

Submission ID #: 72558

Scriptwriter Name: Ruchira Mukherji

Project Page Link: https://review.jove.com/files_upload.php?src=22130238

Title: Paradigm for Social Recognition in Mice for Distinguishing Short- and Long-Term Relationships

Authors and Affiliations:

Anastasia Achziger, Meghan Cum, Albert Li, Nayla Jimenez, Nancy Padilla-Coreano

Department of Neuroscience, University of Florida

Corresponding Authors:

Nancy Padilla-Coreano npadillacoreano@ufl.edu

Email Addresses for All Authors:

anastasiachziger@ufl.edu

mcum@ufl.edu

albert.li@ufl.edu

naylajimenez@ufl.edu

npadillacoreano@ufl.edu

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? **Yes**
All screen recordings have already been uploaded!
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 15

Number of Shots: 31

Interviews

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

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Anastasia Achziger:** We study how mice distinguish familiar from novel individuals using the Four Cups paradigm to uncover mechanisms underlying social recognition and memory.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Anastasia Achziger:** Existing assays inadequately capture social memory; our protocol addresses this gap by simultaneously quantifying recognition of long-term, short-term, and novel social relationships.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.3. **Anastasia Achziger:** Although habituation and consistent environmental conditions minimize variability, researchers should anticipate sex and strain differences in mouse behavioral phenotypes across experiments.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Anastasia Achziger:** This protocol can be combined with electrophysiology, optogenetics, and chemogenetics to investigate neural circuits underlying social recognition.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Anastasia Achziger**: Future studies can adapt this protocol to explore how neural circuits and disease-associated mutations influence different levels of social recognition.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

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

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What is the key takeaway you hope viewers remember after watching your video article?

- 1.6. **Anastasia Achziger, Undergraduate Research Assistant**: (authors will present their testimonial statements live)

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Anastasia Achziger, Undergraduate Research Assistant**: (authors will present their testimonial statements live)

How does the video format of your article help you to share your work with the broader scientific community?

- 1.8. **Anastasia Achziger, Undergraduate Research Assistant**: (authors will present their testimonial statements live)

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC)
at the University of Florida

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Animal Preparation and Housing

Demonstrator: Anastasia Achziger

- 2.1. To begin, habituate adult, same-sex, group-housed mice to handling for at least 2 days before testing [1-TXT]. ~~House mice under a reverse light-dark cycle with ad libitum food and water [3].~~ **NOTE: The VO is edited for the deleted shots and added in a concise way**

2.1.1. ~~WIDE: Shot of animal facility showing same-sex group-housed mice.~~ **NOTE: Not filmed**

2.1.2. Talent habituating the mice by cupping or gently stroking them to minimize stress. **TXT: Maintain a reverse light-dark cycle with ad libitum food and water**

~~2.1.3. Shot of mice being acclimatized to reverse light-dark cycle with unrestricted access to food and water.~~ **NOTE: Not filmed**

- 2.2. Define long-term familiar agents as same-strain, same-sex cage mates, housed with the subject, for a minimum of 3 to 4 weeks [1-TXT]. Maintain same-strain, same-sex short-term familiar and novel agents in different home cages, so that novel agents have no prior exposure to the subject [2]. Pick novel and short-term familiar agents from different home cages to guarantee distinct olfactory cues [3].

2.2.1. Shot of long-term familiar agent mice playing and interacting with test mouse. **TXT Mice - CD-1 Male or Female or C57BL/6 Male**

2.2.2. Shot of novel and short-term familiar agent mice being housed in cages separate from test subject to avoid prior exposure.

2.2.3. Shot of novel and short-term familiar agents being picked from different home cages respectively, to guarantee distinct olfactory cues.

3. Arena Setup

- 3.1. Place a clean, square open-field arena in a quiet room, minimally illuminated using a tall overhead lamp [1-TXT]. Position four identical, tall, heavy, and equally weighted, perforated plastic cups in the corners, with two sides flush against the walls, to standardize access and minimize hiding [2]. Position the camera high enough to capture the entire assay box or arena without blind spots and record continuously [3].

