ACUTE OXYTOCIN ADMINISTRATION AVOID ANXIETY LIKE BEHAVIOR IN MALE AND FEMALE MICE

by
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A thesis proposal presented to the Honors College
of Middle Tennessee State University in partial fulfillment
of the requirements for graduation from the University Honors College

Spring 2023

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ACKNOWLEDGMENTS

Completing a research project is a team effort, and I would like to express my gratitude to all those who have contributed to the successful completion of this project. Firstly, I would like to extend my sincere thanks to my research mentor, Dr. Rogers, for being an outstanding mentor throughout this project. Your expertise, guidance, and support have been invaluable to the success of this project. Your patience, constructive feedback, and encouragement have challenged me to grow as a researcher and have helped me develop skills that will benefit me in my future endeavors.

I would also like to express my gratitude to the lab group for their support and collaboration throughout this project. Thank you to fellow graduate student, Isabela Ramos, and to the following undergraduate students: Alex Ketel, Britney Donovan, Jade Murray, Steven Brown, and Jacob Grones. All of your help completing drug dosing schedules, running behavioral tests, and coding behavior made the completion of this project possible. I feel privileged to have been a part of such a talented and supportive team.

Furthermore, I would like to thank my family and my best friends for their unwavering love and support. Your encouragement and belief in me have been a constant source of inspiration and motivation. Your patience and understanding have enabled me to pursue my passion for research, and I am grateful for the sacrifices you have made to help me achieve my goals. Your faith in me has given me the confidence to pursue my dreams, and I am forever grateful for your unwavering support.

ABSTRACT

Oxytocin is a neurotransmitter and hormone with a well-established role in prosocial behaviors in animals and humans. It is currently being tested in clinical trials for the treatment of social symptoms associated with autism spectrum disorders. However, the behavioral effects of oxytocin treatment in humans have been variable with both prosocial (increased empathy) and antisocial (increased competitiveness) behaviors. Previous studies in our lab have shown increased anxiety-like behaviors in mice treated chronically with oxytocin (12 ug dose per day for 14 consecutive days, data unpublished). The current study aims to see the effect of acute oxytocin administration on anxiety and social behavior in male and female mice to determine if the schedule of oxytocin administration affects behavioral outcomes. Adult C57BL/6J mice will be acutely pretreated with saline or oxytocin (12 μg) via intranasal (i.n.) or intraperitoneal (i.p.) administration an hour before the behavior tests. Mice completed a battery of behavioral tests including the elevated plus maze (EPM), three-chamber sociability task (3C), and free dyadic social interaction (FDSI) after drug administration to determine changes in social behavior and anxiety-like behavior. Human coders coded anxiety-like behaviors, social preference, and social novelty. With acute oxytocin administration, sociability increase as measured by the 3C and FDSI tasks while avoiding increases in anxiety-like behaviors, as measured by the EPM task, associated with chronic administration. Understanding the effects of acute oxytocin administration on anxiety in mice might lead to the development of new treatments for anxiety disorders in humans.

TABLE OF CONTENTS

ABSTRACT……ii

LIST OF TABLES……...v

LIST OF FIGURES……...vii

CHAPTER I: INTRODUCTION……1

Oxytocin……...…1

Animal Models of Social Behavior…….….2

Purpose of the Present Study……...5

CHAPTER II: THESIS STATEMENT……...8

CHAPTER III: METHODS……...10

Animals……..…10

Housing……..10

Sample Size……10

Animal Handling……10

Oxytocin Administration……...11

Behavioral Tests…….11

Data Coding……...13

CHAPTER IV: RESULTS……15

Elevated Plus Maze……15

Three Chamber Sociability Task……...18

Free Dyadic Social Interaction Task……..20

CHAPTER V: DISCUSSION……23

REFERENCES……..28

APPENDICES……...37

Appendix A: Animal handling procedure……...38

Appendix B: Elevated Plus Maze……39

Appendix C: Three-Chamber Social task……...40

Appendix D: Free Dyadic Social Interaction (FDSI) task……..41

Appendix E: IACUC protocol approval……42

LIST OF TABLES

Table 1. Experimental groups of Subject Mice……12

Table 2. EPM all group descriptive…….16

Table 3. Animal handling procedure……...38

LIST OF FIGURES

Figure 1. Time spent in open arms vs closed arms Elevated Plus Maze graph……....17

Figure 2. Sex or drug differences in Elevated Plus Maze graph.……..17

Figure 3. Three-Chamber phase two task graphs……..19

Figure 4. Three-Chamber phase three task graphs……....19

Figure 5. Free Dyadic Social Interaction (FDSI) data……..21

Figure 6. Elevated Plus Maze task……39

Figure 7. Three-Chamber Social task……...40

Figure 8. Free Dyadic Social Interaction test……41

Figure 9. IACUC protocol approval…….42

CHAPTER I
INTRODUCTION

Social interactions significantly influence human behavior, learning, and development throughout the lifespan (Ricceri et al., 2007). When social interactions are impaired by a lack of social skill, serious behavioral consequences emerge. A common characteristic of many neurodevelopmental disorders, such as autism spectrum disorder (ASD), is impaired social behavior. According to the American Psychiatric Association (APA, 2016), ASD is a spectrum of neurodevelopmental disorders that are characterized by deficiencies in social behavior and communication as well as constrained interests and repetitive activities. Recent research indicates sex differences in ASD such that males are greater than three times more likely to have ASD than females (Halladay et al., 2015). Clinical data show that some medicines (such as atypical antipsychotics and selective serotonin reuptake inhibitors) may reduce repetitive behavior in ASDs, but these treatments have not shown to be beneficial for social deficits and have been linked to serious severe side effects (Carrasco et al., 2012; McDougle et al., 2005; McPheeters et al., 2011; Stachnik & Nunn-Thompson, 2007).

Oxytocin

A promising new therapy for neurodevelopmental problems has been proposed: oxytocin, a hormone, and neurotransmitter classically associated with prosocial behavior (Bartz & Hollander, 2008; Ring et al., 2006; Zhang et al., 2015). However, the outcomes in human trials have been conflicting (Tachibana et al., 2013; Neumann & Slattery, 2016). Studies have revealed that administering oxytocin has little therapeutic impact and

may even cause undesirable social behaviors (Shamay-Tsoory & Abu-Akel, 2016; Tachibana et al., 2013). In mammals, the neuropeptide oxytocin (OT) causes uterine contractions during childbirth and milk ejection during lactation (Gainer et al., 2001; Neumann et al., 2000). Oxytocin is mostly recognized for its role in maternal responses, but significant evidence shows that oxytocin promotes the development of social relationships, sexual behavior, and parental skills (Insel, 2010). When oxytocin is released within the brain, it plays the role of neuromodulators. From a variety of locations on the neuronal membrane, oxytocin gets released and has the capacity to aim for distance targets such as the striatum, hypothalamus, hippocampus, amygdala, striatum, and mid- and hindbrain nuclei (Bartz & Hollander, 2008). The administration of oxytocin controls social recognition in both mice and humans (Choleris et al., 2009; Kemp & Guastella, 2011). Oxytocin has been demonstrated to have similar antidepressant effects in animals (Arletti & Bertolini, 1987). It has shown results to make mice more approachable and sociable (Lim et al, 2005; Sala et al, 2011; Winslow and Insel, 2002).

Animal Models of Social Behavior

Rodent models are commonly-used models for examining the neural substrates of social behavior and behavioral effects of pharmacological substances. Many animals have extensive social networks that influence many aspects of their behaviors, from parenting to social hierarchy, making them useful models for studying social behaviors (Ardesch et al., 2019; Dennis et al., 2020). Mammal social groups have a great degree of complexity in both the variety and quantity of social interactions (Choleris et al., 2009).

