STANDARD OPERATING PROCEDURE

TITLE: Ultrasonic Vocalization

CATEGORY: Behavioral Assay

Introduction

Goal: Ultrasonic vocalization is used to quantitatively assess social communication in pups separated temporarily from their mothers. Pups are placed by themselves in a Styrofoam recording chamber attached to a microphone. Calls are recorded and then later analyzed using software from Avisoft Bioacoustics. No prior training is required for the subjects.

Materials

- Subjects – any strain of mouse or rat may be used. Testing can begin on pups as young as two days old.
- Apparatus – A Styrofoam box with an ultrasound microphone (Avisoft UltraSoundGate condenser microphone capsule CM16) inserted via a hole in the lid. The microphone should be placed 20cm above the subject.
- Software – Avisoft Bioacoustics UltraSound Gate 116H, Avisoft SASLab Pro,
- Accessories: plastic drinking cup, thermometer, and scale

Test Settings

- Mice should be housed on a 12L/12D light cycle. Litters chosen should contain at least 5 pups. The day the mice are born should be called postnatal day 0 (PND 0). Pups are recorded on PNDs 2, 4, 6, 8, and 12.
- Testing should be conducted in a very quiet room free from harmonic noises, especially the hum produced by electronic equipment. Lighting should be dim.

Testing procedures

- Bring the subject pup into the testing room in an empty cage with clean bedding at the bottom. Pups not being tested should be left in a separate room so that their calls do not interfere with the test.
- Place the pup in a clean plastic drinking cup, and place the cup inside the Styrofoam container. Close the lid tightly.
- Immediately begin recording with Avisoft UltraSoundGate. Trial length is 5 minutes.
- Once the trial ends, weigh the pup, take its temperature, and mark its tail using the identification system of choice.
- Return the pup to its home cage.
- Clean the cup in between subjects.

Data Analysis

- Calls are analyzed using Avisoft SasLab Pro. Parameters of interest include:
    - Number of calls
    - Duration of calls
    - Maximum and minimum frequency
    - Maximum amplitude
    - A qualitative categorization of calls