



Aug 23, 2025

Ultrasonic Vocalizations test in adult rodents

DOI

<https://dx.doi.org/10.17504/protocols.io.kqdg319x7l25/v1>

Amirah Wright¹, Madison Wypyski², Susan F.Murphy¹, Pamela VandeVord³

¹Department of Biomedical Engineering, Virginia Tech, Blacksburg, VA;

²Graduate Program in Translational Biology, Medicine & Health, Virginia Tech, Blacksburg, VA;

³Salem VAMC/ Virginia Tech

PRECISE-TBI



Monique Surles-Zeigler

University of California, San Diego

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.kqdg319x7l25/v1>

Protocol Citation: Amirah Wright, Madison Wypyski, Susan F.Murphy, Pamela VandeVord 2025. Ultrasonic Vocalizations test in adult rodents. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.kqdg319x7l25/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: August 07, 2025

Last Modified: August 23, 2025

Protocol Integer ID: 224220

Keywords: PRECISE-TBI, Traumatic Brain Injury, TBI, rat, ultrasonic vocalization, ultrasonic vocalizations test in adult rodent, ultrasonic vocalizations test, ultrasonic vocalization, adult rat, adult rodent, rat, therapeutic, clinical translation of therapeutic, clinical translation, microphone

Abstract

This is a protocol to describe the materials and methods utilized to measure ultrasonic vocalizations (USVs) in adult rats. The model utilizes microphones to detect USVs produced by rats while in their home cage. The model utilizes microphones to detect USVs produced by rats while in their home cage to examine different emotional states, such as distress and pleasure.

The posting of this protocol is part of the mission of the PREclinical Interagency reSearch resourceE-TBI (PRECISE-TBI, precise-tbi.org) to improve clinical translation of therapeutics by providing an online catalog and standardized protocols to reduce the variability of model usage between laboratories.

Materials

UltraVox XT Software (Noldus Information Technology), (RRID:SCR_027313)

Sound Attenuation Chamber

Pettersson M500-384 Microphone

General Protocol

- 1 Plug the microphone into the computer (See: Note 1)
- 2 Open Ultravox software
- 3 Under "New Experiment", click "New..." and name your experiment
- 4 Click on "Experiment Settings"
- 5 Under "Experiment location", Select the folder destination where you would like the data to be stored
- 6 Under "Microphone", Select the recording device that is plugged into the computer. (See: Note 2)
- 7 Under the "Setup" tab, click "Trial Control Settings"
- 8 Under "Start acquisition", select "Manually" and under "Stop acquisition", select "After 5 minutes" (See: Note 3)
- 9 Under the "Acquisition" tab, click on "Acquisition"
- 10 Place the animal, inside of its home cage, inside of the sound attenuating chamber. Remove the lid. (See: Note 4)
- 11 Put the microphone inside of the microphone holder and clip it onto the side of the cage. (See: Note 5)
- 12 Close the doors to the chamber then proceed to start the experiment by clicking the red "Record" button in the Ultravox software

- 13 Once the experiment is completed, remove the animal and its cage from the sound attenuating chamber.
- 14 Once all trials are completed, click on "Recording List" to verify that all trials have been recorded correctly.
- 15 Under the "Analysis" tab, click "Call Detection"
- 16 Under "Call Definition", click "Add" to characterize call definitions for detection analysis
- 17 Add two call definition types with the following parameters (See: Note 6):

	A	B	C	D	E	F	G
	Call Definition	Frequency low (Hz)	Frequency high (Hz)	Min amplitude	Min duration (ms)	Max duration (ms)	Min gap (ms)
	22-kHz	18000	36000	50	20	300	10
	50-kHz	40000	60000	50	10	150	10

- 18 Once the call definition settings are set, go to "Save as template", name the template, and click "Save"
- 19 Once the call definitions are added you can either click "Apply" to analyze each trial separately or click "Apply to All" to have the analysis performed on all trials at one time (See: Note 7)
- 20 After the call detection analysis has been completed, click on "Call Statistics"
- 21 Under "Call grouping", select "Name and pattern"
- 22 Under "Statistics", select "Count" and "Mean duration" (See: Note 8)



- 23 Click on "Export", name the file, then select a destination folder for the file be saved to. (See: Note 9)

NOTES

- 24 Note 1: The tester can use more than one microphone but the max is 2
- 25 Note 2: When the microphone has been selected and is operating, there will be a black check mark under "Selected" and a green check mark under "Status".
- 26 Note 3: An external trigger can be utilized if the user chooses to do so. If Ultravox is being used in conjunction with a behavior test (Open Field, Elevated Plus Maze, etc.) the program used for that behavioral assay can be paired with the Ultravox software to be used as an "External program trigger".
- 27 Note 4: The food container can also be removed from the cage if the tester wants to do so.
- 28 Note 5: Make sure the microphone is as close to the animal as possible to get the best audio recordings.
- 29 Note 6: Min amplitude and Min gap were left as the software's default settings but can be changed based on tester preference
- 30 Note 7: While the analysis is being performed, it will show you the count for each call definition, the parameters for each detected call, and the visual calls being detected in the spectrogram
- 31 Note 8: You can add other statistics of interest based on user needs
- 32 Note 9: The default and only allowable file type is a text (.txt) file