Humans and animals learn a wide range of social behaviors throughout development that enable adaptive functioning aimed at survival and reproduction in maturity. The majority of mammalian species, including humans, exhibit social play behaviors. Social play is most prevalent between weaning and puberty (Vanderschuren et al., 2016). Therefore, in rodents, the period of most social play refers to the juvenile phase through mid-adolescence, which corresponds to childhood through early/mid adolescence in humans (Vanderschuren et al., 2016). Social play behavior includes components of aggressive, predatory, and sexual behavior that are adjusted or exaggerated (Pellis & Pellis, 2009). These playful behaviors are anticipated by obvious physical, facial, or verbal cues indicating that the behavior's goal is playful. As a result, social play behavior is often straightforward to identify and quantify. This is especially true for several rodent species, including the rat.

Cooperative behavior, a complex executive function, needs a variety of social and cognitive abilities from individuals, as well as continuous monitoring of ongoing social connections (Tomasello & Vaish, 2013). Rats are sociable creatures with abilities such as recognition, communication, and cognitive flexibility (Schuster & Perelberg, 2004). Mice and rats have quite different social behaviors, with rats having a larger and more complicated repertoire of social behaviors (Jabarin et al., 2022), being less aggressive, and being better rewarded by social interactions (Vanderschuren et al., 2016). Due to the larger genetic toolbox available for mice, which allows the generation of mouse models with genetic alterations mimicking those found in humans, the majority of ongoing research that considers hypotheses regarding the etiology and underlying mechanisms of NDDs is being conducted on mice (Jabarin et al., 2022).

As animals give researchers the ability to regulate the environment, optimize genetic identicality, and use invasive procedures, they encourage strong internal validity for experimental research designs. Due to their morphological, physiological, and genetic closeness to humans, mice have specifically been used as the best species for animal models in scientific research. Around 95% of the 30,000 genes present in both mice and humans are shared by the two species (Bryda, 2013). Research using animal models for the study of the brain and behavior is significantly aided by the fact that mice and humans have brains that have been evolutionarily preserved, demonstrating similarity in brain structure and connection (Semple, 2013). Animal studies have shown links between oxytocin's function in anxiety and social behaviors; for example, when stressed, oxytocin makes rats approach and stay near to other rodents they know (Engelmann et al, 1996). Invasive techniques are needed to examine neurochemical activity, which might affect brain function worldwide and raise the risk of injury to clinical populations. Hence, employing animal models to study psychopharmacological dynamics in fundamental research is suitable.

Many studies have been conducted to demonstrate the sophisticated social behaviors of mice, who are intelligent, friendly, and curious creatures. They naturally like to occupy areas, establish groups, and then develop intricate social structures, including dominance hierarchies, inside these groupings (Williamson, 2016). In mice, social behavior is still complicated, and strain and species differences in behavior naturally occur, allowing for the specialization of models for certain study concerns. For instance, the field is able to examine distinctive parenting and mating behaviors at the genetic level

because prairie voles display monogamous mating, which is connected to the expression of the RS3 334 gene (Ophir et al., 2008).

Mice also exhibit particular maternal and paternal behaviors that are frequently crucial for their offspring's survival and development. The brain network that organizes parental behavior has been studied, and the social behavior variations between parental mice have been demonstrated (Kohl, 2018). As they are influenced by particular socio-ecological selection forces that operated during evolution, sex differences are not identical across species. For instance, female laboratory rats and mice do better in numerous tests than males in terms of activity and anxiety, although this sex difference fluctuates depending on the situation, strain, and age (Palanza & Parmigiani, 2017). Females are more prone to nervousness than males, yet mice models of anxiety reveal inconsistent findings across the sexes. Female also react faster to painful heat stimuli (Reddan et al., 2020).

Purpose of the Present Study

The present study focuses on the effects social behavior in mice following either intranasal (i.n) or intraperitoneal (i.p.) oxytocin administration. Importantly, different routes of administration are used between clinical trials examining oxytocin's therapeutic benefit in ASD and the typical pharmacological experiments using mouse models. This has caused a dispute within the neuroscience field of which route of administration is best in mouse-modeling that shows the greater effects in administration of oxytocin. Intraperitoneal injection is technically straightforward and easy and the most popular option for systemic administration in mice (Bowen & McGregor, 2014; Teng et al.,

2013). In human clinic trials, the intranasal route of administration is typically used, as oxytocin (a relatively large neuropeptide) does not easily cross the blood-brain barrier (Yao & Kendrick, 2022).

Intranasal route of administration is thought to have higher passage across the blood-brain barrier, and it has therefore been used in oxytocin clinical trials to improve the bioavailability of oxytocin in the brain (Yao & Kendrick, 2022). Studies have demonstrated that both i.n. and i.p. administration crosses the blood-brain barrier in mice, although at different levels, such that higher oxytocin levels are noted in the brain following i.n. administration (Mittal et al., 2014). However, what remains to be demonstrated is whether the difference in absorption rates across the two routes of administration are biologically relevant enough to affect behavior. The primary goal of the current research was to determine differences in social behavior following i.n. or i.p. oxytocin administration.

Male and female mice were given acute i.p. and i.n. injection of a standardized of oxytocin to examine the effects of the method of administration on social behavior. By using both male and female mice, we were able to examine any sex differences in social behavior following oxytocin administration. Prior to measuring social behavior, mice were examined for anxiety-like behaviors by using the elevated plus maze (EPM). EPM is one of the assessment techniques used as a standard test of anxiety-like behavior in mice (Szelenyi et al., 2021). The effects of oxytocin administered either i.n. or i.p. on sociability and desire for social novelty were assessed using the three-chamber sociability test (3C) and the free dyadic social interaction task (FDSI). The 3C is a standard test for examining a mouse's desire to interact with another mouse (a social stimulus) by

measuring time spent sniffing. However, in the 3C, the social stimulus mouse is restrained and unable to freely interact naturally with the experimental mouse. In the FDSI task, social approach and social avoidance are measured. Experimental and stimulus mice are able to interact freely with each other in a more natural, reciprocal social interaction. We aimed to identified differences in anxiety-like behaviors, sociability, preference for social novelty, social approach, and social avoidance following either i.n. or i.p. oxytocin administration in male and female mice. A factorial design was utilized with route of administration (i.n. or i.p.), drug (oxytocin or saline control), and sex (male or female) as the three independent variables.

CHAPTER II
THESIS STATEMENT

In Dr. Tiffany Rogers' lab earlier, at Middle Tennessee State University, we looked at the effects of chronic oxytocin treatment (one dose per day for 14 days, Social and Affective Neuroscience (SAN) Lab) delivered either i.n. or i.p. on anxiety in mice. One of the unusual findings that we saw in that data set was that oxytocin, typically related to higher sociability and lower anxiety, was producing higher anxiety in mice compared to saline administration. We were surprised by the result and started to wonder whether this effect is really dependent on oxytocin absorption rates or whether handling differences between the two routes of administration could have affected anxiety-like behaviors.

The goal of this study was to examine whether, the anxiety level in mice change with the change of oxytocin schedule. We change the schedule that the mice getting the oxytocin to acute (one dose an hour before the behavior task), if it is just oxytocin, we would see the same result as the previous study which was increase anxiety in oxytocin group. But if there is something specific about oxytocin with this chronic handling that's a special combination, then we should see different results from the chronic group.
Our team addressed the following research questions:

1. Will increases in anxiety following oxytocin administration be avoided by acute oxytocin administration?
2. Will acute administration of oxytocin still have social behavior effects as in chronic dosing?

