

Submission ID #: 71345

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21760273>

Title: Automated Operant Intermittent Alcohol Drinking in Socially-Housed Mice via the Intellicage Testing System

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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 something similar? **No**
2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done!**
3. **Filming location:** Will the filming need to take place in multiple locations? **Yes, in the same building, just on two different floors (accessible by elevator)**
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: 22

Number of Shots: 52 (26 SC)

Introduction

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. **Lucy Anderson:** Our lab focuses on the cellular and circuit mechanisms mediating compulsive behaviors, specifically individual vulnerability to compulsive alcohol drinking.

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. **Lucy Anderson:** Unlike conventional preclinical models of alcohol use disorder, which rely on singly housing animals, we present a high-throughput social alcohol drinking task.

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

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Lucy Anderson:** Automated, home-cage monitoring systems, such as the IntelliCage, enable long-term assessment of operant, alcohol drinking behaviors among group-housed mice.

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. **Lucy Anderson:** We present code for preliminary analysis of the intermittent alcohol-drinking data, which can be customized for specific research applications.

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. **Lucy Anderson:** This flexible paradigm can be easily adapted to assess drinking in response to variable operant ratios, aversive air-puffs, or other manipulations.

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.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

1.6. Lucy Anderson, former Post-Baccalaureate Fellow, Laboratory on the Neurobiology of Compulsive Behaviors: (authors will present their testimonial statements live)

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

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

1.7. Lucy Anderson, former Post-Baccalaureate Fellow, Laboratory on the Neurobiology of Compulsive Behaviors: (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the National Institutes of Health, Bethesda

Protocol

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

2. IntelliCage Setup for Socially Housed Murine Alcohol Behaviors

Demonstrator: Lucy Anderson

- 2.1. To begin, equip the plastic home cage with 5 compressed cotton fiber pads [1-TXT] and 2 small cotton squares [2-TXT]. Place 4 plastic burrowing structures for bedding and enrichment [3]. Place the top lid with operant corners onto the plastic home cage [4].
 - 2.1.1. WIDE: Talent arranging the compressed cotton fiber pads inside the plastic home cage. **TXT= Fiber pads= 15.24 x 25.4 cm** *Videographer: Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent placing the small cotton squares into the plastic home cage. **TXT: cotton squares= 5.08 x 5.08 cm**
 - 2.1.3. Talent placing the plastic burrowing structures inside the home cage.
 - 2.1.4. Talent positioning the top lid with operant corners over the plastic home cage.
- 2.2. Use provided cables to connect all operant corners to the central microprocessor [1]. Use CAN (*Can*) cables to connect the central microprocessor to a computer preloaded with TSE (*T-S-E*) software [2]. Connect all cages with separate male and female control and limited bedding and nesting, or LBN (*L-B-N*) animal groups serially to the same computer [3].
 - 2.2.1. Talent connecting the operant corners to the central microprocessor with the provided cables.
 - 2.2.2. Talent connecting the central microprocessor to the computer with CAN cables.
 - 2.2.3. Talent showing multiple cages in series connected to the same computer.
- 2.3. Connect the microprocessor to the power source [1]. Ensure that the LED (*L-E-D*) lights in each corner switch on [2] and that all 8 motorized doors move [3].
 - 2.3.1. Talent connecting the microprocessor to the power source.
 - 2.3.2. Talent checking the LED lights switching on in each corner. *Videographer: Please capture the LED lights in each corner* **Videographer's Note: 2.3.2 was broken into multiple shots (A,B,C)**
 - 2.3.3. Talent checking the motorized doors moving. *Videographer: Please capture the shot of the doors moving*
- 2.4. Fill 8 sipper bottles with approximately 250 milliliters each of tap water or another liquid

[1]. Securely press on the caps to ensure a proper seal [2]. Gently twist the sipper bottles into the lickometer openings to ensure a tight connection [3]. Fill the food hopper with rodent chow [4].

