Title: A Multiple Integrated Social Stress Model for Psychiatric Disorders in Female C57BL/6J Mice

Authors and Affiliations:

Xiaojing Zhai[1,2,3], An Xie[1,2,3], Wenxin Zhang[1,2,3], Lin Ai[1,2,3], Hongxing Zhang[1,2,3]

[1]Jiangsu Province Key Laboratory of Anesthesiology, Xuzhou Medical University
[2]Jiangsu Province Key Laboratory of Anesthesia and Analgesia Application Technology, Xuzhou Medical University
[3]NMPA Key Laboratory for Research and Evaluation of Narcotic and Psychotropic Drugs, Xuzhou Medical University

Corresponding Authors:

Hongxing Zhang   (hongxing.zhang@xzhmu.edu.cn)
Xiaojing Zhai   (1031233461@qq.com)

Email Addresses for All Authors:

An Xie   (xiean971005@163.com)
Wenxin Zhang   (wxzhang1999@163.com)
Lin Ai   (1259089256@qq.com)
Hongxing Zhang   (hongxing.zhang@xzhmu.edu.cn)
Xiaojing Zhai   (1031233461@qq.com)

Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

3. Filming location: Will the filming need to take place in multiple locations? **NO**

Current Protocol Length
Number of Steps: 10
Number of Shots: 30

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

REQUIRED:

1.1. **Xiaojing Zhai**: This research aims to understand how chronic social stress leads to psychiatric symptoms in female mice by establishing a multi-integrated social stress model.

   1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.1.1., 3.1.2.*

What research gap are you addressing with your protocol?

1.2. **Xiaojing Zhai:** Most currently available models for psychiatric disorders have been established using male animals. This protocol introduces a new mouse model designed to study these disorders in female mice.

   1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4.1, 3.4.2.*

What new scientific questions have your results paved the way for?

1.3. **Xiaojing Zhai:** This study has developed a reliable animal model that allows us to investigate the neurophysiological mechanisms underlying psychiatric disorders in female mice.

   1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 4.*

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Xuzhou University Animal Care and Use Committee

Protocol

2. **Training for Tube Competition, Winner Screening, and CD1 Aggressor Screening**

    Demonstrator: Xiaojing Zhai

2.1. To begin, place a 10-centimeter-long, 26-millimeter internal diameter acrylic tube in each cage of female subject mice and winner candidates to allow them to acclimate and learn to pass through it over 3 days **[1-TXT]**.

    2.1.1. WIDE: Talent placing an acrylic tube in the cage of a female subject mouse. **TXT: Handle the mice for 1 – 2 min/day for 3 days to reduce stress from unfamiliar experimenters**

2.2. To train mice for tube competition, gently grasp the tail of each mouse and place it on the table **[1]**. Then, hold the mouse by the tail and place it at one end of the tube **[2]**. Release the tail once the mouse enters the tube **[3]** and allow it to pass through voluntarily **[4]**. Now, place the mouse at the opposite end of the tube and repeat the same procedure **[5]**. After repeating the entire process once more, return the mouse to its home cage **[6]**. NOTE: The VO has been edited.

    2.2.1. Talent picking up a female mouse by its tail and placing it gently on the table.

    2.2.2. Talent holding the mouse by the tail and placing it at one end of the acrylic tube.

    2.2.3. Talent releasing the tail as the mouse enters the tube.

    2.2.4. The mouse walks through the tube.

    2.2.5. Talent placing the mouse at the other end of the tube and releasing the tail as the mouse enters the tube.

    2.2.6. Talent placing the mouse back into its home cage. **TXT: Perform this training protocol once daily for 3 consecutive days** NOTE: The onscreen text has been added here.

2.3. For winner screening, prepare a 30-centimeter-long, 26-millimeter internal diameter acrylic tube and draw a visible middle line on the tube **[1]**. Hold both winner candidate mice by their tails and place them at opposite ends of the tube **[2]**. Allow them to enter and meet at the midpoint **[3]**, then simultaneously release them and start the timer **[4]**. Identify the winner as the mouse that pushes the other out of the tube and award it 1 point **[5-TXT]**. NOTE: The VO has been edited.

