Y Maze test

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Ref: Holcomb L 1998 Nature Medicine

Summary:

The Y-maze evaluates memory and special learning in rodents though quantification of spontaneous alternations during an – 8 minute test in the maze (a measure of spatial cognition).

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
White acrylic Y maze (arm length: 35 cm; height: 20 cm)	Noldus or others	
Camera (GigE or WebCams)	Bassler or Microsoft, etc	
Tracking Software (EthoVision XT)	Noldus	
Animal marker -black	Stoelting	50451-1
Lab Coat/Gloves/PPE		
Paper towels		
Disinfectant	Nolvasan 10%	

Protocol:

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

a. Acclimate mice in the testing room at least one hour prior to testing; record time of transfer.
b. Turn the white lights off and turn on the four red lights (60W indirect); turn on the power strip to the camera.
c. Open Ethovision software and the configuration file for the Y maze test and set the maze so that the arms correspond (1, 2, 3) to the software map file. The arm borders should not include the shadow of the vertical wall or the last few cm. at the end of the wall (mouse length) where there is shadow.

> **SUPPLEMENTARY NOTES:**
> - The camera is mounted at an angle of approximately 90 degrees and centered to facilitate the best viewing conditions: Arm 1: red; Arm 2: yellow; Arm 3: green; Arm 4: blue (center).
> - The maze should be 2 feet above the ground and in the same room location for all mice.
> - 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.

2. PROCEDURE

a. Retrieve mouse from home cage, manually click once on "Start" and immediately put the mouse on the center of the Y maze, facing a random corner (between arms).
b. Close the door to the test room and watch the session in the anteroom on the computer. Monitor the recording session patiently and quietly. In case of accidental fallings, cease the session and mark it down in the comments section of the test worksheet.
c. The program stops after 8 minutes as a pre-set automatic function. When the recording ends automatically, a screen will show up to acquire data input (add Mice ID, Experimenter name). Save file in proper folder.
d. Remove mouse from maze and return to home cage. Clean the Y maze in between animals using Nolvasan and paper towels to dry.

3. ANALYSIS (Ethovision)

In the analysis profile the total number of arm entries must be counted, which is a measurement of activity and locomotion during the testing session and is also be used to calculate the percentage of alternation. Alternation is defined as successive entries <u>into the three arms</u>, of which the order does not matter, but that an alternation is only achieved in a *set* of <u>three different arm</u> entries. For example, successive entries into arms a, c and b is considered an alternation, while entries into arms a, b, and a again is not considered an alternation. This measure of spontaneous alternation can be expressed as an absolute number, or as a percentage, and is a measure of short term spatial memory.

$$\text{Spontaneous alternation (\%)} = \frac{\text{no. of spontaneous alternations}}{\text{total number of arm entries - 2}} \times 100$$