2.4.1. Talent filling the sipper bottles with tap water.

2.4.2. Talent pressing caps securely onto the filled sipper bottles.

2.4.3. Talent twisting the filled sipper bottles into the lickometer openings.

Videographer's Note: Added 2.4.3 A Second side and 2.4.3 B Third side

2.4.4. Talent filling the food hopper with rodent chow.

3. Running the Operant, Intermittent Alcohol Drinking Experiment

Demonstrator: Lucy Anderson

3.1. After verifying the readability of RFID (*R-F-I-D*) transponders implanted in experimental mice and recording their body weights, place up to 16 same-sex mice in the prepared IntelliCage apparatus [1-TXT]. Turn on the computer [2]. After opening the **Controller** application [3], load the **Habituation.experiment** file into the “**Controller**” application and start recording to ensure all visits, nosepokes, and licks are being captured whenever a mouse enters the operant corner [4]. Following 24 hours of habituation, choose Experiment to open the **Water Training.experiment** file to check and verify that all mice learnt to nosepoke on an FR-3 (*fixed ratio-three*) schedule to access the sipper bottle nozzles [5].

3.1.1. Talent placing the RFID transponder-implanted mice in prepared IntelliCage apparatus. **TXT: RFID: Radiofrequency Identification**

3.1.2. Talent turning on the computer.

3.1.3. SCREEN: 71345-3.1.3-tk2-(1).mp4. 00:00–00:02; 00:07–00:09

3.1.4. SCREEN: 71345-3.1.4-tk3.mp4. 00:03–00:07; 00:10–00:12; 00:22–00:26

3.1.5. SCREEEN: 71345-3.1.5-tk2.mp4. 00:01–00:03; 00:05–00:10; 00:16–00:19

3.2. To begin intermittent alcohol drinking after successful water training, prepare 20 percent alcohol using 95 percent ethanol, 190 proof, in tap water, volume per volume, at approximately 250 milliliters per bottle [1].

3.2.1. Talent preparing the 20 percent alcohol solution.

3.3. Remove the sipper water bottles in operant corners 2 and 4 [1]. Replace each bottle with approximately 250 milliliters of 20 percent alcohol [2].

3.3.1. Talent removing the sipper bottles from the specified operant corners.

3.3.2. Talent replacing each removed bottle with a bottle containing 20 percent

alcohol.

- 3.4. Stop running the **Water Training.experiment** file [1]. Load the **Intermittent Alcohol.experiment** file [2].
 - 3.4.1. SCREEN: 71345-3.4.1-3.4.2-tk2.mp4. 00:00–00:06
 - 3.4.2. SCREEN: 71345-3.4.1-3.4.2-tk2.mp4. 00:15–00:19; 00:21–00:25
- 3.5. After starting the mice on the "**Water Access Only**" module, verify the system switches to "**Alcohol Access ON**", and that licks are recorded in the operant corners [1]. Throughout the experiment, regularly check that the software records visits, nosepokes, and licks for each mouse [2]. Confirm that all motorized doors are opening properly [3].
 - 3.5.1. SCREEN: 71345-3.5.1-3.5.2.mp4. 00:08–00:11; 00:17–00:20
 - 3.5.2. SCREEN: 71345-3.5.1-3.5.2.mp4. 00:30–00:35
 - 3.5.3. Talent checking that the motorized doors are opening properly. *Videographer: Please capture the opening of the motorized doors.*
- 3.6. Next, replace the alcohol and water bottles while performing the cage changes at least once per week during the "Water Access ONLY" periods [1].
 - 3.6.1. Talent replacing all sipper bottles from the cage.
- 3.7. To assess punishment-insensitive drinking, prepare 0.5 millimolar and 1 millimolar quinine-adulterated 20 percent alcohol [1]. Fill each sipper bottle in the alcohol corners with approximately 250 milliliters of 0.5 millimolar quinine-adulterated 20 percent alcohol solution [2].
 - 3.7.1. Talent preparing the quinine-adulterated 20 percent alcohol solutions. **Videographer's Note: 3.7.1 & 3.7.2 combined**
 - 3.7.2. Talent filling each alcohol-corner sipper bottle with 0.5 millimolar quinine-adulterated 20 percent alcohol solution.
- 3.8. Load the **Intermittent Alcohol.experiment** file and start recording as demonstrated previously [1].
 - 3.8.1. SCREEN: 71345-3.4.1-3.4.2-tk2.mp4. 00:15–00:19; 00:21–00:25
 - ~~3.8.2. SCREEN: Show recording being started in the software.~~
- 3.9. After the first quinine adulterated alcohol day, replace each bottle in operant corner 2 and 4 with approximately 250 milliliters of 1 millimolar quinine-adulterated 20 percent alcohol solution [1]. Keep the experiment running during the bottle switch [2].
 - 3.9.1. Talent replacing each alcohol-corner bottle with 1 millimolar quinine-adulterated 20 percent alcohol solution.
 - 3.9.2. SCREEN: Show that the experiment remains running during the bottle switch.

