



STANDARD OPERATING PROCEDURE

TITLE: Intellicage (Place Learning and Avoidance Learning)

CATEGORY: Behavioral Assay

Introduction

Goal: This document aims to provide the reader information on how to run the Intellicage. The Intellicage is used to evaluate spatial learning and memory retrieval 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 mouse may be used.
- Apparatus – two Intellicages each with four gated, RFID-reading corners. Raised platforms may be placed inside each cage so that animals do not have to stretch up to reach corners.
- Software – IntellicagePlus Designer, Controller, and Analyzer
- Accessories: Bedding that will not get caught in corner gates, plastic shelters (2 per cage centered under the food tray), water bottles (300mL, placed two per corner for a total of eight per cage), water containers (300mL, used to block corners when animals need to be removed and to weight down food tray and prevent animal escape), RFID tags and reader.

Test Settings

- Mice should be housed on a 12L/12D light cycle.
- Mice should be injected with RFID tags prior to placement in the apparatus.
- Multiple mice can be placed in each cage with a maximum of 15 mice per cage.
- Mice should be socially housed prior to the test.

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General Testing procedures:

The following is a sample program timeline with descriptions of modules commonly used:

1. *Habituation (all doors open, 3-4 days)*
 - All doors open at all times, water available from all four corners without nose poke.
2. *Nose poke Learning (all corners with nose poke, 3-4 days)*
 - Water available from all four corners with nose poke.
3. *Corner Learning (one corner with nose poke, 3-4 days)*
 - Water available from least preferred corner (programmed as correct corner based on lick preferences during Habituation/Nose poke Learning) with nose poke. Nose pokes at incorrect corners do not open doors.
4. *Reversal (opposite corner with nose poke, 3-4 days)*
 - Water available from opposite corner with nose poke (ex. if animal had corner 2 programmed as correct during Corner Learning, corner 4 is correct during Reversal). Nose pokes at incorrect corners do not open doors.
5. *Aversive Learning (one corner with air puff, 1-2 days)*
 - Air puff from most preferred corner, i.e. correct corner if Aversive Learning follows Corner Learning or Reversal (ex. if animal had corner 4 programmed as correct during Reversal, animal will receive air puff from corner 4 during Aversive Learning).
6. *Free H₂O (all doors open, 24hrs)*
 - All doors open at all times, water available from all four corners without nose poke.
7. *Corner Learning 2 (one corner with nose poke, 3-4 days)*
 - Water available from least preferred corner with nose poke, i.e. air puff corner if Corner Learning follows Aversive Learning (ex. if animal received air puff from corner 4 during Aversive Learning, corner 4 is programmed as correct for Corner Learning 2). Nose pokes at incorrect corners do not open doors.
 - **General note:** Make sure all animals drink. An animal that does not drink for >36 hrs should be excluded, and all corners should be programmed as correct for that animal. Also, monitor any animals with abnormally few licks.
 - **Monitor any corner preferences:** Biases in visit preference may be due to uneven water flow from bottles, differences in lighting, or bedding distribution.

Specific Testing Procedures:

The following procedures can be used if the experimenter is interested in a specific test using the Intellicage set up. Habituation and nose poke learning should still be performed prior to each test.

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Place Learning and Reversal:

- Place learning consists of 4 consecutive days in which each mouse has access to water in only one corner.
- After the four learning days, all of the mice are removed from the Intellicage for 72 hours.
- Mice are then returned to the Intellicage for a probe trial in which the number of visits to the correct corner are evaluated.
- After the probe trial is complete, animals are left in the cage for 24 hours with the settings used during place learning.
- Following this 24 hour reminder trial, animals are tested in reversal. A new corner is designated as the correct corner, and the animals are tested for 4 days with this new location.

Place Avoidance:

- For place avoidance, animals are trained to avoid a corner where they are met with an aversive stimulus in the form of an air puff.
- After 4 days for training, mice are removed from the Intellicage for 72 hours.
- They are then placed back in the apparatus and tested in a probe trial. There are no air puffs given during the probe trials.
- The percent visits to the previous punishment corner versus the other corners should be measured.

Data Analysis

Parameters of interest during include:

- Percent incorrect and correct visits
- Percent incorrect and correct nose pokes
- Number of errors and correct nose pokes
- General activity as measured by antenna crossings
- Latency to find water