

Validation and Testing of a Social Motivation Task for Mice

 Student Name

Dr. Rogers

Introduction

The current project seeks to develop a measurement for social motivation in mice. While the behavior of mice is often used to inform researchers about the biological mechanisms of human behavior, we do not currently have a standard way to measure social motivation in mice. Two aims will be addressed. First, we will validate a social motivation behavior arena for mice. The social reward task is designed to understand measure social motivation in mice. The arena's structure is divided into one large chamber and two small chambers that are separated by weighted one-way vertical swing doors (reference appendix A for visual of arena). The one large chamber holds the mouse, and the two smaller chambers contain any stimuli. Weights are added to increase the degree of effort required by the mice for the stimuli in the smaller chambers. Specifically, in our project, the arena will hold a social stimulus, such as another mouse, in one chamber and food/novel object in the other chamber. This arena allows for the direct investigation of the effect of the stimuli on the mice's motivation to access it. The second aim will be to manipulate the mouse's level of social motivation pharmacologically by administering oxytocin, a hormone associated with prosocial behavior. Together these aims would demonstrate the validity of the social motivation task as they would demonstrate: 1) preferences for social stimuli (other mice) over novel objects or food, 2) the amount of effort mice are willing to exert to interact with the social stimulus, and 3) that the amount of social motivation can be increased or decreased pharmacologically.

This study will test the hypotheses that:

- 1) a social stimulus is more motivating than food or novel objects, and
- 2). oxytocin administration can increase social motivation. The overall significance of

This project will be to help establish a standard way of measuring social motivation in mice.

Background

The social reward chamber was initially described by Borland and colleagues (2017). They aimed to measure social reward and social motivation in rodents, and they developed the social reward chamber described above as a means to effectively allow rodents to choose between two stimuli and to exert effort to enter the chamber of their choice. In this way both the choice to explore social stimuli and the amount of effort expended to do so can be measured. However, Borland et al. used hamsters in the test and did not attempt to alter motivation in order to validate the arena. The chamber has not been used since its original description despite being a simple and well-designed way to measure social motivation. As the field remains without a standard way to measure social motivation, our project will revisit this chamber and will attempt to pharmacologically manipulate levels of social motivation.

Oxytocin is a neuropeptide that is produced in the hypothalamus and stored in the pituitary gland. Oxytocin plays a crucial role in childbirth and breastfeeding, but more importantly it has been associated with social behaviors in both humans and mice (Lukas, 2011). The oxytocinergic system has shown to be a prime target for treating diseases that involve aberrant social behavior such as autism (Fineberg, 2017). Oxytocin appears to impact dopaminergic activity which is affiliated with reward and motivated behaviors. Dopamine, a neurotransmitter, influences motivational salience, impacting the drive toward certain rewards rather than the pleasure derived from the reward itself. Since dopamine is well known for motivated behaviors and oxytocin interacts with dopamine, this evidence suggests a linked dopamine and oxytocinergic functioning towards social motivation (Love, 2014). This connection allows our research to further investigate the role oxytocin may have on social motivation.

In the last semester, I participated in Dr. Roger's lab to assist with her prior research with mice and oxytocin. I assisted with a project examining behavioral differences between male and female mice receiving either intranasal or intraperitoneal injections of oxytocin. That research is currently being prepared for publication. Also in the fall semester of 2019, I received the opportunity to conduct my own research project in my genetics course and present my results at Scholar's Week. The research involved the revision of genes and I worked in collaboration with my genetics professor and a peer from the course. The genetics research poster is attached in the application as an example of my prior research.

Purpose

The purpose of this study is to validate a social behavior arena using mice and test the correlation between oxytocin and social motivation. This research will help to establish a standard way to measure social motivation in mice. The expected outcomes are that the behavioral arena will be valid to test social motivation, the social stimuli will be more motivating than food/novel objects, and oxytocin administration will increase social motivation for the mice.