I hypothesize that acute oxytocin administration will increase sociability while avoiding anxiety increases associated with chronic administration. However, a smaller effect size with a single dose of oxytocin is expected. Also expected that, as found with previous chronic administration, females would exhibit more anxiety and less modulation of social behavior, and that intranasal administration will produce fewer anxiety behaviors than injections.

CHAPTER III
METHODS

Animals

At the age of nine weeks, sixty-eight C57BL/6J mice (#000664; M = 20, F = 19) mouse obtained from Jackson Laboratory, Nashville, TN. All the mice were between 10 and 14 weeks old when the experiment began. The number of mice to be employed in this investigation, according to *a priori* power assessments, was the minimum required to produce an appropriate projected effect size of 0.60 (see Erdfelder et al., 2005).

Housing

Two to five mice were kept in each home cages, with sex-matched littermates. A 12:12 hour light/dark cycle was used to regulate the environment, which was kept at a constant 22°C with an average humidity level of 55%. Humidity level were controlled by using of humidifier in the winter and dehumidifier in the summer. Food and water were always available. Mice bedding and litter were used in the cages for the mice's comfort and changed every week.

Sample Size

There were 20 male mice, and 19 female mice used in this trial.

Animal Handling

As it is well established that the handling of mice in the research (e.g., picking up the animal) can cause low levels of distress in mice, a procedure was used to habituate

the animals to human handling. The purpose of the handling practice was to gradually up the intensity of the holding method for the drug administration. To adapt individuals to the appropriate drug delivery handling practices, a ten-day handling training (as shown in Table 1) was performed.

Oxytocin Administration

Route of administration (intranasal/i.n. or intraperitoneal injection/i.p.) was randomly assigned by home cage, such that each mouse in a cage received the same route of administration. An hour before measuring each behavior, the mice were put in the behavior room and mice were administered either oxytocin, or saline, the control drug, by either i.p. or i.n. administration. The resulting groups are shown in Table 2. Oxytocin was administered in doses of 12 μg in 12 ml per intravenous administration and 12 μg in 120 ml per intramuscular injection, with identical volumes of saline administered for control comparisons.

Behavioral Tests

Three behavioral tests were used to measure the behavioral effects, of acute oxytocin administration. The elevated plus maze (EPM) measured anxiety. The three-chamber sociability task (3C) measured sociability and preference for social novelty, and the free dyadic social interaction (FDSI) test measured social approach and avoidance.

Table 1
Experimental groups of test mice

Group	Sex	Housing	Drug
1	Male	Group	Saline
2	Female	Group	Oxytocin
3	Male	Group	Oxytocin
4	Female	Group	Saline
5	Male	Group	Saline
6	Female	Group	Saline
7	Male	Group	Oxytocin
8	Female	Group	Oxytocin
9	Male	Group	Saline
10	Female	Group	Saline

One task was performed each day across a three-day period. First, mice were tested on the EPM task, which is a commonly used test to identify anxiety-like mouse behavior. The test is performed in a raised cross-shaped device with four arms (two closed arms and two open arms, see Figure 1). Each test mouse had 10 minutes to investigate the four arms of the maze. As mice naturally prefer closed spaces for safety and yet have a natural propensity to explore novel areas, the EPM tests whether mice's the desire to explore will win out over the desire to stay safe. Increased time in the walled arms is a common indication of higher anxiety time (Yang et al., 2011). The behavior of

each mouse in the EPM was recorded for the 10-minute testing period, and time spent in the closed and open arms of the EPM were recorded.

On the second day of behavioral testing, sociability and preference for social novelty were tested by the 3C task. A three-chambered box with passages between each chamber was used for this task, allowing the mouse to pass between each chamber (left, center, and right). The arena is 24" x 12" in size, with each chamber measuring 8". The task is divided into three ten-minute phases. In first stage, no social indicators (mice) were available; instead, two empty wire pencil cups were there on each side of the chamber. The second stage of the test served as a time to evaluate the subject mouse's friendliness. As a social stimulus, a trained conspecific mouse (a same-sex, same-strain, weight-matched mouse) was placed under one of the wired pencil cups. Because mice are highly social, it is expected that they will explore the chamber with the social stimulus more than the empty chamber. The third stage of the experiment served as a time to analyze the subject mouse's propensity for social novelty. A second conspecific mouse was introduced to the previously empty chamber under a wire pencil cup, giving the subject mouse the option of choosing between the mouse from phase two or the novel mouse just introduced. Because mice have a general preference for novelty, typical mice are expected to seek out the novel social stimulus rather than the familiar one (Yang et al., 2011).

The FDSI task involves the monitoring of social behavior and anxiety in a freely explorable setting (see Figure 4). A novel, sex, age, and weight matched stimulus mouse was put in a 12"x12" white, acrylic, open field apparatus with each test mouse. Ten minutes of behavioral interaction were captured on tape. We analyze the frequency with

which the experimental mouse turned towards the stimulus mouse was defined as social approach, while the frequency with which the test mice turned away from the stimulus mouse was classified as social avoidance.

A Hero Silver 7 GoPro was used to capture behavioral data at 30 and 60 frames per second (see Yang et al., 2011).

Data Coding

All the behavior tasks were recorded, and data were code by hand coding. Coders evaluate and record data such as mouse spending time in the open/closed arm, time spend in each chamber, and the amount of time spent sniffing each stimulus mouse. For FDSI, coders code the frequency of avoidance and approach for test mice to the stimulus mice. Finally, data analysis was conducted through SPSS.

CHAPTER IV
RESULTS

Elevated Plus Maze

Table 2 displays descriptive statistics for all groups. For all analyses, a familywise alpha of 0.05 was employed. The GLM technique in SAS Studio (version 3.80) was used to examine the effects of medication (saline and OT), mode of administration (i.n. and i.p.), and sex (male and female) on the following dependent variables: (1) total number of arm crossings and (2) time spent investigating the open arms.

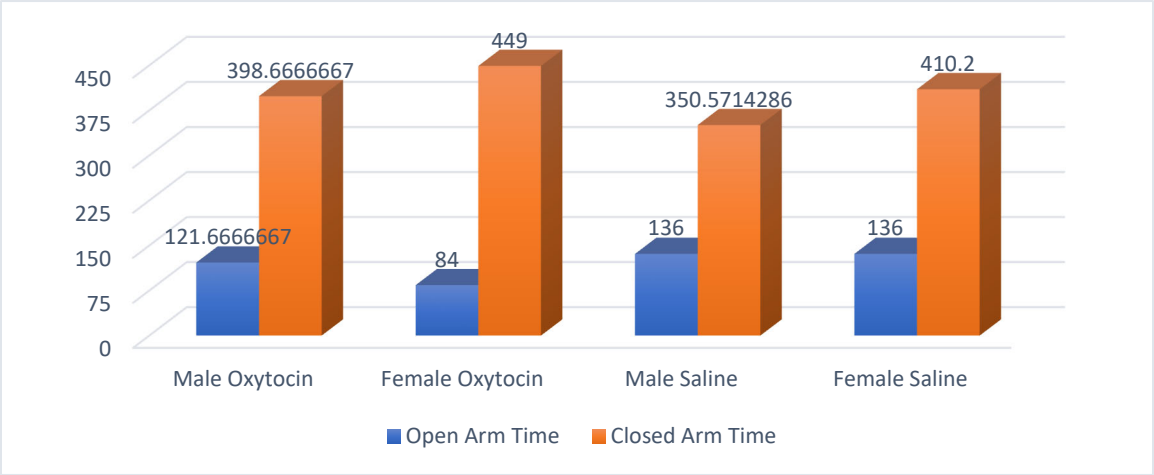
The results of the factorial ANOVA conducted on the open arm time data from the Elevated Plus Maze show that there were no main effects for sex, drug, or route (F (1, 27) = 1.615, p = 0.22), drug (F (1, 27) = 0.433, p = 0.52), or route (F (1, 27) = 2.641, p = 0.12). There were also no significant two-way interaction effects between sex and drug, sex and route, or drug and route (F (1, 27) = 0.65, p = 0.43); sex by route (F (1, 27) = 0.08, p = 0.78); drug by route (F (1, 27) = 0.274, p = 0.61). However, there was a significant three-way interaction effect (F (1, 27) = 7.009, p = 0.01, partial eta squared which informs us the magnitude of the influence of the independent variable(s) on the dependent variable = 0.21), indicating that the relationship between the three factors (sex, drug, and route) was not additive. Pairwise comparisons were used to analyze this three-way interaction, and the results indicated that the majority of groups were not different. However, there were significant differences between male/saline/IN and female/saline/IN (p = 0.03), and two comparisons that were near significance: male/OT/IN and male/OT/IP (p = 0.08) and female/sal/IN and female/sal/IP (p = 0.08). The descriptive statistics for each group showed that the mean open-arm time was highest for male/OT/IP

