



Elevated Plus Maze

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Summary:

The y-maze evaluates assess anxiety behavior. The animal is placed on the + shaped apparatus (80 cm above the floor) with two open arms (no sides) and two closed arms (sides). The animal is allowed to freely explore the maze for the session length. The computer records position, area entries, movement speed, and duration. The preference for being in open arms over closed arms (expressed as either as a percentage of entries and/or a percentage of time spent in the open arms) is calculated to measure anxiety-like behavior.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Plus Maze	Noldus or others	n/a
Overhead red light lamps		
Camera (GigE or WebCam)	Bassler or Microsoft, etc	n/a
Tracking Software (EthoVision XT)	Noldus	
Lab Coat/Gloves/PPE		
Paper towels		
Disinfectant	Nolvasan 10%	

Protocol:

1. SET-UP (acclimation of mice, software setup)

- a. Acclimate mice **outside** the testing room 30 min prior to testing; record time of transfer.
- b. Turn the white lights off and turn on the red lights (60W indirect); set the Plus maze on the testing platform (80 cm above floor). **NOTE: the closed arms are oriented door to wall.**
- c. Open Ethovision software and the configuration file for the Plus maze test and set the maze so that the arms correspond to the software map file as follows:
 - Open Arms Up – Yellow
 - Open Arms Down – Yellow
 - Closed Arms Left – Blue
 - Closed Arms Right – Blue

Center - Red

SUPPLEMENTARY NOTES:

- If testing white mice, paint a black stripe painted from head to rump with washable animal marker. This insures accurate tracking of mice. If the experiment includes mice of various colors, all mice should go through the marking process even if they don't need the mark for tracking.
- Use balanced test order for the experiment. If more than one mouse per cage is going to be tested, place the mice in a clean cage after testing until all are tested, then return to home cage.
- The camera is mounted at an angle of approximately 90 degrees and centered to facilitate the best viewing conditions.

2. PROCEDURE

- If mice are individually housed, test one cage/one mouse at a time. If mice are group housed, take each individual mouse to be tested out of the home cage in the hallway and place in new cage for transport into the test room with the red light. Place on table in corner facing the apparatus. Acclimate animal 3-5 minutes while setting up computer and equipment. Do this for each mouse. Keep the home cage containing the other animals in the hallway!
- Click the "Subjects" icon on the top menu. A window labeled "Subjects Database" will pop up.
- The first line will default to "Subject_01." Click on the edit button (looks like a mouse with a pencil) at the top of the window. An "Edit Subjects" window will appear, and you can change the mouse ID, gender, etc.
- To add additional subjects, click on the icon that looks like a mouse with a green plus sign. Add all necessary information (Mouse ID, gender, etc.)
- Click on the "Scheduler" icon on the top menu.
- The left-hand side labeled "Experiment Subjects" lists all the subjects previously added.
- The right-hand side labeled "Trial Schedule" will which animals are still scheduled for a run (indicated by a green check mark next to the ID) and which ones have been completed (indicated by a red check mark next to the ID).
- When you are ready to acquire data, click the appropriate mouse ID in the "Trial Schedule" window. Click on the "Data Acquisition" icon on the top menu and the "Runtime Viewer" window will appear. Verify that the subject code is the correct animal you want to run.
- A second window will appear labeled "Time Control." This displays the latency for the run (e.g., 8 minutes) and a start/stop button control.
- When ready to begin data collection, hit the start button. When ready to begin data collection, retrieve mouse from its cage, manually click once on "Start" and immediately put the mouse on the center of the plus maze, facing a random corner (between an open arm and a closed arm at an angle). The tracking does not start until the mouse is in the maze, so timing is not critical. Make sure the tracking is working, then turn off the monitor and leave the room. In case of accidental fallings, manually cease the session and mark it down in the comments section of the test worksheet. Save the file as usual and return the animal to its home cage. Do not rerun the animal again.

- k. Once the trial is over, click the save button at the top of the program screen to save data. A “Save As” window will appear. Save the file with the appropriate name (e.g., EPM Cohort 1 07-07-15”).
- l. After each trial, make sure to click the save icon at the top of the screen. This will save all data currently collected for the session.

NOTE: Clean the Plus maze in between animals using 10% Nolvasan spray and paper towels.

3. DATA ANALYSIS

- a. In the Pan Lab SMART program, open the desired experimental file.
- b. Open the “Analysis” tab at the top menu bar. On the left side under “Trial Schedule” click and drag the trial(s) to be exported into the center window. Each trial will show up as a line of data in the center window.
- c. Under the “General Configuration” on the right side, click on the “Zone Definition” tab and find the exact .SMZ file used for testing (i.e., “Smart – PlusMaze 7-7-15.SMZ”). Highlight all trials in the center window and click the red check mark (next to the “zone definition”) to assign the selected zones to the selected trials.
- d. Under “Report Definition,” check the “Summary Report” tab and click on the icon on the right with 3 dots to manage report definitions. A new window named “Report Definitions” will open.
- e. Click on the pencil icon to edit selected reports. A new window will open named “Calculations to include in the Summary Report.”
- f. On the left side under “Available Calculations” there are several tabs of parameters to include in the data report. Under each of the tabs (listed below), highlight the parameters and click on the arrow icon (in the center) to move them to the “Included Calculations” window on the right side.
- g. Standard measures comprise the frequency of open and closed arm entries (arm entry defined as all four paws into an arm), total arm entries, and amount of time spent by the animal in open and closed sections of the maze, % open entries and % time spent in open and closed arms.

4. SUPPLEMENTARY INFO-SOFTWARE CONFIGURATION -SMART Tracking Software

- a. Select the “Standard” tab and select these parameters in this order so the data will output into the correct format for the master dataset.
 - A. Under the “Subject” tab:
 1. Subject Info
 - a. Subject name
 - B. Under the “Session” tab:
 2. Experimental Info
 - a. Exp. File Name
 - b. Exp. File Date
 3. Trial Info
 - a. Trial Date
 - b. Trial Duration (HH:MM:SS,00)
 - c. Trial Name

- d. Trial Session
 - e. Trial Time
 - C. Under the “Data” tab:
 - 1. Tracking Info
 - a. Distance in Zone (%)
 - b. Distance in Zone (sec)
 - c. Total Distance
 - d. Entries in Zone
 - e. Latency 1st entry into Zone (sec)
 - f. Resting Time in Zone (%)
 - g. Resting Time in Zone (sec)
 - h. Time in Zone (%)
 - i. Time in Zone (sec)
 - j. Fast Time in Zone (sec)
 - k. Fast Time in Zone (%)
 - l. Slow Time in Zone (sec)
 - m. Slow Time in Zone (%)
 - n. Max Speed in Zone
 - o. Min Speed in Zone
 - p. Mean Speed in Zone
 - q. Mean Speed w/o Resting in Zone
- b. Once all parameters have been added to the “Included Calculations” window, click the “accept” button and close the “Report Definitions” window.
- c. Highlight all trials in the center window and click the red check mark (next to the “Summary Report” tab) to assign the parameters to the selected trials.
- d. Make sure the “Full Trial” option is checked under the “Time Interval” section.
- e. Click the “Analyze” button at the bottom of the analysis window. Once the data is generated, a new window will open labeled “Export Preview.”
- f. Click the “Export” button at the bottom and an excel spreadsheet will be generated. Save this raw data file to the study folder and import the Excel tab into the master dataset file.