Methods

Experimental mice will be inserted into the one large chamber and the two smaller chambers with weighted doors will contain any stimuli (reference appendix A for visual of arena). The experimental mouse will choose between the two stimuli, social stimulus (another mouse) or food/novel object. The effort exerted by the mouse on the weighted doors (time spent pushing and amount of weight pushed) to access the stimulus will be measured as its social motivation. We will determine the appropriate range of weights and types of stimuli to utilize in the arena. After determining a set of motivating stimuli, it will be tested whether social motivation can be altered by oxytocin. This research will be conducted with 20 mice divided into

two groups of 10 mice in which each group will either receive saline or oxytocin for 14 days prior to the behavioral task. All 20 mice will be male to prevent variability as oxytocin is known to have differential effects on male and female mice. The experiment will be video recorded. The data, such as the time spent pushing, weight pushed by the mice, and the time spent in the chambers, will be obtained through hand coding of the video recordings. Finally, data analysis will be conducted through SPSS.

Timeline

Validation phase (February 8th – 12th): determine appropriate weights and stimuli (food/novel objects to be tested against social stimuli) for arena

Handling/ Habituation phase (February 15th – February 19th): familiarize mice to experimenter handling

Oxytocin administration phase (February 22nd – March 5th): habituate mice with oxytocin

Oxytocin experiment phase (March 8th – March 12th): oxytocin administration to mice for experimentation

Data analysis phase (March 15th – 19th): hand coding & SPSS

Poster (March 22nd – April 2nd): prepare poster for presentation

Collaboration with Faculty Mentor

The faculty mentor, Dr. Rogers, will play a significant role in responding to ideas, providing advice for new directions and resources, discussing the results, and editing drafts of the final report. Through Dr. Roger's prior experience and expertise in this area of research, she will provide guidance, motivation, emotional support, and role modeling. There will be regularly scheduled meetings between myself and the faculty mentor. This project relates to her ongoing research exploring the role oxytocin in the social behaviors of mice.

References

Borland JM, Frantz KJ, Aiani LM, Grantham KN, Song Z, Albers HE. (2017). A novel operant task to assess social reward and motivation in rodents. *J Neurosci Methods*;1; 287:80-88. Doi: 10.1016/j.jneumeth.2017.06.003.

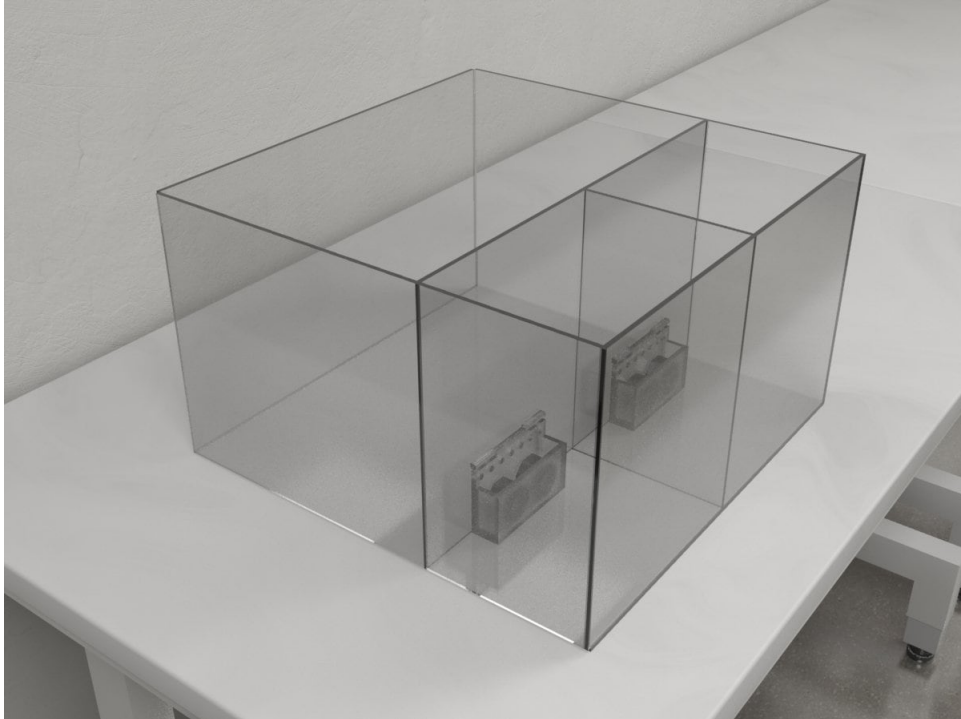
Fineberg, S. K., & Ross, D. A. (2017). Oxytocin and the Social Brain. *Biological psychiatry*, 81(3), e19–e21. <https://doi.org/10.1016/j.biopsych.2016.11.004>

