Confirming Measures of Social Motivation in Background Strains of Mice Using the Weighted Doors Task

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

Fall 2023

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APPROVED:

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Acknowledgements

I would like to express my gratitude to my thesis advisor, Dr. Rogers, for her unconditional support, guidance, and patience throughout the journey of this research project. Her knowledge and mentorship have been invaluable, and I am so grateful to have had such an amazing experience with her. I would also like to express my appreciation to my family and friends for their encouragement, love, and for listening to me talk a little bit too much about the mice. Their support has gotten me through the trying times, and I could not have gotten through it without them. Finally, I would like to thank God for His blessings and for getting me through to the end. This thesis would not have been possible without the collective support and encouragement of these individuals, and for that, I am truly thankful.

Abstract

The weighted doors task is a novel measure of social motivation and is used in the current study to determine the differences in social motivation between C57 and BTBR mice as well as sex differences. It was found that male mice displayed higher social motivation in comparison to the female mice and the C57 mice displayed higher social motivation than the BTBR mice. The social deficits the BTBR mice displayed support their continued use as an Autism Spectrum Disorder (ASD) mouse model. Additionally, this research validated the weighted doors task as a robust and reliable measure of social motivation, allowing future neuroscience research to utilize the weighted doors task as a standardized measure of social motivation. In this way, conditions such as ASD can be investigated and better understood for future interventions.

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List of Abbreviations

Autism Spectrum Disorders (ASD)

BTBR T^+ Itpr3tf/J (BTBR)

C57BL/6J (C57)

Institutional Animal Care and Use Committee (IACUC)

Statistical Package for the Social Sciences (SPSS)

Introduction

Social behavior plays a crucial role in human survival but can be disrupted by various social deficits, which can be manifested in disorders such as autism spectrum disorder, depression, and anxiety (Tsai et al., 2020). These neurodevelopmental disorders impact not only the individual, but their families and loved ones. The symptoms of these disorders include lack of interest in others, little eye contact, trouble relating to others, and sensory sensitivity (Anxiety & Depression Association of America, 2021). Understanding the underlying causes and mechanisms of social deficits in humans is a crucial area of study. Recent research has focused on addressing the social symptoms of these disorders and exploring new treatment options. Additionally, reward is a key factor in the development of social behavior. By examining the relationship between social behavior and reward, researchers can gain a deeper understanding of the underlying mechanisms of these disorders and how they can be treated (Bhanji & Delgado, 2014). This understanding is important for advancing research in the field and improving the lives of those who suffer from these conditions.

In the field of neuroscience, mice are frequently used as a model for investigating various aspects of human social behavior and social interactions due to their complex social behavior. Mouse models allow researchers to investigate the underlying neural mechanisms that drive social behavior that contribute to the development of social disorders. Mouse models are widely used because they have a similar anatomy and physiology to humans and they exhibit many similar behaviors, including social behavior. They also provide a controlled experimental environment, which allows for the manipulation of different variables to understand the impact on social behavior. To better understand social behavior in humans, researchers use mouse models with different genetic backgrounds to represent the diversity of human populations. With the recent development of 16 diverse lab mouse reference genomes, scientists can now explore the relationships between social behavior and reward across different mouse strains (Endo et al., 2019). Mouse models can also be used to study the effects of various treatments, such as pharmacological or behavioral interventions, on social behavior, making them an important tool for developing new treatments for social disorders.

The C57 and BTBR mouse strains are two mouse strains that have been used in social behavior studies due to their distinct social behaviors. Both of the mouse strains are inbred strains commercially available through vendors such as Jackson Laboratories. Inbred strains are commonly used in neuroscience and animal behavior studies as the practice of inbreeding keeps genetic variability to a minimum, allowing for higher internal validity due to a reduction in genetic variance. Variance introduced by environment is also able to be kept to a minimum, increasing internal validity by keeping the housing conditions of the mice, regardless of sex or strain, as identical as possible. These specific strains were selected for their unique traits. C57 mice are known for their high levels of social interaction and preference for social novelty (Moy et al., 2004). Conversely, BTBR mice display reduced social interaction and increased repetitive behavior (Pobbe et al., 2010). BTBR mice are often used as non-genetically modified autism mouse models due to these behavioral traits. Given these social differences between the C57 and BTBR strains, it is plausible that there may be differences when testing social motivation between these strains.

