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Protocol status: In development

We are still developing and optimizing this protocol

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Collection and Analysis of Mouse Open Field Activity

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ABSTRACT

The open field task is used to measure locomotor activity in mice. This protocol describes the setup of the task and the acquisition of open field data. It also covers the use of EthoVision Software to analyze recorded videos in order to calculate output velocity, distance traveled, time spent moving vs not moving, and clockwise vs counter-clockwise rotations.

MATERIALS

- Camera: Doric Behavior Camera (BTC_USB3.0)
- Tripod: Amazon Basics Tripod (Amazon Cat: B00XI87KV8)
- Computer with Ethoision software installed (RRID:SCR_000441)
- Adjustable racks: YOHKOH4-Tier(AmazonCat:B0BLCK26B9)
- Clear acrylic sheet (15”x35”), two acrylic cylinders (26” diameter), and two white lids to fit cylinders
- Large binder clips
- White printer paper
- Paper towels
- Clear or laboratory tape
- 70% ethanol

Keywords: ASAPCRN,
Mouse, Behavior, Open Field,
Analysis

Setup

1 1 hour prior to recording video, bring mice to behavior room to habituate.

2 Take two racks and place the clear acrylic sheet between them.

3 Clip the acrylic to the racks using large binder clips.

4 Tape 4 sheets of white paper together and wrap tightly around the outside of the cylinders and tape in place.

5 Place two cylinders on the acrylic. Use tape to mark locations of the cylinders to keep consistent between trials. Check that the acrylic is flat.

6 Roll up a paper towel and tape between cylinders to catch any urine between cylinders.

7 Place camera on tripod under the acrylic sheet. Ensure both cylinders are in view and in focus, are well lit, and that glares are minimized.

Video acquisition: Procedure for each mouse

8 Write wheel numbers (or mouse IDs) on Post-It notes, and place in corners of acrylic next to each

cylinder — check that this is visible in video frame.

9 Place one mouse into each cylinder and place white acrylic lid on top of cylinder. Do not run mice of the opposite sex together at the same time.

10 Record behavior for desired time.

11 After recording, return mice to their cages. Spray cylinder with 70% ethanol and thoroughly clean.

12 If paper around cylinders or paper between cylinders are soiled with mouse urine, replace.

13 Repeat for as many trials as necessary.

End of day

14 Spray cylinders with MB10/Vimoba, let sit for 10min, and thoroughly wipe down.

Analysis

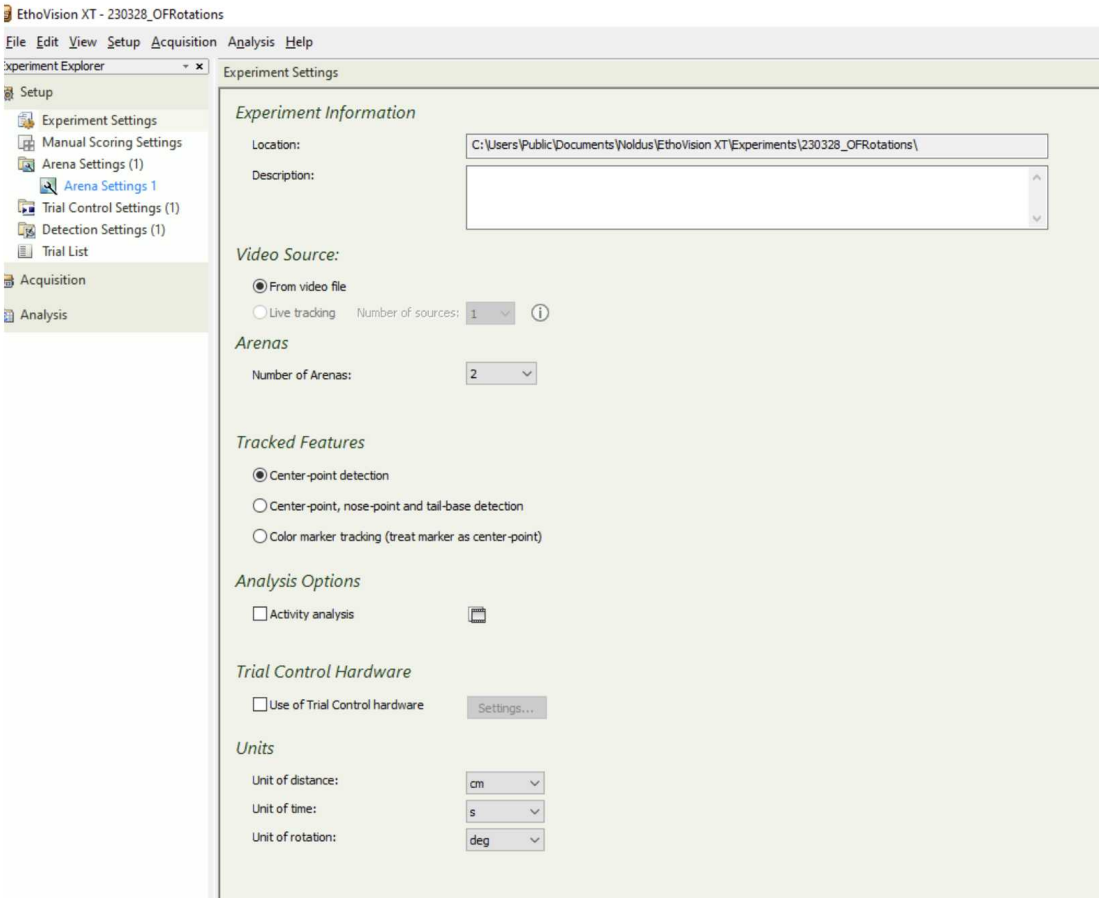
15 Record video data (see above).

