Introduction: The proposed project will explore the social motivation in five different inbred mice on social behavior. The Rogers Lab has been working to establish behavioral measures of social motivation in mice as the field lacks a standard measurement of this behavior. We will compare social motivation measurements to standard measurements of social behavior such as the 3 chamber, tube test, and open chamber test. Performing these tests in five mouse strains will establish the measures as standards to be used by other research labs.

Hypothesis 1: *The five strains of mice will vary in their social motivation, but social motivation will have only a modest correlation with typical social behavior measurements.*

Hypothesis 2: *Measurements of social motivation will be highly correlated with each other.*

Hypothesis 3: *Measurements of motor function, health, and anxiety will not be strongly correlated with social motivation.*

Background: The current study will establish measurements for social motivation in mice for use by other research labs. Mice are an excellent animal model for exploring the neurobiology of social behavior as mice display complex social behaviors. Mice are naturally social and driven to explore new social cues (Nadler et al., 2004). Mice establish hierarchies in their home cage and engage in mating and parenting behaviors (Robbers et al., 2021, Lee & Brown 2002, Kokocińsk et al., 2017).

This study will compare inbred mouse strains and their levels of social motivation. Inbred strains are wildtype strains initially occurring naturally and then inbred in labs to be genetically identical within each strain. The purpose of inbred strains is to use a typical or unaltered mouse or for use as a control to compare against mutant models. Each inbred strain has its own

idiosyncrasies such as a tendency toward retinal degeneration or anxiety. Therefore, different labs have preferences for different inbred strains depending on their research aims and measures. Along with many other studies, we will be using the inbred mouse strain C57BL/6J as they display average levels of social behavior (Netser et al., 2020). While C57BL/6J mice are a standard mouse model, we will include four other inbred mouse strains for comparison and to encourage other labs using other inbred strains to use our measurements of social motivation.

The study of social behavior in mice is well established. Among these measurements, the most common is the three chamber developed by Jacqueline Crawley (Moy et al., 2007). However, typical measurements of social behavior simply ask how interested the mouse is in another mouse. Social motivation is distinct from general social interest and requires effort to access another mouse. The measurements of social motivation developed by our lab require mice to exert effort to access another mouse. The weighted doors task requires mice to push doors with increasing weight to access another mouse. The platform task requires a mouse to climb to a platform that increases in height to access another mouse. Finally, the social operant chamber requires mice to press a lever an increasing number of times to access a social partner.

Purpose: The overall purpose of this experiment is to establish a series of measurements for social motivation using five different inbred mice strains. We will also attempt to establish divergent validity by showing that social motivation measurements are distinct from well-established general sociability measurements. We also expect to establish convergent validitiy by showing that multiple measurements of social motivation are highly correlated with each other. We will also demonstrate that any changes in social motivation performance between mouse strains is not due to motor skill, general health, or anxiety by measuring motor function, health, and anxiety across each strain.

Methods: Both male and female mice from strains C57BL/6J, DBA/2J, FVB/NJ, BALB/cByJ, and BTBR will participate in five different behavioral tasks. These five strains are the most commonly used inbred mice.

Prior to social testing the mice will be habituated to research handling, checked for general health, measured for impairments in motor function, and tested for anxiety using the elevated plus maze (see Figure 1). The elevated plus maze consists of a cross-shaped apparatus suspended approximately two feet off the ground. Two arms of the cross are "open" such that they have a floor but no walls. The other two arms are "closed" as they have a floor and walls. Mice have a natural inclination to explore but also do not like to be at heights. Therefore, mice that are more anxious will stay in the closed arms more while mice that are less anxious will explore the open arms more.

General social behavior will be measured with two behavioral tests. The first test will measure dominance within the cage using the Tube Test (see Figure 2). This will be done by placing a tube down, and then selecting two mice from the same cage. One mouse will be placed in the opening of the tube and the other mouse will be placed in the other side of the tube. Both mice will be let go at the same time. The more dominant mouse will cause the submissive mouse to back out of the tube. This process will be repeated until we have the most dominant mouse from the cage. One week after the Tube Test we will conduct the 3 Chamber Test (see Figure 3). This will allow us to measure the sociability preference of the mice. This has three phases. For the first phase, the test mouse will be put in the middle of an arena that has three chambers. The test mouse is free to move around the entire arna for ten minutes. After ten minutes, we will introduce a novel mouse to the test mouse by placing a mouse under a pencil cup. The test mouse will have the choice of interacting with the novel mouse or freely move around the arena for ten

minutes. After that ten minutes, we will introduce another novel mouse in the third chamber. The test mouse will be given the opportunity to either freely roam the arna, interact with the mouse it already met, or interact with the novel mouse for ten minutes. One week later, we will test the mice for social motivation. We will first use the weighted doors task. The mice will be placed in an arena with a swinging door separating two chambers. The test mouse is placed in one chamber and the door is progressively weighted with increasingly heavy weights. We will measure the amount of weight the mouse is willing to push to access a social partner in the next chamber. Next we will use the platform task in which the mouse is required to climb to a platform of increasing height to access a social partner. Next, we will use the free dyadic social interaction test to measure general social approach (see Figure 4). The test mouse will be placed in an arena with another mouse, and we will measure the amount of time the test mouse sniffs the partner or approaches the partner. Lastly, we will use the social operant chamber that requires the mouse to press a lever an increasing number of times to access a social partner.