(177), followed by male/sal/IN (141.4), male/sal/IP (122.5), female/sal/IP (181.8), female/sal/IN (59.8), male/O/IP (94), female/O/IN (100.75), and female/O/IP (70.6). These results suggest that the three factors (sex, drug, and route) interacted in a complex way to influence open arm time in the Elevated Plus Maze task. Further research may be needed to elucidate the nature of these interactions.

Table 2
All group descriptive

Sex	Drug	Route	Mean	SEM (sec)
m	O	IN	94	27.36
m	O	IP	177	38.7
m	S	IN	141.4	29.97
m	S	IP	122.5	47.39
f	O	IN	100.75	33.51
f	O	IP	70.6	29.97
f	S	IN	59.8	29.97
f	S	IP	181.8	29.97

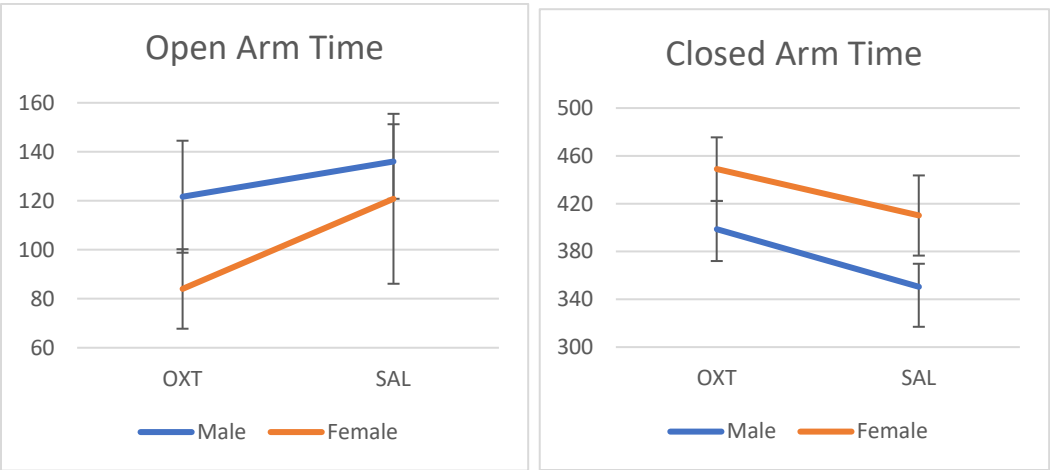
Figure 1
Time spent in open arms vs closed arms Elevated Plus Maze graph



Graph depicting time in seconds of open arm and closed arm exploration during the 10-minute elevated plus maze. Each group spent significantly more time in the closed arms as compared to the open arms.

Figure 2
Sex or drug differences in Elevated Plus Maze (EPM)

A **B**



A. Graph depicting time in seconds of open arm and closed arm exploration during the 10-minute elevated plus maze. No significant differences for sex or drug were found. **B.** Graph depicting time in seconds of open arm and closed arm exploration during the 10-minute elevated plus maze. No significant differences for sex or drug were found.

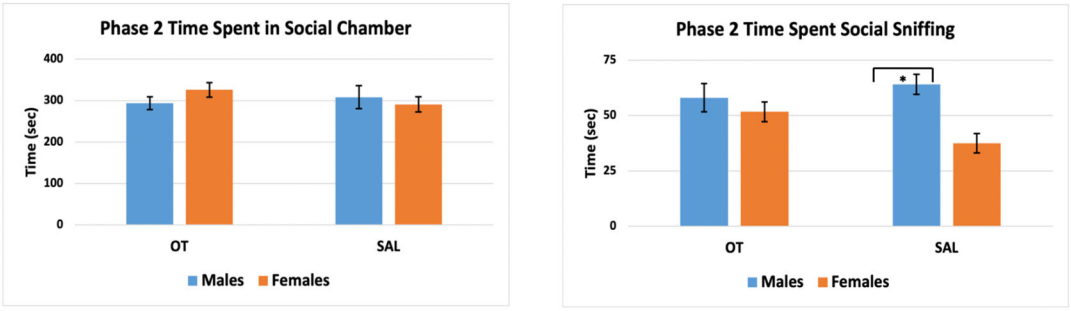
Three-Chamber Sociability Test

Figures 3 and 4 exhibit descriptive statistics for chamber exploration time and social sniffing time, respectively. Four factorial ANOVAs were used to compare time spent in the social/novel stimulus chamber and time spent sniffing the social/novel stimulus in phases 2 and 3 with independent variables of drug (saline, OT), and sex (female, male).

The results of the factorial ANOVA for time in social chamber during phase 2 indicated that there were no significant main effects of drug (F (1, 29) = 0.219, $p = 0.64$), and sex (F (1, 29) = 0.114, $p = 0.74$), and no significant interaction (F (1, 29) = 1.28, $p = 0.27$). The factorial ANOVA for time spent sniffing the social during phase 2, indicated no significant main effect of drug (F (1, 29) = 0.367, $p = 0.55$), but a significant main effect of sex (F (1, 29) = 5.995, p = 0.02), and no significant interaction (F (1, 29) = 2.278, $p = 0.142$). For time spent in the novel chamber during phase 3, there were no significant main effects of drug (F (1, 29) = 1.147, $p = 0.29$), and sex (F (1, 29) = 2.045, $p = 0.16$), and no significant interaction (F (1, 29) = 2.628, $p = 0.12$). Finally, the results of the factorial ANOVA for time spent sniffing the novel social stimulus during Phase 3 indicated that there was no significant main effect of drug (F (1, 29) = 0.056, $p = 0.81$), but a significant main effect of sex (F (1, 29) = 6.12, $p = 0.019$), and no significant interaction (F (1, 29) = 1.127, $p = 0.30$). Importantly, despite having no significant interactions, pairwise comparisons of sniffing time during phase 3 indicated that while males did not demonstrate drug effect, females did show differences between saline and oxytocin groups. This suggests that oxytocin is having a subtle effect that may be sex-specific during phase 3.

Figure 3
Three-Chamber phase two data

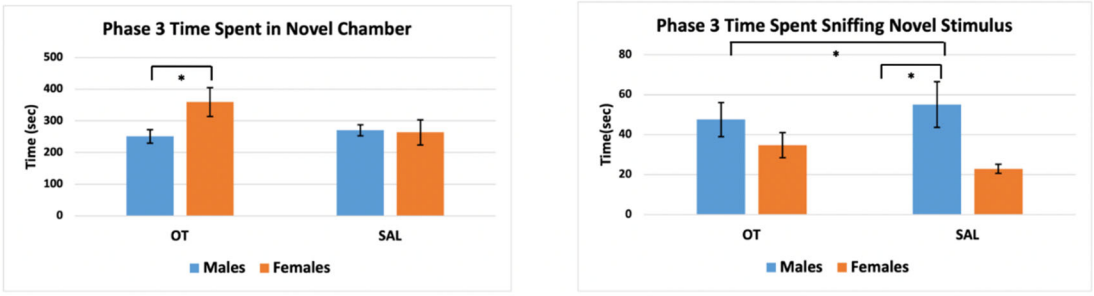
A **B**



A. Graph depicting time spent in the chamber containing the social stimulus in phase 2 of the three-chamber sociability task. No significant differences. **B.** Graph depicting time spent sniffing the social stimulus in phase 2 of the three-chamber sociability task. Males and Females differed within the Saline administration groups ($p < 0.05$).