Love T. M. (2014). Oxytocin, motivation and the role of dopamine. *Pharmacology, biochemistry, and behavior*, 119, 49–60. <https://doi.org/10.1016/j.pbb.2013.06>

Lukas, M., Toth, I., Reber, S. O., Slattery, D. A., Veenema, A. H., & Neumann, I. D. (2011). The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 36(11), 2159–2168. <https://doi.org/10.1038/npp.2011.95>

.011

Appendix A



Psychology Department
MTSU Box 87
Murfreesboro, TN 37132
(615) 898-2706



January 27, 2021

To the URECA committee,

As (Students' Name) research mentor, I write this letter of support to describe her previous research experience in my lab and in her classes. (Students' Name) began working as a research assistant in my neuroscience mouse lab last semester (Fall 2020). (Students' Name) has expressed an interest in writing an honors thesis, and I agreed to be her advisor. Prior to beginning her work on her thesis, we agreed that it would be good to work in the lab to gain hands-on training and to help with ongoing research projects. In the lab, (Students' Name) has been trained on daily care for the mice. She provided food and water to the mice, checked on room conditions, and changed and cleaned the mouse cages. After this training, she began work on a research project that compared routes of administration of oxytocin across male and female mice to determine any differences in social behavior outcomes. (Students' Name) administered oxytocin to the mice either by intraperitoneal injection or by intranasal administration. (Students' Name) then tested the mice in various behavioral tasks. She also video recorded the data to be coded for the project.

(Students' Name) has also had experience with research in her classes. In her Genetics course (Fall 2019), she completed a research project as part of the class assignments including data collection, coding, and analysis. (Student's Name) then presented her findings at Scholar's week in the spring of 2020.

In her training, (Students' Name) has demonstrated her ability to complete tasks in the lab with skill, and she has learned quickly. Her experience in my lab and in her classes has prepared her to advance in her training. She is now applying for the Silver Scholar level of the URECA grant to be able to fund her time and resources needed to progress in her research training. I would be happy to provide any additional information and can be contacted with the information provided below.

Sincerely,

A handwritten signature in black ink that reads "Tiffany Rogers".

Tiffany Rogers, Ph.D.
Assistant Professor



Budget Justification - (Student's Name) URECA Proposal Spring 2021

Item	Quantity	Price per Pkg	Total price	Link to Product	Reason
Oxytocin	2 - 1000 International Units each	\$86.40	\$172.80	https://www.sigmaaldrich.com/catalog/product/sigma/o3251?lang=en&region=US	Drug to be administered to animals
Pipette tips	1 bulk box containing 10 boxes of tips	\$64.50	\$64.50	https://www.amazon.com/BrandTech-732726-Bio-Cert-Pipette-Sterile/dp/B089NN5Q9B/ref=sr_1_4?dchild=1&keywords=20+ul+pipette+tips&qid=1611085334&sr=8-4	Pipettes will be used to administer drug to animals

Explanation of IACUC Approval Process

To aid in the review of the current URECA grant application, we have included the protocol approval for IACUC Protocol 20-3003 and a submission for amendment. The protocol includes working with the mouse species included in the URECA grant and the administration of oxytocin. The protocol does not currently cover the particular behavioral task, but an amendment for approval to use this task has already been submitted to IACUC.

