

Project Page Link: <https://review.jove.com/account/file-uploader?src=21856653>

Authors and Affiliations:

Department of Speech, Language, and Hearing Sciences, Purdue University

Kaitlyn Dwenger kdwenger@purdue.edu

Brooke Rodgers	rodger42@purdue.edu
Allison J. Schaser	aschaser@purdue.edu
Kaitlyn Dwenger	kdwenger@purdue.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 done!**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 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. **No**

Current Protocol Length

Number of Steps: 15

Number of Shots: 30

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. **Kaitlyn Dwenger:** Our research in the Purdue Animal Behavior Core investigates cranial sensorimotor dysfunction underlying voice and swallowing disorders and develops standardized methods to study rat and mouse ultrasonic vocalizations.
 - 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. **Kaitlyn Dwenger:** Many USV paradigms rely on mating behaviors. This protocol uses food reward elicitation methods to reduce social and hormonal confounds.
 - 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. **Brooke Rodgers:** Consistent handling, conditioning, and recording conditions are essential for reliable vocalization elicitation and reproducible results.
 - 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. **Brooke Rodgers:** Recordings can be analyzed using alternative methods and combined with behavioral, histological, molecular, or neurophysiological analyses.
 - 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. **Kaitlyn Dwenger:** Future studies can evaluate vocal changes across disease models, interventions, species, and experimental conditions.
 - 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.

Ethics Title Card

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

Protocol

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

2. Ultrasonic Vocalization Recording Setup for Rodents

Demonstrator: Kaitlyn Dwenger

- 2.1. To begin, conduct the recordings in a darkened room or under red lighting to align with the animals' reverse light cycle [1]. Limit room entry and remain in an adjacent space during sessions to minimize acoustic disturbances [2].
 - 2.1.1. WIDE: Talent placing the cage in the darkened recording room or red lighting.
***Videographer:** Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent exiting the recording room and remaining in an adjacent space.
- 2.2. Use sound-attenuated recording chambers with specific outer and inner dimensions [1] to minimize external noise and electronic interference [2]. Assign four A53T-SynGFP (*ay-fifty-three-tee-sin-jee-eff-pee*) mice to four chambers using a randomized, counterbalanced schedule across all recording days and experimental conditions [3].
 - 2.2.1. Talent setting up the sound-attenuated recording chambers.
 - 2.2.2. TEXT ON A PLAIN BACKGROUND
Recording Chamber Dimensions:
Outer diameter: 60 cm x 60 cm x 82 cm
Inner diameter: 56 cm x 53 cm x 60 cm
 - 2.2.3. Talent assigning animals to recording chambers according to a randomized and counterbalanced schedule.
- 2.3. Record the chamber assignment for each session, including the animal identification, group, chamber number, date, and session type, in a lab notebook [1].
 - 2.3.1. Talent recording chamber assignment details in a lab notebook.

3. Cage Preparation and Recording System Setup

Demonstrator: Kaitlyn Dwenger

- 3.1. ~~Within each recording chamber, use clean polycarbonate cages without bedding to minimize movement-related noise [1].~~ Clean each cage thoroughly with 70 percent ethanol between recordings [2] and allow it to dry completely before the next recording [3].
 - 3.1.1. ~~Talent placing a clean polycarbonate cage inside a recording chamber.~~ **Note:** The

authors removed this shot, as it was shown in 2.2.1.

- 3.1.2. Talent cleaning a polycarbonate cage with 70 percent ethanol.
- 3.1.3. Shot of the cleaned cage drying completely.
- 3.2. Turn on the recording computer [1] and launch the ultrasonic vocalizations acquisition software [2].
 - 3.2.1. Talent turning on the recording computer
 - 3.2.2. SCREEN: 71661_screenshot_1.mp4. 00:04–00:06
- 3.3. Click on **File/Experiment**, then choose **New** to create a new experiment [1]. Under the **Identification** menu, input the experiment name [2]. Next, in the **Species** menu, choose **Mouse** [3], and in the **Cages** menu, retain the number of recording channels as 4 [4], then click on **Save** [5].
 - 3.3.1. SCREEN: 71661_screenshot_1.mp4. 00:08–00:12
 - 3.3.2. SCREEN: 71661_screenshot_1.mp4. 00:14–00:18
 - 3.3.3. SCREEN: 71661_screenshot_1.mp4. 00:27–00:32
 - 3.3.4. SCREEN: 71661_screenshot_1.mp4. 00:33–00:38
 - 3.3.5. SCREEN: 71661_screenshot_1.mp4. 00:44–00:49
- 3.4. Prior to each session, perform a test recording by selecting **Execute Test Session** followed by **Preview Ultrasounds** to open the four recording channels [1]. Click the **Record** icon to confirm that the multi-channel interface is recognized and to verify acceptable signal integrity, channel function, and noise levels [2].
 - 3.4.1. SCREEN: 71661_screenshot_2.mp4. 00:03–00:11
 - 3.4.2. SCREEN: 71661_screenshot_2.mp4. 00:12–00:20
- 3.5. Create a test session by clicking **Design Test Session**, then **New Test Session**, and set the recording duration to 10 minutes [1]. Assign treatment codes and animal identifications to the appropriate chamber channels and verify session information before recording, as the settings cannot be modified after completion [2].
 - 3.5.1. SCREEN: 71661_screenshot_3.mp4. 00:03–00:08
 - 3.5.2. SCREEN: 71661_screenshot_3.mp4. 00:09–00:19