Figure 4
Three-Chamber phase three data

A **B**



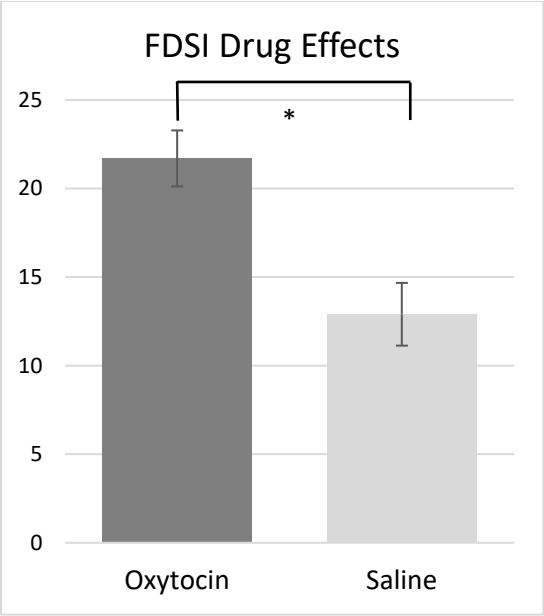
A. Graph depicting time spent in the chamber containing the novel social stimulus in phase 3 of the three-chamber sociability task. Males and females differed within the oxytocin administration groups ($p < 0.05$). **B.** Graph depicting time spent sniffing the novel social stimulus in phase 3 of the three-chamber sociability task. Males and females differed within the saline administration groups ($p < 0.05$), and oxytocin and saline groups differed within the female mice ($p < 0.05$).

Free Dyadic Social Interaction (FDSI)

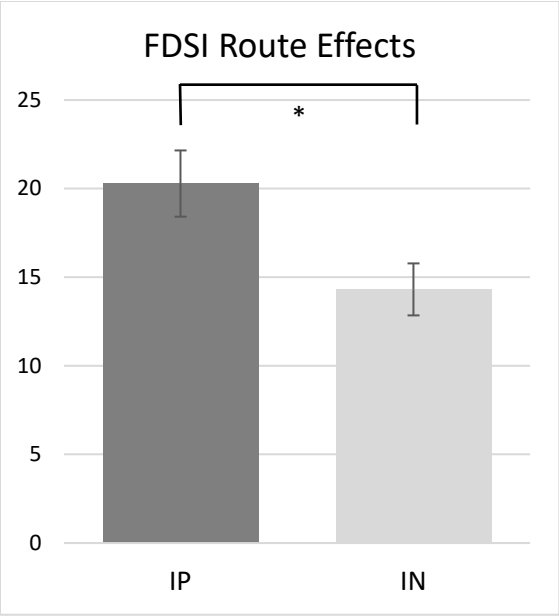
The ANOVA results revealed significant main effects for drug ($F(1, 24) = 13.68$, $p = 0.001$), route ($F(1, 24) = 6.31$, $p = 0.019$), and sex ($F(1, 24) = 7.41$, $p = 0.012$), indicating that oxytocin and IP administration, as well as being male, increased social approach behavior. Furthermore, there was a significant drug-sex interaction ($F(1, 24) = 10.96$, $p = 0.003$), indicating that the influence of oxytocin on social approach behavior varied between men and females, with larger oxytocin-induced behavioral changes occurring in males (male/saline: $M = 12.20$, $SEM = 2.67$, male/oxytocin: $M = 28.87$, $SEM = 2.33$) compared to females (female/saline: $M = 13.60$, $SEM = 2.33$; female/oxytocin: $M = 14.53$, $SEM = 2.14$).