**Animal Use Protocol Amendment
Middle Tennessee State University
Amendment**

Please type all entries. Use this form to request significant changes in animal care and use made to your currently approved protocol. Complete *all fields* in Section I and any applicable questions in Section II.

Submit the *completed* amendment to the Office of Compliance via compliance@mtsu.edu.

Section I: Protocol Information

Protocol #20-3003

Title: Neurochemical and Behavioral Analysis of Social Interaction in Mice

Principal Investigator: Tiffany Rogers

Phone: [REDACTED]

Email: [REDACTED]

Co-Investigator:

Phone:

Email:

Funding Source: MTSU Start Up Funds and FRCAC Grant

Section II: Request Changes

Type of amendment: Please check all that apply and complete the corresponding sections below:

<input type="checkbox"/>	Strain—Only if the phenotype could potentially affect the welfare of the animal
<input type="checkbox"/>	Species addition and/or removal
<input checked="" type="checkbox"/>	Increase in animal numbers
<input checked="" type="checkbox"/>	Animal use procedures (i.e., surgery, euthanasia, blood collection, special diets, etc.)
<input type="checkbox"/>	Use of additional potentially hazardous substances: biologics, radioisotopes, chemicals, drugs, infectious agents, recombinant DNA (including use or generation of transgenics)
<input type="checkbox"/>	Principal Investigator
<input type="checkbox"/>	Other

1. Strain: Describe the phenotype and list any conditions that are not normal in healthy animals. Describe the course of action that will be taken to relieve pain/distress.

A. How will the animals be monitored and by whom?

2. Species: Please describe what species you would like to add to this protocol and provide scientific justification as to why this species is required.

3. Increase in animal numbers from the total numbers approved in the original submission. Please complete Parts 3A and 3B. Only describe the numbers needed beyond those that were approved in the original submission.

A. Animal Number Chart

Species	Number Procured	Number Transferred (include protocol #)	Number Produced
C57/BL6J			144

B. Animal Number Justification. Please justify why you need to use more animals than you were originally approved for. Also describe how these animals will be used on this protocol.

The current amendment includes behavioral tests that require 12 mice per group (see justification for group numbers for behavioral experiments included with original protocol submission). Three tests are proposed each needing 4 groups (factor of sex with 2 levels and factor of drug condition with 2 levels = 4 groups per task) for a total of 144 mice needed.

4. Animal Use Procedures. Please complete 4A, 4B, and 4C as applicable.

A. Description of procedures. Describe the proposed procedure(s) or change in procedures to be performed in addition to those already approved on this protocol. Describe how this related to the original goal of the approved protocol.

- If you need to refer to approved procedures in your description, please explain clearly which is (are) the proposed procedures.

Three behavioral tasks are proposed to be added to the protocol.

1) Social Reward Chamber

The social reward chamber consists of a three-chambered plexiglass box. One chamber is larger and the other two are smaller. The chambers will be separated by clear plexiglass and each of the smaller chambers will have vertical swinging doors that the experimental mouse may push to access the chambers. The doors will also contain plexiglass pockets in which tunsten weights may be progressively added. stimuli (either food or a social stimulus - sex, weight, and age-matched conspecific)

will be separately introduced to the two chambers. The experimental mouse will initially be introduced into the larger chamber and will choose which chamber to enter. The amount of time spent in the chamber, the amount of time spent pushing the door, and the amount of weight pushed will be measured to evaluate social motivation. The length of the test will not exceed 10 minutes. A picture and description of the chamber can be accessed here:
<https://conductscience.com/maze/portfolio/social-reward-chamber/>.

2) Social Motivation Chamber

The social motivation chamber will consist of a clear plexiglass box and will contain two chambers separated by a plexiglass wall. The wall will be of adjustable height and height will progressively be added. Experimental mice will be placed on one side of the wall and a stimulus mouse will be placed on the other. The stimulus mouse will be contained in an inverted wire pencil cup so that it may not climb over the wall. The height of the wall climbed and the length of time spent attempting to climb the wall will be measured to evaluate social motivation. The length of the test will not exceed 10 minutes.