4. Ultrasonic Vocalization Recording Protocol

Demonstrator: Brooke Rodgers

- 4.1. To initiate the session, place the animals into the recording chambers, ensuring the correct chamber assignment [1]. Close the chamber doors [2].

- 4.1.1. Talent placing the animals into the assigned recording chambers.
- 4.1.2. Talent closing the recording chamber doors.
- 4.2. In the recording software, open the corresponding test session by clicking **Design Test Session**, then **Open Test Session [1]**.
 - 4.2.1. SCREEN: 71661_screenshot_4.mp4. 00:02–00:06; 00:11–00:15
- 4.3. Under **Execute Test Session**, choose **Record Ultrasounds**, then click the **Record** button to start recording and monitor the signals in real time **[1]**.
 - 4.3.1. SCREEN: 71661_screenshot_4.mp4. 00:16–00:24
 - 4.3.2. SCREEN: Recording signals being monitored in real time. **Note: This shot is merged with 4.3.1 at the postshoot stage.**
- 4.4. Allow the software to record continuously for 10 minutes **[1]**, and automatically stop at the end of the session **[2]**.
 - 4.4.1. SCREEN: 71661_screenshot_4.mp4. 05:09–05:15
 - 4.4.2. SCREEN: 71661_screenshot_4.mp4. 10:17–10:23
- 4.5. Save the recording immediately to prevent data loss by clicking the **X** button, then **Save [1]**. Store and back up the recorded files after each session for later analysis **[2]**.
 - 4.5.1. SCREEN: 71661_screenshot_4.mp4. 10:30–10:36
 - 4.5.2. SCREEN: 71661_screenshot_5.mp4. 00:18–00:24
- 4.6. Remove the animals from the chambers **[1]** and return them to their home cages **[2]**. Provide a standardized, palatable food reward immediately after returning each animal to its home cage, maintaining consistency across all sessions in the timing of rewards **[3]**.
 - 4.6.1. Talent removing the animals from the recording chambers.
 - 4.6.2. Talent returning the animals to their home cages.
 - 4.6.3. Talent providing a standardized palatable food reward to an animal in its home cage.
- 4.7. Perform recording sessions once daily for 3 to 5 consecutive days following conditioning, maintaining identical timing, handling procedures, and environmental conditions across all sessions **[1]**.
 - 4.7.1. Talent preparing for a daily recording session following conditioning.

Results

5. Results

- 5.1. Representative recordings produced by a 15-month-old Sprague-Dawley female ovariectomized rat demonstrated distinct ultrasonic vocalization or USV (*U-S-V*) spectrograms with well-defined acoustic contours, including flat [1] and frequency-modulated structures, enabling accurate extraction of acoustic parameters [2].
 - 5.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight the simple USV in the processed spectrogram at the top, showing the flat horizontal contour.*
 - 5.1.2. LAB MEDIA: Figure 1. *Video editor: Highlight the complex USV in the processed spectrogram at the bottom, showing the curved frequency-modulated contour.*
- 5.2. Representative classification results from a 10-minute baseline recording of a 3-month-old alpha-synuclein-overexpressing transgenic male mouse demonstrate successful vocal detection with the distribution of calls across simple and complex types [1].
 - 5.2.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the classification bar categories shown in the chart labelled “Identified USV’s”.*
- 5.3. It also shows distinct frequency modulation patterns—including flat [1], upward [2], downward [3], and complex profiles [4]—as well as call distributions across separate duration bins [5].
 - 5.3.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the bar labelled “ΔF ~0”*
 - 5.3.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the bar labelled “ΔF Up”*
 - 5.3.3. LAB MEDIA: Figure 2B. *Video editor: Highlight the bar labelled “ΔF Down”*
 - 5.3.4. LAB MEDIA: Figure 2B. *Video editor: Highlight the bar labelled “ΔF Complex”*
 - 5.3.5. LAB MEDIA: Figure 2C. *Video editor: Highlight the bars labelled “Td <15”, “15<Td<50”, “50<Td<100”, and “Td>100” sequentially.*

