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Title: Chronic Stress Shifts Effort-Related Choice Behavior in a Y-Maze Barrier Task in Mice

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Author Questionnaire

1. Microscopy: Does your protocol demonstrate 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. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **38**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Ben Samuels**: This protocol allows researchers to examine the maladaptive effects of chronic stress on effortful responding in both male and female mice **[1]**.

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

REQUIRED:

1.2. **Andrew Dieterich**: An advantage of this technique is that both male and female mice can simultaneously undergo a validated chronic social defeat paradigm, allowing reward behaviors to be directly compared **[1]**.

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

OPTIONAL:

1.3. **Ben Samuels**: Historically, preclinical research into mood disorders has utilized behavioral tasks that involve avoidance of threatening environments. However, reward and motivation tasks, such as effort-related choice, may offer more translational relevance **[1]**.

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

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Rutgers University.

Protocol

2. Chronic Corticosterone (CORT)

2.1. To prepare the vehicle solution, dissolve 3.375 grams of beta-cyclodextrin in 750 milliliters of tap water in a 1-liter screw-top glass container **[1]**.

2.1.1. WIDE: Talent adding beta-cyclodextrin to water, with beta-cyclodextrin container visible in frame NOTE: Authors did not distinguish between 2.1 and 2.2, got shots showing the beta-cyclodextrin being added to the CORT bottle, so one of those could be used here to show making the Vehicle solution. CORT solution is vehicle solution with CORT powder. *Videographer: Important step*

2.2. To prepare the corticosterone solution, dissolve 3.375 grams of beta-cyclodextrin in 750 milliliters of tap water **[1]** followed by the addition of 26.25 milligrams of corticosterone **[2]**.

2.2.1. Talent adding beta-cyclodextrin to water, with beta-cyclodextrin container visible in frame

2.2.2. Talent adding corticosterone to water, with corticosterone container visible in frame

2.3. Dissolve the corticosterone in solution in an ultrasonic cleaner water bath **[1]** for approximately 30 minutes at 40 kilohertz until the liquid demonstrates a clear appearance **[2]**.

2.3.1. Container being sonicated

2.3.2. Shot of clear solution

2.4. Then fill the water bottles of the vehicle and corticosterone mouse group with the appropriate solutions **[1]** and record the volume of liquid consumed twice a week **[2-TXT]**.

2.4.1. Talent filling water bottle, with mouse cage visible in frame

2.4.2. Talent checking bottle volume **TEXT: Weigh mice in each group weekly**

3. Y-Maze Apparatus Habituation

3.1. To habituate the mice to the Y-maze, the day after food deprivation, place a large number of 20-miligram, grain-based food pellets in the caps of two 50-millilier centrifuge tubes **[1]** and place one cap at the end of each arm of the Y-maze **[2]**.

3.1.1. WIDE: Talent adding pellets to cap
3.1.2. Talent placing cap in arm

3.2. Then place a mouse in the start box of the Y-maze with the start box divider in place for a few seconds **[1]** before removing the divider **[2]** and allowing the mouse to explore the Y-maze for 15 minutes **[3-TXT]**.

3.2.1. Talent placing mouse into box
3.2.2. Talent removing divider
3.2.3. Mouse exploring maze **TEXT: Repeat for each mouse/group**

4. Y-Maze Forced-Choice Training

4.1. After two days of Y-maze habituation, place four pellets in a cap for the high-reward arm **[1]** and place two pellets in a cap for the low reward arm **[2-TXT]**.

4.1.1. WIDE: Talent adding pellets to cap
4.1.2. Talent placing cap into arm **TEXT: Counterbalance which arm is the high-reward arm within each group**

4.2. For a high reward forced-choice trial, block access to the low reward arm **[1]** and place the mouse in the start box for a few seconds **[2]** before removing the divider, allowing the mouse 60 seconds to enter high reward arm to consume the available pellets **[3]**.

4.2.1. Talent blocking LR arm
4.2.2. Talent placing mouse into box
4.2.3. Talent removing divider/mouse entering maze

4.3. For a low reward forced-choice trial, block access to the high reward arm **[1-TXT]**.

4.3.1. Shot of HR arm with cap of pellets in view, then arm being blocked **TEXT: Alternate forced-choice trials until 5 HR and 5 LR trials completed**

5. Y-Maze Free Choice Training

5.1. For free choice training, begin each session with one high and one low arm forced-choice trial **[1]** before placing the mouse into the start box **[2]**. Allow the mouse to select either maze arm **[3]**.

