https://doi.org/10.1542/peds.108.5.1155

Beversdorf, D. Q. (2020). The Role of the Noradrenergic System in Autism Spectrum Disorders, Implications for Treatment. *Seminars in Pediatric Neurology*, *35*, 100834. https://doi.org/10.1016/j.spen.2020.100834

Beversdorf, D. Q., Carpenter, A. L., Miller, R. F., Cios, J. S., & Hillier, A. (2008). Effect of Propranolol on verbal problem solving in autism spectrum disorder. *Neurocase*, *14*(4), 378–383. https://doi.org/10.1080/13554790802368661

*BibleGateway.com: Search for a Bible passage in 71 languages and 225 versions.* (n.d.). Www.biblegateway.com. https://www.biblegateway.com/passage/?s

Bilder, D., Pinborough-Zimmerman, J., Miller, J., & McMahon, W. (2009). Prenatal, Perinatal, and Neonatal Factors Associated With Autism Spectrum Disorders. *PEDIATRICS*, *123*(5), 1293–1300. https://doi.org/10.1542/peds.2008-0927

Biomarkers Definitions Working Group. (2001). Biomarkers and Surrogate endpoints: Preferred Definitions and Conceptual Framework. *Clinical Pharmacology & Therapeutics*, *69*(3), 89–95. https://doi.org/10.1067/mcp.2001.113989

Bishop, S. L., Richler, J., Cain, A. C., & Lord, C. (2007). Predictors of Perceived Negative Impact in Mothers of Children With Autism Spectrum Disorder. *American Journal on Mental Retardation*, *112*(6), 450. https://doi.org/10.1352/0895-8017(2007)112[450:popnii]2.0.co;2

Bodner, K. E., Beversdorf, D. Q., Saklayen, S. S., & Christ, S. E. (2012). Noradrenergic Moderation of Working Memory Impairments in Adults with Autism Spectrum Disorder. *Journal of the International Neuropsychological Society*, *18*(3), 556–564. https://doi.org/10.1017/S1355617712000070

Bojinov, I., Chen, A., & Liu, M. (2020). The Importance of Being Causal. *Harvard Data Science Review*. https://doi.org/10.1162/99608f92.3b87b6b0

Bolla, K. I., Brown, K., Eldreth, D., Tate, K., & Cadet, J. L. (2002). Dose-related neurocognitive effects of marijuana use. *Neurology*, *59*(9), 1337–1343. https://doi.org/10.1212/01.wnl.0000031422.66442.49

Boyd, B. A., McDonough, S. G., & Bodfish, J. W. (2011). Evidence-Based Behavioral Interventions for Repetitive Behaviors in Autism. *Journal of Autism and*

*Developmental Disorders*, *42*(6), 1236–1248. https://doi.org/10.1007/s10803-011-1284-z

Brantigan, C. O., Brantigan, T. A., & Joseph, N. (1982). Effect of beta blockade and beta stimulation on stage fright. *The American Journal of Medicine*, *72*(1), 88–94. https://doi.org/10.1016/0002-9343(82)90592-7

Buxhoeveden, D. P., Semendeferi, K., Buckwalter, J., Schenker, N., Switzer, R., & Courchesne, E. (2006). Reduced minicolumns in the frontal cortex of patients with autism. *Neuropathology and Applied Neurobiology*, *32*(5), 483–491. https://doi.org/10.1111/j.1365-2990.2006.00745.x

Cambridge Dictionary. (2023, November 14). *geopolitically*. Cambridge.org. https://dictionary.cambridge.org/us/dictionary/english/geopolitically

Cashin, A., & Yorke, J. (2016). Overly Regulated Thinking and Autism Revisited. *Journal of Child and Adolescent Psychiatric Nursing*, *29*(3), 148–153. https://doi.org/10.1111/jcap.12152

Cashin, A., & Yorke, J. (2018). The Relationship between Anxiety, External Structure, Behavioral History and Becoming Locked into Restricted and Repetitive Behaviors in Autism Spectrum Disorder. *Issues in Mental Health Nursing*, *39*(6), 533–537. https://doi.org/10.1080/01612840.2017.1418035

Charman, T., & Gotham, K. (2012). Measurement Issues: Screening and diagnostic instruments for autism spectrum disorders - lessons from research and practise. *Child and Adolescent Mental Health*, *18*(1), 52–63. https://doi.org/10.1111/j.1475-3588.2012.00664.x

Charman, T., Taylor, E., Drew, A., Cockerill, H., Brown, J.-A., & Baird, G. (2005). Outcome at 7 years of children diagnosed with autism at age 2: predictive validity of assessments conducted at 2 and 3 years of age and pattern of symptom change over time. *Journal of Child Psychology and Psychiatry*, *46*(5), 500–513. https://doi.org/10.1111/j.1469-7610.2004.00377.x

Chawarska, K., Klin, A., Paul, R., & Volkmar, F. (2007). Autism spectrum disorder in the second year: stability and change in syndrome expression. *Journal of Child Psychology and Psychiatry*, *48*(2), 128–138. https://doi.org/10.1111/j.1469-7610.2006.01685.x

Christensen, D. L., Baio, J., Braun, K. V. N., Bilder, D., Charles, J., Constantino, J. N., Daniels, J., Durkin, M. S., Fitzgerald, R. T., Kurzius-Spencer, M., Lee, L.-C., Pettygrove, S., Robinson, C., Schulz, E., Wells, C., Wingate, M. S., Zahorodny, W., & Yeargin-Allsopp, M. (2016). Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012. *MMWR. Surveillance Summaries*, *65*(3), 1–23. https://doi.org/10.15585/mmwr.ss6503a1

Clayton, J. A. (2015). Studying both sexes: a guiding principle for biomedicine. *The FASEB Journal*, *30*(2), 519–524. https://doi.org/10.1096/fj.15-279554

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Lawrence Erlbaum Associates.

Cohen, S., Conduit, R., Lockley, S. W., Rajaratnam, S. M., & Cornish, K. M. (2014). The relationship between sleep and behavior in autism spectrum disorder (ASD): a

review. *Journal of Neurodevelopmental Disorders*, *6*(1). https://doi.org/10.1186/1866-1955-6-44

Comparan-Meza, M., Vargas de la Cruz, I., Jauregui-Huerta, F., Gonzalez-Castañeda, R. E., Gonzalez-Perez, O., & Galvez-Contreras, A. Y. (2021). Biopsychological correlates of repetitive and restricted behaviors in autism spectrum disorders. *Brain and Behavior*, *11*(10), e2341. https://doi.org/10.1002/brb3.2341

Corbett, B. A., Constantine, L. J., Hendren, R., Rocke, D., & Ozonoff, S. (2009). Examining executive functioning in children with autism spectrum disorder, attention deficit hyperactivity disorder and typical development. *Psychiatry Research*, *166*(2-3), 210–222. https://doi.org/10.1016/j.psychres.2008.02.005

Cortesi, F., Giannotti, F., Ivanenko, A., & Johnson, K. (2010). Sleep in children with autistic spectrum disorder. *Sleep Medicine*, *11*(7), 659–664. https://doi.org/10.1016/j.sleep.2010.01.010

Courchesne, E., Campbell, K., & Solso, S. (2011). Brain growth across the life span in autism: Age-specific changes in anatomical pathology. *Brain Research*, *1380*, 138–145. https://doi.org/10.1016/j.brainres.2010.09.101

Croteau, C., Mottron, L., Presse, N., Tarride, J.-E., Dorais, M., & Perreault, S. (2017). Increase in Psychoactive Drug Prescriptions in the Years Following Autism Spectrum Diagnosis: A Population-Based Cohort Study. *Journal of Population Therapeutics and Clinical Pharmacology*, *24*(3). https://doi.org/10.22374/1710-6222.24.3.2

Cummings, C. M., & Fristad, M. A. (2011). Anxiety in Children with Mood Disorders: A Treatment Help or Hindrance? *Journal of Abnormal Child Psychology*, *40*(3), 339–351. https://doi.org/10.1007/s10802-011-9568-5

Daniels, N., Moerkerke, M., Steyaert, J., Bamps, A., Debbaut, E., Prinsen, J., Tang, T., Van der Donck, S., Boets, B., & Alaerts, K. (2023). Effects of multiple-dose intranasal oxytocin administration on social responsiveness in children with autism: a randomized, placebo-controlled trial. *Molecular Autism*, *14*(1). https://doi.org/10.1186/s13229-023-00546-5

Dawson, G. (2008). Early behavioral intervention, brain plasticity, and the prevention of autism spectrum disorder. *Development and Psychopathology*, *20*(03). https://doi.org/10.1017/s0954579408000370

DeMayo, M. M., Young, L. J., Hickie, I. B., Song, Y. J. C., & Guastella, A. J. (2019). Circuits for social learning: A unified model and application to Autism Spectrum Disorder. *Neuroscience & Biobehavioral Reviews*, *107*, 388–398. https://doi.org/10.1016/j.neubiorev.2019.09.034

