



STANDARD OPERATING PROCEDURE

TITLE: Olfactory Habituation and Dishabituation

CATEGORY: Behavioral Assay

Introduction

Goal: This document aims to provide the reader information on how to run the Olfactory Habituation and Dishabituation Test. The test is useful for detecting deficits in olfactory functioning, memory, and social interest.

Materials:

- Subjects – any strain of mouse may be used.
- Apparatus – An empty cage, cotton swabs
- Accessories: Ovariectomized females (for urine), almond extract, vanilla extract (or other scented extracts of your choice), water, micropipette, stop watch.

Testing set up:

- To collect urine, place each ovariectomized female (OEF) in an empty cage with wire mesh on the bottom for 12 hours in their dark cycle. Collect urine with a pipette from the bottom of the cage. Place each mouse's sample in a different tube.
- Prepare a 1% extract solutions of each non-social scent. OEF urine is left undiluted.

General Testing procedures:

Non-Social Trials:

- Animals should be acclimated to the testing room an hour prior to testing.
- Data can be collected live or through video scoring.

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- Pipette 100 μ L of water onto one end of a cotton swab and place the treated end 1 cm above the cage bottom so that mice can explore the swab without rearing. Attach the cotton swab in manner that is compatible with the cage and lid being used.
- Each mouse should be given a 2 minute trial with a 3 minute inter-trial interval.
- Record the amount of time the mouse spends sniffing the cotton swab. Sniffing is defined as direct physical contact between the nose and the swab. Chewing does not count.
- Following the end of the 2 minute trial, place the mouse into a different cage to wait out the inter-trial interval. Prepare a new cotton swab in the same way as the first and replace the first swab. At the end of the three minute inter trial interval, place the mouse back into the testing cage and allow the mouse to explore the new swab for 2 minutes again.
- Repeat the process with a third cotton swab prepared identically to the first two.
- After three trials with water, run three trials using the same procedure with the almond solution.
- Run another three trials with the vanilla solution.

Social Trials:

- Run the social trials on the following day.
- Each social trial lasts for 2 minutes with a 10 minute inter trial interval.
- The odors should be presented in the same manner as the non-social ones. Each mouse should be given three trials with undiluted urine from the first OEF, followed by three trials with urine from a different OEF.

Data Analysis:

- Compare the sniffing times for each trial. Mice with normal olfaction and memory should spend less time investigating the same smell over each trial. There should be an increase in sniffing time each time a new smell is presented.
- Mice should spend at least 10 seconds investigating the swab in the first trial of each new smell. Shorter sniffing times may indicate issues with either the mouse or the scent sample.
- Time spent sniffing the swab treated with plain water gives a baseline measure with which to compare the sniffing of other odors.