5.1.1. WIDE: Talent blocking one arm *Videographer: Important step*
5.1.2. Talent placing mouse into box *Videographer: Important step*
5.1.3. Mouse selecting arm *Videographer: Important step*

5.2. Once the mouse has traveled to the end of the arm to the cap of pellets, place an arm divider behind the mouse, locking in the animal until it has consumed the pellets **[1]**.

5.2.1. Shot of mouse in end of arm, then divider being placed *Videographer: Important step*

5.3. Record which arm is selected for all 10 free choice trials daily **[1-TXT]**.

5.3.1. Talent watching mouse select arm/recording arm selection **TEXT: Record arm and arm selection latency/trial**

6. Y-Maze Barrier Testing

6.1. Once a mouse has selected the high reward arm for 7 out of 10 trials in a free choice training day, place a 10-centimeter barrier halfway down the high reward arm in the Y-maze **[1-TXT]** and perform at least two high and low arm forced-choice trials with the barrier in place **[2-TXT]**.

6.1.1. WIDE: Talent showing recording data of **TEXT: 7/10 HR arm selection = 70% criterion**

6.1.2. Talent placing mouse into start box, with arm divider visible in frame **TEXT: If mice are resistant to climbing over the barrier, prompt them with a long, thin piece of plexiglass**

6.2. After completing all four trials, place the mouse in the start box **[1]** and allow the mouse to select an arm for 10 free choice trials with the 10-centimeter barrier to the high reward arm in place **[2]**.

6.2.1. Talent placing mouse into box *Videographer: Important step*

6.2.2. Talent lifting divider/mouse exploring maze Author NOTE: Perhaps the mice were a little anxious, but I do not think we got any shots of a mouse leaving the start box and directly choosing and climbing the barrier, so hopefully that can be edited. The main point here is to see that the mice can select and climb over the barrier in the HR side (4 pellets) to consume the greater number of the food pellets. *Videographer: Important step*

6.3. On the following day, habituate and test the mouse as demonstrated but use a 15-centiemter barrier in the high reward arm **[1]**.

6.3.1. Mouse exploring arm/climbing 15-cm barrier

6.4. The next day, test the mouse with a 20-centimeter barrier as demonstrated **[1]**.

6.4.1. Talent placing barrier into arm

7. Chronic Non-Discriminatory Social Defeat Stress (CNSDS)

7.1. For chronic non-discriminatory social defeat stress testing, align the cages of CD-1 (C-D-one) males **[1]** with C57BL/6J (C-fifty-seven-black-six-J) males and females, with CD-1 cages in the front and C57BL/6J cages adjacent to these CD-1 male mice in the social defeat room **[2]**.

7.1.1. WIDE: Talent placing CD-1 cages onto table/bench Author NOTE: The CD-1s are large white males; the C57's are smaller black males and females *Videographer: Important step*

7.1.2. Talent placing C57BL/6J cages *Videographer: Important step*

7.2. To start the trial, place one adult male and one adult female experimental C57BL/6J mouse into the home cage of each CD-1 aggressor male for a 5-minute social defeat session **[1]** and record the attack latency and frequency of attack for both male and female experimental animals **[2]**.

7.2.1. Talent placing mouse/mice into cage *Videographer: Important step*

7.2.2. Talent recording attack latency/frequency Author NOTE: We got plenty of shots of placing the mice and them interacting with each other. No attacks even occurred so don't worry about this during editing! *Videographer: Do not show mouse attacks in shot*

7.3. At the end of the session, transfer the male C57BL/6J mouse into the cage of co-housed CD-1 male separated by a clear, perforated plexiglass barrier **[1]**.

7.3.1. Talent placing mouse into cage Author NOTE: We clearly showed a clear Plexiglas barrier dividing the co-housed CD-1 male (placed directly behind the "attacking" CD-1 male cage), before placing the actual wire-top cage-lid.

7.4. Separate the attacking CD-1 and female C57BL/6J mice with a similar clear, perforated plexiglass barrier **[1-TXT]**.

7.4.1. Mouse being placed into cage **TEXT: Alternate male and female C57BL/6J mouse housing with CD-1 aggressor/d**

7.5. For a control session, place one control female mouse in the home cage of one control male C57BL/6J mouse **[1]**.

7.5.1. Talent placing mouse into cage

7.6. After 5 minutes, place a clear, perforated plexiglass divider between the animals **[1]**.

7.6.1. Divide being placed

7.7. At the end of the first experiment, rotate the mice for the remaining 9 defeat sessions such that each C57BL/6J male and female pair is rotated one cage to the left to provide a new interaction with novel CD-1 mice for each session **[1]**.

