

 THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE	UQ Animal Ethics Committee- Standard Operating Procedure LAB_085 Ultrasonic Vocalizations for Rodent Pups Institutional author: Queensland Brain Institute AEC Reviewed & Approved: September 2025 SOP Expiry: September 2028	Version #1.2 Page 1 of 4
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LAB_085 Ultrasonic Vocalizations (USV) for Rodent Pups (Expiry: Sept 2028)

OBJECTIVE

To describe the procedure for capturing the full acoustic spectrum to process and analyse ultrasonic calls in rodent pups. Rodent pups start emitting ultrasonic vocalizations (USV) shortly after birth and are recorded on brief isolation from their dams. These calls elicit pup retrieval by the dam, maternal licking, and crouching behaviour. USV in rodents are useful in studies on social behaviour, such as autism-related, schizophrenia and anxiety-like behaviours.

NB: The use of (*) indicates this statement is dependent on the facility procedures.

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COMMENTS / RECOMMENDATIONS

- Behavioural assessments are ideally performed in a dedicated behavioural suite.
- The environment should be free from uncontrolled external stimuli that may influence the animal's behaviour, such as human traffic, unnecessary noise, and intense lighting.
- To reduce the circadian cycle's effects on the behaviour, perform behavioural testing at a similar time each day.
- The majority of USV calls from Wild Type mouse pups occur between ages 3-9 days (Thornton *et al.*, 2005) however variations in USVs continue up to 13-15 days of age. Testing of mouse pups should not be performed prior to 3 days of age due to the high risk of cannibalisation of the pups by the dam. If testing is required earlier than 3 days specific justification for this must be provided to the AEC and approval given**.
- In rats, the majority of USV calls in pups are between birth and weaning (at 18-21 days). Testing can commence at day one of age.

SAFETY AND COMPLIANCE

- The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken, and risk assessments have been performed. If unsure, consult your supervisor.
- Facility protocols should be followed.
- Possible risks include mouse/rat bite injury, repetitive task musculoskeletal injury and psychosocial harm.

TRAINING CONSIDERATIONS

- All unsupervised animal procedures must be performed by appropriately trained personnel who have been deemed competent in the procedure.

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EQUIPMENT

- PPE*
Minimum PPE is gloves and gown, P2 mask; additional PPE may be required based on facility or additional risk, e.g. working with infectious animals.
- Appropriate trolley for transporting cages
- Disinfectant (70% Ethanol recommended) and paper towel for cleaning equipment*
- Kimwipes (or other paper towel for the pups to sit on)
- USV apparatus – The USV apparatus comprises a specialised microphone capable of capturing high-frequency calls connected to a computer, a sound-attenuated chamber* and either a plastic container (diameter, 5 cm; height 10 cm) or a facility-specific home cage to contain the pup (Figure A).

PREPARATION

1. Check AEC approvals to ensure the correct procedure and personnel are approved for the planned work**.
2. Prepare equipment, including disinfecting, prior to first use.
3. Ensure the beaker is dry after it has been disinfected and place a clean Kimwipe in the base.
Placing the pup on the Kimwipe instead of the beaker itself reduces the likelihood of any disinfectant getting on the pup and the dam rejecting or cannibalising it.
4. Turn the heating pad ON prior to bringing the litter into the behavioural room.
5. Handling of rodents as per: LAB_006 Handling and Restraint in Mice and Neonates and LAB_039 Handling and Restraint in Rats and Neonates
6. Depending on absorption, drugs/compounds can be given within appropriate times before recording USV. Drug administrations as per the relevant ethics approval and SOP**.

PROCEDURE

1. Begin preparations two days prior to the expected day of birth by isolating pregnant females. Monitor the females for signs of birth every morning and evening, marking the day of birth as P0.
2. On the day(s) of testing (refer to Comments), remove the dam from the home cage and litter and keep her separated from the litter in a separate holding room.
3. Transport the home cage, which contains the litter, and place it on a thermoregulated heating pad, allowing for a five-minute habituation period.
4. Prepare the computer for recording USV.
5. Before handling the pups, gently rub gloved fingertips in the nesting material to acquire the scent profile of the litter.
This practice helps prevent maternal aggression or rejection by ensuring that the pups retain a familiar olfactory signature.