Figure 5
Free Dyadic Social Interaction (FDSI) data

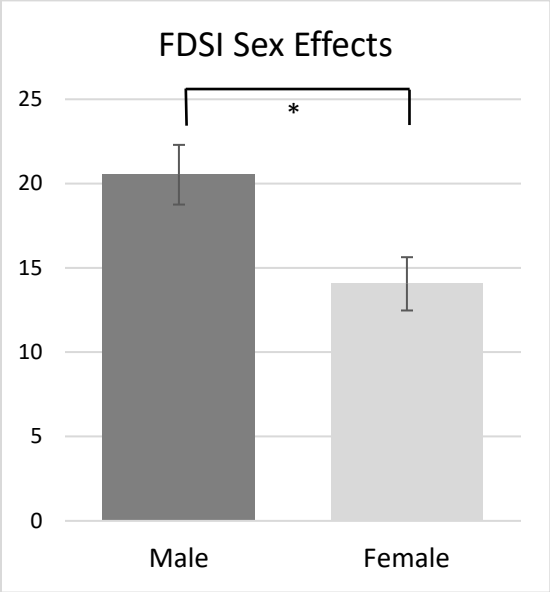
A



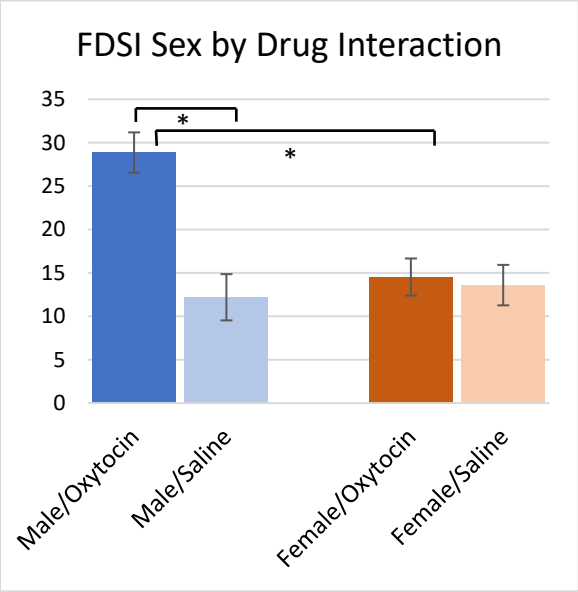
B



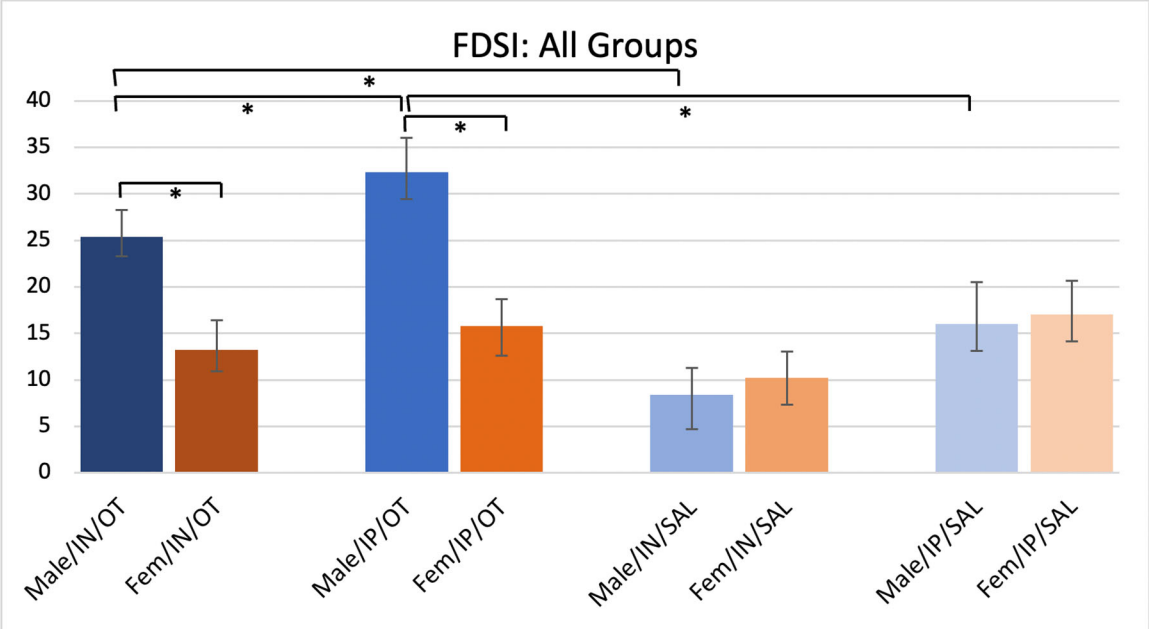
C



D



E



A. Graph depicting difference between saline and oxytocin group for FDSI. There is a significate difference between these two groups ($p = 0.001$) with increased social approach following oxytocin administration compared to saline. **B.** Graph depicting significant difference between i.p. and i.n. administration for FDSI. There is a significant difference between these two groups ($p = 0.019$) with more social approach following i.p. compared to i.n. **C**. Graph depicting sex difference in social approach. There is a significate difference between male and female ($p = 0.012$) with male mice showing more social approach than female. **D.** Graph depicting the significant interaction between sex and drug ($p = 0.003$) with oxytocin significantly increasing social approach in males but not in females. **E.** Graph depicting social approach frequency for each experimental group and pairwise comparisons. While males and females did not differ in saline groups, males were significantly higher in social approach in all oxytocin groups compared to females ($p< 0.05$). Within males, i.p. oxytocin injections produced significantly more social approach than i.n. administration of social approach ($p < 0.05$), and oxytocin elicited more social approach compared to saline given by the same route of administration ($p < 0.05$).

CHAPTER V
DISCUSSION

The purpose of this study was to determine if anxiety-like behavior found during chronic administration of oxytocin would be replicated with the acute administration. This study had three aims. Because there are known expression variations in oxytocin receptors between the sexes, we aimed to assess sex differences following acute administration of oxytocin between male and female mice by including trials with female mice (Bredewold & Veenema, 2018). The second goal was to pharmacologically influence the mouse's social behavior, demonstrating that acute oxytocin will still have an effect even if less pronounced than chronic administration. The final aim was to determine behavioral differences following i.n and i.p administration of oxytocin to determine if the handling methods of each route may also influence social and/or anxiety-like behaviors.

The use of mice in this study provides an important model for understanding the effects of oxytocin on social behaviors. Prior research has proven that mice are a viable model for studying the effects of various therapies on social behaviors (Bryda, 2013), and the findings of this study back up that assumption. The EPM and 3C tests, in particular, were frequently employed in the literature to measure anxiety and social behaviors in mice. Additionally, the use of mice as a model for investigating oxytocin's effects on social behaviors has major translational importance, as the same brain circuits that govern social behaviors in mice are also present in people (Ko, 2017). Such similarities indicate that the findings of the present study can be applicable to human populations, although further research is needed to assess the degree of the translational applicability of these

findings. Overall, the study's use of mice provides a great model for investigating the effects of oxytocin on social behaviors and has substantial translational value for human populations.

In mice, both intranasal (i.n.) and intraperitoneal (i.p.) injection of oxytocin (OT) had relatively little impacts on social behaviors. Given that we employed a single acute dosage of OT, these findings were predicted. It is crucial to note, however, that the effects of OT on social behaviors may vary depending on the dose regimen employed. Some studies, for example, revealed greater effects of OT on social conduct after chronic administration (e.g., daily for many days), but others found no changes between acute and chronic administration. Numerous studies have found that persistent OT treatment can promote social interaction and decrease anxiety-like behaviors in rats (e.g., Olff et al., 2013; Sabihi et al., 2014). Acute treatment of OT, on the other hand, has been found to have varying effects on social behaviors in mice, with some research showing no effects (e.g., Winslow et al., 1993) and others reporting enhanced (e.g., Gabor et al., 2012) or decreased social contact (e.g., Yang et al., 2007). These findings imply that the time and dose of OT administration may be important in determining its effects on social behaviors. As a result, future research may benefit from exploring the impact of different OT dosage regimens on social behaviors in mice.

Numerous earlier research has found gender variations in the effects of oxytocin on mouse behaviors. One study, for example, discovered that acute oxytocin therapy boosted sociability in male mice but had no impact on female mice (Yao & Kendrick, 2022). Another study found that prolonged oxytocin injection improved female mice's social behaviors but had no impact on male mice (Chen et al., 2018). Our findings of a

strong main effect of sex on sniffing time in the social chamber are consistent with earlier findings and highlight the importance of including sex as a factor in oxytocin studies.

Earlier investigations on the effects of oxytocin on anxiety in rats and humans yielded conflicting results. Several studies have shown that oxytocin may have anxiolytic benefits, whereas others have reported no impact or even an increase in anxiety after oxytocin delivery (Walter et al., 2021). Nonetheless, the findings of this study are consistent with prior research that revealed no indication that oxytocin enhances anxiety in mice. For example, one study discovered that oxytocin decreased anxiety-like behaviors in mice during a maze test (McGregor & Bowen, 2012) but another discovered that oxytocin had no effect on anxiety-like behaviors in the elevated plus maze (Chen et al., 2011). Overall, the findings of the present study add to the growing body of data showing acute oxytocin delivery does not raise anxiety in mice.

The results of the EPM test in our present study suggested that route of administration of oxytocin may have sex-specific effects. This is a significant result since prior research has revealed that intranasal delivery may have stronger central nervous system effects than peripheral administration. It is crucial to emphasize, however, that our study only looked at the acute effects of a single dosage of oxytocin. Future research should examine whether at if there are any differences in the effects of various methods of administration over longer time periods or with chronic dosage.

The sex-by-drug interaction found in the 3C is essential for explaining the diverse results with OT shown in clinical investigations. There is mounting evidence that gender variations may contribute to the heterogeneity of OT effects on human behaviors and physiology. Some research, for example, showed that OT enhances social cognition and

emotion control in females but not in males, while others found the reverse tendency (Chen et al., 2018). Moreover, there is evidence that individual variations in OT and other neurotransmitter baseline levels may modify the effects of exogenous OT. A recent study, for example, discovered that subjects with low baseline OT levels improved more in social cognition after intranasal OT treatment than those with high baseline OT levels (Horta et al., 2020).

The present research focused on how oxytocin affected social behavior and anxiety in mice using two distinct administration methods (intraperitoneal and intranasal). Our FDSI findings revealed that oxytocin increased social approach behavior when averaging across all oxytocin and saline groups. We also found sex effects such that males receiving displayed higher social approach than females at baseline (in saline control groups). Furthermore, we discovered a strong drug-sex interaction, demonstrating that oxytocin improved social approach in males more than females. This agrees with previous research showing sex variations in the effects of oxytocin on social behavior in mice (e.g., Dumais and Veenema, 2016; Bowen et al., 2017). Surprisingly, we discovered that the route of administration had a substantial influence on social approach behavior, with intraperitoneal treatment resulting in higher social approach than intranasal administration. This data implies that when examining the effects of oxytocin on social behavior, the route of delivery is an essential factor to consider.