Demetriou, E. A., DeMayo, M. M., & Guastella, A. J. (2019). Executive Function in Autism Spectrum Disorder: History, Theoretical Models, Empirical Findings, and Potential as an Endophenotype. *Frontiers in Psychiatry*, *10*. https://doi.org/10.3389/fpsyt.2019.00753

Distefano, G., Calderoni, S., Apicella, F., Cosenza, A., Igliozzi, R., Palermo, G., Tancredi, R., Tritto, G., Craig, F., Muratori, F., & Turi, M. (2023). *Impact of sleep disorders on behavioral issues in preschoolers with autism spectrum disorder*. *14*. https://doi.org/10.3389/fpsyt.2023.1181466

Dixit, P. V., Sahu, R., & Mishra, D. K. (2020). Marble-burying behavior test as a murine model of compulsive-like behavior. *Journal of Pharmacological and Toxicological Methods*, *102*, 106676. https://doi.org/10.1016/j.vascn.2020.106676

Downes, N., & Mullins, P. (2013). The Development of Myelin in the Brain of the Juvenile Rat. *Toxicologic Pathology*, *42*(5), 913–922. https://doi.org/10.1177/0192623313503518

Dworzynski, K., Ronald, A., Bolton, P., & Happé, F. (2012). How Different Are Girls and Boys Above and Below the Diagnostic Threshold for Autism Spectrum Disorders? *Journal of the American Academy of Child & Adolescent Psychiatry*, *51*(8), 788–797. https://doi.org/10.1016/j.jaac.2012.05.018

Elison, J. T., Wolff, J. J., Reznick, J. S., Botteron, K. N., Estes, A. M., Gu, H., Hazlett, H. C., Meadows, A. J., Paterson, S. J., Zwaigenbaum, L., & Piven, J. (2014). Repetitive Behavior in 12-Month-Olds Later Classified With Autism Spectrum Disorder. *Journal of the American Academy of Child & Adolescent Psychiatry*, *53*(11), 1216–1224. https://doi.org/10.1016/j.jaac.2014.08.004

Elrod, M. G., & Hood, B. S. (2015). Sleep Differences Among Children With Autism Spectrum Disorders and Typically Developing Peers. *Journal of Developmental & Behavioral Pediatrics*, *36*(3), 166–177. https://doi.org/10.1097/dbp.0000000000000140

Espadas, C., Ballester, P., Londoño, A. C., Almenara, S., Aguilar, V., Belda, C., Pérez, E., & Peiró, A. M. (2020). Multimorbidity and psychotropic polypharmacy among participants with autism spectrum disorder with intellectual disability. *Psychiatry Research*, *292*, 113321. https://doi.org/10.1016/j.psychres.2020.113321

Estanislau, C., Veloso, A. W. N., Filgueiras, G. B., Maio, T. P., Dal-Cól, M. L. C., Cunha, D. C., Klein, R., Carmona, L. F., & Fernández-Teruel, A. (2019). Rat self-grooming and its relationships with anxiety, dearousal and perseveration: Evidence for a self-grooming trait. *Physiology & Behavior*, *209*, 112585. https://doi.org/10.1016/j.physbeh.2019.112585

Evans, D. W., Uljarević, M., Lusk, L. G., Loth, E., & Frazier, T. (2017). Development of Two Dimensional Measures of Restricted and Repetitive Behavior in Parents and Children. *Journal of the American Academy of Child and Adolescent Psychiatry*, *56*(1), 51–58. https://doi.org/10.1016/j.jaac.2016.10.014

Faigel, H. C. (1991). The Effect of Beta Blockade on Stress-Induced Cognitive Dysfunction in Adolescents. *Clinical Pediatrics*, *30*(7), 441–445. https://doi.org/10.1177/000992289103000706

Farkouh, A., Riedl, T., Gottardi, R., Czejka, M., & Kautzky-Willer, A. (2019). Sex-Related Differences in Pharmacokinetics and Pharmacodynamics of Frequently Prescribed Drugs: A Review of the Literature. *Advances in Therapy*, *37*(2), 644–655. https://doi.org/10.1007/s12325-019-01201-3

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2), 175–191. https://doi.org/10.3758/bf03193146

FOLSTEIN, S., & RUTTER, M. (1977). Genetic influences and infantile autism. *Nature*, *265*(5596), 726–728. https://doi.org/10.1038/265726a0

Fombonne, E. (2009). Epidemiology of Pervasive Developmental Disorders. *Pediatric Research*, *65*(6), 591–598. https://doi.org/10.1203/pdr.0b013e31819e7203

*Food and Drug Administration | USAGov*. (n.d.). Www.usa.gov. Retrieved April 11, 2023, from https://www.usa.gov/agencies/food-and-drug-administration

Francis, K. (2005). Autism interventions: a critical update. *Developmental Medicine & Child Neurology*, *47*(7), 493–499. https://doi.org/10.1017/s0012162205000952

Frawley, W. (2008). Private speech, cognitive-computational control, and the autism-psychosis continuum. *Behavioral and Brain Sciences*, *31*(3), 269–270. https://doi.org/10.1017/s0140525x08004305

Frazier, T. W., Thompson, L., Youngstrom, E. A., Law, P., Hardan, A. Y., Eng, C., & Morris, N. (2014). A Twin Study of Heritable and Shared Environmental Contributions to Autism. *Journal of Autism and Developmental Disorders*, *44*(8), 2013–2025. https://doi.org/10.1007/s10803-014-2081-2

Fung, T. C., Olson, C. A., & Hsiao, E. Y. (2017). Interactions between the microbiota, immune and nervous systems in health and disease. *Nature Neuroscience*, *20*(2), 145–155. https://doi.org/10.1038/nn.4476

Gardener, H., Spiegelman, D., & Buka, S. L. (2011). Perinatal and Neonatal Risk Factors for Autism: A Comprehensive Meta-analysis. *PEDIATRICS*, *128*(2), 344–355. https://doi.org/10.1542/peds.2010-1036

Geurts, H. M., Corbett, B., & Solomon, M. (2009). The paradox of cognitive flexibility in autism. *Trends in Cognitive Sciences*, *13*(2), 74–82. https://doi.org/10.1016/j.tics.2008.11.006

Goin-Kochel, R. P., Abbacchi, A., Constantino, J. N., & Autism Genetic Resource Exchange Co. (2007). Lack of evidence for increased genetic loading for autism among families of affected females. *Autism*, *11*(3), 279–286. https://doi.org/10.1177/1362361307076857

Goldman, S. E., Surdyka, K., Cuevas, R., Adkins, K., Wang, L., & Malow, B. A. (2009). Defining the Sleep Phenotype in Children With Autism. *Developmental Neuropsychology*, *34*(5), 560–573. https://doi.org/10.1080/87565640903133509

Gregory, S. G., Connelly, J. J., Towers, A. J., Johnson, J., Biscocho, D., Markunas, C. A., Lintas, C., Abramson, R. K., Wright, H. H., Ellis, P., Langford, C. F., Worley, G., Delong, G. R., Murphy, S. K., Cuccaro, M. L., Persico, A., & Pericak-Vance, M. A. (2009). Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. *BMC Medicine*, *7*, 62. https://doi.org/10.1186/1741-7015-7-62

Grether, J. K., Anderson, M. C., Croen, L. A., Smith, D., & Windham, G. C. (2009). Risk of Autism and Increasing Maternal and Paternal Age in a Large North American Population. *American Journal of Epidemiology*, *170*(9), 1118–1126. https://doi.org/10.1093/aje/kwp247

Grillon, C., Cordova, J., Morgan, C. A., Charney, D. S., & Davis, M. (2004). Effects of the beta-blocker Propranolol on cued and contextual fear conditioning in humans. *Psychopharmacology*, *175*(3), 342–352. https://doi.org/10.1007/s00213-004-1819-5

Grimshaw, J., Campbell, M., Eccles, M., & Steen, N. (2000). Experimental and quasi-experimental designs for evaluating guideline implementation strategies. *Family Practice*, *17*(90001), 11S16. https://doi.org/10.1093/fampra/17.suppl_1.s11

Guastella, A. J., Boulton, K. A., Whitehouse, A. J. O., Song, Y. J., Thapa, R., Gregory, S. G., Pokorski, I., Granich, J., DeMayo, M. M., Ambarchi, Z., Wray, J., Thomas, E. E., & Hickie, I. B. (2023). The effect of oxytocin nasal spray on social interaction in young children with autism: a randomized clinical trial. *Molecular Psychiatry*, *28*(2), 834–842. https://doi.org/10.1038/s41380-022-01845-8

Guthrie, W., Swineford, L. B., Nottke, C., & Wetherby, A. M. (2012). Early diagnosis of autism spectrum disorder: stability and change in clinical diagnosis and symptom presentation. *Journal of Child Psychology and Psychiatry*, *54*(5), 582–590. https://doi.org/10.1111/jcpp.12008

