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Title: A Chronic Sleep Fragmentation Model Using Vibrating Orbital Rotor to Induce Cognitive Deficit and Anxiety-Like Behavior in Young Wild-Type Mice

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group?

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **22**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Fengfei Ding**: Does chronic sleep disturbance lead to neurodegenerative disease pathogenesis? This simple, stable, and long-term protocol can be used to model frequent interruptions during human sleep to address this question **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Yi Xie**: This method minimizes handling labor and artificial bias and standardizes the interventions among mice within the same experimental group and between different experiments to produce repeatable behavioral and pathological outcomes **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Simiao Chen**: This method can be used to characterize pathological and behavioral outcomes even in young mice after 1 month of treatment and to test the effects of new therapies on chronic sleep disturbance-related deficits **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.4. **Yi Xie:** This model can be applied for a variety of disease models, such as depression and Alzheimer's disease, to study the impacts of chronic sleep disturbance in the presence of an underlying disease **[1]**.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

Protocol

2. Mouse Screening Preparation

2.1. Begin by randomly assigning three to five 20-28-gram, 8-10-week-old, adult, wild-type, male mice to CSF (C-S-F) modeling **[1-TXT]** and control cages to avoid social isolation stress **[2-TXT]**.

2.1.1. WIDE: Talent placing mouse into CSF cage **TEXT: CSF:** chronic sleep fragmentation

2.1.2. Talent placing mouse into control cage **TEXT: Ear tag animals for monitoring**

2.2. House both groups of cages in the same 21-23-degree Celsius and 35-60% humidity modeling room with a 12-hour light-dark cycle to maintain an identical surrounding environment **[1]**.

2.2.1. Talent pushing cage cart into room/placing cages onto cage rack or similar

2.3. Then provide the mice with sufficient food and water **[1-TXT]**, using long nozzles with ball valve tips on the water bottles to prevent water leakage upon the platform movements **[2]** and fastening the water bottle on top of each cage with a spring to avoid dislocation of the bottles during rotation **[3]**.

2.3.1. Talent adding food to cage(s) **TEXT: Minimize noise and interference while in modeling room**

2.3.2. Shot of water bottle

2.3.3. Bottle being secured to cage with spring *Videographer: Important step*

3. Chronic Sleep Fragmentation (CSF) Modeling

3.1. For chronic sleep fragmentation modeling, program an electrically controlled orbital rotor with a 67- x 110-centimeter platform to run during the 8:00 AM to 8:00 PM light phase **[1]** and use a solid-state timer to set the rotor speed to 110 revolutions per minute for a repetitive 10 seconds on, 110 seconds off cycle **[2]**.

3.1.1. WIDE: Talent setting rotor on cycle *Videographer: Important step*

3.1.2. Talent setting timer speed and rotation cycle *Videographer: Important step*

3.2. Use thick springs to fasten the CSF cages on top of the rotor platform to prevent dislocation of the cages upon platform rotation **[1]** and confirm that the food and water access are maintained during the orbital rotations **[2-TXT]**.

3.2.1. Talent fastening cage to platform *Videographer: Important step*

3.2.2. Shot of cage rotating/food and water being able to be accessed OR Talent checking food and water access on rotating cage *Videographer: Important step*
TEXT: Check/refill food and water every 2 d

3.3. After setting up the model system, observe the cages for least for 1 hour to ensure that the orbital rotor is operating appropriately **[1]**.

3.3.1. Cages on rotator

3.4. During the modeling period, weigh the mice weekly at 8:00 AM when changing the bedding **[1-TXT]** and remove any aggressors from the cages as necessary **[2]**.

3.4.1. Talent placing mouse onto balance *Videographer: Important step*

3.4.2. Talent removing mouse from cage *Videographer: Important step*

3.5. At the end the modeling period, continue to house and feed the mice in the modeling room **[1]**.

3.5.1. Talent adding food and/or water to cage(s)

4. Morris Water Maze (MWM) Test

4.1. To perform a Morris water maze test, between 8:00 AM and 12:00 PM every day for 5 days, release one mouse at a time into a circular tank filled with 20-23-degree Celsius water in one of four quadrants for each trial **[1]**.

4.1.1. WIDE: Talent placing mouse into quadrant

4.2. During the trial, use a video tracking system to record the escape latency of each mouse **[1]** as it attempts to swim to the platform **[2]**.