Various methods are used to measure and assess social behavior in mouse models, including the three-chamber test, the barrier, and the weighted door task. The three-chamber test, as described by Moy, has been validated and has been used to study autistic-like behavior in mice (Moy et al., 2004). However, while the three-chamber test provides a standard measure for social behavior, there is currently no standard measure for social motivation, which is defined as the effort exerted to engage in social behavior.

The weighted doors task is a promising method for assessing social motivation in mice (Figure 1). This task involves placing a mouse in a contraption with a door of varying weights, behind which is another mouse acting as the social stimulus. The mouse is given time to push the door to reach the other mouse, and the weight of the door increases as the trials progress. The weighted doors task has been used to quantify social reward in hamsters and has been validated and used in mouse research at Dr. Rogers' lab at MTSU (Borland et al., 2017).

The primary objective of this thesis is to explore and validate the measures of social motivation in two background strains of mice, C57 and BTBR, through the utilization of the weighted doors task. Developed and validated by the Rogers Lab for the application of social behavior in mice, the weighted doors task will serve as a tool for quantifying and standardizing the measurement of social motivation. By gaining a deeper understanding of these measures and standardizing their measurement, we hope to facilitate advancements in research in the field and make meaningful contributions toward the long-term goal of identifying neurological changes in social motivation.

Thesis Statement

I hypothesize that there will be differences in social motivation between C57 and BTBR mice, as measured by the weighted doors task. The objective of this study is to provide empirical evidence for the validity of the weighted doors task as a robust and reliable measure of social motivation in male and female mice of both the BTBR and C57 strains**.** Having the two different mouse strains that differ in social behavior will demonstrate variability in social motivation scores, and, therefore, help validate the test. This confirmation is important to researchers in the field of social motivation because it can help validate other social behavior tests done in the past as well as improve the quality and credibility of future animal studies testing social behaviors.

Approach/Methods

Two different strains of mice, BTBR T^+ Itpr3tf/J (BTBR; Strain #002282 from Jackson Laboratories) and C57BL/6J (C57; Strain #000664 from Jackson Laboratories, bred at MTSU), were used in this research project. Four groups composed of fifteen males and fifteen females per strain were compared (see Table 1).

Table 1

Experimental Groups

	Sex	
Strain	Male	Female
C57	N = 15	N = 15
BTBR	N = 15	N = 15
	Total N	60

Note. Adult male and female C57 and BTBR mice were used to compare across the variables of sex and strain.

BTBR and C57 mice are inbred strains of mice, meaning the mice of their respective strains have been bred to be genetically identical. The BTBR mice were ordered from the Jackson Laboratory in Bar Harbor, Maine, while the C57 mice were bred in-house in Dr. Rogers' lab at Middle Tennessee State University. At the time of the study, the mice were all aged between 10 and 14 weeks, considered to be adult mice. Before any experimentation, all mice were provided a habituation period to settle into their surroundings. They stayed in a mouse colony room with a controlled environment and a 12-hour light and dark cycle. Humidity was monitored and food and water were available for the mice ad libitum, allowing them free access. All procedures conducted in this study were approved by Middle Tennessee University's Institutional Animal Care and Use Committee (IACUC), adhering to the ethical guidelines and regulations outlined by them.

After the habituation period, mice underwent training and tests to assess their baseline behavior before beginning the actual experiment. In the context of the larger lab project, the mice were tested for potential confounding behaviors. These assessments include motor tests, anxiety tests, and a social behavior test (see Table 2). These tests ensure that any differences observed between groups is due to strain and sex and not due to motor deficits or anxiety. Additionally, the three-chamber test was used to provide a comparison for the social motivation task. The three-chamber test is the current gold standard for measuring social behavior in mice, but it is a general test for sociability (willingness to be social with a freely available social stimulus). In contrast, the weighted doors task used in this study is specific to social motivation. Therefore, comparing the weighted doors task and three-chamber test outcomes allows for divergent validity for the novel weighted doors task.