2.3.1. Talent drawing a visible middle line on the tube.

2.3.2. Talent holding two mice by their tails and positioning them at opposite ends of the tube.

2.3.3. Mice approaching each other at the midpoint inside the tube.

2.3.4. The mouse being released and the talent starting the timer.

2.3.5. One mouse pushing the other out of the tube. **TXT: Reverse the mice's starting positions and score the outcome again**

2.4. Return both mice to their home cage **[1-TXT]**. Rank the mice based on their total scores **[2-TXT]**.

2.4.1. Talent placing both mice back into their home cage. **TXT: Use a round-robin format to match each mouse with other cage mates**

2.4.2. Talent analyzing a pre-prepared ranking chart or table displaying the mice's competition scores. **TXT: The highest-scoring mice will be used for subsequent experiments as the "winners"** *Videographer: Please make sure the computer screen is visible in this shot.* NOTE: The onscreen text has been added here.

2.5. For CD1 *(C-D-one)* aggressor screening, place a C-57 *(C-fifty-seven)* male mouse directly into the home cage of a CD1 male for 3 minutes **[1]**. Record the latency to the first attack, the number of attacks, and the duration of aggressive behaviors within the 3-minute period as indicators of aggressiveness and repeat this screening procedure for two additional days **[2-TXT]**. NOTE: The VO has been edited.

2.5.1. Talent placing a C57BL/6J male mouse into a cage containing a CD1 male.

2.5.2. Talent reviewing the data logging interface (such as a table or a chart) displaying the above-mentioned recorded metrics. **TXT: CD1 mice with attack latency <1 min on at least 2 of 3 screening days will be selected as aggressors** *Videographer: Please make sure the computer screen and the talent reviewing the data both are visible in this shot.* NOTE: The onscreen text has been added here.

3. **Modeling Procedure for Inducing Social Stress in Mice**

3.1. For the social competition failure, place a Multiple Integrated Social Stress or MISS *(Miss)* mouse- and a "winner" mouse at opposite ends of the tube **[1]** so that they walk forward and meet at the midpoint **[2-TXT]**. Switch the positions of the two mice

and repeat the interaction **[3]**. Then, return both the MISS and "winner" mice to their home cages **[4-TXT]**. NOTE: The VO has been edited.

> 3.1.1. Talent placing the MISS mouse and the winner mouse at opposite ends of the tube.

> 3.1.2. The mice walk forward and meet at the midpoint. **TXT: The MISS mouse will be pushed out of the tube by the "winner" mouse** NOTE: The onscreen text has been added here.

> 3.1.3. Talent switching the mice's positions and the mice walk forward and meet at the midpoint.

> 3.1.4. Talent placing both mice back into their home cages. **TXT: Introduce a novel winner mouse and repeat the procedure twice with the same MISS mouse**

3.2. For the modified vicarious social defeat stress modeling, place a female MISS mouse inside a CD1 aggressor's cage, confined beneath a perforated acrylic cover, to allow vicarious observation of aggressive interactions **[1-TXT]**. Introduce a male C-57 intruder mouse into the cage **[2]** to be physically attacked by the CD1 mouse for 10 minutes **[3]**. After 10 minutes, remove the male intruder **[4]** and allow the female MISS mouse an additional 5 minutes of sensory exposure to the CD1 mouse **[5]**.

> 3.2.1. Talent placing a female MISS mouse inside a CD1 aggressor's cage, confined beneath a perforated acrylic cover. **TXT: CD1 aggressors are pre-screened for consistent aggressive behavior**

> 3.2.2. Talent introducing the male C57BL/6J intruder mouse into the cage.

> 3.2.3. The CD1 mouse attacking the male C57BL/6J intruder mouse.

> 3.2.4. Talent removing the intruder mouse.

> 3.2.5. A shot of the MISS mouse staying under the cover inside the cage, observing the CD1 mouse.

3.3. After 5 minutes, replace the CD1 mouse with a novel male C-57 intruder mouse and repeat the entire procedure three times **[1]**. Then, return the MISS mouse to its home cage **[2]**.

> 3.3.1. Talent replacing the CD1 mouse with a novel male C57BL/6J intruder mouse into the cage.

> 3.3.2. Talent returning the MISS mouse to her home cage.