Overall, the study is going to use a total of 150 mice. This is broken down into 10 groups of mice based on two independent variables: strain and sex. Table 1 in the appendix shows the breakdown of each group that contributes to the overall use of 150 mice.

Previous Experience and Application: I have previously worked with Dr. Rogers on experiments examining social behavior in mice in her lab. I have gained experience in conducting behavioral studies with mice and in applying drugs via injections. Other skills I have gained from this lab are daily care of mice, weaning, ear tagging, stereotaxic surgeries, drug administration, anesthesia, euthanasia, and data analysis/coding. My major is Psychology and my career goal is to become a Neuropsychologist to help veterans who have PTSD and traumatic

brain injuries. Working on these projects will give me important skills to be more competitive for graduate schools to pursue these goals.

My Role in the Project: I will participate in the behavioral measures for the mice and the daily care which includes feeding, watering, and changing their cages. I will particularly focus on the social behavior tasks the mice will be participating in, their social interactions, coding the results, and analyzing the data from these tests. I will also take a lead role in the project as I have worked with the mice before in basic handling and care techniques. I will ensure that the project stays on the proposed timeline and help organize other students working on this project in the Rogers Lab.

Collaboration with Faculty Mentors: This collaboration will be facilitated both inside and outside the laboratory. During the training period, Dr. Rogers and I will meet daily so I can gain new skills related to running the behavioral tests. Following the training period, weekly meetings will begin in which Dr. Rogers will provide feedback specifically for this project regarding lab work, analysis, and creating figures to move towards the overall goal of a poster presentation. She will also review the data and writing that I will be working on for the publication of this project. Additionally, I participate in weekly lab meetings for the Rogers Lab, so I will have additional times to meet with Dr. Rogers and fellow students working on the project.

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Appendix

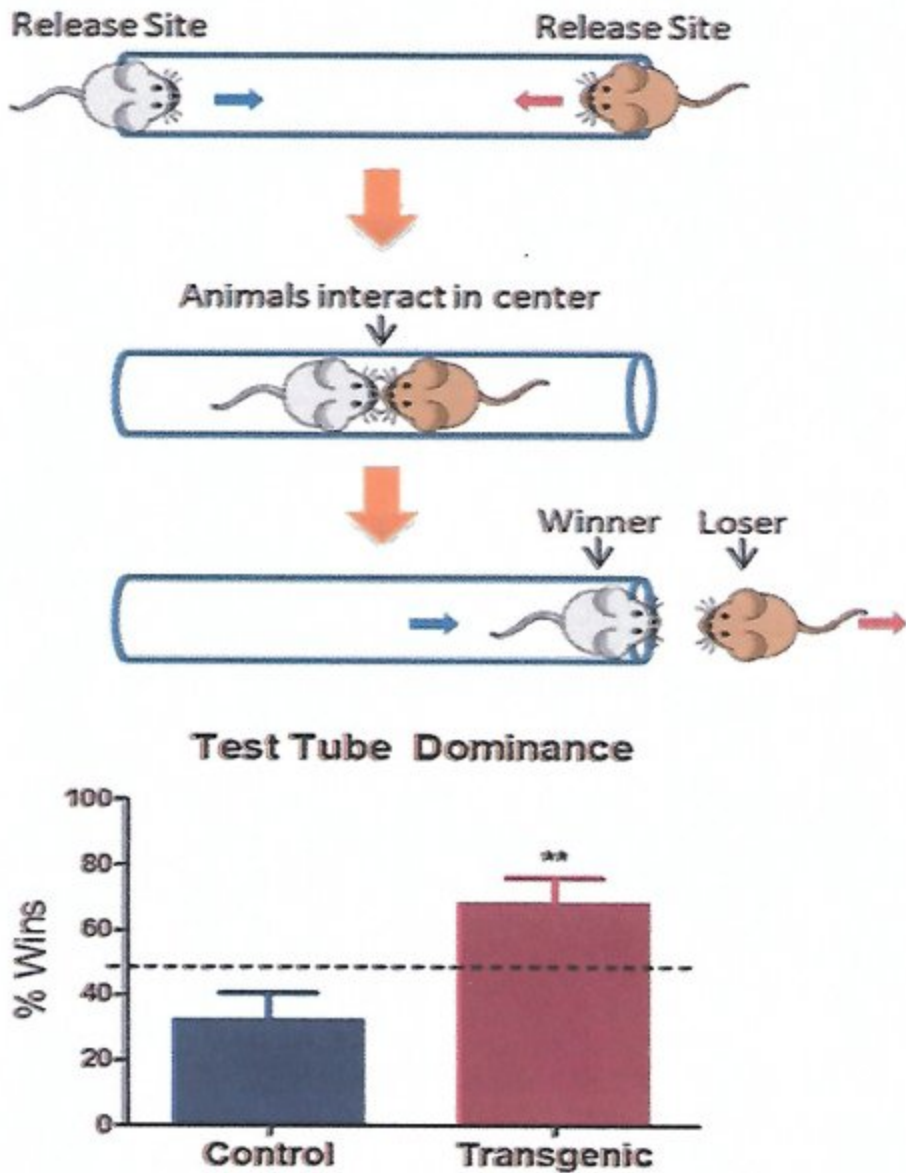Figure 1: Elevated Plus Maze Apparatus. The figure depicts the elevated plus maze apparatus.