Table 2

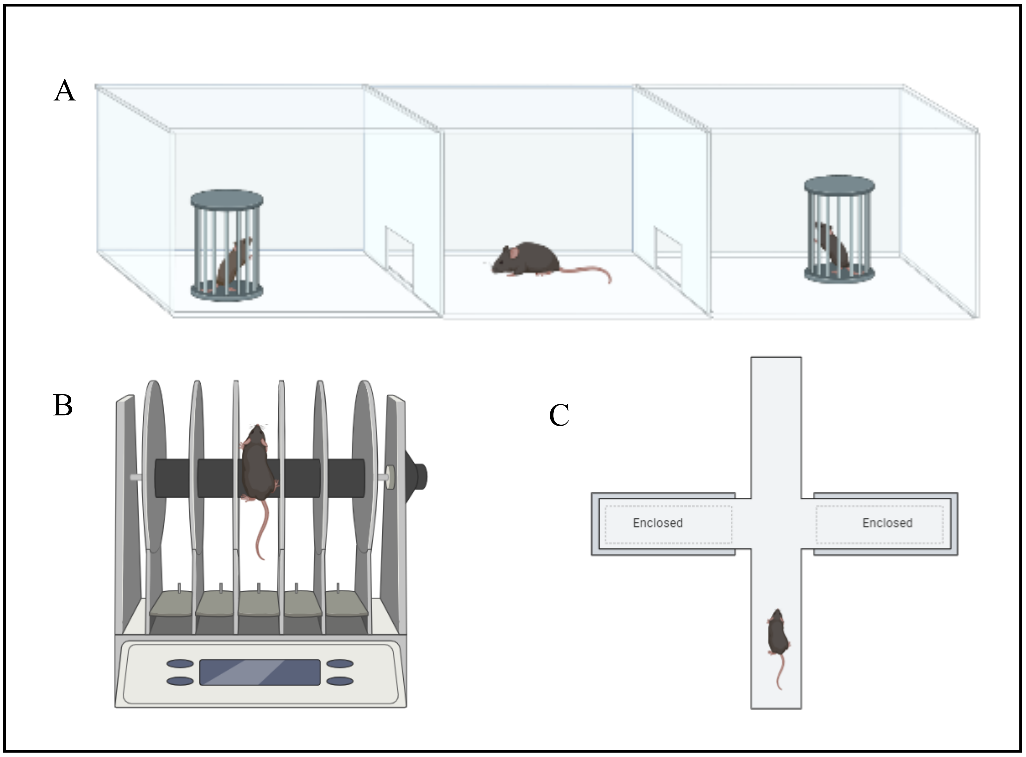
Baseline Behavioral Tests

Tests for Confounding Behaviors	Purpose of Behavioral Test	Description of Behavioral Test
Rotarod Test	Balance and motor coordination	Mouse is placed on elevated rotating rod that accelerates over time. Time before fall into catch is recorded.
Elevated Plus Maze	Anxiety-like behavior	Mouse is placed on elevated plus-shaped platform with two open arms and two walled arms. Time spent in open vs. closed arms is recorded.
Three-Chamber Test	General sociability	Mouse is placed in three-chambered arena. Social stimuli are placed in side chambers one at a time. Time spent sniffing first social stimulus and then second is recorded.

Note. This table describes the baseline behavioral tests conducted by the Rogers Lab in the context of a larger project.

Figure 1

Depictions of Baseline Behavioral Tests



Note. Figure 1 depicts the baseline behavioral tests performed in the context of the larger project conducted in the lab. (A) Three-Chamber Test; (B) Rotarod Test; (C) Elevated Plus Maze. Images created in BioRender.

Over the next two to three weeks, the mice were individually tested using the weighted doors task. In this task, the experimental mouse is expected to open a one-way weighted door, which opens to a room containing a social stimuli (another mouse) in an inverted pencil cup to restrict the movement of the social stimulus. Both mice were of the same sex, size, weight, and strain to reduce aggressive or dominance-based behaviors. The experimental mouse begins by learning the mechanism of the task by going through the door while it is propped open. Once the mouse had familiarized itself with the door mechanism, it completed five trials, progressing from no added weights to 1.33 oz by adding 2 tungsten cubes (0.16 oz per cube) to the door in each subsequent trial. Each experimental mouse was given 3 minutes to complete each trial for a maximum testing time of 15 minutes (Figures 2 and 3). The experimental mouse was tested to see how motivated the experimental mouse was to interact with the mouse behind the weighted door. Social motivation was measured by time spent pushing the weighted door and latency to reach the social stimulus. The data was video recorded and then quantified and coded using Noldus Ethovision software (Figure 4).