Harlow, J. M. (1848). Passage of an Iron Rod through the Head. *The Boston Medical and Surgical Journal*, *39*(20), 389–393. https://doi.org/10.1056/nejm184812130392001

Harris, A. D., McGregor, J. C., Perencevich, E. N., Furuno, J. P., Zhu, J., Peterson, D. E., & Finkelstein, J. (2006). The Use and Interpretation of Quasi-Experimental Studies in Medical Informatics. *Journal of the American Medical Informatics Association*, *13*(1), 16–23. https://doi.org/10.1197/jamia.m1749

Harris, J. (2018). Leo Kanner and autism: a 75-year perspective. *International Review of Psychiatry (Abingdon, England)*, *30*(1), 3–17. https://doi.org/10.1080/09540261.2018.1455646

Harris, N. S., Courchesne, E., Townsend, J., Carper, R. A., & Lord, C. (1999). Neuroanatomic contributions to slowed orienting of attention in children with autism. *Cognitive Brain Research*, *8*(1), 61–71. https://doi.org/10.1016/s0926-6410(99)00006-3

Harrop, C., Gulsrud, A., & Kasari, C. (2015). Does Gender Moderate Core Deficits in ASD? An Investigation into Restricted and Repetitive Behaviors in Girls and Boys with ASD. *Journal of Autism and Developmental Disorders*, *45*(11), 3644–3655. https://doi.org/10.1007/s10803-015-2511-9

Harrop, C., McConachie, H., Emsley, R., Leadbitter, K., & Green, J. (2013). Restricted and Repetitive Behaviors in Autism Spectrum Disorders and Typical Development: Cross-Sectional and Longitudinal Comparisons. *Journal of Autism and Developmental Disorders*, *44*(5), 1207–1219. https://doi.org/10.1007/s10803-013-1986-5

Harrop, C., Sterrett, K., Shih, W., Landa, R., Kaiser, A., & Kasari, C. (2021). Short-term trajectories of restricted and repetitive behaviors in minimally verbal children with autism spectrum disorder. *Autism Research*. https://doi.org/10.1002/aur.2528

Hartley, S. L., & Sikora, D. M. (2009). Sex Differences in Autism Spectrum Disorder: An Examination of Developmental Functioning, Autistic Symptoms, and Coexisting Behavior Problems in Toddlers. *Journal of Autism and Developmental Disorders*, *39*(12), 1715–1722. https://doi.org/10.1007/s10803-009-0810-8

Hiller, R. M., Young, R. L., & Weber, N. (2014). Sex Differences in Autism Spectrum Disorder based on DSM-5 Criteria: Evidence from Clinician and Teacher Reporting. *Journal of Abnormal Child Psychology*, *42*(8), 1381–1393. https://doi.org/10.1007/s10802-014-9881-x

Hirsch, L. E., & Pringsheim, T. (2016). Aripiprazole for autism spectrum disorders (ASD). *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.cd009043.pub3

Hiruma, L., Pretzel, R. E., Tapia, A. L., Bodfish, J. W., Bradley, C., Wiggins, L., Hsu, M., Lee, L.-C., Levy, S. E., & Daniels, J. (2021). A Distinct Three-Factor Structure of Restricted and Repetitive Behaviors in an Epidemiologically Sound Sample of Preschool-Age Children with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-020-04776-x

Hoch, J., Spofford, L., Dimian, A., Tervo, R., MacLean, W. E., & Symons, F. J. (2015). A Direct Comparison of Self-Injurious and Stereotyped Motor Behavior Between Preschool-Aged Children With and Without Developmental Delays. *Journal of Pediatric Psychology*, *41*(5), 566–572. https://doi.org/10.1093/jpepsy/jsv102

Holdman, R., Vigil, D., Robinson, K., Shah, P., & Contreras, A. E. (2021). Safety and Efficacy of Medical Cannabis in Autism Spectrum Disorder Compared with Commonly Used Medications. *Cannabis and Cannabinoid Research*. https://doi.org/10.1089/can.2020.0154

Hong, E., Dixon, D. R., Stevens, E., Burns, C. O., & Linstead, E. (2018). Topography and Function of Challenging Behaviors in Individuals with Autism Spectrum Disorder. *Advances in Neurodevelopmental Disorders*, *2*(2), 206–215. https://doi.org/10.1007/s41252-018-0063-7

Horner, R. H., Carr, E. G., Strain, P. S., Todd, A. W., & Reed, H. K. (2002). Problem Behavior Interventions for Young Children with Autism: A Research Synthesis. *Journal of Autism and Developmental Disorders*, *32*(5), 423–446. https://doi.org/10.1023/a:1020593922901

Houghton, R., Ong, R. C., & Bolognani, F. (2017). Psychiatric comorbidities and use of psychotropic medications in people with autism spectrum disorder in the United States. *Autism Research, 10*(12), 2037–2047. https://doi.org/10.1002/aur.1848

Hu, V. W., Frank, B. C., Heine, S., Lee, N. H., & Quackenbush, J. (2006). Gene expression profiling of lymphoblastoid cell lines from monozygotic twins discordant in severity of autism reveals differential regulation of neurologically relevant genes. *BMC Genomics, 7*(1). https://doi.org/10.1186/1471-2164-7-118

Hull, L., Petrides, K. V., Allison, C., Smith, P., Baron-Cohen, S., Lai, M.-C., & Mandy, W. (2017). "Putting on My Best Normal": Social Camouflaging in Adults with Autism Spectrum Conditions. *Journal of Autism and Developmental Disorders, 47*(8), 2519–2534. https://doi.org/10.1007/s10803-017-3166-5

Hundley, R. J., Shui, A., & Malow, B. A. (2016). Relationship Between Subtypes of Restricted and Repetitive Behaviors and Sleep Disturbance in Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *46*(11), 3448–3457. https://doi.org/10.1007/s10803-016-2884-4

Hus, Y., & Segal, O. (2020). Functional Communication Profiles of Children and Youth with Autism: A Roadmap to Therapeutic and Educational Interventions. *Folia Phoniatrica et Logopaedica*, *73*(3), 205–221. https://doi.org/10.1159/000510056

Hus, Y., & Segal, O. (2021). Challenges Surrounding the Diagnosis of Autism in Children. *Neuropsychiatric Disease and Treatment*, *17*, 3509–3529. https://doi.org/10.2147/NDT.S282569

Hutchinson, J. A., Oluwa Folawemi, Parikshit Bittla, Kaur, S., Vani Sojitra, Zahra, A., & Khan, S. (2023). The Effects of Risperidone on Cognition in People With Autism

Spectrum Disorder: A Systematic Review. *Cureus*. https://doi.org/10.7759/cureus.45524

Jackson, S. J., Andrews, N., Ball, D., Bellantuono, I., Gray, J., Hachoumi, L., Holmes, A., Latcham, J., Petrie, A., Potter, P., Rice, A., Ritchie, A., Stewart, M., Strepka, C., Yeoman, M., & Chapman, K. (2017). Does age matter? The impact of rodent age on study outcomes. *Laboratory Animals*, *51*(2), 160–169. https://doi.org/10.1177/0023677216653984

Jang, J., Dixon, D. R., Tarbox, J., & Granpeesheh, D. (2011). Symptom severity and challenging behavior in children with ASD. *Research in Autism Spectrum Disorders*, *5*(3), 1028–1032. https://doi.org/10.1016/j.rasd.2010.11.008

Jobski, K., Höfer, J., Hoffmann, F., & Bachmann, C. (2016). Use of psychotropic drugs in patients with autism spectrum disorders: a systematic review. *Acta Psychiatrica Scandinavica*, *135*(1), 8–28. https://doi.org/10.1111/acps.12644

Kaland, N., Smith, L., & Mortensen, E. L. (2007). Brief Report: Cognitive Flexibility and Focused Attention in Children and Adolescents with Asperger Syndrome or High-Functioning Autism as Measured on the Computerized Version of the Wisconsin Card Sorting Test. *Journal of Autism and Developmental Disorders*, *38*(6), 1161–1165. https://doi.org/10.1007/s10803-007-0474-1

Keating, J., Van Goozen, S., Uljarevic, M., Hay, D., & Leekam, S. R. (2023). Restricted and repetitive behaviors and their developmental and demographic correlates in 4–8-year-old children: A transdiagnostic approach. *Frontiers in Behavioral Neuroscience*, *17*. https://doi.org/10.3389/fnbeh.2023.1085404

Kenworthy, L., Anthony, L. G., Naiman, D. Q., Cannon, L., Wills, M. C., Luong-Tran, C., Werner, M. A., Alexander, K. C., Strang, J., Bal, E., Sokoloff, J. L., & Wallace, G. L. (2013). Randomized controlled effectiveness trial of executive function intervention for children on the autism spectrum. *Journal of Child Psychology and Psychiatry*, *55*(4), 374–383. https://doi.org/10.1111/jcpp.12161

