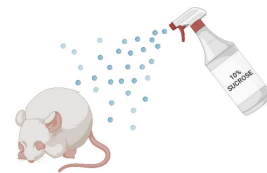


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Splash test in adult rats

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We use this protocol and it's working

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Disclaimer

None

Abstract

This is a protocol to describe the materials and methods utilized to perform the splash test for assessment of grooming behaviors as a measure of self-care in adult rats. The assessment uses an atomized spray bottle to coat the animal's back fur in a 10% sucrose solution before being placed into a small testing box for observation of grooming behaviors. Alterations in total time spent grooming, frequency of grooming bouts, or latency to begin grooming may suggest depression-like behavior or compulsive-like behavior, depending on the directionality of behavioral alterations.

The posting of this protocol is part of the mission of the PREclinical Interagency reSearch resourceE-TBI (PRECISE-TBI, precise-tbi.org) to improve clinical translation of therapeutics by providing an online catalog and standardized protocols to reduce the variability of model usage between laboratories.

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Materials

Ethovision XT [RRID:SCR_000441],
Atomized spray bottle,
sucrose,
distilled water,
matte black box (27.7 cm × 36.8 cm),
open field arena (80cm x 80cm),
timer,
towel,
camera

Pre-Testing Preparation

- 1 Prepare 150 mL of 10% (w/v) sucrose solution and fill atomized spray bottle with solution (see Note 1).
- 2 Place a small matte black box in the corner of an open field arena and tape the bottom of the testing box to secure it in place.
- 3 Prepare camera and Ethovision XT software prior to testing (see Note 2). The camera should be placed directly above the testing box, at a height where the entire testing area is clearly visible.
- 4 Turn off fluorescent overhead lights in the behavior room and turn on red lights prior to beginning the test.

General Protocol

- 5 Place the animal in the testing box to begin the habituation trial (see Note 3). Before leaving the room, begin camera recording on Ethovision XT and set a timer for five minutes (see Note 4).
- 6 After the five-minute habituation trial, reenter the behavior room.
- 7 Pick up the animal and using the atomized spray bottle, spray the 10% sucrose solution twice onto its back fur, then place the animal back in the testing box.
- 8 Immediately after placing the animal back into the testing box, begin the camera recording for the test trial and quickly exit the behavior room. Set a timer for ten minutes for this trial.
- 9 At the conclusion of the ten-minute test trial, reenter the behavior room and remove animal from testing box.
- 10 Wipe the animal's fur with a damp towel and dry off the animal before returning the rat to its home cage.
- 11 Clean the testing box and open field arena before testing a new animal.



- 12 Using Ethovision XT software, review video recordings of the test trials and manually score the start and stop times of all grooming bouts throughout the ten-minute trial period. Export grooming behavior metrics of interest, such as total time spent grooming, frequency of grooming bouts, and/or latency to begin grooming.

Notes

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Note 1: Combine 15 g sucrose with 150mL distilled water in the spray bottle and mix until dissolved.

Note 2: A behavior tracking system with camera recording is ideal for this assessment. Grooming behavior will be recorded on the Ethovision XT software while investigators step out of the room, and the videos will be manually scored using the software later. However, this assessment could be conducted without such software - with videos recorded on a standalone camera (ex. GoPro) and behavior scored by hand upon reviewing the videos.

Note 3: The habituation trial serves as an opportunity for the animal to become familiar with the testing room and testing box prior to beginning testing activities. This allows the animal to get acquainted with the testing environment before any assessments are conducted.

Note 4: If using Ethovision XT or similar software, set the duration of the trial recording prior to testing so that camera recording automatically ends at the conclusion of the trial.