



STANDARD OPERATING PROCEDURE

TITLE: Three-chamber Sociability & Social Novelty Test

CATEGORY: Behavioral Assay

Introduction

Goal: This document aims to provide the reader information on how to conduct the Three-chamber Sociability & Social Novelty Test. The test is used to evaluate social recognition and preference in mice. 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. No prior training is required, though subjects should be acclimated to testing environment and experimenter before testing. Normal 5-day handling should be supplemented with 3 days of 10min/day habituation to pencil cup.
- *Object mice:* two per subject, no previous interaction with subject. Object mice may also require handling.
- *Apparatus:* two 20 x 40.5 x 22cm three-chamber Plexiglass apparatuses with 10.2 x 5.4cm infrared-equipped portals between chambers.
- *Steel pencil cups (8):* 10.4cm diameter, 11cm high, steel bars spaced 1cm apart. Two used as empty cups, six used to hold objects.
- *Plastic drinking cups (4):* placed on top of steel pencil cups to prevent subject from climbing on top.
- *Video cameras on tripods (2):* positioned one in front of each apparatus and angled so that video is taken from slightly above.
- *Software:* automated tracking system (ex. EthoVision).
- *Privacy blind:* placed around apparatus to eliminate external room cues.
- *Standing lamps with white light bulbs (2):* aimed away from apparatus to provide diffuse light.
- *Light meter:* used to measure illumination in apparatus.
- *Water:* used to clean apparatus between trials.
- *20% ethanol:* used to clean apparatus and cups between subjects.

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Test Settings

- *Lighting*: consistent illumination throughout apparatus (approximately 200lux).

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*: none required.

Testing procedures:

1. Habituation

- Doors to side chambers are closed and animal is placed in center chamber. With two apparatuses, two subjects may be run simultaneously. Recording begins and doors are opened.
- After 10min of habituation, recording is stopped, subject is guided back to center chamber, and doors are closed. Urine is soaked up, fecal pellets are removed, and side chambers are cleaned with water.

2. Sociability

- One steel pencil cup is added to each side chamber. Object mouse #1 is placed under cup in left chamber and novel object is placed under cup in right chamber. Plastic cups are placed on top of steel pencil cups to prevent subject from climbing on top. Recording begins and doors are opened.
- After 10min Sociability trial, recording is stopped, subject is guided back to center chamber, and doors are closed. Cups are removed for cleaning and object mouse #1 is returned to home cage. Urine is soaked up, fecal pellets are removed, and side chambers are cleaned with water.

3. Social Novelty

- New steel cups are added, one to each side chamber. Object mouse #1 is placed under cup in right chamber and object mouse #2 is placed under cup in left chamber. Same plastic cups are placed on top of steel cups. Recording begins and doors are opened.
- After 10min Social Novelty trial, recording is stopped, subject is guided back to center chamber, and doors are closed. Subject is returned to home cage, cups are removed for cleaning, and object mice are returned to home cages. All cups and chambers are cleaned with 20% ethanol between subjects.

Data Analysis

- The following parameters are collected for analysis:
 - Time in each chamber
 - Entries into chambers
 - Time spent sniffing each cup