- 3.1.1. Talent preparing a clean square arena in a minimally illuminated room. **TXT-Arena Dimensions: 49.5 cm × 49.5 cm × 49.5 cm; Illumination: 8 – 20 lux**
- 3.1.2. CU: Talent placing four identical, tall, heavy, and equally weighted, perforated plastic cups with two sides flush against the wall in the corners of the arena.
- 3.1.3. Talent positioning the camera in a manner that the entire arena is captured clearly with minimum glare.
- 3.2. Between trials, disinfect [1] and rinse the arena and cups to remove residual odors [2], then let them dry completely [3].
 - 3.2.1. Talent applying disinfectant to the arena floor and cups to remove residual odors. *Videographer: Please obtain multiple reusable takes of 3.2.1 as these will be used later in the protocol.*
 - 3.2.2. Talent rinsing the arena floor and cups with plain water. *Videographer: Please obtain multiple reusable takes of 3.2.2 as these will be used later in the protocol.*
 - 3.2.3. Talent leaving everything to dry out completely before starting with the next trial. *Videographer: Please obtain multiple reusable takes of 3.2.3 as these will be used later in the protocol.*

4. Habituation to Arena at Day 0

- 4.1. Place the subject and its cage mates in the arena, with four empty perforated cups for 20 minutes of free exploration [1], then return them to their home cage [2]. Simultaneously, habituate all social agents from the same home cage to individual cup restraint for 10 minutes [3].
 - 4.1.1. Talent placing the subject and its cage mates, along with empty cups, in the arena for free exploration.
 - 4.1.2. Talent carefully returning all mice to their respective home cage.
 - 4.1.3. Talent habituating mice to restraint by placing each of them under a perforated cup for 10 minutes simultaneously.
- 4.2. Clean the arena and cups afterward [1].
 - 4.2.1. *Reuse 3.2.1 and 3.2.3*

5. Baseline Setting and Short-Term Acquisition

- 5.1. Permit the subject to explore the arena with four empty cups for 10 minutes [1].
 - 5.1.1. Talent releasing the subject mouse to freely explore the arena for 10 minutes.

- 5.2. Confine the subject under a weighted, perforated cup in the center of the arena [1]. Confine the short-term familiar agent under one designated corner cup in the arena, leaving the other three corner cups empty [2]. Release the subject and permit 10 minutes of free exploration [3].

- 5.2.1. Talent placing the subject in the arena center under a weighted perforated cup.
- 5.2.2. Talent placing the short-term familiar mouse under a designated weighted, perforated cup in one randomly chosen corner of the arena.
- 5.2.3. Talent releasing the subject mouse to explore the arena freely for 10 minutes.

6. Inter-Trial Interval

- 6.1. Return the short-term familiar agent to its home cage [1] and let the subject explore the arena alone for 10 minutes [2]. Prepare the novel [3] and long-term familiar agents for testing [4].
 - 6.1.1. Talent returning the short-term familiar mouse back to its original cage.
 - 6.1.2. Talent releasing the subject mouse to explore the arena for 10 minutes.
 - 6.1.3. Talent preparing the novel-agent for the trial by gentle coaxing.
 - 6.1.4. Talent preparing the long-term familiar agent for the trial by gentle coaxing.

7. Social Recognition Phase

- 7.1. Confine the subject under a weighted perforated cup at the center of the arena [1], while placing the long-term familiar, short-term familiar, and novel-agent mice under separate cups [2]; leave the fourth cup empty as a control [3]. Release the subject from the arena center and permit 10 minutes of free exploration [4].
 - 7.1.1. Talent placing the subject in the arena center under a weighted perforated cup.
 - 7.1.2. Talent placing long-term familiar, short-term familiar, and novel agent mice under separate cups randomly.
 - 7.1.3. Shot of one corner cup left empty, with no mouse, to be used as control.
 - 7.1.4. Talent releasing the test subject from the central cup to let it explore the arena freely.
- 7.2. Then, stop recording [1], return all mice to their home cages [2], and thoroughly clean the arena and cups before the next trial [3].

- 7.2.1. Shot of talent pressing the stop recording button on the camera.
- 7.2.2. Shot of mice being returned to their respective home cages.
- 7.2.3. *Reuse 3.2.1 and 3.2.3*

8. Behavioral Scoring

- 8.1. Import videos into the Behavioral Observation Research Interactive Software [1]. Define the behavioral matrix before scoring [2]. Define an investigation bout as the subject positioning its nose within 1 centimeter of a cup while maintaining an investigatory posture, such as chewing or gnawing [3]. Annotate the total investigation time for each cup during the 10-minute social recognition test [4]. Export the data for statistical analysis [5].
 - 8.1.1. Shot of talent sitting at the computer, launching BORIS.
 - 8.1.2. SCREEN: 72558_Screen-1_8.1.2-8.1.3.mp4: 00:15-00:22, 01:48-01:56
 - 8.1.3. SCREEN: 72558_Screen-1_8.1.2-8.1.3.mp4: 02:00-02:11, 03:39-03:40
 - 8.1.4. SCREEN: 72558_Screen-2_8.1.4.mp4: 00:15-00:20, 03:06-03:17 *Video Editor: Please emphasizing the small box at the bottom right-hand corner labeled 'Event 8.2 CD1 F 4 Cups'.*
 - 8.1.5. Talent at the computer, exporting data from BORIS for further statistical analysis.
-