Pronunciation Guide:

1. Ultrasonic

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

IPA: /ˌʌl.trəˈsɔː.nɪk/

Phonetic Spelling: uhl·truh·saa·nik
2. Vocalization

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

IPA: /ˌvoʊ.kə.ləˈzeɪ.ʃən/

Phonetic Spelling: voh·kuh·luh·zay·shuhn

3. Rodents
Pronunciation link: <https://www.merriam-webster.com/dictionary/rodent>
IPA: /'rɒð.dənt/
Phonetic Spelling: roh·duhnt
4. Acoustic
Pronunciation link: <https://www.merriam-webster.com/dictionary/acoustic>
IPA: /ə'kuː.stɪk/
Phonetic Spelling: uh·koos·tik
5. Sound-attenuated
Pronunciation link: <https://www.merriam-webster.com/dictionary/attenuate>
IPA: /ə'ten.ju.ɪ.tɪd/
Phonetic Spelling: uh·ten·yoo·ay·tuhd
6. Electronic
Pronunciation link: <https://www.merriam-webster.com/dictionary/electronic>
IPA: /ɪ.lek'trɔː.nɪk/
Phonetic Spelling: ih·lek·traa·nik
7. A53T-SynGFP
Pronunciation link: No confirmed link found
IPA: No confirmed IPA found
Phonetic Spelling: ay·fif·tee·three·tee·sin·jee·eff·pee
8. Randomized
Pronunciation link: <https://www.merriam-webster.com/dictionary/randomize>
IPA: /'ræn.də.maɪzd/
Phonetic Spelling: ran·duh·myzd
9. Counterbalanced
Pronunciation link: <https://www.merriam-webster.com/dictionary/counterbalance>
IPA: /ˌkaʊn.tə'bælənst/
Phonetic Spelling: kown·ter·bal·uhnst
10. Polycarbonate
Pronunciation link: <https://www.merriam-webster.com/dictionary/polycarbonate>
IPA: /ˌpɑː.li'kɑːr.bə.nɛt/
Phonetic Spelling: pol·ee·kaar·buh·nayt
11. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /'eθ.ə.nɔːl/
Phonetic Spelling: eth·uh·nawl
12. Acquisition
Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>
IPA: /ˌæk.wə'zɪʃ.ən/
Phonetic Spelling: ak·wuh·zish·uhn
13. Species
Pronunciation link: <https://www.merriam-webster.com/dictionary/species>

IPA: /'spiː.ʃiːz/

Phonetic Spelling: spee·sheez

14. Ultrasounds

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

IPA: /'ʌl.trəˌsaʊnd/

Phonetic Spelling: uhl·truh·sownd

15. Multi-channel

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

IPA: /ˌmʌl.ti'tʃæn.əl/

Phonetic Spelling: mul·tee·chan·uhl

16. Interface

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

IPA: /'ɪn.t̬əˌfeɪs/

Phonetic Spelling: in·ter·fays

17. Signal

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

IPA: /'sɪɡ.nəl/

Phonetic Spelling: sig·nuhl

18. Integrity

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

IPA: /ɪn'teg.rə.t̬i/

Phonetic Spelling: in·teg·ruh·tee

19. Treatment

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

IPA: /'triː.t.mənt/

Phonetic Spelling: treet·muhnt

20. Conditioning

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

IPA: /kən'dɪʃ.ənɪŋ/

Phonetic Spelling: kuhn·dish·uh·ning

21. Standardized

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

IPA: /'stæn.dəˌdaɪzd/

Phonetic Spelling: stan·der·dyzd

22. Palatable

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

IPA: /'pæl.ə.t̬ə.bəl/

Phonetic Spelling: pal·uh·tuh·buhl

23. Consecutive

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

IPA: /kən'sek.jə.t̬ɪv/

Phonetic Spelling: kuhn·sek·yuh·tiv