Figure 2

Depiction of the weighted doors task. Image created in BioRender.

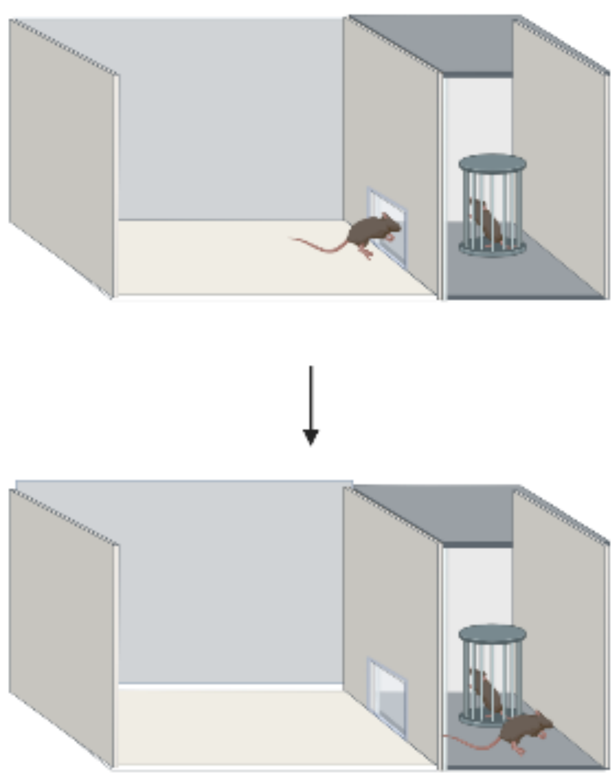


Figure 3

Steps to Complete Weighted Doors Task

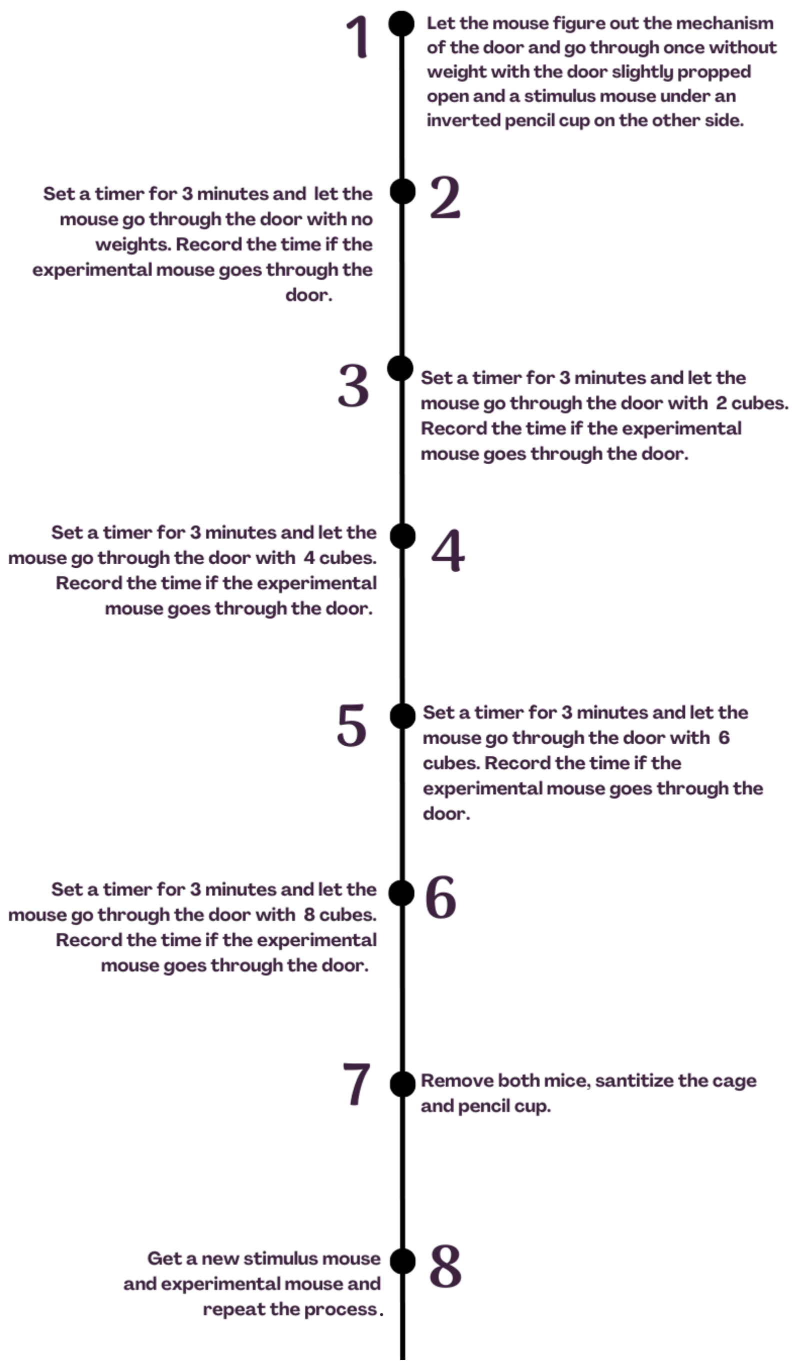
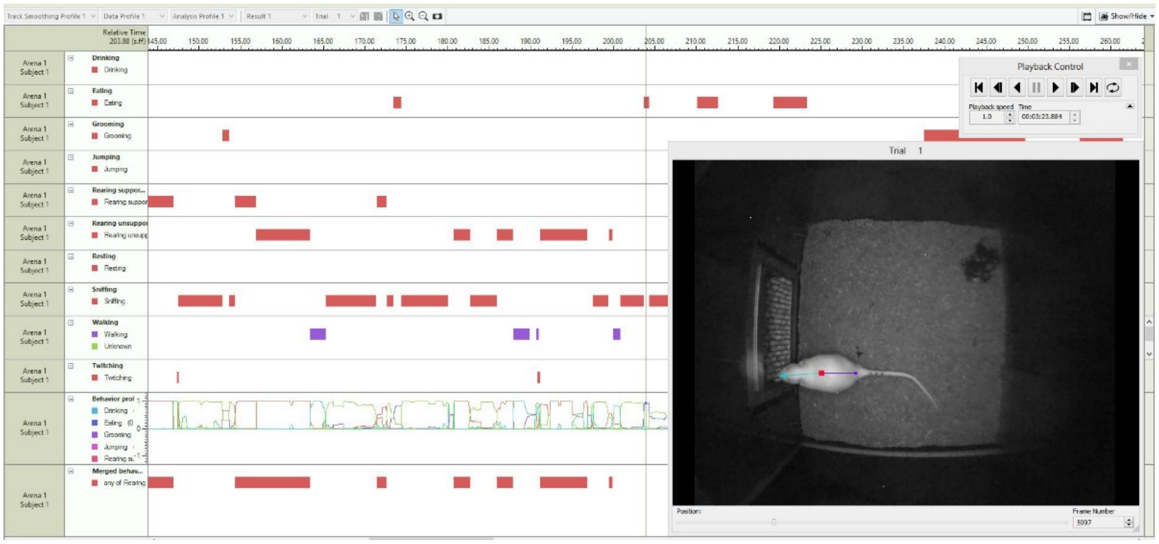


Figure 4

Depiction of Noldus Software



Note. The Noldus Ethovision software is a behavior recognition software that was used to analyze the mouse's social motivation and behavior. Image from https://www.noldus.com/ethovision-xt/behavior-recognition.

Noldus Ethovision is a software program used in behavioral research, specifically animal behavior. Using Noldus was essential in this study, providing precise data collection important for confirming measures of social motivation in our mice. Noldus tracked and recorded the movements and interactions of the experimental mice during the weighted doors task. Specifically, the amount of time each mouse spent pushing the door as it reached the stimulus mouse on the other side of the door. The software recorded critical variables such as the time taken for each interaction, the movements and patterns of the mouse, and the frequency of door pushes for each trial. The output given included these variables, which allowed for analysis of social motivation within the conditions of the experiment, such as strain and gender.

To analyze the data recorded during the weighted doors task, we used the Statistical Package for the Social Sciences (SPSS). Within SPSS mixed-methods ANOVAs was conducted with time spent pushing the weighted door and latency to reach the social stimulus entered as dependent variables in two separate ANOVAs. Weight across trials was entered as a within-subjects variable as each mouse was measured across multiple trials. The between-subjects factors were strain and sex. The alpha level selected for all analyses was $\alpha = 0.05$.