The present study has two potential applications. First, as researchers select routes of administration (i.n. or i.p.) and schedules of administration (chronic or acute), these new data will help to identify potential behavioral impacts of these methods. The availability of this information will also help consumers of oxytocin research in mouse

models understand the influences of each methodological choice. Second, the data contribute to a larger effort to identify the sources of variability of oxytocin treatment for developmental disorders such as ASD. Ultimately, the data may contribute to the identification of novel therapeutic treatments for ASD and disorders with similar social symptoms.

In conclusion, our research contributes to the expanding body of knowledge about the effects of oxytocin on social behavior and anxiety and emphasizes the need of considering both route of administration and sex effects when examining the effects of oxytocin in mice. Our results might contribute to the ongoing efforts to develop new treatments for the social deficits associated with a variety of psychological disorders and for anxiety disorders.

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APPENDICES

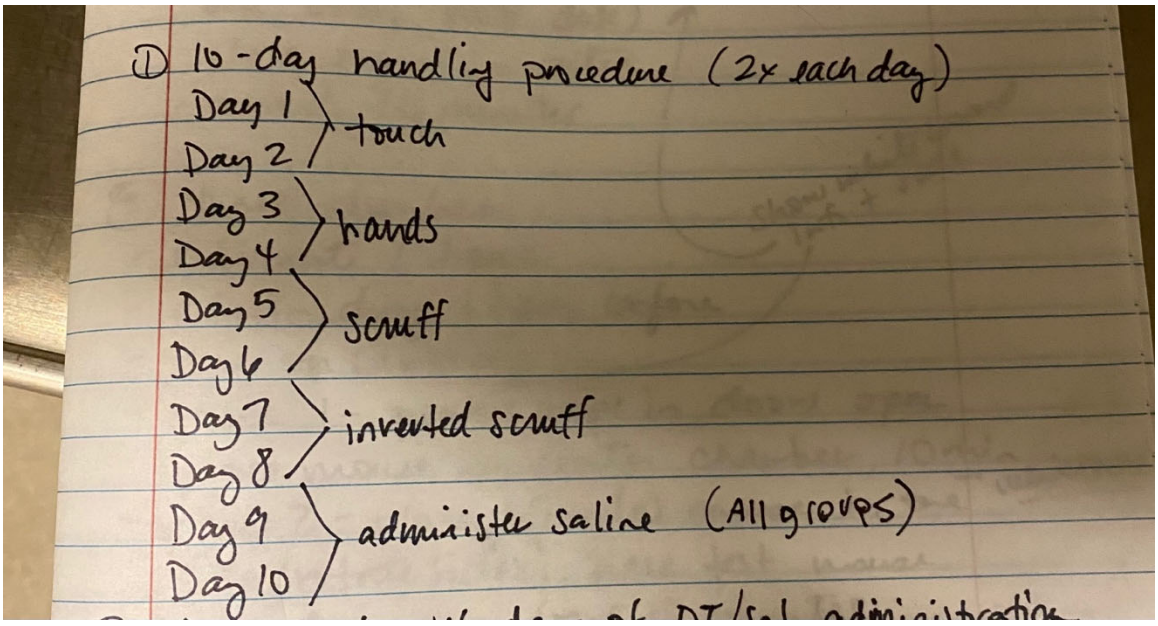
APPENDIX A: ANIMAL HANDLING PROCEDURE

Table 3
Animal handling procedure

A

Day	Description of Animal Handling Procedure
1	Pick up via tail; Hold in hand/on lab coat for 1 minute; Weigh
2	Pick up via tail; Hold in hand/on lab coat for 1 minute; Weigh
3	Pick up via tail; Hold in hand/on lab coat for 1 minute; Weigh
4	Pick up via tail; Hold in hand/on lab coat for 1 minute; Weigh
5	Lightly neck scruff while in hands; Weigh
6	Lightly neck scruff while in hands; Weigh
7	Neck scruff and invert mouse to expose stomach
8	Neck scruff and invert mouse to expose stomach
9	Neck scruff and i.p. or i.n. administration of saline; Neck scruff and intranasal administration of saline
10	Neck scruff and i.p. or i.n. administration of saline; Neck scruff and intranasal administration of saline

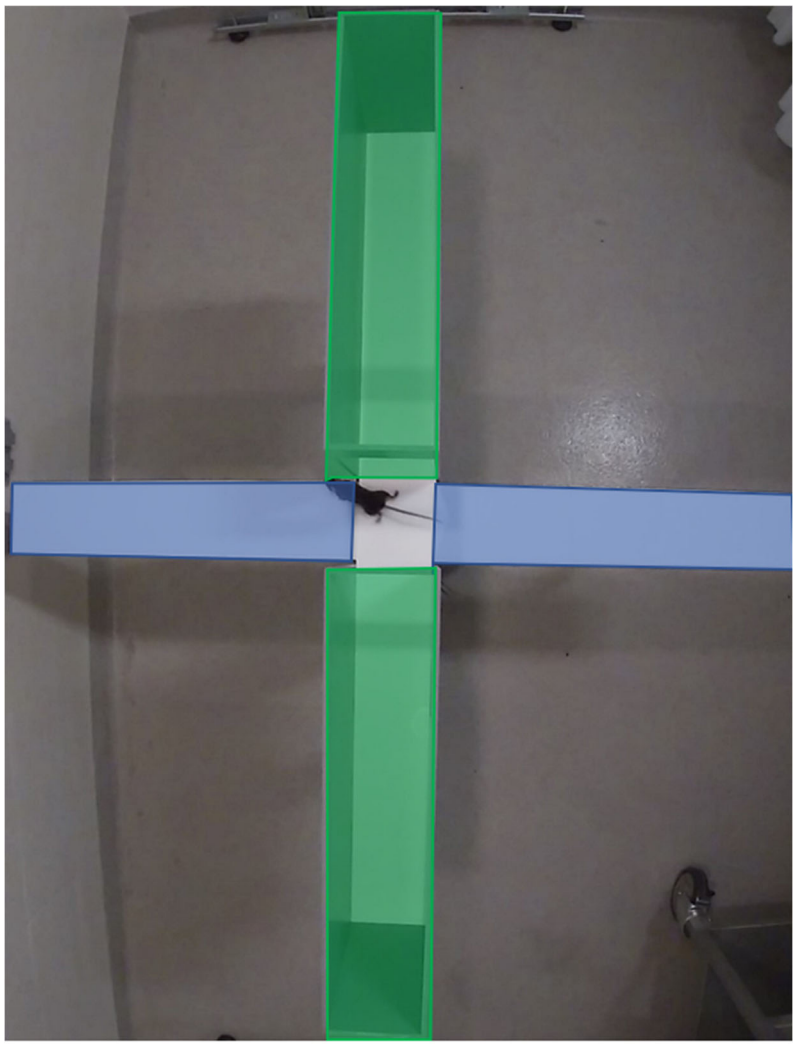
B



A. Depicts ten-day animal handling training procedure. **B.** The animal handling we used for present study.

APPENDIX B: ELEVATED PLUS MAZE TASK.