3) Measuring Social Motivation with an Operant Conditioning Chamber

A two-chambered shuttle box will be modified to allow the measurement of social motivation. The box will be divided into two chambers (a test chamber and a target chamber) by a programmable guillotine door. Wire mesh will be mounted to the door to create a complete barrier between the chambers while allowing limited social interaction. The door will be opened by lever press from the mouse. Alternatively, the mouse will also be able to lever press for a liquid food reward (15 ul of sweetened condensed milk). The experimental mouse will be placed in the test chamber and a social stimulus will be placed in the target chamber. The experimental mouse will be able to lever press for food or social reward. The number of times the mouse lever presses for each stimulus will be recorded and the length of the test will not exceed 10 minutes. The chamber and a description of methods can be found here:

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4598097/>.

B. Qualifications. Indicate the personnel that have the relevant qualifications for the specific procedures proposed in this amendment.

Dr. Rogers has experience in running these and similar behavioral experiments. Dr. Rogers will train all students included on the protocol for the use of these behavioral tasks prior to students using them.

C. Literature Search. For new proposed procedures that have the potential to cause pain/distress, a literature search is required.

A. Was a literature search conducted? Yes No

5. Use of Additional Potentially Hazardous Substance(s). Describe and justify any proposed changes in the use, or the addition of potentially hazardous substances.

6. **Principal Investigator.** Please explain the reason for changing the PI.

7. **Other.** Please describe and justify any additional changes you would like to make to this protocol.

For Compliance Office Use Only

Review.

Type of review: DMR Full

Decision.

Decision made: Approved Approve Pending Clarification Refer to Full Committee
 Not Approved

Explanation:

IACUC

INSTITUTIONAL ANIMAL CARE and USE COMMITTEE

Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129



IACUCN006: FCR PROTOCOL APPROVAL NOTICE

Thursday, May 14, 2020

Senior Investigator **Tiffany Rogers** (ROLE: Principal Investigator)
Co-Investigators NONE
Investigator Email(s) [REDACTED]
Department Psychology

Protocol Title ***Neurochemical and Behavioral Analysis of Social Interaction in Mice***
Protocol ID **20-3003**

Dear Investigator(s),

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 met on 5/12/2020 to determine if your proposal meets the requirements for approval. The Committee determined through a majority of vote that this REVISED protocol meets the guidelines for approval in accordance with PHS policy. In view of the current COVID-19 crisis, the IACUC also introduced a few restrictions. 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	5/31/2021	
Number of Animals	192 (ONE HUNDRED and NINETY TWO)	
Approved Species	C57BL/6J Mice (Jackson Laboratories)	
Category	<input type="checkbox"/> Teaching <input checked="" type="checkbox"/> Research	
Subclassifications	<input type="checkbox"/> Classroom <input checked="" type="checkbox"/> Laboratory <input type="checkbox"/> Field Research <input type="checkbox"/> Field Study <input type="checkbox"/> Laboratory <input checked="" type="checkbox"/> Handling/Manipulation <input type="checkbox"/> Observation	
	Comment: NONE	
Approved Site(s)	MTSU Vivarium: Rooms SCI1170L (housing) and SCI 1170K (procedures)	
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 COVID19	
Comments	NONE	

This approval is effective for three (3) years from the date of this notice **till 5/31/2023**. The investigator(s) 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 **5/31/2021** 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	4/30/2021	NONE
Second year report	4/30/2022	NONE
Final report	4/30/2023	NONE

Post-approval Amendments:

Date	Amendment	IACUC Notes
NONE	NONE	NONE

Post-approval Actions:

Date	Amendment	IACUC Notes
05/14/2020	(co-PI's name), Psychology graduate student) is added as a co-investigator. CITI training and health screening are confirmed	Initial FCR approval

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.

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)