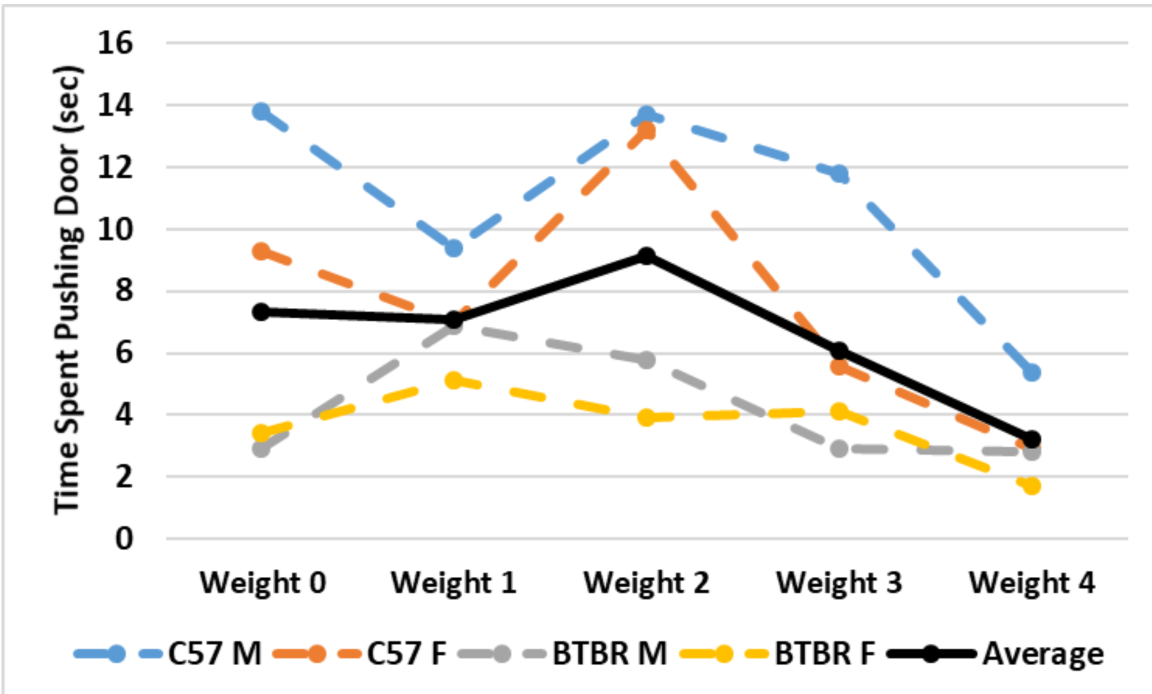
While the larger study conducted in the Rogers Lab will analyze the results of the motor, anxiety, and general sociability measurements, the results of those comparisons are not included here as this thesis study was limited to comparing sex and strain difference within the weighted doors task only.

Results

Using the software SPSS, we tested for sphericity using Mauchly's Test prior to the ANOVA. The Assumption of Sphericity was not met (χ^2 (9) = 34.13, $p < 0.001$). Therefore, the Greenhouse-Geisser Correction was applied. A mixed-method ANOVA was conducted, with door weight as a within-subjects factor and sex and strain as between-subjects factors and using the time spent pushing the door (seconds) as the dependent variable. The within-subjects factor of weight was found to be significant ($F(2.67, 93.60) = 5.805$, $p = 0.002$). Post-hoc analysis revealed that time spent pushing decreased as weight increased (Figure 5). Both the between-subjects factors of sex and strain were significant ($F(1, 35) = 4.46$, $p = 0.04$; $F(1, 35) = 33.49$, $p < 0.001$, respectively). C57 mice displayed higher push times (M = 9.21 sec, SEM = 0.65) compared to BTBR mice (M = 3.95 sec, SEM = 0.63), and males demonstrated higher push times (M = 7.54 sec, SEM = 0.63) than females (M = 5.62 sec, SEM = 0.65; see Figure 6). All interactions were not statistically significant.