Knutsen, J., Crossman, M., Perrin, J., Shui, A., & Kuhlthau, K. (2018). Sex differences in restricted repetitive behaviors and interests in children with autism spectrum disorder: An Autism Treatment Network study. *Autism*, 136236131878649. https://doi.org/10.1177/1362361318786490

Kong, X., Liu, J., Cetinbas, M., Sadreyev, R., Koh, M., Huang, H., Adeseye, A., He, P., Zhu, J., Russell, H., Hobbie, C., Liu, K., & Onderdonk, A. B. (2019). New and Preliminary Evidence on Altered Oral and Gut Microbiota in Individuals with Autism Spectrum Disorder (ASD): Implications for ASD Diagnosis and Subtyping Based on Microbial Biomarkers. *Nutrients*, *11*(9), 2128. https://doi.org/10.3390/nu11092128

Kuzminskaite, E., Begeer, S., Hoekstra, R. A., & Grove, R. (2020). Short report: Social communication difficulties and restricted repetitive behaviors as predictors of anxiety in adults with autism spectrum disorder. *Autism, 24*(7), 136236132093421. https://doi.org/10.1177/1362361320934218

Lai, M.-C. ., Lombardo, M. V., Suckling, J., Ruigrok, A. N. V., Chakrabarti, B., Ecker, C., Deoni, S. C. L., Craig, M. C., Murphy, D. G. M., Bullmore, E. T., & Baron-Cohen, S. (2013). Biological sex affects the neurobiology of autism. *Brain*, *136*(9), 2799–2815. https://doi.org/10.1093/brain/awt216

Lai, M.-C., Baron-Cohen, S., & Buxbaum, J. D. (2015). Understanding autism in the light of sex/gender. *Molecular Autism*, *6*(1). https://doi.org/10.1186/s13229-015-0021-4

Lake, C. R. (1977). Increased Norepinephrine Levels and Decreased Dopamine-β-Hydroxylase Activity in Primary Autism. *Archives of General Psychiatry*, *34*(5), 553. https://doi.org/10.1001/archpsyc.1977.01770170063005

Launay, J.-M., Bursztejn, C., Ferrari, P., Dreux, C., Braconnier, A., Zarifian, E., Lancrenon, S., & Fermanian, J. (1987). Catecholamines metabolism in infantile autism: A controlled study of 22 autistic children. *Journal of Autism and Developmental Disorders*, *17*(3), 333–347. https://doi.org/10.1007/bf01487064

Leekam, S. R., Prior, M. R., & Uljarevic, M. (2011). Restricted and repetitive behaviors in autism spectrum disorders: A review of research in the last decade. *Psychological Bulletin*, *137*(4), 562–593. https://doi.org/10.1037/a0023341

Lehnhardt, F.-G., Falter, C. M., Gawronski, A., Pfeiffer, K., Tepest, R., Franklin, J., & Vogeley, K. (2015). Sex-Related Cognitive Profile in Autism Spectrum Disorders Diagnosed Late in Life: Implications for the Female Autistic Phenotype. *Journal of Autism and Developmental Disorders*, *46*(1), 139–154. https://doi.org/10.1007/s10803-015-2558-7

Leigh, J. P., & Du, J. (2015). Brief Report: Forecasting the Economic Burden of Autism in 2015 and 2025 in the United States. *Journal of Autism and Developmental Disorders*, *45*(12), 4135–4139. https://doi.org/10.1007/s10803-015-2521-7

Leskovec, T. J., Rowles, B. M., & Findling, R. L. (2008). Pharmacological Treatment Options for Autism Spectrum Disorders in Children and Adolescents. *Harvard*

*Review of Psychiatry*, *16*(2), 97–112. https://doi.org/10.1080/10673220802075852

Li, A., MacNeill, B., Curiel, H., & Poling, A. (2017). Risperidone in combination with other drugs: Experimental research in individuals with autism spectrum disorder. *Experimental and Clinical Psychopharmacology*, *25*(5), 434–439. https://doi.org/10.1037/pha0000144

London, E. B., Yoo, J. H., Fethke, E. D., & Zimmerman-Bier, B. (2020). The Safety and Effectiveness of High-Dose Propranolol as a Treatment for Challenging Behaviors in Individuals With Autism Spectrum Disorders. *Journal of Clinical Psychopharmacology*, *40*(2), 122–129. https://doi.org/10.1097/JCP.0000000000001175

*Luke 8:2 - Bible Gateway*. (n.d.). Www.biblegateway.com. Retrieved October 19, 2023, from https://www.biblegateway.com/verse/en/Luke%208%3A2

Lyall, K., Croen, L., Daniels, J., Fallin, M. D., Ladd-Acosta, C., Lee, B. K., Park, B. Y., Snyder, N. W., Schendel, D., Volk, H., Windham, G. C., & Newschaffer, C. (2017). The Changing Epidemiology of Autism Spectrum Disorders. *Annual Review of Public Health*, *38*(1), 81–102. https://doi.org/10.1146/annurev-publhealth-031816-044318

Macaskill, G. (2021). The Bible, Autism and Other Profound Developmental Conditions: Regulating Hermeneutics. *Journal of Disability & Religion*, 1–25. https://doi.org/10.1080/23312521.2021.1881024

MacDuffie, K. E., Munson, J., Greenson, J., Ward, T. M., Rogers, S. J., Dawson, G., & Estes, A. (2020). Sleep Problems and Trajectories of Restricted and Repetitive

Behaviors in Children with Neurodevelopmental Disabilities. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-020-04438-y

Maenner, M. J. (2020). Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016. *MMWR. Surveillance Summaries*, *69*(4). https://doi.org/10.15585/mmwr.ss6904a1

Maenner, M. J. (2023). Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2020. *MMWR. Surveillance Summaries*, *72*(2). https://doi.org/10.15585/mmwr.ss7202a1

Maenner, M. J., Shaw, K. A., Bakian, A. V., Bilder, D. A., Durkin, M. S., Esler, A., Furnier, S. M., Hallas, L., Hall-Lande, J., Hudson, A., Hughes, M. M., Patrick, M., Pierce, K., Poynter, J. N., Salinas, A., Shenouda, J., Vehorn, A., Warren, Z., Constantino, J. N., & DiRienzo, M. (2021). Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2018. *MMWR. Surveillance Summaries*, *70*(11), 1–16. https://doi.org/10.15585/mmwr.ss7011a1

Magiati, I., Ong, C., Lim, X. Y., Tan, J. W.-L., Ong, A. Y. L., Patrycia, F., Fung, D. S. S., Sung, M., Poon, K. K., & Howlin, P. (2015). Anxiety symptoms in young people with autism spectrum disorder attending special schools: Associations with gender, adaptive functioning and autism symptomatology. *Autism*, *20*(3), 306–320. https://doi.org/10.1177/1362361315577519

Mandy, W., Chilvers, R., Chowdhury, U., Salter, G., Seigal, A., & Skuse, D. (2011). Sex Differences in Autism Spectrum Disorder: Evidence from a Large Sample of Children and Adolescents. *Journal of Autism and Developmental Disorders*, *42*(7), 1304–1313. https://doi.org/10.1007/s10803-011-1356-0

Maneeton, N., Maneeton, B., Puthisri, S., Woottiluk, P., Narkpongphun, A., & Srisurapanont, M. (2018). Risperidone for children and adolescents with autism spectrum disorder: a systematic review. *Neuropsychiatric Disease and Treatment*, *Volume 14*, 1811–1820. https://doi.org/10.2147/ndt.s151802

Mannion, A., Leader, G., & Healy, O. (2013). An investigation of comorbid psychological disorders, sleep problems, gastrointestinal symptoms and epilepsy in children and adolescents with Autism Spectrum Disorder. *Research in Autism Spectrum Disorders*, *7*(1), 35–42. https://doi.org/10.1016/j.rasd.2012.05.002

Mano-Sousa, B. J., Pedrosa, A. M., Alves, B. C., Galduróz, J. C. F., Belo, V. S., Chaves, V. E., & Duarte-Almeida, J. M. (2021). Effects of Risperidone in Autistic Children and Young Adults: A Systematic Review and Meta-Analysis. *Current Neuropharmacology*, *19*(4), 538–552. https://doi.org/10.2174/1570159x18666200529151741

Marcus, R. N., Owen, R., Manos, G., Mankoski, R., Kamen, L., McQuade, R. D., Carson, W. H., & Findling, R. L. (2011). Safety and Tolerability of Aripiprazole for Irritability in Pediatric Patients With Autistic Disorder. *The Journal of Clinical Psychiatry*, *72*(09), 1270–1276. https://doi.org/10.4088/jcp.09m05933

Maskey, M., Warnell, F., Parr, J. R., Le Couteur, A., & McConachie, H. (2012). Emotional and Behavioural Problems in Children with Autism Spectrum