Figure 2: Mouse Tube Test. The figure depicts how the tube test is conducted and an example of potential findings.

Figure 3: 3 Chamber Sociability Test. The figure demonstrates the three phases of the three-chamber sociability test and the apparatus.
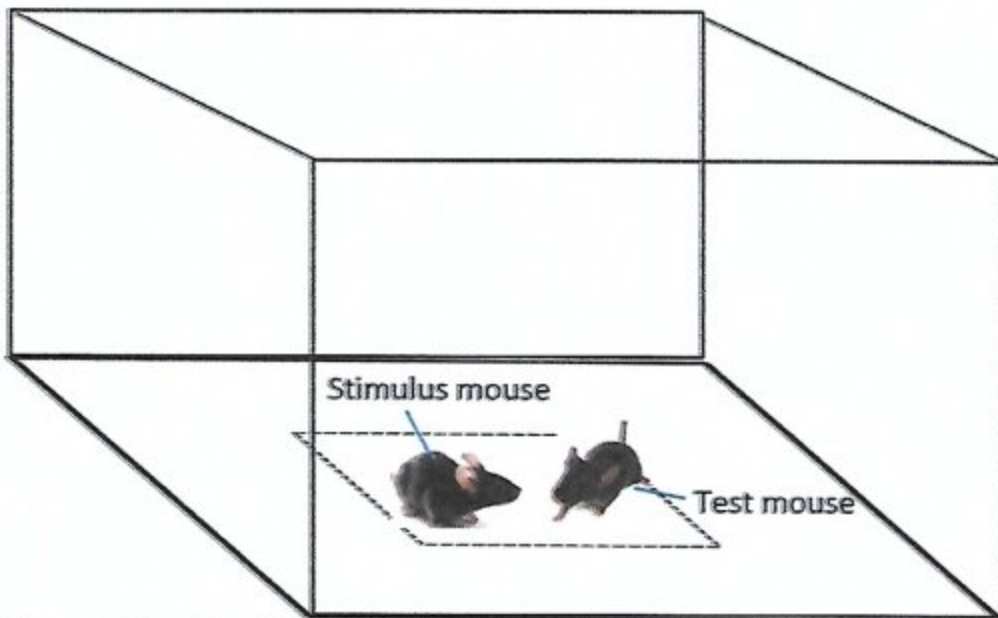
Figure 4: Free Dyadic Social Interaction Test. The figure shows the how the free dyadic social interaction test is conducted.

Sex	Breed	TOTAL
Male	C57BL/6J x 15	**150 mice**
	DBA/2J x 15	
	FVB/NJ x 15	
	BALB/cByJ x 15	
	BTBR x 15	
Female	C57BL/6J x 15	
	DBA/2J x 15	
	FVB/NJ x 15	
	BALB/cByJ x 15	
	BTBR x 15	

Table 1: The number of mice used in each group to get to the total of 150 mice being used.

Timeline

Week 1: Handling Mice

Week 2: General health tests

Week 3. Motor function tests

Week 4: Elevated Plus Maze

Week 5: Tube Test

Week 6: 3 Chamber Test

Week 7: Weighted Doors Test

Week 8: Platform Task

Week 9: Free Dyadic Social Interaction Test

Week 10: Social Operant Chamber Test

Week 11-12: Data Analysis

Week 13: Writing results and figure creation to prepare for poster presentation

100 total hours/13 weeks = approximately 7.5 – 8 hours/week