3.4. Next, to model inescapable overcrowding stress, place an opaque sleeve inside a standard cage containing bedding **[1]**. Introduce 12 to 15 female mice, either all MISS mice or mixed with other females, into the sleeve for 30 minutes daily **[2]**.

    3.4.1. Talent positioning the opaque sleeve inside a cage with bedding.

    3.4.2. A shot of a group of female mice into the sleeve.

3.5. For social isolation, house MISS mice individually when they are not undergoing social competition failure, vicarious defeat stress, or overcrowding stress, for the remainder of the 10-day modeling period **[1]**.

    3.5.1. Talent placing a single MISS mouse into an individual cage, isolating it from other mice.

Results

---

4. **Representative Results**

4.1. The sucrose preference test, which evaluates anhedonia by measuring preference for a sweet solution over water **[1]**, showed that sucrose preference was significantly lower in MISS-exposed mice compared to naïve controls **[2]**.

 4.1.1. LAB MEDIA: Figure 3B.

 4.1.2. LAB MEDIA: Figure 3B, 3C. *Video Editor: Highlight the blue bar in 3C*.

4.2. Total liquid intake in the sucrose preference test, including both water and sucrose, did not differ significantly between MISS and naïve mice, indicating that the reduced sucrose preference was not due to altered fluid consumption **[1]**.

 4.2.1. LAB MEDIA: 3D.

4.3. The tail suspension test, which assesses behavioral despair by measuring passive immobility, revealed that **[1]** MISS-exposed mice had significantly longer immobility times than naïve mice **[2].**

 4.3.1. LAB MEDIA: Figure 3E.

 4.3.2. LAB MEDIA: Figure 3E, 3F. *Video Editor: Highlight the blue bar in 3F*.

4.4. Additionally, the open field test, used to evaluate anxiety by measuring the time spent in the center of an open arena, showed that **[1]** MISS-exposed mice spent significantly less time in the central zone compared to naïve mice **[2]**.

 4.4.1. LAB MEDIA: Figure 4A,

 4.4.2. LAB MEDIA: Figure 4A, 4B. *Video Editor: Highlight the blue bar in 4B.*

4.5. The elevated plus maze, which measures anxiety through avoidance of open arms, showed that **[1]** MISS-exposed mice spent significantly less time in the open arms than naïve controls **[2]**.

 4.5.1. LAB MEDIA: Figure 4D.

 4.5.2. LAB MEDIA: Figure 4D, 4E. *Video Editor: Highlight the blue bar in 4E.*

Pronunciation Guides:

1. **Acrylic**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/acrylic

- **IPA**: /əˈkrɪlɪk/
- **Phonetic Spelling**: uh-kril-ik

2. **CD1**
- **Pronunciation link**: No confirmed link found
- **IPA**: /si di wʌn/
- **Phonetic Spelling**: see-dee-one

3. **Anhedonia**
- **Pronunciation link**: https://www.merriam-webster.com/dictionary/anhedonia
- **IPA**: /ˌænhiˈdoʊniə/
- **Phonetic Spelling**: an-hee-doh-nee-uh

4. **Immobility**
- **Pronunciation link**: https://www.merriam-webster.com/dictionary/immobility
- **IPA**: /ˌɪmoʊˈbɪləti/
- **Phonetic Spelling**: ih-moh-bil-uh-tee

5. **Vicarious**
- **Pronunciation link**: https://www.merriam-webster.com/dictionary/vicarious
- **IPA**: /vaɪˈkɛriəs/
- **Phonetic Spelling**: vy-kair-ee-uhs

6. **Perforated**
- **Pronunciation link**: https://www.merriam-webster.com/dictionary/perforated
- **IPA**: /ˈpɜːrfəˌreɪtɪd/
- **Phonetic Spelling**: pur-fuh-ray-tid

7. **Opaque**
- **Pronunciation link**: https://www.merriam-webster.com/dictionary/opaque
- **IPA**: /oʊˈpeɪk/
- **Phonetic Spelling**: oh-payk

8. **Sucrose**
- **Pronunciation link**: https://www.merriam-webster.com/dictionary/sucrose
- **IPA**: /ˈsuːkroʊs/
- **Phonetic Spelling**: soo-krohs