7.7.1. Talent rotating mouse cage placement *Videographer: Difficult step*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?

2.1., 5.1., 5.2., 6.2., 7.1., 7.2.

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

7.7.

Results

8. Results: Representative Chronic Stress-Induced Effort-Related Choice Behavior Analyses

8.1. Chronic corticosterone and chronic non-discriminatory social defeat stress mice both exhibit a reduced mean body weight **[1]** compared to vehicle and control animals **[2]**. These mice also consume less mean home cage lab chow **[3]**.

8.1.1. LAB MEDIA: Table 1 *Video Editor: please emphasize CORT and SDS Body Weight data cells*
8.1.2. LAB MEDIA: Table 1 *Video Editor: please emphasize Vehicle and Control Body Weight data cells*
8.1.3. LAB MEDIA: Table 1 *Video Editor: please emphasize CORT and SDS Daily Food Given data cells*

8.2. Vehicle and corticosterone-administered mice consume a similar volume of liquid over 4 weeks of treatment and 3 weeks of behavior testing **[1]**.

8.2.1. LAB MEDIA: Figure 1B *Video Editor: please emphasize black and grey data bars*

8.3. Chronic non-discriminatory social defeat stress produces a maladaptive phenotype in susceptible mice **[1]** compared to either resilient **[2]** or control mice not exposed to chronic non-discriminatory social defeat stress **[3]**, displaying a reduction in time spent in the interaction zone containing a novel CD-1 mouse **[4]**.

8.3.1. LAB MEDIA: Figure 1D *Video Editor: please emphasize dark grey data bar*
8.3.2. LAB MEDIA: Figure 1D *Video Editor: please emphasize light grey data bar*
8.3.3. LAB MEDIA: Figure 1D *Video Editor: please emphasize white data bar*
8.3.4. LAB MEDIA: Figure 1D

8.4. Both chronic corticosterone and chronic non-discriminatory social defeat stress mice **[1]** produce a shift in effortful responding **[2]** when the barrier height increases to 15 and 20 centimeters **[3]**.

8.4.1. LAB MEDIA: Figures 2B and 2C
8.4.2. LAB MEDIA: Figures 2B and 2C *Video Editor: please emphasize grey 15 and 20 CM barrier data bars in Figure 2B*
8.4.3. LAB MEDIA: Figures 2B and 2C *Video Editor: please emphasize light and dark grey 15 and 20 CM barrier data bars in Figure 2C*

8.5. High and low reward arm latency with the 15-centimeter barrier **[1]** is not impacted by

corticosterone administration **[2]** and is similar for both groups with both low and high reward arms **[3]**.

8.5.1. LAB MEDIA: Figure 3
8.5.2. LAB MEDIA: Figure 3 *Video Editor: please add brackets with "n.s." text over LR arm data bars and HR arm data bars*
8.5.3. LAB MEDIA: Figure 3 *Video Editor: please emphasize add bracket with "n.s." text over both groups of data bars*

8.6. Importantly, if chronic corticosterone or chronic non-discriminatory social defeat stress impairs learning of the Y-maze barrier task **[1]**, these mice may fail to reach the criterion in free choice training sessions, impacting subsequent barrier result interpretation **[2]**.

8.6.1. LAB MEDIA: Figure 4 *Video Editor: please add/emphasize brackets and asterisks*
8.6.2. LAB MEDIA: Figure 4

8.7. In addition, the social interaction task can be used **[1]** to stratify the mice into resilient **[2]** and susceptible populations **[3]**, as well as by sex **[4]**.

8.7.1. LAB MEDIA: Figure 5B
8.7.2. LAB MEDIA: Figure 5B *Video Editor: please emphasize Res data bar in left graph*
8.7.3. LAB MEDIA: Figure 5B *Video Editor: please emphasize Sus data bar in left graph*
8.7.4. LAB MEDIA: Figure 5B *Video Editor: please emphasize right graph*

Conclusion

9. Conclusion Interview Statements

9.1. **Andrew Dieterich**: When conducting the Y-maze barrier task, it is critical to record both the selected arm and the latency to reach the food pellet reward in each individual trial **[1]**.

9.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (5.3.)

9.2. **Ben Samuels**: Other important and translationally relevant reward-related behaviors, such as outcome devaluation or progressive ratio, can be performed following the chronic corticosterone, social defeat, or non-discriminatory social defeat protocols **[1]**.

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