Disorder. *Journal of Autism and Developmental Disorders*, *43*(4), 851–859. https://doi.org/10.1007/s10803-012-1622-9

Mathew, S. K., & Pandian, J. D. (2010). Newer insights to the neurological diseases among biblical characters of old testament. *Annals of Indian Academy of Neurology*, *13*(3), 164–166. https://doi.org/10.4103/0972-2327.70873

Matson, J. L., Dempsey, T., & Fodstad, J. C. (2009). Stereotypies and repetitive/restrictive behaviours in infants with autism and pervasive developmental disorder. *Developmental Neurorehabilitation*, *12*(3), 122–127. https://doi.org/10.1080/17518420902936730

Matson, J. L., Mahan, S., Hess, J. A., Fodstad, J. C., & Neal, D. (2010). Progression of challenging behaviors in children and adolescents with Autism Spectrum Disorders as measured by the Autism Spectrum Disorders-Problem Behaviors for Children (ASD-PBC). *Research in Autism Spectrum Disorders*, *4*(3), 400–404. https://doi.org/10.1016/j.rasd.2009.10.010

Mauldin, R. L. (2020, January 15). *8.2 Quasi-experimental and pre-experimental designs*. Uta.pressbooks.pub; Mavs Open Press. https://uta.pressbooks.pub/foundationsofsocialworkresearch/chapter/8-2-quasi-experimental-and-pre-experimental-designs/

McCanlies, E. C., Fekedulegn, D., Mnatsakanova, A., Burchfiel, C. M., Sanderson, W. T., Charles, L. E., & Hertz-Picciotto, I. (2012). Parental Occupational Exposures and Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *42*(11), 2323–2334. https://doi.org/10.1007/s10803-012-1468-1

McDougle, C. J., Stigler, K. A., & Posey, D. J. (2003). Treatment of aggression in children and adolescents with autism and conduct disorder. *The Journal of Clinical Psychiatry*, *64 Suppl 4*, 16–25. https://pubmed.ncbi.nlm.nih.gov/12672261/

McKinnon, C. J., Eggebrecht, A. T., Todorov, A., Wolff, J. J., Elison, J. T., Adams, C. M., Snyder, A. Z., Estes, A. M., Zwaigenbaum, L., Botteron, K. N., McKinstry, R. C., Marrus, N., Evans, A., Hazlett, H. C., Dager, S. R., Paterson, S. J., Pandey, J., Schultz, R. T., Styner, M. A., & Gerig, G. (2019). Restricted and Repetitive Behavior and Brain Functional Connectivity in Infants at Risk for Developing Autism Spectrum Disorder. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *4*(1), 50–61. https://doi.org/10.1016/j.bpsc.2018.09.008

*Medical Marijuana States 2023*. (n.d.). Worldpopulationreview.com. https://worldpopulationreview.com/state-rankings/medical-marijuana-states

MEDINA, K. L., HANSON, K. L., SCHWEINSBURG, A. D., COHEN-ZION, M., NAGEL, B. J., & TAPERT, S. F. (2007). Neuropsychological functioning in adolescent marijuana users: Subtle deficits detectable after a month of abstinence. *Journal of the International Neuropsychological Society*, *13*(05). https://doi.org/10.1017/s1355617707071032

Memari, A. H., Ziaee, V., Shayestehfar, M., Ghanouni, P., Mansournia, M. A., & Moshayedi, P. (2013). Cognitive flexibility impairments in children with autism spectrum disorders: Links to age, gender and child outcomes. *Research in Developmental Disabilities*, *34*(10), 3218–3225. https://doi.org/10.1016/j.ridd.2013.06.033

Merriam Webster. (2019). *Definition of PHARMACOLOGY*. Merriam-Webster.com. https://www.merriam-webster.com/dictionary/pharmacology

Miller, M., Sun, S., Iosif, A.-M., Young, G. S., Belding, A., Tubbs, A., & Ozonoff, S. (2021). Repetitive behavior with objects in infants developing autism predicts diagnosis and later social behavior as early as 9 months. *Journal of Abnormal Psychology*, *130*(6), 665–675. https://doi.org/10.1037/abn0000692

Miyake, A., Friedman, N. P., Emerson, M. J., Witzki, A. H., Howerter, A., & Wager, T. D. (2000). The Unity and Diversity of Executive Functions and Their Contributions to Complex "Frontal Lobe" Tasks: A Latent Variable Analysis. *Cognitive Psychology*, *41*(1), 49–100. https://doi.org/10.1006/cogp.1999.0734

Mustonen, A., Niemelä, S., Nordström, T., Murray, G. K., Mäki, P., Jääskeläinen, E., & Miettunen, J. (2018). Adolescent cannabis use, baseline prodromal symptoms and the risk of psychosis. *The British Journal of Psychiatry*, *212*(4), 227–233. https://doi.org/10.1192/bjp.2017.52

Narayanan, A., White, C. A., Saklayen, S., Scaduto, M. J., Carpenter, A. L., Abduljalil, A., Schmalbrock, P., & Beversdorf, D. Q. (2010). Effect of Propranolol on Functional Connectivity in Autism Spectrum Disorder—A Pilot Study. *Brain Imaging and Behavior*, *4*(2), 189–197. https://doi.org/10.1007/s11682-010-9098-8

Newschaffer, C. J. (2002). Heritable and Nonheritable Risk Factors for Autism Spectrum Disorders. *Epidemiologic Reviews*, *24*(2), 137–153. https://doi.org/10.1093/epirev/mxf010

NIH. (2017, January 31). *Medication Treatment for Autism*. Http://Www.nichd.nih.gov/. https://www.nichd.nih.gov/health/topics/autism/conditioninfo/treatments/medication-treatment

Ongono, J. S., Béranger, R., Baghdadli, A., & Mortamais, M. (2020). Pesticides used in Europe and autism spectrum disorder risk: can novel exposure hypotheses be formulated beyond organophosphates, organochlorines, pyrethroids and carbamates? - A systematic review. *Environmental Research*, *187*, 109646. https://doi.org/10.1016/j.envres.2020.109646

*Organophosphate - an overview | ScienceDirect Topics*. (n.d.). Www.sciencedirect.com. https://www.sciencedirect.com/topics/earth-and-planetary-sciences/organophosphate

*Organophosphate Pesticides | Biomonitoring California*. (n.d.). Biomonitoring.ca.gov. https://biomonitoring.ca.gov/chemicals/organophosphate-pesticides

Orsolini, L., Tomasetti, C., Valchera, A., Vecchiotti, R., Matarazzo, I., Vellante, F., Iasevoli, F., Buonaguro, E. F., Fornaro, M., Fiengo, A. L. C., Martinotti, G., Mazza, M., Perna, G., Carano, A., De Bartolomeis, A., Di Giannantonio, M., & De Berardis, D. (2016). An update of safety of clinically used atypical antipsychotics. *Expert Opinion on Drug Safety*, *15*(10), 1329–1347. https://doi.org/10.1080/14740338.2016.1201475

Ozonoff, S., Macari, S., Young, G. S., Goldring, S., Thompson, M., & Rogers, S. J. (2008). Atypical object exploration at 12 months of age is associated with autism in a prospective sample. *Autism*, *12*(5), 457–472. https://doi.org/10.1177/1362361308096402

Park, S., Cho, S.-C., Cho, I. H., Kim, B.-N., Kim, J.-W., Shin, M.-S., Chung, U.-S., Park, T.-W., Son, J.-W., & Yoo, H. J. (2012). Sleep problems and their correlates and comorbid psychopathology of children with autism spectrum disorders. *Research in Autism Spectrum Disorders*, *6*(3), 1068–1072. https://doi.org/10.1016/j.rasd.2012.02.004

Paul, R., Chawarska, K., Cicchetti, D., & Volkmar, F. (2008). Language outcomes of toddlers with autism spectrum disorders: a two year follow-up. *Autism Research*, *1*(2), 97–107. https://doi.org/10.1002/aur.12

Pazos, A., Engel, G., & Palacios, J. M. (1985a). β-Adrenoceptor blocking agents recognize a subpopulation of serotonin receptors in brain. *Brain Research*, *343*(2), 403–408. https://doi.org/10.1016/0006-8993(85)90766-8

Pazos, A., Engel, G., & Palacios, J. M. (1985b). β-Adrenoceptor blocking agents recognize a subpopulation of serotonin receptors in brain. *Brain Research*, *343*(2), 403–408. https://doi.org/10.1016/0006-8993(85)90766-8

Perez-Fernandez, C., Matamala Montoya, M., Morales-Navas, M., Guardia-Escote, L., Cabré, M., Colomina, M. T., Giménez, E., & Sánchez-Santed, F. (2022). Influence of Gestational Chlorpyrifos Exposure on ASD-like Behaviors in an fmr1-KO Rat Model. *Molecular Neurobiology*, *59*(9), 5835–5855. https://doi.org/10.1007/s12035-022-02933-0