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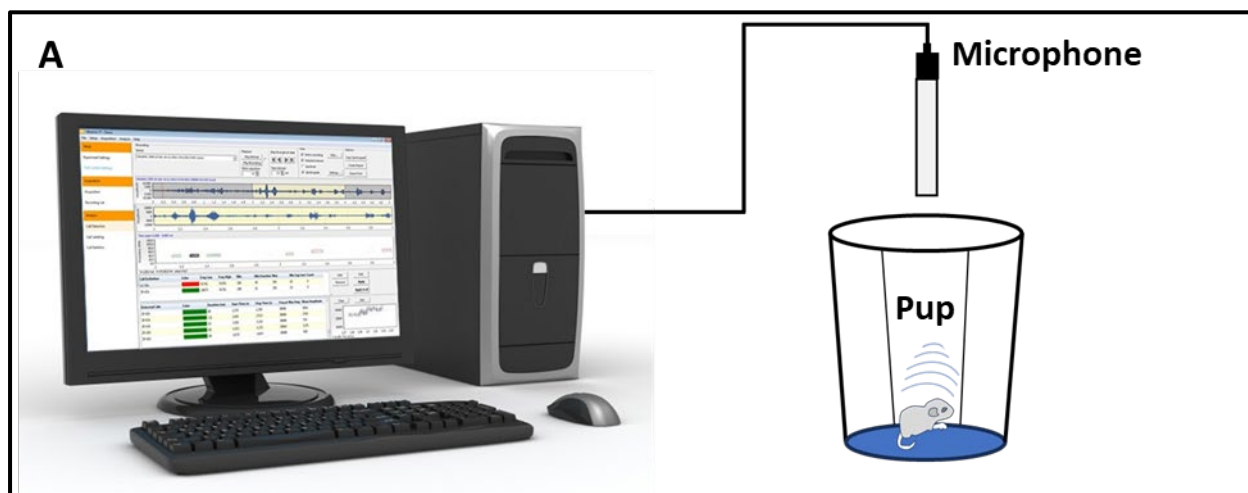
6. Place a pup on the Kimwipe inside the beaker located within the sound-attenuating box* and record USV during a test session lasting three to five minutes.
Do not exceed five minutes of USV recording for individual pups due to loss of body temperature.
7. Ensure the microphone is positioned approximately 10-15 cm above the test chamber floor, directly above the pup.
8. At the conclusion of the recording session, perform the following tasks for each pup:
 - Weigh
 - record the pup's sex using the anogenital distance method
 - if required, identify the pup using a non-toxic marker such as a Sharpie permanent marker***Perform these tasks gently and efficiently as it is important that the pups are returned to the nest as soon as possible without getting too cold.*
9. Subsequently, return the pup to the litter.
10. Configure the computer for the next pup and repeat the aforementioned steps ensuring that the beaker is cleaned with disinfectant between use.
11. While the trials are ongoing, the researcher should remain in the testing room and monitor USV from the computer screen.
12. Maintain a quiet environment during the trials to minimize background noise, which can interfere with the spectrogram. Therefore, it is crucial to conduct USV recordings in a quiet room and/or within a sound-attenuated chamber.
13. Once testing is completed, transport the entire litter to the holding room ensuring it is covered with the scent of the bedding and reunite the dam.
14. For longitudinal USV recordings on the same pups, a gap of one day between recordings should be implemented. If more frequent recordings are required justification for this must be provided and AEC approval is required**

ANALYSIS

1. Data is analysed using a spectrum analysis program*.
2. Total number of calls, call frequency, amplitude and call duration are typically analysed.
3. Calls can be selected visually in a spectrogram, and similar calls can be categorised based on frequency, amplitude, duration, and time gaps.

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REFERENCES

Baharnoori, M., et al. (2012). "Neonatal behavioral changes in rats with gestational exposure to lipopolysaccharide: a prenatal infection model for developmental neuropsychiatric disorders." *Schizophr Bull* **38**(3): 444-456.

Thornton, L. M., et al. (2005). "Genetic and Developmental Influences on Infant Mouse Ultrasonic Calling. III. Patterns of Inheritance in the Calls of Mice 3–9 Days of Age." *Behavior Genetics* **35**(1): 73-83.

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Version #	Reviewing AEC	AEC Review Date	Outcome
1.2	Health Sciences AEC, Laboratory Biomedicine AEC, Molecular Biosciences AEC, Anatomical Biosciences AEC	September 2025	Approved

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