Figure 5

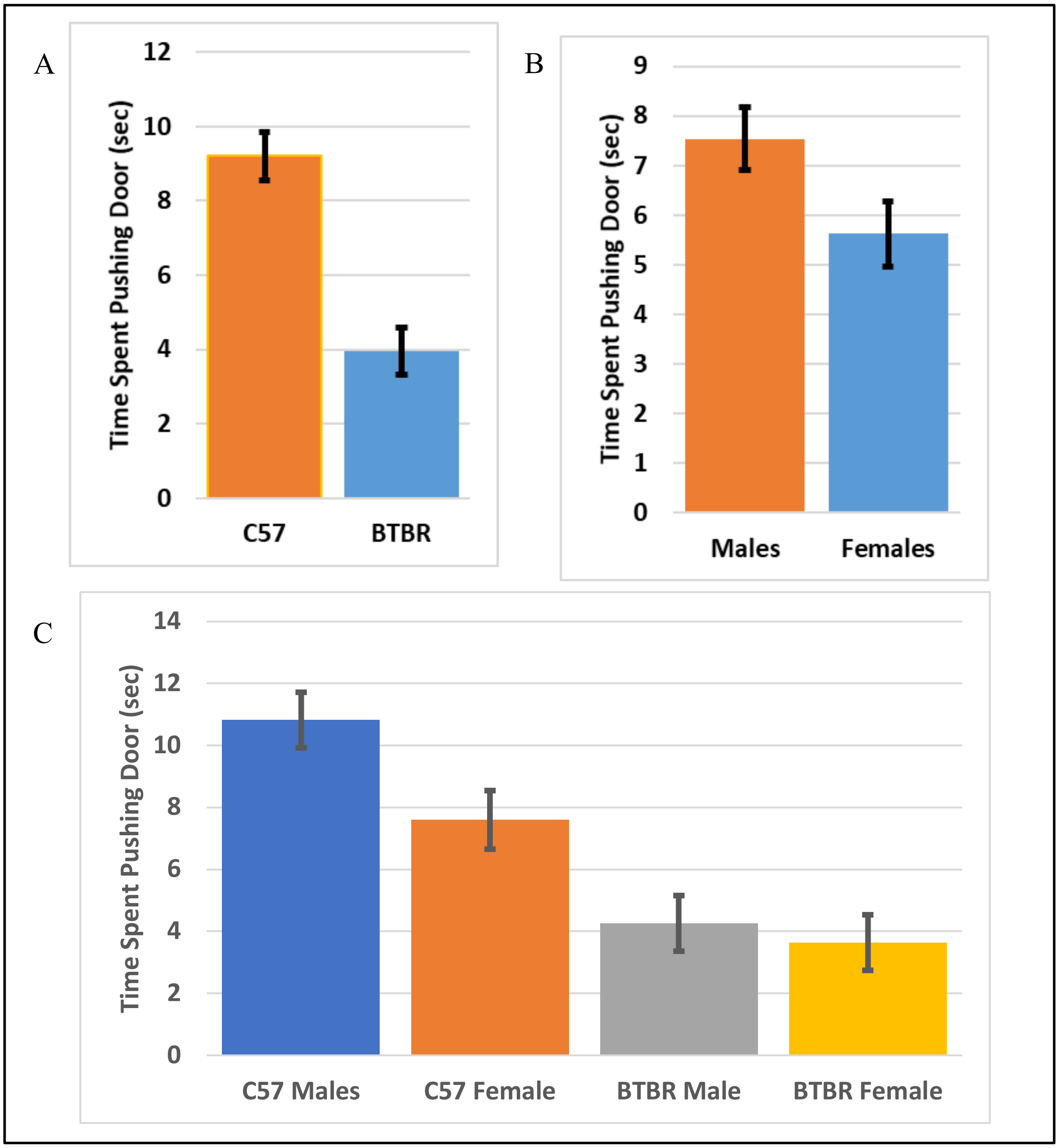
Time Spent Pushing the Social Door across Each Weight



Note. Time spent pushing the door to gain access to the social stimulus across each weight is depicted. Weight was a statistically significant factor in a within-subjects ANOVA ($p = 0.002$). Time spent pushing the door decreased as the weight of the door increased for all groups.

Figure 6

Between-subject differences of sex and strain.



Note. Figure 6 depicts the between-subject differences in sex and strain. (A) C57 mice displayed more social motivation than BTBR, $p < 0.05$. (B) Males displayed more social motivation than females, $p < 0.05$. (C) Average time spent pushing weighted doors across each of the four experimental groups.