Petrou, A. M., Parr, J. R., & McConachie, H. (2018). Gender differences in parent-reported age at diagnosis of children with autism spectrum disorder. *Research in Autism Spectrum Disorders*, *50*, 32–42. https://doi.org/10.1016/j.rasd.2018.02.003

Pope, H. G., Gruber, A. J., Hudson, J. I., Cohane, G., Huestis, M. A., & Yurgelun-Todd, D. (2003). Early-onset cannabis use and cognitive deficits: what is the nature of the association? *Drug and Alcohol Dependence*, *69*(3), 303–310. https://doi.org/10.1016/s0376-8716(02)00334-4

Posar, A., & Visconti, P. (2020). Sleep Problems in Children with Autism Spectrum Disorder. *Pediatric Annals*, *49*(6), e278–e282. https://doi.org/10.3928/19382359-20200511-01

*Propranolol: medicine for heart problems, anxiety and migraine*. (2019, January 14). Nhs.uk. https://www.nhs.uk/medicines/Propranolol/#:~:text=Propranolol%20slows%20do

Ratey, J. J., Bemporad, J. R., Sorgi, P., Peter Anthony Bick, Polakoff, S., O'Driscoll, G. A., & Mikkelsen, E. J. (1987). Brief report: Open trial effects of beta-blockers on speech and social behaviors in 8 autistic adults. *Journal of Autism and Developmental Disorders*, *17*(3), 439–446. https://doi.org/10.1007/bf01487073

RAVIZZA, S. M., SOLOMON, M., IVRY, R. B., & CARTER, C. S. (2013). Restricted and repetitive behaviors in autism spectrum disorders: The relationship of attention and motor deficits. *Development and Psychopathology*, *25*(3), 773–784. https://doi.org/10.1017/S0954579413000163

Reichenberg, A., Gross, R., Weiser, M., Bresnahan, M., Silverman, J., Harlap, S., Rabinowitz, J., Shulman, C., Malaspina, D., Lubin, G., Knobler, H. Y., Davidson, M., & Susser, E. (2006). Advancing Paternal Age and Autism. *Archives of General Psychiatry*, *63*(9), 1026. https://doi.org/10.1001/archpsyc.63.9.1026

*Restrictive and Repetitive Behavior*. (2013). Kennedykrieger.org; Kennedy Krieger Institute. https://www.kennedykrieger.org/patient-care/conditions/restrictive-and-repetitive-behavior

Reynolds, A. M., Soke, G. N., Sabourin, K. R., Hepburn, S., Katz, T., Wiggins, L. D., Schieve, L. A., & Levy, S. E. (2019). Sleep Problems in 2- to 5-Year-Olds With Autism Spectrum Disorder and Other Developmental Delays. *Pediatrics*, *143*(3), e20180492. https://doi.org/10.1542/peds.2018-0492

Rodrigues, R., Lai, M., Beswick, A., Gorman, D. A., Anagnostou, E., Szatmari, P., Anderson, K. K., & Ameis, S. H. (2020). Practitioner Review: Pharmacological treatment of attention-deficit/hyperactivity disorder symptoms in children and youth with autism spectrum disorder: a systematic review and meta-analysis. *Journal of Child Psychology and Psychiatry*. https://doi.org/10.1111/jcpp.13305

Rogge, N., & Janssen, J. (2019). The Economic Costs of Autism Spectrum Disorder: A Literature Review. *Journal of Autism and Developmental Disorders*, *49*(7). https://doi.org/10.1007/s10803-019-04014-z

Rubenstein, E., Wiggins, L. D., & Lee, L.-C. (2014). A Review of the Differences in Developmental, Psychiatric, and Medical Endophenotypes Between Males and Females with Autism Spectrum Disorder. *Journal of Developmental and Physical Disabilities*, *27*(1), 119–139. https://doi.org/10.1007/s10882-014-9397-x

Rylaarsdam, L., & Guemez-Gamboa, A. (2019). Genetic causes and modifiers of autism spectrum disorder. *Frontiers in Cellular Neuroscience*, *13*(13). https://doi.org/10.3389/fncel.2019.00385

Rynkiewicz, A., Schuller, B., Marchi, E., Piana, S., Camurri, A., Lassalle, A., & Baron-Cohen, S. (2016). An investigation of the “female camouflage effect” in autism using a computerized ADOS-2 and a test of sex/gender differences. *Molecular Autism*, *7*(10), 10. https://doi.org/10.1186/s13229-016-0073-0

Sagar-Ouriaghli, I., Lievesley, K., & Santosh, P. J. (2018). Propranolol for treating emotional, behavioural, autonomic dysregulation in children and adolescents with autism spectrum disorders. *Journal of Psychopharmacology*, *32*(6), 641–653. https://doi.org/10.1177/0269881118756245

Saniee, S., Pouretemad, H. R., & Zardkhaneh, S. A. (2019). Developing set-shifting improvement tasks (SSIT) for children with high-functioning autism. *Journal of Intellectual Disability Research*, *63*(10), 1207–1220. https://doi.org/10.1111/jir.12633

Schmidt, R. J., Tancredi, D. J., Ozonoff, S., Hansen, R. L., Hartiala, J., Allayee, H., Schmidt, L. C., Tassone, F., & Hertz-Picciotto, I. (2012). Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. *The American Journal of Clinical Nutrition*, *96*(1), 80–89. https://doi.org/10.3945/ajcn.110.004416

Schuck, R. K., Flores, R. E., & Fung, L. K. (2019). Brief Report: Sex/Gender Differences in Symptomology and Camouflaging in Adults with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *49*(6), 2597–2604. https://doi.org/10.1007/s10803-019-03998-y

Sellick, T., Ure, A., & Williams, K. (2021). Repetitive and restricted behaviours and anxiety in autism spectrum disorder: protocol for a systematic review and meta-analysis. *Systematic Reviews*, *10*(1). https://doi.org/10.1186/s13643-021-01830-2

Shadish, W. R., Cook, T. D., & Donald Thomas Campbell. (2002). *Experimental and Quasi-experimental Designs for Generalized Causal Inference*. Cengage Learning.

Shelton, J. F., Tancredi, D. J., & Hertz-Picciotto, I. (2010). Independent and dependent contributions of advanced maternal and paternal ages to autism risk. *Autism Research*, n/a-n/a. https://doi.org/10.1002/aur.116

Siafis, S., Çıray, O., Wu, H., Schneider-Thoma, J., Bighelli, I., Krause, M., Rodolico, A., Ceraso, A., Deste, G., Huhn, M., Fraguas, D., San José Cáceres, A., Mavridis, D., Charman, T., Murphy, D. G., Parellada, M., Arango, C., & Leucht, S. (2022). Pharmacological and dietary-supplement treatments for autism spectrum disorder: a systematic review and network meta-analysis. *Molecular Autism*, *13*(1). https://doi.org/10.1186/s13229-022-00488-4

Sifre, R., Berry, D. J., Wolff, J. J., & Elison, J. T. (2021). *Longitudinal change in restricted and repetitive behaviors from 8-36 months*. *13*(1). https://doi.org/10.1186/s11689-020-09335-0

Soldin, O. P., Chung, S. H., & Mattison, D. R. (2011). Sex Differences in Drug Disposition. *Journal of Biomedicine and Biotechnology*, *2011*, 1–14. https://doi.org/10.1155/2011/187103

Spencer, D., Marshall, J., Post, B., Kulakodlu, M., Newschaffer, C., Dennen, T., Azocar, F., & Jain, A. (2013). Psychotropic Medication Use and Polypharmacy in

Children With Autism Spectrum Disorders. *Pediatrics*, *132*(5), 833–840. https://doi.org/10.1542/peds.2012-3774

Steenen, S. A., van Wijk, A. J., van der Heijden, G. J., van Westrhenen, R., de Lange, J., & de Jongh, A. (2015). Propranolol for the treatment of anxiety disorders: Systematic review and meta-analysis. *Journal of Psychopharmacology*, *30*(2), 128–139. https://doi.org/10.1177/0269881115612236

Stone, W. N. (1973). Anxiety and β-Adrenergic Blockade. *Archives of General Psychiatry*, *29*(5), 620. https://doi.org/10.1001/archpsyc.1973.04200050033005

Supekar, K., & Menon, V. (2015). Sex differences in structural organization of motor systems and their dissociable links with repetitive/restricted behaviors in children with autism. *Molecular Autism*, *6*(1). https://doi.org/10.1186/s13229-015-0042-z

Szatmari, P., Liu, X., Goldberg, J., Zwaigenbaum, L., Paterson, A. D., Woodbury-Smith, M., Georgiades, S., Duku, E., & Thompson, A. (2011). Sex differences in repetitive stereotyped behaviors in autism: Implications for genetic liability. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *159B*(1), 5–12. https://doi.org/10.1002/ajmg.b.31238

