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Open Field V.2

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Protocol status: Working

We use this protocol and it's working



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Abstract

Protocol for general locomotion in rodents using open field arenas and Ethovision video tracking.

Purpose: Assess cognitive, spontaneous motor activity, and anxiety

Note that there are many variations of this test. We perform this test during the mouse's dark cycle (i.e. their active phase).

Materials

- Open field boxes (usually we use 4 boxes and test 4 mice at a time) – measurement 40 cm x 40 cm – solid color (depending on mouse fur for contrast), not transparent
- 70% ethanol and water spray bottles for cleaning
- Med Associates Infrared Detection: <https://med-associates.com/product-category/operant-conditioning-general-behavior/infrared-detection/>
- Ethovision Software: <https://noldus.com/ethovision-xt>

Before start

Note that our lab performs this assay in the dark phase to reduce stress and to test animals during their active cycle. Our animals are kept on an inverted light cycle.

Protocol for spontaneous activity measurement:

- 1 *Days 1-2 – training*
The purpose of the training days is to reduce to the minimum the influence of cognitive and anxiety states on motor activity by acclimating the mice to the equipment. Additional training days can be added if preferred.
- 2 Acclimate mice to the behavior room for 1-2 hours before starting.
- 3 The mouse is gently carried and placed in the center of the open field (OF) box.
- 4 Let mouse explore the environment for 5-10 min (keep consistent across experiments).
- 5 Record motor activity using:
 - 5.1 A set of camera + Ethovision software OR
 - 5.2 Med. Associates infrared beam system.
- 6 At conclusion, return mouse to the home cage and clean OF boxes with water-EtOH-water sequence solutions. (Sequence is done to reduce strong odor of ethanol).
- 7 Start with another mouse, etc...
- 8 *Day 3 - testing*
- 9 Acclimate mice to the behavior room for 1-2 hours before starting.
- 10 The mouse is gently carried and placed in the center of the OF box.

- 11 Let mouse explore the environment for 30 min (keep consistent across experiments).
- 12 Record motor activity using a set of camera + Ethovision software.
 - 12.1 A set of camera + Ethovision software OR
 - 12.2 Med. Associates infrared beam system.
- 13 At conclusion, return mouse to the home cage and clean OF boxes with water-EtOH-water sequence solutions.
- 14 Start with another mouse, etc....
- 15 Main outcome measures:
 - Assess distance and time travelled/moved in the box within 30 min trial of day 3; resting time; vertical time and rearing episodes; ambulation in the periphery and center of the box; etc..
 - Comparison of movements (between periphery and center distance, time, center crosses) can be used as indication of anxiety behavior. We usually use the data from day #1.

Protocol for cognitive performance and anxiety:

- 16 *Days 1-2 - AM*
- 17 Acclimate mice to the behavior room for 1-2 hours before starting.
- 18 The mouse is gently carried and placed in the center of the OF box.
- 19 Let mouse explore the environment for 5-10 min (keep consistent across experiments).



- 20 Record motor activity using a set of camera + Ethovision software.
- 20.1 A set of camera + Ethovision software OR
- 20.2 Med. Associates infrared beam system.
- 21 At conclusion, return mouse to the home cage and clean OF boxes with water-EtOH-water sequence solutions.
- 22 Start with another mouse, etc...
- 23 *Days 1-2 – PM: After a trial interval time of~ 4 hours, repeat testing as above described.*
- 24 Main outcome measures:
 - Assess cognitive performance quantifying distance and time travelled/moved in the box within 5-10 min trials over the 4 trials. These should decrease over time in normal mice with no cognitive deficits as measure of learning capability.
 - Comparison of movements (between periphery and center distance, time, center crosses) can be used as indication of anxiety behavior. We usually use the data from trial #1, but performance over the 4 trials can also be compared.