Results

9. Results

- 9.1. In the four-cup social-recognition test, CD-1 male mice showed greater discrimination toward both the novel conspecific [1] and the long-term familiar cage mate [2] than toward the short-term familiar conspecific [3]. Discrimination did not differ much between the novel mouse and the cage mate [4].
 - 9.1.1. LAB MEDIA: Figure 2 **Video Editor:** *Please highlight the green bar in the graph.*
 - 9.1.2. LAB MEDIA: Figure 2 **Video Editor:** *Please highlight the brown bar in the graph.*
 - 9.1.3. LAB MEDIA: Figure 2 **Video Editor:** *Please highlight the central bar in the graph.*
 - 9.1.4. LAB MEDIA: Figure 2 **Video Editor:** *Please highlight the green and brown bars in the graph at the same time.*
- 9.2. In contrast to males, CD-1 female mice showed no differences in discrimination among the novel [1], short-term familiar [2], and long-term familiar conspecifics [3]. Females investigated all three social agents at comparable levels, indicating broad sociability without a preference based on familiarity [4].
 - 9.2.1. LAB MEDIA: Figure 3 **Video Editor:** *Please highlight the green bar in the graph.*
 - 9.2.2. LAB MEDIA: Figure 3 **Video Editor:** *Please highlight the yellow bar in the graph.*
 - 9.2.3. LAB MEDIA: Figure 3 **Video Editor:** *Please highlight the brown bar in the graph.*
 - 9.2.4. LAB MEDIA: Figure 3 **Video Editor:** *Please highlight all three bars in the graph at the same time.*
- 9.3. During their 10-minute social recognition phase, C57BL/6 (*C-fifty-seven black six*) males showed a trend toward greater discrimination of novel [1] than short-term familiar conspecifics [2], with no significant differences between novel and cage mate discrimination [3].
 - 9.3.1. LAB MEDIA: Figure 4 **Video Editor:** *Please highlight the green bar in the graph.*
 - 9.3.2. LAB MEDIA: Figure 4 **Video Editor:** *Please highlight the central bar in the graph.*
 - 9.3.3. LAB MEDIA: Figure 4 **Video Editor:** *Please highlight the green and brown bars in the graph at the same time*

1. Habituate
Pronunciation link: <https://www.merriam-webster.com/dictionary/habituate>
IPA: /həˈbɪtʃuˌeɪt/, /həˈbɪtʃuˌweɪt/
Phonetic Spelling: huh·BIT·choo·ayt / huh·BIT·choo·wayt
2. Ad libitum
Pronunciation link: <https://www.merriam-webster.com/dictionary/ad%20libitum>
IPA: /ˌæd ˈlɪbɪtəm/
Phonetic Spelling: ad·LIB·ih·tuhm
3. Olfactory
Pronunciation link: <https://www.merriam-webster.com/dictionary/olfactory>
IPA: /ɑːlˈfæktəri/, /oʊlˈfæktəri/
Phonetic Spelling: aal·FAK·tuh·ree / ohl·FAK·tuh·ree
4. Perforated
Pronunciation link: <https://www.merriam-webster.com/dictionary/perforated>
IPA: /ˈpɜːfəˌreɪtɪd/
Phonetic Spelling: PUR·fuh·ray·tid
5. Habituation
Pronunciation link: <https://www.merriam-webster.com/dictionary/habituation>
IPA: /həˈbɪtʃuˌeɪʃən/, /həˈbɪtʃuˌweɪʃən/
Phonetic Spelling: huh·bit·choo·AY·shuhn / huh·bit·choo·WAY·shuhn
6. Behavioral
Pronunciation link: <https://www.merriam-webster.com/dictionary/behavioral>
IPA: /bɪˈheɪvjərəl/
Phonetic Spelling: bih·HAYV·yuh·ruhl
7. Investigatory
Pronunciation link: <https://www.merriam-webster.com/dictionary/investigatory>
IPA: /ɪnˈvestəɡəˌtɔːri/
Phonetic Spelling: in·VES·tih·guh·tor·ee
8. Conspecific
Pronunciation link: <https://www.merriam-webster.com/dictionary/conspecific>
IPA: /ˌkɑːnspeɪˈsɪfɪk/
Phonetic Spelling: kon·spuh·SIF·ik
9. Discrimination
Pronunciation link: <https://www.merriam-webster.com/dictionary/discrimination>
IPA: /dɪˌskrɪməˈneɪʃən/
Phonetic Spelling: dih·skrim·uh·NAY·shuhn
10. Sociability
Pronunciation link: <https://www.merriam-webster.com/dictionary/sociability>
IPA: /ˌsoʊʃəˈbɪləti/
Phonetic Spelling: soh·shuh·BIL·uh·tee