4.2.1. Talent starting recording/shot of recording system being initiated

4.2.2. Mouse swimming to platform

4.3. If the mouse is unable to locate the platform within 60 seconds, guide the animal to the platform **[1]** and allow it to remain on the platform for 15 seconds **[2]** before returning it to its cage **[3]**.

4.3.1. Mouse being guided to platform

4.3.2. Mouse on platform

4.3.3. Talent placing mouse into cage

4.4. On the sixth day after training, remove the platform from the water tank **[1]** and release the mouse from the northeast quadrant for one final 60-second swim period per animal **[2]**.

4.4.1. Talent removing platform

4.4.2. Mouse being released/swimming

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.3., 3.1., 3.2., 3.4.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?

In step 3, it is crucial to set vibrating cycle and duration parameters, meanwhile regular monitor animal status with minimized disturbance to the modeled mice as regard to the housing environments and experimenter's interventions.

Results

5. Results: Representative Effects of CSF Modeling on Young Wild-Type Mouse Cognitive Abilities and Anxiety-Like Behaviors

5.1. After CSF modeling **[1]**, no differences in the weights of mice between the control and experimental groups are observed **[2]**.

5.1.1. LAB MEDIA: Figure 1B
5.1.2. LAB MEDIA: Figure 1B *Video Editor: please emphasize data lines*

5.2. The CSF group displays a reduced escape ability **[1]** in locating the platform over a period of 5 training days in the Morris water maze behavioral trial **[2]** compared to the control animals **[3]**.

5.2.1. LAB MEDIA: Figure 2A
5.2.2. LAB MEDIA: Figure 2A *Video Editor: please emphasize red data line*
5.2.3. LAB MEDIA: Figure 2A *Video Editor: please emphasize black data line*

5.3. In the probe test **[1]**, the CSF mice spend significantly less time proportionally in the targeted quadrant **[2]**, crossing the previous platform location fewer times **[3]** without an observable swimming speed difference **[4]**.

5.3.1. LAB MEDIA: Figures 2B-2D
5.3.2. LAB MEDIA: Figures 2B-2D *Video Editor: please emphasize red data bar in Figure 2B*
5.3.3. LAB MEDIA: Figures 2B-2D *Video Editor: please emphasize red data bar in Figure 2C*
5.3.4. LAB MEDIA: Figures 2B-2D *Video Editor: please emphasize red data bar in Figure 2D*

5.4. In the familiar phase of the novel object recognition test, there is no significant difference in the total exploration time between the CSF and control groups **[1]** or in the exploration time between objects A1 and A2 in either group **[2]**.

5.4.1. LAB MEDIA: Figure 3 *Video Editor: please add/emphasize ns text in Figure 3A*
5.4.2. LAB MEDIA: Figure 3 *Video Editor: please add/emphasize ns texts and brackets in Figure 3B*

5.5. In the test phase, the Discrimination Index of the CSF mice is significantly reduced **[2]** compared to that of the control animals **[3]**.

5.5.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize CSF data bar in Figure 3C*
5.5.2. LAB MEDIA: Figure 3 *Video Editor: please emphasize Control data bar in Figure 3C*

5.6. In these representative open field test and forced swimming test evaluations **[1]**, the CSF group spent less time in the central zone during the test **[2]** than did the control group **[3]**.

5.6.1. LAB MEDIA: Figure 4
5.6.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize CSF data bar in Figure 4A*
5.6.3. LAB MEDIA: Figure 4 *Video Editor: please emphasize Control data bar in Figure 4A*
5.6.4. LAB MEDIA: Figure 4

5.7. CSF mice also exhibited a longer total distance of movement within the tank **[1]**, suggesting increased spontaneous activity after modeling **[2]**.

5.7.1. LAB MEDIA: Figure 4 *Video Editor: please emphasize CSF data bar in Figure 4B*
5.7.2. LAB MEDIA: Figure 4

5.8. Nevertheless, this CSF modeling did not induce depression-like behavior, as verified by non-significant differences in the immobility time between the two groups subjected to forced swimming test **[1]**.

5.8.1. LAB MEDIA: Figure 4 *Video Editor: please add/emphasize ns text in Figure 4C*

Conclusion

6. Conclusion Interview Statements

6.1. **Yi Xie**: Selecting an appropriate vibrating cycle and modeling duration and a proper timeline for the treatment and phenotype identification are important for the success of the experiment **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.1.)

6.2. **Fengfei Ding**: Traditional sleep deprivation protocols require a great deal of work and are mostly for acute or short-term modeling. This protocol allows the study of the pathological mechanisms of mild but long-lasting sleep disturbance **[1]**.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera