



STANDARD OPERATING PROCEDURE

TITLE: Five-trial Social Memory

CATEGORY: Behavioral Assay

Introduction

Goal: This document aims to provide the reader information on how to conduct the Five-trial Social Memory Test. The test is used to evaluate social memory and determine whether a mouse can distinguish between different novel intruders. As this is a general description of standard materials, test settings, and procedures, variations may be made to fit specific needs.

Materials

- *Subjects:* any strain of mice. Subjects require training and should be acclimated to testing environment and experimenter before testing.
- *Ovariectomized females (OEFs):* two per subject for testing and one per day of 5-day habituation, no previous interaction. OEFs may also require handling.
- *Opaque barriers (4):* manila folders or paper, placed between adjacent home cages.
- *Clear plastic covers:* placed over home cages
- *Video cameras on tripods (2):* positioned to each capture two cages and angled so that video is taken from slightly above.
- *Software:* automated tracking system (ex. EthoVision).
- *Privacy blind:* placed around apparatus approximately to eliminate external room cues.
- *Standing lamps with red light bulbs (2):* aimed away from apparatus to provide diffuse light.
- *Light meter:* used to measure illumination in apparatus.
- *20% ethanol:* used between trials to eliminate visual and olfactory residue in arena.

Test Settings

- *Lighting:* dim, consistent illumination throughout apparatus.

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Detailed Standard Operating Procedure

Before testing:

- *Acclimation*: subjects in home cage are placed in testing room for at least 1hr before testing to minimize effects of stress on behavior during testing.
- *Subject training*: 5-day habituation to OEFs to extinguish sexual behavior.
 - One randomly selected OEF is placed in subject home cage for 24hrs. Procedure is repeated for total of 5 days of habituation, using new OEF each day.
 - Animals that display extended sexual behavior are excluded. OEFs are removed 1hr prior to testing.

Testing procedures:

1. Trial 1

- Four subject home cages are placed together with opaque dividers separating adjacent cages. Cage tops are removed and clear plastic covers are placed on top of cages.
- Recording begins and OEF is placed in subject cage. With four cages, four subjects may be run simultaneously.
- After 1min, OEF is removed and recording is stopped.

2. Trials 2-4

- Trials 2-4 are run as in Trial 1, using same OEF and 10min between start of trials for each subject.

3. Trial 5

- Trial is run as in Trials 1-4, except using novel OEF for each subject.
- After Trial 5, cage tops are replaced, and clear plastic covers are cleaned with 20% ethanol.

Data Analysis

- Interaction with each OEF (defined as sniffing and/or head within 1cm of object) is collected for analysis.