Szczepanski, Sara M., & Knight, Robert T. (2014). Insights into Human Behavior from Lesions to the Prefrontal Cortex. *Neuron*, *83*(5), 1002–1018. https://doi.org/10.1016/j.neuron.2014.08.011

Szeleszczuk, Ł., & Frączkowski, D. (2022). Propranolol versus Other Selected Drugs in the Treatment of Various Types of Anxiety or Stress, with Particular Reference to Stage Fright and Post-Traumatic Stress Disorder. *International Journal of Molecular Sciences*, *23*(17), 10099. https://doi.org/10.3390/ijms231710099

Teh, E. J., Chan, D. M.-E., Tan, G. K. J., & Magiati, I. (2017). Continuity and Change in, and Child Predictors of, Caregiver Reported Anxiety Symptoms in Young People with Autism Spectrum Disorder: A Follow-Up Study. *Journal of Autism and Developmental Disorders*, *47*(12), 3857–3871. https://doi.org/10.1007/s10803-017-3136-y

Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L. A., & Paylor, R. (2009). Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology*, *204*(2), 361–373. https://doi.org/10.1007/s00213-009-1466-y

Tian, J., Gao, X., & Yang, L. (2022). Repetitive Restricted Behaviors in Autism Spectrum Disorder: From Mechanism to Development of Therapeutics. *Frontiers in Neuroscience*, *16*. https://doi.org/10.3389/fnins.2022.780407

Tillmann, J., Ashwood, K., Absoud, M., Bölte, S., Bonnet-Brilhault, F., Buitelaar, J. K., Calderoni, S., Calvo, R., Canal-Bedia, R., Canitano, R., De Bildt, A., Gomot, M., Hoekstra, P. J., Kaale, A., McConachie, H., Murphy, D. G., Narzisi, A., Oosterling, I., Pejovic-Milovancevic, M., & Persico, A. M. (2018). Evaluating Sex and Age Differences in ADI-R and ADOS Scores in a Large European Multi-site Sample of Individuals with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *48*(7), 2490–2505. https://doi.org/10.1007/s10803-018-3510-4

Townsend, J., Courchesne, E., Covington, J., Westerfield, M., Harris, N. S., Lyden, P., Lowry, T. P., & Press, G. A. (1999). Spatial Attention Deficits in Patients with Acquired or Developmental Cerebellar Abnormality. *The Journal of*

*Neuroscience*, *19*(13), 5632–5643. https://doi.org/10.1523/jneurosci.19-13-05632.1999

Troyb, E., Knoch, K., Herlihy, L., Stevens, M. C., Chen, C.-M., Barton, M., Treadwell, K., & Fein, D. (2015). Restricted and Repetitive Behaviors as Predictors of Outcome in Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders*, *46*(4), 1282–1296. https://doi.org/10.1007/s10803-015-2668-2

Tsai, L., Stewart, M. A., & August, G. (1981). Implication of sex differences in the familial transmission of infantile autism. *Journal of Autism and Developmental Disorders*, *11*(2), 165–173. https://doi.org/10.1007/bf01531682

Tudor, M. E., Hoffman, C. D., & Sweeney, D. P. (2012). Children With Autism. *Focus on Autism and Other Developmental Disabilities*, *27*(4), 254–262. https://doi.org/10.1177/1088357612457989

Tural Hesapcioglu, S., Ceylan, M. F., Kasak, M., & Sen, C. P. (2020). Olanzapine, risperidone, and aripiprazole use in children and adolescents with Autism Spectrum Disorders. *Research in Autism Spectrum Disorders*, *72*, 101520. https://doi.org/10.1016/j.rasd.2020.101520

U.S. Government Accountability Office. (2019). *U.S. Government Accountability Office (U.S. GAO)*. Gao.gov. https://www.gao.gov/

Uljarević, M., Arnott, B., Carrington, S. J., Meins, E., Fernyhough, C., McConachie, H., Le Couteur, A., & Leekam, S. R. (2017). Development of restricted and repetitive behaviors from 15 to 77 months: Stability of two distinct subtypes? *Developmental Psychology*, *53*(10), 1859–1868. https://doi.org/10.1037/dev0000324

Van Wilder, L., Devleesschauwer, B., Clays, E., Pype, P., Vandepitte, S., & De Smedt, D. (2022). Polypharmacy and Health-Related Quality of Life/Psychological Distress Among Patients With Chronic Disease. *Preventing Chronic Disease*, *19*. https://doi.org/10.5888/pcd19.220062

Vasa, R. A., & Mazurek, M. O. (2015). An update on anxiety in youth with autism spectrum disorders. *Current Opinion in Psychiatry*, *28*(2), 83–90. https://doi.org/10.1097/yco.0000000000000133

Voineagu, I., Wang, X., Johnston, P., Lowe, J. K., Tian, Y., Horvath, S., Mill, J., Cantor, R. M., Blencowe, B. J., & Geschwind, D. H. (2011). Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature*, *474*(7351), 380–384. https://doi.org/10.1038/nature10110

Wainwright-Sharp, J. A., & Bryson, S. E. (1993). Visual orienting deficits in high-functioning people with autism. *Journal of Autism and Developmental Disorders*, *23*(1), 1–13. https://doi.org/10.1007/bf01066415

Wang, J., Li, K., Li, A., Dai, K., Yin, Y., & Li, J. (2021). Shaking table test of a 1:10 scale thermal power plant building equipped with passive control systems. *Engineering Structures*, *232*, 111804–111804. https://doi.org/10.1016/j.engstruct.2020.111804

Ward, F., Tharian, P., Roy, M., Deb, S., & Unwin, G. L. (2013). Efficacy of beta blockers in the management of problem behaviours in people with intellectual disabilities: A systematic review. *Research in Developmental Disabilities*, *34*(12), 4293–4303. https://doi.org/10.1016/j.ridd.2013.08.015

Weimer, K., Gulewitsch, M. D., Schlarb, A. A., Schwille-Kiuntke, J., Klosterhalfen, S., & Enck, P. (2013). Placebo effects in children: a review. *Pediatric Research, 74*(1), 96–102. https://doi.org/10.1038/pr.2013.66

Weinberger, A. H., Platt, J., & Goodwin, R. D. (2016). Is cannabis use associated with an increased risk of onset and persistence of alcohol use disorders? A three-year prospective study among adults in the United States. *Drug and Alcohol Dependence, 161,* 363–367. https://doi.org/10.1016/j.drugalcdep.2016.01.014

Werling, D. M., & Geschwind, D. H. (2013). Sex differences in autism spectrum disorders. *Current Opinion in Neurology*, *26*(2), 146–153. https://doi.org/10.1097/wco.0b013e32835ee548

Windham, G. C., Zhang, L., Gunier, R., Croen, L. A., & Grether, J. K. (2006). Autism Spectrum Disorders in Relation to Distribution of Hazardous Air Pollutants in the San Francisco Bay Area. *Environmental Health Perspectives, 114*(9), 1438–1444. https://doi.org/10.1289/ehp.9120

Wolff, J. J., Botteron, K. N., Dager, S. R., Elison, J. T., Estes, A. M., Gu, H., Hazlett, H. C., Pandey, J., Paterson, S. J., Schultz, R. T., Zwaigenbaum, L., & Piven, J. (2014). Longitudinal patterns of repetitive behavior in toddlers with autism. *Journal of Child Psychology and Psychiatry*, *55*(8), 945–953. https://doi.org/10.1111/jcpp.12207

Xu, G., Strathearn, L., Liu, B., O'Brien, M., Kopelman, T. G., Zhu, J., Snetselaar, L. G., & Bao, W. (2019). Prevalence and Treatment Patterns of Autism Spectrum Disorder in the United States, 2016. *JAMA Pediatrics, 173*(2), 153. https://doi.org/10.1001/jamapediatrics.2018.4208

Yamasue, H., Okada, T., Munesue, T., Kuroda, M., Fujioka, T., Uno, Y., Matsumoto, K., Kuwabara, H., Mori, D., Okamoto, Y., Yoshimura, Y., Kawakubo, Y., Arioka, Y., Kojima, M., Yuhi, T., Owada, K., Yassin, W., Kushima, I., Benner, S., & Ogawa, N. (2018). Effect of intranasal oxytocin on the core social symptoms of autism spectrum disorder: a randomized clinical trial. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-018-0097-2

Zamzow, R. M., Ferguson, B. J., Stichter, J. P., Porges, E. C., Ragsdale, A. S., Lewis, M. L., & Beversdorf, D. Q. (2016). Effects of Propranolol on conversational reciprocity in autism spectrum disorder: a pilot, double-blind, single-dose psychopharmacological challenge study. *Psychopharmacology*, *233*(7), 1171–1178. https://doi.org/10.1007/s00213-015-4199-0