Discussion

This study demonstrates that social motivation differs between sexes and mouse strains. Notably, male mice displayed higher social motivation than their female counterparts, and BTBR mice were found to have lower social motivation compared to the C57 mice. This is an expected finding as BTBR mice have been documented to have reduced frequencies and durations of social interactions. For example, in the research of Bolivar et al. (2007) studying autism-like behavior in different mouse strains, the researchers found clear differences in social interactions among the strains. Specifically, the BTBR mice were found to have spent the least amount of time engaging in social behavior.

Due to these social traits, BTBR mice, while an inbred strain, are often used as mouse models for Autism Spectrum Disorder (ASD). Moy et al. (2007) conducted a research study in the context of autism, focusing on the phenotypes of 10 inbred mouse strains, including BTBR. Their research involved employing various behavioral tasks, such as the three-chamber test, to demonstrate how this strain of mice models ASD by emulating core symptoms of autism. The BTBR mice exhibited reduced sociability, displayed less preference for social novelty, had lower sniffing times, and showed deficits in social communication. Our findings extend this work by demonstrating lower social motivation in the context of the weighted doors task, in which mice must exert effort to reach a social stimulus. This suggests that differences in sociability might be directly linked to social motivation in this model.

In our study, males were found to have more social motivation than females. Similarly, another study investigating social reward in juvenile mice found that the male mice presented higher social play and interaction levels in comparison to the female mice (Panksepp & Lahvis, 2007). There are also often sex differences in various drug disorders, including addiction to drugs like cocaine and opioids. Male mice frequently show greater motivation for drug self-administration and are more vulnerable to addicted-related behaviors than the female mice (Fattore et al., 2014). This correlates with our findings regarding the male mice exhibiting more social motivation than the female mice.

The weighted doors task proved to be useful for the objective of our study but also lays the foundation to be used in future neuroscience research involving mouse models, namely in research in the realm of ASD. Social motivation is a key factor when considering ASD behavior and can be very helpful in understanding ASD as well as determining interventions. BTBR mice are commonly used ASD mouse models due to their autistic-like behaviors and the validation of the weighted doors task supports the application of using this test in other ASD models. ASD consists of many symptoms and genetic heterogeneity, therefore having a validated test like the weighted doors task allows the opportunity to compare studies across different mouse models.

While this study's findings are important and relevant to current ASD literature, the limitations of the study should also be noted. These limitations allow for future research as well as a more comprehensive understanding of the findings of this study. One limitation is that the variability between sexes in our study may warrant larger sample sizes for more robust conclusions. In our study, we exclusively used the BTBR strain as the model with low sociability, and while we did get results, it limits generalization to other ASD mouse models. In this specific study, the only test used was the weighted doors task. However, comparing this task with other validated social tests like the ladder task would allow for more of a comprehensive analysis of social motivation and a comparison of convergent validity. Lastly, physical traits such as weight could be a potential confounding variable and may predict push time.

Future studies should investigate the brain pathways of social motivation and focus on determining the neurotransmitters or neuroanatomical regions responsible for variations in social motivation. For example, exploring the role of oxytocin, which is known for being involved with social bonding and social motivation, using the weighted doors task could shed more light on oxytocin's involvement in social motivation. Additionally, it could be valuable to compare various autism mouse models that represent different genes and phenotypes related to social motivation. Extending this research to rats, which exhibit more elaborate social behaviors than mice, can allow a broader perspective of social motivation, especially in the context of ASD.

Conclusion

This research revealed significant differences in social motivation between sexes and between the mouse strains C57 and BTBR. Specifically, the male mice displayed higher levels of social motivation compared to the females, and the C57 mice exhibited higher levels of social motivation in comparison to the BTBR mice. These findings are consistent with the BTBR mice's use as an Autism Spectrum Disorder model for neuroscience research.

These results are important as they provide empirical evidence to support the use of the weighted doors task as a robust and reliable measure of social motivation, validating the weighted doors task for future neuroscience research and assisting in standardizing the measurement of social motivation. This outcome provides credibility for future animal studies investigating social behaviors.

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Appendix

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

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	☒ Laboratory ☐ Field Research ☐ Field Study ☒ 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)

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

- **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