16 Open EthoVision on PC.

17 Set the tracking settings on the 'Setup' menu

17.1 **Experiment Settings:**

- Video Source: from video file
- Number of Arenas: will change depending on experiment; e.g. 2 arenas for 2 cylinders
- Tracked Features: Center-point detection or multipoint detection
- All other settings: default



Screenshot of 'Experiment Settings' tab on EthoVision. These settings should be used to analyze a previously recorded video file.

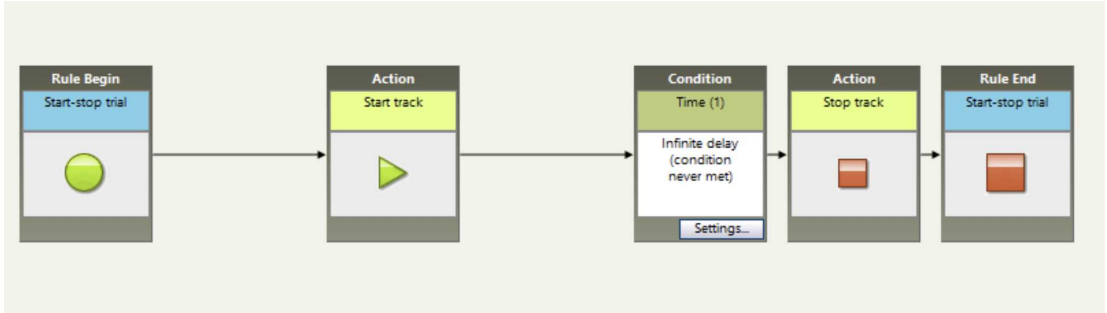
17.2 Skip Manual Scoring Settings

17.3 **Arena Settings**:

- Check Arena Settings for each video in case cylinders have moved. You can make a new Arena Settings by duplicating the previous settings.
- Select one video file and ‘Grab’ a background image; Ideally this image is taken when no mice are present, but it’s OK if mice are present
- Highlight Arena from popup window and use ellipse tool to draw Arena(s)
- Calibrate by clicking ‘Calibrate Scale’ and drawing a line across the diameter of the cylinder and entering the diameter of the cylinder in cm (e.g. 26 cm)
- Click ‘Validate Arena Settings’ to ensure settings are valid

Screenshot of 'Arena Settings' tab on EthoVision.

17.4 **Trial Control Settings**



Screenshot of "Trial Control Settings" tab on EthoVision.

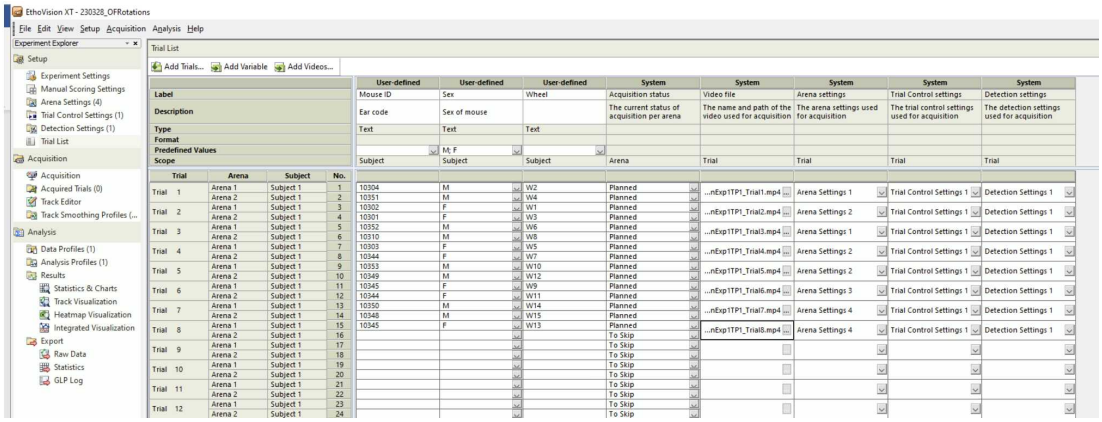
17.5 **Detection Settings** (for black mice with white background)

- Method: Gray scaling
- Video sample rate should match frame rate from video (e.g. 30 fps)

- Detection: Adjust range to highlight mouse in yellow and remove any orange background noise
- All other settings default

17.6 Trial List

- Copy and paste metadata (mouse ID, sex, wheel, etc.)
- Add appropriate number of trials.
- Assign appropriate video file, arena settings, trial control settings, and detection settings for each trial.
- Assign videos you want analyzed as ‘Planned’



Screenshot of 'Trial List' tab on EthoVision.

18 Run the video tracking using the 'Acquisition' tab

- Select ‘Track all planned trials’
- Check ‘Detection determines speed’
- Click green button to start

19 Analyze tracks using the 'Analysis' tab

19.1 Data Profiles

- If necessary, add a filter to reduce tracks so that only relevant trials are analyzed
- Add a ‘Time’ nest to restrict analysis to a consistent time frame; This is necessary if you record a few extra seconds
- If you want data broken into intervals (e.g. every 5 minutes), click ‘Result’ box, check ‘Results per time bin’ and set length; check ‘ignore last time bin if incomplete’ and ‘Apply to all results’
- These parameters can be easily changed and rerun once acquisition is complete

20 Analysis Profiles

- Select outcome measures; ‘Distance moved’ and ‘Velocity’ are pre-selected.

21 Results

- Statistics & Charts – click ‘Calculate’ to run analysis; click ‘Export Data’ to export Excel sheet. Export trial statistics, not group statistics.
- Track Visualization – can be used to see tracks in time bins; click camera icon to export images.