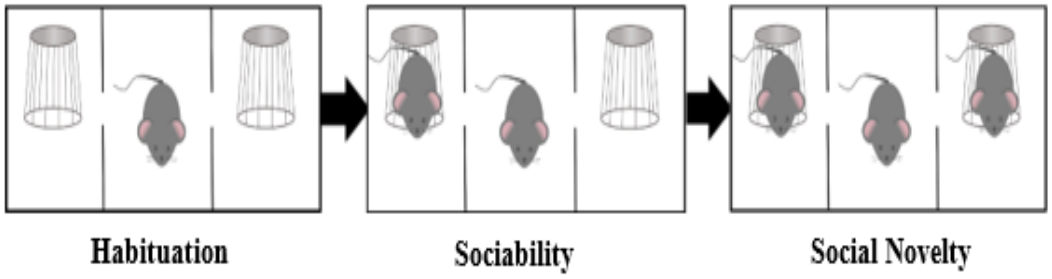
Figure 6
Elevated plus maze task



The blue highlighted arms symbolize the maze's open arms, while the green highlighted maze represents the maze's closed arms**.**

APPENDIX C: THREE-CHAMBER SOCIAL TASK

Figure 7
Three-chamber social task



Three-chamber social task (reprinted from Meadows et al, in preparation). The following diagram represents the three stages of the task: Habituation consists of two empty chambers, Sociability consists of one empty chamber and one room with stimulus mice, and Social Novelty consists of the familiar mouse chamber and a new stimulus mouse chamber.

APPENDIX D: FREE DYADIC SOCIAL INTERACTION TASK

Figure 8
Free Dyadic Social Interaction (FDSI) task

Test in the open field. Mice are put on opposite chamber sides at the start of the trial. The test mouse is indicated in blue, whereas the stimulus mouse is marked in green.

APPENDIX E: IACUC PROTOCOL APPROVAL

Figure 9

IACUC protocol approval

IACUC
INSTITUTIONAL ANIMAL CARE and USE COMMITEE
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

MIDDLE TENNESSEE STATE UNIVERSITY

IACUCN006: FCR PROTOCOL APPROVAL NOTICE

Friday, July 01, 2022

Senior Investigator	**Tiffany Rogers** (ROLE: Principal Investigator)
Co-Investigators	Isabela Ramos, Steven Brown, Kyle Hennsley, and Fatima Razzaq
Investigator Email(s)	*tiffany.rogers@mtsu.edu; igr2a@mtmail.mtsu.edu*
Department	Psychology
Protocol Title	*Optogenetic and Behavioral Analysis of Social Interaction in Mice*
Protocol ID	**22-3011**
Funding	NONE

Dear Dr. Arbour,

The MTSU Institutional Animal Care and Use Committee has reviewed the REVISED animal use proposal identified above under the ***Full Committee Review (FCR) mechanism***. The IACUC initially met on 5/10/2022 to determine if your proposal meets the requirements for approval. The IACUC has determined through an unanimous vote that your animal use protocol meets the guidelines for approval in accordance with PHS policy. In view of the current COVID-19 restrictions, the IACUC also introduced a few policies to protect students and junior staff. A summary of the IACUC action(s) and other particulars of this this protocol are tabulated below:

IACUC Action	**APPROVED for one year**	
Date of Expiration	**5/31/2023**	
Number of Animals	ONE HUNDRED AND TWENTY EIGHT (128)	
Approved Species	***C57BL/6g (Jackson Laboratories)***	
Category	☐ **Teaching**	☒ **Research**
Subclassifications	☐ Classroom	☒ Laboratory ☐ Field Research ☐ Field Study
	☐ Laboratory	☒ Handling/Manipulation ☐ Observation
	Comment: NONE	
Approved Site(s)	SCI 1170 K (house) and SCI 1170 J (Use)	
Restrictions	**1. Must comply with all FCR requirements;** **2. Mandatory compliance with CDC guidelines during COVID-19; Social distancing guidelines are made by the Dean of CBAS.** **3. The PI must make alternative plans to ensure proper animal care, including euthanasia if needed, in the event the research team is quarantined due to COVID-19**	
Comments	NONE	

This protocol expires on **5/31/2023** and it can be extended for THREE years until **5/31/2025** by requesting a continuing review by submitting annual progress reports. The investigator(s)

IACUCN006 Version 1.3 Revision Date 05.03.2016

Appendix E: IACUC protocol approval

Figure 9 (cont.)

IACUC protocol approval

MUST file a Progress Report annually updating the status of this study. Refer to the schedule for Continuing Review shown below; NO REMINDERS WILL BE SENT. A continuation request (progress report) must be **approved** by the IACUC prior to **4/1/1931** for this protocol to be active for its full term. Once a protocol has expired, it cannot be continued and the investigators must request a fresh protocol.

Continuing Review Schedule:

Reporting Period	Requisition Deadline	IACUC Comments
First year report	4/31/23	NONE
Second year report	4/31/24	NONE
Final report	4/31/25	NONE

Post-approval Protocol Amendments:
A new amendment may not be started while a current amendment request is still pending. Amendments will NOT be entertained when the protocol has less than 31 days for expiration.

Date	*Amendment(s)*	*IRB Comments*
06/30/2022	The following students are added to this protocol with vivarium access: 1. Steven Brown (swb3e – M10661633); 2. Kyle Hensley (knh5r – M01552830); 3. Fatima Razzaq (fr2r – M10764624).	 IACUCA2022-32 IACUCA2022-33 IACUCA2022-34

Other Post-approval Actions:
The following actions are done subsequent to the approval of this protocol on request by the PI/FA or on recommendation by the IRB or by both.

Date	*IRB Action(s)*	*IRB Comments*
07/01/2022	The health screening for the three students added on 06/30/2022 was confirmed from 20-3003	IACUCA2022-32-34

MTSU Policy defines an investigator as someone who has contact with live or dead animals for research or teaching purposes. Anyone meeting this definition must be listed on your protocol and must complete appropriate training through the CITI program. Addition of investigators requires submission of an Addendum request to the Office of Research Compliance.

The IACUC must be notified of any proposed protocol changes prior to their implementation. Unanticipated harms to subjects or adverse events must be reported within 48 hours to the Office of Compliance at (615) 494-8918 and by email – compliance@mtsu.edu.

All records pertaining to the animal care be retained by the MTSU faculty in charge for at least three (3) years AFTER the study is completed. In addition, refer to MTSU Policy 129: Records retention & Disposal (https://www.mtsu.edu/policies/general/129.php) for Tennessee State requirements for data retention. Please be advised that all IACUC approved protocols are subject to audit at any time and all animal facilities are subject to inspections at least biannually. Furthermore, IACUC reserves the right to change, revoke or modify this approval without prior notice.

COVID-19 Management:
The PI must follow social distancing guidelines and other practices to avoid viral exposure to the workers and others who come in contact with the animals.

- The study must be stopped if a student/employee or others should test positive for COVID-19. This must be reported to the IACUC as an "adverse event."
- The MTSU's "Return-to-work" questionnaire found in Pipeline must be filled by the investigators on the day of each animal activity prior to physical contact.
- PPE must be worn if coworkers would be within 6 feet from the each other.
- Physical surfaces that will come in contact with the investigators must be sanitized between use

Appendix E: IACUC protocol approval

Figure 9 (cont.)

IACUC protocol approval

- **PI/FA's Responsibility:** The PI/FA is given the administrative authority to make emergency changes to protect the wellbeing of the animals and student researchers during the COVID-19 pandemic. However, the PI/FA must notify the IACUC after such changes have been made. The IACUC will audit the changes at a later date and the PI/FA will be instructed to carryout remedial measures if needed.

Data Management & Storage:
All research-related records (logs, charts, investigator training, etc.) must be retained by the PI or the faculty advisor (if the PI is a student) at the secure location. The data must be stored for at least three (3) years after the study is closed. Additional Tennessee State data retention requirement may apply (*refer "Quick Links" for MTSU policy 129 below*).

Sincerely,

Compliance Office
(On behalf of IACUC)
Middle Tennessee State University
Tel: 615 494 8918
Email: iacuc_information@mtsu.edu (for questions) and
iacuc_submissions@mtsu.edu (for sending documents)

Quick Links:
- MTSU Policy 129: Records retention & Disposal: https://www.mtsu.edu/policies/general/129.php