Zeestraten, E. A., Gudbrandsen, M. C., Daly, E., de Schotten, M. T., Catani, M., Dell'Acqua, F., Lai, M-C., Ruigrok, A. N. V., Lombardo, M. V., Chakrabarti, B., Baron-Cohen, S., Ecker, C., Murphy, D. G. M., & Craig, M. C. (2017). Sex differences in frontal lobe connectivity in adults with autism spectrum conditions. *Translational Psychiatry*, *7*(4), e1090–e1090. https://doi.org/10.1038/tp.2017.9

Zuvekas, S. H., Grosse, S. D., Lavelle, T. A., Maenner, M. J., Dietz, P., & Ji, X. (2020). Healthcare Costs of Pediatric Autism Spectrum Disorder in the United States, 2003–2015. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-020-04704-z

Zwaigenbaum, L., Bauman, M. L., Choueiri, R., Kasari, C., Carter, A., Granpeesheh, D., Mailloux, Z., Smith Roley, S., Wagner, S., Fein, D., Pierce, K., Buie, T., Davis, P. A., Newschaffer, C., Robins, D., Wetherby, A., Stone, W. L., Yirmiya, N., Estes,

A., & Hansen, R. L. (2015). Early Intervention for Children With Autism Spectrum Disorder Under 3 Years of Age: Recommendations for Practice and Research. *PEDIATRICS*, *136*(Supplement), S60–S81. https://doi.org/10.1542/peds.2014-3667e

# APPENDIX A: MEMORANDUM OF UNDERSTANDING

## The University of Mississippi Medical Center

### Memorandum of Understanding for Care and Use, and Oversight of Animal Subjects between Collaborating Institutions

This is a Memorandum of Understanding (MOU) between The University of Mississippi (UMMC) and the collaborator noted below for the performance of animal research, testing, or teaching. This MOU is entered into by both institutions' programs of animal care & use, and sets forth the agreed terms and conditions in accordance with which both institutions shall collaborate on an animal care & use activity.

**APPLICABILITY:** This MOU applies only to the protocol(s) specifically listed below.

**NAME OF INSTITUTION A: University of Mississippi Medical Center (UMMC)**

**USDA Registration Number**: 65-R-0102
**OLAW Assurance Number:** D16-00174 (A3275-01)
**AAALAC Accreditation Date**: 06/30/2021
**AAALAC Accreditation Status**: Full Accreditation
**IACUC Administrative Contact:** Amanda Kinslow, iacuc@umc.edu, 601-815-5006

**Principal Investigator Name:** Amy Kohtz, PhD
**Protocol Number:** 2022-1244
**Protocol Approval Date:** 01/05/2023
**Protocol Title:** Developmental Exposure to Organophosphates on Adult Behavior in the Rat

**NAME OF INSTITUTION B: Liberty University (LU)**

**USDA Registration Number**: 52-R-0137
**OLAW Assurance Number:** D20-01061
**AAALAC Accreditation Date**: N/A
**AAALAC Accreditation Status**: N/A
**IACUC Administrative Contact:** Connor Bryant, iacuc@liberty.edu, 434-582-2827

**Student Name**: Melanie Berry
**Student Status**: Doctoral Student
**Expected Graduation Date**: 12/2024
**Project Title**: Inhibiting Repetitive and Restricted Behaviors in Sprague-Dawley Rats Using Propranolol: A Look At Sex Differences

| INSTITUTION A | | Indicate affirmative agreement or N/A to each of the terms below | INSTITUTION B | |
|---|---|---|---|---|
| Yes | N/A | | Yes | N/A |
| **Program-wide Acknowledgements** | | | | |
| X | | The parties acknowledge that each institution maintains an independent program of animal care and use qualified to perform animal care and use activities in compliance with all applicable federal and state animal welfare laws, regulations and policy. **Both institutions agree to notify the other party promptly of any changes in such status.** | X | |
| X | | The parties agree to share any information necessary to comply with regulatory requirements related to program procedures or activities associated with this MOU, upon request. | X | |
| **Ownership & Oversight** | | | | |

| | | | | |
|---|---|---|---|---|
| X | | The parties acknowledge that ownership of animals related to this MOU resides with INSTITUTION A. No transfer of ownership is implied unless otherwise described in another agreement between the parties. | X | |
| X | | The parties acknowledge that each institution is responsible for appropriate care and oversight of animals while in their possession, and the provision of appropriate husbandry, peri-procedural care, pain management, and methods of disposition. | X | |
| X | | All animal activities related to this MOU will be reviewed and approved by INSTITUTION A's IACUC prior to the initiation of those activities. | X | |
| X | | The parties acknowledge that each has a process for monitoring on-going animal related activities on their property, including established procedures for identifying and reporting potential adverse events and any non-compliance associated with animal care and use at its facility. | X | |
| **Investigation and Reporting** | | | | |
| X | | The parties agree that INSTITUTION A is responsible for notification, investigation, and reporting of serious or continuing incidents of noncompliance and protocol suspension to OLAW, the accrediting body, or regulatory agency (*e.g.*, AAALAC, USDA) in accordance with reporting requirements. Copies of these reports and any responses received are forwarded to the other INSTITUTION. | X | |
| X | | INSTITUTION A agrees to promptly notify INSTITUTION B, within 30 days of identification, any potentially reportable events, specific to the protocol related to this MOU and/or any significant programmatic deficiencies occurring during the conduct of the activities associated with this MOU that directly impact animal welfare or well-being. | X | |
| **Other Requirements Not Addressed Above** | | | | |
| | | | | |

**Signatures of Animal Program Representatives**
**(IACUC Chair, AV, IACUC Administrator, or IO)**

This MOU becomes effective upon the date of last signature, and will remain in effect for three (3) years or for the duration of dissertation project related to this MOU unless sooner terminated by either party on notice to the other. Either party may terminate this MOU without cause upon sixty (60) days written notice.

[redacted]

**Date:** 10/27/2023
**Name:** Amanda Kinslow
**Title:** Director, Office of Animal Welfare
**E-Mail:** amurray@umc.edu/iacuc@umc.edu
**Phone:** 601-815-5006

[redacted]

**Date:** 11/3/2023
**Name:** Connor Bryant
**Title:** Admin. Chair for Animal Research
**E-Mail:** cabryant@liberty.edu / iacuc@liberty.edu
**Phone:** 434-582-2827

# APPENDIX B: MARBLE BURYING PROTOCOL

Marble Burying Protocol

**Summary and Purpose.** Provide accurate and sensitive assays of repetitive and compulsive-like behaviors in rodents. Future applications should extend these tests to the screening of new treatments for human conditions such as OCD and ASDs.

**Apparatus and Location**

The marble burying test will be performed using CCR standard rat cages with fitted filtered-top covers. The testing will be performed in room TR09-06 using rat cages with fresh unscented bedding that is 5 cm deep. The rats will be moved to the testing room 60 minutes before the start of testing. The testing will be performed in white light between 10 and 11 am. A university computer with a webcam attachment per IACUC standards be used to film the testing sessions. An IACUC approved camera will be attached to the rat's cages to record the testing process. Standard glass toy marbles (assorted styles and colors, 15 mm in diameter) will be gently placed on the surface of the bedding in 5 rows of 4 marbles. One rat will be placed into a cage as far from the marbles as possible and the filtered-top will be put on. Food and water will be withheld during the testing time. The rat will remain undisturbed for 15 minutes. After 15 minutes, the rat will be removed and returned to her home cage taking extreme care to not move or dislodge the marbles. A photograph is taken of the cage with the marbles buried.

**Data Analysis and Recording**

A marble will be scored as buried if two-thirds of its surface area is covered by bedding. An average score of the number of marbles buried for each rat will be recorded. The number of marbles 1/3 buried, 2/3 buried, and fully buried are recorded. The video is scored for the total time spent burying and total time spent grooming.

**All video files and images of the buried marbles will be labelled with the rat identifier and date. Eg. MBB-23-F01 08302023 and placed in a folder for the particular experiment on the Kohtzlab drive.

# APPENDIX C: RAW DATA TEMPLATE

| | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
| 1 | **CPF-23-FX VEHICLE** | | | A1 | | | | | |
| 2 | MARBLE BURYING | | | | | | | | |
| 3 | | burying percentage | burying average | burying total | TOTAL TIME BURYING | grooming percentage | grooming average | grooming total | TOTAL TIME GROOMING |
| 4 | Vehicle | | | | | | | | |
| 5 | | # | # | # | # | # | # | # | # |
| 6 | Propranolol I.P. injection-5mg/kg @ .weight | | | | | | | | |
| 7 | | # | # | # | # | # | # | # | # |
| 8 | Propranolol I.P. injection-10mg/kg @ .weight | | | | | | | | |
| 9 | | # | # | # | # | # | # | # | # |
| 10 | Propranolol I.P. injection-20mg/kg @ .weight | | | | | | | | |
| 11 | | # | # | # | # | # | # | # | # |
| 12 | | | | | | | | | |
| 13 | | | | | | | | | |