Note: 3.9.2 was captured by the videographer.

3.10. After the second quinine-adulterated alcohol day, remove sipper bottles containing adulterated alcohol [1]. Replace each bottle with approximately 250 milliliters of unadulterated 20 percent alcohol and return mice to intermittent alcohol drinking or free-access alcohol drinking phase [2].

3.10.1. Talent removing sipper bottles containing adulterated alcohol. **Videographer's**

Note: 3.10.1 & 3.10.2 combined; Added Mouse B-Roll per author request

3.10.2. Talent replacing each removed bottle with unadulterated 20 percent alcohol.

4. Data Analysis of Drinking Behaviors in Control and LBN Mice

Demonstrator: Roland Bock

4.1. Compile all data, including zip files output by the **Controller** application and the CSV (C-S-V) file containing mouse body weight data [1].

4.1.1. SCREEN: 71345-4.1.1.mp4. 00:08–00:10; 00:12–00:14; 00:16–00:20; 00:28–00:30

4.2. Download the R project template from the GitHub repository [1]. Rename the project to match the experiment [2].

4.2.1. SCREEN: 71345-4.2.1-tk2.mp4. 00:08–00:10; 00:19–00:23

4.2.2. SCREEN: 71345-4.2.2-tk2.mp4. 00:23–00:26; 00:30–00:33

4.3. Open the data/C1 subfolder containing the sample data files [1]. Replace the archive files with the personalized data archives [2].

4.3.1. SCREEN: 71345-4.3.1-tk3.mp4. 00:08–00:13

4.3.2. SCREEN: 71345-4.3.2-tk2.mp4 00:09–00:13; 00:16–00:21

4.4. Launch RStudio [1] and open the project [2]. **Note:** The timestamps for these shots are combined

4.4.1. SCREEN: 71345-4.4.1-tk2.mp4. 00:04–00:08

4.4.2. ~~SCREEN: Show the project opening in RStudio.~~

4.5. Update the **Cohort Background** [1] and, after verifying the groups, update the **Experimental Timeline** code chunks [2] to match the experimental details [3]. **Note:** The narration and numbers are adjusted for the added shot.

4.5.1. SCREEN: 71345-4.5.1-tk2.mp4. 00:20–00:25

4.5.2. SCREEN: 71345-4.5.2.1.mp4. 00:16–00:23; 00:40–00:44

Added Shot: 71345-4.5.2.2.mp4. 0:09–0:13

4.6. Verify that all animals are being loaded properly in the **Subject Overview [1]**, and that animal weights remain consistent throughout the experiments **[2]**.

4.6.1. SCREEN: 71345-4.6.1-tk2.mp4. 00:18–00:20; 00:24–00:27

4.6.2. SCREEN: 71345-4.6.2-tk2.mp4. 00:42–00:49

4.7. In the **IC Data Wrangling** section **[1]**, verify the accuracy of day **[2]** and drink assignment labels **[3]**. View individual animal performance data and exclude specific animals, as necessary **[4]**.

Added Shot: SCREEN: 71345-4.7.1-tk2.mp4. 00:00–00:02

4.7.1. SCREEN: 71345-4.7.1.2-tk1.mp4. 00:19–00:24

4.7.2. SCREEN: 71345-4.7.2-tk2.mp4. 00:02–00:04; 00:13–00:16

4.7.3. SCREEN: 71345-4.7.3-tk2.mp4. 00:13–00:23

~~4.7.4. SCREEN: Show exclusion of specific animal ID.~~

4.8. Perform a preliminary analysis to explore the data, write new code, and draft the analysis section as required **[1]**.

4.8.1. SCREEN: 71345-4.8.1-tk2.mp4. 02:12–02:18; 03:14–03:18

Results

5. Results

- 5.1. Across all days of intermittent alcohol drinking, both control [1] and LBN-reared mice showed wide variations in the daily alcohol licks relative to their body weights [2].
 - 5.1.1. LAB MEDIA: Figure 6A. *Video Editor: Please emphasize the left side of the graph showing lines with gray points along with the heading "Control"*
 - 5.1.2. LAB MEDIA: Figure 6A. *Video Editor: Please emphasize the right side of the graph showing lines with pink points along with the heading "LBN"*
- 5.2. LBN-reared mice generally drank more alcohol per gram of body weight [1] than controls during intermittent drinking [2].
 - 5.2.1. LAB MEDIA. Figure 6A. *Video Editor: Please emphasize the regions corresponding to X-axis value 6 and beyond in both "Control" and "LBN" panels simultaneously*
- 5.3. Similarly, in punishment-insensitive drinking, control mice showed a general reduction in mean alcohol licks per day [1], whereas the LBN-reared mice didn't show a significant difference in alcohol intake [2].
 - 5.3.1. LAB MEDIA. Figure 6B. *Video Editor: Please emphasize the heights of the lines within the yellow highlighted range in "Control" panel*
 - 5.3.2. LAB MEDIA. Figure 6B. *Video Editor: Please emphasize the heights of the lines within the yellow highlighted range in "LBN" panel.*

Pronunciation Guide:

1. Operant
 Pronunciation link: <https://www.merriam-webster.com/dictionary/operant>
 IPA: /'ɑ:pərənt/
 Phonetic Spelling: ah·puh·ruhnt
2. Microprocessor
 Pronunciation link: <https://www.merriam-webster.com/dictionary/microprocessor>
 IPA: /,maɪkroʊ'prɑ:sesər/
 Phonetic Spelling: my·kroh·prah·seh·ser
3. IntelliCage
 Pronunciation link: No confirmed link found
 IPA: No verified American IPA found
 Phonetic Spelling: in·teh·lee·kayj

4. Habituation
Pronunciation link: <https://www.merriam-webster.com/dictionary/habituation>
IPA: /həˌbɪtʃuˈeɪʃən/
Phonetic Spelling: huh·bich·oo·ay·shuhn
5. Nosepoke
Pronunciation link: No confirmed link found
IPA: No verified American IPA found
Phonetic Spelling: nohz·pohk
6. RFID
Pronunciation link: <https://www.merriam-webster.com/dictionary/RFID>
IPA: /ˌɑːr ɛf aɪ ˈdiː/
Phonetic Spelling: ar·ef·eye·dee
7. Transponder
Pronunciation link: <https://www.merriam-webster.com/dictionary/transponder>
IPA: /trænˈspɑːndər/
Phonetic Spelling: tran·spon·der
8. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /ˈeθəˌnɔːl/
Phonetic Spelling: eth·uh·nawl
9. Quinine
Pronunciation link: <https://www.merriam-webster.com/dictionary/quinine>
IPA: /ˈkwainɪn/, /kwiˈniːn/
Phonetic Spelling: kwy·nine; kwee·neen
10. Adulterated
Pronunciation link: <https://www.merriam-webster.com/dictionary/adulterated>
IPA: /əˈdʌltəˌreɪtɪd/
Phonetic Spelling: uh·duhl·tuh·ray·tid
11. Lickometer
Pronunciation link: No confirmed link found
IPA: No verified American IPA found
Phonetic Spelling: lik·om·uh·ter
12. Millimolar
Pronunciation link: <https://www.merriam-webster.com/medical/millimolar>
IPA: /ˌmɪliˈmoʊlər/
Phonetic Spelling: mil·uh·moh·ler
13. RStudio
Pronunciation link: No confirmed link found

IPA: No verified American IPA found

Phonetic Spelling: ar·stoo·dee·oh

14. GitHub

Pronunciation link: No confirmed link found

IPA: No verified American IPA found

Phonetic Spelling: git·hub

15. Cohort

Pronunciation link: <https://www.merriam-webster.com/dictionary/cohort>

IPA: /'kɒɹ̩,hɔːrt/

Phonetic Spelling: koh·hort

16. Wrangling

Pronunciation link: <https://www.merriam-webster.com/dictionary/wrangling>

IPA: /'ræŋɡlɪŋ/

Phonetic Spelling: rang·gling

17. Repository

Pronunciation link: <https://www.merriam-webster.com/dictionary/repository>

IPA: /rɪ'pɑːzə,tɔːri/

Phonetic Spelling: rih·pah·zuh·tor·ee

18. Serially

Pronunciation link: <https://www.merriam-webster.com/dictionary/serially>

IPA: /'sɪriəli/

Phonetic Spelling: seer·ee·uh·lee

19. Experimental

Pronunciation link: <https://www.merriam-webster.com/dictionary/experimental>

IPA: /ɪk,sperə'mentəl/

Phonetic Spelling: ik·spear·uh·men·tl

20. Preliminary

Pronunciation link: <https://www.merriam-webster.com/dictionary/preliminary>

IPA: /prɪ'limə,neri/

Phonetic Spelling: prih·lim·uh·nair·ee